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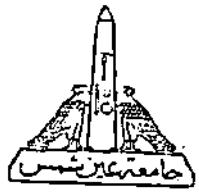

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EFFECTS ON THE MICELLAR SOLUBILIZATION OF ORGANIC COMPOUNDS BY SURFACTANT MICELLES. II. NATURE AND POSITION OF SUBSTITUENT GROUPS IN AROMATIC ACIDS.

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ABSTRACT

The semiequilibrium dialysis method has been used to investigate the equilibrium solubilization of o-, m- and p-toluic acids by 0.1N HCl aqueous solutions of the cationic surfactant 1-hexadecylpyridinium chloride (cetylpyridinium chloride), throughout a range of concentrations of the toluic acids and surfactant. Values of the apparant solubilization constant, K, of the neutral acids have been correlated with mole fractions of the acid in the micelle X_A, where $K = X_A$ /[monomeric acid]. The activity coefficients of both acid and surfactant were obtained, consistent with the Gibbs-Duhem equation.

Several conclusions can be drawn from the results: (a) For each of the three toluic acid - cetylpyridinium chloride systems, K is found to vary nearly linearly with X_A, throughout the investigated range of acid concentration. (b) The presence of both the methyl and carboxyl groups as substituents in the benzene ring

enhances the solubilization much more than the additive effect of both groups, when present separately. (c) The position of the substituent groups with respect to each other, will affect the extent of solubilization of the investigated acids. (d) The difference in the values of K for the three acids show a contradiction to a group contribution model developed recently.

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INTRODUCTION

The solubilization of organic compounds in surfactant micelles has been studied utilizing various techniques such as vapor pressure (1), gas chromatography (2), nmr-spin-echo technique (3), micellar enhanced ultra filtration (MEUF) and the semiequilibrium dialysis method (SED) which has been recently developed and used extensively (4-9). Some important factors are known to influence the extent of solubilization as, for example, bulk solution effects (10,11), possible Laplace pressure effects (12,13), and the effect caused by the interaction between the electrical potential at the ionic micellar surface and polar or polarizable nonelectrolyte molecules solubilized within the micelle (14). In many applications of micellar- based separation methods, the concentration of organic solutes in micelles may be expected to be relatively small. Thus, it will be very helpful to be able to predict the partition coefficient of organic molecules in aqueous surfactant micelles (the solubilization constant).

As a contribution to the accumulating information about the solubilization of a variety of organic compounds in different surfactants, we investigated in Part I of this series (15) the effect of inserting a $(CH2)_n.COO$ group between the hydrogen and the phenyl group of benzene, on the extent of solubilization of benzene in the micelles of hexadecylpyridinium chloride (CPC). The study enabled also the comparison of the solubilization of the three acids. To add still more to the accumulating information of this subject, we studied, in the present work, the solubilization of o- , m- , and p- toluic acids in CPC. Several conclusions could be drawn from the results of such a study when compared with previous results obtained for toluene and for

benzoic acid, such as the effect of the nature and position of the substituent groups present on the extent of solubilization. Also, the results of the present study could be used to test the applicability of a new approach made recently (16) attempting to predict the value of the solubilization constant, based on a group contribution model depending only on the nature of the organic compound and on the groups present as substituents or branches.

EXPERIMENTAL

The SED technique has been described in detail previously (4). A 0.1M HCl solution containing known concentrations of the surfactant and solute was placed on one side of the membrane (retentate), and the other side (permeate) contained 0.1M HCL solution. The initial surfactant concentration is chosen to be much higher than the critical micelle concentration (cmc), to ensure that most of the surfactant micelles are present in micellar form. The HCl solution is used instead of an aqueous solution to reduce the concentration of free hexadecylpyridinium ion in the retentate solution, and also to supress the ionization of the organic acids used, hence, minimizing any complications in the final computations. Equilibrium is usually reached within 18-24 hours, at which time the activity of free organic solute is the same in the retentate and permeate.

Molalities were used to assure maximum accuracy in final calculations. The initial molality of the organic solute in the retentate varied from 0.0103 - 0.129 for o-toluic acid (OTA), 0.0102 - 0.1022 for m-toluic acid (MTA) and 0.0109 - 0.1135 for p-toluic acid (PTA). The initial CPC concentration was 0.05 to 0.2 molal, The cmc of CPC is 0.00080M (17).

All organic acids used (Aldrich 99.5%) and hexadecylpyridinium chloride monohydrate (Hexcel), were used as received.

The permeate solution was analyzed using UV spectroscopy. Extinction coefficients (absorptivities) were determined at 10 nm intervals across the major CPC, OTA, MTA, and PTA absorbance peaks, assuming that Beer's law applies to the very dilute solutions of the individual components. The concentrations of the organic solute and CPC in the permeate were calculated simultaneously, using a nonlinear least squares computer program (4). Retentate concentrations were obtained by correcting the original concentrations for the small amount of solute transferred into the permeate side.

DATA ANALYSIS AND RESULTS

The detailed methods of analysis of the data obtained were given previously (5, 7-9). The ionization of the acids has been neglected, since it was minimized by using 0.1M HCl, as mentioned before. The relationships leading to the calculation of the solubilization constants of the organic acids used in CPC, and the calculation of the activity coefficients of the organic acids and surfactant can be summarized in the following equations.

$$[A]_{tot} = \gamma_A X_A c_A^\circ + X_A/(1-X_A)\,[CPC]_{mic} \quad (1)$$

$$[CPC]_{tot} = \gamma_{CPC}(1-X_A)\, c_{CPC}^\circ + [CPC]_{mic} \quad (2)$$

$$K = X_A / c_A \quad (3)$$

$$K = K_o (1-b X_A) \quad (4)$$

$$\gamma_A = 1 / (K c_A^\circ) \quad (5)$$

$$\gamma_A = a / (1 - b X_A) \quad (6)$$

$$\ln\gamma_{CPC} = \{1/(1-b)\}\{b \ln(1-X_A) - \ln(1-b X_A)\} \quad (7)$$

$$K = (1-X_A)\{[A]_{tot}^{ret} - [A]_{tot}^{per}\} / \{[A]_{tot}^{per}[CPC]_{mic}^{ret} - [A]_{tot}^{ret}[CPC]_{mic}^{per}\} \quad (8)$$

$[A]_{tot}$ and $[CPC]_{tot}$ denote the total concentration of the organic acid and surfactant, respectively. $[CPC]_{mic}$ is the molar concentration of the surfactant in the micelle, X_A is the intramicellar mole fraction of organic acid, and γ_A and γ_{CPC} are activity coefficients based on the pure component standard state for the organic acid and the pure micelle standard state for the surfactant. c°_A is selected to approximately equal to the concentration of monomeric organic acid at which the partial pressure or fugacity of acid is equal to that of pure acid at the same temperature. c°_{CPC} is the concentration of monomeric surfactant in the bulk phase in the absence of added organic solute.

Table I lists all of the primary results for the o-, m- and p-toluic acid solutions in CPC. The first 2 columns list the experimental values for both acid and surfactant concentrations in the retentate solution. Columns 3 and 4 list the values of the acid and surfactatnt concentrations in the permeate solution. The last column lists the value of the concentration of the acid in the permeate solution, as predicted from the model.

Table II lists the values of the least-squares parameters (Ko and b) derived by fitting all of the $[A]^{per}_{tot}$ data for a given toluic acid with the above mentioned model. The table also includes values for c°_A and c°_{CPC} used in the analysis. The relative root-mean-square deviations tabulated are those in $[A]^{per}_{tot}$ for each system.

Figures 1-3 show the dependence of γ_A and γ_{CPC} on X_A for the three CPC-toluic acid systems. The 3 figures are quite similar in shape, γ_A increases with increasing the acid mole fraction into the micelle, whereas γ_{CPC} decreases in the same direction.

Figures 4-6 are plots of K against X_A, the straight lines corresponding to the values of K and b for each system. The points indicate values of K calculated point by point by a method similar to that described previously (5), making the assumption that the value of X_A in the permeate solution is equal to that in the retentate.

DISCUSSION AND CONCLUSIONS

The most important factors influencing the extent and region of solubilization of organic solutes into surfactant micelles, are refered to in the "Introduction", and can be summarized as:

1- Bulk solution effects

2- Possible Laplace pressure effects

3- The effect caused by the interaction between the electrical potential at the ionic micellar surface and polar or polarizable nonelectrolyte molecules solubilized within the micelle.

The expected effects of these factors have been discussed previously (7, 15, 18), explaining the assumed structure of the ionic micelles, and the subsequent effect of this structure on the solubilization of different organic compounds. Accordingly, benzene, the parent compound of the substances investigated in the present study, is distributed, when present as the organic solute, almost uniformly throughout the surface and core regions of the CPC micelle (19).

In Part I of this series (15), it was found that K for benzoic acid is considerably larger than that for benzene (72 M^{-1} as compared with 40M^{-1}), which was attributed to the fact that the -COOH group is intensily charged and capable of hydrogen bonding with water, together with considering the 3 factors mentioned above. Also, when a methyl group, -CH3, is introduced into benzene, in toluene, the aliphatic character in the molecule is increased, in addition to the already present aromatic character of the benzene molecule, resulting in an apparent alteration of the 3 factors, such that K for toluene is much higher than that for benzene (8), (125 M^{-1} as compared with 40 M^{-1}).

In the present study, the compound used contains a carboxylic acid group and a methyl group, both introduced in the same benzene ring (toluic acid), where its o-, m- and p- substituents were investigated.

It is clear from Table II that the values of the apparent solubilization constant K_s for the 3 acids are all higher than that for either benzene or toluene. This indicates that the presence of both groups, as substituents into the benzene ring at the same time, not only does not contradict the effect of either of them on the factors influencing the solubilization, but actually enhances the solubilization to a larger extent than the algebric addition of the separate effect of both the -COOH and -CH3 groups with respect to the benzene molecule.

In comparing the values of K for the three acids, as can be seen from Table II, it can be clearly seen that the values of K vary in the order m->p->o- substituents. The lowest value for the o- substituent can be explained on the basis that the presence of the -COOH and -CH3 groups, ortho to each other, will impose some steric hinderence of one on the other, thus decreasing the expected effect for each group, when present separately, on the extent of solubilization. The fact that the value of K for the p-substituent is less than that for the m-substituent, although there is no steric hinderence in both, can be explained on the basis that when the two substituent groups are present 'para' to each other, there tends to be a competing effect between surface solubilization due to the -COOH group, and core solubilization due to the -CH3 group, resulting in the noticed lower value for K than that for the m-substituent, where there is neither the steric effect nor the competing effect of the substituent groups.

The apparent difference in the value of K for the three acids is in clear contradiction to a group contribution model developed recently (16). According to this model, all 3 values of K should be, theoretically, the same since the model is based on the assumption that the value of K is dependent only on a certain contribution from the groups present, according to their nature and irrelevant of their position. A similar contradiction to the model was also shown in Part I of this series, where, according to the model, values of K were expected to differ because the substituents are different, but the results showed that the K values are almost the same, indicating that the extent of solubilizaton will definitely depend on several factors based on both nature and position of the substituents.

It can be generally seen, from Figs. 1-6, that, for all three toluic acids, the solubilization constants decrease, and the activity coefficients increase, as the mole fraction of the acid solubilized in the micelle increases. This is in accordance with previous results for highly polar and aliphatic solutes, solubilized by ionic surfactant micelles (5-9, 15).

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Table I. Experimental and Calculated Values of Toluic Acid Concentrations in the Permeate for Known Retentate Solutions Containing CPC and Acid.[a]

o-Toluic Acid

Retentate Solution		Permeate Solution		
[A]	[CPC]	[A]	[CPC]	$[A]_{pred.}$[b]
1.2876E-01	2.2185E-01	3.3736E-03	8.6239E-05	3.5368E-03
1.2887E-01	2.2185E-01	3.2479E-03	7.9830E-05	3.5407E-03
6.4434E-02	1.1349E-01	3.1397E-03	0.0000E+00	3.3475E-03
6.4590E-02	1.1349E-01	2.9836E-03	0.0000E+00	3.3568E-03
5.9827E-02	1.1097E-01	2.9743E-03	5.6945E-05	3.1522E-03
5.9854E-02	1.1098E-01	2.9483E-03	5.1155E-05	3.1535E-03
2.7838E-02	5.3831E-02	2.6304E-03	3.4645E-05	2.8308E-03
2.7835E-02	5.3836E-02	2.6334E-03	2.9494E-05	2.8302E-03
1.1015E-01	2.1708E-01	2.8257E-03	0.0000E+00	3.0006E-03
1.1021E-01	2.1905E-01	2.7689E-03	3.3370E-05	3.0029E-03
5.3449E-02	1.0864E-01	2.5774E-03	0.0000E+00	2.8396E-03
5.3445E-02	1.0863E-01	2.5812E-03	1.5236E-05	2.8398E-03
5.0312E-02	1.0594E-01	2.4549E-03	4.3225E-05	2.7293E-03
5.0320E-02	1.0588E-01	2.4475E-03	0.0000E+00	2.7275E-03
1.9210E-02	4.2859E-02	2.1492E-03	1.4885E-06	2.3509E-03
1.9226E-02	4.2860E-02	2.1329E-03	0.0000E+00	2.3530E-03
8.6024E-02	2.2079E-01	1.9654E-03	5.4336E-05	2.2587E-03
8.6033E-02	2.2080E-01	1.9563E-03	4.5223E-05	2.2590E-03
4.2293E-02	1.1100E-01	1.9386E-03	2.2083E-05	2.1435E-03
4.2308E-02	1.1099E-01	1.9235E-03	3.1197E-05	2.1446E-03
6.1099E-02	2.2288E-01	1.3986E-03	7.4127E-05	1.5445E-03
6.1109E-02	2.2288E-01	1.3895E-03	6.9170E-05	1.5447E-03
2.8082E-02	1.0476E-01	1.2916E-03	2.7510E-05	1.4636E-03
4.3658E-02	2.1674E-01	1.0603E-03	1.1233E-04	1.1139E-03
2.0106E-02	1.0209E-01	9.5785E-04	6.9573E-05	1.0563E-03
2.0106E-02	1.0210E-01	9.5734E-04	6.0634E-05	1.0563E-03
2.0510E-02	2.1033E-01	4.5237E-04	9.6693E-05	5.2558E-04
2.0492E-02	2.1032E-01	4.7047E-04	1.0724E-04	5.2514E-04
1.0444E-02	1.0918E-01	4.3899E-04	6.4735E-05	5.0314E-04
1.0391E-02	1.0919E-01	4.4391E-04	6.1587E-05	5.0051E-04

[a] All concentrations in $mol\text{-}l^{-1}$

[b] Calculated results obtained by fitting the activity coefficient with equations 5 & 7, using constants in Table II.

m-Toluic Acid

Retentate Solution		Permeate Solution		
[A]	[CPC]	[A]	[CPC]	$[A]_{prod.}^{b}$
6.0672E-02	1.0608E-01	2.1718E-03	5.2337E-05	2.3042E-03
2.9406E-02	5.3030E-02	1.9730E-03	5.0245E-05	2.1324E-03
2.9453E-02	5.3031E-02	1.9252E-03	2.9002E-05	2.1363E-03
1.0217E-01	2.3602E-01	1.4580E-03	1.1923E-04	1.7225E-03
1.0214E-01	2.3600E-01	1.4858E-03	1.3877E-04	1.7221E-03
4.4031E-02	1.0338E-01	1.3711E-03	8.1008E-05	1.6522E-03
4.4018E-02	1.0339E-01	1.3840E-03	7.3743E-05	1.6515E-03
8.8854E-02	2.2120E-01	1.3055E-03	1.0754E-04	1.5836E-03
8.8821E-02	2.2116E-01	1.3397E-03	1.4185E-04	1.5832E-03
4.3217E-02	1.0915E-01	1.2773E-03	6.5946E-05	1.5280E-03
4.3219E-02	1.0914E-01	1.2759E-03	7.0792E-05	1.5281E-03
6.3776E-02	2.2923E-01	9.8033E-04	1.2429E-04	1.1133E-03
6.3988E-02	2.2024E-01	9.7732E-04	1.3620E-04	1.1135E-03
3.0951E-02	1.0841E-01	1.0329E-03	8.5278E-05	1.0722E-03
3.0985E-02	1.0842E-01	9.9873E-04	7.6905E-05	1.0734E-03
4.4084E-02	2.1958E-01	6.8983E-04	1.2254E-04	7.5253E-04
4.4092E-02	2.1958E-01	6.8125E-04	1.2232E-04	7.5269E-04
2.3382E-02	1.1792E-01	6.6701E-04	8.5341E-05	7.3151E-04
2.3397E-02	1.1793E-01	6.5237E-04	7.7548E-05	7.3194E-04
2.1191E-02	2.1639E-01	2.9095E-04	1.1593E-04	3.5797E-04
2.1189E-02	2.1639E-01	2.9362E-04	1.1797E-04	3.5773E-04
1.0201E-02	1.0555E-01	2.7970E-04	7.3614E-05	3.4687E-04
1.0201E-02	1.0556E-01	2.7931E-04	6.9182E-05	3.4686E-04

[a]All concentrations in $mol\text{-}l^{-1}$

[b]Calculated results obtained by fitting the activity coefficient with equations 5 & 7, using constants in Table II.

Table 1. (continued)

p-Toluic Acid

Retentate Solution		Permeate Solution		
[A]	[CPC]	[A]	[CPC]	$[A]^{b}_{pred.}$
5.9056E-02	1.0439E-01	2.3729E-03	9.7068E-04	2.0995E-03
5.9066E-02	1.0490E-01	2.3816E-03	9.8480E-04	2.0988E-03
2.8073E-02	5.1118E-02	2.0769E-03	8.3285E-04	1.9853E-03
2.8016E-02	5.1095E-02	2.1340E-03	8.5599E-04	1.9834E-03
1.1348E-01	2.2861E-01	2.1136E-03	9.4812E-04	1.9088E-03
1.1347E-01	2.2862E-01	2.1162E-03	9.3232E-04	1.9087E-03
5.1662E-02	1.0566E-01	2.0360E-03	9.8157E-04	1.8507E-03
5.1741E-02	1.0583E-01	1.9575E-03	8.1530E-04	1.8507E-03
8.7189E-02	2.2224E-01	1.5006E-03	6.7697E-04	1.5421E-03
8.7191E-02	2.2225E-01	1.4982E-03	6.6713E-04	1.5421E-03
4.3473E-02	1.1283E-01	1.4754E-03	6.1475E-04	1.4933E-03
4.3416E-02	1.1237E-01	1.5064E-03	6.2413E-04	1.4969E-03
6.2199E-02	2.1447E-01	1.1768E-03	5.9241E-04	1.1652E-03
6.2206E-02	2.1451E-01	1.1692E-03	5.5409E-04	1.1651E-03
3.0466E-02	1.0661E-01	1.1227E-03	5.0697E-04	1.1291E-03
3.0463E-02	1.0662E-01	1.1252E-03	4.9463E-04	1.1289E-03
4.2103E-02	2.1457E-01	7.4325E-04	4.1847E-04	8.0511E-04
4.2094E-02	2.1457E-01	7.5193E-04	4.1024E-04	8.0495E-04
2.1863E-02	1.1292E-01	7.1352E-04	3.5840E-04	7.8198E-04
2.1863E-02	1.1294E-01	7.1295E-04	3.3595E-04	7.8185E-04
2.1994E-02	2.1620E-01	3.4920E-04	2.4446E-04	4.2658E-04
2.1984E-02	2.1619E-01	3.5957E-04	2.6244E-04	4.2640E-04
1.0947E-02	1.0915E-01	3.4302E-04	2.2256E-04	4.1300E-04
1.0945E-02	1.0916E-01	3.4533E-04	2.1921E-04	4.1291E-04

[a]All concentrations in $mol\text{-}l^{-1}$

[b]Calculated results obtained by fitting the activity coefficient with equations 5 & 7, using constants in Table II.

Table II: Least Squares Parameters for Toluic Acids in 1-Hexadecylpyridinium Chloride at 25°C.

Solute	$K_o(M^{-1})$[a]	b[b]	C_A^o	C_{CPC}^o	10^{-5} RMSD[c]
o- Toluic Acid	185	1.244	0.0475	0.88 mm	4.768 $mol\text{-}l^{-1}$
m- Toluic Acid	275	1.241	0.0261	0.88 mm	6.542 $mol\text{-}l^{-1}$
p- Toluic Acid	229	0.754	0.0573	0.88 mm	3.298 $mol\text{-}l^{-1}$

[a]Intercept of a plot of the solubilization constant, K, vs. the mole fraction of acid in the micelle.

[b]Parameter in equation 4.

[c]Root mean square deviation in acid concentration in the permeate solution, fitted with model described.

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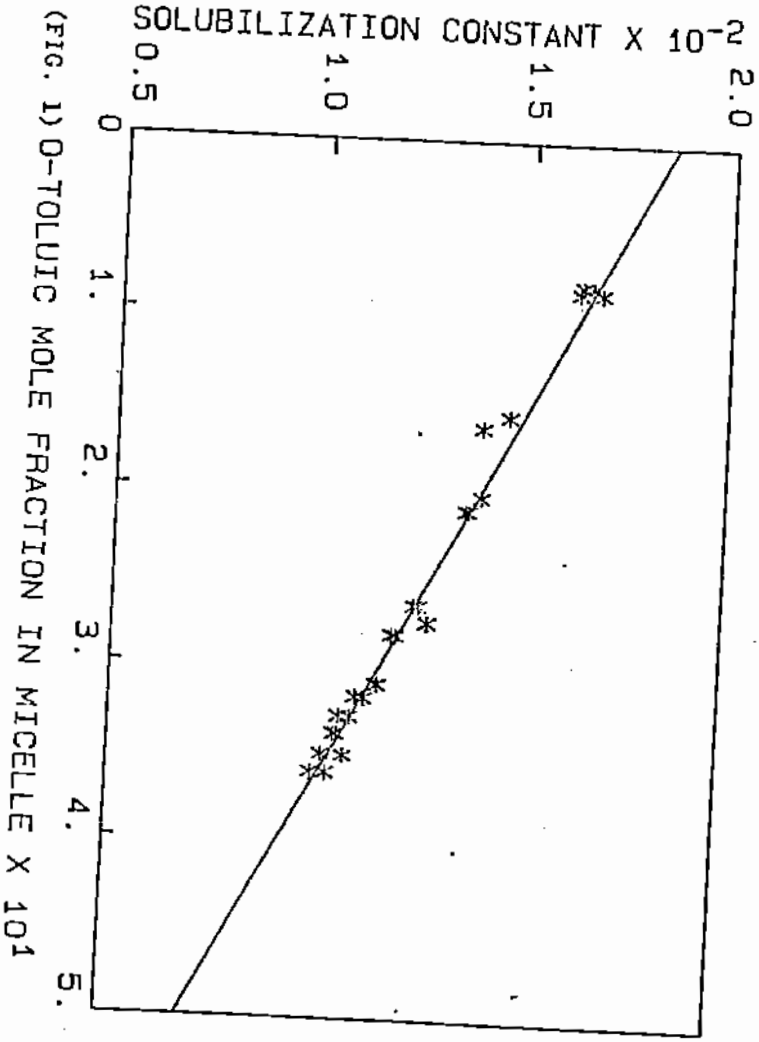
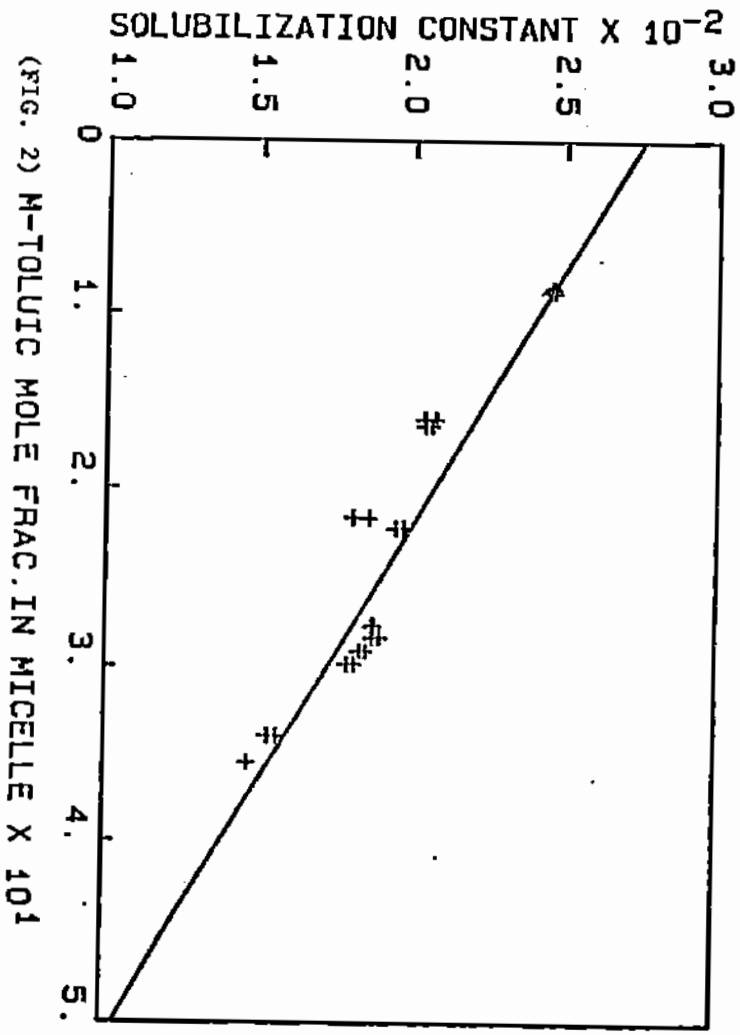

SOLUBILIZATION CONSTANT X 10^{-2}
0.5
1.0
1.5
2.0
0
1.
2.
3.
4.
5.
(FIG. 1) O-TOLUIC MOLE FRACTION IN MICELLE X 10^{1}


SOLUBILIZATION CONSTANT X 10^{-2}
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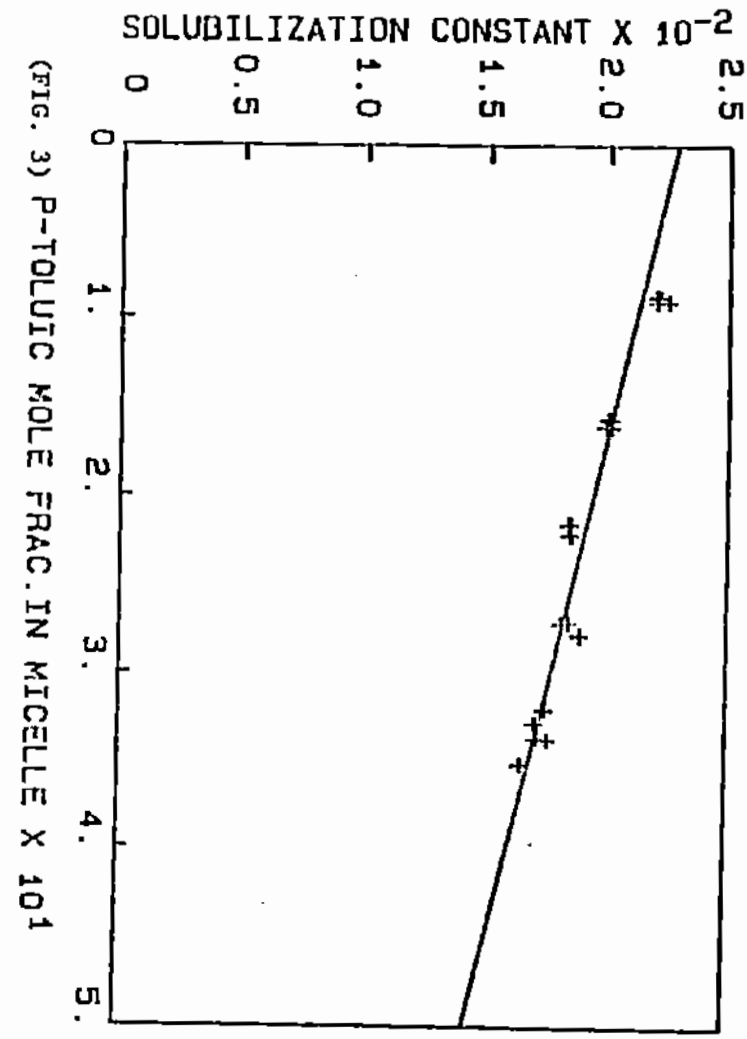
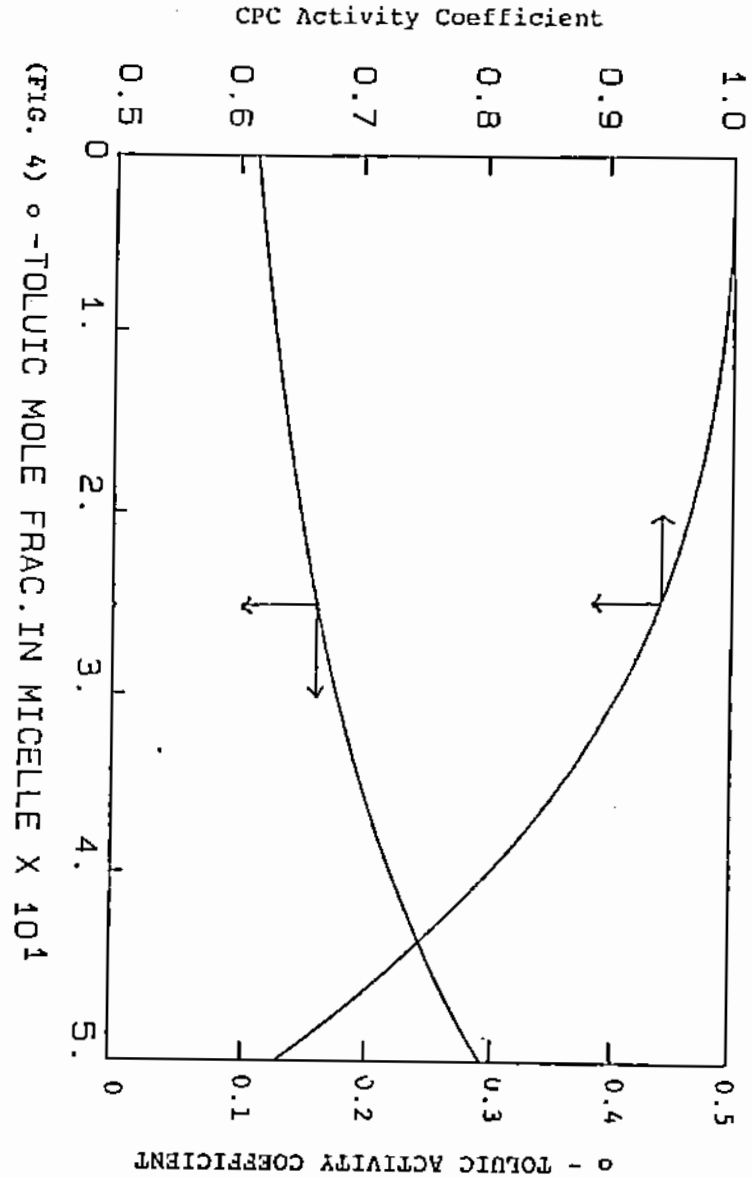
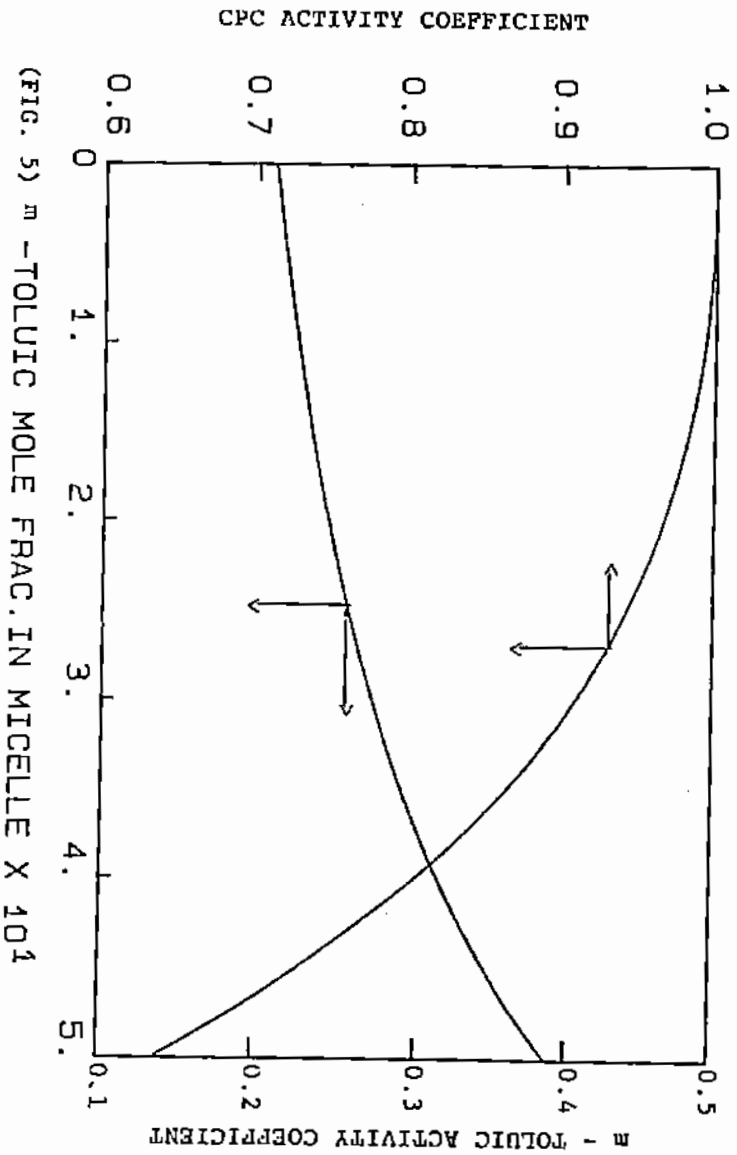
1.5
2.0
2.5
3.0
0
1.
2.
3.
4.
5.
(FIG. 2) M-TOLUIC MOLE FRAC. IN MICELLE X 10^{1}

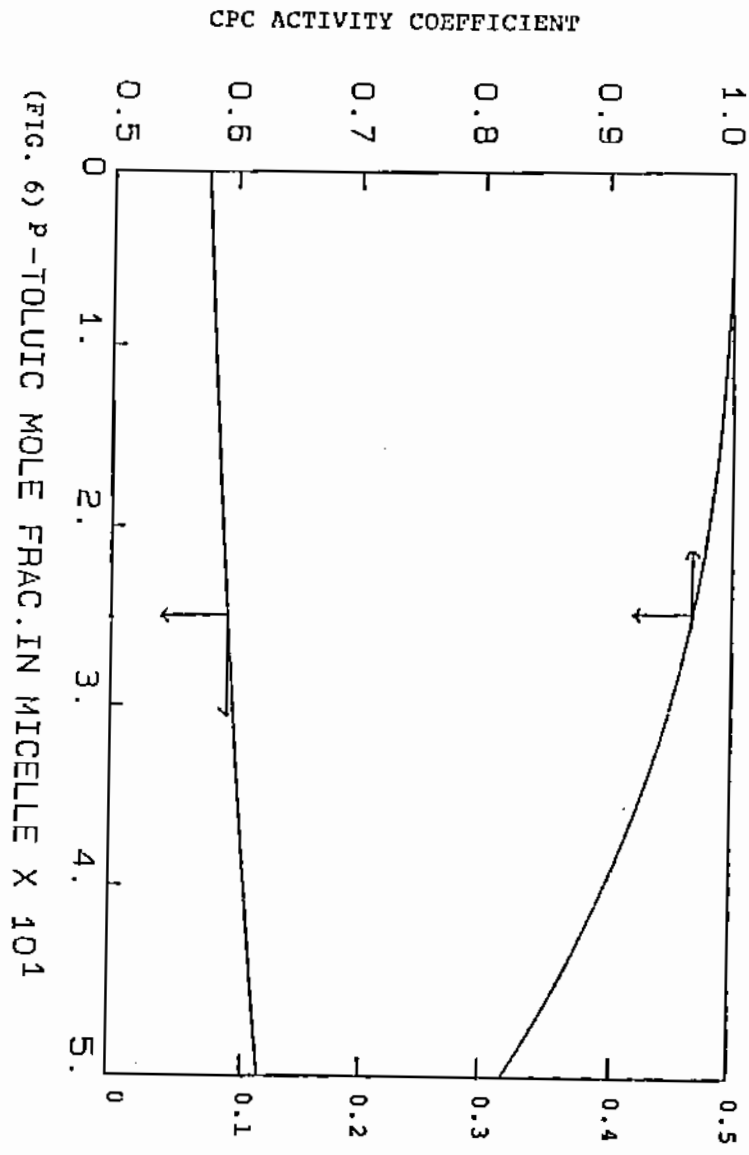

SOLUBILIZATION CONSTANT X 10^{-2}
0
0.5
1.0
1.5
2.0
2.5
0
1.
2.
3.
4.
5.
(FIG. 3) P-TOLUIC MOLE FRAC. IN MICELLE X 10^{1}



(FIG. 4) o -TOLUIC MOLE FRAC. IN MICELLE X 10¹



(FIG. 5) m -TOLUIC MOLE FRAC.IN MICELLE X 10¹



(FIG. 6) P-TOLUIC MOLE FRAC.IN MICELLE X 10¹

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بسم الله الرحمـــن الرحـــــيم

العوامل المؤثرة على اذابة المركبات العضوية بواسطة المبلات

٢ ـ تاثير طبيعة ومكان المجموعات المستعاضة في الاحماض العضويـــة

د٠ وفاء صلاح حجـــازى ، د٠ فائق محمد زكريـــا
قسم الكيمياء ـ كلية البنـات ـ جامعة عين شمـــــس

يتناول هذا البحث استخدام طريقة الديلزة شبة المتزنة لتعيين ثوابت الاذابة لاحماض " الاورثو ، والضياوالبارا تولويك " فى المحاليل المائية المحضة للمنشط السطحـــى كلوريد الهكساديكـــل بريديتيم ٠

وقد أجريت هذه الدراسة على تركيزات مختلفة من الاحماض وأمكن استنتاج علاقة بـــين ثوابت الاذابة للاحماض المختلفة وبين تركيزاتها فى المبلات التكوسه وهذه العلاقة تكاد تكـــون خطية بالنسبة لمدى التركيزات المستخدمة٠

كما أمكن ايضـا الوصول الى النتائج التاليـــة :

١ ـ أن وجود مجموعة الشبيل والكربوكسيل معا على حلقة البنزين فى هذه الاحماض يزيـد اذابتهـا بدرجة اكبر من مجموع تأثيرهما منفردين ٠

٢ ـ مكان تواجد كل من المجموعتين المذكورتين بالنسبة للاخرى يؤثر على درجة الاذابه للاحمـــاض المعنيـــة ٠

٣ ـ الفرق بين قيم ثوابت الاذابه للاحمـاض الثلاثة تظهر بعض التعارض مع الاتجاه المتوقع تبعـــا لنموذج نظرى حديث بنى على اساس مساهمـة المجموعات الموجودة فى الجزئ بدون اجراء تجارب عملية٠

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ELECTROCHEMICAL PARAMETERS FOR Mo ALLOYED STEEL IN CHLORIDE SOLUTIONS

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ABSTRACT

The electrochemical parameters for a type of Mo alloyed stainless steel (containing 3.15%) was studied in chloride solutions (0.1 - 5.0 M) at different pH values and 30°C. The results show that the electrochemical parameters were dependent on the concentration and pH of the test solution.

The Mo alloyed steel containing 3.15% Mo can be passivated in chloride solutions having molarity from 0.1 to 3.0 M at pH from 1.0 to 3.0 and the passive range become wide with increase of pH value. The alloys dissolve actively from their corrosion potentials, and can not passivated in 5.0 M chloride solution at all.

The scanning rate of which the potential of the alloy is varied, dV/dt has a considerable influence on the shape of the polarization curve. E_{corr} and E_c decrease and E_{pp}, I_{crit} and I_{pit} increase with increasing dV/dt.

INTRODUCTION

The electrochemical behaviour of Mo alloyed steel in sulphate and sodium chloride solutions were previously studied in this laboratory[1-6]. The literature contains contraversal results to pitting potentials and corrosion parameters[2,7,8-20]. Because of the well established usage of molybdenum alloying for increasing the resistance of steinless steel to pitting corrosion interest

was developed in extending potentiodynamic study of the 3.15% Mo steel containing 16.98% Cr and 11.29% Ni in sodium chloride solutions (0.1 - 5.0 M) at different pH values at 30°C. The effect of the rate of which the potential of the alloy is varied, dV/dt on the corrosion parameters of the alloy has been also studied. The chemical composition of the steel used is shown in Table 1.

Table 1. Composition of steel.

Element	C	S	Cr	Ni	Mo	Mn	Si	P	Cu
wt%	0.052	0.014	16.98	11.29	3.15	1.66	0.40	0.022	-

EXPERIMENTAL

The steel is produced by Avesta (Sweden) in the shape of rods with a diameter of 20 mm. It was heated for 20 min at 1050°C and then was quenched in water.

PRETREATMENT OF SPECIMEN

Circular specimens of 15 mm ϕ were embeded in a plastic resin ground with emery paper and polished with diamond paste (1 um). Finally the specimens were cleaned in bidistilled water and ethyl alcohol.

To avoid crevice corrosion Glyptal Protecting Lacque was applied directly after polishing around the edges of the specimens (1 mm around the edges).

The specimens were prepolarized in the actual NaCl solutions (from 0.1 to 5.0MNaCl)at different pH values (from 1.0 to 3.0) at -700 mV_{SCE} for 1 hr.

CONDITIONS AND PROCEDURE

Experiments were carried out at 30°C in sodium chloride solutions with concentration varying from 0.1 to 5.0 M. at different pH (from 1.0 to 3.0). The cell was continously

purged with purified nitrogen to get rid of oxygen. The reference electrode was a saturated calomel electrode, connected by a salt bridge and Haber Lyggin Capillary approximately 0.5 mm from the specimen surface. The specimens were placed in a specimen holder as shown in Fig. 1. The counter electrode was a sheet of Pt placed in a separate cell containing the same used electrolyte connected with the measuring cell by a salt bridge.

For potentiodynamic measurements, the potential was controlled by Tormatic Potentiostat P_2 and the diagrams were recorded by means of Sevogor RE 511 Recorder.

The experimental procedure consisted of :

(1) Bubbling with nitrogen gas at a potential of -700 mV_{SCE} (for 1 hr) in chloride solutions.

(2) Changing the potential in noble direction using a scanning rate of 5 mV/min in (0.1 - 5.0 M NaCl at pH from 1.0 to 3.0) or at different dV/dt (from 5 to 100 mV/min in 1M NaCl at pH 2). The potential was stopped when the total current through the system reached 160 uA, this means a current density of 85 uA/Cm^2.

DISCUSSION

Typical potentiodynamic curves for the steel used are shown in Fig. 2 and 3. The active region exhibited maxima in dilute sodium chloride solution (0.1 M) at different pH values. The active region exhibited two current maxima in concentrated solutions from 0.5 to 5 M-NaCl and especially at lower pH values (1 and 1.4). These data are shown in Figs. 4, 5, 6, 7 and 8 where a pronounced second current maximum is observed in most of the figures at different potentials. The amplitude of this

current maximum increases with the decrease of pH values.

It is clear from the Figs. (2-8), that this type of steel can be passivated in chloride solutions having molarily from 0.1 to 3 M at pH values 1-3.0 and the passive range become wide with increase of pH value. It dissolve actively from its corrosion potential and is not passivated in 5.0 M NaCl at all.

The electrochemical parameters for this type of Mo alloyed steel are given in tables (2-4).

Table 2. The electrochemical parameters for Mo alloyed steel containing 3.15% Mo in chloride solutions C_{Cl^-} from 0.1-5 M at pH 1-2.

M NaCl	pH	$E_{pp.}$ $mV_{SCE.}$	$I_{crit.}$ $\mu A/cm^2$	$I_{p.}$ $\mu A/cm^2$	I_{pit} $\mu A/cm^2$	$b_{cathode}$ mV/decade	$E_{corr.}$ $mV_{SCE.}$
0.1	2	-265	9.5	2.5	3.5	95	-405
0.1	1	-220	28	15	37	280	-275
0.5	2	-285	20	3,4	9,75	150	-415
0.5	1.4	-295	26.5	3,5	11	125	-410
0.5	1	-280	95	10.5	14	135	-345

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Table 3.

M NaCl	pH	$E_{pp.}$ $mV_{SCE.}$	$I_{crit.}$ $\mu A/cm^2$	$I_{p.}$ $\mu A/cm^2$	I_{pit} $\mu A/cm^2$	$b_{cathode}$ mV/decade	$E_{corr.}$ $mV_{SCE.}$
3	3	-370	5.5	1.8	5	150	-415
3	2	-330	17.5	3	10	105	-410
3	1	-315	16	33	20	115	-395

Table 4.

M NaCl	pH	$E_{pp.}$ $mV_{SCE.}$	$I_{crit.}$ $\mu A/cm^2$	$I_{p.}$ $\mu A/cm^2$	I_{pit} $\mu A/cm^2$	$b_{cathode}$ mV/decade	$E_{corr.}$ $mV_{SCE.}$
5	1	-290	240	110	70	105	-405
5	1.4	-340	77.5	5.5	19.0	100	-415
5	2	-320	215	65	30	115	-420

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It is clear from these tables how the critical current ($I_{crit.}$) varied with pH and chloride concentrations at 30°C. The results show an increase in $I_{Crit.}$ with decreasing pH due to the increase of corrosion rate with acidity and conductivity of the electrolyte. Similar observation was also found for the same type of steel in sulphate solutions[2]. The critical current increased by more than two orders of magnitude (From 9.5 to 215 $\mu A/cm^2$) as chloride concentration was increased from 0.1 to 5.0 M at pH 2. From the anodic diseolution parameters for this type of steel which are listed table (2-4), corrosion potentials ($E_{Corr.}$) increased with decreasing the pH of the solution and usually were more active the smaller the magnitude of $I_{Crit.}$

Samples with respectively increasing $I_{Crit.}$ required a correspondingly longer time to passivate, and in some cases never passivated at all as in concentrated chloride solutions in 5M at pH 1 and 2 (as in Fig. 8).

It is clear from tables (2-4) that I_{Pit} increases with increase of chloride ion concentration especially at lower pH values.

Tables (2-4) show that $b_{cathode}$ (Tafle slope) lies within the range 100 - 150 mV/log i for most specimens investigated, excluding the results in 0.1M NaCl at pH 1., which yield a higher value (280 mV/log i). This Tafel slope may suggest a slow discharge step (Volmer mechanism). The deviation from 118 mV/decade may be ascribed to changes in the symmetry of the energy barrier (a depart from 0.5).

It is clear that the current density I_p in the passive region of steel increases with increasing chloride

concentration (especially at lower pH values). The results indicate the progressive increase in the corrosion rate in higher concentrated chloride solutions (3 and 5 M NaCl at pH 1 and 1.4). We found all potentials decreased as would be expected with increasing chloride ion concentration.

The effect of the rate of which the potential of the specimen is varied dV/dt has a considerable influence on the shape of the polarization curve. On the other hand, investigations carried out by Greene and Leornard[22] indicate that it is of no importance whether the potential is varied in steps or continuously. The continuous potentiodynamic method is nowadays the most widely used and was preferred in this study. Fig. (9) illustrates some characteristic values of the polarization curve for the used steel in 1M NaCl solution at pH 2 against dV/dt. It is clear from Fig. 9 that E_{corr} and E_c (pitting potential) where as E_{pp} (protection potential from pitting), I_{Crit} and I_{Pit} increase with increasing dV/dt. Littlewood[23] reported that a low transverse rate of polarization give rise to an etching effect, whereas in the present study it was not the case. Probably the higher value of E_{Corr} at low dV/dt are due to enrichment of more noble alloying elements just below $E_{Corr.}$. Similar observation has been found by lars Troselius[27] on AISI 304 in 1M-H_2SO_4 solution.

The actual polarization curves obtained at the highest and lowest scanning rates indicate that the scanning rates affect several aspects of the measurements. The higher scanning rate gives rise to a higher active anodic dissolution peak, which is shifted toward more noble potentials. It has been reported that the faster scans give more noble pitting[25,26], but the opposite we found in this work on molybdenum containing alloy. This may be due to the increase of the passive film thickness

with time at a potential in the passive range. Thus the passive film would be thicker the lower the speed of the scanning. As the film thickness increases, a low fraction of the potential difference between metal and solution would be expected to occur within the film and thus not be available at the film - solution interface to assist in the entry of chloride ions into the film. A similar effect was reported by Bond and Lizlovs[27] for the 17.6% Cr 13.5% Ni 2.9% Mo in 1M NaCl solution.

The lower values of E_{Crit} at low dV/dt are attributable to a combination of these effects. Similar observation has been observed by Lars Troselius[24]. The polarization curve measured for a metal alloy is the resultant of the superimposed polarization curves for the alloying elements, E_{pp} does not have the same significance as the flade potential of a pure metal. The latter is the potential at which the entire metal surface is passivated because an oxide is thermodynamically stable or oxygen is chemisorbed. E_{pp}, on the other hand is a potential at which the resultant polarization curve has a maximum and its position is dependent upon the surface fractions of the included alloying elements.

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LEGEND :

Fig. 1. Specimen holder: 1- PVC, 2- Oring, 3- Resin, 4- Specimen, 5- Brass road, 6and7- PVC, 8- Copper road and 9- Electric connection.

Fig. 2. Potentiodynamic anodic polarization for the 3.15% Mo alloyed steel in 0.1M NaCl (pH 1).

Fig. 3. Potentiodynamic anodic Polarization for the 3.15% Mo alloyed steel in 0.1M-NaCl (pH 2).

Fig. 4. Potentiodynamic anodic polarization for the 3.15% Mo alloyed steel in 0.5M-NaCl (pH 1).

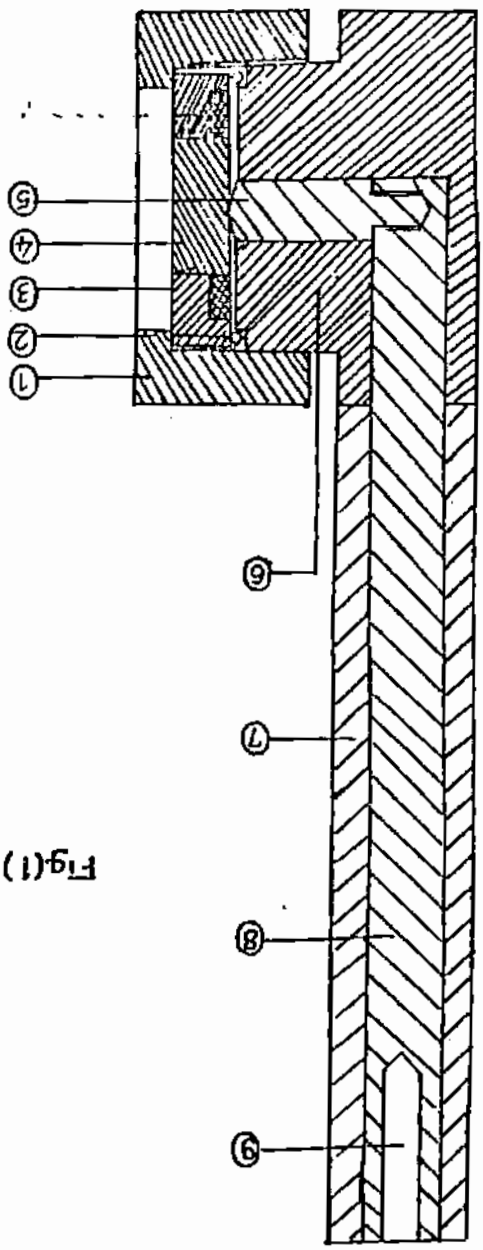
Fig. 5. Potentiodynamic anodic polarization for the 3.15% Mo alloyed steel in 0.5M NaCl (pH 1.4).

Fig. 6. Potentiodynamic anodic polarization for the 3.15% Mo alloyed steel in 3M NaCl (pH 1).

Fig. 7. Potentiodynamic anodic polarization for the 3.15% Mo alloyed steel in 3M NaCl (pH 3).

Fig. 8. Potentiodynamic anodic polarization for the 3.15% Mo alloyed steel in 5M NaCl (pH 1).

Fig. 9. Electrochemical parameters for 3.15% Mo alloyed steel in 1M NaCl (pH 2) using different dV/dt.



Fig(1)

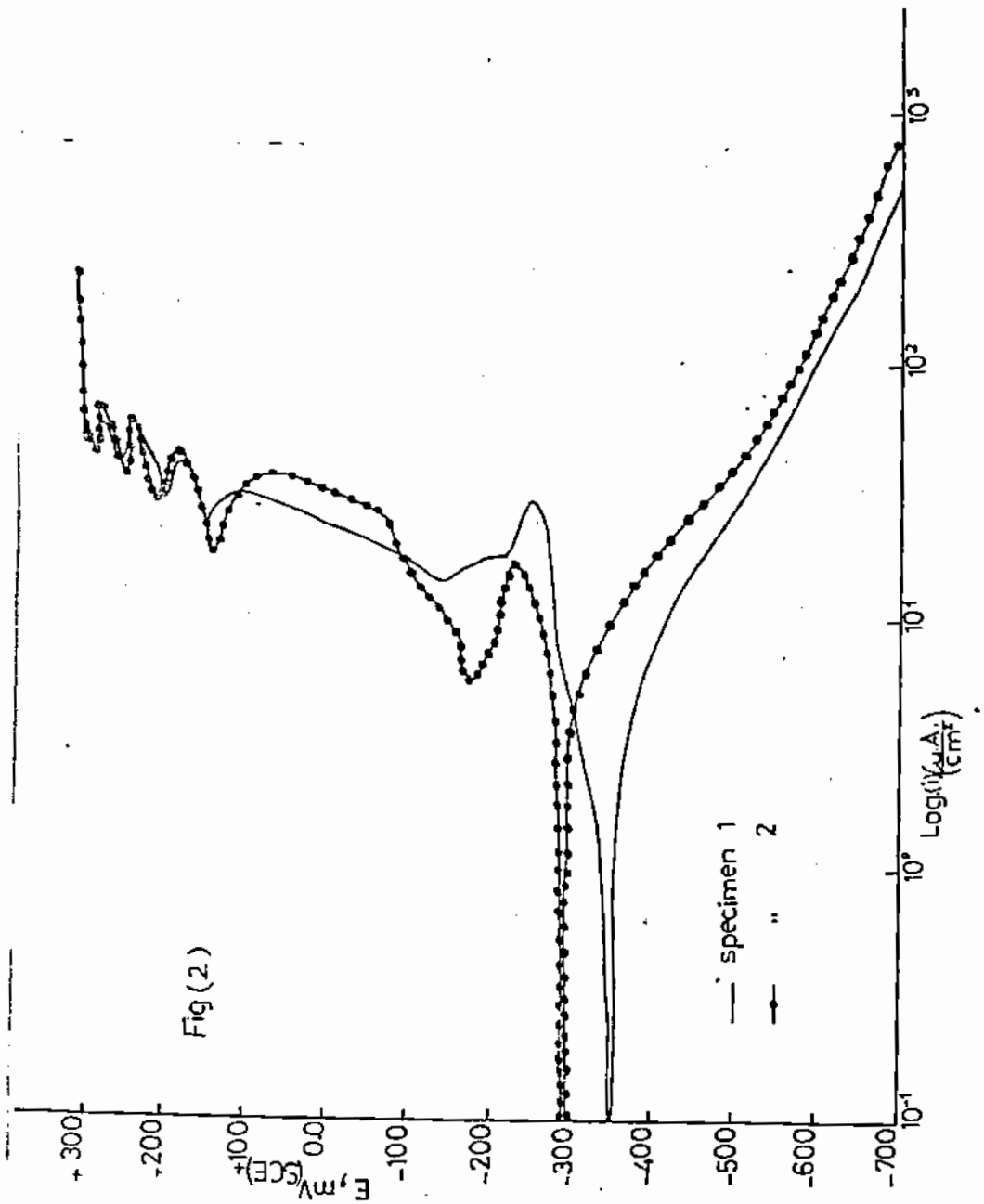

+300
+200
+100
0.0
-100
-200
-300
-400
-500
-600
-700
E, mV (SCE)
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
Log i (μA/cm²)
specimen 1
" 2

Fig (2)

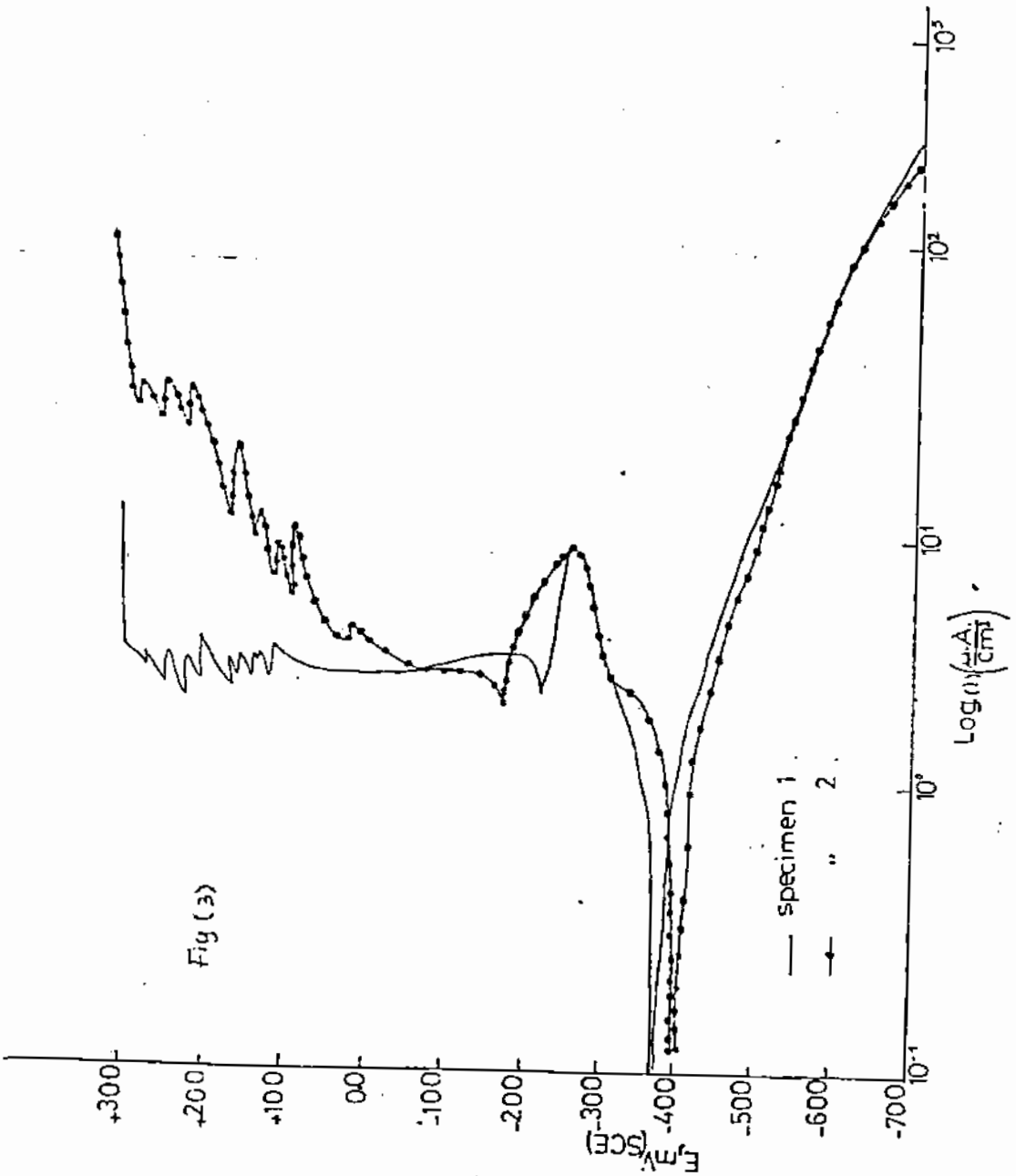
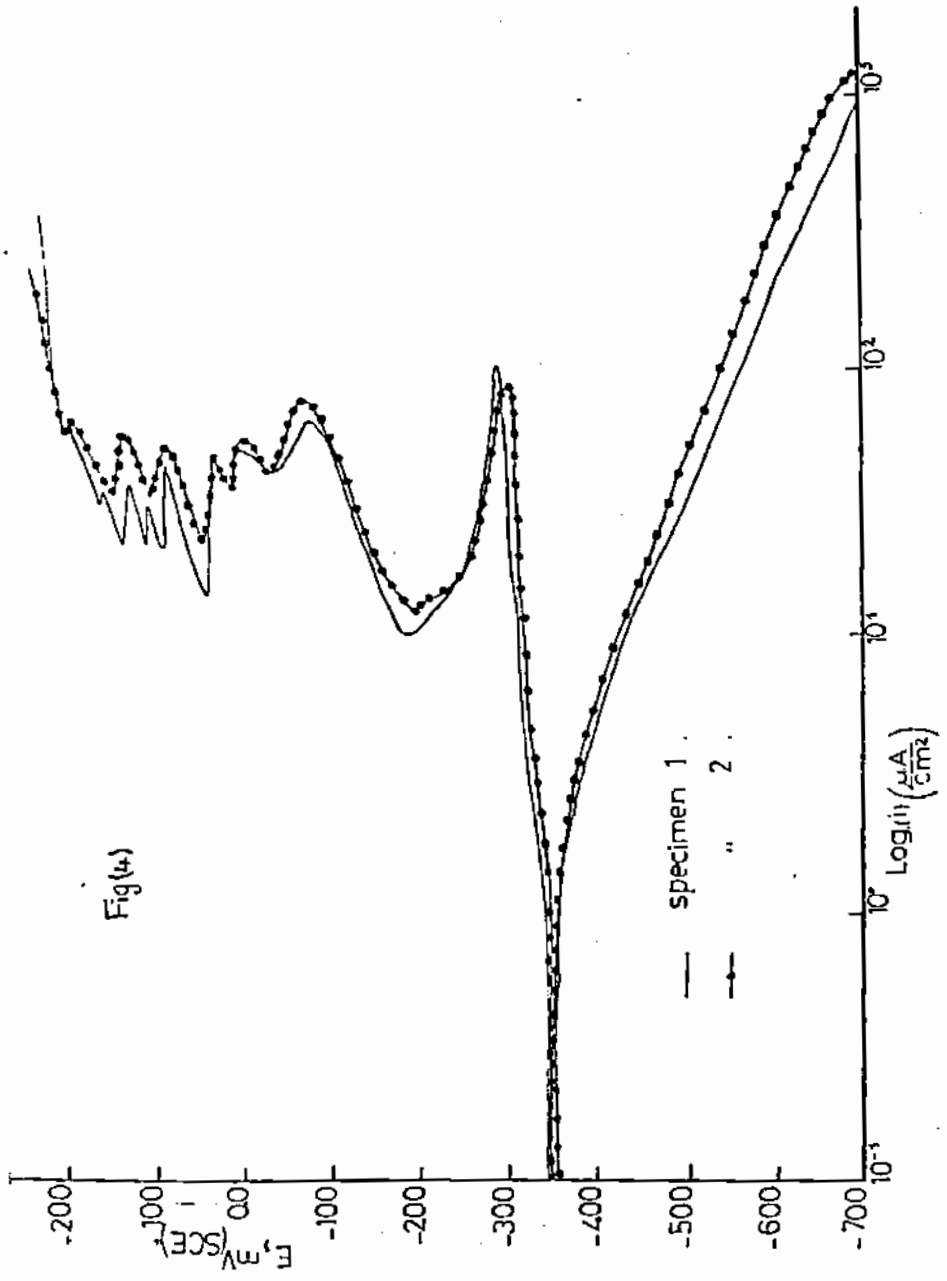
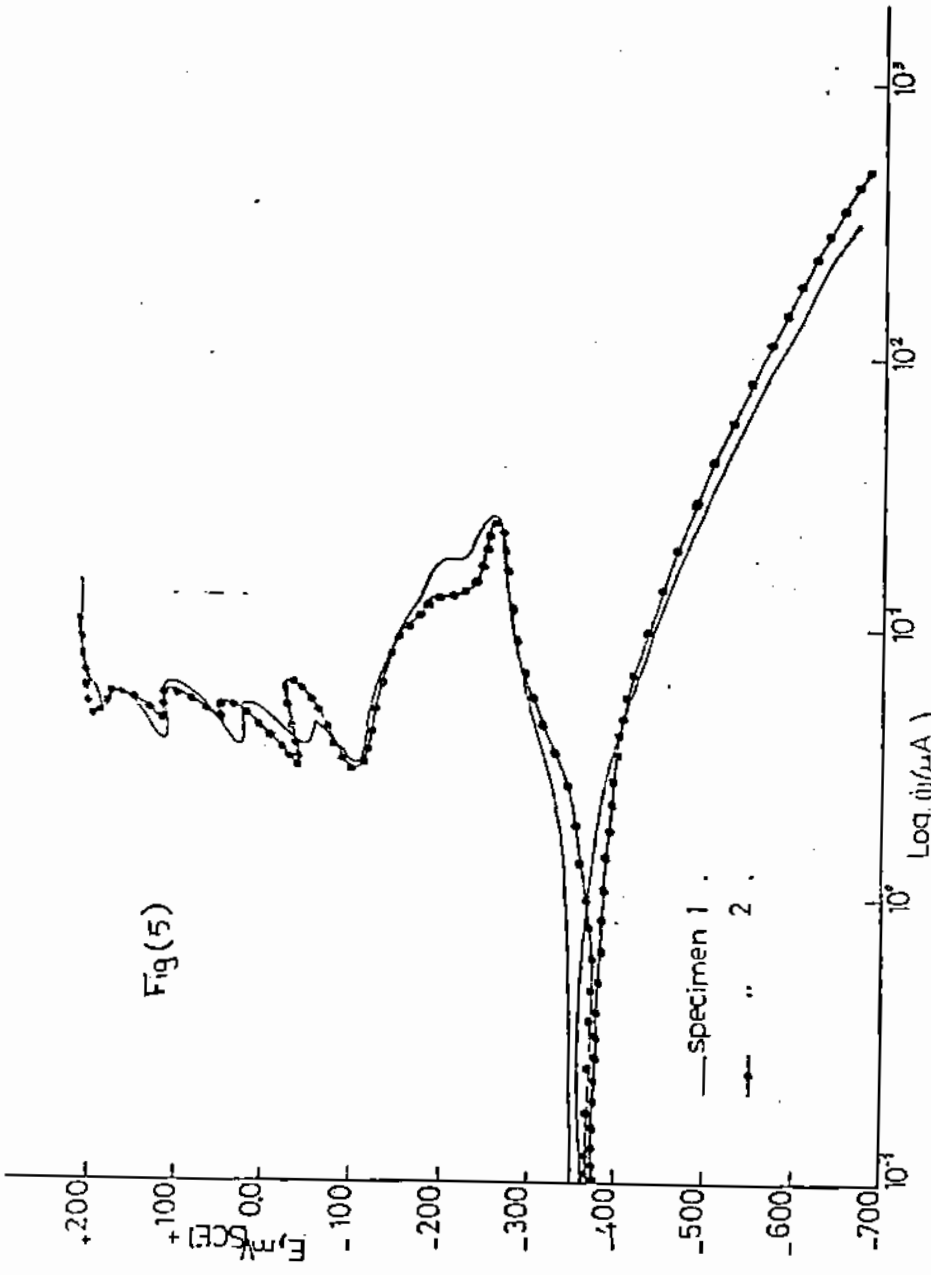

+300
+200
+100
0.0
-100
-200
-300
-400
-500
-600
-700
E, mV (SCE)
10⁻¹
10⁰
10¹
10²
10³
Log (i) (μA/cm²)
specimen 1
" 2

Fig (3)


Fig(4)
E, mV (SCE)
-200
-100
0
-100
-200
-300
-400
-500
-600
-700
specimen 1
" 2
Log i (μA/cm²)
10⁻¹
10⁰
10¹
10²
10³


+200
+100
0.0
-100
-200
-300
-400
-500
-600
-700
E_{rest} (SCE)
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
Log. (i) $(\frac{\mu A}{cm^2})$
—Specimen 1 .
— " 2 .

Fig(5)

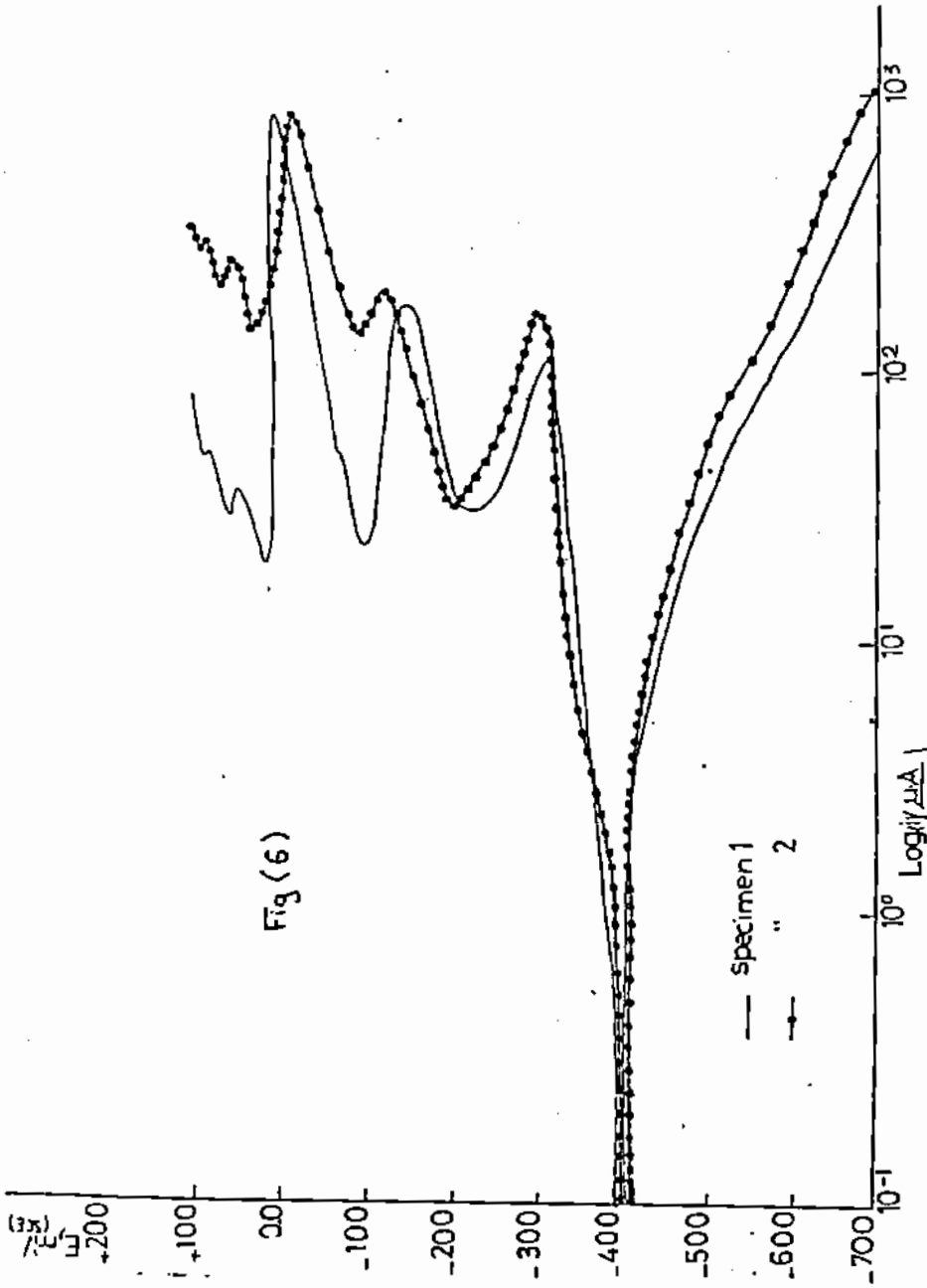
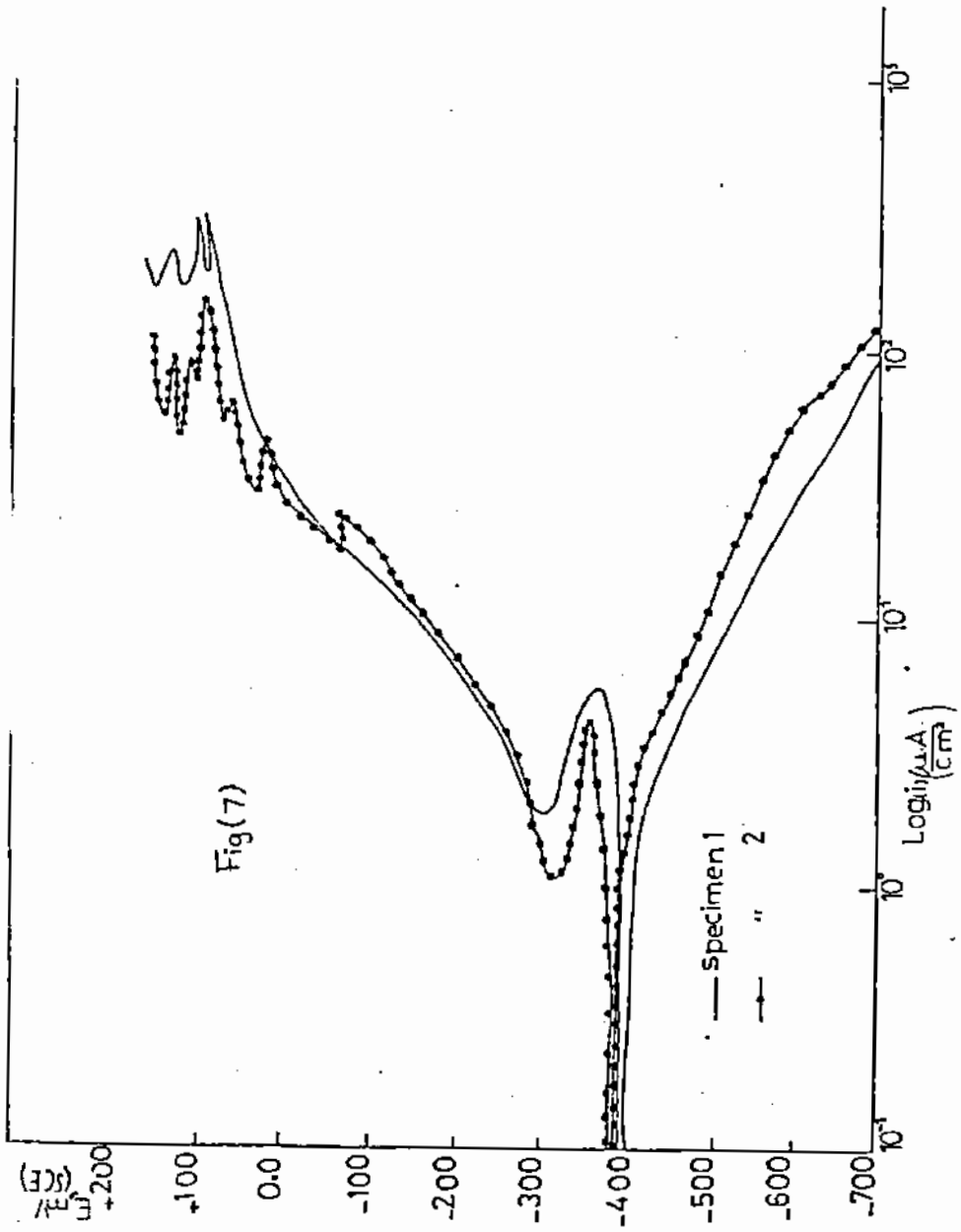

+200
+100
0.0
-100
-200
-300
-400
-500
-600
-700
10³
10²
10¹
10⁰
10⁻¹
Log₁₀(μA/cm²)
Specimen 1
" 2

Fig (6)


Fig(7)
Specimen 1
" 2
Log10(μA/cm²)
(SCE)
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+100
0.0
-100
-200
-300
-400
-500
-600
-700

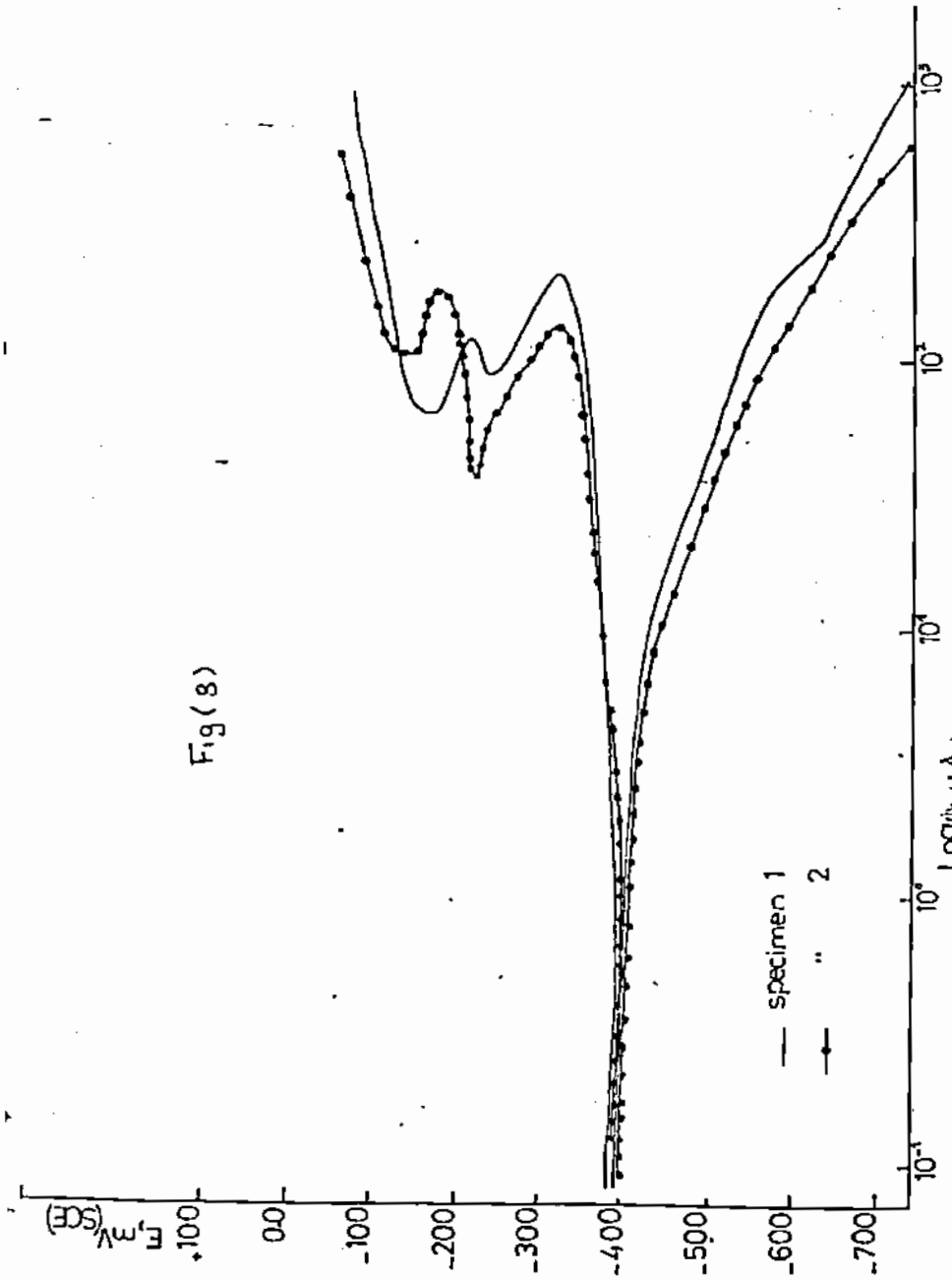
E, mV (SCE)
+100
0.0
-100
-200
-300
-400
-500
-600
-700
Log i (μA/cm²)
10⁻¹
10⁰
10¹
10²
10³
specimen 1
" 2

Fig (8)

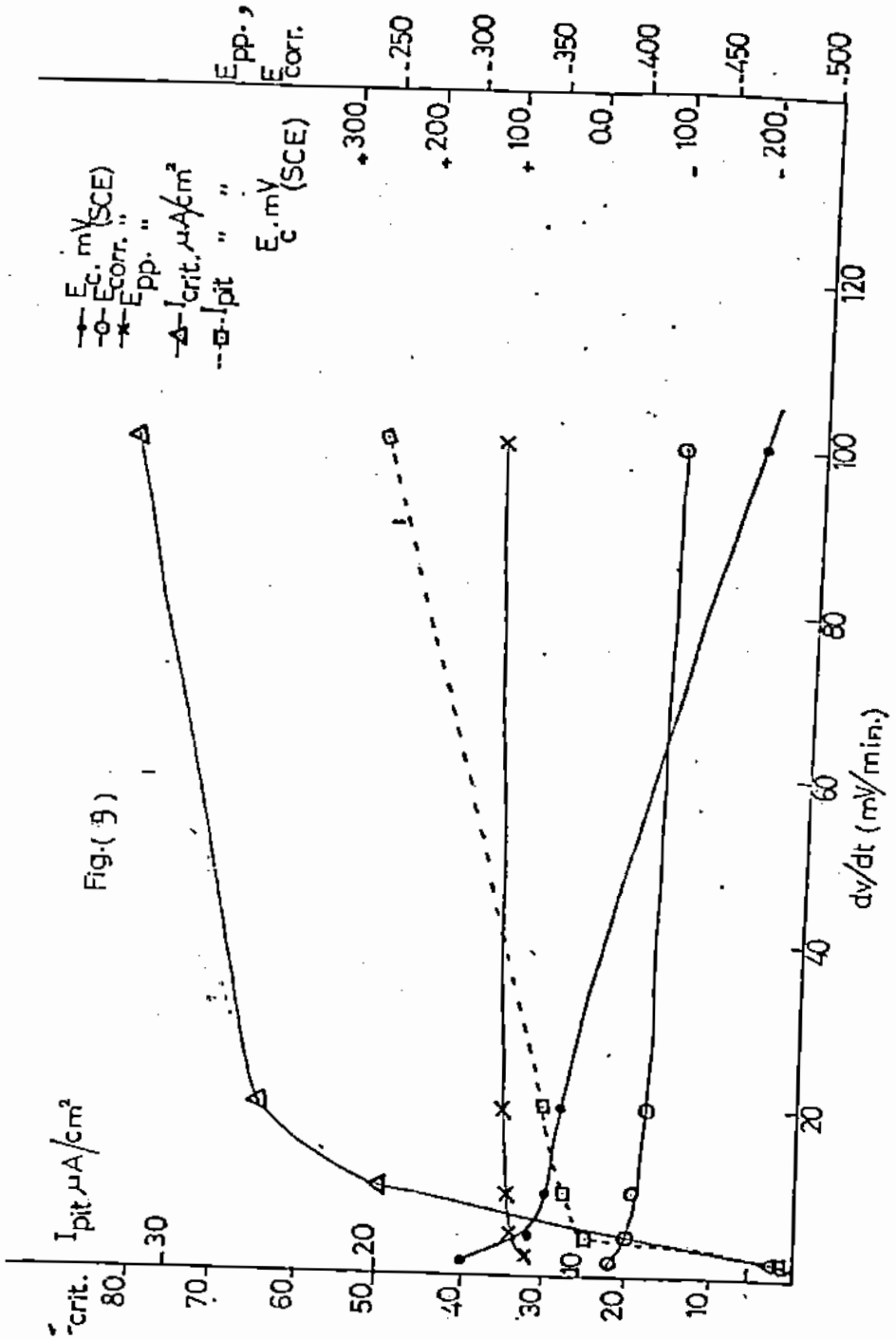
E_c. mV (SCE)
E_corr. "
E_pp. "
I_crit. μA/cm²
I_pit " "
E_c. mV (SCE)
E_pp., E_corr.
+300
+200
+100
0.0
-100
-200
-250
-300
-350
-400
-450
-500
I_crit. I_pit μA/cm²
80
70
60
50
40
30
20
10
30
20
10
dv/dt (mV/min.)
120
100
80
60
40
20

Fig.(9)

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THE BEHAVIOUR OF NICKEL AMALGAMS IN PHOSPHORIC ACID SOLUTIONS

By

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Abstract :

Nickel amalgams of varying concentrations were anodically oxidized galvanostatically in phosphoric acid solutions at 25°C. Two different oxidation patterns were distinguished. The first described the behaviour of the nickel amalgams in concentrated acid solutions (1.0 and 3.0 N H_3PO_4) and the second gave the behaviour of nickel amalgams in dilute acid solutions (0.1 N H_3PO_4). The oxidation curves in concentrated solutions showed regions for the charging of the anodic double layer, dissolution of nickel as Ni^{++} followed by its oxidation to NiO, formation of mercurous and mercuric phosphate, Ni_3O_4 and Ni_2O_3 which is oxidized further to NiO_2 before oxygen evolution.

With dilute solutions the oxidation curves revealed that, the charging of the anodic double layer, dissolution of Ni as Ni^{++}, and its oxidation to NiO as in concentrated phosphoric acid solutions,

was followed by a region of oscillations due to the consecutive formation and dissolution of mercurous and mercuric phosphate. The NiO formed at the early stages of passivity is then oxidized to Ni_3O_4 , Ni_2O_3 then NiO_2 before oxygen evolution.

The relation between the polarizing current, i , and the time of passivation, τ , waa found to follow the following equation :

$$\text{Log } \tau = A - n \log i$$

where A and n are constants. The results indicate that the diffusion of nickel within the amalgam to the amalgam / electrolyte interface was mainly rate the determining in the process of passivation.

The effect of temperature on the time of passivation of nickel amalgams in phosphoric acid solution was also investigated.

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Introduction :

The chemical literature is rich with information regarding the oxidation and passivation of metallic alloys. Certain rules are put to explain and predict the beneficial or determintal effects resulting from incorporating traces of one metal in the bulk of another. Little of such work is directed, however, to study of alloys in which mercury forms the main constituent.

Moreover, nickel and its alloys possess excellent corrosion resistance in a wide variety of environments. This naturally rises considerable interst in investigating the cause of passivity of nickel. Several techniques have been employed to characterize the nature and the thickness of the surface film[1-24].

Therefore the main objective of this study was to investigate the composition of tha film formed on a nickel amalgam electrode and throw more light on the mechanism leading to the electrode passivation.

Experimental :

The electrical cell and experimental details were similar to those given previously[25]. The amalgam electrode was in the form of a small cup of 1.67 cm internal diameter, provided with a sealed platinum wire. The cathode was in the form of an auxillary electrode with a sealed platinum contact. In order to avoid the contamination of the tested solution with the cathode products, the cathode was isolated from the electrolytic solution. This was achieved by auxillary electrode in a special compartment fitted with a sintered - glass disk of medium porosity.

The potential of the working electrode was measured against a saturated calomel electrode. All potentials are referred to the reversible hydrogen electrode scale.

The amalgam of the electrodes were prepared electrolytically in situ from the acid plating baths having the following composition: 90 g. $NiSO_4 . 7 H_2O$, 30 g. $NiCl_2 . 6 H_2O$, 16 g. $(NH_4)_2SO_4$, 30 ml H_3PO_4 and 4.5 g. NH_4Cl / L.

Aknown amount of solution was introduced into the electrode cup containing 1.5 ml of doubly distilled mercury and electrolysis was carried out with fixed

current 50 m.A/electrode for nickel with mercury acting as the cathode. The electrode area was 2.30 cm^2. The mercury and electrolyte were continuously stirred. Complete plating of the metal was marked by the brisk evolution of hydrogen gas from the surface of the electrode. Electrolysis was continued for a further 30 min. period. The amalgam was washed several times with doubly distilled water, and then dried with narrow strips of filter paper. Complete deposition of nickel was ascertained by atomic absorption analysis.

Amalgams varying in concentration between 0.6448×10^{-2} to 3.8557×10^{-2} wt% nickel were thus prepared. Orthophosphoric acid solution was used as testing media to prepare 3.0, 1.0 and 0.1 N solutions.

The results and analysis indicate that the nickel amalgam electrodes prepared in this manner have the following composition (Table 1.)

Table 1. Amalgams Electrode Composition :

Electrode No.	Ni solution, ml	wt-% Ni	Mole fraction
I	0.5	0.6448×10^{-2}	2.2615×10^{-3}
II	2.0	2.5738×10^{-2}	8.6965×10^{-3}
III	3.0	3.8557×10^{-2}	12.9880×10^{-3}

Results and Discussion :

Anodic and cathodic polarization curves for nickel amalgams with increasing metal content were traced in 0.1, 1.0 and 3.0 N - H_3PO_4 solutions at 25°C. For each amalgam and solution composition number of constant polarizing currents were applied to gain insight into the mechanism of passivation.

The results of the present investigation on the anodic behaviour of nickel amalgams in phosphoric acid solutions showed two different oxidation patterns. The first describes the behaviour of the nickel amalgams in concentrated acid solutions (1.0 and 3.0 N H_3PO_4) and the second gives the behaviour of these amalgams in dilute acid solutions (0.1 N H_3PO_4).

The behaviour of nickel amalgams in concentrated acid solutions :

Under a wide range of constant polarizing current densities and solution concentrations(1 and 3N H_3PO_4), nickel amalgams (electrode I, II and III) yield polarization curves which exhibit practically the same features.

Figs. (1,2) represent the potential - time curves of nickel amalgam electrodes (Electrode III and I) in 3.0 and in 1.0 N H_3PO_4 solutions respectively. It can

be seen that, the primary anodic process in the polarization curves represents the charging of the anodic double layer, then the Ni of the amalgam dissolves as Ni^{+2} along region (i) (showing no inflection) according to the following equation :

$$Ni = Ni^{+2} + 2e \qquad E^{o} = -0.25\ V \qquad (1)$$

This reaction is considered for solid nickel electrode, however for nickel amalgams under consideration the reduction in the nickel atom activity must also be accounted for. As a fair approximation the activity of nickel in mercury could be set equal to its mole fraction χ_{Ni} which for 2.2615×10^{-3} and 12.988×10^{-3} wt - % Ni amalgam amounts to 0.64448×10^{-2} , 2.5738×10^{-2} and 3.8557×10^{-2} respectively.

Substitution of these values in the basic Nernst equation :

$$E = E^{o} + 0.059\ pH - 0.029 \log\ \chi_{Ni} \qquad (2)$$

gave for the three amalgams in 0.1, 1, and 3 N-H_3PO_4, values varying from -0.093 to -0.133 V. Formation of Ni^{+2} along this region (i), in the polarization curves corresponds to the first period of activity of nickel amalgams, which is then oxidized to NiO (region ii) showing no inflection also.

Formation of NiO along region ii was also reported by previous authors[10] who reported that, by the galvanostatic technique, small constant c.d. causes the potential of nickel electrode to increase suddenly to a value that corresponds to the reversible NiO/Ni potential. Similar observations were also found by Besson[21] upon his study on the anodic oxidation of nickel electrode in 6N - KOH solution by Rollet's method reported that, the equilibrium transformation which occurs together with the oxidation reduction potential (in V) determined at the points of inflection of the anodic potential-time curves were :

$$Ni_2O \rightleftharpoons Ni_3O_4 \quad \text{(about 0.47 V)},$$

$$Ni_3O_4 \rightleftharpoons NiO \quad \text{(about 0.0 V)},$$

$$\text{and} \quad NiO \rightleftharpoons Ni \quad \text{(no inflection)}$$

The formation of NiO along region (ii) was followed by five clear arrests. Along arrests (I) and (II), mercurous and mercuric phosphatewere formed and the surface of the amalgam electrode was covered with a white deposit.

Evidence that mercurous and mercuric phosphate were formed during arrests I and II in the polarization curvee can be arrived at, by consideration of Fig. (3)

in which pure mercury is oxidized in 1 N-H_3PO_4 acid under the same experimental conditions (the same current density, solution concentration and temperature).

Further information for the formation of mercurous and mercuric phosphate along arrests I and II, respectively, was also confirmed by measuring the open circuit potential that arises from these salts on the surface of a clean doubly distilled mercury pool surface, where values of 625 and 675 mV. were obtained for mercurous and mercuric phosphate, respectively.

Due to the solubility of mercurous and mercuric phosphates in acids, an elongation of the step of formation of mercuric phosphate occurs, Fig. (1); and oscillations occur in the potential-time curve (specially in dilute solutions C.F fig. (4)) due to the continuous formation and dissolution of the salt layer.

Owing to the liquid nature of the electrode, the primary passivating film contained fissures and cracks which allowed the amalgam to come in contact with the solution and the nickelous oxide NiO formed at the first stage of passivity (region ii), would be oxidized to higher oxides along steps III and IV according to

$$NiO + H_2O \longrightarrow Ni_3O_4 + 2H^+ + 2\bar{e}\,; \quad E = 0.897 - 0.059\,pH \quad (3)$$

$$Ni_3O_4 + H_2O \longrightarrow 3Ni_2O_3 + 2H^+ + 2\bar{e}\,; \quad E = 1.305 - 0.059\,pH \quad (4)$$

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Ni_2O_3 which is formed along step IV is further oxidized to NiO_2 along arrest V, according to the following reaction[26]:

$$Ni_2O_3 + H_2O = 2NiO_2 + 2H^+ + 2e^- ; \quad E^o = 1.434 - 0.059\ pH \qquad (5)$$

before finally the potential rises to oxygen evolution values.

These results are in agreement with those obtained by previous authors on solid nickel electrodes in sodium phosphate solution[17]. They suggested that the passive film on nickel electrode has a duplex structure consisting of NiO and Ni_3O_4. At high potential NiO_2 was produced on top of the initial film.

Cathodic polarization curves were traced by reversing the polarizing currents when the potential was at oxygen evolution values (curve C, Figs. 1,2), so as to make the working electrode the cathode.

Three arrests appeared in the cathodic polarization curve. The first two corresponds to the reduction of mercuric and mercurous phosphate according to the following reactions.

$$Hg_3(PO_4)_2 + H_2 = Hg_2HPO_4 + Hg + H^+ + e \qquad (6)$$

$$Hg_2HPO_4 + H_2 = H^+ + H_2PO_4^- + 2Hg \qquad (7)$$

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Then reduction of NiO_2 to NiO took place along the third arrest before finally the potential drops to hydrogen evolution values.

Anodic decay curves obtained upon interrupting the polarizing current when the electrode was at oxygen evolution values (curve D, Figs. 1,2), indicated that the potential dropped directly to values corresponding to the system Hg_3PO_4/Hg_2HPO_4 where the potential remains constant thereafter.

The behaviour of nickel amalgams in dilute phosphoric acid solutions :

Quite interesting is the oxidation characteristics of nickel amalgams in dilute solutions. In Fig.(4) are shown the anodic polarization curves for (2.5738×10^{-2} wt.% Ni) in 0.1 N - H_3PO_4 acid. As indicated from the shapes of the anodic polarization curves(Fig. (4)); it can be seen that, the charging of the anodic double layer and dissolution of Ni as Ni^{++}, followed by its oxidation to NiO as mentioned before in concentrated phosphoric acid solutions (1 and 3 N) was followed by a region of oscillations which may probably be due to the fact that, the formed mercurous and mercuric phosphate are soluble in acid phosphate solutions(18). The formed layers in these dilute solutions once formed

readily dissolve, and fluctuations occur in the anodic polarization curves due to the formation and breakdown of these salts at the first stages of passivity. After certain time the NiO formed at the early stages of passivity is then oxidized to Ni_3O_4 along step I in the polarization curve, (Fig. 4) which is then oxidized to Ni_2O_3 then NiO_2 along steps III and IV as given before in case of concentrated phosphoric acid solutions.

Relation between the polarizing current, i (μA) and time of passivation, τ (min) :

In order to gain further insight on the nature of the process that eventually leads to the electrode passivation (film formation), the relation between the polarizing current, i (μA) and time of passivation τ (min) was considered. Plots of these two variables(C.F. Fig. 5)for electrode III in 0.1, 1.0 and 3N H_3PO_4 solutions, gave almost parallel straight lines with slopes of -2 in excellent agreement with n = 2, predicted by Muller's empirical formula and Sand's equation for electrolysis with constnat current (chronopotentiometry)[27]. Similar observations were also found for electrode I and II in phosphoric acid solutions.

As seen from these curves (Fig. 5), the passivation of the different nickel amalgams in the examined solutions are satisfactorily represented by the relation

$$\text{Log } \tau = A - n \log i \qquad (8)$$

where A and n are constants

In table (2) the values of the constants A and n of relation (7) are grouped.

Table (2) :

Values of the constants A and n in relation (7).

Solution	Electrode No.	A	n
3N H_3PO_4	I	7.49	2
	II	7.80	2
	III	7.89	2
1N H_3PO_4	I	7.51	2
	II	7.65	2
	III	7.69	2
0.1N H_3PO_4	I	6.40	2
	II	6.47	2
	III	7.18	2

From the results of the present investigation the passivation of nickel amalgams in H_3PO_4 solutions appears to be largely governed by the rate of diffusion of nickel atoms to the electrode / electrolyte interface.

Furthermore, it is readily seen from Fig. 6 (electrode III in 0.1, 1.0 and 3N H_3PO_4 solutions) that the value of $i\tau^{1/2}$ is almost constant (for one and the same concentration of amalgam) as the current density was varied. The same results were also obtained for electrode I and II in phosphoric acid solutions.

Consideration of the theoretical relations underlying the process of electrolysis at controlled current can reveal the exact nature of the diffusing ion which participates in the formation of the passivity film. For the product $i\tau^{1/2}$, the term passivation index is here suggested since it gives a clear understanding of the process of passivation.

Effect of temperature on the time of passivations of nickel amalgams in phosphoric acid solution :

The results of the anodic polarization of 2.5738×10^{-2} wt% nickel amalgam in 1N-H_3PO_4 solution at different temperatures are show diagramatically in

Fig. (7). The polarizing current density was 261 $\mu A/cm^2$.

As can be seen from these curves, the time of passivation increases with rise of temperature. Since the potential - time curves are analogous in shape at higher and lower temperatures, save for the increased time of passivation at higher temperatures, hence, the effect of temperature may be accounted for, as due to the increased solubilities of the layers initially present or anodically formed on the electrode surface, therefore, the time needed for the passivation of the electrode greatly increases.

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Fig. 1 : Anodic, Cathodic and Decay Polarization of Electrode III of Ni - Amalgam in 3N-H_3PO_4.

—o— c.d. 600 $\mu A/Cm^2$ —x— c.d. 522 $\mu A/cm^2$

—Δ— c.d. 435 $\mu A/Cm^2$ —•— c.d. 348 $\mu A/cm^2$

—o— c.d. 261 $\mu A/Cm^2$

Fig. 2 : Anodic, Cathodic and Decay Polarization of Electrode I of Ni-Amalgam in 1N-H_3PO_4.

—o— c.d. 435 $\mu A/Cm^2$ —x— c.d. 348 $\mu A/cm^2$

—Δ— c.d. 304 $\mu A/Cm^2$ —•— c.d. 260 $\mu A/cm^2$

—□— c.d. 174 $\mu A/Cm^2$

Fig. 3 : Anodic, Cathodic and Decay Polarization of Hg in 1N-H_3PO_4 , c.d. = 261 $\mu A/cm^2$.

Fig. 4 : Anodic, Cathodic and Decay Polarization of Electrode II of Ni - Amalgam in 0.1N-H_3PO_4

—o— c.d. 435 $\mu A/Cm^2$ —x— c.d. 348 $\mu A/cm^2$

—Δ— c.d. 304 $\mu A/Cm^2$ —•— c.d. 260 $\mu A/cm^2$

Fig. 5 : Log i - Log τ Relation for Electrode III in :

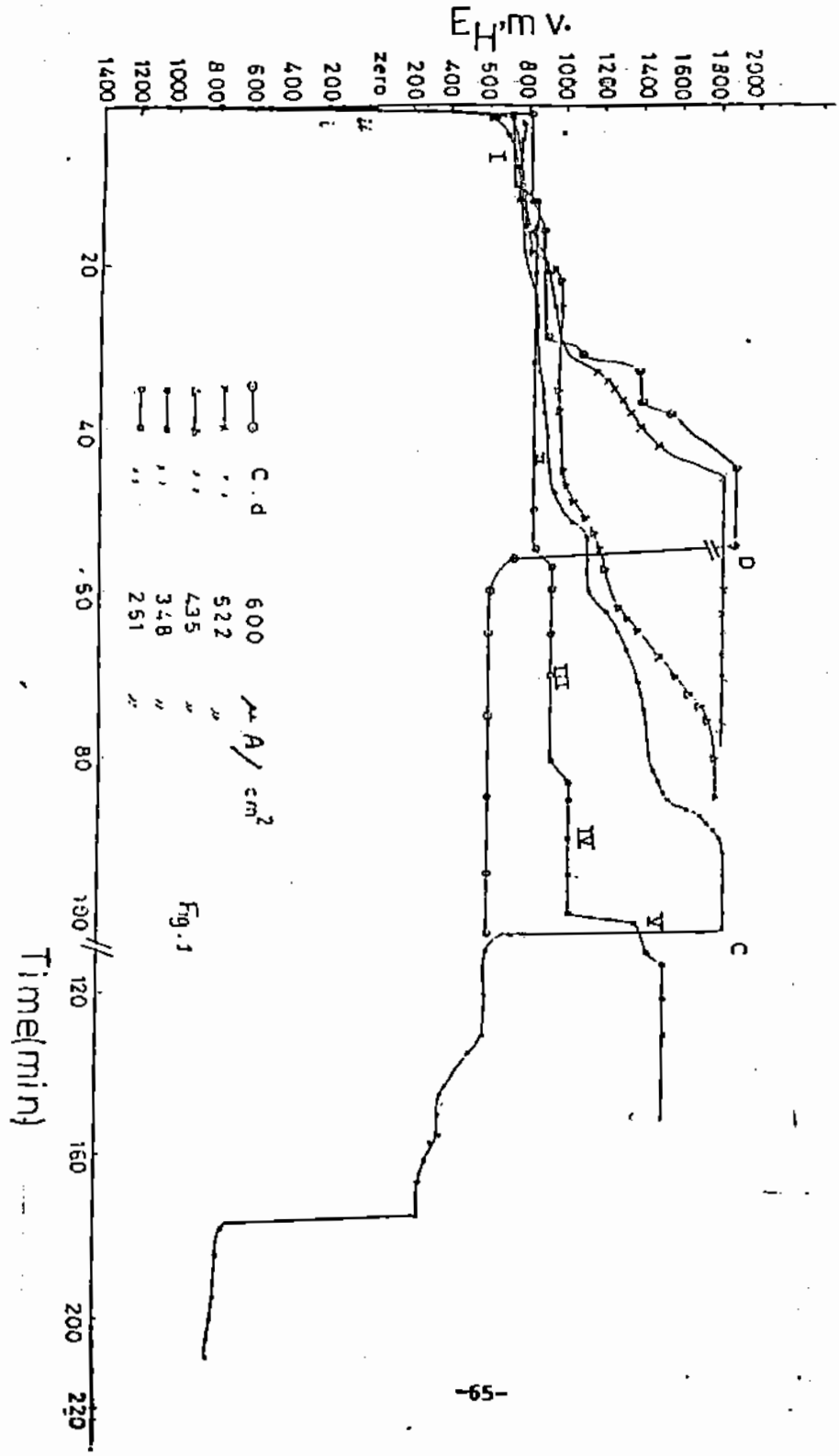
—o— 3.0N-H_3PO_4 —x— 1.0N-H_3PO_4

—•— 0.1N-H_3PO_4

Fig. 6 : Relation between $i\tau^{1/2} \times 10^3$ and i (μA) for Electrode III in :

Fig. 7 : Effect of Temperature in Electrode II in :
1.0N-H_3PO_4 , c.d. 261 mA/cm^2

45°C 35°C 30°C
20°C 10°C


E_H m v.
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1200
1000
800
600
400
200
zero
I
II
III
IV
V
D
C
20
40
60
80
100
120
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180
200
220
Time(min)
C.d 600 μA/cm²
522 "
435 "
348 "
261 "

Fig.1

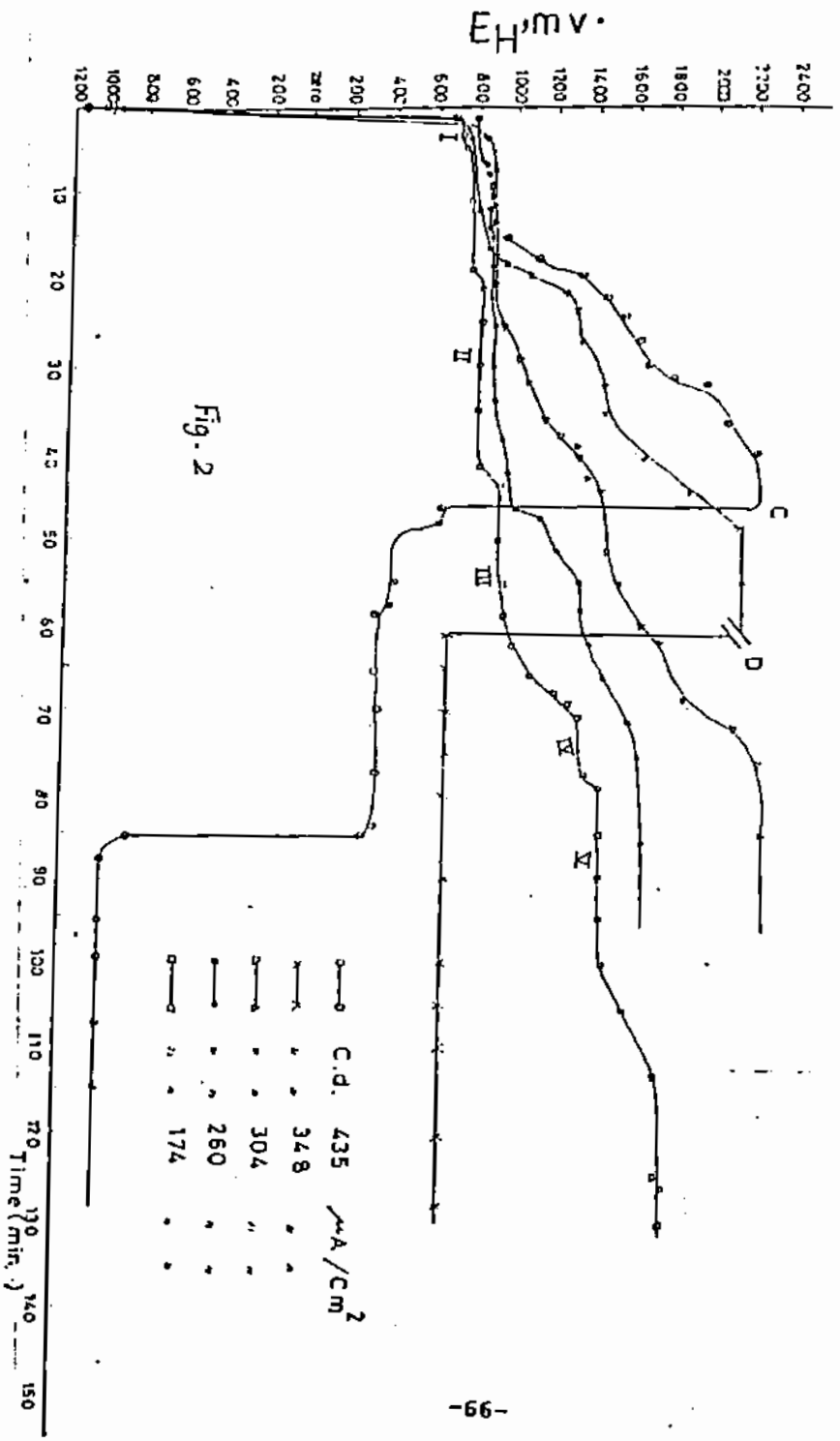
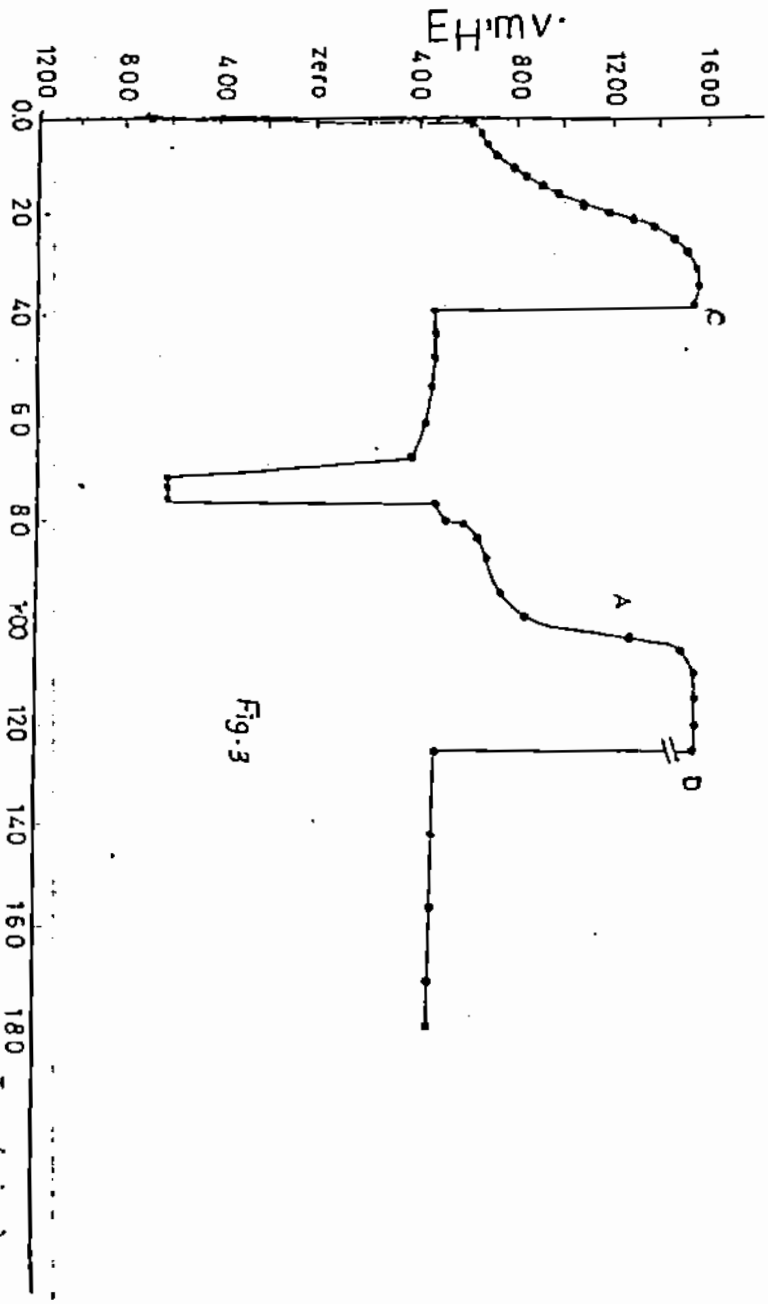


Fig. 2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800
400
zero
400
800
1200
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40
60
80
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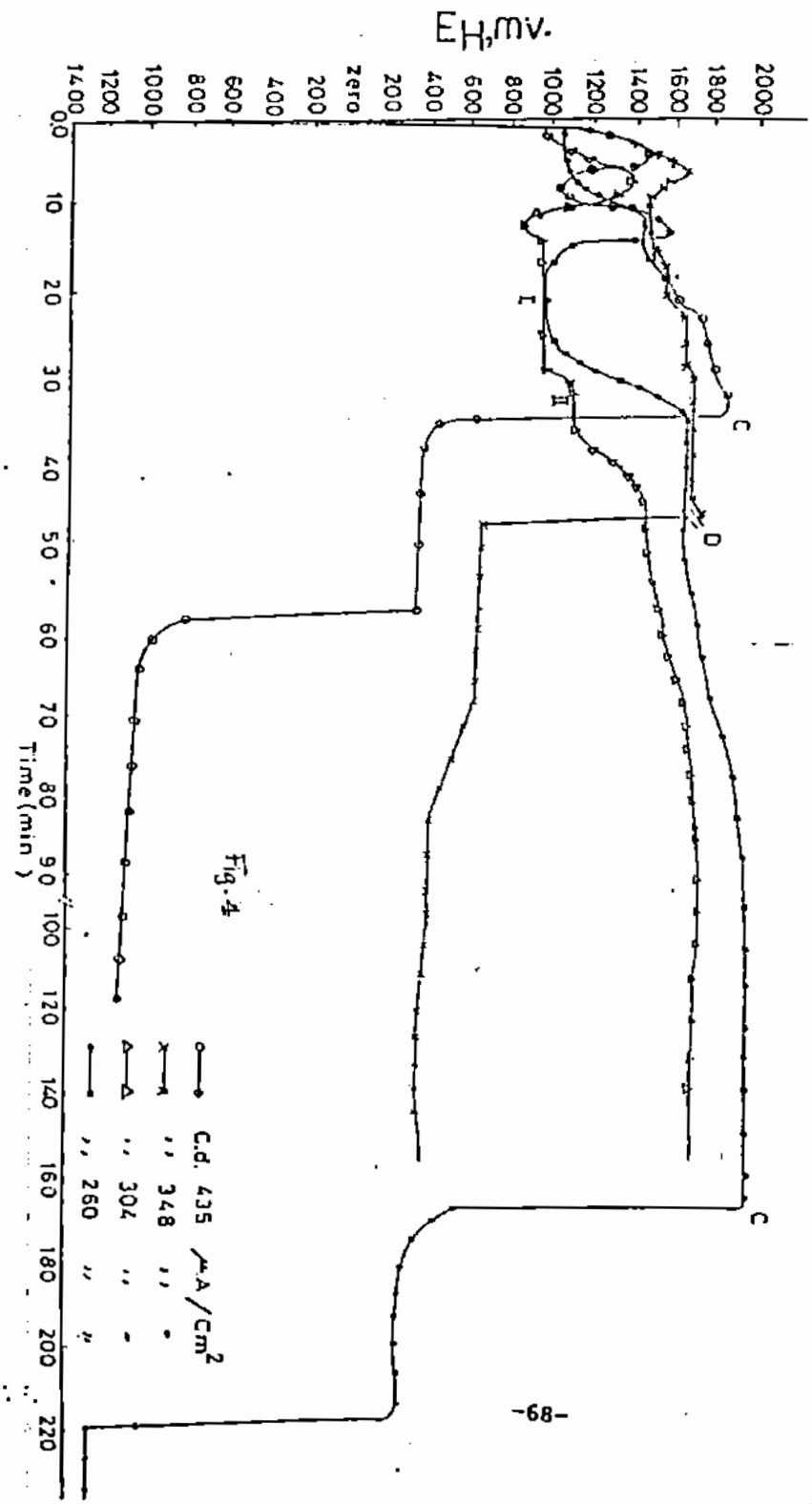
120
140
160
180
Time (min.)
A
C
D

Fig.3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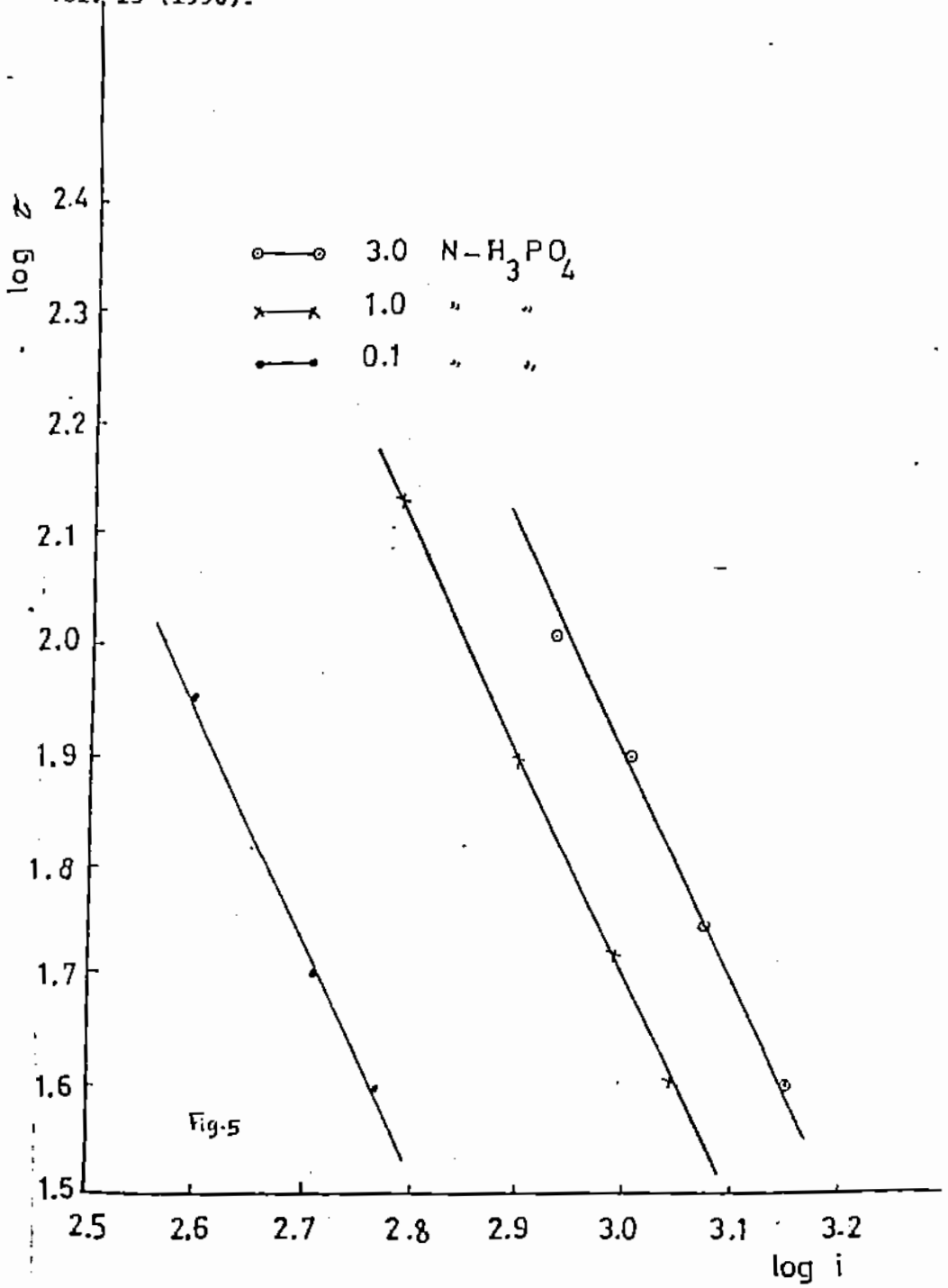
10
20
30
40
50
60
70
80
90
100
120
140
160
180
200
220
Time(min)
I
II
C
D
C
C.d. 435 μA/Cm²
,, 348 ,,
,, 304 ,,
,, 260 ,,

Fig. 4


log ℓ
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2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
3.0 N-H3PO4
1.0 " "
0.1 " "
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2
log i

Fig.5

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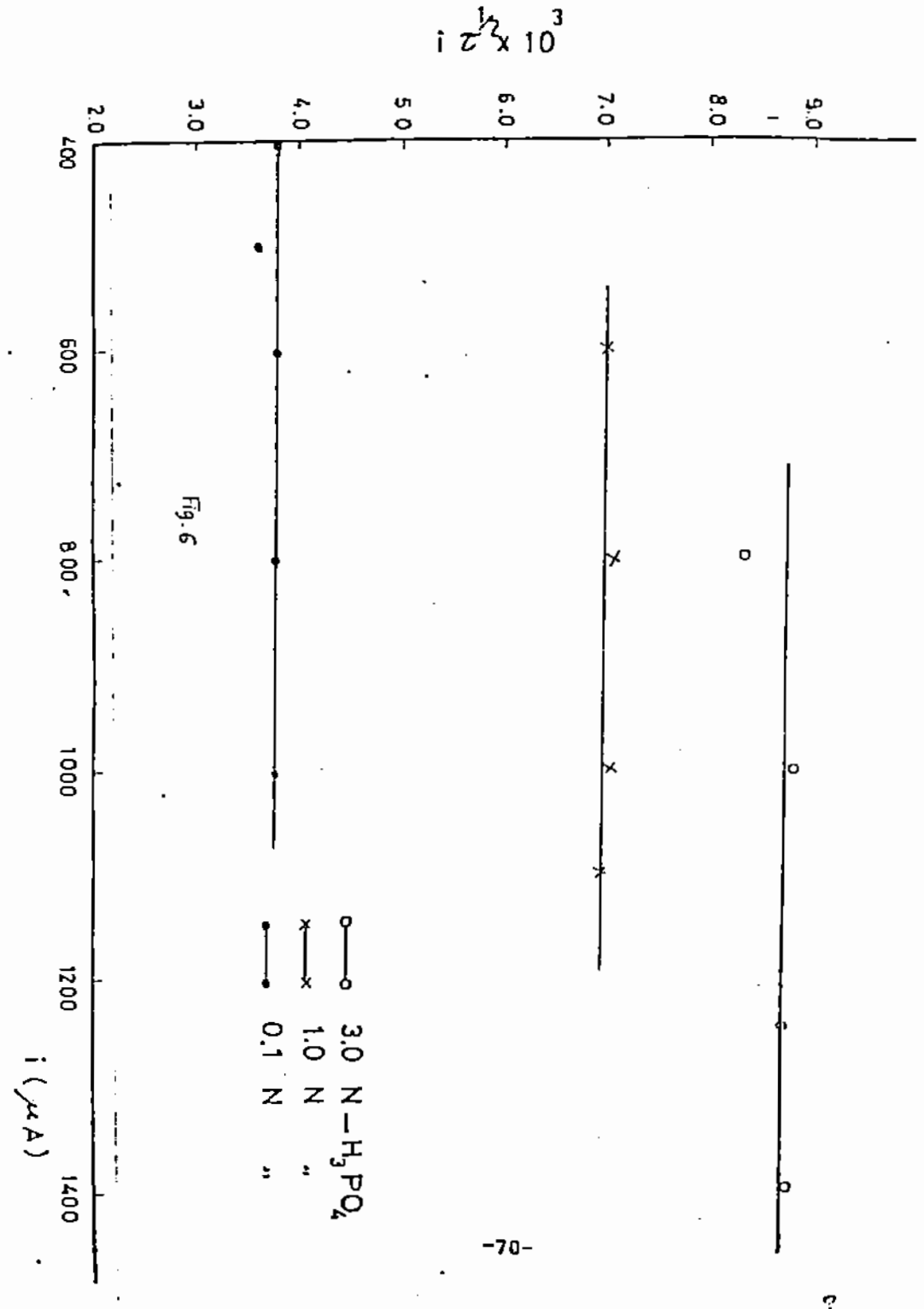
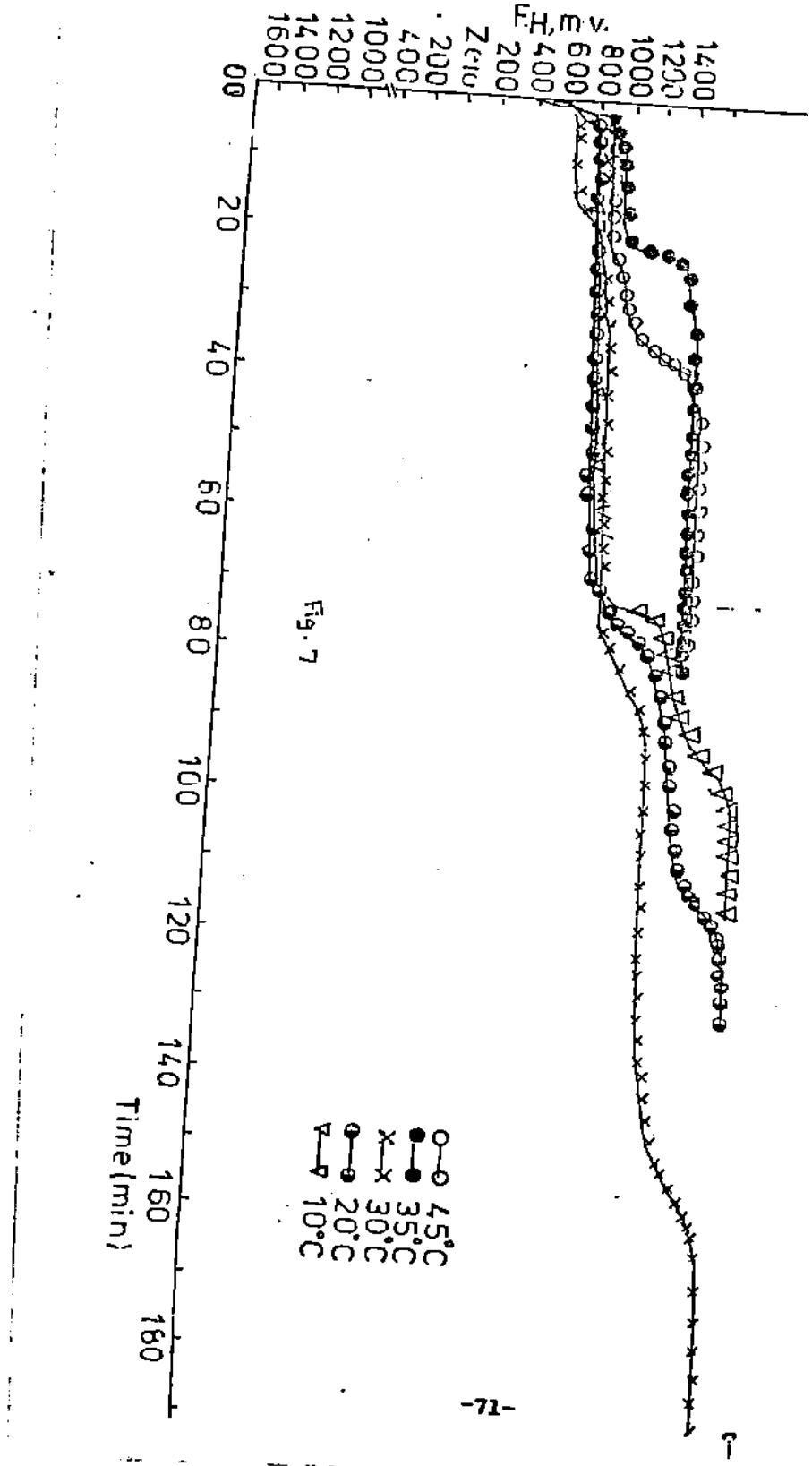


Fig. 6


EH, m v.
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Zero
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1200
1400
1600
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40
60
80
100
120
140
160
180
Time (min)
45°C
35°C
30°C
20°C
10°C

Fig. 7

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CHARACTERISTICS DEPENDANCE ON TEMPERATURE FOR GRIDDED STREAMER DETECTOR

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Ain Shams University

ABSTRACT

Effect of temperature variation on the characteristics of gridded streamer detector is investigated. Relative detection efficiency is represented, which is very useful for practical use of gridded corona streamer counter.

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INTRODUCTION

Temperature is an effective parameter on the efficiency of corona detectors[1-10]. No detailed investigation of the effect of temperature on spark detectors sensitivity has been made[1-5]. Recently an interesting study of characteristics dependance on temperature for wire-plane streamer detectors has been carried out.[6,8]

A trial is presented in this work to clarify the effect of temperature variation on the characteristics of gridded streamer counters.[7]

EXPEREMENT

The streamer counter system was described elsewhere[7] so that only a brief account may be given here.

The form of the gridded single-wire anode streamer detector and block diagram of electronic circuit are shown in Fig.(1). A highly polished stainless steel plate (82 mm effective length) served as cathode. Molybdenum anode and grid wires of diameter ϕ = 0.32 mm were used. The following conditions were chosen: a spacing of 3 mm between grid wires, 17 mm grid-to-cathode spacing, 7 mm anode-to-cathode spacing, 5 MΩ anode load resistance and 10 MΩ grid load resistance.

Also a wide beam of alpha-particles emitted from 239 pu source (25 mm Ø) which was limited by passing through a rectangular plexiglass slit (25 mm length X 4.5 mm width x 8.3 mm thickness) was used.

The detector was operated inside an air-tight thermostat supplied with a temperature control to within ± 0.5°C. A steady stream of dry air free from carbon dioxide was possible to pass through this thermostat. A hygrometer and a thermometer were fixed inside the thermostat to show the relative humidity and temperature during the measuring of the experimental results

EXPERIMENTAL RESULTS

Fig.(2) shows the corrected counting characteristics for various temperatures extending from 10°C to 60°C keeping grid voltage constant at 2250 Volts. Variation in relative humidity (R.H.) due to change in temperature was removed by the possibility of introducing a stream of dry air inside the thermostat. Besides, curves of Fig.(2) were corrected for small deviations from 35% R.H. by using the relative efficiency vs R.H. curves[9]. The results indicate that the sensitivity of the gridded streamer detector is affected by temperature variation. Also it shows that the length of the plateau is somewhat decreased with increasing temperature.

However a small positive plateau slope is noticed at 10°C, while at 50°C and 60°C a small negative plateau slope is noticed.

The corona current characteristics are shown in Fig.(3). These curves show that the corona streamer current I_a increases non-linearly as the anode voltage V_a increases according to equation (1)

$$I_a = a + b V_a + C V_a^2 \quad \ldots\ldots \quad (1)$$

where a, b and c are constants.

Applying the least - squares fits to the present experimental results in Figure (3) , the values of a, b & c are calculated.The computed empirical formulae of current I_a are represented on fig (3) by continuous curves. These curves show good agreement with the experimental reseults which are shown in Fig.(3) by different types of dots.

Fig.(4) represents the relative efficiency "E_r" [$E_r = N(X°c)/N(10°c)$] i.e. the average plateau counting rate relative to the average plateau counting rate at temperature 10°c as a function of the temperature. Fig.(4) indicates that the relative efficiency of the gridded streamer detector increases gradually with the increase of temperature up to ~30°c, after which it increases sharply.

It is clear from fig (3) that the gridded corona streamer detector is more temperature dependent than the wire-plane streamer detector.[6,7]

The internal resistance "r_d" can be determined as function of effective anode voltage "V_{ae}" for different values of temperature by equations (2) & (3)

$$r_d = \frac{V_{ae}}{I_t} \qquad (2)$$

where

$$V_{ae} = V_a - I_a R_a - R_t I_t \qquad (3)$$

Fig (5) shows the results. It is obvious that "r_d" decreases with the increase of the temperature and that

a small variation in "r_a" is observed with the change in temperature at high values of "V_{ae}".

Fig.(6) represents the a.c. resistance "r_a" vs."V_{ae}" for different values of temperatures i.e. 10, 20, 30, 40 & 50°c, as given by equ.(4).

$$r_a = \frac{dV_{ae}}{dI_t} \qquad (4)$$

It is clear that, "r_a" decreases with the increase of temperature, while at large values of V_{ae} a smaller variation in "r_a" is noticed.

Fig.(7) shows the threshold voltage "V_o" vs."T" which is constructed from the curves in Fig.(1). It is clear that as the temperature increases the threshold voltage increases also.

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توقف المنحنيات المميزه على درجة الحرارة للكواشف الفيضية ذات الشبكه

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ملخص البحث

درس تأثير تغير درجة الحرارة علىخصائص العدادات الهالية الفيضيه ذات الشبكه ورسمت المنحنيات المختلفة التى تربط المتغيرات . حددت الكفاءة النسبية ، وهى ذات أهمية كبيرة لاستخدام الكواشف الهالية فى الاغراض العملية .

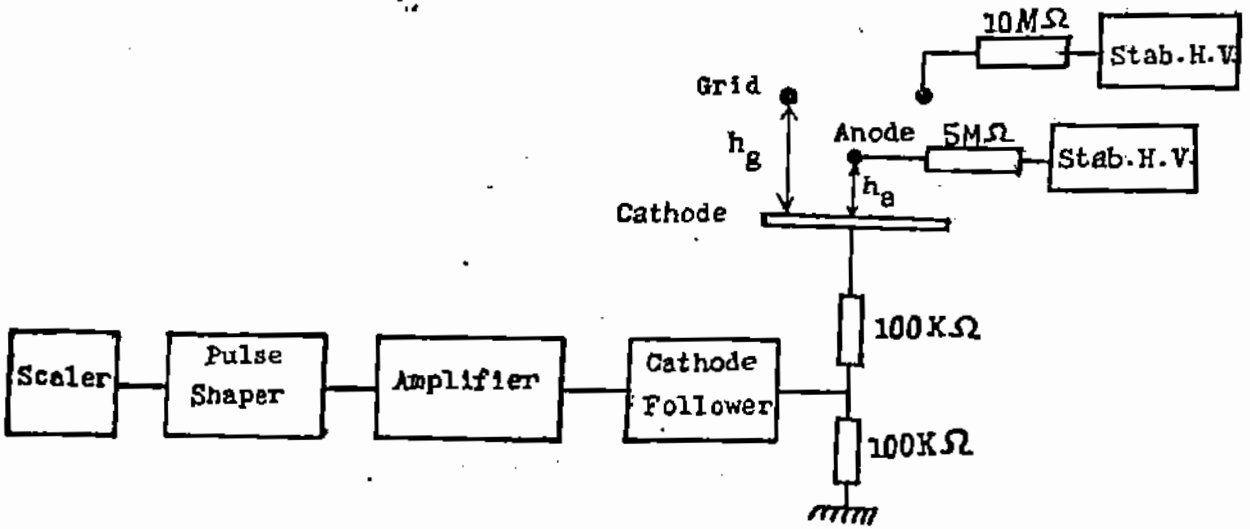

FIG (1) THE CIRCUIT FOR PRODUCING AND RECORDING PULSES DUE TO INCIDENT ALPHA PATICLES.

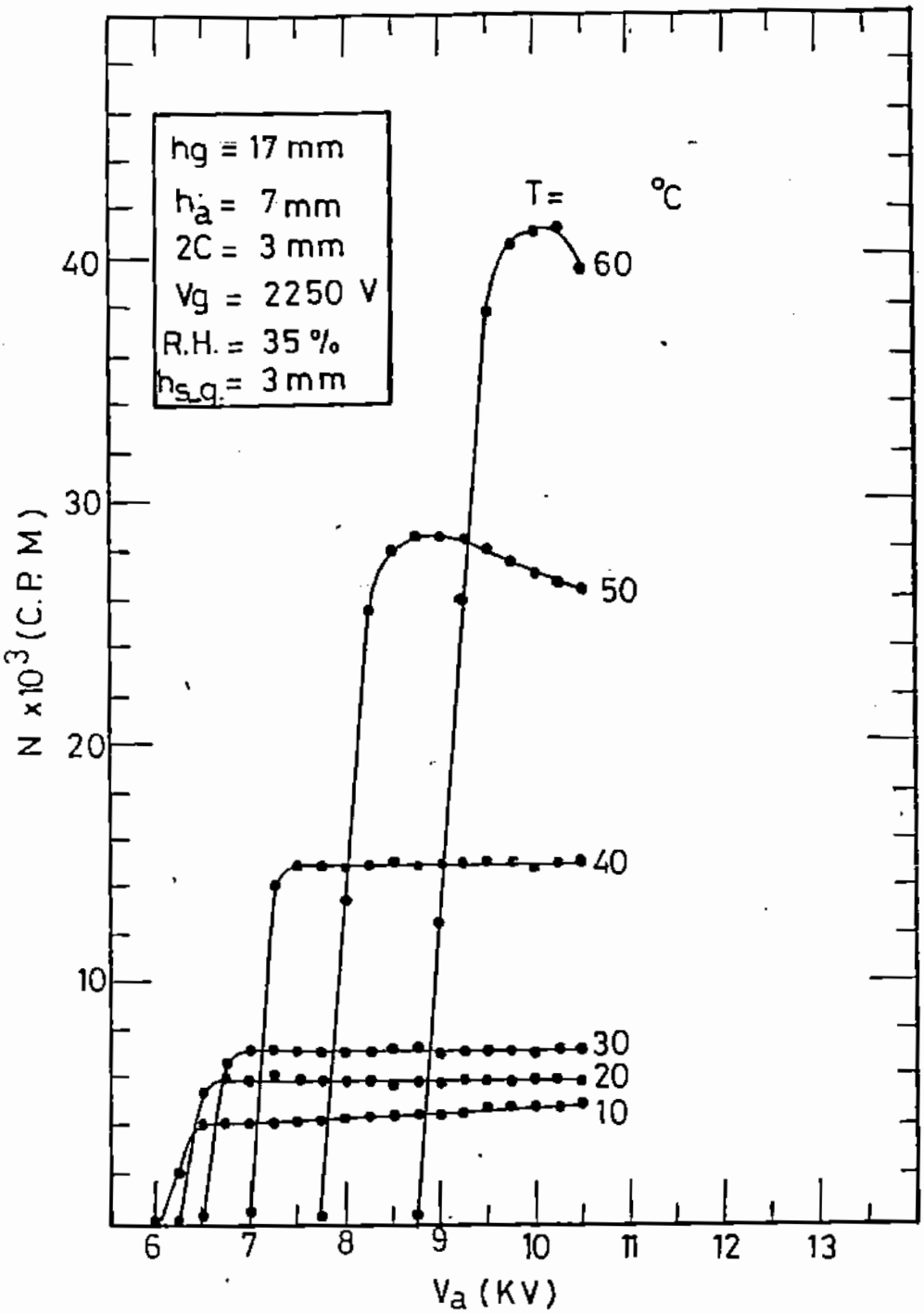

FIG.(2) COUNTING CHARACTERISTICS FOR DIFFERENT VALUES OF TEMPERATURE.

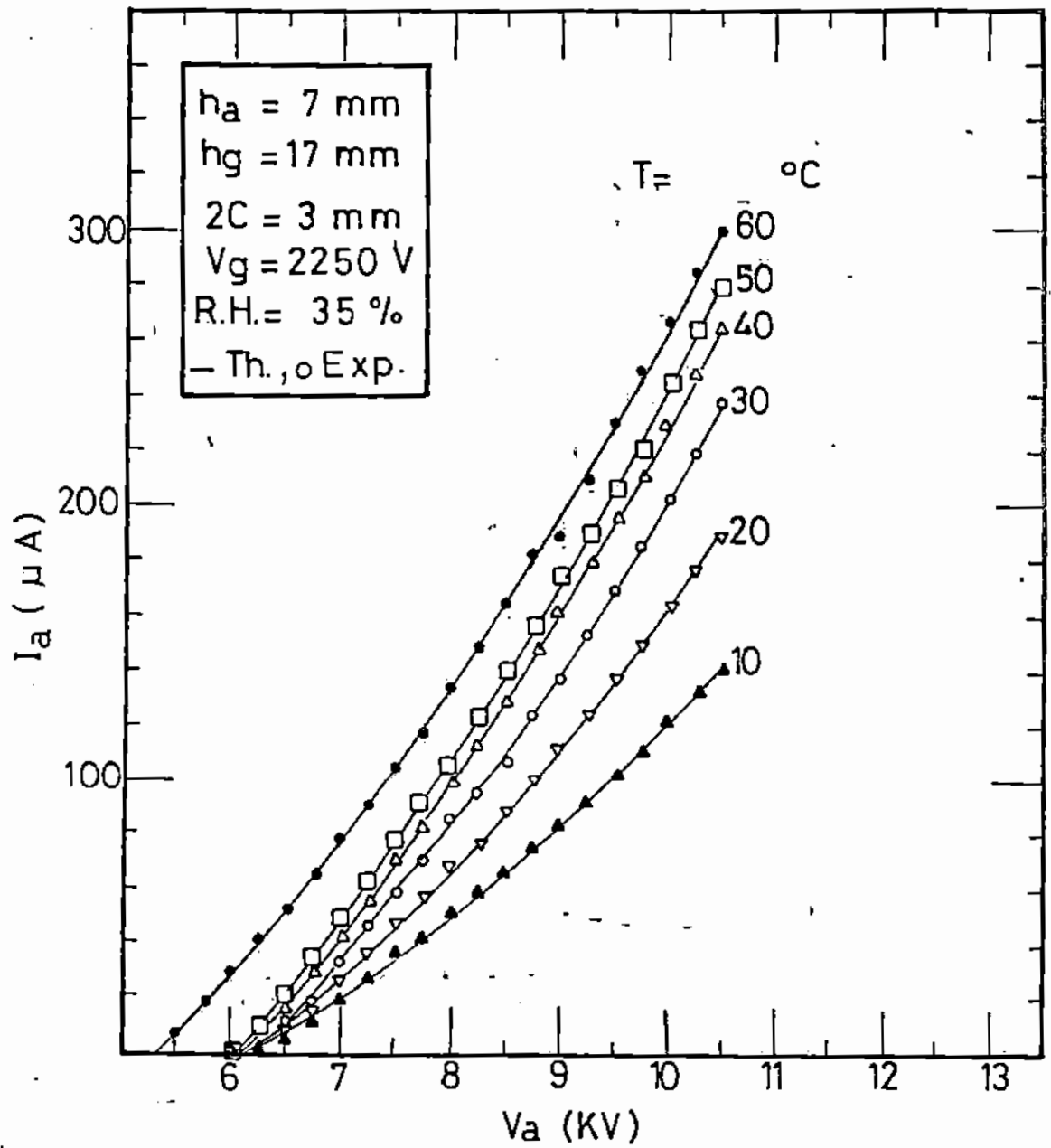

FIG. (3) CORONA CHARACTERISTICS FOR DIFFERENT VALUES OF TEMPERATURE.

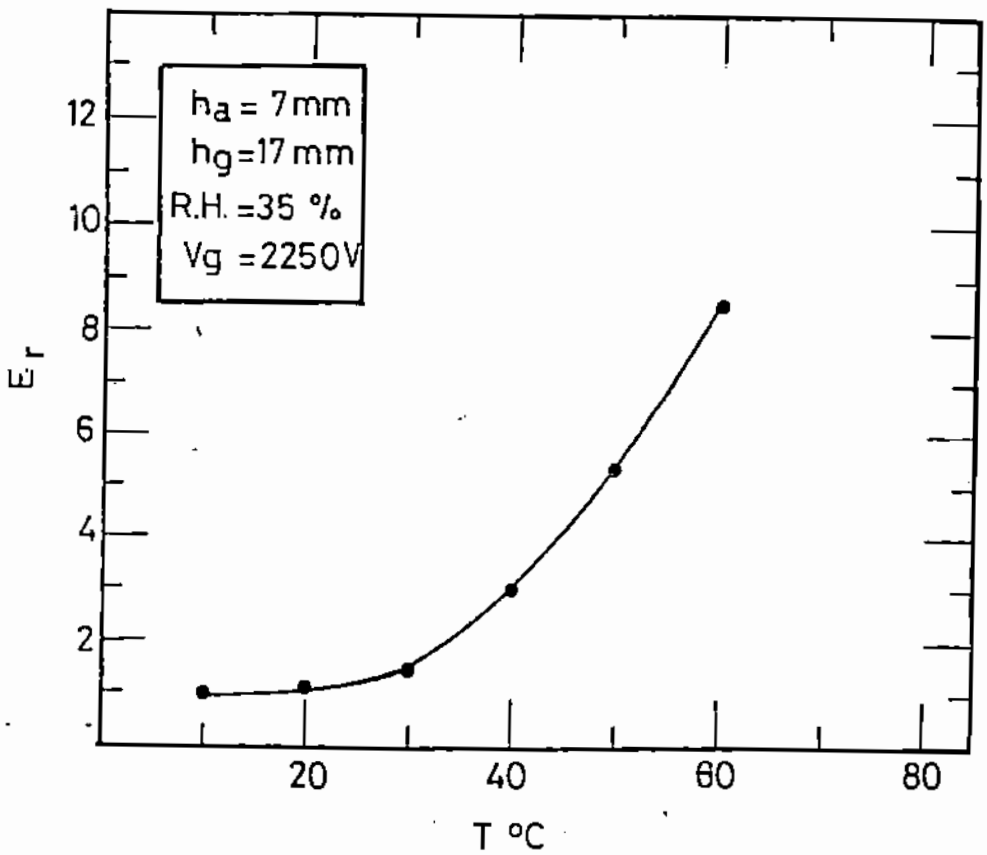

FIG.(4): CALCULATED VALUES OF RELATIVE EFFICIENCY E_r vs. T.

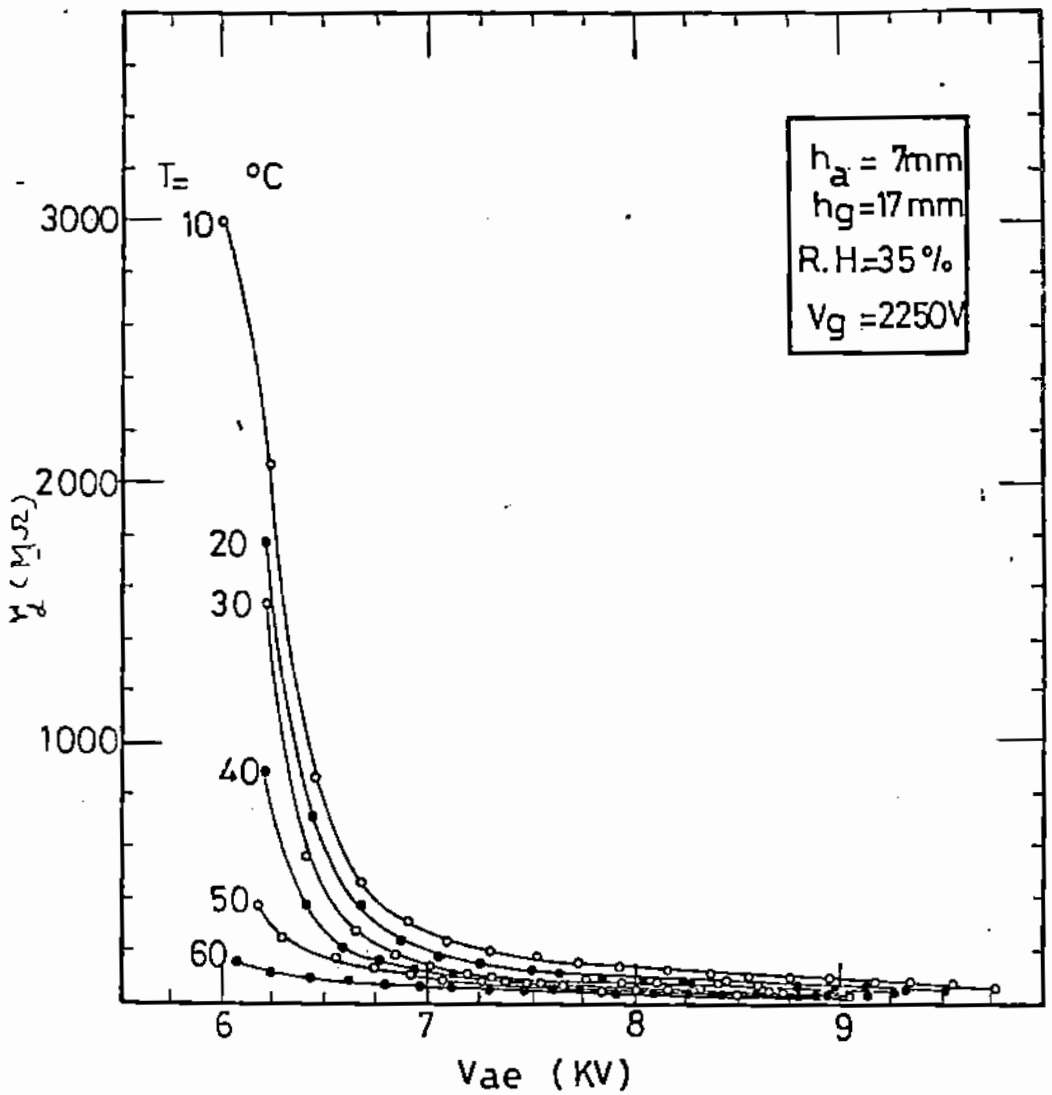

FIG.(5) INTERNAL RESISTANCE r_d vs V_{ae} FOR DIFFERENT VALUES OF T.

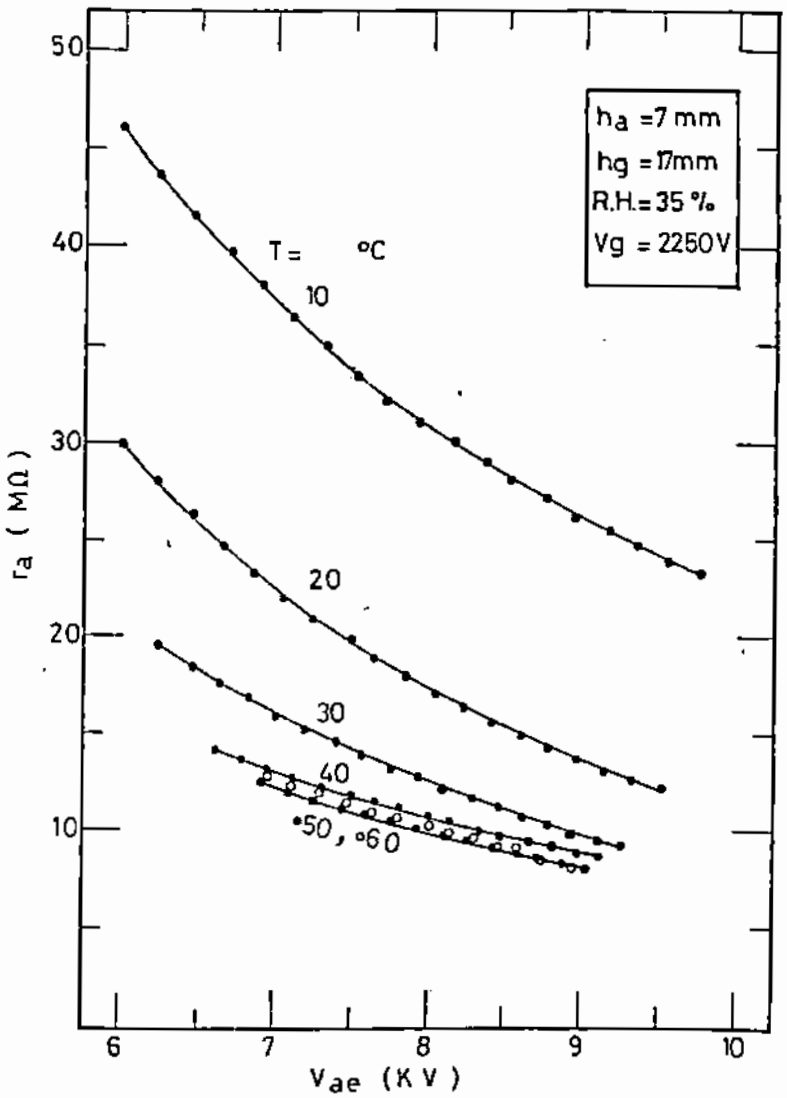

FIG.(6) ra Vs. Vae FOR DIFFERENT VALUES OF T.

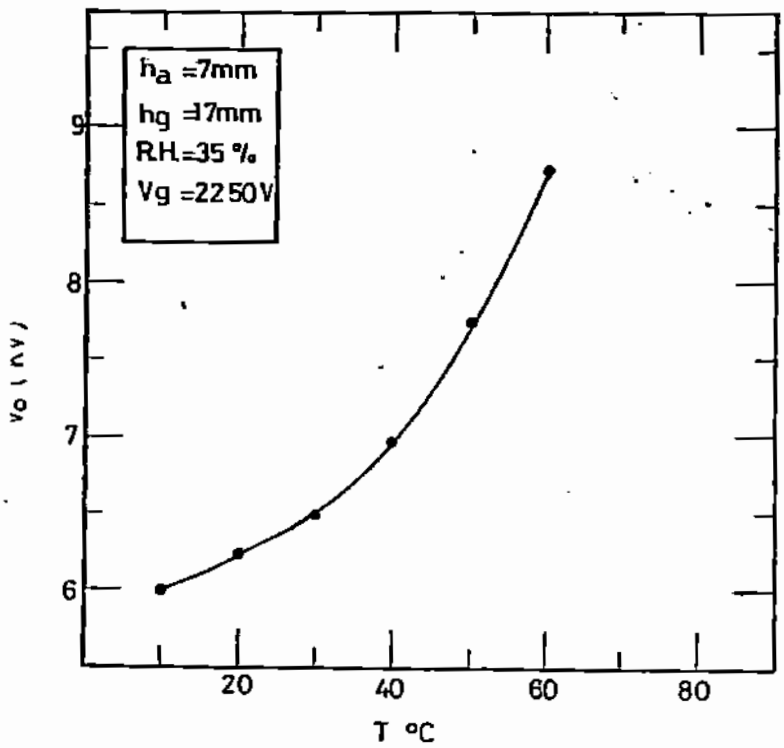

FIG.(7) THRESHOLD VOLTAGE V_o vs. T.

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EFFECT OF SALINE CONDITIONS AND GROWTH REGULATORS ON GERMINATION AND GROWTH OF FIVE SOYBEAN CULTIVARS PLANT.

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ABSTRACT

Seeds of five Glycine max cultivars (Columbus, Caland, issex, Clark, and Crewford) were subjected to saline irrigation water (0,3000,6000, and 9000 ppm NaCl), and presowing seed soaking in either (50 and 100 ppm GA_3), or (500 and 750 ppm CCC).

Saline irrigation water at the lower level of 3000 ppm increased the germination percent of the tested soybean cultivar seeds except Glycine max cv. Crawford, while the medium and high salinity levels (6000 and 9000 ppm), seed germination of five soybean cultivars was markedly reduced.

All tested aspects of growth including height, fresh and dry weight of the five soybean cvs. seedlings were markedly depressed with the increase of NaCl level in irrigation water.

Under control conditions, presowing seed soaking in both GA or CCC. solutions increased the germination precent of Clark seeds and decreased it for the other varieties. However, under saline conditions, seed sooking treatments improved the germination of Clark and Crawford only, and the treatments relatively reduced the harmeful effects of salinity on seedling growth.

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INTRODUCTION

One of the major problemes which interferes with crop growth and production is salinity conditions: of soil or irrigation water. High salinity levels, usually reduce water absorption which in turn inhibits the most physiological processes, resulting in either reduction or complete failure of seed germination. Several investigators, e.g., Bernstein and Hayward (1958), Strogonov (1962), Tailakov (1967), Petrosovits (1968), Merri and Poljakoff (1970), and, Rai (1977), studied the differential response of seed germination with respect to Kind and salt concentration, as well as the emparing effect of these factors on growth and yield aspects of different crops. Mainwhile, the use of growth regulators including gibberellic acid and cycocel is Known to increase the tolerance of plants to the adverse effect of soil salinity (Nieman and Bernstein 1959). The response of plants to such substances varies according to the nature of the used substance, its concentration, and the growth stages of treated plants (uppel and sing 1976). Also, it was reported that presowing treatments or hardening of plant seeds induced good germination (Cocks and Donald 1973), increased tolerance of plants to salinity as measured by more active coleopeile growth (Hafeez and Hudson 1964), and, development of bigger root system (Chanduri and Weibe 1964).

Nowadays, the current national plan calls for cultivate economical crops in the newely reclaimed lands by using the available water sources, such as the underground and dranaige water. Under such conditions, it is desirable to evaluate the salinity degree of irrigation water which may not adversly reduce seed germination and also try to minimize or counteract the harmeful effect of saline water by several means. Therefore the aim of thie investigation was to study the influece of chloride salinity and presowing seed treatments with GA_3 or ccc., upon seed germination, height, and weight of seedling shoot for five saybean cultivars plant.

MATERIALS AND METHODS

A pot experiment was conducted under green house conditions at N.R.C., Dokki, Cairo, Egypt, to study the effect of different levels of sodium chloride, presowing seed treatment with GA_3 or CCC solutions upon germination percentage, seedling height, fresh and dry weight (aerial parts), for five soybean cultivars (Columbus, Caland, Issex, Clark, and,Crawford). Plastic pots, 15 cm., in diameter supplied with drainage hole were used as seeds bed, and each pot was filled with air dried pure sand, previously treated with Con. hydrochloric acid and washed throughly with water. Twenty treatments were applied for each Spp., and each treatment consist of ten pots. The treatments were four salinity levels of irrigation water (0,3000, 6000, and 9000 ppm. NaCl), and their combination with

presowing seed soaking for 6 hrs., in either GA_3 (50 and 100 PPM.), or CCC (500 and 750 PPM.), solutions. Concerning the salinity treatments, the seeds were presowing soaked also for the same period in tap water. In all cases, the soaked seeds in either tap water or growth regulator solulions were air-dried on filter paper before sowing at a constant depth in the pots (10 seeds/pot). After sowing all treatments (each of 10 pots), were arrangeal in complete randomized block design, and the pots of each treatment recieved the proper saline irrigation water in sufficient volume to allow dripping, and then at 2 days interval throughout the experimntal period. The number of germinated seeds per each pot waa daily recorded until aconstant number of seedlings was obtained and the germination percentage was then calculated. The seedlings were then allowed to grew, and for seedlings fertilization, one g., from each of Ammonium nitrate and Superphosphate were added to each pot. After six weeks from starting the experiment, seedling height, fresh, and, dry weight (at 70 C°), of seedling aerial parts were determined.

RESULTS AND DISCUSSION

1- Effect of saline Irrigation water and Presowing seed soaking on Germination percent:

The data of germination percent for the seeds of different soybean species as affected by saline irrigation water and presowing seed treatment as well as their inter-

action are presented in table (1). It is obvious from these data that the lowest salinity level of irrigation water increased the germination percent of the tested seeds, with one exception, being the seeds of Cv.Crawford, where its germination percent value decreased than the control. Similar results were obtained by Abichandani and Bhatt (1965) with Pennesetum typhoides and Sorghum vulgere, and, Mandour ebal (1985) with Brassica alba seeds. The medium chloride salinity of irrigation water, (6000 ppm), however showed a slight and insignificant reduction in seed germination of soybean cvs. (Columbus and caland), while seed germination of the other species was markedly decreased . Seed germination of all tested Glycine max cvs. plant was sharply depreseed at the higher salinity level of irrigation water (9000ppm), where the lowest germination percentages were recorded. The obeerved decrease in seed germination of soybean CVS. plant due to high salinity level are in agreement with thoee obtained by Uprety and sarin (1974), Rai (1977), Khadr ebal (1980), and Mandour et al (1985).

Regarding the effect of presowing seed soeking in GA_3 or CCC. solutions, the obtained data (table 1), reveal that under control conditions (Zero salinity), the concentrations used of these substances decreased seed germination of the tested soybean CVS., except seed germintation of CV. Clark, where presowing soaking treatment with the different

concentrations of both substances increased clearly its germination percent. Moreover, the obtained data show that the presowing soaking treatments decreased slightly the germination percent of Crawford in comparison to soybean CVS. (Columbus, Caland, and Issex).

The intereaction effect between saline irrigation water and presowing seed soaking treatments in GA_3 or CCC solutions on seed germination showed various responces table (1). Generally, the presowing soeking treatments in the adopted concentrations of both subetances failed to improve seed germination percentage of CVS. Columbus, Caland, and Issex speeics when seeded under the different levels of saline irrigation water. On the other hand, soaking treatments developed clearly the germination percentage of both CVS. Clark and crawford, when seeded under such conditions of salinity. The above mentioned results indicate that only the germination of CVS. Clark and crawford was positivily affected by preeowing soaking treatments wether they seeded under saline or non saline conditions. In this respect, Soliman (1979), observed promotion effect of CCC solution on seed germination of Egyptian berseem seeded under saline conditions, whereas GA_3 did not overcome this conditions, mainwhile El-Fouly (1972), observed that CCC solution did not improve the germination of wheat seeds under saline conditions.

2- Effect of saline Irrigation water and Presowing seed Soaking on seedling Growth:

The mean values of seedling height (cm.), fresh, and, dry weight of seedling shoot, together with their standard error values are presented in tables 2 and 3.

A. seedling Height

Data presented in table (2) showed a general decreasing effect on seedling height by increasing NaCl conc., a result which may be due to the disturbance in metabolic pathway of plants, resulting from the adverse effect of salinity on enzyme activity (Strogonov 1962).

Comparing the dsta given for GA_3 and CCC growth regulators, it is obvious that, soaking seeds in GA_3 100 ppm, increased the seedling height of soybean CVS. (Colombus, Caland, Issex, Clark and Crawford. The results also indicate that CCC treatments had a general decreasing effect on seedling height of the tested seeds except CV.Clark.

The interaction between salinity treatments and growth substances showed that GA_3 treatment 50,and 100 ppm. increased seedling height of the tested seeds grown under different levels of NaCl compared with their counterparts grown under the same levels of salinity but did not soaked previously in GA_3.

Regarding the effect of CCC upon seeds subjected to saline conditions, the previous table showed that the conc., of 750 ppm CCC depressed seedling height of CVS. (Caland and Crawford) and slightly increased the seedling height of CV. Clark however the 500 ppm., conc. did not exert appreciable effects.

B - Moisture Content:

Saline irrigation generally induced increase in the moisture content of columbus, Caland and Issex seedlings and remarkable decrease in moisture content of clark and Crawford seedling grown under the different levels of NaCl (Table 2).

The concentrations used of GA_3 and CCC reduced the moisture content of CVS. Clark and Crawford under saline and non-saline conditions. The increaaed moisture content of soybean CVS. seedlings grown under saline conditions might be attributed to a decreased rate of transpiration (Strogonov, 1962).

C. Fresh and Dry Weight

Data preeented in Table (3) revealed that both fresh and dry matter production decreased by incressing NaCl concentration. Pre Soaking seeds in CCC regulators at conc. of 500 and 750, increased both fresh and Dry Weight

production of CVS. Caland, Issex and Clark, a result which is in harmony with those obteined by KishK and Shalaby (1983), and Khafagi et. al (1984).

The obtained data indicate clearly that, at least one of the used concentrations of GA_3 or CCC stimulated fresh and dry weight production of the five soybeam CVS. subjected to seline conditions.

The interaction between salinity conditions and pre-sowing soaking treatments showed in most cases a general reduction in both fresh and dry weight of seedlings. Nevertheless, soaking treatments seems to be usefull, where these treatments enables the seedlings to survive under the highest level of saline water (9000 ppm). Generally, it could be concluded that clark and Crawford seeds seemed to be more salt tolerance, while soybean cultivars Columbus, Caland and Issex eeeds are relatively salt sensitive. However the imbalonce in nutritive cations in tissues of salt affected plants mey also be responsible for the depression of growth.

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Table (1): Effect of saline condition and seed treatment on germination percent of soybean CVS. Columbus, Caland, Issex, Clark and Crawford.

NaCl ppm	Growth Subs.ppm.	Columbus		Caland		Issex		Clark		Crawford	
		germ. %	germ. % as % of the cont.	germ. %	germ. % as % of the cont.	germ. %	germ. % as % of the cont.	germ %	germ. % as % of the cont.	germ. %	germ. % as % of the cont.
0	Control	77.0	100.0	78.3	100.0	93.3	100.0	68.8	100.0	91.0	100.0
	$GA_3$50	53.0	68.8	54.3	69.3	69.3	74.8	80.8	117.4	86.0	94.5
	$GA_3$100	66.6	86.4	67.0	85.5	82.0	87.8	84.3	122.5	88.0	96.7
	CCC 500	54.5	70.7	55.5	70.8	61.0	65.3	94.7	137.6	85.0	93.4
	CCC 750	70.0	90.9	73.0	93.2	77.0	82.5	94.0	136.6	91.0	100.0
3000	Control	90.0	100.0	79.6	100.0	98.3	100.0	81.6	100.0	81.2	100.0
	GA_3 50	57.0	63.3	58.5	73.5	65.0	66.1	83.0	101.7	83.7	103.0
	GA_3 100	62.0	68.8	63.4	79.6	70.0	71.2	86.0	105.3	86.0	105.9
	CCC 500	64.0	71.1	65.0	81.6	72.0	73.2	83.2	101.9	82.0	100.9
	CCC 750	61.0	67.7	62.0	77.8	69.0	70.1	85.0	104.1	87.0	107.1
6000	Control	74.0	100.0	76.0	100.0	82.0	100.0	58.0	100.0	61.2	100.0
	GA_3 50	51.0	68.9	53.0	69.7	59.0	71.9	66.0	113.7	68.0	101.1
	GA_3 100	48.0	64.8	50.0	65.7	56.0	68.2	73.0	125.0	71.0	116.4
	CCC 500	52.0	70.2	53.4	70.2	58.0	70.7	72.0	124.1	67.0	109.5
	CCC 750	48.0	64.8	49.5	65.1	54.0	65.8	76.0	131.0	73.5	120.0
9000	Control	61.0	100.0	62.0	100.0	69.0	100.0	43.0	100.0	42.0	100.0
	GA_3 50	35.0	57.3	36.5	58.8	43.0	62.3	48.0	111.6	44.0	104.8
	GA_3 100	40.0	65.5	42.0	67.7	48.0	69.5	53.0	123.2	50.3	120.0
	CCC 500	41.0	67.2	43.4	70.0	49.0	71.0	54.0	125.5	48.0	114.3
	CCC 750	39.0	63.9	40.0	64.5	47.0	68.1	56.0	130.2	52.5	125.0

Table (2)*: Effect of the interaction between salinity and growth substances on shoot height (cm) and moisture content (%) of five Glycine max cultivars.

Treatments	Columbus		Caland		Issex		Clark		Crawford	
Growth subs. ppm	Shoot height cm	Moisture content %	Shoot height cm	Moisture content %	Shoot height cm	Moisture content %	Shoot height cm	Moisture content %	Shoot height cm	Moistur content %
Control	10.5±1.9	79.2	14.0±3.4	81.4	15.0±2.3	82.7	14.9±2.5	86.2	15.2±3.7	82.5
GA_3 50	8.9±1.7	81.9	17.0±3.1	83.3	13.0±2.9	78.5	19.2±3.0	84.6	14.3±2.2	75.7
GA_3 100	12.4±1.5	82.8	21.0±2.2	84.7	17.0±2.2	83.5	19.3±1.4	83.2	17.4±3.6	75.6
CCC 500	8.9±0.7	77.5	11.0±2.2	81.9	14.0±1.6	80.8	18 ±1.7	80.7	14.5±3.1	74.3
CCC 750	11.9±1.1	77.4	10.0±1.9	80.7	16.0±1.1	82.0	18.5±2.0	81.8	14.0±2.1	82.4
Control	9.2±1.4	83.3	9.6±1.4	85.5	13.7±2.0	85.6	14.2±1.3	84.4	14.8±1.8	80.6
GA_3 50	9.6±0.8	84.2	11.9±1.2	82.3	14.1±3.0	83.0	17.2±3.2	80.3	14.0±2.0	72.7
GA_3 100	13.3±1.5	82.0	16.4±1.4	83.6	17.7±5.2	83.7	16.6±2.6	83.2	16.0±0.3	70.5
CCC 500	9.0±1.7	81.3	7.6±1.2	81.5	13.4±1.2	82.6	15.2±2.8	81.8	13.0±1.9	72.5
CCC 750	11.3±20	80.4	8.6±1.6	81.7	15.7±4.1	83.8	16.6±3.0	82.3	13.3±2.1	74.5
Control	8.0±0.3	83.3	7.4±0.6	84.2	12.5±1.8	81.6	13.5±1.8	83.0	13.2±2.1	70.0
GA_3 50	11.4±0.7	82.6	9.7±1.3	81.8	15.9±3.8	77.7	16.3±3.0	80.6	14.2±1.6	72.3
GA_3 100	12.9±2.0	83.7	13.1±0.8	83.5	16.9±3.5	81.3	16.2±3.0	81.8	16.9±2.1	71.2
CCC 500	9.1±1.7	73.3	6.9±0.3	82.5	13.6±1.6	74.2	14.5±1.2	80.4	12.7±2.0	71.4
CCC 750	9.6±1.9	77.5	5.9±1.1	80.0	14.1±1.1	78.2	15.2±2.6	79.0	13.9±1.8	71.0
Control	--	--	--	--	--	--	--	--	--	--
GA_3 50	11.0±1.5	85.7	8.3±0.7	81.8	15.0±2.6	76.3	14.3±1.5	80.5	13 ±1.6	66.0
GA_3 100	13.2±2.1	85.0	9.8±1.4	82.7	17.0±3.6	75.0	15.6±2.3	81.4	15.3±3.2	62.5
CCC 500	9.1±1.8	80.0	4.8±0.9	80.5	13.1±4.4	61.7	13.6±4.1	79.6	12.3±1.2	60.0
CCC 750	11.0±1.9	72.5	3.0±0.4	81.3	15.0±2.6	68.3	14.3±1.5	80.0	13.3±1.0	60.6

* Means and standard deviation of 20 plants.

Table (3)*: Effect of the interaction between salinity and growth substances on fresh weight(g/plant) and dry weight (g/plant) of five Glycine max cultivars.

Treatments		Columbus		Calnnd		Issex		Clark		Crawford	
Nacl ppm	Growth subs. ppm	Fr.wt. g/plant	Dry wt. g/plant	Fr.wt. g/plant	Dry.wt. g/plant	Fr.wt. g/plant	Dry wt. g/plant	Fr.wt. g/plant	Dry wt. g/plant	Fr.wt. g/plant	Dry wt g/plan
0	Control	2.40±0.2	0.5	2.10±0.1	0.39	2.20±0.12	0.38	3.19±0.18	0.44	3.25±0.4	0.57
	GA_3 50	2.21±.03	0.4	2.40±0.3	0.40	2.60±0.4	0.56	3.20±0.37	0.49	2.80±0.21	0.60
	GA_3 100	2.92±.0.2	0.6	2.30±0.3	0.35	2.80±0.46	0.46	2.88±0.4	0.47	2.24±1.0	0.56
	CCC 500	1.82± 0.1	0.41	2.21±0.4	0.40	2.50±0.2	0.48	3.01±0.29	0.58	2.10±0.14	0.54
	CCC 750	2.30±.21	0.52	2.29±0.4	0.44	2.90±0.1	0.52	3.09±0.3	0.56	3.24±0.3	0.57
3000	Control	1.50±.12	0.25	1.80± .01	0.26	2.50±.21	0.36	3.33±0.08	0.52	3.04±5.0	0.58
	GA_3 50	1.90±.02	0.30	2.20± .34	0.41	2.53±1.4	0.43	4.23±0.5	0.50	2.20±0.23	0.60
	GA_3 100	2.00±.04	0.36	2.50± .32	0.41	2.95±0.85	0.48	3.12±0.06	0.52	1.90±0.01	0.56
	CCC 500	1.39±.32	0.26	1.90± .25	0.35	2.48±1.2	0.43	2.65±0.04	0.48	2.00±0.21	0.55
	CCC 750	1.58±.51	0.31	2.30± .4	0.42	2.91±1.0	0.47	3.00±0.03	0.53	2.20±0.42	0.56
6000	Control	0. 9±.01	0.15	1.46±0.5	0.22	1.96±0.04	0.36	2.83±0.02	0.48	1.80±0.12	0.54
	GA_3 50	1. 4±.25	0.24	2.42±0.2	0.44	1.84±0.1	0.41	2.84±0.3	0.55	2.02±0.98	0.56
	GA_3 100	1. 6±.43	0.26	2.36±1.2	0.39	2.24±0.21	0.42	2.76±1.2	0.50	1.98±1.2	0.5
	CCC 500	0. 6±.02	0.16	1.95±0.3	0.34	1.82±0.05	0.47	2.30± .25	0.45	1.85±0.88	0.5
	CCC 750	0. 8± 0.1	0.18	1.60±0.1	0.32	1.70±0.03	0.37	2.0 ±1.0	0.42	1.97±0.92	0.5
9000	Control	--	--	--	--	--	--	--	--	--	--
	GA_3 50	0. 7±.002	0.10	1.60± .61	0.29	1.35±0.01	0.32	1.80±0.9	0.35	1.50±0.3	0.4
	GA_3 100	0. 8±.04	0.12	1.91± .09	0.33	1.32±0.12	0.33	2.10±1.2	0.39	1.20±0.11	0.4
	CCC 500	0. 4±.03	0.08	1.95± .3	0.38	0.94±0.03	0.36	2.10±0.32	0.44	1.10±0.19	0.4
	CCC 750	0. 4±.02	0.11	1.50± .01	0.28	1.20±0.015	0.38	1.70±0.15	0.34	1.32±0.21	0.5

* Means and standard deviation of 20 plants.

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ASSOCIATIVE EFFECT OF AZOSPIRILUM LIPOFERUM AND AZOTOBACTER CHROOCOCCUM WITH RHIZOBIUM SPP. ON MINERAL COMPOSITION AND GROWTH OF CHICK PEA (CICER ARIETINUM) ON SANDY SOILS.

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Summary

The interactions between Azospirillum lipoferum, Azotobacter chroococum and Rhizobium spp. were assessed on the growth patern and mineral concentration of chickpea (Cicer arietinum cv. Giza 2) on loamy sand soil and on sandy soil. Seeds were inoculated with Rhizobium and with either Azospirillum or Azotobacter or with mixture of both inoculants. Growth of chickpea was improved by association of Azospirillum and/or Azotobacter with Rhizobium in both soil types used. However, Rhizobium with both inoculants was found to be the most responsive.

Cross sections of chick pea root nodules showed that the two diazotrophs plus Rhizobium improved nodule branching over the Rhizobium inoculated treatments in both soil types.

Generally the values of nutrient concentration in chickpea tissues were higher in plants grown in loamy sand soil than those grown in sandy soil inspite of the plant age and the applied treatment.

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Introduction

Several studies in recent years have explored the interactions of bacterial diazotrophs which are able to colonize the root zones of leguminous or nonleguminous plants and subsequently fixing nitrogen either in symbiotic or in associative manner.

There have been reports that mixed cultures of either Azotobacter spp. or Azospirillum spp. and Rhizobium strains when used as inoculants for several legumes increased nodule number, nodule dry weight (Abou Bakr et al. 1987, Burns et al., 1981, Iruthyathas et al, 1983 Rai, 1983) improved grain yield, nitrogenase activity and increased dry matter accumulation in plant parts (Djordjevic et al., 1982, El-Mokadem et al., 1986, Kumar Rao et al., 1976, Singh and Subba Rao, 1979, Plazinski et al., 1984; Sarig et al., 1986).

There is evidence that, equal growth does not necessarily indicate functional equivalence since there may be nutritional and metabolic differences. Furthermore, there are few reports describing alteration in the chemical composition of some crop species by inoculation with Azospirillum or Azotobacter (Kapulnik et al., 1985, Lin et al., 1983, Okon, 1982) who suggested that inoculation with Azospirillum enhanced the uptake of nitrate, phosphate and potassium.

The objective of the present study was to determine the interaction of Azospirillum lipoferum and/or Azotobacter chroococcum and Rhizobium on nodulation, growth and nutrient concentration on growing chick pea (Cicer arietinum CV. Giza 2) in two different soil types (loamy sand soil and sandy soil). Determinations done throughout plant development stages were compared with rhizobia inoculated control plants of similar growth stages and soil type.

Material & Methods

Biological materials:

Chick pea (Cicer arietinum cv. Giza 2) seeds were obtained from Crop Research Institute, Ministry of Agriculture; Azospirillum Lipoferum, strain isolated from Fayoum Egyptian soil and identified by Girgis (1985), was cultured in maltate medium, (Dobereiner, 1978), Azotobacter chroococcum R, isolated and identified by Elwan and El-Naggar, (1969) was cultured in nitrogen deficient medium (Elwan and ElNaggar, 1971), Rhizobium sp. local isolate, obtained from Egyptian ministry of Agriculture was cultured in yeast mannitol medium (Vincent, 1970).

Experimental design:

To study the above mentioned interactions, a field plot experiment with (2 x 2 m.) factorial design was carried out on either loamy sand textured soil or sandy soil.

Mean values of some soil characteristics are given in table 1. The physico-chemical properties of soil were determined according to Jackson (1967). Treatments in both soil types were as follows:

a) Seeds inoculated with Rhizobium spp. (control).

b) Seeds inoculated with Rhizobium spp.+ Azospirillum lipoferum.

c) Seeds inoculated with Rhizobium spp.+ Azotobacter chroococcum.

d) Seeds inoculated with Rhizobium spp.+mixed culture of Azospirillum and Azotobacter.

Treatments were replicated thrice and were completely randomized.

The average number of cells per ml. varied from $5x10^7$ of Rhizobium spp. to 6×10^9 of Azotobacter chroococcum and 12.5×10^8 of Azospirillum lipoferum. Inoculation of seeds were accomplished by moistening seeds with 10% sugar solution and mixing the inoculum with seeds immediately before sowing. Inoculation treatments received an additional application of Azospirillum and Azotobacter 15 days and one month after sowing. Stringent precautions were taken to avoid transfer of inoculant strains between treatments (Young and Mytton, 1983).

Growth Conditions :

Plants were grown under regular winter conditions and sufficiently irrigated.

Evaluations and assays:

Plant height, number of nodules, dry weight of roots and shoots, number of branches and leaves were recorded in each treatment at 30, 60 and 90 days old. At full maturity pods were collected and seeds were subject to analysis.

For macro and micro nutrient determinations, whole plant samples after harvest were thoroughly washed, dried at 70°C to constant weight, ground and digested in nitric, perchloric and sulfuric acid . Phosphorus was analysed using the vanadatomolybdate colorimetric method (Chapman and Prott, 1978) Ca, Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectrophotometry. K, Na were estimated by flame photometry (Jackson, 1967).

Nitrogen was measured by Kjeldahl method. Protein were estimated by multiplying the total nitrogen content by the factor 6·25 (A.O.A.C 1975). Total carbohydrate constituent were determined according to the method of Malik and Sing (1980).

Preparation of root nodules for observation by light microscope:

Root nodules of 90 days old plants were fixed in Carnoys, dehydrated, embedded and thin longitudinal sections of the nodule central region were prepared (Drury & Wallingtons, 1967) and examined with light microscope to observe nodule morphology.

Results

Effect of inoculation upon plant growth:

Data given in table 2 , reveal that, growth of chickpea was improved by association of Azospirillum lipoferum and/or Azotobacter chroococcum with Rhizobium spp. in both soils used.

It was found that inoculation with Azospirillum or Azotobacter either alone or in combination increased plant dry weight over Rhizobium inoculated control during plant growth (30, 60, & 90 days old). In general, the shoot/root ratio (S/R) of inoculated plants was higher than that of Rhizobium inoculated ones, specially in case of dual inoculation on loamy sand soil, the magnitude of increase ranges from 20% - 87% over their control while differences between treatments were slight in plants grown on sandy soil.

Inoculation with Azospirillum, Azotobacter or a combination of both inoculants produced more leaves/plant than Rhizobium inoculated control plants (Table 2).

The beneficial effect of inoculation were manifested also on the dimensions (height) of the plants; plant height was, in general, proportional to the number of leaves (Table 2). Dual inoculation with both inoculants resulted greater plant height than those inoculated with Rhizobium. Separate inoculation with Azospirillum and Azotobacter produced similar responses in plant height and both treatments produced taller plants than the control. There was little difference between Azospirillum and Aotobacter treatment in plants grown in sandy soil.

Nodulation:

Nodules were formed in all treatments, and mostly of pink interior, suggesting that nitrogen fixation took place. Presence of Azospirillum lipoferum and/or Azotobacter chroococcum caused increase in nodulation (Table 2). The increase in root nodules ranged from 5% to a maximum of 107% (Table 2). In general nodules were bigger and more branched (lobed) in soil inoculated with Azosirillum and Azotobacter. Dual inoculation resulted in the highest response followed by Azospirillum then Azotobacter in both soils used. The internal anatomical response of root nodules to inoculation showed that the two diazotrophs plus Rhizobium

improved nodule branching over the Rhizobium inoculant treatments in both soils (Fig. 1)

Effect of inoculation on nutrient concentration in plant tissues:

From tables 3,4 & 5 it can be seen that, in general the values of nutrient concentrations in chick pea tissues were higher in plants grown in loamy sand soil than those grown in sandy soil inspite of the plant age and the applied treatment.

a) On loamy sand soil, inoculation of Azospirillum and Azotobacter either alone or in combination favours nitrogen and protein accumulation in plant tissues (Table 3), the response was higher with dual inoculation.

The presence of Azospirillum or Azotobacter has shown to have positive influence on phosphorus, potassium, calcium and magnesium content of inoculated plants throughout development.

However, accumulation of sodium in plant tissue was favoured by inoculation at the early stage of growth (30 days old). Azospirillum and Azotobacter inoculation favours the relative uptake of micronutrient like Mn, Zn & Cu in chick pea plants, as compared to Rhizobium treatment.
Lower carbohydrates content was shown in inoculated plants.

b) In sandy soil, differences between treatments in terms of nutrient concentration can be contrasted (Table 4).

The presence of Azospirillum or Azotobacter with Rhizobium has shown to have positive influence on nitrogen, protein and phosphorus content of inoculated plants. Inoculation did not significantly affect the concentration of K and Na in plant tissue. However, treatments induced a decrease in the concentration of Ca & Mg in chickpea.

Plant dry matter accumulation was positively correlated with percentage of total carbohydrate and negatively correlated with Ca & Mg. In general micronutrient contents per gm. dry wt. of Zn and Cu were relatively at higher rates at 30 days old then showed a tendency to decline with age till 90 days. Micronutrients were taken up rapidly during the early stage of growth, then the rate decreased.

Nutrient concentration of seeds:

Effect of Azospirillum & Azotobacter inoculation on nutrient constituents in chickpea seeds in both soils is given in Table, 5 . The differences in nutrient concentrations for fully developed chickpea seeds were less pronounced than the differences in plant tissue at the developing stages (Tables 3 & 4). This an indication that the alteration of nutrients due to inoculation with Azospirillum and/or

Azotobacter may occur probably mainly in vegetative structure or there is every possibility of reachy effect of the alteration of the nutrient constituent of the mature seeds.

Discussion

The experiments conducted indicated that the inoculation of chickpea with Azospirillum lipoferum and/or Azotobacter chroococcum in association with Rhizobium spp. increases the number of nodules, plant height, number of leaves and branches and above and below ground biomass. Plants inoculated with both organisms or Azospirillum grew better than those inoculated with Azotobacter or control plants in loamy sand soil and sandy soil.

This also reflects the involvement of the plant in response to the three interacting bacterial species. However, such growth responses are variable (Table 2) depending on the initial fertility of soil (Lehri et al., 1978 & Subba Rao et al., 1980).

In most experiments there were positive responses of dry matter and/or nitrogen content of chickpea to inoculation of Azospirillum & Azotobacter. The bacterial effect on the S/R ratio found in this study also merit some comments. One of the ways to control the distribution of biomass between shoot and root is based on the feed-

back effect of shoot plant nutrients on the flow of the photosynthetic products to the heterotrophic parts of the system. However another mechanism is also possible to explain the effect of Azospirillum and Azotobacter which are known to synthesize and exude into the medium plant hormones (Tien et al.,1979), which can alter the hormonal balance within the plant, thereby affecting the S/R ratio.

The present results, together with previous reports (Abou Bakr et al., 1987, Burns et al., 1981; Iruthayathas et al., 1983; Rai, 1983) clearly indicate that inoculation of Azospirillum and Azotobacter enhances nodulation and plant growth.

The favourable effect of Azospirillum and/or Azotobacter on agricultural crops is at present attributed to multiple action. They can affect plant growth not only by fixing nitrogen but also by altering microbial balance, supression of pathogenic microorganism, mobilisation of soil phosphate or by providing metabolites that stimulate plant development (Brown 1974, Cooper 1959, Mishustin & Naumova 1962, Shende, et al. 1975; Brown 1974; Brown and Walker 1970 and Davies et al., 1964).

It can be seen that on inoculation with Azospirillum and Azotobacter, more root hairs become susceptible to rhizobial infection. Okon (1984) and Patriquin et al., (1983)

showed that Azospirillum inoculation shortened time appearance and increased root hair formation in roots of wheat and other grasses. Moreover, perhaps Azospirillum cells produce an excretable compound (S) which create new infection sites (Plazinski & Rolfe 1985). Gross-sections of inoculated nodules showed increased nodule branches(lobes). These effects on nodule morphology may be due to the production of plant growth promoting substances by the colonizing bacteria (Azotobacter and Azospirillum or by the plant as a reaction to colonization. Morphological changes may have a physiological effect on inoculated roots were postulated by Okon & Kapulnik 1986.

One of the objective of this experiment was the evaluation of the role of Azospirillum and Azotobacter in association with Rhizobium on chickpea nutrition in low fertilized loamy sand, and weak sandy soil. Data shown in tables 3, 4 & 5 showed that nutrient concentrations also varied among soil type through plant development. Nutrients were taken up rapidly during the early stages of growth in presence of Azospirillum and Azotobacter. This emphasizes the potential role of both inoculants in increasing the efficiency of mineral assimilation and respectively plant growth. Enhaced nutrient uptake following Azospirillum and Azotobacter inoculation suggests that these rhizosphere bacteria increase the availability of nutrient through altering root surface characteristics involved in nutrient

uptake (Lin et al., 1983 and Helimish et al., 1986). Other mechanisms not involving nitrogen fixation have been cited to explain inoculation responses. Enhanced uptake of nitrate, phosphate and potassium by excised root sections of maize and sorghum inoculated with Azospirillum brasilense have been observed (Kapulnik, et al., 1985, Lin, et al., 1983, and Okon, 1982).

Villas and Döbereiner (1981), suggested that Azospirillum may stimulate nitrate assimilation by plant. Moreover, it has been found that in grasses inoculated with Azospirillum there was an increase in mineral and water uptake by the roots and greater accumulation of dry matter in plant parts(Kapulnik, et al., 1983; Kapulnik, et al., 1981; Sarig, et al., 1984 and Yahalom, et al., 1984) resulting in increase in root and shoot biomass.

Our studies have shown that the presence of Azospirillum and/or Azotobacter could influence the Rhizobium—legume symbiotic interaction. Relationships involving physiological compatibility in the tripartite association are unknown and may match in importance the ability of the three associatives to tolerate independently a common set of environmental and edaphic factors. Extended studies of versatile tripartite associations are appreciated for recomendation of their combined use in practice.

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Table (1) : Soil analysis and soil water tension

(1)	granuolometric analysis	Sandy soil	loamy sand soil
	Gravel %	10.4%	8.5%
	Coarse sand	50.8	31.2
	Fine sand	38.0	54.6
	silt	6.7	3.3
	clay	4.5	10.9
(2)	Chemical analysis		
	E.C. (μ mhos = S)	2.8×10^{-3}	4.8×10^{-3}
	pH	7.00	7.10
	Organic matter %	0.38	0.67
	Nitrogen %	0.036	0.055
	Chlorides %	0.20	0.10
(3)	Soil water tension (centibar)		
	before irrigation	33.0	30.0
	after irrigation	10.0	10.0

Table (2) * Interaction between Azospirillum lipoferum, Azotobacter chroococcum and Rhizobium spp.and their effects on growth and biomass distribution of chickpea (Cicer arietinum cv. Giza 2)

Treatments	Plant age	S/R ratio dry wt	dry wt (g/ Plant)	plant height (cm)	no. of nodules plant	no. of branches plant	no. of leaves plant	no. of flower buds/plant
	Chick pea on loamy soil							
Control (Rhizobium)	30 days	3.7	0.14 ± 0.8	17.8 ± 1.8	4.5 ± 0.9	3.0 ± 0.6	4.5 ± 1.0	-
	60 days	6.9	0.56 ± 0.4	38.3 ± 1.2	7.8 ± 2.0	3.0 ± 0.2	24.8 ± 2.4	21
	90 days	6.6	1.06 ± 1.6	42.5 ± 1.1	7.6 ± 0.5	4.0 ± 1.0	27.0 ± 6.4	-
Azospirillum	30 days	5.0	0.17 ± 0.4	20.4 ± 2.4	7.1 ± 1.6	3.3 ± 0.4	8.3 ± 0.4	-
	60 days	9.9	1.09 ± 0.4	44.0 ± 0.9	10.75± 1.4	4.0 ± 0.7	47.0 ± 4.4	32
	90 days	7.8	3.18 ± 1.2	59.2 ± 1.2	11.0 ± 0.9	7.2 ± 2.7	55.0 ± 7.0	-
Azotobacter	30 days	4.7	0.18 ± 1.7	22.9 ± 1.7	8.9 ± 1.1	2.9 ± 1.1	8.0 ± 1.2	-
	60 days	9.8	0.69 ± 1.4	43.9 ± 2.6	9.2 ± 2.1	4.0 ± 0.6	36.7 ± 4.2	32
	90 days	8.1	2.56 ± 1.3	63.8 ± 1.4	10.8 ± 1.3	5.6 ± 0.8	42 ± 3.9	-
Azospirillum ± Azotobacter	30 days	7.0	0.22 ± 1.5	26.0 ± 2.1	9.3 ± 1.0	2.7 ± 0.5	9,6 ± 1.7	-
	60 days	8.0	1.28 ± 0.1	46.5 ± 2.2	16.8 ± 2.1	5.0 ± 0.8	71.5 ± 9.5	59
	90 days	12.3	5.86 ± 1.0	75.0 ± 1.8	15.0 ± 2.1	14.0 ± 2.6	97.6 ± 1.0	-
	Chick pea on sandy soil							
Control (Rhizobium)	30 days	3.9	0.18 ± 0.7	22.9 ± 2.2	5.2 ± 0.73	1 ± 0.25	8.8 ± 0.5	-
	60 days	7.5	0.79 ± 1.5	45.3 ± 4.1	10.6 ± 1.5	3 ± 0.32	39.5 ± 5.7	58
	90 days	11.0	5.4 ± 1.4	61.0 ± 1.5	15 ± 1.1	12.4 ± 1.7	52.6 ±10.9	-
Azospirillum	30 days	4.1	0.25 ± 1.4	22.3 ± 2.9	5.5 ± 0.67	3.42± 0.4	8.7 ± 1.5	-
	60 days	6.9	1.47 ± 1.4	50.5 ± 1.5	18 ± 3.2	4 ± 0.2	55 ± 8.0	82
	90 days	12.7	6.08 ± 1.6	60.2 ± 1.9	16 ± 3.5	11 ± 3.4	52.2 ± 8.3	-
Azotobacter	30 days	4.6	0.18 ± 0.9	23.8 ± 2.5	8.1 ± 1.35	2.92± 0.2	8.3 ± 0.8	-
	60 days	4.7	0.63 ± 1.1	51.8 ± 1.9	13.4 ± 2.1	3 ± 0.2	31 ± 4.2	55
	90 days	15.7	5.08 ± 0.8	67.6 ± 3.3	17.2 ± 3.3	11.2 ± 3.3	77.8 ±13.4	-
Azospirillum ± Azotobacter	30 days	4.0	0.25 ± 0.8	23.2 ± 0.8	10.75± 2.7	3.63± 0.67	9.3 ± 1.0	-
	60 days	8.0	1.7 ± 0.9	48.7 ± 1.4	15.7 ± 3.3	4 ± 0.52	60.3 ± 8.0	60
	90 days	9.9	6.06 ± 1.3	62.6 ± 1.6	18.8 ± 3.1	7 ± 2.2	62.2 ± 9.0	-

* Means and standard deviation of 30 plants from three experimental field plots.

Table (3)*: Interaction between *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Rhizobium Spp.* and their effect on some nutrient constituents of chickpea (*Cicer arietinum*) Var. giza 2. Plants grown in Loamy soil.

Loamy soil		mg / g dry wt							Mg / g dry wt			
Date	Treatment	Nitrogen	protein	total carbohy.	P	K	Ca	Mg	Na	Mn	Zn	Cu
30 days	Control	35.7	223.7	86.0	2.0	24.8	5.4	2.5	1.1	43.2	38.7	17
	Azospirillum	43.1	269.4	61.8	4.3	51.0	9.0	3.7	1.9	106.8	87.0	22
	Azotobacter	40.9	255.6	52.5	5.2	49.5	8.1	3.3	1.7	68.4	73.8	18
	Azospirillum + Azotobacter	45.8	286.3	41.3	4.6	52.5	9.3	3.7	1.9	73.8	70.5	28
60 days	Control	29.1	181.9	40.0	2.8	27.0	18.8	4.2	0.9	127.8	68.1	25
	Azospirillum	29.9	186.9	67.5	4.6	46.5	17.5	4.6	1.4	104.4	60.3	21
	Azotobacter	22.7	141.9	55.5	5.3	30.0	27.5	4.5	1.0	155.4	72.0	38
	Azospirillum + Azotobouter	36.1	200.6	57.9	5.5	26.2	18.8	4.5	0.8	131.4	83.4	26
90 days	Control	23.8	148.8	86.6	3.2	30.0	18.8	4.4	0.8	77.4	76.2	7
	Azospirillum	28.1	175.6	74.5	3.7	34.5	15.0	4.2	0.8	81.0	99.0	12
	Azotobacter	27.0	168.8	75.0	3.5	36.5	13.8	4.3	0.8	83.4	81.0	9
	Azospirillum + Azotobacter	29.5	184.8	62.0	3.9	36.8	15.0	4.5	0.6	75.0	97.2	9

* Values are means of three determinations.

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Table (4)*: Interaction between Azospirillum lipoferum, Azotobacter chrodcoccum and Rhizobium Sp. and their effect on some nutrient constituents of chickpea (Cicer arietinum) Var. giza 2. Plants grown in sandy soil.

Sandy soil		mg / g dry wt								Mg / g dry wt		
Date	Treatment	Nitrogen	protein	total carbohy	P	K	Ca	Mg	Na	Mn	Zn	Cu
30 days	Control (Rlizobium)	35.6	222.5	51.9	2.7	47.3	11.4	4.7	1.5	93.0	66.9	17
	Azospirillun	35.3	220.6	48.0	4.0	48.8	9.9	3.9	1.4	74.7	75.6	23
	Azotobacter	34.2	213.8	45.2	3.9	50.3	8.7	3.7	1.4	63.6	81.3	37
	Azospirillun + Azotobacter	37.5	234.3	58.5	3.5	43.5	10.5	4.4	1.5	78.0	70.0	20
60 days	Control (Rhizolium)	23.0	143.8	66.7	4.6	36.0	23.8	5.4	1.1	165.6	97.5	38
	Azospirillum	29.6	185.0	63.1	4.3	45.0	15.0	2.8	0.9	80.4	71.1	18
	Azotobactor	26.6	166.3	57.4	3.7	39.8	16.3	3.7	0.9	68.4	78.0	20
	Azospirillum + Azotobouter	24.1	150.6	65.9	3.9	37.5	15.0	5.8	0.8	79.2	75.6	21
90 days	Control (Rhizobium)	33.7	210.6	78.8	4.1	39.0	20.0	3.8	0.9	73.8	86.7	10
	Azospirillum	25.1	156.9	42.0	3.4	33.0	15.0	3.7	0.9	63.0	81.0	9
	Azotobacter	26.5	165.6	81.3	3.5	36.8	15.0	3.4	0.8	60.6	92.7	10
	Azospirillum + Azotobacter	33.1	206.9	85.3	3.8	34.5	15.0	3.4	0.7	57.0	72.0	6

* Values are means of three determinations.

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Table (5) Interaction between Azospirillum lipoferum, Azotobacter chroococcum and Rhizobium spp and their effect on some nutrient constituents in the seeds of chickpea (Cicer arietinum) var giza 2

Soil type	Treatment	mg/ g dry wt.								Mg/ g dry wt.			
		Nitrogen	Protein	Total carbohy.	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Loamy soil	Control(Rhizobium.)	32.0	200.0	294	5.7	16.5	1.6	1.5	0.7	85.8	27.0	67.5	6
	Azospirillun	32.6	203.8	200.5	5.5	15.5	2.2	1.5	0.7	92.3	36.6	65.1	3
	Azotobacter	30.4	190.0	286.8	4.8	13.5	1.5	1.3	0.6	48.1	28.2	54.9	6
	Azospirillum + Azotobacter	33.4	210.0	239.3	5.6	15.0	1.8	1.3	0.6	53.3	28.2	64.5	5
Sandy soil	Control (Rhizobium.)	27.8	173.8	271.1	4.9	17.3	1.8	1.8	0.5	57.2	30.0	60.6	1
	Azospirillum	32.6	203.8	215.0	5.5	15.0	1.5	1.4	0.5	44.2	25.8	68.7	8
	Azotobacter	25.1	156.9	205.3	4.8	15.0	1..5	1.7	0.5	49.4	19.8	58.5	3
	Azospirillum + Azotobacter	28.6	178.8	273.8	5.2	12.0	1.6	1.0	0.7	58.2	24.6	60.0	1

* Values are means of three determinations

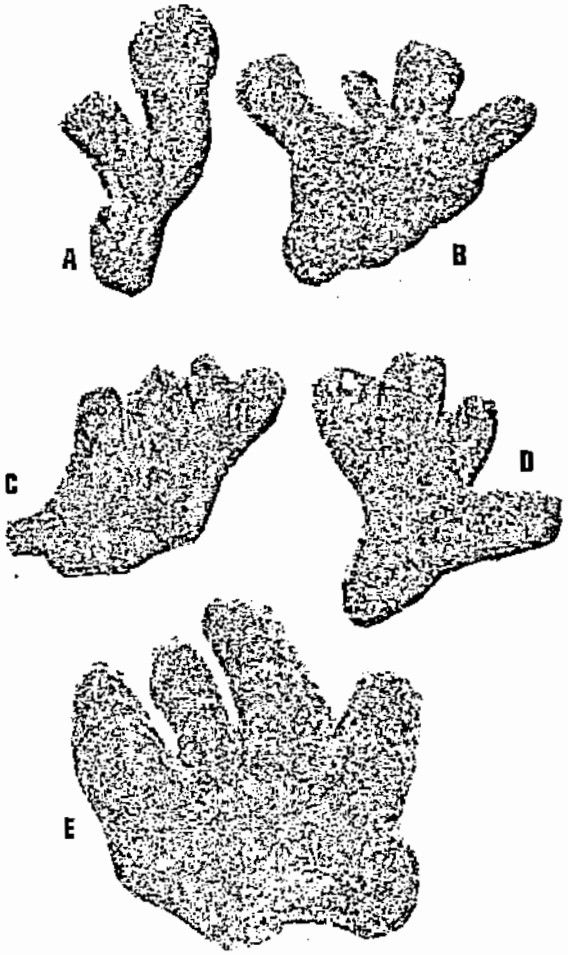

Fig. 1. Cross section of chick pea nodules X 5

A) Control, sandy soil B) Azotobacter, sandy soil.

C) Control, loamy soil D) Azosprillum, loamy soil.

E) Azospirillum + Azotobacter, loamy soil.

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DIFFERENTIAL RESPONSES OF TWO CULTIVARS OF FLAX TO DIFFERENT LEVELS OF SALINITY .I. CHANGES IN CERTAIN GROWTH CRITERIA AND ENDOGENOUS CROWTH HORMONES

BY

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ABSTRACT

Application of different levels of salinity in irrigation water (0, 2000, 4000 & 6000 ppm) to Giza 5 & Reina cultivars of flax plant, resulted in a differential responses in two different directions particularly at the highest level of salinity (6000 ppm).

The plants of Reina cultivar responded positively and exhibited stimulation in the growth criteria and yield, whereas the plants of Giza 5 cultivar followed a reverse situation. On the other hand, the plants of the two cultivars responded similarly to the low and moderate levels of salinity (2000 & 4000 ppm) but differed in the magnitude of the response.

It is suggested that, the plants of Reina cultivar are more tolerant to

salinity than those of Giza 5 cultivar. The difference in the magnitude of response between the plants of the two cultivars was discussed on the basis of the differential changes in the contents and activity levels of the endogenous growth hormones.

INTRODUCTION

Soil salinity is one of the most important factors affecting growth and yield of plants. It represents one of the main problems facing those who are interested in agriculture and plant physiology (Mass and Nieman, 1978).

Through the last few years, the problem of salinity has recieved much attention in Egypt due to the lack of good quality of water obtained from the River Nile owing to the reduction of its water level. On the other hand, the newely reclaimed areas which are far from the main course of the Nile or one of its branches, depends mainly on water of low quality pumped either from wells or collective drains. All of these water sources contain high levels of salinity.

The effect of salinity on the growth and yield of many plants have been reported by many investigators. In certain plant species, the response may be shown as a marked stimulation of the growth and yield. In this connection, we can

refer to the work of: Bakr Ahmed et.al.(1970); Ahmed et. al. (1977, 1978); Khalil et. al. (1978); Singh et.al. (1982), Ahmed et. al.(1983). Al-Rawi and Lazim (1983) ; Shalaby et. al. (1983) and Wassif et.al. (1983). On the other hand, the response may be reflected in the form of a severe reduction of the growth and yield of plants as reported by some authors. Balba (1960); Strogonov (1962); Hutton (1971); Lashin & Atansiu (1972). Shalhavet & Yaron (1973). Abaza et.al. (1974); Ghazi (1976); Heikal(1976); Heikal et.al. (1980 a, b); and Aly (1987). Generally, whatever the response of plants to the salinization treatments, the magnitude of such response varied according to the level of salinity and the species of plant.

Regarding the changes in the endogenous hormones (auxins, gibberellins & cytokinins) as influenced by salinization treatment and the reflection of such change on the growth and development of plants, limited work was carried out in this field (Itai and Vaadia, 1965. Itai et.al.1968, Browning, 1973; Zabadal, 1974 and Ghazi, 1976).

The present paper presents the differential responses of two cultivars of flax to salinization treatments for evaluating their tolerance to soil salinity and to bring into focus the change in growth and yield of plants in relation to the concomitant changes in the endogenous growth hormones in response to the salinization treatments.

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MATERIALS AND METHODS

Two cultivars of flax plant (Linum usitatissimum. L) were used in the present investigation. They were :

1. Giza 5 : local variety, started to be released from 1978. It was originated from the cross between Giza 4 and Precederia in Field Crops Research Institute, Agricultural Research Center.

2. Reina : a fiber type variety imported from Holland.
A pure lot of the seeds of both cultivars was kindely obtained from Agricultural Research Center, Giza, A.R. Egypt.

Time Course Experiment :

Pot experiment was conducted in an open-air wirehouse at the Faculty of Education during the growing season. Seeds of both cultivars were sown on November 20th 1987 in 320 large unglazed pots(40 cm Ø) containing equal amounts of homogenous soil collected from the upper 30 cm layer from the Botanical Garden of the Faculty of Education, Ain Shams University. The pots were irrigated with equal volumes of tap water. Two weeks later, thinning was done so that ten uniform seedlings were left in each pot for experimentation. The plants were exposed to normal day length and natural illumination.

The pots of each cultivar (160 pots in each) were divided into 4 groups, 40 pots each. The plants of the 1st group were irrigated with tap water to serve as control, whereas

those of the 2nd, 3rd & 4th groups were irrigated with 2000, 4000 & 6000 ppm of salinity levels respectively. The saline solution was composed of a mixture of Nacl + $Cacl_2$ (2:1 by weight). The plants of all treatments were irrigated occasionally with certain volume of tap water to keep the salinity level constant.

When the capsules of the differently treated and untreated plants attained full maturity, the following parameters were analysed in the shoot system :

1. Total length of stem (cm).
2. Technical length of stem (cm).
3. Straw yield / feddan (in tons).
4. Fiber yield / feddan (in tons).
5. Fiber percentage.

The data of the different treatments were statistically analysed using the least significant difference (L.S.D) at 1% and 5% levels of probability.

Extraction, separation and bioassay of growth hormones

Extraction : The method of extraction of the growth substances was essentially similar to that described by Zaky (1985).

Separation : Strip loading of the plant extract, each representing 0.5 g dry weight of plant tissue, was carr-

ied out on Whatman No. 1 paper chromatography and developed using isopropanol : ammonia : water (10: 1:1 V/V) in a descending manner. The dried chromatograms were cut into 10 equal strips which were eluted overnight at 5°C with distilled water.

Bioassay : The method used to assay the activity of auxins and growth inhibitors was the straight growth test of Hordeum coleoptile as adopted by Foda and Radwan (1962). The method used for the bioassay of gibberellins and gibberellin-like substances was similar to that adopted by Bently - Mowat (1966). The method used for the bioassay of cytokinins was essentially that of Esashi & Leopold (1969).

The results of the bioassays were analysed statistically using the method of the least significant difference (L.S.D) at 5% level of probability. The significant activities of auxins and growth inhibitors, gibberellins and gibberellin - like substances, and cytokinins are represented by shaded areas.

RESULTS

I. Changes in growth and yield components :

A- Total length of shoots :

It is obvious from Table 1 that, the shoot length of Giza 5 cultivar of flax plant was found to be either

significantly increased in the plants irrigated with the lowest concentration used of salinity (2000 ppm), or highly significantly increased in the plants irrigated with the medium concentration used of salinity (4000 ppm) over that of the control. On the other hand, the shoot length of the plants irrigated with the highest concentration used (6000 ppm) was found to be significantly decreased below that of the control.

Regarding the response of Reina cultivar to the same levels of salinity, it is evident that the shoot length of the treated plants followed a different trend as compared with that of Giza 5 cultivar. At all levels of salinity used (2000. 4000 & 6000 ppm), the shoot length was highly significantly increased.

B- Technical length of shoots :

The technical length of Giza 5 cultivar shoots was found to be highly significantly increased in plants treated with 2000 & 4000 ppm of salinity levels as compared with that of the control. On the other hand, in response to treatment with the highest level of salinity (6000 ppm), the technical length of the shoot appeared to be non significantly affected.

As concerning the change in the technical length of

Reina cultivar plants, it was found that at all salinity levels used, the technical length was remarkably increased over that of the corresponding control. This increase was found to be either highly significantly increased at 2000 & 4000 ppm of salinity level or significantly increased at the highest concentration used (6000 ppm).

C- Straw yield / feddan :

The changes in the straw yield / feddan of both Giza 5 and Reina cultivars in response to treatment with various levels of salinity were found to follow the same pattern of change as the technical length of the shoots (Table 1).

D- Fiber yield / feddan :

As regards Giza 5 cultivar, the fiber yield/feddan was found to be highly significantly increased at the relatively low levels of salinity (2000 & 4000 ppm), whereas the same character was highly significantly decreased in plants irrigated with the highest level of salinity (6000 ppm).

In Reina cultivar plants, the pattern of change in fiber yield/feddan was quite different. It was highly significantly increased at all levels of salinity.

E- Fiber percentage :

The fiber percentage of both Giza 5 and Reina cultivars showed highly significant increases in response to the irrigation of plants with the relatively low levels of salinity (2000 & 4000 ppm). On the other hand, the same character was found to be highly significantly decreased in Giza 5 cultivar and non significantly affected in Reina cultivar due to treatment with the highest level of salinity (6000 ppm).

II. Changes in the endogenous growth hormones :

A: Changes in auxins and growth inhibitors contents :

The pattern of changes in auxins and growth inhibitors in the control plants and those irrigated with the different levels of salinity are illustrated in fig.(1).

It is apparent that the extract of the control plants of Giza 5 cultivar contained at least 4 growth promoting zones having significant auxin activity, the R_f of which are at : 0.0 - 0.2, 0.2 - 0.4, 0.4 - 0.6 and 0.8 - 1.0. The last two zones appeared likely to be indole acetic acid (IAA) and indole acetonitrile (IAN) since they produced the same positive colour reactions with the reagents testing for indole substances as the authentic compounds. In addition the same extract appeared to contain one significant growth inhibiting zone (R_f: 0.6 - 0.8)

which appeared to be unsaturated lactone since it gave a positive colour reaction with diazotized p - nitroaniline reagent.

The extracts of plants irrigated with the lowest level of salinity (2000 ppm), showed a marked increase in the activity levels of the growth promoting zones which appeared to be unchanged in its number but their R_f values were different as compared with those of the corresponding control. Their R_f values ranged between : 0.0 - 0.2, 0.3 - 0.5, 0.5 - 0.7 and 0.7 - 0.9.

The extract of plants treated with the moderate level of salinity (4000 ppm) contained the highest number of significant growth promoting zones which reached their maximum activity levels as compared with that of the untreated plants and that of the treated ones.

On the other hand, the highest level of salinity (6000 ppm) caused a sharp decrease in the content and the activity levels of the growth promoting zones. The zones of IAA sharply decreased and IAN disappeared completely and two significant growth inhibiting zones (R_f:0.1 - 0.2 & 0.7 - 0.9) appeared in the extract of plants treated with that highest level of salinity.

Concerning auxins and growth inhibitors of Reina cultivar, it is clearly shown from the histogram illus-

trated in fig.(1) that, the extract of the untreated plants contained 4 growth promoting zones having significant auxin activity, all of which appeared to contain indole compounds. The R_f values of which ranged between: 0.1 - 0.3, 0.3 - 0.5, 0.6 - 0.8 and 0.8 - 0.9. On the other hand, only one growth inhibiting zone was found in the same extract (R_f: 0.5 - 0.6).

The content and the activity levels of growth promoting zones which have the significant auxin activity were progressively and markedly increased as the level of salinity was increased. However, this increase in the auxin activity was accompanied by complete disappearance of the growth inhibitors at 2000 and 4000 ppm salinity level. On the other hand, the highest level of salinity used (6000 ppm) increased slightly the auxin activity as compared with that of the control.

It is of interest to mention that in all treatments of salinity, both IAA and IAN did not disappear from the extracts of the treated Reina plants.

B: Changes in gibberellin contents :

The results of changes in gibberellins and gibberellin-like substances of differently salinized - treated and untreated plants of both Giza 5 and Reina cultivars are

illustrated in fig. (2).

It is clearly shown that the extract of the untreated plants of Giza 5 cultivar contained at least 4 growth promoting zones having significant gibberellin activities, the R_f values of which ranged between : 0.0 - 0.1, 0.1 - 0.3, 0.4 - 0.6 and 0.7 - 0.9 respectively. All of the previously detected zones appeared likely to contain gibberellin compounds since they produced a positive colour reaction with the reagent testing for gibberellins. Treating plants with the relatively low levels of salinity (2000 & 4000 ppm) caused marked and progressive increase in the content and activity levels of the zones having significant gibberellin activities without any change in their number. On the other hand, the highest level of salinity (6000 ppm) did not cause any obvious change in the biological activity of the extract since the content and the activity levels of the detected zones having gibberellin activities appeared to be more or less comparable to those appeared in the extract of the control plants.

It is of worthy mentioning that all zones detected in extracts of the differently treated plants of Giza 5 cultivar appeared to contain gibberellin-like substances, since they produced negative colour reactions with the reagent testing for gibberellins with the exception of the zone detected at R_f ranged between : 0.6 - 0.8 in the extract of plants treated with the lowest level of salinity (2000

ppm) which exhibited gibberellin nature since it gave positive colour reaction with the previous reagent.

With regard to Reina cultivar plants, it is clear that treatment with various levels of salinity resulted in marked and progressive increases in the content and the activity levels of the promoting zones having significant gibberellin activities which reached their maximum in the extracts of plants treated with the moderate level of salinity (4000 ppm). In the extract of the plants treated with the highest level of salinity (6000 ppm), the biological activities and the contents of the significant gibberellins were found to be lowered slightly as being compared with those detected in the extracts of plants treated with the relatively lower levels of salinity (2000 & 4000 ppm), but still higher than those detected in the corresponding control.

It is of interest to mention that, the observed marked progressive increases in the gibberellin contents of the Reina cultivar plants in response to salinization treatments, were accompanied by the appearance of gibberellin compounds in the extracts of these plants on one hand, since all zones detected gave a positive colour reaction with the reagent testing for gibberellins, and the increase in the content and the activity level of the zone detected at R_f 0.6 - 0.8 which was corresponding to GA_3

on the other hand.

C: Changes in cytokinin contents :

The results of the changes in cytokinins due to salinization treatments of Giza 5 and Reina cultivars are illustrated in fig. (3).

It is clearly shown that, the fractionated extract of the control plants of Giza 5 cultivar exhibited cytokinin activity represented by 4 significant zones. The R_f of which are nearly at : 0.1 - 0.2, 0.3 - 0.5, 0.5 - 0.7 and 0.7 - 0.9 respectively. The colour test of the previously mentioned zones indicates that these zones are occupied by cytokinin compounds of purine nature. On the other hand, the extracts of the corresponding control of Reina cultivar exhibited cytokinin activity represented by only 3 significant zones. Their R_f values are nearly at : 0.2 - 0.4, 0.4 - 0.5 and 0.6 - 0.8 respectively, all of which are of purine nature since they gave positive colour reactions with the reagent testing for purines. The activity levels of the significant zones which appeared in the fractionated extract of Reina cultivar were found to be lower than those of Giza 5 cultivar.

As regards the changes in cytokinin content of Giza 5 and Reina cultivar plants in response to salinization treatments, it was found that such changes followed more or

less similar pattern (Fig. 3). It is clearly shown that the responses of the test objects due to the different levels of salinity showed increases in the content and the activity levels of the cytokinin compounds at all concentrations applied as compared with those of the corresponding controls. At the same time, the moderate concentration of salinity (4000 ppm) induced the maximum level of cytokinin compounds in both Giza 5 and Reina cultivar plants without any change in the number of the significant zones having cytokinin activity.

DISCUSSION

The results of the present investigation reveal that the two cultivars of flax plant (Giza 5 & Reina) exhibited a differential response by applying the highest level of salinity. The growth characters, yield and the changes in the endogenous growth hormones (auxins, gibberellins & cytokinins) were found to be negatively affected in response to the salinization treatments of Giza 5 cultivar particularly when the highest level of salinity was used (6000 ppm). On the other hand, the same characters of Reina cultivar were found to be positively affected at all levels of salinity, where such characters exhibited either significant or highly significant increase over the values of the corresponding controls (Table 1).

Regarding the effect of the low and moderate levels of

salinity (2000 & 4000 ppm) on the growth characters and yield of Giza 5 & Reina cultivars, it was found that, the shoot length, technical length of shoot, straw yield, fiber yield and fiber % were found to be stimulated over those of corresponding controls. The magnitude of stimulation was higher in plants of Reina cultivar than that observed in plants of Giza 5 cultivar. In this connection, similar findings were reported by several investigators, among them we may refer to the work of Ahmed et. al. (1977, 1978) working on cotton and safflower plants; Khalil et. al. (1978) working on flax plant; Singh et. al.(1982) working on Capsicum; Al-Rawi and Lazim (1983) working on flax plant, Shalaby et. al. (1983) working on wheat plants and El-Gayar (1988) working on flax plant.

Based on the preceeding results, it appears that at the low and moderate levels of salinity , the plants of the two studied cultivars (Giza 5 & Reina), not only could tolerate the salinization treatments, but also exhibited a significant stimulation in the different criteria of growth and yield of plants. Such stimulation in growth criteria may be attributed to the stimulatory effect of the low and moderate levels of salinity on the endogenous growth hormones including auxins, gibberellins and cytokinins (Figs 1,2&3). In this regard, it could be concluded that the tolerance of plants of the two studied cultivars to the low and moderate levels of salinity and their significant stimulated growth and yield might be caused through the enhancement of such levels of salinity on the synthesis of the

endogenous growth hormones to considerable activity levels which enable plants to cope with water deficits and finally exhibit a growth rate surpassing that of the control plants. This conclusion is supported by the findings of Miller & Kramer (1965) and Fischer (1970).

On the other hand, it was found that the highest level of salinity (6000 ppm) induced a different responses in plants of Giza 5 and Reina cultivars respectively. Whereas such high level of salinity caused either non significant or highly significant decline in the parameters of growth and yield of plants of Giza 5 cultivar, the same level of salinity (6000 ppm) induced either significant or highly significant increases in the parameters of the growth and yield of plants of Reina cultivar. Both negative and positive responses of plants of Giza 5 and Reina cultivars respectively as a result of treatment with the highest level of salinity, were found to be correlated to a great extent to the changes in the endogenous growth hormones. In this connection, it is suggested that the changes in the hormonal level evoked certain concurrent sequence of events leading to water unavailability for plants of Giza 5 cultivar and water availability for plants of Reina cultivar. This suggestion is supported by the findings reported by Ouda & Bandurski (1984) and Reid and Wample (1985).

Concerning the changes in auxins and growth inhibitors content of plants in response to salinization treatments with

the low and moderate levels (2000 & 4000 ppm), it was found that the plants of Giza 5 and Reina cultivars followed a more or less similar pattern. In their extracts, there was a marked increase in the content and activity levels of the significant growth promoting zones having indole nature which reached their maximum when the moderate level of salinity was used (4000ppm). It is of worthy mentioning that the magnitude of the content and activity level of growth promoters in the extract of plants of Reina cultivar were found to be relatively higher than those of plants of Giza 5 cultivar. On the other hand, the extract of plants of Giza 5 cultivar which were treated with the moderate level showed the presence of growth inhibiting substance, whereas the extract of plants of Reina cultivar treated with the same level of salinity was found to be completely devoid from the growth inhibitors (Fig. 1). When the highest level of salinity was used (6000 ppm), the extract of plants of Giza 5 cultivar showed a marked decline in the content and activity levels of the indolic auxins and an increased number of the significant growth inhibiting zones, whereas the extract of plants of Reina cultivar showed an increased content in the growth promoters over that present in the extract of the control plants. From such results, it appears that the plants of the two studied cultivars differs in their magnitude of response to high salinization treatment and that difference depends- to a great extent - on the potentiality of plants of each cultivar not only to synthesize adequate levels of the growth promoters, but also maintained such levels from enzymatic destruction by IAA -

oxidase, so the growth promoting substances can serve as modulators of plant response. Our results offer a great support for the previous suggestion.

It is worthy to refer to the complete disappearance of both IAN and sharp decrease of IAA in the extract of plants of Giza 5 cultivar which were treated with the highest level of salinity, while both substances IAA & IAN showed high activity levels in the extracts of plants of Reina cultivar treated with the same level of salinity.

The relative high levels of auxins especially IAA and IAN in the extract of Reina cultivar plants grown in the soil irrigated with the highest applied level of salinity (6000 ppm) may play an important role in increasing the active water uptake (non-osmotic absorption) against the osmotic gradient (Audus, 1972; Wareing & Phillips, 1981; Wilkins, 1984).

This may be one of the mechanisms by which the plants of Reina cultivar were shown to be relatively more tolerant to the high level of salinity than those of Giza 5 cultivar. In this regard, Livine and Vaadia (1972); Hsiao (1973); El-Beltagy & Hall (1974) and Reid & Wample (1985) reported that the endogenous hormonal levels are considered to be a link between water potential and growth responses. They also added that the adaptive

mechanisms of plants to water stress may involve regulation through changes in hormone balance including growth components which serve as modulators or turners for the alterations in water status .

As regards the changes in gibberellins and cytokinins in response to salinization treatments, it was found that the gibberellins of both Giza 5 and Reina cultivars followed a pattern of change more or less comparable to that of auxins (Fig. 2 & 3). On the other hand, the content and activity levels of cytokinins in plants of the two cultivars were found to be nearly comparable and increased over that of the control plants. These results appear to be in accordance with those obtained by Mizrahi et. al. (1971) and in contradiction with the findings of Itai and Vaadia (1965); Itai et al (1968); Blumenfeld (1970) and Itai & Ben - Zioni (1976).

In conclusion, the overall obtained results in the present investigation suggest that plants of Giza 5 and Reina cultivar responded differently to the salinization treatments. The plants of Reina cultivar were found to be more tolerant to high salinity than those of Giza 5 cultivar. The difference in the magnitude of response between the plants of the two cultivars may be attributed to the changes in the contents and activity levels of the endogenous growth hormones and / or the operation of certain adaptive control mechanism leading to the alterations in water status.

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Fig(1) Effect of different levels of salinity on auxin and inhibitor contents of Giza 5 and Reina cultivars of Linum usitatissimum plants

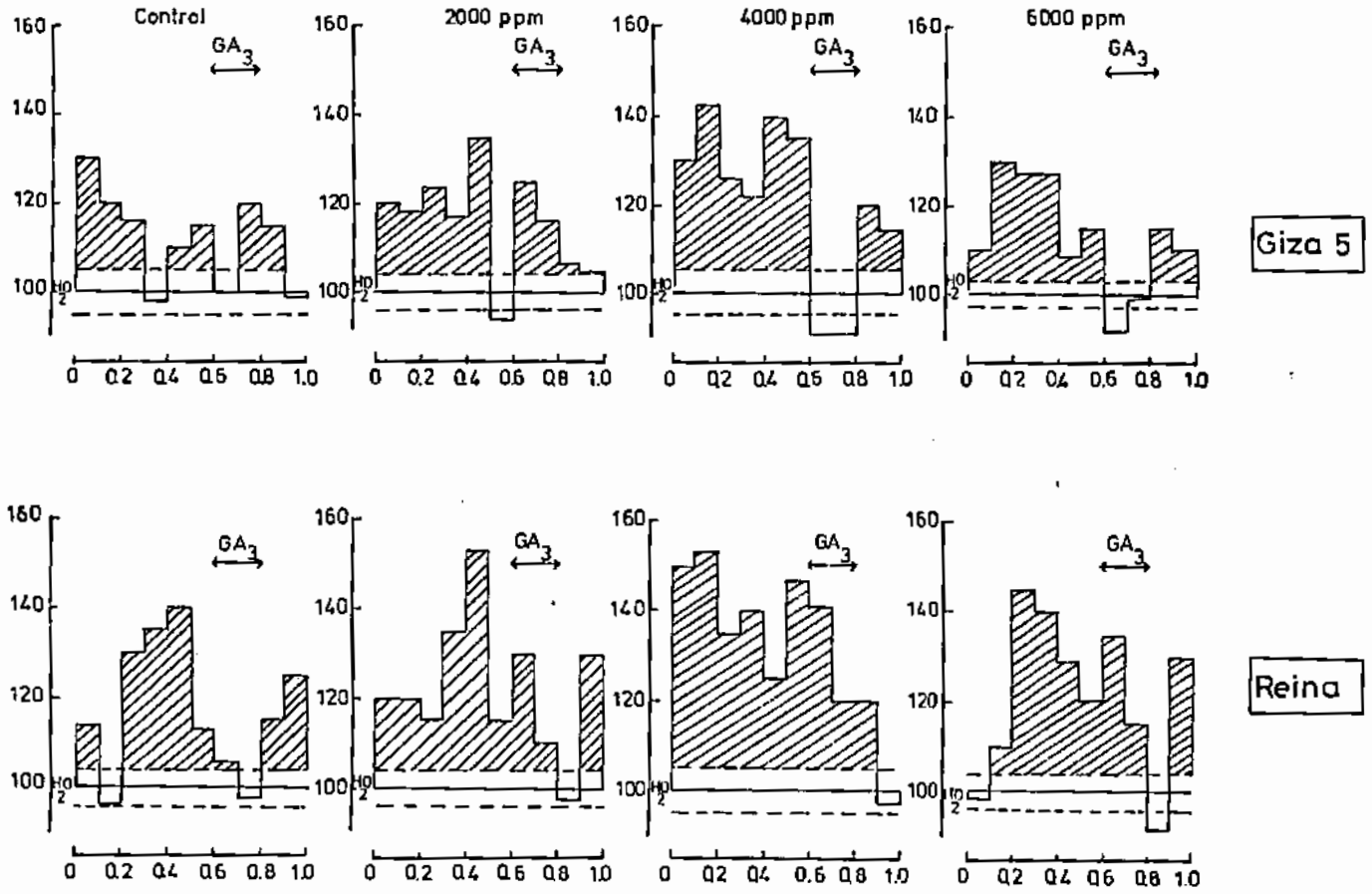

Fig.(2) Effect of different levels of salinity on gibberellin and gibberellin-like substance contents of Giza 5 and Reina cultivars of Linum usitatissimum plants.

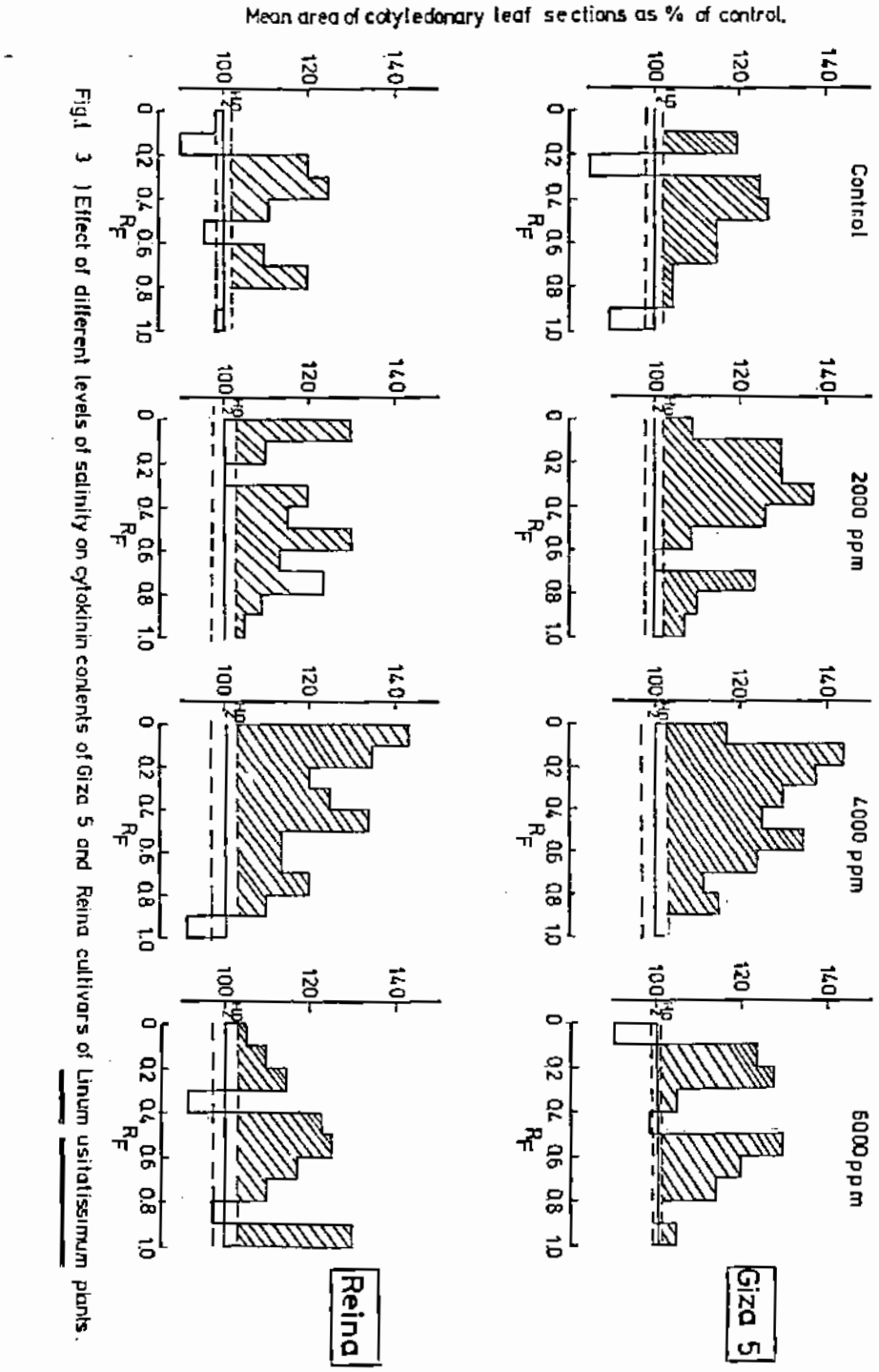

Fig. 3) Effect of different levels of salinity on cytokinin contents of Giza 5 and Reina cultivars of Linum usitatissimum plants.

Table(1): Effect of different levels of salinity on growth and yield components of Giza 5 and Reina cultivars of Linum usitatissimum plants.

Cultivar of flax	Salinity concentration ppm	Total length (cm)	Technical length (cm)	Straw yield in tons/feddan	Fiber yield in tons/feddan	Fiber%
Giza 5	0 (Control)	82.09	73.99	2.80	0.354	12.66
	2000	84.95 + S	78.85 + HS	3.08 + HS	0.412 + HS	13.38 + HS
	4000	90.28 + HS	83.18 + HS	3.26 + HS	0.454 + HS	13.92 + HS
	6000	79.03 - S	73.93 NS	2.70 NS	0.298 - HS	11.02 - HS
	L.S.D at 5% level L.S.D at 1% level	2.17 3.18	3.12 4.13	0.12 0.16	0.006 0.011	0.12 0.16
Reina	0 (Control)	97.44	90.02	1.97	0.330	16.77
	2000	101.91 + HS	96.08 + HS	2.17 + HS	0.386 + HS	17.78 + HS
	4000	106.58 + HS	100.16 + HS	2.34 + HS	0.417 + HS	17.82 + HS
	6000	102.36 + HS	94.01 + S	2.12 + S	0.356 + HS	16.79 Ns
	L.S.D at 5% level L.S.D.at 1% level	3.76 4.07	3.16 4.76	0.11 0.18	0.010 0.013	0.13 0.16

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ANTIMICROBIAL ACTIVITIES OF SOME PLANTS USED IN FOLK MEDICINE IN SINAI EGYPT.

- 8 -

By

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ABSTRACT

Sinai's medicinal plants were screened for their antimicrobial activities against ten different microorganisms. The ethanolic extracts of the shoot system of 41 plant species belonging to 20 families were examined against gram-positive, gram-negative bacteria, a yeast, dermatophytes and a filamentous fungus. 75.6% of the plant species showed activities against the test organisms . Of them 19.5% were active against gram-positive bacteria , 12.3% active against gram-positive and gram-negative bacteria, 12.1% active against gram-positive bacteria and fungi, and 31.7% showed broad spectrum activity.

A preliminary phytochemical screening of *Ricinus communis* shoots showed the presence of tannins; unsaturated sterols and terpenes; alkaloids; flavonoids; glycosides and/or carbohydrates. An antimicrobial agent (Rc 18) was isolated from fresh petiols and stems of *Ricinus communis* L. Its physical, physio-chemical and biological characterstics were studied.

INTRODUCTION

Infectious diseases are of ancient origin. Mankind has a venerable history of use of higher plant extracts for the therapy of such infections. Nothing was reliable known about the nature of infectious diseases untill the 1800s.

The modern antibiotic era were opened with the first clinical trail of penicilin in early 1941. The success with penicilin prompted the search for other antibiotic substances in nature and was also extented to cover the higher plants.

Several individual articles have been published giving the result of large screening studies. The beginning of this new period of intensive search is marked by the work of Osborn (1943), who screened 2300 species from 166 families against *Staphylococcus aureus* and *Echerichia coli*. As a result 63 genera were shown to contain antimicrobial substances which inhibited the growth of at least one of the tested bacteria. Later Huddleson et al. (1944), studied the antimicrobial activities of 23 genera belonging to 15 families against *E. coli*, *S. aureus* and *Brucella abortus*. Hayes (1946) found that 46 plant out of 231 were active against at least one of the test organisms, *S. aureus*, *E. coli*, *Erwinia cartovora* and *Phytomonas tumefaciens*. Carlson et al. (1948) found 115 of 550 plants to have antibacterial activities against *S. aureus* and *E. coli*. Sporoston et al. (1948) found *Impatiens biflora* to be the most active of 73 vermont plants. Atkinson (1949) studied 1200 Australian flowering plants and reported 50 species active against *S. aureus* end 4 active against *Salmonella typhi*. Mariam George and Pandalai (1949) found the aqueous and ethanolic extract of 90 indian plants had antibacterial activity (in vitro); and so on (see for example freerksen and Bonicke, 1951; Hughes, 1952; Tanabe, 1954; Maruzzella and Freundlich, 1959; Celayeta, 1960; Abdou et al, 1972; El-hissy and Ahmed, 1973; Ross et al, 1980; Wat et al, 1980; Mishenkova, et al, 1985; and Nishino et al,, 1970). These screening reports illustrated that it is reasonable to find useful chemotherapeutic

agents from higher plants.

In addition, individual papers from time to time describing study of individual plants for antimicrobial activity. These papers are evidence of a continued interest in antimicrobial agents from higher plants.

In this work we wish to report on the antimicrobial activity of plants used in folk medicine in Sinai.

MATERIALS AND METHODS

Plant material

Samples of the shoot system of 41 plant species belonging to 20 families were collected from sites throughout Sinai. These were wild and cultivated plants which are usually used in folk medicine by the Sinai bedouins.

Herbarium specimens have been deposited in the Botany Department's hebarium, Faculty of Science, Suez Canal University.

extraction procedures :

Fresh plant materials were macerated in a mechanical macerator at room temperature with redistilled methanol. Each extract was then filtered and the process repeated until the plant was exhausted .

The total extract from each plant material was concentrated under reduced pressure in a rotary evaporator at 40 ° C. Care being taken to avoid total dryness which led to loss of active material due to polymerisation.

Determination of Antimicrobial Activity:

I-Diffusion Methods:

Disc Diffusion Method:

Crude extracts were tested for antimicrobial activity against gram-positive, gram-negative bacteria, a yeast dermatophytes, and a filamentous fungus as listed (Table 1) Cultures of bacteria were maintained at 27 °C on Nutrient agar slants, dermatophytes and yeasts on Sabouraud agar and filamentous fungi on Czapek's dox agar.

The activity of crude extracts were assayed by dipping 6 mm. diameter paper discs into the test sample, draining then transferring the discs to the surface of an agar plate previously seeded with the test organism.

For quantitative bioassays, known volumes of the test solution measured onto the paper disc with a micropipette. A solvent blank paper disc were included in all bioassays.

Plates were pre-incubated at 4 ° C for an hour to permit maximum diffusion of the substances. After 24-72 hours of incubation at 27 ° C, the diameter of the inhibition zone were measured and used to estimate the minimum inhibitory concentration (MIC).

II- Dilution methods:

Turbidimeter method (Skinner, 1955):

The two-fold serial dilution of the antibiotic were made in 10 ml Nutrient broth, Sabouraud broth and Czapek Dox broth were used for bacteria, yeast, dermatophytes and filaments fungi respectively. The tubes were inoculated with the test organisms and incubated at 27 °C for 24-72 hours then examined. The minimum inhibitory concentration (MIC) was taken as the dilution showing 50% inhibition of turbidity.

Table 1: Organisms used in screening higher plants for antimicrobial activity.

No	Organism	Classification
Bacteria:		
1	*Bacillus subtilis* ATCC 6633	Gram positive
2	*Staphylococcus aureus* ATCC 25923	Gram positive
3	*Klebsella pneumonia* ATCC 13883	Gram negative
4	*Escherichia coli* ATCC 25922	Gram negative
5	*Proteus vulgaris* ATCC 13315	Gram negative
6	*Pseudomonas aeruginosa* ATCC 27853	Gram negative
Fungi:		
7	*Candida albicans* DSM 70014	Yeasts
8	*Microsporum canis*	Dermatophytes
9	*Trichophyton mentagraphytes*	Dermatophytes
10	*Aspergillus niger* ATCC 9642	Filamentous fungi

The bioautography is often the only way to detect antibiotics that have been separated on paper chromatography (Wallhausser, 1969), or thin layer chromatography.

A preliminary phytochemical screening was carried on the plant material of *Ricinus communis* collected from north Sinai. It was tested for volatile oils, tannins, alkaloids and flavonoids according to Balbaa et al. (1981) methodology, unsaturated sterols and triterpenes (Brieskorn et al. 1961); saponins (Balbaa et al, 1981) and glycosides and/or carbohydrates (Vogel, 1978).

The median lethal dose (LD 50) is determined according to Ahmed, (1979) method.

RESULTS AND DISCUSSION

I- Screening the antimicrobial activity of the selected plants:

Plants still provide an important source of the world's pharmaceuticals (Lewis and Elvin Lewis, 1977) and still seems to be a potentially rich source of antimicrobial agents (Betina, 1983).

Only 5-10% of the world's plant resources have been evaluated for pharmacological activity (McCallin et al. 1982) and very few for the production of antimicrobial substances (Betina, 1983).

Sinai's unique flora has a high proportion of medicinal plants and endemic species and has been the subject of a number of chemical studies which have been summarized in Sinai Peninsula Informative Abstracts of Researchers, Studies and News 1960-1980, (1982).

Very few of Sinai's unique flora have been examined from the antimicrobial point of view. There are many studies in Suez Canal

antimicrobial point of view. There are many studies in Suez Canal University to determine the active principles of Sinai's medicinal plants through a project number 830511 which concerned the assessment of medicinal plants in Suez Canal zone (Bacha , 1984; Soliman, 1985; Gazara, 1986; and Moustafa, 1986). To complete these studies, we are following folklore medicine and are currently investigating Sinai's medicinal plants for antimicrobial activity.

The chosen plants are usually used in folkloric medicine by the Sinai bedwins. These plants are used medicinally as diuretic, cardiotonic, antidiabetic, antitumor, expectorant, stimulant, anthelmintic, antiseptic, stomachia, antiinflammatory agent, ..etc. They are also used commercially for the production of soaps, lubricants, liqueurs, perfumes, fertilizers, etc.

Antimicrobial activities of these plants were examined by extracting their shoot system with ethanol and testing them against gram-positive, gram-negative bacteria, yeasts, dermatophytes,and a filamentous fungus. The results proved that :

1. None of the extracts were active against the filamentous fungus *Aspergillus niger* ATCC 9642.
2. 24.4% of the species were inactive.
3. 75.6% of the species were active against the test organism.
4. 19.5% of the species were active against gram-positive bacteria.
5. The species active against gram-positive and gram-negative bacteria represented 12.3%.
6. The species active against gram-positive bacteria and fungi represented 12.1%.
7. The species showing broad spectrum activity represented 31.7%.

8. Consequently, the species showing broad spectrum activity represented the highest percentage among the other groups.

Reviewing the above results in relation to available literature on the antimicrobial activity of higher plants, it could be concluded that: this study showed that 25 plant species are recorded for the first time to show antimicrobial activity against at least one of the test organisms. These 25 plant species are marked with an asterisk.(Table 2).

II-Isolation, purification and characterization of the antimicrobial agent (Rc18).

Ricicnus communis L. was selected for further studies, since it is widely distributed in north Sinai,. It is tolerant to different severe environmental conditions. Although the plant has many medicinal use, there are no systematic study for its importance as producer of antimicrobial agents. So, it was found reasonable to select this particular plant for isolating its antimicrobial principle and studying its characters.

The preliminary phytochemical screening of the shoot system of *Ricinus communis* L. shows the presence of tannins, unsaturated sterols, terpenes, alkaloids, flavonoids, glycosides, and\or carbohydrates negative results were obtained for volatile oils and saponins.

The results revealed that the antimicrobial activities of *Ricinus communis* L. extracts were detected only in fresh stems, petioles and flowers. Only the miscible solvents succeeded to extract the antimicrobial principle. The highest microbial activity of the plant was reported in spring for all parts of seedling and adult plant namely stem, leaf, petiole and flower (Histogram 1)

Table (2): Antimicrobial activity of the selected plant extracts.

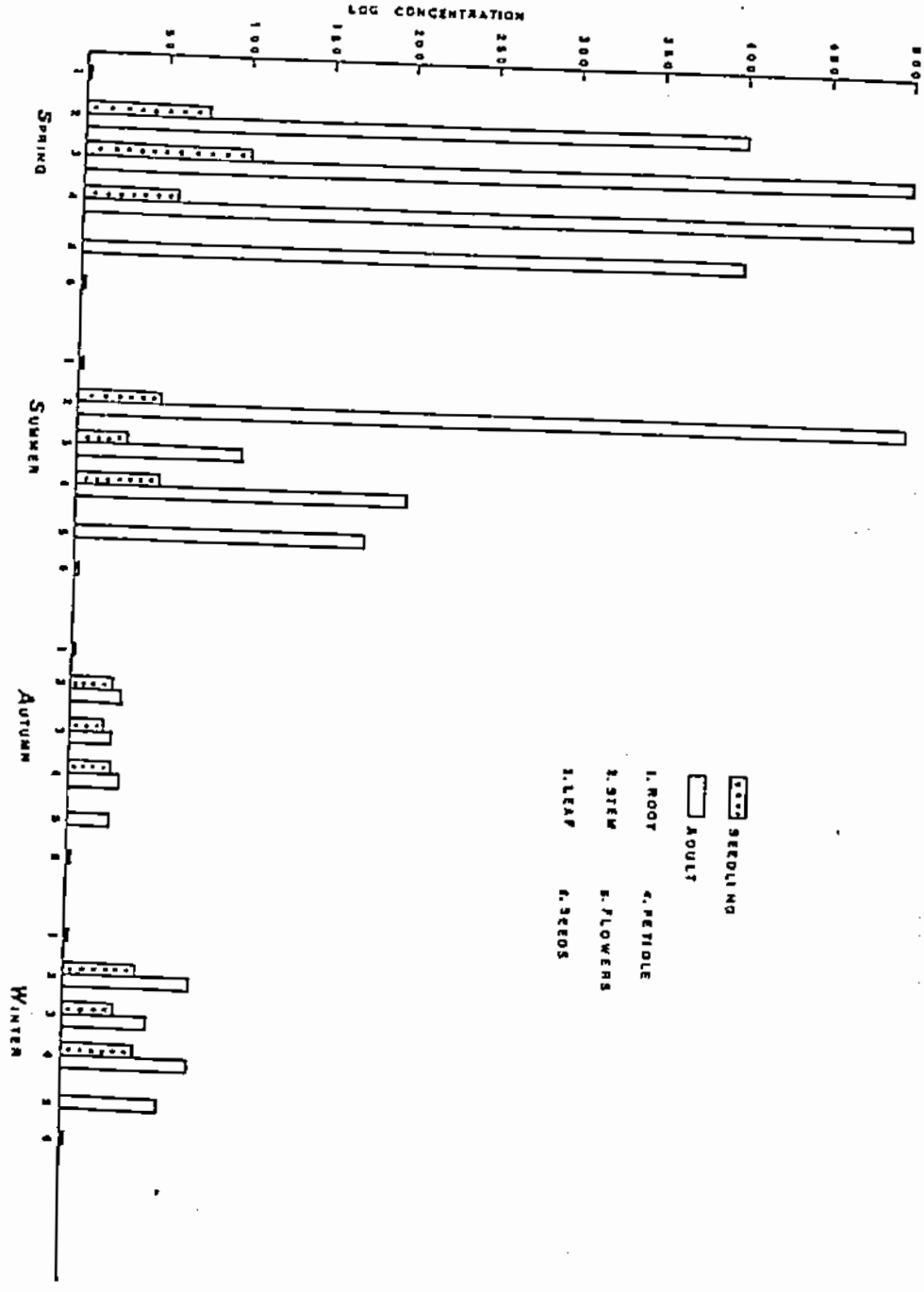
Plant species	Organism number table (1)										Remark
	1	2	3	4	5	6	7	8	9	10	
Apocynaceae:											
1* Nerium oleander L.	++	++	-	-	-	-	-	-	-	-	a
2* Vinca rosea L.	++	++	++	-	-	-	-	-	-	-	a
Capparaceae:											
1 Capparis spinosa L.	-	-	-	-	-	-	-	-	-	-	i
Chenopodiaceae:											
1* Arthrocnemum glaucum Del.	+++	+++	+++	+++	+++	++	+++	+++	+++	-	a
2 Chenopodium murale L.	-	-	-	-	-	-	-	-	-	-	i
3 Cornulaca monacantha Del.	-	-	-	-	-	-	-	-	-	-	i
4 Halocnemum strobilaceum (Fall.) M. Bieb.	-	-	-	-	-	-	-	-	-	-	i
Cleomaceae:											
1* Cleome droseritolia (Forssk.) Del.	++	++	++	++	++	++	++	++	++	-	a
Compositae:											
1 Artemisia herba alba Asso	+++	+++	-	-	-	-	++	++	-	-	a
2* Artemisia judaica L.	+++	+++	-	-	-	-	++	++	-	-	a
3 Artemisia monosperma Del.	+++	+++	+++	+++	++	++	+++	++	++	-	a
4* Conyza dioscoridis Del.	++	++	-	++	+	+	+	+	-	-	a
5* Echinops spinosa L.	+	+	-	-	-	-	+	-	-	-	a
6* Santolina chamaecyparissus L.	++	++	++	++	++	++	++	++	++	-	a
7* Senecio desfontainei Druce	+	+	+	-	-	-	+	-	-	-	a
8* Sonchus oleraceus L.	++	++	++	++	++	++	++	++	++	-	a
Convolvulaceae:											
1 Convolvulus arvensis L.	-	-	-	-	-	-	-	-	-	-	i
Euphorbiaceae:											
1* Ricinus communis L.	+++	+++	+++	+++	++	+++	+++	+++	+++	-	a
Gramineae:											
1 Phragmites australis (Cav.) Trin. ex steudel	-	-	-	-	-	-	-	-	-	-	i

* Species showimg antimicrobial activity for the first time.
+++ highly active
++ active
\+ weakly active
\- inactive

a : active
i : inactive

Table 2 (contd.)

Juncaceae:											
1 Juncus rigidus C.A.Mey	-	-	-	-	-	-	-	-	-	-	i
Labiatae:											
1* Mentha microphylla C.Koch.	++	++	-	-	-	-	-	-	-	-	a
2 M.piperita L.	++	++	++	++	++	++	++	-	-	-	a
3 Ocimum basilicum L.	++	++	-	-	-	-	-	-	-	-	a
4* Origanum syriacum L.	++	++	-	-	-	-	-	-	-	-	a
5* Phlomis aurea Decne	++	++	-	-	-	-	-	-	-	-	a
6* Stachys aegyptiaca Pers.	++	++	-	-	-	-	-	-	-	-	a
7 Teucrium polium L.	++	++	++	++	++	++	++	++	++	-	a
Leguminosae:											
1* Acacia nilotica (L.) Willd. ex. Del.	+	-	-	-	-	-	-	-	-	-	a
2 Melilotus indica (L.) All.	-	-	-	-	-	-	-	-	-	-	i
Myrtaceae:											
1* Eucalyptus globulus Labill.	++	++	++	++	++	++	++	++	++	-	a
Nitrariaceae:											
1* Nitraria retusa (Forssk.) Asch.	++	++	-	++	+	+	++	++	++	-	a
Orobanchaceae:											
1* Cistanche phelypaea (L.) Cout.	++	++	++	++	+	+	++	++	-	-	a
Resedaceae:											
1* Ochradenus baccatus Del.	++	++	+	+	+	+	++	-	-	-	a
Solanaceae:											
1 Datura stramonium L.	++	-	-	-	-	-	-	-	-	-	a
2 Hyoscyamus muticus L.	-	-	-	-	-	-	-	-	-	-	i
Tamaricaceae:											
1* Tamarix aphylla (L.) Karst.	+	-	-	-	-	-	-	-	-	-	a
Thymelaeaceae:											
1 Thymelaea hirsuta (L.) Endl.	-	-	-	-	-	-	-	-	-	-	i
Umbelliferae:											
1* Ammi majus L.	++	++	-	-	-	-	-	-	-	-	a
Zygophyllaceae:											
1* Peganum harmala L.	++	++	-	++	-	-	-	-	-	-	a
2* Zygyphyllum album L.f.	+	+	-	-	-	-	-	-	-	-	a
3* Z. coccineum L.	+	+	+	-	-	-	-	-	-	-	a



HISTOGRAM(1) SEASONAL DISTRIBUTION OF *R.c.18* IN DIFFERENT PARTS OF *RICINUS COMMUNIS* L.

Extraction and purification of the active principle:

The systematic fractionation scheme of extraction and purification of the active principle is illustrated in Fig. 1.

The isolated pale yellew powder was subjected to further purification by thin layer chromatography using silica gel G plates and ethyl acetate: methanol (2:1) as solvent system. The pure active principle (Rc18)was obtained as pale yellow crystals (m. p. 203-205 ^{0} C). It is soluble in acetone, butanol, ethanol, ethyl acetate and water, but insoluble in petroleum-ether, ether or chloroform. The ultraviolet absorption spectrum of the antimicrobial agent (Rc 18) in methanol exhibits two maximum peaks at λ *max* 255 and 313 nm. (Fig.2). These two regions are in agreement with those of flavones and flavonoids. The infra-red spectrum showed 1 characteristic peaks at 530, 1110, 1660 3480 cm^{-1}. (Fig.3).

The purity of these crystals was ensured by bidimensional chromatographic technique using different solvent systems.

The absorption at 530 cm was assigned to benzene ring, the strong absorption at 1110 cm^{-1} to a single bond stretching; the peak at 1660 cm to C=C, C=N, C=O and that 3480 cm^{-1} to NH or OH stretching (Williams and Fleming 1980). Since the elemental analysis showed the absence of nitrogen, the peak at 1660 cm^{-1} may be due to C=C or C=O and that at 3480 cm^{-1} may be due to OH stretching only which characterize the phenolic compounds.

These physical and physico chemical characteristics lead to the conclusion that the antimicrobial agent Rc 18 isolated from *Ricinus communis* L. may be a flavonoid .

This is in agreement with the fact that numerous flavonoids of plant

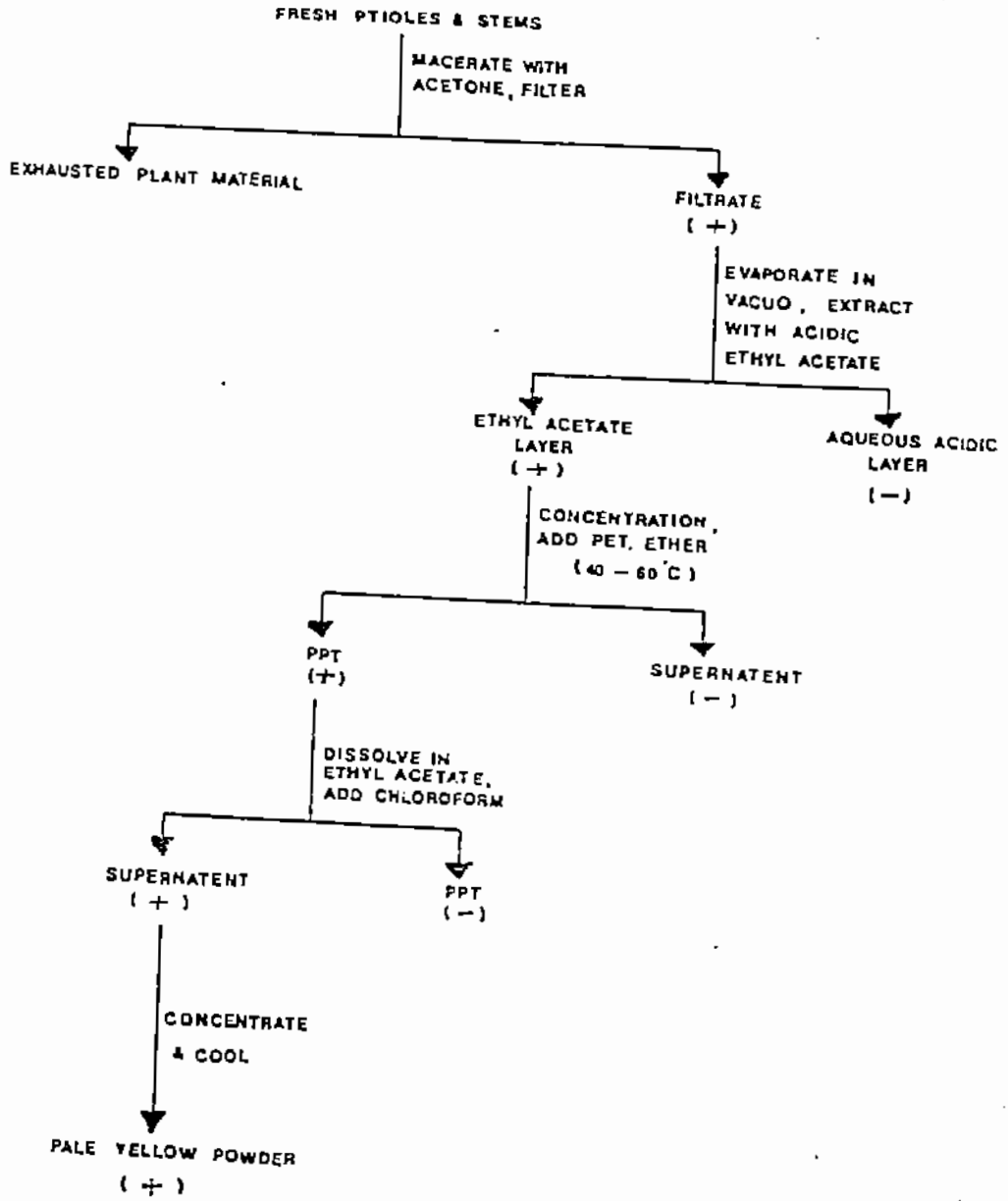


FIG (1) SCHEMATIC DIAGRAM FOR ISOLATION OF ANTIMICROBIAL AGENT FROM *RICINUS COMMUNIS* L.

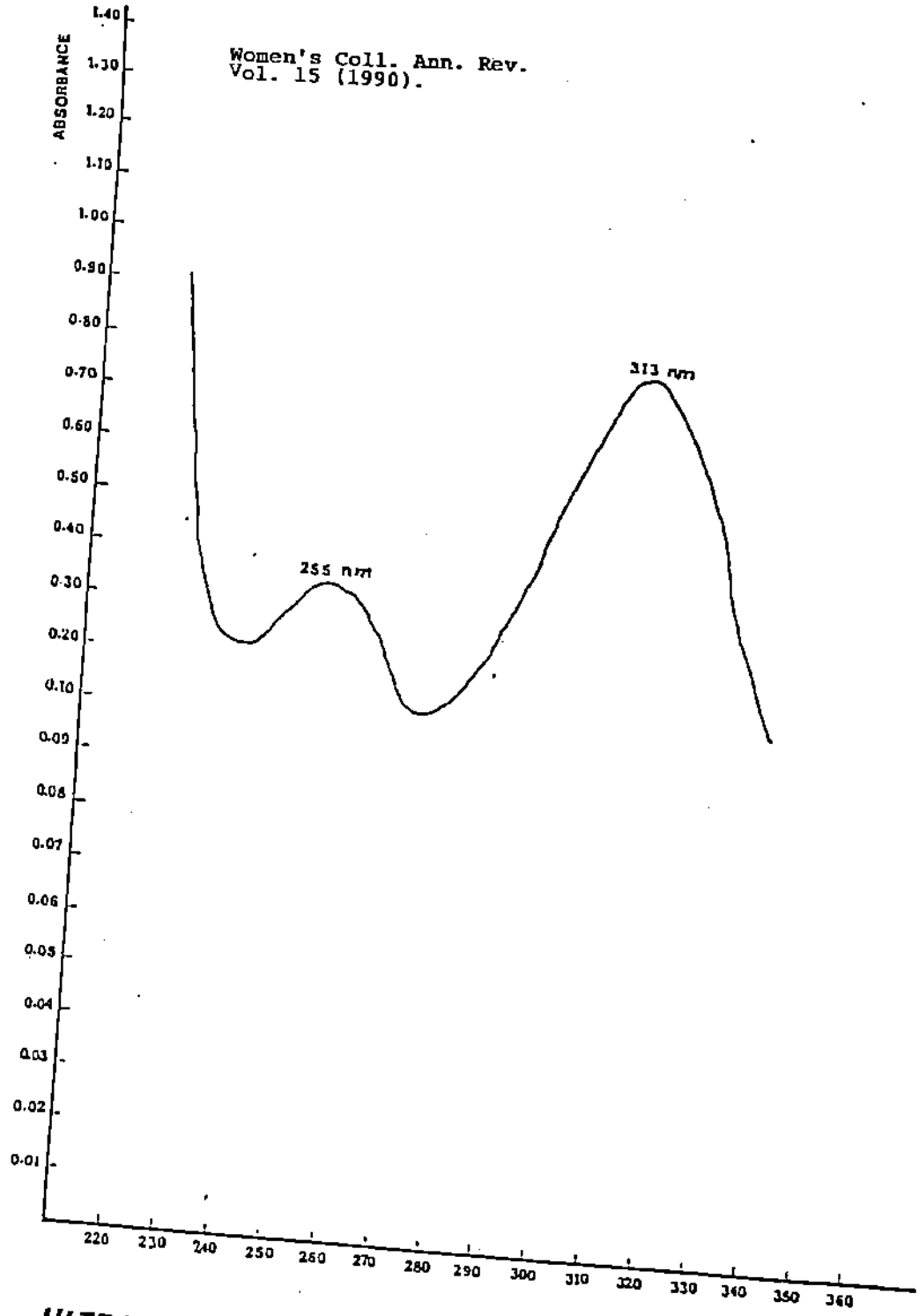


fig.'2' **ULTRAVIOLET ABSORPTION SPECTRUM OF R.C.18**

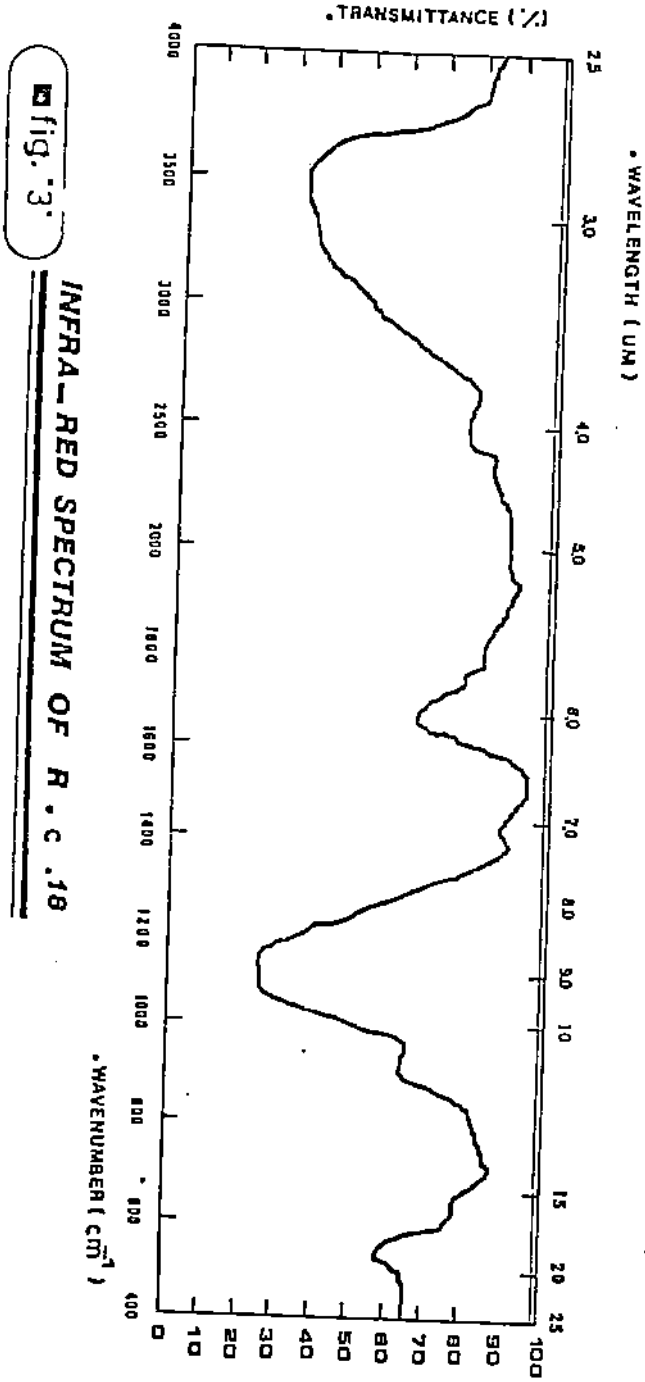


fig. 3.

INFRA-RED SPECTRUM OF R . C .18

origin exhibited strong antibacterial and antifungal activities. (Freerksen and Bonike 1951, and Kuhnan 1976).

The antimicrobial activity of Rc 18 acting against a variety of microorganisms (MIC) showed strong inhibitory activity against gram-positive bacteria and yeasts at concentration of 6.25μg. /ml. It also showed activity against gram- negative bacteria and dermatophytes in the range of 6.25- 25μg./ml. On the other hand, no important activity was detected on filamentous fungi (Table 3).

Determination of LD 50 of Rc 18 revealed that this antimicrobial agent is nontoxic when applied in concentrated dose as high as 350 mg./kg.of body weight.

Table (3): Antimicrobial activity of the antimicrobial agent Rc 18 .

Bacteria / Fungi	Minimum inhibitory concentration (μg/ml)
Gram-negative bacteria:	
Escherichia coli ATCC 25922	12.50
Klebsella pneumoniae ATCC 13883	06.25
Proteus vulgaris ATCC 13315	12.50
Pseudomonas aeruginosa ATCC 27853	25.00
Gram-positive bacteria:	
Bacillus subtilis ATCC 6633	06.25
Staphyllococcus aureus ATCC 25923	06.25
Fungi:	
Aspergillus niger ATCC 9642	> 100
Candida albicans DSM 70014	06.25
Microsporum canis	25.00
Trichophyton mentagrophytes	25.00

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INTERRELATION BETWEEN THE COMMONLY ASSOCIATED RICINUS COMMUNIS AND SESBANIA SESBAN

-9-

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SUMMARY

Growth substances exuded from the seeds and roots of Ricinus communis and Sesbania sesban plants which are commonly associated together were isolated by paper chromatography technique. The fractionated exudates of either Ricinus or Sesbania seeds contained one or two growth promotors of auxin type and a number of growth inhibitors. The exudates of Ricinus communis seeds contained no gibberellins, where as Sesbania sesban seeds showed only gibberellin-like substance. The seed exudates of both plants contained purine and non purine compounds having cytokinin activity, also some amino acids and sugars.

The root exudates of both plants contained auxin, growth inhibitors, gibberellins, cytokinins, amino acids and sugars.
The quantity and quality of the above mentioned substances markedly changed according to the age of these plants.

The different concentrations used of non fractionated Sesbania sesban seed or root exudates generally exhibited significantly increase in the linear growth of the roots and shoots of Ricinus communis at low concentrations. The reverse occurred at higher concentrations. The same results were obtained on using non fractionated Ricinus communis seed or root exudates.

INTRODUCTION

Ricinus communis and Sesbania sesban are commonly seen growing associated together. This observation led us to investigate if some chemical substances are released into the soil from each plant that

may affect the subsequent growth of the another. Reviews on liberation of different organic substances from some higher plants and the effects of one plant upon another include those of Borner (1960), Garb (1961), Street et al. (1964), Hurtt and Foy (1965), Rovira (1969), Tukey (1969) and Raifa (1971).

Mishustin and Naumova (1955) demostrated that the roots of 3or4 years old Lucerne exuded saponins which retarded the growth of cotton but not of wheat. Street et al. (1964) detected an indole compound of auxin nature, released into the culture medium during the growth of excised tomato roots. Vancura (1964) detected B-indole acetic acid in root exudates of barley and wheat. Morris et al. (1969) have shown that radio-active IAA tended to accumulate in the developing lateral roots, while there was no evidence that IAA reached the primary root. Raifa (1971) reported the presence of a number of growth promotors of auxin type and a number of growth inhibitors in root exudates of certain Egyptian weeds. Elliot and Green wood (1974) isolated IAA from the exudates of Zea mays roots. Mohga (1979) investigated the nature and contents of the growth substances present in the exudates of Zea mays and Corchorus olitorius seeds. She reported that the action of the root exudate upon growth of other plants depends mainly on the concentration and chemical nature of the substances present in this exudate. She also found that relatively higher concentrations of root exudates of Corchorus olitorius markedly decreased the shoot and the root length of Zea mays whereas the relatively low concentrations increased the shoot and root length.

Presence of gibberellins in either intact or excised roots was referred to by Murakami (1960, 1968), showed that certain grass roots (including rice) contain gibberellin-like activities at levels comparable to the shoot. Raifa (1971) detected many gibberellin-like substances in the root exudates of certain Egyptian weeds. Reid et al. (1972) demonstrated that tomato roots exuded certain gibberellins which decreased when the tomato roots were flooded. Frydman and Wareing (1975) demonstrated gibberellin by bioassay of root extracts of Hedera helix. Torrey (1976) reported that the evidence for the presence of gibberellins in roots comes largely from indirect evidence based on collection by diffusion and extraction combined with bioassay of materials from roots or from activity measured by bioassay from root exudates.

Concerning presence of cytokinin in plant root exudates, Vardjan and Nitsch (1961) isolated a substance having a cytokinin activity from the root extract of Cichorium. Similar substances were isolated from many plants by several investigators e.g. sunflower (Weiss and Vaadia 1965), Pisum sativum (Short and Torrey (1972), Zea mays (Feldman 1975), tomato (Carnes et al., 1975) and Coleus blumei. Impatiens Wallerana, Phaseolus vulgaris and Protea compacted(Kende 1964). Wareing & Phillips (1970) and Torrey (1976) reported that cytokinin are synthesized in the roots and can be detected in the root exudates of many plants. Koda and Okazawa (1978) reported that root tips have been considered as a major site of cytokinin synthesis in higher plants as they have observed an accumulation of cytokinins in the surrounding medium in which root tips are

cultured and Van Stadan (1976a), reported secretion of cytokinin into the culture medium from roots of corn seedlings. Radi and Maeda (1986), showed that the effect of kinetin and benzyladenin on rice excised root growth is inhibitory in the high and moderate concentration and a slight stimulation was observed with the relatively low concentrations. The inhibitory effect of both cytokinins and auxins may be due to their stimulation of ethylene production and a synergism between auxin and cytokinins was suggested by Adams and Young (1981).

Different inorganic and organic constituents were detected in seed and root exudates. Katznelson et al. (1955) stated that the dessication and subsequent rewetting of the sand in which tomatoes, soybean, barley or oats were grown resulted in the excretion of certain amino acids such as glutamic acid, aspartic acid, leucine, alanine, cysteine, glycine, lysine, phenylalanine and proline and a reducing compound of R_F value identical with glucose. Vancura (1964), analysed root exudates of barley and wheat in their initial phases of growth and identified four type of oligosaccharides in addition to two amino sugars. Fourteen amino acids were identified in root exudates of barley whereas eighteen were identified in root exudates of wheat and seven organic acids were identified in both exudates. He (1967), also reported that germinating seeds of both maize and cucumber exuded different amino acids and sugars during seeds germination at seedling stage. Youssef and Khairia (1971) analysed seed and root exudates of two cotton variaties and ten amino acids and three sugars were identified. Raifa (1971)

analysed root exudates of certain Egyptian weeds, Melilotus, Cypereus, Chenopodium, Urospermum, Amaranthus and Euphorbia and detected a number of amino acids, sugars and organic acids.

The present work was conducted to investigate the growth substances that may be present in the exudates of both, Ricinus communis and Sesbania sesban which are commonly associated together and to study the effect of seed and root exudates of each plant on germination and seedling growth of the another one.

Material and Methods:

Ricinus communis and Sesbania sesban seeds used in this investigation were collected from the Botanical Garden of the Women's College. Ain Shams University, Cairo. Grains of Hordeum vulgare Cv. Giza 120 and Sorghum (Andropogon sorghum Cv. Giza 142) and Xanthium brasilicum Hell seeds were obtained from the Egyptian Ministry of Agriculture.

Extraction of the growth regulating substances is done in a way similar to the method adopted by El-Ghobashy (1968).

Seed and root exudates of each plants were fractionated into its major components of growth promotors and inhibitors by paper chromatography. The extract was chromatographed in descending manner using 80% isopropyl alcohol: 20% distilled water as a running solvent. The chromatograms after developed, were dried in cold air, examined for fluorescence by ultraviolet chromatolite lamps, prepared for bioassay and sprayed with chemical reagent. The amount

of the exudates loaded in each case was equivalent to that obtained from 0.1 g dry weight of the plant tissue.

Each chromatogram, after air drying was cut transversely into 10 equal portions, each of which was eluted in 5 ml. distilled water in a thoroughly small Petri-dish. The dishes were kept in refrigerator overnight, and then the solution were ready for biological test.

The method used to assay promotors and inhibitors was the straight growth test of Hordeum coleoptile. It was applied according to the method adopted by Foda and Radwan (1962). The method used to assay gibberellins and gibberellin-like substances was the Sorghum first leaf test (Bently-Mowat (1966) with certain modification). The method used to assay the cytokinin was the Xanthium cotyledonary leaf sections (Esashi & Leopold (1969)).

The results of each test was represented graphically by a histogram of 10 columns representing the different R_FS of the chromatograms. The mean length of the control (coleoptile, first leaf and cotyledonary leaf grown in distilled water) was indicated in the histograms by a horizontal line.

The dried chromatograms were examined by an ultraviolet chromatolite lamp. They were subjected to some chemical test, where a longitudinal strip from the chromatogram was sprayed with certain reagent to test for indole compounds (Kefford, 1955 and Powell, 1959), reducing substances, amino acids (Hunt, 1959 and Smith, 1962), hydroxyl groups (Swain, 1953), gibberellins (Jones et al., 1963 and Kagawa et al. 1963), unsaturated lactones (Swain, 1953)

and cytokinin substances containing purine ring (Reguera and Ascinow, 1950).

The free amino acids of the root exudates were spotted on Whatman filter paper No. 1 Authentic amino acid mixture was simultaneously loaded with each chromatogram. The solvent system used for running was n. butanol: acetic acid: water (4:1:5 v/v/v) (Hunt, 1959, Smith 1962). The chromatograms after development were dried and sprayed with ninhydrin reagent (Block et al. 1958).

The method used for identification of the carbohydrates was similar to that of Partridge (1948). The chromatograms were sprayed with aniline hydrogen phthalate reagent.

Effect of seed or root exudates of Ricinus communis on the germination and the linear growth of Sesbania sesban and the reverse:

Five different concentrations of seed or root (15 days old)exudates of each of Ricinus communis or Sesbania sesban plants were prepared, starting with the exudates obtained from fresh seeds and roots of 10 g dry weight in 10 ml of distilled water. The different concentrations used are referred to as 1, 1/2. 1/4. 1/8 and 1/16, water being used as a control. Replicate samples from each concentration were prepared.

Ten seeds of Ricinus communis were sown in Petri-dishes on filter paper moisted with each of the different concentrations mentioned above of either seed or root exudates of Sesbania sesban. All dishes were then incubated at 25°C and the percentage of germination at the different treatments was calculated after 48 hours.

After ten days, the mean linear growth of the developed seedlings was measured.

The data were statistically analyzed according to the procedure recorded by Snedecor and Colchran (1969).

RESULTS AND DISCUSSION

It is clear from Figure 1(a) that the exudate of Ricinus communis contained only one growth promotor zone (R_F 0.1-0.2) and produced positive reactions with indole reagents and two growth inhibitor zones produced positive colour reactions with diazotized-p-nitro-aniline which may show that they are unsaturated lactones (R_F 0.2-0.3 and 0.4-0.5). On the other hand the exudate of Sesbania sesban contained at least two growth promotor zones, one of them (R_F 0.7-1.0) of indole nature and one growth inhibiting zone (R_F 0.4-0.7) may contain a phenolic lactone compound according to their chemical colour reactions.

With regard to the effect of seedling age on the root exudate, it has been shown (Fig. 2 a&b) that the root exudate of seven days old Ricinus communis seedlings was found to contain three promoting zones, two of them (R_F 0.5-0.6 and 0.7-0.9) having auxin activity of indole nature and three growth inhibitory zones (R_F 0.0-0.1, 0.1-0.3 and 0.4-0.5) the first growth inhibitor appeared likely to contain unsaturated lactone compound, while the third may contain a phenolic lactone compound. On the other hand the root exudate of seven days old Sesbania sesban seedlings contained at least three promoting zones, two of them (R_F 0.0-0.2 and 0.3-0.6) of indole

nature and one growth inhibiting zone (R_F 0.8-1.0) appear to be unsaturated lactone. Increasing the age of the two plants to 15 days, increased the level of auxins exuded from roots of both plants, while growth inhibitors disappeared completely. The root exudate of 15 days old Ricinus communis seedlings was found to contain at least four promoting zones (R_F 0.0-0.2, 0.3-0.5, 0.5-0.8 and 0.8-1.0), the first, the third and the fourth zones having auxin activity of indole nature. On the other hand the root exudate of 15 days old Sesbania sesban seedlings was found to contain five growth promoting zones, three of them (R_F 0.0-0.2, 0.4-0.6 and 0.8-1.0) of indole nature. These may bear some agreement with those of Bonner (1950), Borner (1960), Garb (1961), Rovira (1969), Tukey (1969), Raifa (1971) and Mohga (1979). Street et al. (1964) detected an indole compound of auxin nature liberated into the cultural medium from excised tomato roots. El-Gindy (1976) detected indole auxin compounds in the root exudates of cotton and maize plants.

Concerning gibberellins content, the exudates of Ricinus communis seeds contained no gibberellins (Fig. 1b), whereas Sesbania sesban seed exudates showed only one gibberellin-like substance. On the other hand, the root exudates of both plants contained more than one gibberellin and gibberellin-like substances. The exudate of seven days old Ricinus communis seedlings (Fig. 3a), contained at least three zones having gibberellins activity (R_F 0.0-0.2, 0.4-0.5 and 0.7 - 0.8). Increasing the age (15 days old) the number of gibberellin zones detected increased to four (R_F 0.0-0.2, 0.3-0.5, 0.6-0.7 and 0.8-1.0). On the other hand, the exudate of seven

days old Sesbania sesban (Fig. 3b) contained also three zones having gibberellins activity (R_F 0.0-0.2, 0.3-0.5 and 0.6-0.7). Increasing the age to 15 days, gibberellin zones increased to four (R_F 0.0-0.2, 0.4-0.5, 0.6-0.7 and 0.9-1.0). These results may agree with those obtained by Raifa (1971) and Mohga (1979). They also reported that gibberellin content increased in the root exudate of Melilotus, Zea mays and Corchorus olitorius by increasing age.

Concerning the cytokinins content, the exudates of Ricinus seeds (Fig. 1 c), contained four promoting zones having cytokinin activity (R_F 0.0-0.2, 0.4-0.5, 0.6-0.7 and 0.9-1.0), the second zone seems likely to contain a purine compound, while the others contained non-purine substances since they produced negative colour reactions with the reagents testing for purine substances. On the other hand exudates of Sesbania sesban seeds contained three promoting zones having cytokinin activity (R_F 0.0-0.2, 0.4-0.5, 0.6-0.7 and 0.9-1.0), the second zone seems likely to contain a purine compound, while the others contained non-purine substances since they produced negative colour reactions with the reagents testing purine substances. On the other hand exudates of Sesbania sesban seeds contained three promoting zones having cytokinin activity (R_F 0.0-0.1. 0.6-0.7 and 0.8-1.0), the second zone seems to contain non-purine substance, while the others contained purine compounds. The root exudates of seven days old Ricinus communis seedlings (Fig. 4a) were found to contain two promoting zones (R_F 0.2-0.5 and 0.8-1.0), and purine compound, having cytokinin activities. while the root exudates of seven days old Sesbania sesban contained three promoting zones (Fig. 4b), having cytokinin activity (R_F 0.0-0.1,

0.2-0.4 and 0.8-1.0). The second zone seems to contain non-purine substance while the others contained Purine compounds. The root exudates of fifteen days old Ricinus communis contained two promoting zones (RF 0.0-0.2 and 0.7-1.0), having purine compounds of cytokinin activities, while exudates of 15 days old Sesbania sesban (Fig. 4b) contained four promoting zones (R_F 0.0-0.2, 0.2-0.4, 0.4-0.6 and 0.8-1.0). The second zone seems to contain non purine compounds, while the others contained purine compounds of cytokinin activities. It is clear that increasing the age, increased the cytokinins exuded and this may be due to the increase in the number of root tips. Koda and Okazawa (1978) reported that root tips have been considered as a major site of cytokinin synthesis in higher plants. They observed, an accumulation of cytokinins in the surrounding medium in which root tips are cultured. Our results also agree with those obtained by Kende (1964), Wareing & Phillips (1970), Wheeler (1971), Torrey (1976), Goodwin & Morris (1979) and Radi & Maeda (1986).

Concerning the amino acids, the exudates of 7 days old Ricinus seedling was found to contain five amino acids, namely glycine, lycine, proline, cystine and ornithine. while exudates of seven days old Sesbania seedlings, contained glutamic acid only. The exudates of fifteen days old Ricinus seedlings contained six amino acids namely glycine, cystine, threonine, lysine, proline and aspartic acid. The exudates of fifteen days old Sesbania seedlings contained four amino acids, namely glycine, lysine, alanine and glutamic acid. Many investigators also identified the amino acids present in some plants (Vancura 1964, Youssef & Khairia 1971, Raifa 1971 and Mohga 1979).

Concerning the sugar contents, the exudates of seven days old Ricinus seedlings contained glucose only, while the exudates of seven days old Sesbania seedlings contained glucose and fructose. The exudates of fifteen days old Ricinus seedlings contained three sugars, namely galactose, glucose and rhamnose, while the exudates of fifteen days old sesbania seedlings contained three sugars, namely glucose, fructose and sucrose.

It is evident that seed and root exudates of Ricinus communis and Sesbania sesban contained auxins, growth inhibitors, gibberellins, gibberellin-like substances, cytokinins, amino acids and sugars. These raise the question whether substances exuded from each species plants which commonly grow in association influence the growth of each other. It is obvious that low concentrations of non fractionated seed or root exudates of Sesbania sesban (1/16, 1/8 and 1/4) induced significant increase in the linear growth of Ricinus communis seedlings (Table 1), while concentrations (1/2 and 1) showed non significant effect and decreased the linear growth as compared with the control (untreated plants). On the other hand the seed and root exudates of Ricinus communis induced significant increase in the linear growth of Sesbania sesban at low concentrations (1/16, 1/8, 1/4), whereas high concentration (1/2, 1) caused non-significant decrease in the linear growth of Sesbania sesban (Table 2). Mohga (1979) reported that the action of the root exudate upon growth of other plants depends mainly on the concentration and the chemical nature of the substances present in this exudate. She found that the relatively higher concentrations of root exudates of 20 days old zea mays

plants, markedly inhibit the growth of Corchorus olitorius. These inhibitory actions were obviously and gradually decreased by dilutions. Adams and Young 1981 suggested that the inhibitory effect may be due to both cytokinins and auxins which stimulate the ethylene production and a synergism between them.

In conclusion it appears that there is an apparent role of exudates of each species on the growth of the other. Both species are being naturalized and are gaining ground in several habitats. In addition to the prominant causal factor for association of species i.e. similarity of essential ecological requirements, the possible effects of interaction between the two studied species gains some evidence from the present study.

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Table (1): Effect of different concentrationsof each of seed or root exudate of Sesbania sesban on shoot and root length of Ricinus communis seedlings

Relative conc. of seed exudate	Root length cm.	t	Shoot length cm.	t	Relative conc. of root exudate	Root length cm.	t	Shoot length cm.	t
Control	10.0±0.16		8.2±0.22		Control	10.0±0.16		8.2±0.22	
1/16	10.4±0.16	4.0 S	9.1±0.69	2.77 S	1/16	10.5±0.16	5 HS	9.4±0.30	7.36 HS
1/8	10.7±0.36	4.01 S	9.9±0.41	8.15 HS	1/8	11.2±0.23	9.6 HS	10.0±0.41	8.58 HS
1/4	11.0±0.33	5.84 HS	10.5±0.38	11.65 HS	1/4	11.8±0.2	15.79 HS	11.0±0.79	7.62 HS
1/2	9.8±0.38	0.87 NS	7.8±0.22	-2.83 NS	1/2	10.0±0.16	0.0 NS	8.2±0.22	0.0 NS
1	9.6±0.25	-2.98 NS	7.5±0.16	-5.72 NS	1	9.6±0.25	-2.98 NS	8.0±0.94	-4.65 NS

Each value; a mean of five replicate

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Table (2): Effect of different concentrations of each of seed or root exudate of Ricinus comminus on shoot and root length of Sesbania sesban

Relative conc. of seed exudate	Root length cm.	t	Shoot length cm.	t	Relative conc. of root exudate	Root length cm.	t	Shoot length cm.	t
Control	7.1±0.25		5.0±0.36		Control	7.1±0.25		5.0±0.36	
1/16	8.0±0.38	4.45 S	6.0±0.38	4.26 S	1/16	8.2±0.28	6.52 HS	6.9±0.2	10.3 HS
1/8	8.7±0.25	10.02 HS	6.4±0.23	7.28 HS	1/8	9.2±0.12	18.26 HS	7.0±0.38	8.53 HS
1/4	8.4±0.16	9.83 HS	5.6±0.14	3.46 S	1/4	8.5±0.16	10.58 HS	5.7±0.16	4.0 S
1/2	7.0±0.41	-0.46 NS	5.3±0.16	1.7 NS	1/2	7.1±0.25	0.0 NS	5.2±0.14	1.15 NS
1	6.5±0.38	-2.95 NS	4.7±0.16	-1.7 NS	1	6.9±0.14	-1.56 NS	4.8±0.14	-1.15 NS

Each value:a mean of five replicates

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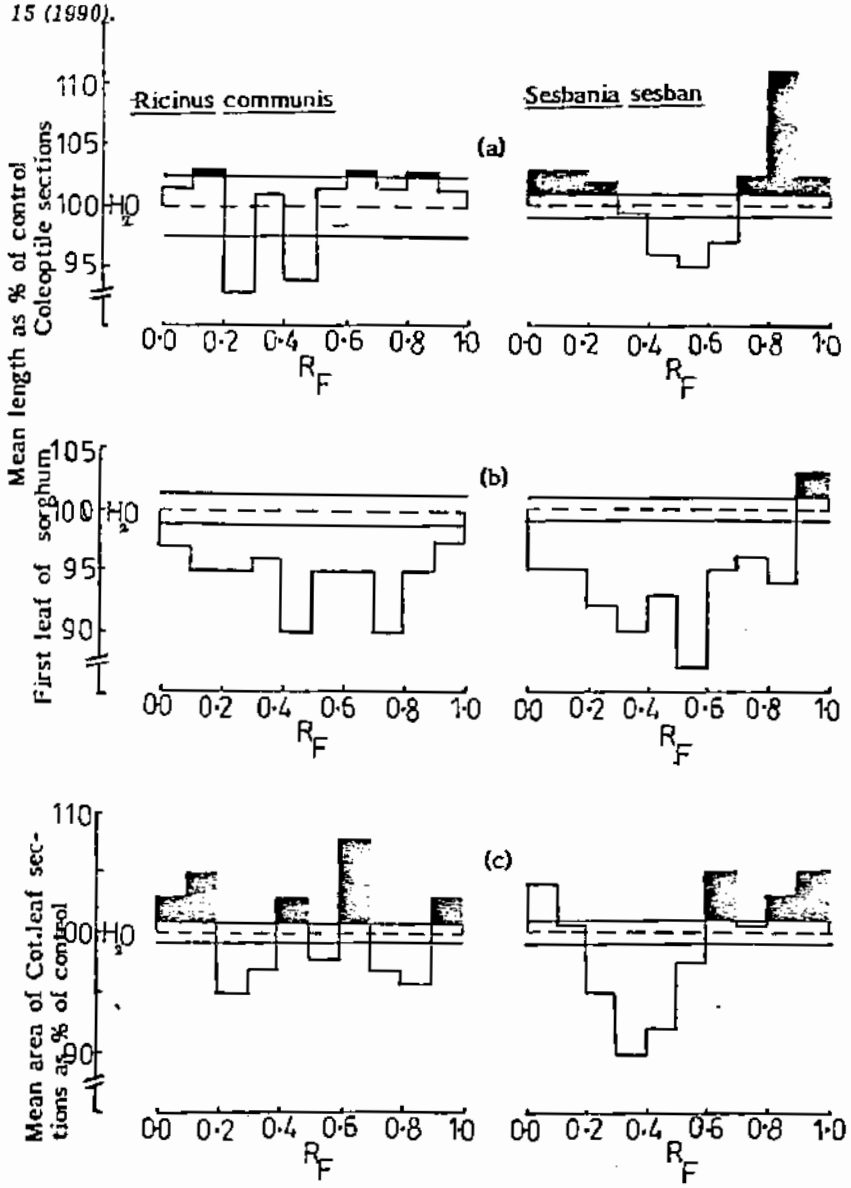

Fig. (1): Growth Regulators Exuded from Mature Seeds of Ricinus communis and Sesbania sesban.

a) Auxins and inhibitors
b) Gibberellins and gibberellin-like substances.
c) Cytokinins

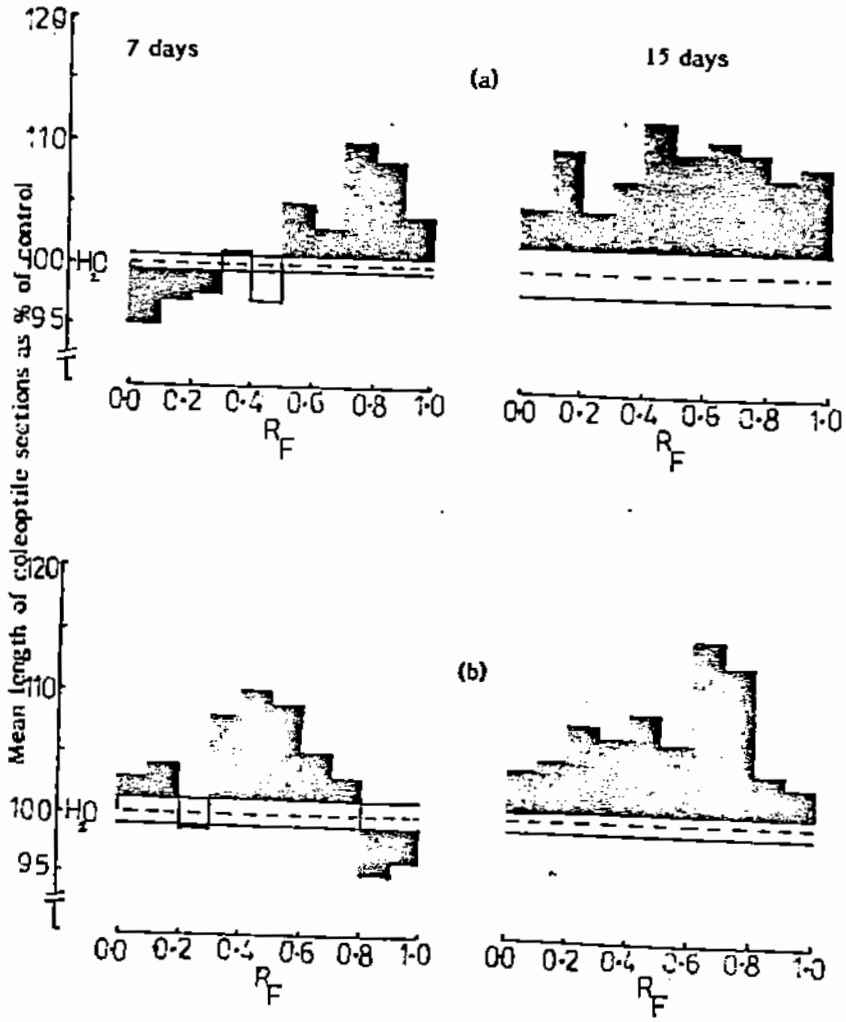

Fig. (2): Auxins and growth inhibitors exuded from:

a) Ricinus communis roots at 7 and 15 days age.
b) Sesbania sesban roots at 7 and 15 days age.

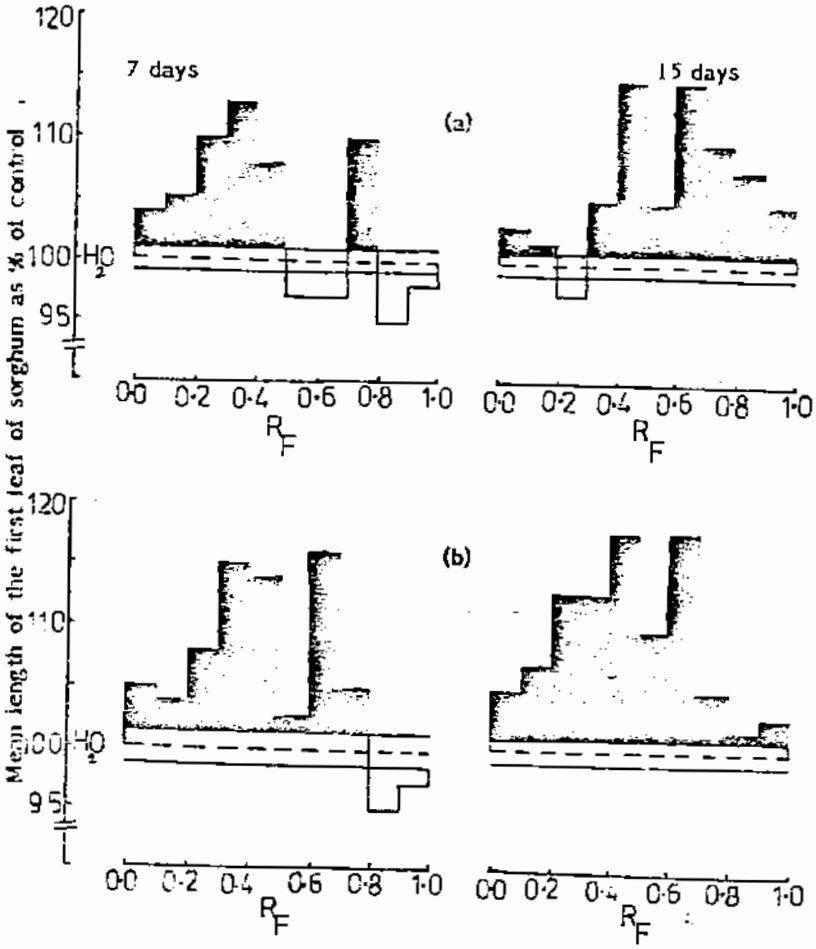

Fig. (3): Gibberellins and gibberellin-like substances exuded from:
a) Ricinus communis roots at 7 and 15 days age.

b) Sesbania sesban roots at 7 and 15 days age.

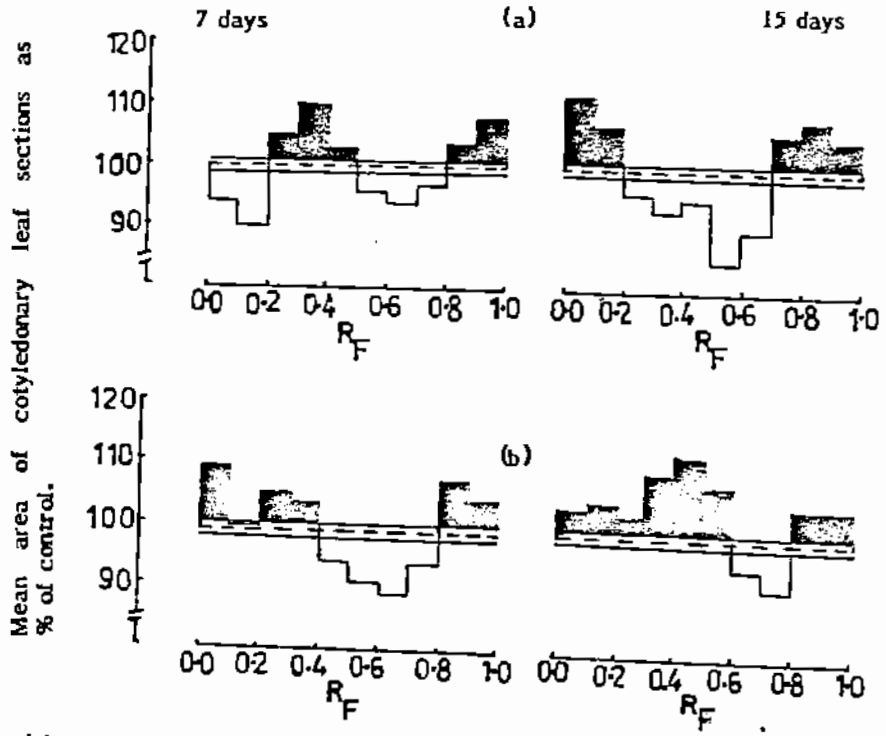

Fig. (4): Cytokinins exuded from:

a) Ricinus communis roots at seven and fifteen days age.

b) Sesbania sesban roots at seven and fifteen days age.

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CHEMOTAXONOMIC STUDY OF THREE *ARTEMISIA* SPECIES GROWING IN SINAI, EGYPT.

_10_

By

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ABSTRACT

A comparative chemotaxonomic study of three *Artemisia* species, *A. monosperma*, *A. judaica*, *A. herba alba* is presented. Eight forms of *A. monosperma*, growing in Wadi El- Arish (North Sinai) and the other two species growing in Wadi El-Shiekh and Wadi El-Talaa (Saint Catherine, South Sinai) respectively, were collected in the same growth season to eliminate the effect of ecological factors.

The chemical study comprised the preliminary phytochemical screening, investigation of total, water-soluble and acid-insoluble ash; carbohydrates; total nitrogen and amino acids; lipids; fatty acids and flavonoids. The results revealed that the eight forms of *A. monosperma* were greatly similar in their chemical composition. On the other hand, they differed qualitatively and quantitatively from the other two species, viz. *A. herba alba* and *A. judaica*. Therefore, the phytochemical results fully justified the systematic treatment.

INTRODUCTION

The genus *Artemisia* is of common use in folk medicine and in pharmaceutical preparations (Boulos, 1983), and several compounds were isolated from its tissues. Most of these compounds are of medicinal interest. In this regard Fahmy *et al.* (1960) isolated four crystaline compounds from powdered leaves and the flowering tops of *A. monosperma* . Maksudov *et al.* (1962) determined the essential oils, organic acids, tannins, sugars, ash and tars in blooms of *A. scoparia*

Many authors identified and isolated a great number of flavone compounds from different *Artemisia* species : Rodrigues *et al.* (1972) from seven *Artemisia* taxa ; Segal *et al.* (1973) from *A. herba alba* ; Khafagy *et al.* (1979) from *A. monosperma*; Ghazouly *et al.* (1984) and Bacha (1984) from *A. judaica* and Saleh *et al.* (1985) from *A. monosperma* and *A. herba alba* . Sayed *et al.* (1979) studied the fatty acids of *A. absinthium* , while Laivant and Proskurnikova (1965) studied the amino acids of the protiens of *A. rhodantha* , qualitatively ,quantitatively, and their seasonal fluctuations during the developmental phases. Garrone *et al.* (1973) examined the level of free amino acids in *A. vulgaris* and *A. verlotorm*. Also, Khamdamov and Chamsrkov (1976) studied qualitatively the amino acids in *A. diffusa* , *A. halophila* and *A. turanica* . Hammouda *et al.* (1978) isolated an acetophenone derivative and coumarins from *A. monosperma*.

Alekseeva (1962) studied the metabolism of carbohydrates in *A. turanica* in various soils under desert conditions. Maki (1968) isolated hemicellulose from leaves of *A. capillaris* . On the other hand, Garrone *et al.* (1973) examined the levels of soluble carbohydrates in *A. vulgaris* and *A. verlotorum* .

A critical taxonomical revision was realized by Gazara (1987) for *Artemisia* species growing in Sinai and known earlier by Tackholm (1974). In this revision , it was possible to distinguish between different *Artemisia* species according to vegetative, head as well as floral characters. The following key was made by Gazara (1987).

1.a Heads homogamous, oblong, tapering at base.....*A. herba alba*

1.b Heads heterogenous, hemispherical to oblong-ovate not tapering at base 2

2.a Involucral bracts hairy , bisexual flowers fertile, 15-29 per head ..... *A. judaica*

2.b Involucral bracts glabrous, bisexual flowers sterile, 3-9 per head 3

3 Heads ovate, 4 mm long, female flowers 2-6 per head .....*A. monosperma*

In the present study a chemotaxonomical investigation was carried out to compare the three *Artemisia* species. namely *A. monosperma*, *A. judaica* and *A. herba alba*, and between the eight different forms of the first species.

MATERIALS AND METHODS

The material used in the present investigation was obtained from *A. judaica*, *A. herba alba* and *A. monosperma* growing naturally in Sinai. The two first species were collected from South Sinai (Saint Catherine area) . *A. judaica* was collected from Wadi El-Shiekh and *A. herba alba* from Wadi El-Talaa . The eight different forms of *A. monosperma* (A1-A8) were collected from Wadi El-Arish, North Sinai. The plant samples of the three species were collected at the same growth season. The shoots were manually cleaned, dried in an oven at 50C and reduced to fine powder.

The preliminary phytochemical screening was carried out on the powdered dried shoots of the three different species of *Artemisia*. This included testing for volatile oils and tannins (Balbaa *et al.* 1981), unsaturated sterols (Brieskorn *et al.* 1961) , flavonoids (Wall *et al.* 1954 and Balbaa *et al.* 1981), glycosides and\or carbohydrates (Vogel

1978), and saponins (Wall *et al.* 1954 and Abd El Maksoud 1983).

The total ash, water-soluble ash as well as acid-insoluble ash were determined according to E. P. (1984) methodology, using two grams of the powdered air-dried shoots of the eight different of *A. monosperma* (A1-A8), as well as *A. judaica* and *A. herba alba.*

The total carbohydrates were determined according to Karawya and Afifi (1979) method, and the sugar content was expressed as gram dextrose per 100 gram dry weight. The qualitative investigation of the free and combined sugars was realized according to Karawya and Afifi (1979) methods of analysis.

Allen *et al.* (1974) methodology was followed for the investigation of nitrogen, amino acids and lipid contents. Flavonoids were investigated according to Bacha (1984) methodology. Finally, fatty acids were studied using gas-liquid chromatography according to Karawya *et al.* (1979) method. The analysis was done by GCV chromatograph using the following conditions:

-Column	10% PEGA
-Column temp.	70 ° C (initial temp.)
-Rate	8 ° C/min.
-Final temp.	190 ° C
-Final time	20 min.
-Chart speed	2 min./cm.
N_2	30 ml/min.
H_2	33 ml/ min.
Air	330 ml/ min.

RESULTS AND DISCUSSION

Investigation of Plant Constituents:

The principal chemical constituents were studied in order to compare between the eight forms of *A. monosperma* on one hand and between the three *Artemisia* species on the other hand. It can be concluded from Table (1) that all the three species of *Artemisia* contained volatile oils, carbohydrates and/or glycosides, flavonoids, sterols and saponins. Negative results were obtained for tannins and alkaloids in all of them.

Results presented in Table (2), revealed that the percentage of total ash content were approximately similar in different forms of *A. monosperma* ranging between 7.5 and 5.5 g.% in *A. judaica* and *A. herba alba* respectively. It is clear also that water-soluble ash contents in *A. monosperma were* approximately similar in different forms ranging between 3.5 and 4.0 g.%. Obviously, the records were 4.0 and 2.5 g.% in *A. judaica* and *A. herba alba* respectively. Results also clerified that the acid-insoluble content of different forms of *A. monosperma* ranged between 0.5 and 1.0 g.%. *A. judaica* and *A. herba alba* on the other hand had higher values of 2.8 and 2.3 g.% respectively (Table 2).

It is clear that the eight forms of *A. monosperma* had similar contents of total ash as well as water-soluble and acid-insoluble ash contents . These contents differed from those of the other two *Artemisia* species. Although the total ash and water-soluble ash of *A. judaica* were similar to those of *A. monosperma*, the acid-insoluble ash was much higher. The contents of the three types of ash in *A. herba alba* differed from those of the other two species.

Results presented in Table (2) clearly show that the total carbohydrate contents of the studied *Artemisia* species belonging to *A.*

Table (1): Preliminary phytochemical screening of shoots of three species of *Artemisia*.

Test	Species		
	A. monosperma (A1-A8)	*A. judaica*	*A. herba alba*
Volatile oils	+ve	+ve	+ve
Tannins	-ve	-ve	-ve
Unsaturated sterols	+ve	+ve	+ve
Alkaloids	-ve	-ve	-ve
Flavonoids	+ve	+ve	+ve
Glycosides and/or carbohydrates	+ve	+ve	+ve
Saponins	+ve	+ve	+ve

TABLE (2): Quantitative analysis of the three Artemisia species.

Species	Artemisia monosperma								A. judaica	A. herba-alba
Character	A1	A2	A3	A4	A5	A6	A7	A8		
Total ash (g %)	7.5	7.5	7.5	8.0	7.5	8.0	8.0	7.5	7.5	7.5
Water soluble ash (g %)	4.0	4.0	3.5	4.0	4.0	4.0	4.0	4.0	4.0	2.5
Acid insoluble ash (g %)	0.5	1.0	1.0	0.5	0.5	1.0	1.0	0.5	2.8	2.3
Total carbohydrates (g glucose %)	0.87	0.83	0.83	0.83	0.87	0.87	0.87	0.83	2.42	2.60
Total nitrogen (g/100 g plant material)	0.286	0.276	0.286	0.286	0.286	0.276	0.276	0.276	0.332	0.350
Alcohol extract (crude flavones %)	3.28	3.31	3.37	3.24	3.28	3.28	3.37	3.28	0.50	0.40
Total lipid content (g%)	15.4	15.4	14.2	15.4	14.2	15.4	14.2	14.2	10.2	8.2

monosperma (A1-A8) attained values that ranged between 0.83 and 0.879/100 g. dry matter. On the other hand, data collected for *A. judaica* and *A. herba alba* indicated higher values (2.42 & 2.60 g.% respectively).

The qualitative study of sugers presents in the three studied species using paper chromatography (Table 3) revealed that *A. monopserma* with all different forms contained galactose. The other two species, namely *A. judaica* and *A herba alba* contained mannose. All forms of *A. monosperma* differed from the other two *Artemisia* species.

Results in table (2) also show that the amounts of the total nitrogen content attained their maximum values in *A. judaica* (0.332 g.%) and *A. herba alba* (0.350 g.%) that decreased remarkably in the eight forms of A. monosperma (0.276- 0.286 g.%).

The results of total lipid content (Table 2) estimated quantitatively in the different forms of *A. monosperma* (A1-A8) indicated values that ranged between 14.2 to 15.4 g.%. On the other hand, both *A. judaica* and *A. herba alba* indicated lower values of 10.2 and 8.2 g.% respectively.

The percentages of the alcoholic extract, containing total flavones, were also compared (Table 2). It is evident that the crude total flavonoid content of the eight forms of *A. monosperma* were similar, ranging from 3.24 to 3.31 g.%; however, *A. judaica* contained only 0.5 g.% ; neverthless *A. herba alba* contained the least amount of flavonoids (0.14 g.%).

The results of the qualitative study of amino acids in the three studied species using paper chromatography (Table 3) revealed that *A. monosperma* with all its different forms contained lysine, asparagin, aspartic acid, glutamic acid, alanine, tyrosine, methionine and leucine.

Table (3): Qualitative analysis of carbohydrates, amino-acids, flavonoids and fatty acids of the three Artemisia species.

Species	---- Artemisia monosperma ----								A.	A.
Character	A1	A2	A3	A4	A5	A6	A7	A8	judaica	herba-alba
Carbohydrates										
Galactose	+	+	+	+	+	+	+	+	-	-
Mannose	-	-	-	-	-	-	-	-	+	+
Amino acids										
Cystine	-	-	-	-	-	-	-	-	+	+
Lysine	+	+	+	+	+	+	+	+	+	+
Aspargine	+	+	+	+	+	+	+	+	-	+
Aspartic acid	+	+	+	+	+	+	+	+	+	-
Glutamic acid	+	+	+	+	+	+	+	+	+	-
Serine	-	-	-	-	-	-	-	-	-	+
Alanine	+	+	+	+	+	+	+	+	+	+
Tyrosine	+	+	+	+	+	+	+	+	+	-
Methionine	+	+	+	+	+	+	+	+	-	-
Valine	-	-	-	-	-	-	-	-	+	+
Leucine	+	+	+	+	+	+	+	+	+	-
Isoleucine	-	-	-	-	-	-	-	-	-	+
Flavonoids ‡										
Quercetin 3- glucoside	+	+	+	+	+	+	+	+	-	+
Quercetin 3- rurinoside	+	+	+	+	+	+	+	+	-	+
Quercetin 5- glucoside	+	+	+	+	+	+	+	+	-	+
Isorhamnetin 5- glucoside	+	+	+	+	+	+	+	+	-	-
Patuletin 3- glucoside	+	+	+	+	+	+	+	+	-	+
Patuletin 3- rutinoside	+	+	+	+	+	+	+	+	-	+
Acacetin 7- glucoside	-	-	-	-	-	-	-	-	-	+
Acacetin 7- rutinoside	+	+	+	+	+	+	+	+	-	-
Isovitexin	+	+	+	+	+	+	+	+	-	-
Vicenin -2	+	+	+	+	+	+	+	+	-	+
Schaftoside	-	-	-	-	-	-	-	-	-	+
Isoschaftoside	-	-	-	-	-	-	-	-	-	+
Lucenin -2	+	+	+	+	+	+	+	+	-	-

‡ Identified by Bacha (1984) and Saleh et. al.

Table 1.3 (contd.

Methylated aglycone	+	+	+	+	+	+	+	+	−	+
Chrysoerial 7- rutinoside	−	−	−	−	−	−	−	−	+	−
Leutulin	−	−	−	−	−	−	−	−	+	−
Cirstakogenin	−	−	−	−	−	−	−	−	+	−
Apigenin	−	−	−	−	−	−	−	−	+	−
Fatty acid esters										
Octanoic	+	+	+	+	+	+	+	+	+	−
Capric	+	+	+	+	+	+	+	+	+	+
Undecanoic	−	−	−	−	−	−	−	−	−	−
Lauric	+	+	+	+	+	+	+	+	+	+
Tridecanoic	+	+	+	+	+	+	+	+	+	+
Myristic	+	+	+	+	+	+	+	+	+	+
Pentadecanoic	+	+	+	+	+	+	+	+	+	+
Palmitic	+	+	+	+	+	+	+	+	+	+
Stearic	+	+	+	+	+	+	+	+	+	+
Oleic	+	+	+	+	+	+	+	+	+	+
Linoleic	+	+	+	+	+	+	+	+	+	+
Linolenic	+	+	+	+	+	+	+	+	−	+
Arachidic	+	+	+	+	+	+	+	+	−	−

On the other hand, *A. judaica* contained lysine, aspartic acid, glutamic acid, alanine, tyrosine, valine and leucine; while *A. herba alba* contained cystine, lysine, asparagin, serine, alanine, valine and isoleucine.

Considering the qualitative investigation of flavonoids (Table 3) *A. monosperma* contained quercetin 3-glucoside, quercetin 3-rutinoside, quercetin 5-glucoside, isorhamnetin 5-glucoside,patuletin 3-rutinoside, acacetin 7-glucoside, acacetin 7-rutinoside, vicenin-2, lucenin and methylated aglycones as reported by Saleh *et al.*(1985). Differently, *A. judaica* contained chrysoerial 7-rutinoside, leutulin, cirstakogenin (Bacha, 1984). Finally, *A. herba alba* contained quercetin 3-glucoside, quercetin 3-rutinoside, patuletin 3-glucoside, patuletin 3-rutinoside, isovitexin, vicenin-2 schaftoside, isoschaftoside and methylated aglycones (Saleh *et al.* 1985). These results clarify the presence of different flavones in the three *Artemisia* species. The results also show that the eight forms of *A. monosperma* contain the same flavones, and differ from the other two species.

The results presented in Table (4) reveal the presence of the following fatty acids in the eight forms of *A. monosperma* : octanoic, capric, lauric, tridecanoic, myristic, pentadecanoic, palmitic, stearic, oleic, linolenic and arachidic acids. It is also clear that *A. judaica* contained a group of fatty acids similar to that of *A. monosperma*, with the difference that undecanoic acid is present, while octanoic and arachidic acids are absent. On the other hand, *A. herba alba* contained octanoic, capric, lauric, tridecanoic, stearic, oleic, linoleic acids, and was free of undecanoic, linolenic and arachidic acids. These results show that the percentage of some fatty acids varies not only in the three

Table (4): Percentages of fatty acid esters of the three studied Artemisia pecies.

Species	Artemisia aonosperma								A.	A.
Character	A1	A2	A3	A4	A5	A6	A7	A8	judaica	herba-alba
Octanoic	5.84	0.82	9.09	22.57	5.75	6.85	4.85	0.82	-	25.87
Capric	26.16	9.33	12.12	4.27	16.09	21.27	25.22	9.33	24.31	7.71
Undecanoic	-	-	-	-	-	-	-	-	2.78	-
Lauric	1.11	0.27	0.22	1.65	0.81	2.95	0.33	0.27	8.10	8.81
Tridecanoic	0.47	1.51	2.98	5.37	2.01	5.32	3.49	1.51	2.31	12.11
Myristic	3.96	0.96	1.50	2.20	2.76	11.82	2.18	0.96	3.86	5.50
Pentadecanoic	4.42	16.58	9.20	34.17	9.58	14.65	4.80	16.58	27.39	4.41
Palmetic	50.49	15.79	17.86	11.30	21.86	24.82	16.38	15.79	1.93	2.75
Stearic	0.32	1.57	2.71	1.65	1.53	0.83	0.76	1.57	3.70	1.47
Oleic	0.26	9.02	8.44	3.05	7.66	0.59	15.07	9.02	4.63	17.61
Linoleic	3.47	36.84	18.99	8.79	25.21	0.89	16.81	36.84	7.41	13.76
Linolinic	2.24	5.26	12.99	3.67	3.58	2.92	8.73	5.26	13.58	-
Arachidic	1.26	2.05	3.90	1.22	3.16	7.09	1.64	2.05	-	-

Artemisia species, but also in the forms of *A. monosperma* (Table 4). It must be noted that the qualitative estimation of fatty acids esters by Gas Liquid chromatography is strict since it depends on comparing the fatty acids by authentic samples. On the other hand, the quantitative estimation of some fatty acids by the same method may differ since the peak shape differs in operating conditions and injection technique.

It can be concluded that the eight forms of *A. monosperma* contain the same fatty acids, which differ from those of the other two species viz. *A. judaica* and *A. herba alba*.

From these results, it is clear that the eight forms of *A. monosperma* are greatly similar in their chemical compisition. They differ qualitatively and quantitavely from the other two species, viz. *A. judaica* and *A. herba alba*. In this regard, the phytochemical study fully justifies the systematic treatment.

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ALTERATIONS IN NUCLEIC ACIDS, PROTEIN CONTENT AND MITOTIC DIVISION OF *Vicia faba* ROOT TIP CELLS AS AFFECTED BY MALATHION AND TAMARON INSECTICIDES

-11-

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SUMMARY

The effect of malathion and tamaron insecticides on *Vicia faba* root tip cells was studied. The results showed that malathion increased MI% after 4 and 6 hrs treatments, while tamaron decreased MI% severely. Both insecticides altered mitotic phases percentages and induced a number of chromosomal anomalies.

The two insecticides with the concentrations and time durations applied increased protein content of the root tips. Malathion decreased DNA content, while tamaron showed nearly the same values as the control.

INTRODUCTION

Chemical pest control is widely spread due to various reasons, among them are, the ease of preparation and the somewhat low costs as compared with other methods of control, such as biological control, modifications of methods of cultivation, crop rotation and the extensive plant breeding studies.

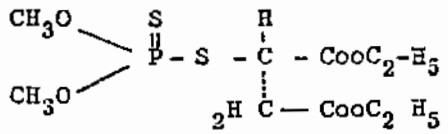
The vital processes of the crop itself may be affected by the use of pesticides. The most serious injury is that on the cytological level, where the DNA molecule and consequently the gene is altered. Regular application of pesticides to crop plants has been cited as a possible source of genetic damage leading to instability (Unrau & Larter, 1952; Suneson & Jones, 1960; Liang et al., 1969; Lee et al., 1974; Adam, 1975 and Reddy & Rao, 1982).

Here in this study we tried to test the effect of two organophosphorus insecticides namely malathion and tamaron on mitosis and some metabolic processes of Vicia faba root tip cells.

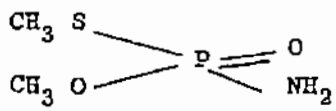
MATERIALS AND METHODS

The test plant was Vicia faba (Var. Giza 2). The chemical formulae of the two used insecticides were as follows and they are considered phorsphoric acid esters.

Malathion



Tamaron



The active ingredients in these insicticides have a number of properties in common :

1. Combined activity against chewing and sucking insects.
2. Inhibition of cholinesterase, a vital enzyme present in blood and tissue.

The germinated seeds (1.5-2 cm radicle length) were immersed in aqueous solutions of the insecticides at concentrations of 250, 500 and 1000 ppm in addition to the control (radicles immersed in tap water) for 2, 4, 6 & 8 hrs. The treated radicles were then washed with water, fixed in Carnoy's fixative (Ethyl alcohol: Glacial acetic acid; 3:1), then stained with leuco basic fuchsin, then smeared in 45% glacial acetic acid and examined.

Nucleic acids were extracted according to Guinn (1966) method and according to Ogur & Rosen (1950). RNA and DNA were determined by using UV. Spectrophotometer at 260 mμ.

Protein was determined photometrically by folinphenol reagent as reported by Lowery et al., (1951).

RESULTS AND DISCUSSION

The data recorded in the following tables showed that both insecticides malathion and tamaron has a preprophasic and a post-prophasic action although they acted differently on Vicia faba root tip cells.

Malathion treatments increased MI% especially after 4 & 6 hrs, while tamaron decreased MI% as compared to the control (Table 1). It is clear from the table also that the effect of tamaron in reducing cell division was more pronounced by increasing concentration, this may indicate that tamaron interfere with the normal sequence of cell cycle to reduce the number of cells starting to divide at interphase. It is also possible to suggest that the reduction in mitotic activity was due to the inhibition of the DNA synthesis which is considered one of the major prerequisites for a cell to divide.

Table (1) Mitotic index of Vicia faba roots treated with different malathion and tamaron concentration under different exposure times.

Time of exposure (hrs.)	Control	Malathion (p p m)			Tamaron (p p m)		
		250	500	1000	250	500	1000
2	53.28	57.21	53.43	44.29	54.28	40.90	28.30
4	52.66	57.77	59.86	61.83	33.11	44.15	31.50
6	50.75	59.93	59.73	64.37	32.86	37.65	30.87
8	56.76	Toxic	Toxic	Toxic	20.53	38.12	45.32

In this connection, Heiner (1971) found that diethyl sulphate caused a blockage of DNA synthesis which in turn induced a substantial mitotic delay. Also, Davids (1973) reported mitotic index inhibition was accompanied by DNA synthesis inhibition and

similar relation was reported by Swietlinska et al., (1974).

On the other hand, the application of malathion tended to increase the mitotic index of root tip's of Vicia faba. Since that increase was not accompanied by an increase in DNA content, the effect of the insecticide could not be attributed to a consequent effect on DNA molecule.

In this respect, the reduced mitotic rate may in part be explained by chromosome aberrations and/or an altered metabolic rate - the data in Table (2) showed that protein contents were sharply increased by tamaron treatments.

Table (2) Protein, RNA and DNA concentrations in Vicia faba root tips treated with malathion and tamaron pesticides at different exposure times.

Treatments	Exposure time (hr.)	Protein mg/100 g F.w	RNA mg/100 g F.w	DNA mg/100 g F.w
Control	2	285	75.00	26.00
	4	265	82.50	30.00
	6	320	93.75	29.50
	8	430	100.00	31.00
Malathion 1000 ppm	2	255	58.75	27.00
	4	280	68.75	28.25
	6	335	61.25	21.50
	8	435	80.00	15.25
Tamaron 1000 ppm	2	345	75.00	26.50
	4	375	83.75	28.25
	6	400	80.00	28.25
	8	510	100.00	27.00

F.W. = Fresh weight

In addition, Vant-Hoff (1968) explained the drop in mitotic activity which is not accompanied by inhibition of DNA synthesis by an increase in the G_2 period. Also Shalaby et al. (1986) reported the same trend where MI changes were not correlated with changes in DNA content in Vicia faba root tip cells treated with growth regulators under different salinity conditions.

Changes in mitotic activity was recorded and discussed by some authors as Shehab & Adam (1981 & 1983); Lazar & Keul (1983); Adam & Rashad (1984) and Amer et al., (1987).

Concerning the percentages of the different mitotic phases Table (3) shows that the general effect of malathion and tamaron in increasing the percentage of metaphase and ana-telaphase was accompanied by a rise in protein content of the treated root tips (Table, 2). This may be explained on the basis that spindle formation required active protein synthesis to form the micro-tubules (Spindle Sub-units). This data showed that tamaron effect was more pronounced in this respect.

Table (3) Percentages of abnormalities in each phase for Vicia faba roots treated with different malathion and tamaron concentrations under different exposure times.

Mitotic phases	Time of treat-ment (hrs.)	Control	Malathion (p p m) 250	500	1000	Tamaron (p p m) 250	500	1000
Prophase	2	85.50	77.96	73.17	77.94	77.73	86.20	63.52
	4	87.58	75.69	52.67	72.59	66.26	88.45	59.73
	6	86.91	62.59	51.20	80.54	87.69	85.53	58.93
	8	85.34	Toxic	Toxic	Toxic	75.00	77.24	58.26
Metaphase	2	9.48	16.85	21.85	14.50	3.39	5.99	29.37
	4	8.40	17.79	29.44	18.62	26.90	3.63	20.64
	6	8.22	21.27	30.37	15.46	4.70	13.16	24.98
	8	9.30	Toxic	Toxic	Toxic	18.40	14.97	21.61
Ana-telophase	2	5.02	5.19	4.98	7.56	18.28	10.68	7.11
	4	3.52	6.52	17.89	8.79	6.84	7.92	19.63
	6	4.87	16.13	18.43	4.00	7.61	2.53	16.09
	8	5.36	Toxic	Toxic	Toxic	6.60	7.79	20.13

Malathion and tamaron induced a number of mitotic abnormalities (Table, 4). The percentages of total abnormalities were higher in case of malathion than tamaron treatments as shown in Table (4).

Table (4) Percentage of total abnormalities in Vicia faba root tips treated with different malathion and tamaron concentrations under different exposure times.

Time of treatment (hrs.)	Control	Malathion (p p m)			Tamaron (p p m)		
		250	500	1000	250	500	1000
2	10.43	34.26	32.31	64.92	34.06	27.26	21.63
4	10.47	34.87	44.73	32.89	23.72	28.98	22.64
6	10.97	38.75	45.26	33.31	21.80	27.34	27.35
8	11.84	Toxic	Toxic	Toxic	12.62	30.44	34.38

The most dominant types of abnormalities scored (Table, 5) were the following in a descending order:
Stickiness (Fig., 1), abnormal prophases, Spindle disturbance (Figs, 2 & 3). In addition malathion and tamaron induced other types of clastogenic anomalies such as lagging (Fig, 4), bridges (Fig., 5), despiralization, binucleate cell (Fig.6) contraction and Asynchronization of chromosome movements.

The types of abnormalities scord in this investigation resembles those reported by Ravindran(1971), Shaikh& Godward (1972) (1972), Anantha (1980), Amer & Mikhael (1983), Somashekar & Goda (1984) and Amer et al., (1987).

The data showed that toxicity appeared after 8 hrs treatment with malathion only that means that malathion was more toxic to Vicia faba root tip cells than tamaron. In support of this view was the high percentages of total abnormalities brought about by malathion as compared with those resulted from tamaron treatments.

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Table (5)- Percentage of different abnormality types in Vicia faba roots treated with malathion and tamaron concentrations under different exposure times.

Insecticide (p p m)		Exposure time (hrs.)	Types of abnormalities								
			Stickiness	Abnormal prophase	Spindle. dis.	Despiralizatio-n	Lagging	Bridge	Break	Asynchronizat-ion	Contract-ion
Malathion	250	2	45.71	25.22	15.54	16.32	2.09	1.13	—	—	—
		4	51.32	22.29	10.98	11.16	3.25	0.99	—	—	—
		6	48.48	17.18	17.28	9.08	4.93	3.14	—	—	—
		8	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	500	2	55.11	25.57	5.84	7.78	3.57	1.05	0.46	—	0.65
		4	48.93	17.87	12.66	8.71	7.20	1.35	0.29	0.38	2.44
		6	45.18	19.91	11.40	11.78	8.66	1.39	—	—	1.67
		8	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	1000	2	41.92	34.36	17.5	14.42	2.70	0.45	0.52	—	—
		4	43.33	30.46	10.46	13.38	0.85	1.03	—	—	0.45
		6	41.23	33.33	8.34	15.07	0.45	1.05	—	—	0.45
		8	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
Tamaron	250	2	66.69	10.83	12.99	6.08	0.96	0.95	0.5	1.43	—
		4	24.42	41.84	10.93	12.09	0.84	—	0.88	—	—
		6	80.99	10.44	4.75	3.78	—	—	—	—	—
		8	60.77	14.47	12.77	12.50	—	—	—	—	—
	500	2	72.47	8.57	2.62	4.50	1.09	—	—	0.44	0.36
		4	75.98	13.33	3.61	4.25	1.13	1.06	—	1.18	—
		6	65.09	26.65	6.04	1.65	2.91	—	—	—	—
		8	54.39	25.50	9.98	7.53	4.11	—	—	—	0.42
	1000	2	54.84	14.50	14.56	10.46	—	1.19	—	—	2.02
		4	49.80	15.55	11.56	13.84	5.93	2.68	0.66	—	—
		6	47.27	22.33	12.08	7.92	6.35	3.70	—	—	—
		8	53.09	19.70	11.64	8.09	4.91	2.57	—	—	—

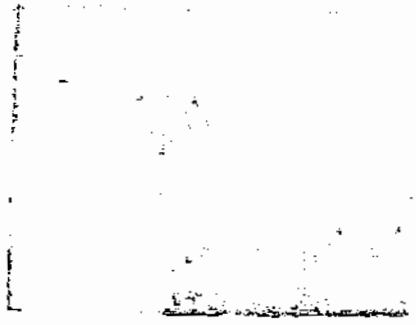

Fig. (1)
Sticky and irregular prophase after treatment with malathion 250 ppm for 2 hrs.

Fig. (2)
Partial C-metaphase after treatment with tamaron 500 ppm for 2 hrs.

Fig. (3)
Disturbed anaphase after treatment with tamaron 250 ppm for 2 hrs.

Fig. (4)
Lagging chromosome after treatment with malathion 1000 ppm for 2 hrs.

Fig. (5)
Double bridged anaphase after treatment with malathion 250 ppm for 2 hrs.

Fig. (6)
Binucleate (micro) cell-after treatment with malathion 500 ppm for 4 hrs.

Also, RNA and DNA contents were decreased by malathion application, while they were more or less the same in case of tamaron.

So we can come to the conclussion that the insecticidal treatments with the used concentrations and time durations affected Vicia faba root tip cells preprophasic (DNA, RNA and protein synthesis period) and postprophasic (chromosome and spindle movements, formation and function).

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Effect of Nitrogen, Phosphorus Fertilization and Rhizobial Inoculation on Yield and Nitrogen Content of Lentil Crop in Newly Reclaimed Area.

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ABSTRACT

Two field experiments were carried out in Kalabsha, experimental station (120 k.m. South West of Aswan), National Research Centre, to evaluate the effect of Nitrogen and Phosphorus fertilization as well as rhizobial inoculation of Lentil.

Diffsrent levels of calcium superphosphate (16.5 % P_2O_5) and Nitrokima (31 % Nitrogen) were used. The best treatment which increased seed yield, nitrogen content as well as harvest index was that of 200 kg. Calcium superphosphate with 60 kg nitrokima/fed.

Half rates of mineral fertilization were sufficient to produce the highest yield when combined with symbiotic fertilization.

INTRODUCTION

Lentil (Lens esculanta L.) is one of the most nutritive food of the legume crops which grow well under the conditions of upper Egypt.

This crop is consumed as an important source of protein, Seed emergence particularly with sensitive legumes crop such as lentil grown in newly cultivated soils, needs the earlier supply of nitrogen to establish uniform crop stand and early time of maturity. The existence of available phosphate is also important for enhancing the biological nitrogen fixation by symbiosis with the growing legume (Hamdi, 1981).

The adequate supply of fertilizers with suitable irrigation was reported by (Sakhon et al., 1981, Verma and Kalra, 1981, 1983). Sharma et el. (1984) reported that the highest seed yield with higher nitrogen and phosphorus content were obtained by using of 20 kg N and 60 kg, P with two irrigations. However, Verma and Kalra (1981) stated that the economic fertilizer rate for lentil crop was 24 - 28 kg/N and 79 - 89 kg P_2O_5.

A linear increase in both dry weight as well as nodules number was recorded by phosphorus fertilizers (Saraf and Biatha- 1982). Eweida et al. (1988) mentioned that plant height, number of pods and number of seed yield and straw yield/fed. were obtained by the application of 22.5 kg N + 40 kg P_2O_5/fed. They also mentioned that N, P fertilizers resulted in higher protein percentage as compared to control plants.

Mohamed et al. (1981) stated that soybean plants responded markedly to N and P application. The maximum yield was produced when N and P were applied, together and the optimum rates were 45 kg N + 15 kg P_2O_5/fed.

Hussein et al. (1984) also mentioned that (32 kg P/fed.) and inoculation of lentil seeds with specific rhizobia, increased the number of pods per plant and nitrogen content. However, rhizobial inoculation seemed to give higher seed yield than uninoculated treatments.

The aim of this study is to evaluate the effect of rhizobial inoculation in combination with phosphatic fertilization on the biological nitrogen fixation by lentil crop under newly reclaimed areas.

MATERIALS AND METHODS

Two field experiments were carried out at Kalabsha, Agricultural Research Station of National Research Centre, 120 km South of Aswan during 1984 - 1985 and 1985 - 1986 growing winter seasons to study the effect of Nitrogen, Phosphorus fertilization and Rhizobial inoculation on yield and nitrogen content of lentil, crops in newly reclaimed area. The soil is sandy with pH 8, its chemical and mechanical analyses are represented in Table (1). Lentil seeds variety Giza 9 were inoculated with specific rhizobia (Rhizobium leguminasum) and sown in field plots (18 m^2) at 1-10 Nov. (1985 - 1986) in two successive seasons.

Commercial inoculum (okadin) was obtained from Agricultural research Centre Dept. of soil Microbiology Containing four effective strains of Rhizobia leguminasum.

The seeds were mixed with arabic gum 48 % then okadin was added to the seeds in a shadow place by the rate of 2 bags to 45 kg. seed for the cultivation of one feddan.

The coated seeds were then sown in the most soil immediately. Superphosphate (16.5 % P_2O_5) and nitrokima (31 % N) were used for nitrogen and phosphorus fertilization. Potassium sulphate (100 kg/fed.) was applied at the same rate for all treatments.

A complete randomized block design, with four replicates were used.

The treatments were as follows :

Zero N P + 0 inoculation.
" P + 30 kg. Nitro kima.
" P + 60 kg. " " .
" P + 90 kg. " " .

100 kg P + 0 kg. " " .
" " P + 30 kg. " " .
" " P + 60 kg. " " .
" " P + 90 kg. " " .

200 kg P + 0 kg. Nitro kima.
" " P + 30 kg. " " .
" " P + 60 kg. " " .
" " P + 90 kg. " " .

The samples were collected from each treatment after 7 weeks of sowing the roots were throughly washed with running tap water, the nodules on roots were isolated and dried at 70, Cito determine its dry weight.

All treatments were harvested at the end of the experiment, the following data are recorded.

1. Seed yield in ardab/fed. (on ardab seeds = 160 kg.).
2. Straw yield (tons/fed.).
3. Total N and P, crude protein content of lentil.
4. N uptake was calculated.
5. Harvest index (calculated as = $\frac{\text{Seed yield/plant}}{\text{Total biological yield}} \times 100$).

RESULTS AND DISCUSSION

Results in Table (2) showed that the yield of straw and seeds ae well as the total biological yields were increased by adding nitrogen fertilizer as compared with control plants. Such results lend more support to those recorded by Sharma et al., (1971).

On the other hand, Hussein (1977) recorded that the application at 8.7 and 15 kg N/fed. had no significant effect on seed and straw yield. The obtained data also revealed a linear increase in the straw and seed yield by increasing nitrogen fertilization from zero up to 90 kg/fed.

However, the highest straw yield and total biological yield was obtained by adding 60 kg.N/fed. Harvest index decreased gradually by increasing nitrogen fertilizers this may be due to the role of nitrogen in favouring plant growth which consequantly increased the total biological yield to seed yield. Such results are in agreement with those obtained by Iftikhar et al. (1971).

Nitrogen and Phosphorus fertilization showed a significant effect on seed yield straw yield, total biological yield as well as, harvest index at both seasons. Applying 100 kg.P/fed. + 60 kg N gave the highly significant increase in seed yield.

Rizk (1979) reported that phosphorus applied to lentil at the rate of 80 kg P_2O_5/hr. gave significantly higher seed yield.

The experimental results showed that the increase in nitrogen over 60 kg showed decreases in yield. This may be due to the role of phosphorus in metabolic processes.

However, 30 kg nitrokima + 100 kg phosphorus was sufficient to increase the average straw yield and total biological yield at both seasons.

The obtained data also demonstrated that harvest index gave maximum value with the addition of 100 kg phosphorus + 60 kg nitrogen/fed. In this connection it may be mentioned that Mohamed et al. (1981), stated that soybean responded to N and P application when applied together.

Increasing phosphorus fertilization to 200 kg/fed. also increased seed and straw yield as well as total biological yield and harvest index as compared with control plants. However, N, P fertilization at the rate of 200 kg P + 60 kg N/fed. gave the optimum increase effect of seed and straw yield as well as total biological yield and harvest index. Additional supply of nitrogen to 90 kg Nitrokima tended to decrease this increase in spite of the absence of significance in some cases.

2. Effect of different doses of superphosphate and nitrogen on nodule-dry weight, N content and N-uptake by lentil seeds :

Data in Table (3) showed that seed inoculation with specific rhizobia precultivation in a virgin soil, significantly increased the number and dry weight of nodules over the control treatment

which did not show any nodulation on the roots. Nitrogen fertilization without phosphorus addition to this virgin soils slightly increased nodulation on the roots of these treatments.

This may be due to the poor content of organic matter and available nitrogen in these virgin soils, needed for the growing seedlings.

The combined effect of N and P fertilization had significantly increased the nodulation of lentil roots by producing satisfactory nodular tissues. Increasing dose of phosphate fertilization had significant effect on nodulation in the presence of nitrogen fertilization, as the best treatment showed the highest significant nodule dry weight was 200 kg P in combination will 60 kg Nitrokima/fed. These results are in harmony with the finding of Mohemed et al., (1981) on soybean plants.

Data presented in Table (3) also revealed that application of Nitrokima without phosphorus at the different doses used (30, 60 and 90 kg/fed.) significantly increased lentil seed yield as well as its total nitrogen content than the control plants. However, the higher dose (90 kg Nitrokima/fed.), showed a decreasing effect, as compared with the other treatment. This may be due to the contraverse effect of these high doses of N on the activity of biological-N fixation.

This assumption may be confirmed by the lower nodule dry weight obtained by this treatment.

This effect could be also noticed in the presence of phosphate fertilization with the two levels used (100 and 200 kg/fed.). The application of Ca superphosphate with the two levels used (100 & 200 kg/fed.) in combination with N fertilization with Nitrokima insignificantly increased seed yield and its N content as well as N-uptake compared to the treatments received N-fertilizer only.

On the other hand, the higher dose of superphosphate (200 kg/fed.) increased the seed yield and N-content than the other levels in the presence of N-fertilization.

The optimum seed yield, N-content and N-uptake was obtained when lentil plants were fertilized by 200 kg superphosphate + 60 kg Nitrokima.

These results in agreement with the findings of Sekhon et al. (1981). It can be concluded that successful cultivation of lentil under newly reclaimed soils of upper Egypt needs continuous inoculation of the seeds with specific rhizobium in combination with N and P fertilization. The recommended dose of mineral fertilization is 60 kg N/fed. as Nitrokima added at earlier stages of growth and 200 kg P_2O_5/fed. as Ca-superphosphate.

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Table (2): Effect of nitrogen and phosphate fertilization on seed yield, straw yield and harvest index of lentile crop. (Average of two seasons).

Treatments	Seed yield Erdab/ fed.	Straw yield Ton/ fed.	Total biological yield kg/fed.	Harvest index
1. Control	0.98	0.670	827	19.00
2. OP + 30 kg N.	1.56	1.079	1328	18.75
3. OP + 60 kg N.	1.61	1.119	1376	18.68
4. OP + 90 kg N.	1.93	1.019	1328	23.27
5. 100 kg P + 0 kg N	2.15	0.993	1337	25.73
6. 100 kg P + 30 kg N	3.20	1.175	1687	30.35
7. 100 kg P + 60 kg N	3.40	0.956	1500	36.27
8. 100 kg P + 90 kg N	2.79	0.773	1219	36.59
9. 200 kg P + 0 kg N	3.33	0.803	1336	39.895
10. 200 kg P + 30 kg N	3.85	0.829	1445	42.63
11. 200 kg P + 60 kg N	4.29	1.052	1738	39.47
12. 200 kg P + 90 kg N	3.83	0.907	1520	40.33
L.S.D. at 5 %	0.029	0.018	-	-

Table (3): Effect of different doses of superphosphate and nitrokima on nodules dry weight, N-content and N_2-uptake by lentil seeds. (kg/fedden).
(Average of two seasons).

Treatments	Nodule dry wt. mg/10 plants	N-content of seeds as %	Seed kg/fed.	N-uptake kg/fed.
1. Control	0.00	2.89	156.8	4.53
2. OP + 30 kg N	135	3.63	248.8	9.03
3. OP + 60 kg N	180	3.85	256.8	9.89
4. OP + 90 kg N	166	3.47	308.8	10.72
5. 100 kg P + 0 kg N	225	3.82	344.0	13.14
6. 100 kg P + 30 kg N	388	4.24	512.0	21.71
7. 100 kg P + 60 kg N	478	4.36	544.5	23.74
8. 100 kg P + 90 kg N	410	4.26	416.1	19.00
9. 200 kg P + 0 kg N	304	3.84	532.8	20.46
10. 200 kg P + 30 kg N	585	4.34	616.0	26.73
11. 200 kg P + 60 kg N	821	4.60	686.4	31.57
12. 200 kg P + 90 kg N	535	4.37	612.8	26.78
L.S.D. at 5 %	148	0.06	-	-

Table (4) : Chemical and mechanical analyses of Kalabsha soil.

Depth (cm.)	Chemical analyses										
	pH in (1:2.5 susp.)	$CaCO_3$ (%)	E.C. in (1.5 mmhos/cm)	Cations (meq/100 gm soil)				Anions (meq/100 gm soil)			
				Na^+	K^+	Ca^{++}	Mg^{++}	CO_3	HCO_3	Cl^-	SO_4^{--}
0-30	8.1	5.31	0.65	2.10	0.28	1.80	0.30	-	0.40	2.0	1.95
30-60	8.3	0.17	0.37	0.75	0.17	2.10	0.20	-	0.30	1.8	2.00

Mechanical analyses

Organic matter (%)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture
0.24	21.9	63.9	12.0	1.2	Sandy soil

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Effect of Different Levels of Nitrogen Fertilization and Irrigation Intervals on Barley Plants Productivity in Newely Reclaimed Areas.

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By

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ABSTRACT

The present work was carried out during two successive seasons at Kalabsha Agricultural Research Station, to study the effect of different levels of nitrogen 0, 15, 30 and 45 kg/fed. on growth characters and yield of Barley plants under different irrigation intervals, 4, 8 and 12 days. Number of leaves, plant height, number of spikes as well as fresh weight of leaves and stems were increased by nitrogen fertilization. The highest number of tillers at first season was obtained by 45 kg N/fed. The highest significant increase in growth characters, straw and grain yield were obtained by using 30 kg N/fed. Plant height, number of leaves, and panicles as well as fresh weight of leaves, panicles and stems increased with shortening the irrigation frequancy to 4 days. Prolonging the irrigation intervals from 4 to 8 and 12 days decreased growth characters, straw and grain yield/fed. at both seasons. The interaction between irrigation and nitrogen fertilization showed positive

effect on growth characters, straw and grain yield/fed. Increasing nitrogen level up to 30 kg/fed. was more effective under the shorter irrigation frequency than those under the wider ones.

INTRODUCTION

Barley is one of the most important cereal crop in Egypt, the average yield per feddan is low, this may be due to irrigation regime and Nitrogen application. Lynch et al. (1979) working on spring 5 barley cv. reported that there was an average increase in grain yield of 11 kg/ha. for each kg/ha. Nitrogen application. Risk and others (1984) also mentioned that barley plants responded to nitrogen application up to 150 kg/ha. by increasing the number of fertile tillers. Ammonium nitrate 120 kg/ha. markedly improved grain yield and size of spring barley (Pain et al., 1978). Similar results were obtained by El-Kholei and Bayoumi (1982). On the other hand, Lynch and Others (1979) and Kahnt (1981) reported that, the increase in the rate of nitrogen application have little effect on grain yield. Similar results were obtained by Pedersen and Jorgensen (1976).

The maximum average yield of barley grain was obtained by application of 35 kg N/ha. as mentioned by Kuizenga (1977). Similar results were obtained by Kandera (1976).

J hansson (1977) revealed that irrigation increased grain yield irrespective of method of N application. However Malhotra and Cheema (1977) concluded that pre-sowing irrigation alone or with two irrigation during the growth period increased the average grain yield. Hooda, and Kalra (1981) also mentioned that dry matter production increased with increasing nitrogen rate for irrigated barley plants than for rainfed crops. Similar results were obtained by Pesant and Cheng (1984). However, Radnev and Vasilev (1973). Concluded that the highest average grain yield was obtained by two irrigations applied compared with plants received only one irrigation.

The aim of this study is to evaluate the effect of different levels of Nitrogen on growth characters and yield of barley plants under different irrigation frequencies.

MATERIAL AND METHODS

Two field experiments were carried out at Kalabsha Agricultural Research Station N.R.C. at High Dam Lake 1983 - 1984 and 1984 - 1985 growing winter seasons to study the effect of nitrogen and irrigation on barley plants cultivar (Giza 121).

Barley grains were sown at 24 November of 1984 and 1985 two successive seasons. The soil was sandy chemical and mechanical analyses of the soil are illustrated in Table (A).

A split plot design with 4 replicates were used in both seasons. Calcium superphosphate, and potassium sulphate by the rates of 200 and 100 kg/fed. were added before sowing. Nitrogen was added broadcasting as 3 equal portions after 1,2 and 3 months from sowing. The following irrigation interval treatments were : 4, 8 and 12 days.

The soil fertilization treatments with each irrigation interval were :

1. Unfertilized (control).
2. 15 kg N/fed. as (Ammonium nitrate).
3. 30 " " " (" ").
4. 45 " " " (" ").

The obtained data were statistically analyzed according to Snedicor and Cochran (1967).

Four plants were chosen from every treatment at time of emergency, panicles and the following measurements of growth were taken.

1. Plant height (in cm).
2. Number of leaves.
3. Fresh weight of leaves (in g).
4. Fresh weight of panioles (g).
5. Fresh weight of stems (g).

Yield :

1. Number of tillers.
2. Number of panicles.
3. 5 panicles weight.
4. Weight of grain of 5 panicles.
5. Straw yield.
6. Grain yield.

RESULES AND DISCUSSION

Data of the vegetative growth and straw as well as grain yields of barley plants as affected by irrigation, nitrogen and the interaction are shown in Tables (1,2 and 3) respectively.

1. Vegetative growth :

Data presented in Table (1 A) showed differences in plant height as well as number of leaves at both seasons due to irrigation intervals. The highest value for plant height and number of leaves were obtained when the irrigation interval was 4 days. Prolonging irrigation interval depressed both number of leaves and plant height. Similar results was obtained by Johanson (1977) on barley.

Results in Table (2 A) showed that plant height and number of leaves/plant generally increased by increasing N level up to 30 kg/fed. However, applying more than

30 kg/fed. N reduced this increase. In this connection it may be mentioned that Finkner and Vernal (1971), reported that nitrogen increased plant height of wheat plants.

The interaction between irrigation and N fertilization as shown in Table (3 A) revealed that the higher values of plant height and number of leaves were recorded by adding 30 kg N/fed. at 4 days irrigation interval at both season.

The increase in plant height and number of leaves by shortening irrigation intervals, and increasing N fertilization was recorded by El-Zeiny (1971) and Hussein (1968) on maize plants.

Number of panicles increased at both seasons when the irrigation intervals shorted to 4 days, increasing irrigation intervals from 8 - 12 days showed insignificant decreasing effect. On the other hand extending irrigation intervals, had no significant effect on number of tiller.

Increasing N levels up to 45 kg/fed. increase significantly number of tiller while 30 kg/fed. was sufficient to increase number of panicles at the first season. However, applying Nitrogen more than 30 kg/fed. was not sufficient to cause any aditional significant effect. It seems from the data recorded, that nitrogen fertilization not only enhance vegetative growth of barley plants represented

plant height and number of leaves but also accelerate reproductive growth through the increase in number of tiller and number of panicles. Similar results were obtained by Hagrass (1972) on wheat.

The interaction between irrigation and N level as shown in Table (3 A) revealed that 30 kg N/fed. was sufficient to increase both number of tillers and panicles in both seasons, when irrigation interval was reduced to 4 days (Kirby, 1969). Increasing irrigation interval to 8 or to 12 days, the level of 45 kg N/fed. was effective in increasing number of tillers to the highest values at both seasons.

Fresh weight :

As shown in Table (2) increasing N level up to 30 kg/fed. increased significantly the fresh weight of leaves at both seasons as compared with the control treatment. Moreover, increase in N level up to kg/fed. reduced this increase. These results are in agreement with those of Hussein and Firgany (1978) and Thalooth (1978) on wheat plants.

The increase of nitrogen level resulted in increases in the fresh weight of stems and panicles in spite of the abscence of significante in some cases at both seasons, the highest values were recorded by adding N fertilizers

at 15 kg/fed. However, any increase in nitrogen above 15 kg/fed. reduced that increases.

The interaction between irrigation and N fertilization react significantly on fresh weight of leaves, stems and panicles, 30 kg/fed. was suffficient when plants were irrigated every 4 days, whereas at longer irrigation intervals, 45 kg was effective.

Weight of 5 panicles and their grains :

Data in Table (1 b) showed that prolonging irrigation intervals up to 12 days, decreased significantly weight of 5 panicles as well as weight of their grains in both seasons, However, the highest values were obtained when irrigation interval was shortened to 8 days.

As shown in Table (2) weights of 5 panicles with increased by adding nitrogen fertilization as compared with control plants.- However, in the first season the weight was not significantly increased with increasing nitrogen level over 15 kg/fed. and for 30 kg/fed. in the second season. The increase in weight of 5 panicles with N level was found to be related to grain weight of the 5 panicles.

The interaction between irrigation and N fertilizers shown in Table (4) revealed that increasing N level from

0 - 45 kg/fed. increased weight of 5 panicles and their grains. In spite of the absence of significance in some cases, the data show that whereas the highest weight was obtained by adding 30 kg/fed. with irrigation frequancy of 4 days, prolonging the irrigation interval showed a significant decreasing effect.

Straw yield :

It is clear from the results in Table (2 b) that extending irrigation interval from 4 to 12 days, significantly decreased straw yield in both seasons while increasing N level from 0 to 45 kg/fed. increased straw yield significantly. This increase may be due to the role of N in increasing the vegetative growth. Kandera (1974) and Kristan (1974) came to the same conclusions.

Concerning the interaction between irrigation and nitrogen fertilization, data in Table (3) showed an increase in straw yield by increasing the nitrogen level. However, the highest straw yield were obtained with irrigation interval of 4 days with 45/fed. at both seasons. At the longer irrigation interval 8 and 12 days the level of 30 kg/fed. was sufficient to increase straw yield at both seasons.

Grain yield :

Significant differences in grain yield were obtained in both seasons due to extending irrigation intervals. However, grain yield decreased significantly by prolonging irrigation intervals from 4 to 8 and 12 days at both seasons. The linear relation between barley grain yield and the amount of water was found by Power et al. (1973). Senlivy (1971) demonstrated that irrigation increased sugar content of the over wintering barley plant by 15.20 times; compared with barley grown under rain fed conditions as water stress may be depress the activity of several enzymes in the leaves.

Grain yield was significantly affected by nitrogen fertilizers as the highest value was obtained by using 30 kg N/fed. in both seasons. This results are in agreement with Dilz et al. (1975) who mentioned that, 30 kg N/fed. was the best for barley. Moreover, the increase in N decreased this increase significantly. Similar results were obtained by Kuizenga (1977) who mentioned that the maximum grain yield of barley was obtained by application of 30 kg N/fed.

Interaction between irrigation treatments and nitrogen fertilization (Table 3) showed that, increasing nitrogen dose significantly increased grain yield whatever the irrigation interval used as compared with the unfertilized plant

at both seasons of experiment. However, the highest yield was obtained by adding 30 kg N/fed. and irrigation interval of 4 days at both seasons, this level was less effective by increasing irrigation intervals to 8 and 12 days.

It might be concluded from these results that grain yield is highly affected by water stress. Increasing nitrogen level up to 45 kg N/fed. slightly decreased this increase when the plants were irrigated every 8 or 12 days at both seasons.

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Table (1,A) : Effect of irrigation of growth and yield of barley.

Irrigation intervals	Plant height in cm.		No. of leaves/ plant		No. of panicles		No. of tillers		Fresh weight/plant (g)					
									Leaves		Panicles		Stems	
	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985
4	75.97	73.95	18.22	18.05	2.22	2.25	3.62	3.50	3.40	3.30	1.82	1.73	9.37	9.90
8	67.92	67.35	17.35	17.42	2.12	2.05	3.30	3.37	2.72	2.67	2.45	2.42	8.01	7.06
12	64.67	64.00	13.87	14.72	1.97	1.60	3.62	3.48	3.22	3.15	2.87	2.72	10.77	10.95
L.S.D. 5 %	8.95	6.50	2.53	1.40	0.42	0.44	0.58	0.41	0.081	0.059	0.09	0.077	0.29	0.32

Table (1,B) :

Irrigation intervals	Yield characters/plant				Yield/fed.			
	5 panicles weight (g)		Grains of 5 panicles (g)		Straw ton.		Grain kg.	
	1984	1985	1984	1985	1984	1985	1984	1985
4	6.60	6.22	4.50	4.32	1.556	1.419	1110.725	1035.64
8	6.72	6.42	5.25	5.09	0.944	0.913	800.400	8090.20
12	5.50	5.35	4.80	4.59	0.625	0.631	662.610	649.06
L.S.D. 5 %	0.434	0.280	0.280	0.225	0.1147	0.045	49.32	50.28

Table (2,A) : Effect of nitrogen fertilizers on growth characters of barley.

N Fertilizers kg N/fed.	Plant height in cm.		No. of Leaves /plant		No. of tillers		No. of panicles		Fresh weight/plant: Leaves		Panicles		Stems	
	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985
Unfertilized	63.00	61.83	11.90	12.06	2.83	3.00	1.90	1.66	1.63	1.56	1.93	1.78	7.00	6.37
15	75.50	73.36	12.23	17.06	3.15	3.16	2.63	2.56	3.13	3.00	3.50	3.40	12.42	12.07
30	73.92	73.86	19.56	19.40	3.91	3.73	2.66	1.90	4.10	4.03	2.36	2.30	10.50	10.84
45	66.30	66.40	17.23	18.40	4.16	3.66	1.73	1.73	3.60	3.56	1.95	1.70	7.59	7.09
L.S.D. 5 %	10.33	7.51	2.67	1.61	0.67	0.47	0.51	0.42	2.60	0.068	0.06	0.09	0.33	0.38

Table (2,B) :

N Fertilized kg N/fed.	5 panicles weight (g)		Grains of 5 panicles (g)		Straw yield/fed. ton.		Grain yield/fed. kg	
	1984	1985	1984	1985	1984	1985	1984	1985
Unfertilized	5.96	5.83	4.76	4.32	0.5077	0.4919	572.0	561.7
15	6.87	6.20	6.10	4.99	1.2290	1.0882	1024.3	1005.6
30	6.83	6.26	5.70	5.09	1.1820	1.1550	1080.5	1032.5
45	6.00	5.90	4.43	4.27	1.2370	1.2156	746.9	712.6
5 %	0.5019	0.321	0.321	0.082	0.132	0.213	56.95	58.06

Table (3 a) : Interaction between irrigation and nitrogen fertilization on barley yield and its components.

Irrigation	Fertilization	Plant height (cm)		No. of leaves /plant		No. of pahioles/ plant		No. of tillers/ plant		Fresh weight g /plant					
										Leaves		Panicles		Stems	
		1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985
4	0	64.6	62.5	10.5	10.0	2.0	2.0	2.5	2.7	1.5	1.4	1.6	1.4	7.80	6.82
	15	86.7	81.6	18.5	19.0	2.2	2.5	3.0	3.2	3.4	3.3	2.0	2.0	14.00	14.35
	30	79.2	76.9	28.7	28.2	3.0	2.5	5.5	4.7	5.6	5.5	2.0	1.8	10.32	13.60
	45	73.4	74.6	15.2	15.0	1.7	2.0	3.5	3.2	3.1	3.0	1.7	1.7	5.32	4.85
8	0	65.2	64.2	13.5	13.5	2.5	2.0	2.5	2.7	2.0	2.0	2.6	2.5	6.25	5.62
	15	65.8	65.2	14.5	14.2	3.0	3.2	3.7	3.7	2.6	2.4	4.0	4.0	9.37	8.45
	30	65.7	65.1	12.0	12.2	2.0	2.0	2.7	2.7	2.6	2.6	1.3	1.3	7.97	6.52
	45	62.0	61.5	19.2	19.2	1.0	1.0	4.5	4.2	3.7	3.7	1.9	1.9	8.42	7.65
12	0	59.2	58.8	11.7	12.7	1.2	1.0	3.5	3.5	1.4	1.3	1.6	1.4	6.95	6.67
	15	74.0	73.3	18.7	18.0	2.7	2.0	2.7	2.5	3.4	3.3	4.5	4.2	13.90	13.42
	30	74.9	74.2	18.0	18.0	2.5	2.2	3.7	3.7	4.1	4.0	3.8	3.8	13.22	12.42
	45	63.6	63.1	21.0	21.0	1.5	1.2	4.5	4.2	4.0	4.0	1.6	1.5	9.03	11.22
L.S.D. 5 %		17.90	13.01	4.63	2.8	0.446	0.736	1.17	0.825	4.5	0.119	0.18	0.155	0.583	0.698

Table (3 b) : Interaction between irrigation and nitrogen fertilization on barley yield and its components.

Irrigation	Fertilization	5 Panicles weight (g)		Grains of 5 panicles wt. (g)		Straw yield/fed. ton.		Grain yield/fed. kg.	
		1984	1985	1984	1985	1984	1985	1984	1985
4	Unfertilized	6.80	5.60	4.80	4.20	0.6372	0.6250	591.2	588.90
	15	7.10	6.60	4.50	4.50	1.8775	1.5195	1056.6	1000.77
	30	7.50	7.70	7.90	7.80	1.7165	1.6130	1546.9	1367.00
	45	7.00	7.00	4.80	4.60	1.9960	1.9440	1234.4	145.77
8	Unfertilized	6.00	7.00	3.80	3.50	0.5760	0.5790	645.9	634.92
	15	7.10	6.30	5.90	5.50	1.0960	0.9852	958.5	963.40
	30	7.30	6.50	5.90	6.10	1.1227	1.1515	981.4	1022.50
	45	6.50	5.90	5.40	5.10	0.9845	0.9385	606.8	616.70
12	Unfertilized	5.10	4.90	5.70	5.10	0.3100	0.2917	479.2	460.10
	15	6.40	5.70	4.90	5.90	0.7520	0.7600	1058.0	1052.75
	30	7.70	6.60	5.50	5.20	0.7090	0.7100	713.4	707.95
		4.50	4.20	3.10	3.00	0.7320	0.7645	399.6	375.45
L.S.D. 5 %		0.869	0.55	0.14	0.25	0.2295	0.095	98.65	100.57

Table (A) : Chemical and mechanical analyses of Kalabsha soil.

Depth (cm.)	Chemical analyses										
	pH in (1:2.5 susp.)	$CaCO_3$ (%)	E.C. in (1.5 mmhos/cm)	Cations (meq/100 gm soil)				Anions (meq/100 gm soil)			
				Na^+	K^+	Ca^{++}	Mg^{++}	CO_3	HCO_3	Cl^-	SO_4^{--}
0-30	8.1	5.31	0.65	2.10	0.28	1.80	0.30	-	0.40	2.0	1.95
30-60	8.3	0.17	0.37	0.75	0.17	2.10	0.20	-	0.30	1.8	2.00

Mechanical analyses

Organic matter (%)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture
0.24	21.9	63.9	12.0	1.2	Sandy soil

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INTRA CELLULAR MINERAL CONTENT OF THE CYANOBACTERIUM ANABAENA ORYZAE IN RESPONSE TO SALINE MEDIA

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Summary

Anabaena oryzae (Egyptian strain) grows over the external salinity range from 50 to 400 mM NaCl in nutrient media. Cultural biomass, heterocyst frequency, intracellular mineral and total carbohydrate content have been determined.

Cultural biomass did not decrease by increasing NaCl and showed only clear reduction at 350 and 400 mM NaCl. Heterocyst frequency was diminished at higher salinity.
The alga responded to increased osmolarity in the medium by an elevation of the internal concentration of carbohydrate content.

The most pronounced effect of NaCl was a trend of increasing the intracellular concentration of Na with increasing extracellular salt which reached 387 fold at 350 and 400 mM NaCl over their corresponding control.

Intracellular P, K, Ca, Mg, Mn and Zn contents were diminished throughout the tested salinity range, while Fe and Cu showed minor changes.

The sensitivity of the N2-fixing system of the alga comprised a gradual decrease in concentration of intra and extracellular nitrogen in response to increasing salinity associated with the increased trend in carbohydrates.

Introduction

The wide distribution and bloom-forming disposal of cyanobacteria have been attributed in part to the fact that they include many species which can fix atmospheric nitrogen. Nitrogen-fixation is commonly limited by certain conditions including salinity. Many species of cyanobacteria thrive in saline environments and were found to exhibit certain degree of adaptability (Fogg et al, 1973; Tel-Or, 1980 a,b; Melamed-Harel and Tel-Or, 1981). The potential mechanisms employed by cyanobacteria for salt adaptation are yet uncompletely clear. Significant ion accumulation has been reported for several micro-algae as a function of increasing salinity (Ahmad and Hellebust, 1984; Ehrenfeld and Coussin, 1984; Gyinzburg, 1981, a,b) which may lead to inhibition of growth and metabolism.

Hellebust, 1985 suggested that, the role of inorganic ions in osmoregulation of micro-algae is uncertain, since technical difficulties in determining ion content in cells in highly saline media have yielded conflicting values (Ahmad and Hellebust, 1984; Ehrenfeld and Coussin, 1984; Gimmler and Shirling, 1978; Ginzburg, 1981 and Munns et al, 1983). Other authors ascertain the important role of inorganic ions in osmoregulation (Brown & Borowitzka, 1979; Brown Hellebust, 1980; Kauss, 1978 and Setter & Greenway, 1983).

However, more recent studies of osmotic adjustment in cyanobacteria have demonstrated that organic osmotic rather than inorganic ions, may play a major role in the maintainance of positive turgor under conditions of salt-stress (Borowitzka et al, 1980; Blumwald and Tel-Or, 1982 a,b; Reed and Stewart, 1983; Reed et al, 1984).

The aim of the present study is to estimate the internal concentration of inorganic mineral and total carbohydrates of the cyanobacterium Anabaena oryzae (isolated from Egyptian soil) over a range of external salt concentration (50 to 400 mM NaCl.) Biomass accumulation and heterocyst frequency were also

determined.Residual macro and microelements were detected by direct assay of the culture supernatant as a result of the algal growth.

Material and Methods

Organism: Anabaena oryzae, Egyptian strain was provided from the Agriculture Research Centre, Giza, Egypt.

Culture media and conditions: Batch cultures were grown photoautotrophically under continuous fluoresent illumination (20 Watt/m^2) at 28°C in (Watanabe et al, 1959) medium. Inocula were grown to mid-exponential-phase. Aliquots of (5 ml) cell suspensions were inoculated ints 500 ml Erlenmeyer flasks containing 200 ml of medium, 5-10 flasks were used for each treatment, sodium chloride, was added to the flasks to the desired concentrations. Experiments were repeated at least twice.

Growth: Cultures were harvested in the late-logarithmic phase of growth The organisms were separated from the medium by centrifugation at 10000 g. for 10 min. at 4°C, the harvested cells were washed twice with distilled water.

Growth was determined in terms of culture biomass estimates (mg dry cells/ml culture) following drying for 24 h. at 70°C (Layzell et al, 1985).

Heterocyst frequency: Cells were fixed in 2.5% (V/V) gluteraldehyde in phosphate buffer, pH 7.2; at beast 1000 cells were counted under light microscope for each treatment and the frequencies of differentiated cells were expressed as a percentage of the total vegetative cell population (Sharma, 1984).

Carbohydrate concentrations: Carbohydrates were estimated by the phenol sulphuric acid test relative to standard solution of glucose. (Dubois et al, 1956).

Analysis of intracellular mineral content: Intracullular ion concentrations were analyzed from the dried algal cells which were powdered and digested in nitric-perchloric-sulphuric acid. Phosphorus was analyzed using the vanadatomolybdate colorimetric method (Chapman and Prott, 1978).

Ca, Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectrophotometry. K and Na were estimated by flame photometry. (Jackson, 1967).

Nitrogen was measured by kjeldahl method (Jackson, 1967).

Residual ions of the culture suprenatant were directly assayed.

Results and Discussion

Response of Anabaena oryzae cells to saline media:

The growth of A.oryzae was tested as a function of external salinities with (0.05 to 0.4 M. NaCl) in the culture media. It is interesting to note from Table 1 that, cell growth was reduced by 0.35 M and 0.4 M. NaCl while cultures were adapted to 0.05-0.3 M NaCl and showed tolerance to this condition and were similar to the control, which indicates the algal capacity to osmoregulation. This is in line with the data presented by Mahmoud et al, (1980) and Alla-El-Din et al, (1980), who suggested that, growth of Nostoc calcicola, N.muscorum, Anabaena variabilis and A.oryzae were stimulated by the addition of up to 9000 pp.m. salt.

Table 1 illustrates the microscopic examination of algal filaments and shows that, the proportion of heterocysts was greatest with the low NaCl concentration (0.05 to 0.2 M)amounted to 10.7 to 8.1% of the cells, while heterocysts proportion amounted to 5.5 to 4.1% with 0.25 to 0.4 μ NaCl. This indicates the relationship between salinity and heterocyst frequency (Fig. 1).

However, since heterocysts are the sole site of aerobic N_2-fixation in filamentous cyanobacteria

(Haselkorn, 1978, Stewart, 1980 and Wolk, 1982), a change in number or quality of heterocysts is bound to affect nitrogenase activity.

Nitrogen and carbohydrate content in relation to NaCl concentration:

Since A.oryzae can grow and adapt to NaCl concentration ranging from 0.05 to 0.4 M, we checked its adaptation to different NaCl concentration in regard to intracellular nitrogen, carbohydrates and mineral content.

Table 2, shows that, salt-grown cells contained much lower nitrogen content amounting to 6.1 - 55% of the control cells, nitrogen decreased linearly with increased salt concentration. The observed decrease in nitrogen content under such saline condition is explained to be mainly due to reduction in nitrogenase activity as compared with control cultures (Table 2) It is obvious that, this change is parallel to the pattern of heterocysts differentiation response to salinity as shown in table 1. It has been postulated that, appearance of nitrogenase activity is concomitant with the development of heterocysts (Bradley and Carr, 1976; Fleming and Haselkorn, 1974; Murry and Benemann, 1979; Neilson et al, 1971)

Total carbohydrate content increased with increasing NaCl concentration (Table 2). In 400 mM NaCl, total carbohydrate amounted to 176.6 mg/g. dry wt. compared with 109.8 mg/g.dry wt. for the control cells. Carbohydrate accumulation during salt-stress suggests that, carbohydrate may play a role in osmotic regulation in Anabaena oryzae cells. Blumwald and Tel-Or (1982b) adaptation to environmental stress requires a larger investment of energy for biosynthesis and reorganization by Nostoc muscorum. Probably A.oryzae cells meet these requirements, by enhanced photosynthetic activity which is an immediate response to salt-stress and resulted in higher carbohydrate content.

Intracellular mineral content:

Elemental analysis of the dried cells revealed that the alga exhibited changes in intracellular elements with increase of external salt concentration (Table 3). Addition of NaCl to the growth media decreased intracellular P, K, Mg and Ca content of A.oryzae and increased the residual minerals in the culture supernatant (Table 4), Accumulation of Mn and Cu in response to various NaCl concentrations was lesser than the previously mentioned set of elements. Moreover, Fe, Zn did not show clear dependance on NaCl concentration.

A most interesting observation was the drastic increase in intracellular Na content of cells over the full range of salinity, increasing with increasing extracellular salt concentration and reached 387 fold at 350 mM and 400 mM NaCl over their corresponding control cells.
The steep increase in Na content per gram dry cells is expected to play an essential role in the osmoregulatory mechanism of this alga.

These results lead us to believe that Na^+ ions are responsible for the adaptation of the cells to the saline medium and have specific ionic effect beside osmotic stress. Studies of Blumwald and Telor, 1982 a, b and Watad, 1983, support these findings. Reed et al, 1985, mentioned that Na^+ exporting system is the limiting process for salt resistance of cyanobacteria from hyper saline environments. Other cyanobacteria responsed to changes in external water status by adjusting intracellular K^+ concentration (Miller et al, 1976; Yopp et al, 1978; Reed et al, 1984). Recently a variety of osmoregulants, employed by cyanobacteria, have been reported, e.g. significant ion accumulation (Ahmad and Hellebust, 1984; Blumwald et al, 1983; Ehrenfeld and Coussin, 1984; Ginzburg, 1981, Reed et al, 1985), synthesis of sucrose (Erdmann, 1983, Blumwald

et al, 1983), accumulation of osmoprotective compounds: glucosylglycerol (Hagemann et al, 1987); glycerol (Hagemann et al, 1987); glycerol derivatives (Borowizka et al, 1980; Tel-Or et al, 1986) and increasing concentration of prolein content (Ahmad and Hellebust, 1984).

From the results of Tables 3 and 4 it is clear that A.oryzae possesses a marked capacity to take up and accumulate ions with different rates from the external medium. This accumulation of ions appears to be almost sufficient to counterbalance the external salt concentration in cells grown at different salinity.

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Table 1. Cultural biomass and heterocyst frequency in batch cultures of Anabaena oryzae grown at a variety of NaCl concentrations in the media

NaCl Concentration	Culture biomass (mg dry cells/ ml culture)	Heterocyst frequency (% Heterocysts/ veg. Cells)
Control	1.06 ± 0.04	12.8 ± 2.0
50 mM	1.05 ± 0.06	10.7 ± 1.8
100 mM	1.11 ± 0.20	11.1 ± 1.2
150 mM	1.18 ± 0.08	9.7 ± 1.8
200 mM	1.17 ± 0.31	8.1 ± 0.7
250 mM	1.16 ± 0.23	5.5 ± 1.0
300 mM	1.14 ± 0.09	5.3 ± 0.8
350 mM	0.78 ± 0.11	5.2 ± 0.6
400 mM	0.48 ± 0.05	4.1 ± 0.7

Table 2. Effect of NaCl on the intracellular carbohydrate and nitrogen content of Anabaena oryzae. Cultures were grown with and without NaCl until late logarthmic phase of growth.

NaCl content mM/l	Total carbohydrates mg/g. dry wt.	Total nitrogen mg/g.dry wt.
Control	109.8	60.4
50 mM	115.3	50.7
100 mM	122.8	51.1
150 mM	151.2	54.8
200 mM	146.3	51.3
250 mM	159.6	44.8
300 mM	158.3	42.7
350 mM	169.5	23.9
400 mM	176.6	27.0

Table 3: Effect of increasing NaCl concentration on the accumulation of intracellular macro and micro elements in Anabaena oryzae. Each value is a mean of at least duplicate determinations

Concentration of NaCl	mg/g dry weight					µg/g dry wt.			
	P		Na	Ca	Mg	Fe	Mn	Zn	Cu
Control	5.4	3.0	0.1	8.8	3.1	172.40	64.80	57.00	19.57
50 mM	6.2	2.0	5.4	2.0	3.0	132.37	20.88	86.26	14.20
100 mM	5.4	1.7	7.7	2.0	2.6	113.99	13.68	95.76	11.52
150 mM	5.1	1.3	8.1	2.2	2.4	125.29	13.68	43.94	10.24
200 mM	4.5	1.7	12.6	1.7	1.8	233.75	12.96	55.72	12.8
250 mM	2.7	1.1	18.0	0.7	0.9	104.72	9.36	32.68	6.04
300 mM	2.1	1.2	30.7	1.0	0.1	207.57	14.4	66.26	11.52
350 mM	1.0	1.2	38.7	1.1	0.4	134.64	17.28	27.74	6.4
400 mM	1.2	1.2	38.7	1.0	0.3	269.28	33.12	60.42	10.24

Table 4 : Changes in macro and microelements concentration of the culture supernatant as a result of growth of Anabaena oryzae.

NaCl Concentration	N	P	K	Na	Ca	Mg	Fe	Mn	Zn	Cu
			µg/ml					p.p. m		
Watanabe medium	Zero	49.92	201.86	0.72	16.0	20.98	0.57	0.56	0.05	0.05
Control	0.042	21.12	187.44	3.6	12.0	5.95	0.27	0.23	0.02	0.05
50 mM NaCl	0.012	18.56	201.86	1020.3	28.0	7.73	0.27	0.24	0.02	0.05
100 mM NaCl	0.003	24.96	201.86	1637.85	40.0	11.47	0.38	0.29	0.02	0.04
150 mM NaCl	0.003	25.60	187.44	2040.6	44.0	11.66	0.30	0.30	0.02	0.05
200 mM NaCl	Zero	28.16	187.44	2309.1	48.0	13.15	0.19	0.33	0.05	0.03
250 mM NaCl	0.006	32.64	187.44	2685.0	58.0	16.90	0.11	0.36	0.05	0.04
300 mM NaCl	0.001	35.84	187.44	2738.7	58.0	18.24	0.19	0.41	0.03	0.03
350 mM NaCl	0.001	40.96	187.44	3275.7	62.0	20.40	0.23	0.36	0.05	0.03
400 mM NaCl	0.003	42.88	187.44	3714.6	66.0	21.07	0.11	0.39	0.05	0.03

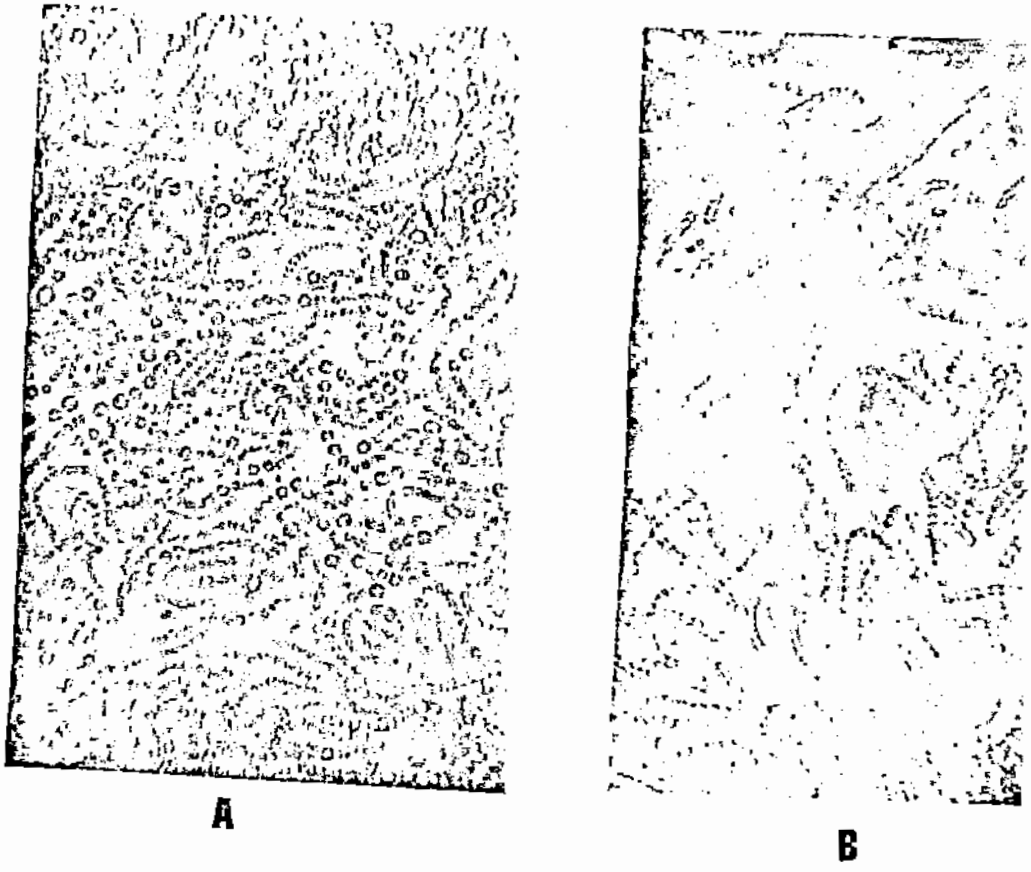

Fig. 1: Anabaena oryzae filaments
A control
B 400 mM NaCl
showing reduction in heterocyst frequency and change in colour

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Cytogenetic Effect of Sulphur Dioxide on Vicia faba plant.

_15_ II

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Introduction

Atmospheric emissions of SO_2 are a major pollution problem, that has been continually growing due to the increasing use of fossil fuels "coal and tar" to generate electricity.

Regulations have been passed in many countries to set legal limits to SO_2 emissions. High levels were permitted within the industrial workplaces. Stokinger (1972) mentioned that, 5 ppm SO_2, is the justified industrial limit of SO_2 emission.

Compliance has been made difficult, due to, the relatively short supply of "clean" low sulphur fuels and the cost of the alternative methods of SO_2 control (Collins 1971, Dunham et al., 1974 and Abelson 1975).

In Egypt, SO_2 levels are very high when compared with other parts of the world. In the polluted Abu Zaal area (40 km north east Cairo), it ranged from 20 to 1[illegible] 5 ppm at 50, 500 and 1000 meters away respectively (Kord 1981).

Kord (1981) found that, treating different plants with SO_2 (5, 10 and 25 ppm) caused retardation of growth. She found also that, fumigation of Raphanus sativus and Eruca sativa with 5 and 10 ppm sulphur dioxide (^{35}S-labalied sodium sulphite, specific activity = 53 mCi/g) for 30 and 60 minutes, increased the sulphur content of the fumigated plants.

Fumigation of Vicia faba plants with 5 ppm SO_2 for a period of 5 hours caused the induction of a highly significant percentage of abnormal PMCs/plant. Such effect increased steadly with the increase of the gas concentration and the period of fumigation (Amer et al. 1987-I).

In the present investigation, the cytogenetic effect of SO_2 after fumigation of Vicia faba plants with different concentrations of the gas (5 - 30 ppm) for 4 hrs /day X 4 days has been studied on the meiosis and pollen viability. Such effect has been followed in groups of treated plants which were left to recover for 6 and 12 days.

Materials and Methods

Vicia faba (V. Giza 2) plants, were fumigated for 4 hrs/day X 4 successive days with different concentrations of SO_2 (5 - 60 ppm). Compare Amer et al. (1987-I) for the detailed method.

A group of the treated plants were left to recover for 6 and 12 days.

Results

Pollen mother cells :

Fumigation of Vicia faba plants with 5 ppm SO_2 4 hrs/day X 4 days affected the induction of 26 % abnormal PMCs/plant. Such percentage increased to a great extent as the concentration of the gas was increased. A high depression in the percentage of abnormal PMCs/plant occurred when the treated plants were left to recover for 6 and 12 days (Fig. 1).

The highest percentage of the induced abnormalities was observed in the anaphase stages. The percentage of abnormal PMCs in meiotic division II was higher than that in meiotic division I (Table I).

Table (II) represents, the different types of abnormalities observed in the PMCs of the plants fumigated with SO_2 and those which were left to recover for 6 and 12 days.

Tetrads :

The percentage of abnormal tetrads was 8.6 % after fumigation with 5 ppm SO_2, and reached 61 % after fumigation with 30 ppm of the gas. Such percentage decreased to a great extent after recovery for 6 and 12 days (Table I).

The presence of more than tetra-group of cells (pentads, hexads and heptads) and dyads (e.g. Fig. 2) instead of the usual tetrads dominated. Its percentage reached 20 % and 27 % of the scored tetrads respectively after fumigation of the plants with 30 ppm SO_2. Tryads were observed in a lower frequency, its percentage did not exceed 6 % of the scored tetrads after fumigation with the different concentrations of SO_2. Tetraploid monads (Syncytes e.g. Fig. 2) were observed (4 %) only after fumigation of Vicia faba plants with 30 ppm of the gas.

Pollen grains :

The percentage of abnormal PGs was generally, much lower than that of abnormal tetrads after fumigation of Vicia faba plants with the different concentrations of SO_2 (Table I).

The nonviable PGs and the small ones were the dominating types of abnormalities observed after treatment with the different concentrations of the gas. The percentage of the small PGs reached 4 % of the scored PGs after fumigation of the plants with 30 ppm SO_2. Tetraploid pollen monads (Syncyte pollen formations e.g. Fig. 4), pollen dyads (e.g. Fig. 3) and tryads as well as deformed PGs were also observed, but, in a lower frequency.

Discussion

Repeated fumigation (4 hrs/day X 4 days) with the different concentrations of SO_2 (5 - 30 ppm) affected the induction of a high percentage of abnormal PMCs/ Vicia faba plant. Rapid was the increase in this percentage with the increase of the gas concentration. Almost all the PMCs were abnormal after fumigation with 30 ppm SO_2, and some dead PMCs were observed in

the flower buds of the plants fumigated with this concentration of SO_2. Thus, a dose about 20 ppm can be considered as the most tolerable concentration for 4 hrs/day X 4 days fumigation of Vicia faba plants with SO_2.

The percentage of abnormal tetrads was high, especially after fumigation of the plants with the higher concentrations of SO_2, and was proportional to the gas concentration.

More than tetra-group of cells was the dominant type of abnormalities observed in this stage after fumigation with 5 ppm SO_2. Such aggregates of cells may result from multipolar telophase II cells as Amer and Farah (1976) suggested in their study on the effect of the carbamate pesticides "IPC," "Rogor" and "Duphar" on Vicia faba plants.

The diploid dyads, unequal dyads and tryads, seemed to be formed as a result of the observed irregular distribution of the chromosomes in the different meiotic stages.

Small pollen grains seemed to be formed as a result of the induction of multipolar or unequal distribution of the chromosomes. Syncyte pollen formation seemed to be formed as a result of the absence cytokinesis.

Sudharshan and Jagadishchandra (1981) reported on the occurrence of syncyte formation and other meiotic irregularities during microsporogenesis in a wild population of Cymbopogan caesius (Poaceae). Montezuma-De-Carvalho (1973) in his study on the effect of nitrous oxide (N_2O) on meiosis of Tradescentia paludosa reported that, nitrous oxide under pressure inhibited the meiotic spindle and gave rise to restitution nuclei (C-interphase). When such cells were allowed to recover, they entered in the 2nd division of meiosis and the spindle was reformed again. Such cells would form diploid pollen dyads.

It is worthy to mention that, a much higher percentage of abnormal PMCs; tetrads and nonviable PGs was observed in the plants fumigated with SO_2 for 4 hrs/day X 4 days than that observed in the plants fumigated with SO_2 for 5 hrs (Amer et al. 1987-I). Such effect may be attributed to the greater ability to take up SO_2 displayed by young leaves (Surrounding the flower buds) than mature or senescent leaves (Guderian 1970).

Vicia faba plants fumigated with SO_2 did not recover after 12 days. A statistically significant percentage of abnormal PMCs and nonviable PGs/plant was still observed, even, in the plants fumigated with the lowest concentration of SO_2 (5 ppm). In this concern it is worthy to

mention that, after daily exposure of the grossly sulphite oxidase-deficient rats to 10 and 30 ppm SO_2 for 6 h/day, 99 days, the testes of the rats were severely atrophied and devoid of spermatogenic cells. There was no evidence for recovery 4 wk following the termination of exposure (Gunnison et al. 1987).

Taking chromosome fragmentation as the criterion for mutagenic potential, it was found that, the percentage of PMCs with fragments increased progressively with the increase of SO_2 concentration (Fig. 5).

It is evident from this study that, repeating exposure to different concentrations of the common air pollutant SO_2 caused adverse genetic hazards for Vicia faba plants. The gas may cause hazardous effects for other cultivated plants. So, arise the need, for more developed not expensive methods for SO_2 emission conrol, for the protection of the genetic information from a mutagen contaminating the biosphere.

Summary

In the present investigation, the cytogenetic effect of fumigation of Vicia faba plants for 4 hrs/day X 4 days with different concentrations of SO_2 (5-30 ppm), has been

studied on the meiosis and pollen viability. Such effect has been followed in groups of treated plants which were left to recover for 6 and 12 days.

Repeated fumigation (4 hrs/day X 4 days) with the different concentrations of SO_2 (5 - 30 ppm) affected the induction of a high percentage of abnormal PMCs/ Vicia faba plant. The highest percentage of the induced abnormalities was observed in the anaphase stages. The percentage of abnormal PMCs in meiotic division II was higher than that in meiotic division I.

The percentage of abnormal tetrads was high, especially after fumigation of the plants with the higher concentrations of SO_2. The presence of more than tetra-group of cells (pentads, hexads and heptads) and dyads instead of the usual tetrads dominated.

The percentage of abnormal PGs was generally, much lower than that of abnormal tetrads after fumigation of the plants with the different concentrations of SO_2. The nonviable PGs and the small ones dominated.

Vicia faba plants fumigated with 5-30 ppm SO_2 for 4 hrs/day X 4 days did not recover after 12 days. A statistically significant percentage of abnormal PMCs

and nonviable PGs/plant was still observed, even in the plants fumigated with the lowest concentration (5 ppm).

Taking chromosome fragmentation as the criterion for mutagenic potential, it was found that the percentage of PMCs with fragments increased progressively with the increase of SO_2 concentration.

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Table I : Percentage of abnormal PMCs, tetrads and nonviable PGs/plant, after fumigation with different concentrations of SO_2 for 4 hrs/day X 4 days and recovery for a period of 6 and 12 days.

Conc. (ppm)	Period of recovery	PMCs			Tetrads		PGs	
		Scored No.	% abn.		Scored No.	% abn.	Scored No.	Abn.PGs
			1st div.	2nd div.				% viable	Mean % + S.E. nonviable
30	-	3665	89.51	93.02	1500	60.53	18000	11.66	6.917 ± 0.820**
	6 days	3595	25.26	33.14	1800	8.33	6000	3.09	3.583 ± 0.300**
	12 days	4345	10.40	14.02	2400	2.29	6000	1.43	1.400 ± 0.245**
20	-	7290	59.90	71.10	3100	40.29	12000	7.29	4.383 ± 0.520**
	6 days	4370	16.71	22.78	2400	3.75	6000	1.24	2.333 ± 0.247**
	12 days	5750	6.45	9.26	2400	1.67	6000	0.69	0.983 ± 0.079**
10	-	5350	36.39	42.88	3460	21.67	12000	4.47	1.883 ± 0.260**
	6 days	6050	9.20	16.61	2400	1.67	6000	0.77	0.900 ± 0.155**
	12 days	5355	4.03	5.13	2400	0.71	6000	0.25	0.417 ± 0.060**
5	-	4932	26.96	26.25	2250	8.58	6000	1.70	0.683 ± 0.100**
	6 days	5800	5.56	9.44	2400	0.33	6000	0.35	0.366 ± 0.049**
	12 days	5260	2.15	2.31	2400	0.08	6000	0.17	0.133 ± 0.033**
Cont.	-	4390	1.17	2.86	2400	1.04	6000	0.57	0.100 ± 0.040
	6 days	4580	0.66	1.30	2400	0.08	6000	0.07	0.083 ± 0.017
	12 days	4040	0.42	0.65	2400	-	6000	0.02	0.050 ± 0.022

** Significant from control at 0.01 level (t-test).

Table II : Number and percentage of the occurring abnormalities in the meioses of *Vicia faba* plants, after fumigation with different concentrations of SO_2 for 4 hrs/day X 4 days and recovery for a period of 6 and 12 days.

Period of recovery	Conc. (ppm)	No. abn. PMCs	Relative to the number of abnormal PMCs: % of the different types of abn.												% of PMCs with multiple type of abn.
			Stick.	Brid.	Desp.	Frag.	Brid. with frag.	Dist.	Retard. sep.	Unequal dist.	Multi. polar.	Tetra-ploid.	Lag.	Micro-nu.	
-	30	3363	16.09	10.35	4.67	15.79	5.53	35.56	0.56	2.85	9.76	0.09	17.54	0.18	15.97
	20	4959	7.06	12.16	1.65	15.11	2.44	29.54	0.54	5.71	16.94	0.24	24.54	0.48	13.79
	10	2109	18.07	10.34	0.33	18.40	2.28	12.00	0.66	7.35	16.83	-	29.30	0.96	14.53
	5	1310	8.18	18.86	-	17.41	3.45	8.14	0.95	2.97	18.56	-	26.13	0.87	5.66
6 days	30	1046	8.90	18.08	2.58	9.56	11.17	11.86	0.76	1.82	24.75	-	19.49	-	8.26
	20	846	8.37	18.08	2.74	10.74	11.00	12.40	0.57	1.65	20.09	-	24.02	-	9.09
	10	764	10.77	14.02	3.25	9.90	7.05	16.47	0.63	2.46	19.40	-	25.90	-	9.09
	5	431	4.58	27.59	0.84	9.15	5.38	7.00	-	3.23	15.07	-	35.26	-	7.41
12 days	30	533	14.07	27.39	-	7.51	4.32	7.69	-	0.75	25.33	-	20.26	-	6.54
	20	455	13.41	24.18	-	4.62	7.69	8.79	-	2.42	22.20	-	21.98	-	4.76
	10	240	14.58	23.75	-	5.00	4.17	5.42	-	2.92	28.33	-	28.33	-	11.50
	5	117	5.98	34.19	-	7.69	4.27	1.71	-	-	17.09	-	35.90	-	6.45

* The sum of the percentages of the different types of abnormalities is more than 100, because the PMCs which have more than one type of abnormalities was recorded under those types in the same time.

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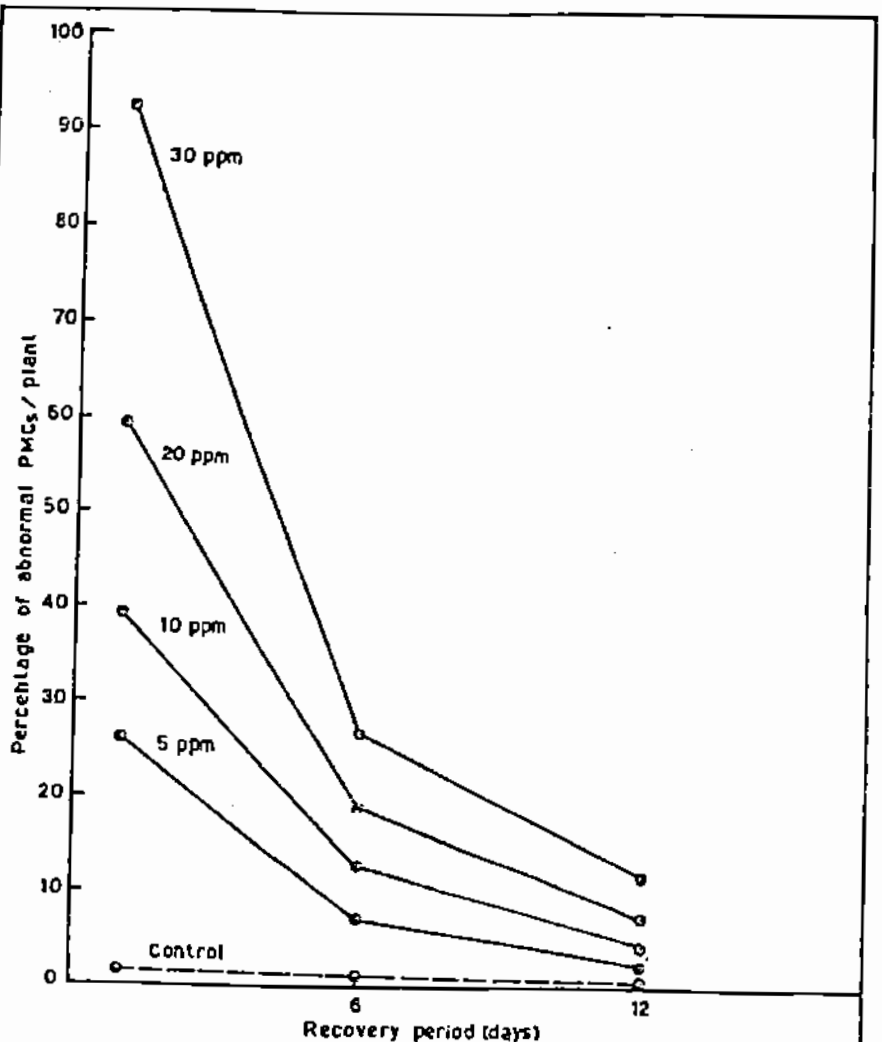

Fig. 1 : Percentage of abnormal PMCs/ Vicia faba plant, after fumigation with different concentrations of SO_2 for 4 hrs/day X 4 days and recovery for 6 and 12 days.

Fig. 2

Fig. 3

Fig. 4

Figs. 2-4 : Dyad consisting of two equal cells (left) and other consisting of two unequal cells (right) and tetraploid monad (Syncyte formation) below (Fig. 2) pollen dyad (Fig. 3) and tetraploid pollen monad (Syncyte pollen formation, Fig.4), after fumigation of Vicia faba plants with:10 ppm (Fig. 2) and 30 ppm (Figs. 3 and 4) SO_2 for 4 hrs/ day X 4 days.

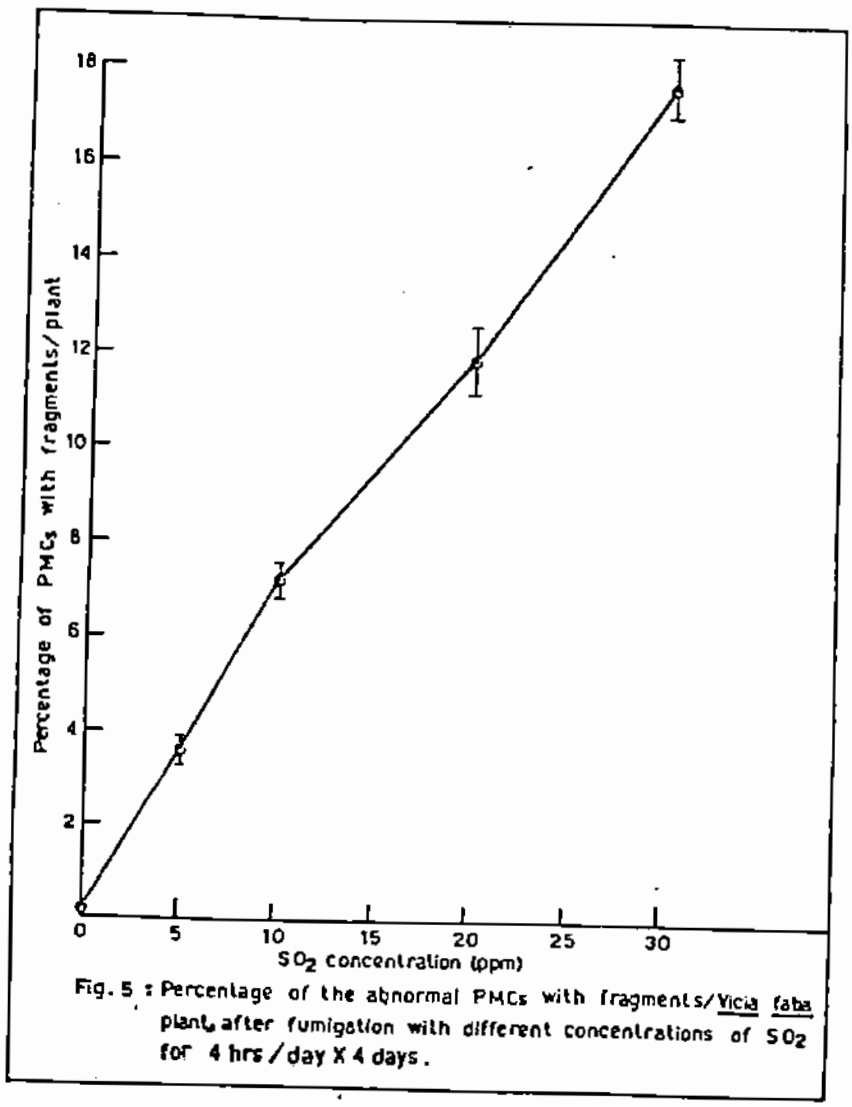

Fig. 5 : Percentage of the abnormal PMCs with fragments/Vicia faba plant after fumigation with different concentrations of SO_2 for 4 hrs / day X 4 days.

Legends

Fig. 1 : Percentage of abnormal PMCs/*Vicia faba* plant after fumigation for 4 hrs/day X 4 days with different concentrations of SO_2 and recovery for 6 and 12 days.

Figs.2-4: Dyad consisting of two equal cells (left) and other consisting of two unequal cells (right) and tetraploid monad (Syncyte formation) below (Fig. 2) pollen dyad (Fig. 3) and tetraploid pollen monad (Syncyte pollen formation, Fig. 4); after fumigation of *Vicia faba* plants with:10 ppm (Fig. 2) and 30 ppm (Figs. 3 and 4) SO_2 for 4 hrs/day X 4 days.

Fig. 5 : Percentage of the abnormal PMCs with fragments/ *Vicia faba* plant, after fumigation for 4 hrs/day X 4 days with different concentrations of SO_2.

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_16_ EFFECT OF SODIUM CHLORIDE SALINITY ON FREE AMINO ACIDS OF SOIL STREPTOMYCETES

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Abstract

Actinomycetes were isolated from a number of saline and non saline salheya soils. From these isolates two isolates of streptomycetes were selected to assess their physiological response to salinity. Intracellular concentrations of the free amino acid pool increased in response to salt stress. Whereas the neutral free amino acids alanine and gamma aminobutyrate accumulated as salinity increased. Accumulation of free amino acids by streptomycetes under salt stress suggests a response typical of procaryotes-although the specific amino acids involved differ from those associated with other gram positive bacteria.

Introduction

The metabolic activities of micro-organisms vary considerably with changes in their growth environments. One of the parameters affecting microbial physiology is the medium osmolarity. Because salinity has become an ever increasing problem in irrigated agriculture,

it stimulated research on the mechanisms of response of microorganisms to salt stress. Sodium chloride concentration in medium has a gross effect on the intracellular levels of free amino acids in many bacterial species (Britten and McClure, 1962, Brown, 1976, Measure, 1975, Tempest and Meers 1970). Gram positive bacteria have been reported to accumulate the neutral amino acids proline (Brown, 1978, Measures, 1975 and Tempest and Meers, 1970) and gamma amino butyrate (Measures, 1975) in response to increasing salt stress, whereas gram negative bacteria accumulate glutamate (Britten and McClure, 1962, Hua et al, 1982, Makemson and Hastings, 1979). In contrast to the accumulation of free amino acids in procaryotes, glycerol, have been found to occur as osmotic agents in eucaryotic fungi and algae (Brown, 1978).

Streptomycetes are strictly gram positive bacteria of the order actinomycetales, because their morphology is sometimes considered to be transitional between the simpler eubacteria and fungi, Harris (1980) posed the question of wheather actinomycetes use free amino acids as heterotrophic procaryotes or as heterotrophic eucaryotes as inducible compatible internal solutes.

There is evidence to suggest that actinomycetes are of particular importance in saline soils (Gupta and Bajpai, 1974) and in environment of high alkalinity (John stone, 1947). Meiklejohn (1957)found that they become an increasingly dominant components of the soil microbiota during prolonged draught.

In this study an investigation was done on the occurrence of actinomycetes in a number of salheya soils of varies salinities. From these soils, two indigonous streptomycetes were selected to asses their physiological response to salinity. The effect of sodium chloride on the growth of these isolates and on composition and concentration of their internal solutes were reported. The data are discussed in relation to osmoregularity mechanisms and to the ecology of actinomycetes in saline soils.

Material and Methods

Soils: Saline surface soils (0-10 cm) were sampled from six agricultural sites in salheya district. A non saline soil was similarly sampled from an agricultural tract of Cairo - Ismaillia. Soils were throughly mixed and sieved before analysis. Values for water soluble salts were determined by evaporating a

well known volume of soil extract solution and weighing the residue (Jackson, 1967). Soil pH was measured with a 1:1 distilled water soil slurry and a glass pH electrode of pH meter (Tacussel). Total soil organic carbon content was determined using Walkley's rapid method (Jackson, 1967).

Microbial counts and actinomycetes isolation: Micro-organisms were counted by the dilution technique using starch-nitrate media of the composition: Soluble starch log., Na No_3 1 g., K_2HPO_4 0.3g, Nacl 0.5 g. Agar, 15 g. distilled water 1 litre (Waksman, 1967). To select for actinomycetes. For each soil dilution, five plates were inclubated at 30°C for 4 days and then counted. Two streptomycetes isolates were preliminary identified by morphological examination and subcultured on to fresh media for use as batch culture inocula.

Preparation of cells:

An early stationary-phase cells, were harvested, centrifuged at 2400 r.p.m. for ten minutes and washed three times using isotonic solution, and then recentri-fuged, the supernatant was discarded, and the resulting pellet were lyophilized using a Beta freez dryer (West Germany).

The amino acids from the lyophilized pellets were extracted by suspending in 5 ml 3.5% trichloroacetic acid (TCA) at 4°C for 24 hr. with occasional blending in a vortex mixer. The suspensions obtained were used for amino acid analysis.

Analytical procedures :

Free amino acids were determined both qualitatively and quantitatively using a Beckman amino acid analyzer, model 118/119 CL with a single column (6 x 460mm) packed with W3 resin citrate buffers of pH 2.83, 3.70 and 3.75 were used. Amino acid concentrations were determined by reaction with ninhydrin reagent (flow rate 22ml/h.).

Results

Plate counts from the six soils (Table 1) indicated no clear relationship between soil salinity and number of actinomycetes.

Table 1: Soil characteristics

Soil No.[a]	pH	Chloride (mg)/ g. soil	Organic carbon (%)	Water soluble salts (mg)/ g.soil	No.of actimo-mycetes per 9 (dry wt.) of soil x 10^8
1	6.1	142	0.9	0.009	2.08
2	6.0	284	2.7	0.011	4.42
3	5.6	710	1.1	0.014	21.12
4	6.0	142.5	2.5	0.017	35.17
5	6.0	177.5	1.59	0.035	16.84
6	5.8	400	0.7	0.044	24.10

a Listed in order of increasing salinity. Soil No.1 nonsaline, soil Nos.2 through 6 saline.

Streptomycete No.1 isolated from soil No.5 was identified by morphology tests as a common actinomycetes in all the saline soils. A second streptomycete isolate, streptomycete No.2. was found in soil No.3. These two streptomyctes were selected to assess their physiological response to salt stress.

Increasing salt concentration reduced the growth as manifested by g/dry weight (untabulated) of the two streptomycetes. This growth reduction was more pronounced in streptomycete No.2 than with streptomycete No.1. In fact, cells of streptomycete No.2 failed to grow at 1% NaCl medium (Table 3).

The free amino acid pool of cells grown in the basal meduim was comprised largely of the acidic amino acids, glutamic and aspartic and neutral amino acids alanine and gamma amino butyric acid .With increasing salt stress there was marked change in both the concentration and composition of the free amino acids in both streptomycetes (Tables 2 & 3). The results indicated that most of the amino acids showed great reduction with salt stress. This reduction was more pronounced in streptomycete No.1 (Table 2) even at the low salt concentration, while in streptomycete No.2, this reduction was observed only at 1% NaCl salinity where cells did not grow. Acidic amino acids such as glutamic acid was greatly affected by salt stress, neutral amino acids such as alanine and gamma amino butyric acid were increased in response to NaCl salinity. Valine was not detected in the basal medium whereas at different levels of salinity considrable amounts were noticed especially in streptomycete No.2. Ammonia shows the same trend as amino acids, where it decreaseswith increase of NaCl salinity in streptomycete No.1 and increases with the increase of NaCl in streptomycete No.2. The intracellular concentrations of the assayed free amino acids cited in Tables 2 and 3 (glycine, leucine and isoleucine, Tyrosine, phenylalanine, Histidine, Lysine, arginine, serine and threonine, cystine, methionine and ornithine) showed the same trend whether in streptomycete No.1 or

streptomycete No.2. Total free amino acids, decrease or increase in response to NaCl salinity corresponding to streptomycete varities.

Discussion

One of the goals of this study was to analyze the amino acid pool of two streptomycetes indigenous to saline soils, grown in media containing different amounts of NaCl and to assess their response to salinity. Growth of the two streptomycetes decreased as NaCl concentration increased. However streptomycetes No.1 could grow up to 1%, while the other one grow up to 0.5% only. These results were in full agreement with the results obtained by Kilham and Firestone (1984), who found that the specific growth yield of two streptomycetes indogenous to saline soils decreased with salt concentration increase . They also found that the yield reduction due to NaCl were consistently greater than those resulting from KCl stress. Moreover the intracellular concentrations of free amino acid did not consistently vary between the two salts. On the other hand, Hua et al. (1982) claimed that the growth rate of Rhizobium spp. strain WR1001 decreased with NaCl increased from 300 to 500 mM, however the bacterium could grow and survive in 500mM NaCl, although

most Rhizobuim strains were very sensitive to salt and minimal media containing 45mM NaCl slows down the growth rate to a greater extent in some variants of R.japonicum (Upchurch and Elkan, 1977).

The observed accumulation of total free amino acids is similar to responses observed in other salt stress studies with gram positive bacteria (Brown, 1976 and Measures 1975). Intracellular accumulation of free alanine under salt stress, however, has previously been demonstrated in streptomyces griseus and streptomyces califoricus by Kilham and Firestone, (1984), whereas the occurrence of gamma aminobutyrate as inducible compatible solute has been reported to accumulate in salt stressed streptococci (Measures, 1975). Betaine, which can also function as a compatible solute in Procaryotes (GalinisKi and Triiper, 1982) was not detected in cell extract.

Gram positive bacteria was found to accumulate the neutral amino acids proline (Brown, 1978 and Tempest and Meers, 1970) and gamma amino butyrate, (Measures, 1975), in response to increasing salt stress, whereas gram negative bacteria accumulate glutamate (Nakamura, 1979 and Hua et al, 1982) while, streptomycetes indogenous

to saline soils were found to accumulate the neutral amino acids alanine which was not found before as a compatible solute in streptomycetes (Kelham and Firestone, 1984). Similarly experiments were done on some higher plants by Cusido et al. (1987) who found that some amino acids increased with NaCl increased in leaves and roots, of Nicotiana plants, those were aspartic, glutamic, ornithine, arginin and proline, the increase was significant especially in proline. In streptomycetes spp. glutamate was the dominant free amino acid in cells grown in the basal meduim, but it decreased with increasing salt stress. These streptomycetes therefore had several features of responses to salt stress that have not been found in other gram positive bacteria.

The reduced cell yields did not result only from the energy cost of amino acids synthesis. Apparently some of the energy cost of response to NaCl stress was specific to the Na ion.

There are numerous factors which might affect the accumulation of amino acids. The solubility of the amino acids may limit continued accumulation, although proline and alanine could have accumulated to far greater concentrations if solubility were the only factor

controlling intracellular solute accumulation. Another factor may affect accumulation of amino acids especially proline to concentration which are in some way inhibitory (Kilham and Firestone, 1984).

Generally in streptomycetes indogenous to saline soil, tolerance to high salinity is associated with intracellular accumulation of free neutral amino acids, alanine and gamma aminobutyrat. To relate this finding to the growth and survival of actinomycetes in saline environment, the energy costs of osmoregulatory solute control must be quantified under the conditions of energy availability characteristic of soil.

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Table (2): Influence of medium Nacl concentrations on the intracellular free amino acid composition of Streptomyces no.1.

	Free amino acids	Amino acid concn(Umol/g.dry wt.)with NaClconcn(%)			
		0	0.25	0.50	1.0
1 -	Glycine	16.013	17.856	3.323	2.707
2 -	Alanine	21.723	7.072	3.448	1.964
3 -	Valine	25.880	6.780	5.827	7.0
4 -	Leucine and Isoleucine	30.654	10.234	9.703	7.571
5 -	Tyrosine	7.550	2.599	3.494	4.139
6 -	Phenylalanine	6.903	2.236	1.888	1.734
7 -	Histidine	4.906	1.082	0.608	1.0
8 -	Aspartic acid	22.409	5.283	-	-
9 -	Glutamic acid	34.679	10.764	15.343	21.234
10-	γ-Amino-n-butyric acid	4.017	1.404	1.162	2.881
11-	Lysine	11.107	4.243	3.541	3.515
12-	Arginine	10.101	3.484	1.396	1.202
13-	Serine and Threorine	28.860	7.561	11.841	12.500
14-	Cystine	1.864	0.333	Traces	0.193
15-	Methionine	4.953	1.768	0.554	0.587
16-	Ornithine	0.702	-	0.710	-
17-	Ammonia	5.569	4.337	-	0.954
Total free amino acids concn(Umol/gm dry wt.of cells).		237.890	86.133	62.837	69.190

Table (3): Influence of medium Nacl concentration on the intracellular free amino acid composition of Streptomyces no.2.

	Free amino acids	Amino acid concn(Umol/gm dry wt.)with Nacl concn(%)			
		0	0.25	0.50	1.0
1 -	Glycine	14.638	28.60	31.524	- [a]
2 -	Alanine	27.976	41.964	65.65	-
3 -	Valine	-	47.762	68.826	-
4 -	Leucine and Isoleucine	22.724	56.498	43.212	-
5 -	Tyrosine	4.407	12.376	12.324	-
6 -	Phenylalanine	4.121	13.416	9.079	-
7 -	Histidine	1.872	5.011	7.394	-
8 -	Aspartic acid	18.110	20.748	58.437	-
9 -	Glutamic acid	50.169	21.307	11.739	-
10-	γ-Amino-n-butyric acid	6.591	11.063	21.746	-
11-	Lysine	5.525	19.448	22.370	-
12-	Arginine	0.936	9.542	2.465	-
13-	Serine and Threonine	24.01	31.863	74.973	-
14-	Cystine	1.261	4.03	3.151	-
15-	Methionine	3.198	10.465	6.365	-
16-	Ornithine	0.624	1.664	1.870	-
17-	Ammonia	10.218	30.953	54.318	-
Total free amino acids concn (Umol/gm dry wt. of cells.		196.38	367.451	495.443	-

a- Cells did not grow.

Table(4): Percentage difference from contorl. Data were given from table 2.

	Free amino acids	o	0.25	0.50	1.0
1 -	Glycine	16.013	+11.5	-79.3	-83.1
2 -	Alanine	21.723	-67.5	-84.1	-90.9
3 -	Valine	25.880	-73.8	-77.5	-72.9
4 -	Leucine and Isoleucine	30.654	-66.6	-68.3	-75.3
5 -	Tyrosine	7.550	-65.6	-53.7	-45.2
6 -	Phenylalanine	6.903	-67.6	-72.6	-74.9
7 -	Histidine	4.906	-77.9	-87.6	-79.6
8 -	Aspartic acid	22.409	-76.4	-	-
9 -	Glutamic acid	34.679	-68.9	-55.8	-38.8
10-	δ-Amino-n-butyric acid	4.017	-65.1	-71.1	-28.3
11-	Lysine	11.107	-61.8	-68.1	-68.4
12-	Arginine	10.101	-65.5	-86.2	-88.1
13-	Serine and Threonine	28.860	-73.8	-58.9	-56.6
14-	Cystine	1.864	-82.1	traces	-89.6
15-	Methionine	4.953	-64.3	-88.8	-88.1
16-	Ornithine	0.702	-	+ 1.2	-
17-	Ammonia	5.565	-22.1	-	-82.8
		237.890	-63.8	-73.6	-70.9

Table (5): Percentage difference from control. Data were given from table 3.

	Free amino acids				
		o	0.25	0.50	1.0
1 -	Glycine	14.638	+95.4	+115.4	-[a]
2 -	Alanine	27.976	+50.0	+134.7	-
3 -	Valine	-	—	—	-
4 -	Leucine and Isoleucine	22.724	+148.6	+90.2	-
5 -	Tyrosine	4.409	+180.8	+179.6	-
6 -	Phenylalanine	4.121	+255.6	+120.3	-
7 -	Histidine	1.872	+210.4	+295.0	-
8 -	Aspartic acid	18.110	+14.5	+222.5	-
9 -	Glutamic acid	50.169	-57.5	-76.6	-
10-	γ-Amino-n-butyric acid	6.591	+67.9	+230.0	-
11-	Lysine	5.525	+252.0	+304.9	-
12-	Arginine	0.936	+919.4	+163.4	-
13-	Serine and Threonine	24.01	+32.7	+212.3	-
14-	Cystine	1.261	+219.6	+149.9	-
15-	Methionine	3.198	+227.2	+99.0	-
16-	Ornithine	0.624	+166.7	+199.7	-
17-	Ammonia	10.218	+202.9	+431.6	-
		157.950	+147.7	+223.0	-

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SENSITIVITY OF ANABAENA ARYZAE AND TOLYPOTHRIX TENUIS (CYANOBACTERIA) TO POLYTRIN INSECTICIDE

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Summary

The effect of increasing concentration of the insecticide Polytrin (0.063 ------1.5 recommended field rate) on the growth of cyanobacteria Anabaena oryzae and Tolypothrix tenuis under asceptic conditions was studied. Percentage dry weight compared to control, total carbohydrates and intracellular nutrient mineral content have been estimated.

addition of Polytrin to growing cultures of A.oryzae and T.Tenuis resulted in a slight reduction of growth at low concentrations, but rapid decrease in growth was observed at higher rates for both strains. The growth of Tolypothrix tenuis was inhibited completely at 1.5 field rate.

Nitogen content of both strains were gradually reduced by increasing the insecticide concentration, while carbohydrate content of algal cells increased.

The intracellular K content of A. oryzae showed slight rise at low insecticide concentrations but a marked reduction at higher concentrations while a marked reduction in K content of T.tenuis was noticed even at the least used concentration.

Slight rise in P and Mg content was observed at low rate followed by decrease at higher applcations in both strains. Na did not show wide change by the insecticide. Zn, Cu and Mn content may show some little rise with Polytrin concentration in both organisms.

Introduction

The successful use of algal inoculation as in India and Japan demonstrates that blue-green (BGA) can serve as an alternative or supplemental source of nitrogen for rice cultivation, (Agrwal, 1979; Alyer et al, 1972; Watanabe, 1967. and Roger & Kuhsooriya, 1980). Blue-green algae may give some advantages not necessarily associated with nitrogen-fixation, such as through the production of growth-promotive substances (Roger and Kulasooriya, 1980).

From the various agronomic practice adopted along the cultivation cycles which may directly influence the growth of blue green algae is the application of

pesticides. The resistance to high levels of pesticides seems to be more characterisitc of BGA than of the eukaryotic algae (Watanabe, 1967, venkataraman & Kajyalakshm, 1971, 1972;Singh, 1978). But some pestisides like chloropicrin may affect all algae without discrimination(Ishizawa and Musuguchi, 1966). Laboratory experiments showed that, metabolic products of the insecticides Aldrin, Dieldrin and endrin are inhibitory to blue-green algae. (Battino-Viterbo et al, 1973). Also, Wright et al, (1977) showed that, 5 ppm of the herbicide propanil prevented the growth of Anabaena cylindrica, Tolypothrix tenuis and Nostoc endophytum in flask cultures.

Although a number of studies have investigated the growth responses of cyanobacteria to pesticides, available literature shows that, there are only few attempts to define systems for the study of the effect of pesticide on the intracellular mineral constituents of cyanobacteria.

The purpose, of the present study was to assess the changes in growth, intracellular carbohydrates, macro-and microelement of the cyanobacteria Anabaena oryzae and Tolypothrix tenuis when subjected to different concentrations of the foliar pyrethroid insecticide, Polytrin, in batch culture grown photoautotrophically in continuous illumination.

Material and Methods

Cyanobacteria:

Anabaena aryzae, Egyptian strain and Tolypothrix tenuis, Japanese strain were provided from the Agriculture Research Centre Giza, Egypt.

Insecticide:

Plytrin, common same Cypermethrin CGA 55' 186, 200 Emulsifiable concentrate (200 EC) was provided from Ciba-Geigy Limited, Cairo, Egypt. It is a foliar pyrethroid insecticide, its imperical formula is $C_{22}H_{19}Cl_2NO_3$.

Experimental procedure:

Cyanobacteria were grown in batch cultures under sterile conditions. Inocula were grown to mid-exponential phase. Aliquots of (5 ml) cell suspensions were inoculated into 500 ml Erlenmeyer flasks containing 200 ml of (Watanabe et al, 1959) medium. Cultures were grown under conditions of continuous illumination at an incident light intensity of 20 W/m^2 provided by white fluoresent lamps. The insecticide Polytrin was added at time-zero to cyanobacterial cultures, the concentrations used were 0.063(1/16), 0.125 (1/8), 0.25 (1/4) 0.5(1/2), 1.0 and 1.5 recommended field rate (0.5 ml 200 EC/L). Experiments were repeated at least twice.

Cells were harvested by centrifugation at the end of the exponential growth phase, the harvested cells were washed twice with distilled water.
Growth was determined in terms of (mg dry wt/100 ml culture) following drying for 24 hours at 70 °C.

Analytical methods:

Total carbohydrates were estimated by the phenol sulphuric acid reagent and calibrated against glucose standard (Dubois et al, 1956).
Intracellular ion concentrations were analyzed from the dried algal cells which were powedered and digested in nitric-perchloric-sulphuric acid. Phosphorus was analyzed using the vanadatomolybdate colorimetric method, (Chapman and Prott, 1978).

Mg, Fe, Mn, Zn, and Cu were determined by atomic absorption spectrophotometry. K and Na were estimated by flame photometry (Jackson, 1967).
Nitrogen was measured by kjeldahl method (Jackson, 1967).

Results

Effect of Polytrin on the growth of algae:

Growth of Anabaena oryzae and Tolypothrix tenuis in the presence of different insecticide concentrations in the medium (Table 1) showed that, growth was slightly

reduced at low insecticide concentratoins (0.063 & 0.125 of the recommended field rate) 80.4% - 72.2% of control culture, but rapid decrease in growth was observed at higher concentrations. Minimal growth of 20.3% and 28.5% in dry weight was obtained at 1.5 and 1.0 field rate for A.oryzae, while the corresponding values for T.tenuis amounted only to 0% and 9.5%. (Table 1). The insecticide caused also yellowing of some Anabaena cells after growth at 1.5 field rate.

Effect of Polytrin on cell composition of carbohydrates and nutrient elements:

Polytrin had a detectable effect on the growth of A.oryzae and T. tenuis at concentrations greater than 0.25 field rate. Analysis of organisms exposed to all used concentration of insecticide (Tables 2,3 & 4) revealed marked differences in the content of carbohydrate, macro and micro-elements as compared to untreated organisms in the exponential phase of growth.

Table 2. shows that Polytrin caused inhibition of nitrogen fixation. The nitrogen content of both strains were gradually reduced by concentration and amounted to 56.7% and 0% of the control for A. oryzae and T. tenuis respectively at 1.5 recommended field rate.

Total carbohydrate content of algal cells increased with increasing the insecticide concentrations (Table2) reaching about twice the control value at 1.5 field rate in A.oryzae, while growth of T.tenuis was inhibited completely at that concentration.

Analysis of the intracellular macroelements of A.oryzae (Table 3) showed a slight rise in K content at low concentration and a marked reduction at higher concentrations from 0.25 recommended field rate upwards, while the marked reduction in K content with T.tenuis began from the least concentration 0.063 field rate.

Slight rise in P and Mg content was observed at low concentration followed by decrease at higher application in both strains.
Na did not show clear dependence on the insecticide concentration. Zn, Cu and Mn content showed some rise with Polytrin concentrations in both studied organisms.

Discussion

A comparison of the effect of used concentrations of Polytrin on cell growth and adaptation in A.oryzae and T.tenuis reveals that Anabaena oryzae is more tolerant to the used concentrations than Tolypothrix tenuis. Inhibition was observed at concentrations greater than

0.25 field rate. This result is similar to some previous observations on ther pesticides. Wright et al, (1977) showed that 5 ppm. of the herbicide propanil prevented the growth of Anabaena cylindrica, Tolypothrix tenuis and Nostoc endophytum in flask cultures. Venkatoraman & Rajyalakshmi (1972) observed that, among 10 Anabaena strains tested for their resistance to Ceresan, 9 could tolerate 100 ppm but 1 was inhibited by concentrations higher than 1 ppm. Cylindrospermum sp. was observed to be less resistant to insecticides than aulosira fertillissima and Plectonema boryanum Singh, (1973).

Under Egyptian conditions (El-Nawawy et al, 1962; Hamdi et al, 1970; Ibrahim, 1972, and Shalan et al, 1984) showed that most Anabaena strains and Tolypothrix tenuis were inhibited by high concentrations of various pesticides not including Polytrin.

The diminution of nitrogen content of A.oryzae and T.tenuis with Polytrin indicates a decrease in nitrogenase activity of these organisms. This loss of activity could reflect a high turn over for some protein (s) involved in N_2 fixation (Romos et al, 1985). Pesticides generally appear to limit N_2-fixing capacities of blue-green algae, thereby affecting the overall nitrogen economy of soils (DA Silva et al, 1975; Eid et al, 1962;

El-Nawawy and Hamdi, 1975; Huang, 1978; Ibrahim, 1972 and Inger, 1970).

Total carbohydrate content of A.oryzae and T.tenuis rose with increasing Polytrin concentration in the growth media. This means that, the cells did not probably completely lose the capacity for photosynthesis in the presence of the insecticide. Hamdi et al (1970),stated that, chlorophyll synthesis was stimulated by low levels of some herbicides. Increased levels of carbohydrates were typically found in nitrogen starved cyanobacteria (Allen & Smith, 1969 and Romos, 1985). Accumulation of carbohydrates could somehow counteract the negative effect of the active nitrogenous metabolites, as it seems to be the case for the control of nitrate utilization in the unicellular cyanobacterium Anacysts (Flores et al, 1983; Espin et al, 1982; Merrick, 1982). El-Mokadem et al (1988) in a previous work have found more accumulation of carbohydrate ossociated with loss of nitrogen content in Anabaena oryzae grown under more saline conditions.

The elemental analysis of the dried cells revealed that, the algae exhibited changes in intracullular elements with increasing the insecticide. Lyzell et al, (1985), had stated that N:P ratio affects the growth rate of cyanobacteria.

It is recommended that for application of Polytrin insecticide in near by cotton or other crop fields, the rate would be adjusted in a manner not to harm the advantageous cyanobacteria added to rice as biofertilizer for nitrogen fixation.

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Table 1:Growth of Anabaena oryzae and Tolypothrix tenuis cultures at different concentrations of Polytrin insectcide.

Polytrin Concentration	Anabaena oryzae		Tolypothrix tenuis	
	mg.drywt 100/ml.cul.	Growth percent	mg dry.wt/100ml. culture	Growth percent
Control	158 ± 19	100%	138 ± 17	100%
0.063 R.f.r.	127 ± 14	80.4%	109 ± 8	79%
0.0125 " "	114 ± 18	72.2%	78 ± 9	56.6%
0.25 " "	88 ± 10	55.7%	57 ± 4	41.3%
0.5 " "	74 ± 9	41.5%	40 ± 7	29%
1.0 " "	45 ± 7	28.5%	13 ± 3	9.5%
1.5 " "	32 ± 5	20.3%	Dead	0

Growth percent: calculated with reference to control growth as 100%; values based on mg. dry wt./100 ml culture the values are the mean of three independent observations.

R.f.r. : Recommended field rate.

Table 2: Effect of Polytrin insecticide on the intracullular carbohydrate and nitrogen content of Anabaena oryzae and Tolypothrix tenuis. (Cultures grown with and without Polytrin untill rate logarthmic phase).

Polytrin concentration	Anabaena oryzae		Tolypothrix tenuis	
	Carbohydrates	Total nitrogen	Carbohydrates	Total nitrogen
Control	13.4	5.82	21.2	7.38
0.063 R.f.r	17.8	5.31	26.3	6.39
0.125 " "	15.3	5.3	28.3	5.42
0.25 " "	19.5	5.3	28.1	5.28
0.5 " "	22.7	4.91	30.4	5.52
1.0 " "	25.0	4.41	29.8	4.16
1.5 " "	26.3	3.35	---	---

R.f.r Recommended field rate

Table 3: Effect of Polytrin insecticide on the intracellular macro & micro elements of Anabaena oryzae.

Treatment	P	K	Na	Mg	Fe	Mn	Zn	Cu
	mg./g .dry weight				µg./g. dry weight			
Control	11.6	14.8	0.14	6.9	192.2	83.95	82.0	24.0
0.063 R.f.r	13.9	17.7	0.14	7.5	128.1	62.1	57.6	25.3
0.125 " "	12.6	16.5	0.28	7.2	138.1	73.0	57.0	20.0
0.25 " "	7.2	0.7	0.14	5.2	73.0	87.9	161.0	32.0
0.5 " "	6.6	0.8	0.14	3.8	64.4	120.5	116.2	34.7
1.0 " "	5.5	0.1	0.14	4.0	61.9	151.5	140.6	61.3
1½ " "	4.9	0.2	0.24	4.2	48.3	93.5	116.0	49.3

R.f.r : Recommended field rate.

Table 4: Effect of Polytrin insecticide on the intracellular macro and micro elements of Tolypothrix tenuis

Treatment	P	K	Na	Mg	Fe	Mn	Zn	Cu
	mg./g .dry wt.				µg./g. dry wt.			
Control	11.1	3.5	0.21	4.5	222.	58.4	51	50
0.063 R.f.r	14.7	0.4	0.28	5.8	291.	98.1	131	62
0.125 " "	14.2	0.5	0.56	6.4	277	98.1	127	64
0.250 " "	4.7	0.5	0.56	1.9	96.1	65.4	117	50
0.500 " "	5.1	0.3	0.38	2.1	117	62.5	164	80
1.00 " "	N. D.					N. D.		
1.5 " "	--	--	--	--	--	--	--	--

R.F. r: Recommended field rate

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Cytological Effects of Water Extracts of Medicinal Plants in Egypt.

Mitotic Disturbances Induced by Water Extract of Cymbopogon proximus (Halfa barr) on Vicia faba.

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Introduction

Because of the ever increasing use of water extracts of medicinal plants in curing diseases instead of the synthetic drugs, care must be taken to avoid harmful effect which may arise from wrong or extra usage. The cytological effects of the crude water extracts of medicinal plants may cause undesired heritable changes. Some cytologists tried to investigate the response of dividing cells to plant extracts, among them Keck and Hoffmann (1951), Kato (1957), Tarkowska (1971), Shehab and Adam (1981,1983), Adam and Rashad (1984, 1985) and Adam and El-Nahas (1988).

In this survey, the mitotic effect of Cymbopogon proximus extract was studied on roots of Vicia faba. Cymbopogon proximus (Halfa barr), is a perennial aromatic grass belonging to family

gramineae. It is widely grown in upper Egypt (Täckholm 1974). The grass extract is used by the inhabitants as carminative, diuretic and urinary antiseptic.

Materials and Methods

The plant extract was prepared by boiling 1,2 and 3 gm of Cymbopogon proximus in 100 ml tap water for 10 minutes. The evaporated water was replaced by tap water to original volume (100 ml). The extract was decanted while hot.

Vicia faba seeds, v. Giza 2, were sawn in saw dust. When the roots were 1.5 - 3 cm in length, they were treated with the different concentrations of the extract (1,2 and 3 %), for 4 hours. Tap water was used for the control experiment.

Another group of Vicia faba roots were immersed in 1 % extract for 24 and 48 hours. The 1 % concentration is commonly used for preparation of Cymbopogon proximus beverage (tea spoonful/200 ml water).

Three replicates, three roots/each were used for each treatment. After treatments, the roots were cut and fixed in Carnoy's fixative. Observations were made from permanent leuco basic fuchsin stained preparations. Mitotic index (MI)

was calculated as the number of dividing cells/1000 counted cells. The data were analyzed according to the t-test.

Results and Discussion

The data scored in Table (1) showed that all treatments affected MI negatively, the decline was not significant in short hours of treatment (4 hours). The effect was about significance in roots treated for 24 hours and highly significant after 48 hours treatment with 1 % extract. Other plant extracts proved to be mitodepressive such as water extracts of : Sonchus, Chenopodium, Crisum and Medicago (Bukolova and Stepanova. 1972), Lupinus termis extract (Shehab and Adam. 1981) and Datura innoxia and Hyoscyamus muticus extract (Shehab et al. 1983).

The results show that the reduced mitotic activity may be ascribed to partial blockage of DNA synthesis, thus minimizing the number of cells entering mitosis rather than hindering spindle formation. Table (1), showed also that analysis of frequency of mitotic phases, points to a preprophasic action. It manifested itself in the decrease of the number of nuclei entering prophase. This effect leads to increase in percentage of metaphases and anatelophases over those of the control in some treatments.Kubink (1966), ascribed the increased percentages of phases to be due to, prolonged duration of it.

Table (2) indicates that, all treatments with the extract induced significant percentages of abnormal dividing cells, its values increased with increase of concentration and time of treatments. The maximum percentage of abnormal dividing scored was 36.71 % after 48 hour treatment with 1 % extract, and the least was 13.89 % after 4 hour treatment with 1 % extract. It was also obvious that, metaphase stage was the most effected and the least was prophase stage.

These results led us to opine that, Cymbopogon proximus extract has an accumulative effect in induction of chromosomal abnormalities. From Table (3) it is evident that, the most frequent types of irregularities, occured in the form of sticky chromosomes (Fig. 1) resulting from, liquifaction of the chromatin material. This effect leads to hinderance of normal separation. A maximum value attained (72.39 %) followed 48 hour treatment with 1 % extract. Disturbancy of chromosome orientation, either in metaphase (Fig. 2) or anaphase, was also a common abnormality which may be the result of, affecting the centromere activity or the spindle fibres. Similar results were reported by Adam and Rashad (1985) treating Vicia faba root tip cells with Ammi visnaga water extract. Selim et al. (1981) refered this type of abnormality to be due to, spindle interruptions caused by treatment. Lagging chromosomes in metaphase (Fig. 3) and

anaphase (Fig. 4) and chromosome bridges were also recorded but with lower frequencies. Despiralization, diagonal orientation of chromosomes and chromosome fragmentation were scarcely scored in some treatments (Table 3).

The present results revealed that, Cymbopogon proximus extract resembles other plant extracts in induction of mitotic irregularities specially on increasing concentration and time of treatment. In this respect it resembles the so called mitotic poisons(D'Amato 1954).

Summary

The present investigation concern the influence of the water extract of Cymbopogon proximus (Hamfa barr) on the mitosis in roots of Vicia faba. All the used concentrations caused mitotic delay, the action of the long durations was significant. The extract also brought about significant percentage of abnormal dividing cells.

The effect of the extract may be determined as preprophasic. The number of prophases decreased while metaphases and anatelophases increased. The extract induced a number of chromosomal irregularities such as stickiness, disturbancy, lagging chromosomes and bridges.

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Table (1) : Mitotic index and frequency of the mitotic stages in Vicia faba roots treated with Cymbopogon proximus extract.

Treatment	Interphase cells			No. of dividing cells	MI ± S. E.	Mitotic stages		
	Total	No. abn.	% abn.			Prophase %	Metaphase %	Ana-telophase %
I. Root-treatment for 4 hrs. with :								
1. 3 % ext.	9751	521	5.31	819	77.44 ± 13.46	43.03	24.99	31.97
2. 2 % ext.	9235	122	1.31	818	81.26 ± 4.15	42.13	23.89	33.99
3. 1 % ext.	9150	56	0.61	780	78.50 ± 5.66	44.49	27.38	28.13
Control	9569	68	0.67	1024	96.60 ± 4.04	45.89	22.25	31.86
II. Root-treatment with 1 % ext. for :								
1. 24 hrs.	9000	12	0.13	532	55.80 ± 2.21	37.87	24.04	38.10
Control	9000	7	0.08	789	80.48 ± 7.60	45.81	24.43	29.75
2. 48 hrs.	9000	3	0.03	368	39.27** ± 2.83	28.77	31.14	40.09
Control	9000	2	0.02	648	67.15 ± 2.25	40.07	22.93	37.00

** Significant to control at 0.01 level of probability (t-test).

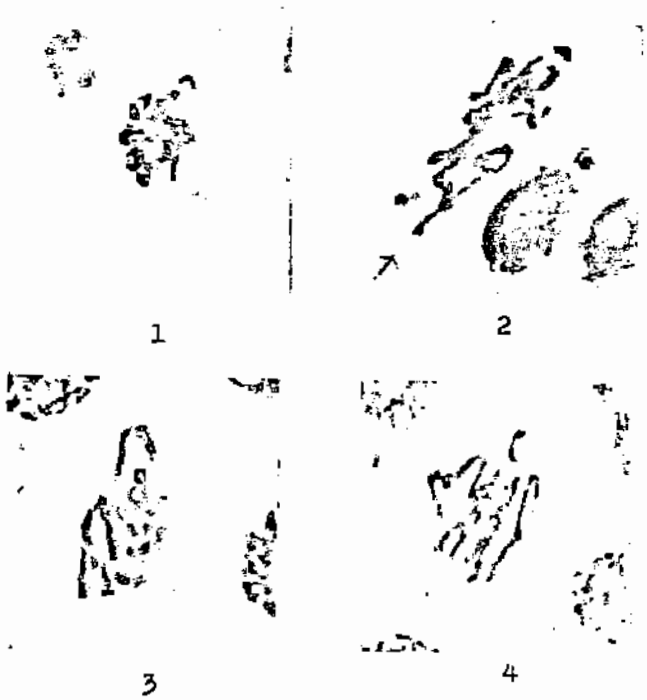
Table (2) : Total percentage of abnormalities and its distribution in the mitotic stages of Vicia faba treated with Cymbopogon proximus extract.

Treatment	Dividing cells				% of abn. in mitotic stages		
	Total	No. abn.	% abn. ± S.E.		Prophase	Metaphase	Ana-telophase
I. Root-treatment for 4 hrs. with :							
1. 3 % ext.	819	156	20.27* ± 4.78		1.62	33.38	33.86
2. 2 % ext.	818	151	18.70** ± 1.93		0.55	32.58	31.42
3. 1 % ext.	780	108	13.89** ± 1.70		0.55	27.80	21.22
Control	1024	17	1.67 ± 0.23		0.18	3.58	2.78
II. Root-treatment with 1 % ext. for :							
1. 24 hrs.	532	162	30.62** ± 2.20		16.24	48.68	33.60
Control	789	36	4.61 ± 0.50		1.43	7.45	8.06
2. 48 hrs.	368	134	36.71** ± 2.48		21.32	69.96	23.01
Control	648	31	4.84 ± 2.00		4.56	4.01	5.69

* Significant to control at 0.05 level of probability (t-test).
** Significant to control at 0.01 level of probability (t-test).

Table (3) : Percentage of the different types of abnormalities relative to the number of abnormal dividing cells in Vicia faba after treatment with Cymbopogon proximus extract.

Treatment	No. of abn. dividing cells	Stick. %	Dist. %	Lag. %	Brid. %	Despiral. %	Diagonal anaph. %	Frag. %
I. Root-treatment for 4 hrs. with :								
1. 3 % ext.	156	69.23	16.03	7.05	7.05	-	-	0.64
2. 2 % ext.	151	29.80	45.03	13.25	9.93	-	-	1.99
3. 1 % ext.	108	57.41	25.93	10.19	6.48	-	-	-
II. Root-treatment with 1 % ext. for :								
1. 24 hrs.	162	54.32	23.46	2.47	3.70	16.10	-	-
2. 48 hrs.	134	72.39	15.67	0.75	0.75	3.73	6.72	-



Figs. 1-4 : Sticky metaphase (1), disturbed metaphase (2) and lagging chromosome in metaphase (3) and anaphase(4) after :

1. Root treatment for 4 hours with :
   a) 1 % extract (Fig. 4).
   b) 2 % extract (Figs. 2,3).
2. Root treatment for 48 hours with 1 % extract (Fig. 1).

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Growth and Development of Beet Seedlings Cultured in Media of Different Levels of N and P Ions.

-19-

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Abstract: Studies were made to elucidate the effect of varying N/P ratios in the culture media on growth, uptake and accumulation of nutrient ions, and assimilation of nitrate. Increasing nitrate or phosphate ion concentration and keeping the concentration of other ions constant in the culture media, resulted in increased growth accompanied by increased uptake and accumulation of nutrient ions in the seedlings except Ca and NO_3 ions. The assimilation of nitrate ions also increased.

The stimulation of all these processes were more enhanced with nitrate than with phosphate concentrations although the concentration of PO_4 ion was much higher than that of NO_3 ion in the culture media.

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Introduction

The effect of different concentrations of N and P in the nutritive media on growth and metabolism of several plants have been studied by many workers. Thus Cole et al.(1963), studying the effects of N on P uptake and metabolism of corn seedlings, suggested a connection between P uptake and N metbolism, and they were of the opinion that the increase in P uptake rates may reflect higher levels on N-intermediates. Nosseir and Spiridinov (1965) found that the progressive increase of N over P concentration in the nutritive media of Phaseolus seedlings stimulated the uptake of P_{32} as well as N-metabolism of seedlings. Also Nosseir and Hathout (1970) found that increases in N concentration in the culture media of sweet potato tuber discs stimulated the rate of P uptake. Dumbroff and Michel (1967) found that increasing of P in the nutritive media of pine seedlings stimulated the uptake of N and other ions with simultaneous increases in growth and development and that such increases were dependent on the increases of N and P levels in the nutritive media.

Truog et al. (1947) and Nosseir (1972) found a positive correlation between P and Mg contents of pea seedlings under different nutritional conditions, thus

supporting the theory that Mg serves as carrier of P within the plant. In contrast, Dumbroff and Michel (1967) found no relationship between Mg and P uptake and accumulation in pine seedlings.

The purpose of the present study was to determine the effect of different levels of N and P in the nutritive media on some physiological activities, viz., growth, uptake and accumulation of nutrient elements.

Materials and Methods

Seeds of beet (Beta vulgaris cultivar "Bettrave") were provided by the Ministry of Agriculture, Egypt. Seven lots of beet seed baches were used, each of which were washed in running water for 8 hr, rinsed in 70% ethyl alcohol for surface sterilization (Hatata et al. 1979) washed several times with sterile distilled water and then distributed in large sterilised Petri-dish containing two filter papers pre-moistened with 20 ml sterile distilled water. The dishes were transferred to a lightened, thermostatically controlled incubator at a constant temperature (25°C) and light intensity (6000 Lux) (Nosseir, 1968). At the 10th day of the commencement of the experiment 12 samples each composed of 50 beet seedlings were transferred to 12 sterilised

glass vessels each fitted with sintered glass bubbler and containing 500 ml water or different culture solutions according to the follwing scheme.

a) Normal Hoagland designated as $N_{15}P_1$ solution.

b) Normal Hoagland + 0.002 M $NaNO_3$ designated as $N_{17}P_1$ solution.

c) Normal Hoagland + 0.005 M $NaNO_3$ designated as $N_{20}P_1$ solution.

d) Normal Hoagland + 0.002 M NaH_2PO_4 designated as $N_{15}P_3$ solution.

e) Normal Hoagland + 0.004 M NaH_2PO_4 designated as $N_{15}P_5$ solution.

Samples were distributed in their cultured media as follows:

Sample No	Treatment
1 : 2	500 ml of distilled water
3 : 4	500 ml of $N_{15}P_1$ solution
5 : 6	500 ml of $N_{17}P_1$ solution
7 : 8	500 ml of $N_{20}P_1$ solution
9 : 10	500 ml of $N_{15}P_3$ solution
11 : 12	500 ml of $N_{15}P_5$ solution

At the end of the experiment which lasted 7 days, the seedlings of each sample were taken out, washed with distilled water, dried gently with blotting paper then laid on squared paper for estimation of their linear growth. The samples were then air dried and weighed. After that, the samples were ground to a fine powder from which weights were taken and used to estimate their nutrient elements. Also the media were sampled at the end of the experiment and used for the analysis of their mineral composition. Phosphate ion was determined calorimetrically using molybdinum reagent together with $SnCl_2$ solution (Nosseir, 1968). For nitrate determination a known weight of the powdered dry matter (100 mgm) is dissolved in 20 ml of 2% acetic acid solution. The mixture is then filtered and the filtrate is completed to 40 ml from which samples were determined calorimetrically using disulphophenolic acid (Peterburgski, 1954).

Potassium ion was determined by means of the flame photometer.

Ca and Mg were determined by titration against trilon according to the method adopted by Verugina (1956).

Results

Growth and development of beet seedlings aerated in culture media having different levels of NO_3 and PO_4 ions.

The total dry weight and the mean length of 50 beet seedlings cultured for 7 days in distilled water or Hoagland solution alone and in combination with different NO_3 and PO_4 levels are shown in Fig.(1). It is clear from this figure that beet seedlings cultured in Hoagland solution alone and in combination with different levels of NO_3 or PO_4, ions have aquired much greater dry weights or linear growth rates over those of control seedlings and that the increase due to the presence of excess NO_3 ions was much higher than that due to the presence of excess PO_4 ions in the external culture media. In seedlings cultured in control solution ($N_{15}P_1$) the total dry weight recorded for 50 beet seedlings was 288.7 mgm as a mean value of 4 replicate samples while the mean length of these 50 beet seedlings was 5.8 cm. Increasing the concentration of nitrate ions in the culture media by 13% and 26% and keeping the concentrations of all other ions caused increased of 28.4% and 35.2% in the total dry weight of the seedlings respectively over that of control seedlings and this was accompained by highly significant increases

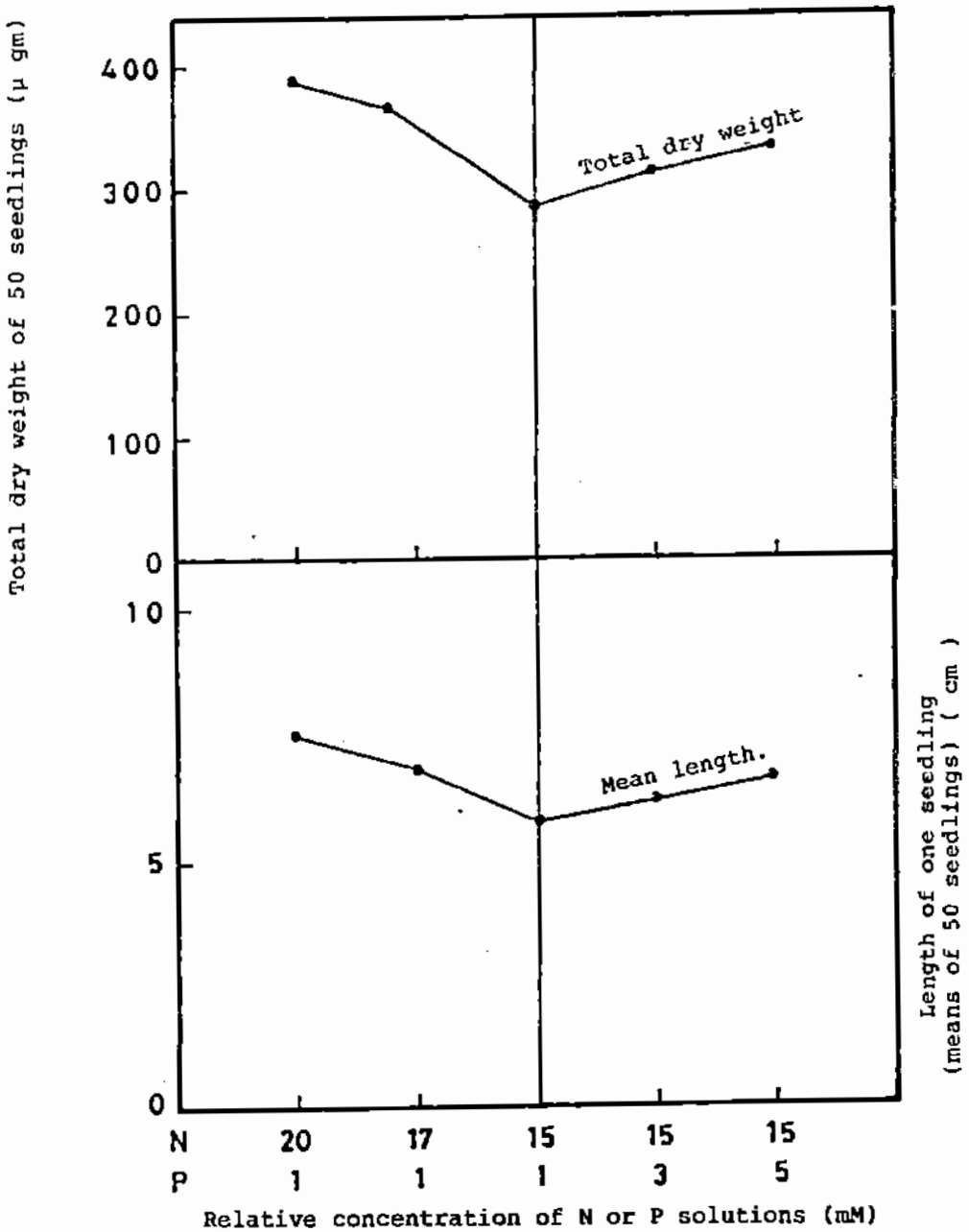

Fig.(1): Total dry weight (in mgms) and mean length (in cm) of 50 beet seedlings cultured for 7 days in 500 ml distilled water or Hoagland solution alone and in combination with different No_3 & PO_4 levels.

of 19.0% and 29.3% in the linear growth of the seedlings. Also increasing the concentration of phosphate ions in the culture media by 200% and 400% and keeping the concentration of all other ions remaining constant, caused increases of 7.5% and 17.9% in the total dry weight of the seedlings respectively over that of control samples and this was accompanied by significant increases of 6.9% and 13.8% in the linear growth of the seedlings.

Thus with both nitrate and phosphate increments in the culture media, the total growth rates of beet seedlings were increased. Such increases were enhanced by further increase in concentration of either of these ions in the nutritive media and that the increases due to increments of nitrate ions were more pronounced than those due to PO_4 ion increments in spite of the presence of much higher concentrations of the latter ions over those of the former ions in the culture media.

Uptake and accumulation of nutrient elements and assimilation of nitrate as affected by different levels of NO_3 and PO_4 ions in the nutritive media.

Fig. (2) shows the relation between the relative concentration of NO_3 or PO_4 ions in the culture media and the element uptake per total dry weight of 50 beet

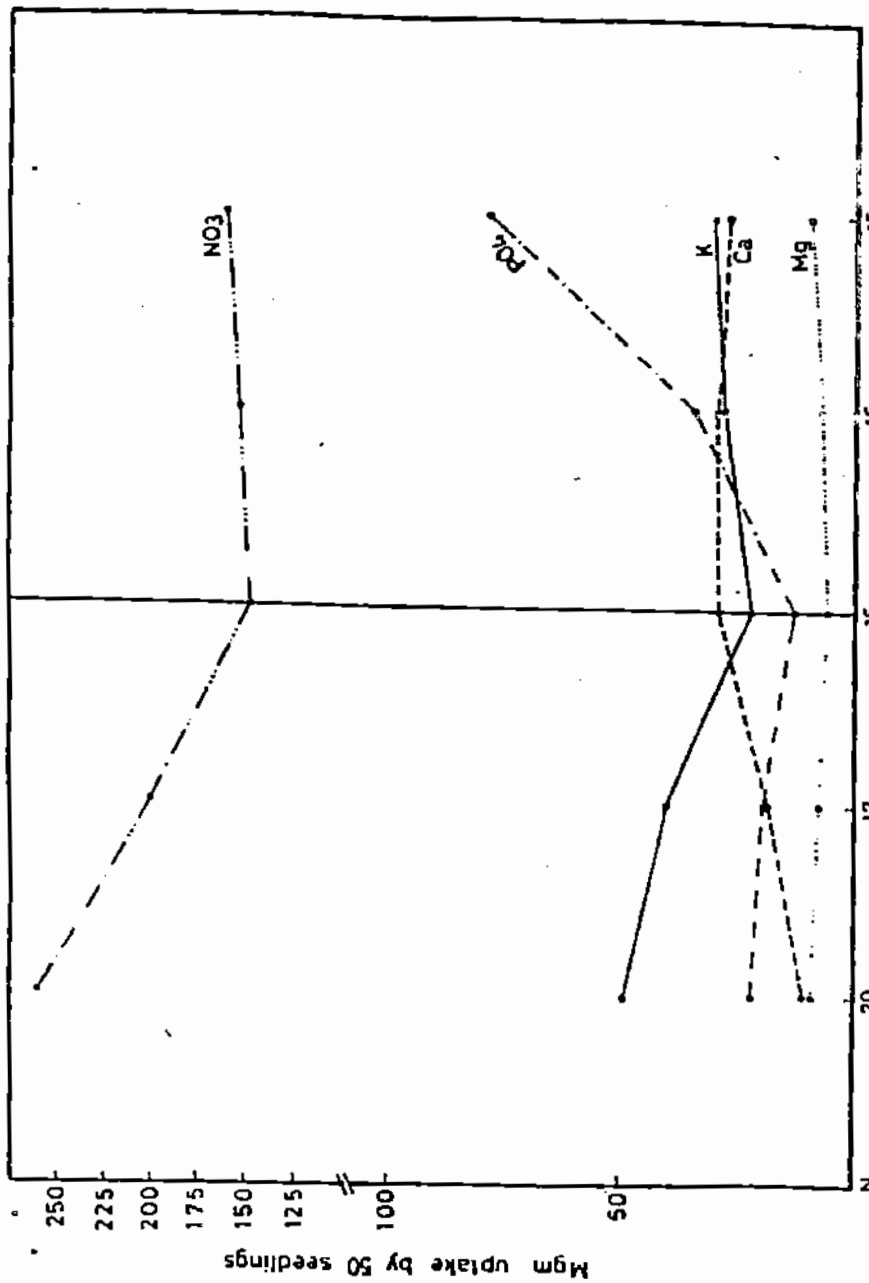

Fig. (2) Uptake of nutrient elements by 50 beet seedlings cultured for 7 days in 500 ml. of Hoagland solution alone and in combination with different nitrate and phosphate levels.

seedlings. It is clear that increasing the concentration of NO_3 ions in the nutritive media by 13% and keeping the concentration of all other ions remaining constant, resulted in increases in uptake of K, Mg, PO_4 and NO_3 ions by 82.8% 34.4%, 39.9% and 34.7% respectively over those of control seedlings cultured in $N_{15}P_1$ solution with a simultaneous reduction in Ca uptake by 37.4% below that of controls. By further increase in NO_3 ion concentration in the culture media by 26%, further increase in uptakes of K, Mg, PO_4 and NO_3 ions reaching 126.7%, 54.1% 62.5% and 75.6% respectively over those of controls were obtained with simultaneous further reduction in Ca uptake by 63.6% below that of control seedlings. On the other hand, increasing the concentration of phosphate ions in the nutritive media by 200% resulted in increases in uptakes of K, Mg, PO_4 and NO_3 ions by 26.7%, 27.8%, 153.7% and 3.4% respectively over those of control in $N_{15}P_1$ solution with a simultaneous reduction in Ca uptake by 3% below that of controls. By further increase in PO_4 ion concentration in the culture media by 400% further increases in uptakes or K, Mg, PO_4 and NO_3 ions reaching amounts of 36.2%, 28.6%, 160.8% nd 8.3% respectively over those of control in $N_{15}P_1$, solution with a simultaneous further reduction in Ca uptake by 7.8% below that of controls were obtained. From the above results, it can be concluded that the

increases in uptake of nutrient elements and the simultaneous decrease in Ca uptake were proportional to the concentration of either nitrate or phosphate ions in the culture media and that the increases due to presence of NO_3 ions were greater than those due to the presence of PO_4 ions in spite of the much higher concentration of the latter over those of the former ions in the culture media.

As regards the interrelationship among nutrient ions as being occurred in beet seedlings consequent to their feeding with culture media having different concentrations of NO_3 and PO_4 ions and hence different N/P rations, Fig.(3) was designated to show the mineral composition and changes in mgms per total dry weight of 50 seedlings as compared with controls cultured in $N_{15}P_1$ solution. From Fig. (3), it is clear that increasing nitrate or phosphate ion concentration in the culture media, the accumulation resulting from absorption of all nutrient elements by beet seedlings were increased except Ca and nitrate ions which were decreased. Such changes were shown to be proportional to the concentration of NO_3 and PO_4 ions in the culture media. Fig. (4) represents the relation between N/P ratios in beet seedlings and the same ratio in the corresponding nutritive media. It is clear that N/P ratio found in control seedlings was 9.9 corresponding to ratio of

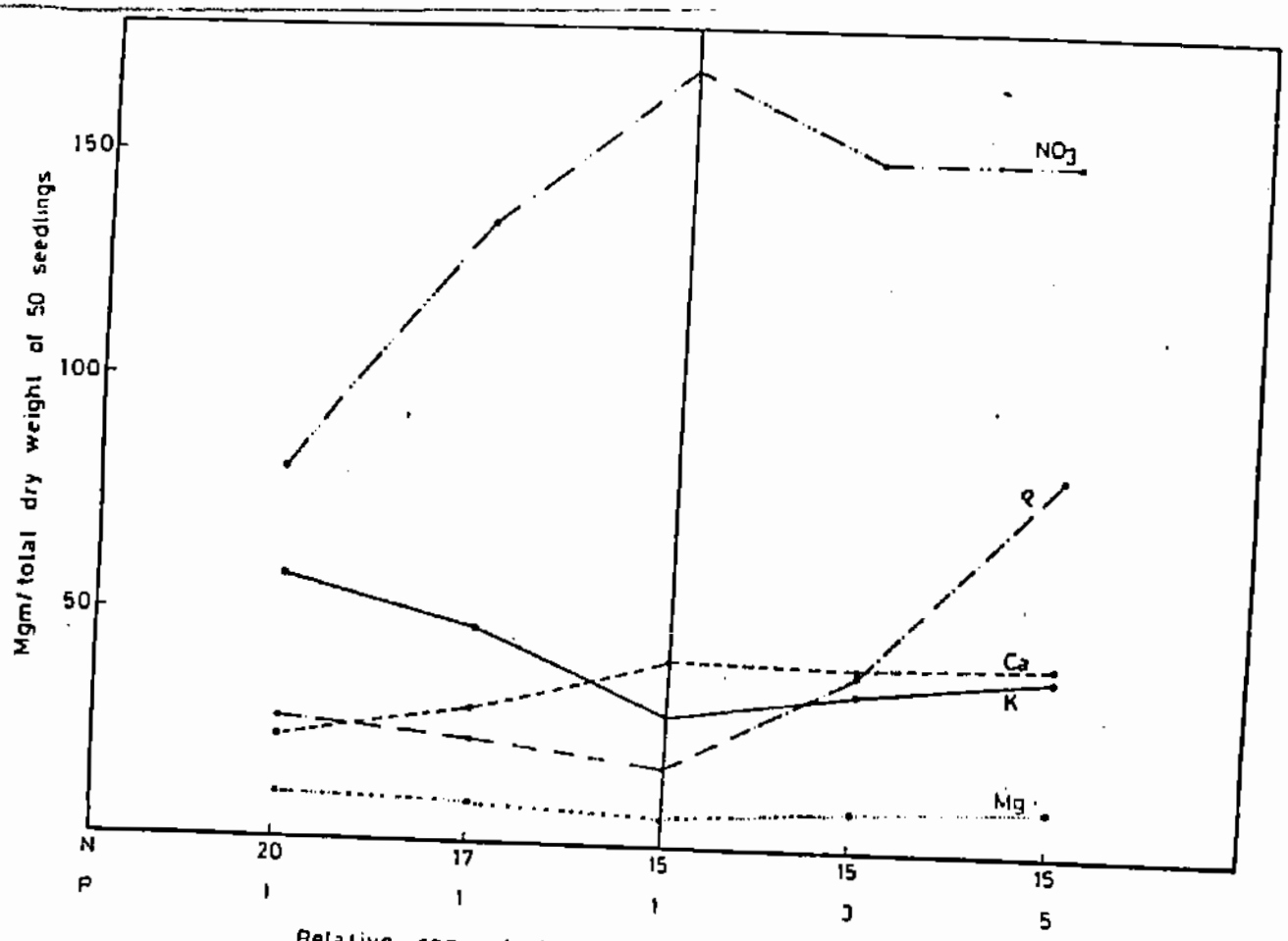

Fig. (3) Mineral composition of total dry wight of 50 beet seedlings aerated for 7 days in 500 ml. of water or Hoagland solution alone and in combination with different nitrate and phosphate levels in the culture media. (Mean values of 4 replicates mgm element per total dry wt. of 50 seedlings).

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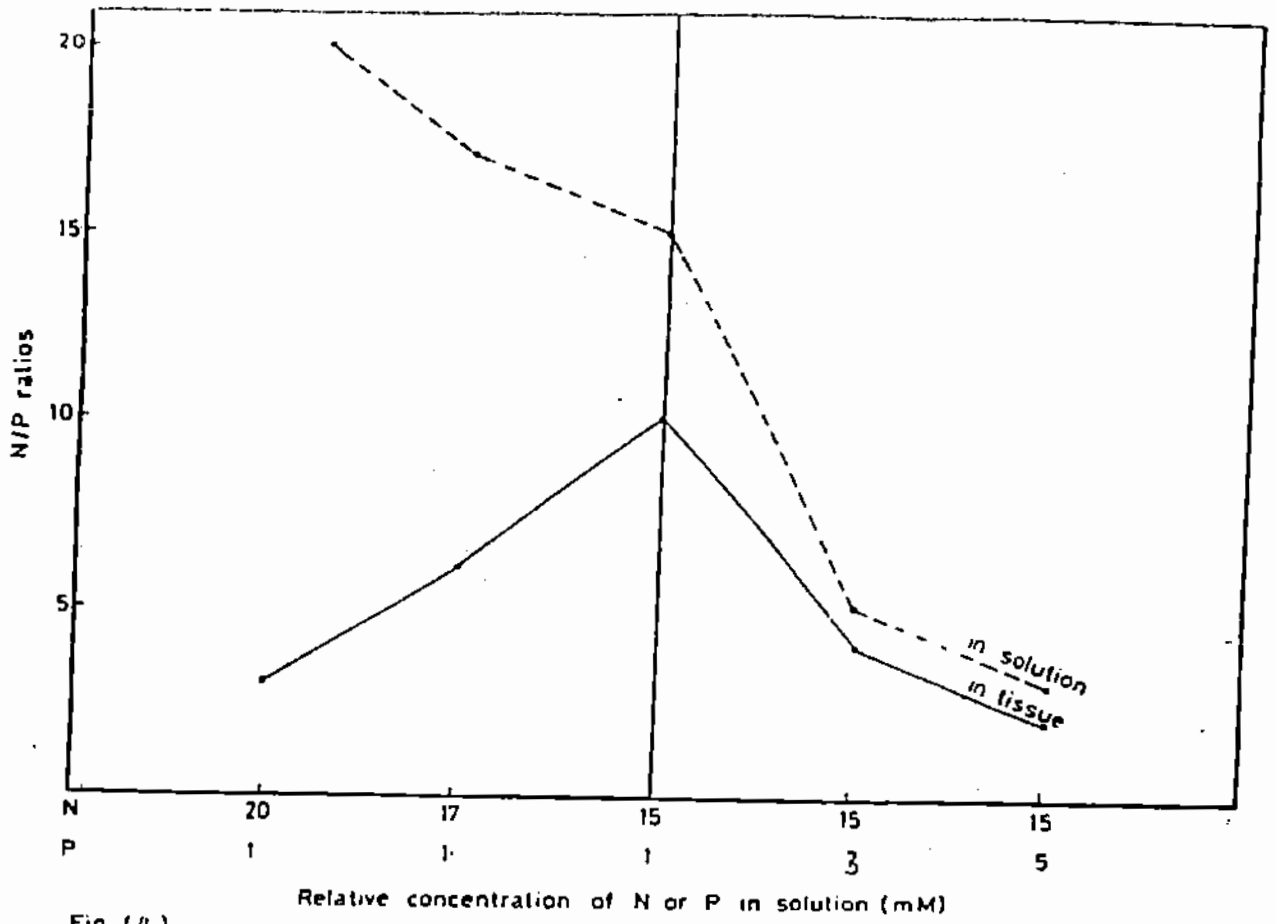

Fig (4)

Relation between N/P ratios in the culture media of 50 beet seedlings aerated for 7 days in Hoagland solution alone or in combination with different NO_3 or PO_4 levels in the culture media and corresponding ratios found in the tissues. (Means of 4 replicate samples).

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15/1 found in the respective media. Increasing the concentration of NO_3 ion in the external solution and keeping the concentration of all other ions remaining constant led to high N/P ratio in the media but to low N/P ratios in the respective beet seedlings. On the other hand increasing the PO_4 ion concentration in the culture media under similar conditions, led to low N/P ratio in the nutritive media accompanied by parallel ratios in the respective media indicating that the accumulation of NO_3 or PO_4 ions in beet seedlings was dependent on the concentration of these ions in their culture media. However, the divergent relationship between N/P ratios in beet seedlings and their respective culture media having progressive increases in NO_3 ions might be due to the variations that took place in nitrate assimilation under such nutritional conditions. The nitrate assimilated by beet seedlings cultured in media having different N/P ratios is represented by Fig. (5) in mgms per total dry weight of 50 beet seedlings. It also shows the increases in the assimilated nitrate ions due to the presence of different concentrations of nitrate or phosphate ions in the culture media. It is clear that control beet seedlings assimilated 31.9 mgms nitrate per total dry weight of 50 seedlings. Increasing the concentration of nitrate ion in the culture media by 13% and 26% and keeping the concentration of all other ions remaining constant resulted in increases

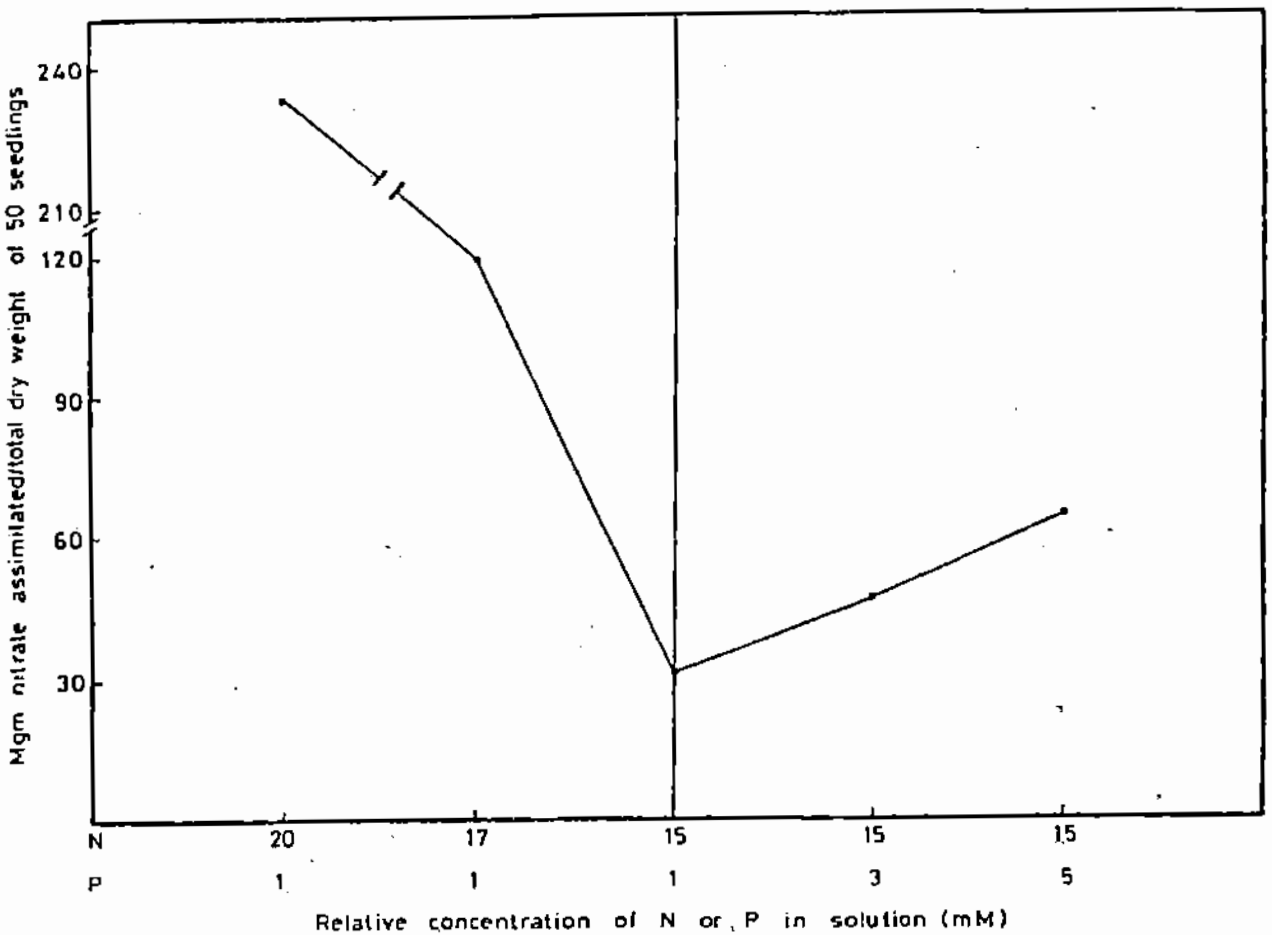

Fig. (5): Assimilation of nitrate ions and the total dry wt. of 50 beet seedlings cultured for 7 days in Hoagland solution alone and in combination with different levels of nitrate or phosphate ions. (Mean values of 4 replicates in mgm nitrate per total dry wt. of 50 seedlings).

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of 274% and 632% respectively in nitrate assimilation over that of control seedlings leading to low accumulation of nitrate ions in the tissues and hence the low N/P ratios in the tissues in spite of high N/P ratios in the culture media.

In such case, it seems that the rate of nitrate assimilation goes faster than the rate of its accumulation. Also increasing the concentration of PO_4 ions by 200% and 400%, under similar conditions, causes 78% and 99% increases in nitrate assimilations over that of control seedlings. Here the rate of nitrate assimilation goes parallel to the amount absorbed.

Discussion

Growth and development of beet seedlings aerated in culture media having different levels of PO_4 and NO_3

The close relationship that existed between total growth and nitrate or phosphate as well as other nutrient elements can be seen by comparing the total growth of the seedlings cultured in different levels of NO_3 or PO_4 with those of control seedlings cultured in normal nutrient solution ($N_{15}P_1$). For both nitrate and phosphate ions, the increases in total growth of the seedlings were stimulated by the increase of concentrations of

either of these ions in the nutritive media. Here growth was observed to be related to the uptake and accumulation of nutrient ions expect Ca and nitrate. Ca is not shown, in the present work, to be related to growth, since increases in growth rates were accompanied by low accumulation of Ca in the tissues. Also nitrate accumulation was low in spite of its high uptake from the nutrient solution. The low accumulation of nitrate ion in the tissues is actually due to its assimilation and its incorporation in the synthesis of organic nitrogenous compounds. The above results were found concordant with the results of many workers.

Cole et al.(1963) found that N or P increments in the nutritive media caused significant increases in the growth of corn seedligs suggesting a connection between N and P metabolism and that increases in P levels in the tissues may reflect higher levels of N-intermediates. Nosseir and Hathout (1970) found that increases in the N concentration of the culture media stimulated the uptake and accumulation of P in sweet-potato tuber discs. However, Dumbroff and Michel (1967) found that increments of P in the nutrient solution of pine seedlings stimulated growth and development of these seedlings with simultaneous accumulation of N and other ions especially Mg and K. Also growth of

these seedlings was dependent on the increases of N & P levels in the nutritive media.

Uptake and accumulation of nutrient elements and nitrate assimilation as affected by different levels of NO_3 and PO_4 in the nutritive media.

It is clear from Fig. (2) that increasing the concentration of NO_3 ions in the nutritive media by 13% and 33% over controls and keeping the concentration of other ions remaining constant, resulted in progressive increases in uptakes of K, PO_4, Mg and NO_3 ions, and in progressive decreases in Ca uptake. The increases in uptake of nutrient ions and simultaneous decrease in Ca uptake were proportional to the concentration of the NO_3 ions in the nutritive media. These results not only indicate that the absorption of nitrate was dependent on its concentration in the nutritive media, but also indicate the connection between N uptake and uptake of other ions.

On the other hand, increasing the concentration of PO_4 ion in the nutritive media by 200% and 400% over controls resulted in progressive increases in K, Mg, PO_4 and NO_3 uptakes and in progressive decrease in Ca uptake. All these processes were found dependent on the concentration of PO_4 ions in the nutritive media.

These results indicate the connection between PO_4 uptake and the uptake of other ions sharing in the metabolism of beet seedlings. From Fig. (3) it is clear that the accumulation of all ions was increased over controls except Ca and nitrate ions which were decreased. But the changes due to the presence of excess NO_3 ions were more pronounced than due to the presence of excess PO_4 and that these changes were proportional to the concentration of PO_4 or NO_3 ions in the nutritive media. The decrease in nitrate accumulation in spite of the associated increase in its absorption is presumably due to its assimilation and disappearance from the tissue consequent to its incorporation in the synthesis of nitrogenous compounds. These results might receive emphasis from the work of Vichery et al. (1936) who found that the NO_3-N supply to beet plants resulted in the formation of organic nitrogenous compounds mainly proteins. Also Said & El Shishiny (1949) found that Nitrate-N plays the major role in the synthesis of protein in radish root tissues. Nosseir (1972) came to the same conclusion using pea seedlings. The positive correlation between uptake and accumulation of N and those of P by beet seedlings clearly show the connection between the uptake and metabolism of N and P. Such correlation was also shown by Nossier and Spiridinov (1965) using Phaseolus seedlings, Nosseir & Hathout

(1970) using sweet potato and Nosseir (1972) using pea seedlings.

From Fig. (4) it is clear that N/P ratio found in control seedlings is 9.9 corresponding to 15/1 found in the respective culture media. Increasing the concentration of NO_3-N in the external media and keeping the concentration of the other ions remaining constant led to high N/P ratio in the media but to low N/P ratios in the respective beet seedlings. On the other hand, increasing the PO_4 ion concentration in the nutritive media under similar conditions led to low N/P ratios in the nutritive media accompanied by parallel ratios in the respective seedlings.

In this respect, beet seedlings behaved like pine seedlings since Dumbroff and Michel (1967) found parallel correlation between N/P ratios in the pine seedlings and their culture media.

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Further studies on the potency of certain digestive enzymes prepared from the white rat treated with the chemical insecticide "tamaron"

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Introduction

The present work is a part of a series of investigations which deal with the effect of the chemical insecticides on the physiology of digestion. Gabr and Said (1972, A,B,C.) on a study which concerns the effect of some chemical insecticides (DDT, lindane and malathione) on the peptic and oxyntic cells in mammals, found that these insecticides have a hazardous effect on the structure and function of these cells. In 1972, Gabr et al. studied the effect of the previously mentioned insecticides on the potency of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic lipase) in the white rat Rattus rattus. They found the potencies of those enzymes showed a considerable reduction. Said (1979), carried out experiments on the white rat Rattus rattus to study the effect of DDT, lindane and malathione on the potency of certain digestive enzymes (maltase and dipeptidase). He

found a reduction in the potencies of these enzymes. In 1981, Said experimented the previously mentioned insecticides on the potency of certain digestive enzymes prepared from the domestic pigeon Columba livia domestica. The enzymes are liver esterase, ileum esterase and pancreatic lipase. The doses applied were 1/10, 1/100 & 1/1000 of the approximate lethal dose (high dose) for each of these chemical insecticides. The enzymes of the 1/10 high dosed treated animals showed a considerable reduction in their potencies. The enzymes of the 1/1000 high dosed treated animals showed a lower reduction in their potencies.

In this work it is aimed to see the effect of the chemical insecticide "tamaron" on the potency of certain digestive enzymes (pancreatic lipase, ileum dipeptidase and ileim maltase) in the white rat Rattus rattus.

Material and Methods

A- Preparation of enzyme solution:

The animals used through this study were adult male white rats (Rattus rattus) weighing about 130 g each. All the animals were kept on the same normal diet (bread, milk(NIDO) and water), during the experiment, to avoid

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* The trem potency designates the change in the activity of the enzyme prepared from insecticide treated animals.

the probable interference of the effect of food kind. The animals were kept in the laboratory in cages about one week before use. In each experiment not less than 30 animals were used after killing them by a blow on their heads. Certain hydrolytic enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase) were prepared from these rabbit animals. Water extracts of fresh pancreatic extract (1:10) and intestinal mucosa extract (1:10) were used.

B- Buffers:

A number of different buffer solutions were used in the present work. The nature of each buffer used in the different experiments is indicated in the tables giving the experimental results. The measurement of the pH values was done by the B.D.H. capillator. The error in this method is about 0.05 pH units.

C- Methods of measurement of the enzyme activity:

1. Pancreatic lipase

The method of Willstatter et al.(1923) was used in the present work. The digestive mixture (total volume 1.5 c.c.) had the following composition.

0.140	c.c.	olive oil
0.500	c.c.	0.2N veronal-acetate buffer
0.025	c.c.	2 % $CaCl_2$
0.025	c.c.	3 % egg albumen
0.310	c.c.	dist. H_2O
0.500	c.c.	enzyme solution
1.5	c.c.	Total volume

The incubation time was one hour at 37 °C.

2. Ileum dipeptidase:

The method of Willstätter and Waldschmidt-Leitz 1921, and Willstätter et al. 1926, wss used for measuring dipeptidase activity. The digestive mixture had the following composition.

1.0	c.c.	2 % glycyl glycine solution
1.0	c.c.	0.1N veronal-acetate-HCl buffer
0.5	c.c.	dist. H_2O
1.0	c.c.	enzyme solution
3.5	c.c.	Total volume

The incubation time was one hour at 37 °C.

3. Ileum maltase:

The method of Hagedorn and Jensen (1922), was used in the present work. The digestive mixture had the following composition.

2.0 c.c. 0.4 % maltose solution

0.8 c.c. 0.1N veronal-acetate-HCl buffer

0.8 c.c. dist. H_2O

0.4 c.c. enzyme solution

4.0 c.c. Total volume

The digestive mixture was incubated at 37°C for exactly 10 minutes. The titration sample was 0.1 c.c.

D. Estimation of the optimal pH values of the enzymes:

All the experiments of the present study were carried out at the optimal pH of the enzymes. The optimal activities of pancreatic lipase, ileum dipeptidase and ileum maltase were at 7.2, 8.1 and 7.3, respectively (Said 1979).

E. Effect of the chemical insecticide on the digestive enzymes:

For studying the effect of tamaron on the potency of the hydrolytic enzymes, 3 doses (1/10, 1/100 and 1/1000 high dose) were tested. The high dose in mg/Kg. body weight/day was 30. This high dose is very near to the LD_{50} for tamaron (Hassan, 1983). The animals were given daily the 1/10 high dose, the 1/100 high dose or the 1/1000 high dose orally for 60 days.

Results

1. Pancreatic lipase:

Table 1 displays the date which concern the potency of pancreatic lipase prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 0.67 to 0.12 (= 82 % reduction), the 1/100 high dose reduced the potency from 0.65 to 0.39 (= 40 % reduction) and the 1/1000 high dose reduced the potency from 0.63 to 0.43 (= 32 % reduction).

2. Ileum dipeptidase:

Table 2 indicates the data which concern the potency of ileum dipeptidase prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 1.27 to 0.70 (=45% reduction) and the 1/100 high dose reduced the potency from 1.28 to 0.87 (= 32 % reduction), while the 1/1000 high dose reduced the potency from 1.25 to 0.99 (= 21 % reduction).

3. Ileum maltase:

Table 3 shows the data which concern the potency of ileum maltase prepared from tamaron treated animals as compared with those prepared from normal animals. The

1/10 high dose reduced the potency from 0.108 to 0.051 (= 53 % reduction), the 1/100 high dose reduced the potency from 0.106 to 0.066 (= 38 % reduction) and the 1/1000 high dose reduced the potency from 0.105 to 0.076 (= 28 % reduction).

Discussion

The results of the present investigation clearly show the reduction in the potencies of the digestive enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase) extracted from tamaron treated animals. These results could be explained as follows:

a) The enzymes of the 1/10 high dose treated animals showed a considerable reduction in the potencies. It is clear that the pancreatic lipase was the most reduced by this chemical insecticide, while ileum dipeptidase showed the least reduction.

b) The enzymes of the 1/100 high dose treated animals, showed the lowest reduction in their potencies as compared with their correspondings for 1/10 high dose treated animals.

c) The enzymes of the 1/1000 high dose treated animals, showed a slight reduction in their potencies as compared with their correspondings for 1/10 and 1/100 high dose treated animals.

The previously mentioned results were in agreement with those obtained by other workers dealing with the effect of certain chemical insecticides on the potency of the digestive enzymes (Dombrovskii et al., 1965; Platonova, 1970; Gabr and Said, 1972A; Gabr et al., 1972; Amitabha and Konar, 1973; Zakirov et al., 1974, 1975; Said, 1979 and 1981).

Summary

1. The enzymes of 1/10 high dose treated animals with tamaron showed a considerable reduction in their potencies. The potencies of pancreatic lipase and ileum maltase were the most reduced by tamaron, while that of ileum dipeptidase is of a lower reduction.

2. The enzymes of 1/100 high dose treated animals, showed a lower reduction in their potencies than that of 1/10 high dose treated animals. The potencies of pancreatic lipase and ileum maltase were the most reduced by tamaron, while that of ileum dipeptidase is still of a lower reduction.

3. The enzymes of 1/1000 high dose treated animals, showed a lowest reduction in their potencies as compared with their correspondings for 1/10 and 1/100 high dose treated animals.

4. The present study is of importance for clarifying one of the probable causes of digestive troubles widely observed nowadays.

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Table I

The potency of pancreatic lipase of tamaron treated white rat

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Digestive mixture 1.5 c.c. containing: 0.5 c.c. pancreatic extract (1:10), 0.125 gm (= 0.14 c.c.) olive oil, 0.5 c.c. 0.2N veronal-acetate buffer, 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % eggalbumen and 0.31 c.c. distilled water. Time of digestion one h. Temperature 37°C. pH value 7.2.

Condition	Titration (c.c. 0.1N KOH)		Potency c.c.0.01N KOH
	After 0 hour	After One hour	
Normal (control of 1/10 high dose)	1.65	2.32	0.67
Treated 1/10 high dose	1.68	1.80	0.12
Normal (control of 1/100 high dose)	1.70	2.35	0.65
Treated 1/100 high dose	1.73	2.12	0.39
Normal (control of 1/1000 high dose)	1.62	2.25	0.63
Treated 1/1000 high dose	1.68	2.11	0.43

Table 2

The potency of ileum dipeptidase of tamaron treated white rat

Digestive mixture 3.5 c.c. containing: 1.0 c.c. extract of ileum mucosa (1:10), 1.0 c.c. 2 % glycyl-glycine, 1.0 c.c. 0.1N veronal acetate - HCl buffer and 0.5 c.c. dist. H_2O.

Titration sample 0.5 c.c. Mean pH value 8.1. Temperature 37°C. Time of digestion one h.

Condition	Titration (c.c. 0.01N KOH		Potency c.c. 0.01N KOH
	After 0 hour	After One hour	
Normal (control of 1/10 high dose)	1.85	3.12	1.27
Treated 1/10 high dose	1.88	2.58	0.70
Normal (control of 1/100 high dose)	1.86	3.14	1.28
Treated 1/100 high dose	1.90	2.77	0.87
Normal (control of 1/1000 high dose)	1.96	3.21	1.25
Treated 1/1000 high dose	1.89	2.88	0.99

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Table 3

The potency of ileum maltase of tamaron treated white rat

Digestive mixture 4.0 c.c. containing: 0.4 c.c. extract of ileum mucosa (1:10), 2.0 c.c. 0.4 % maltose, 0.8 c.c. 0.1M ' veronal- acetate- HCl buffer and 0.8 c.c. dist. H_2O.

Titration sample 0.1 c.c. pH value 7.3. Temperature 37 37°C. Time of digestion 10 minutes.

Condition	Digestion(mg glucose) after 0 minute	Digestion(mg glucose) After 10 minutes	Potency mg glucose
Normal (control of 1/10 high dose)	0.165	0.273	0.108
Treated 1/10 high dose	0.170	0.221	0.051
Normal (control of 1/100 high dose)	0.158	0.264	0.106
Treated 1/100 high dose	0.160	0.226	0.066
Normal (control of 1/1000 high dose)	0.160	0.265	0.105
Treated 1/1000 high dose	0.168	0.244	0.076

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PATHOLOGICAL CHANGES OF BIOMPHALARIA ALEXANDRINA DURING ASEXUAL CYCLE OF SCHISTOSOMA MANSONI INFECTION

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A B S T R A C T

Several days after exposure to S.mansoni miracidia, many snails appeared inactive and partly withdrawn. By 15 days post infection, the daughter sporocysts concentrated in the region of the digestive gland and to some extent in the ovotestis. There was also infection in the various anteriorly situated organs like tentacles, head-foot and oesophagus. By 30 days post infection, daughter sporocysts increased in size and

number which occupied almost all of the interlobular connective tissue of the digestive gland and ovotestis. Necrosis of glandular tissues was noted when glandular lobules were compressed and invaded by granulomatus tissue formed around daughter sporocysts and cercariae. Atrophy, degeneration and ulceration of glandular epithelium then resulted. By 45 days post infection displacement of the digestive gland tubules by the sporocysts was observed. Ovotestis were most severly damaged, the acini lost their regular shape and were occupied by the parasite.

INTRODUCTION

Schistosomiasis is one of the most important public health problems in many countries in the tropics and subtropics. It is a water - borne disease which is contracted by the cercarial stages of the parasites peneterating the skin of individuals who comes in contact with infested water.

Schistosomiasis is still considered as one of the most important endemic diseases in Egypt, its impact on health and economy is trem ndous (Faroog and Samaan, 1967) . During the last few decades, there has been a steady and significant change in the epidemiology and pattern of distribution of both species of schistosomes and their respective intermediate host with S.mansoni and its snail host B.alexandrina spreading into new areas which have hitherts free of the parasite and its snail intermediate host. In view of this change and due to the higher morbidity of Shistosoma mansoni , wide interest and attention are now directed to the study of S.mansoni and its relationships with the snail intermediate host B.alexandrina.

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The histopathological effects of the parasites on the snail tissues have been studied by many authors, Pelseneer (1906) , Lebour (1911) , Faust (1920), Rees (1936), James (1965), Malek and Cheng (1974), Jordan and Webbe (1982) , Kamel et al. (1986) . The most commonly encountered histopathological changes in molluscs, especially gastropods , are there associated with the presence of larval trematodes (sporocysts and / or rediae). This range from extremely severe to minor alterations. This topic has been comprehensively reviewed by Cheng and Snyder, (1962), Malek (1980) and only a few additional studies have been reported since these reviews.

The present work has been planned to investigate the pathological changes of the snail Biomphalaria alexandrina during asexual cycle of Schistosoma mansoni infection.

MATERIAL AND METHODS

Biomphalaria alexandrina snails were obtained from laboratory inbread stock of approximately same size (6-8 mm in shell diameter) . Snails were classified into two groups. The first was infected with S.mansoni miracidia while the second was left as a control group. Snails were infected individually, the eggs of S mansoni were isolated from the intestine of mice with mature infection of S.mansoni according to the method used by Mohamed (1987).

Each snail was placed with a little distilled water in 2.5 cm cup about 10 mm in diameter and 15 mm in depth. Snail then was exposed to 6-8 newly hatched miracidia of S.mansoni.

The containers were left 3-4 hours to ensure the penetration of miracidia.

For histopathological examinations, infected snails were randomly selected after 15,30 and 45 days post infection. The soft parts of infected and control specimens were removed from their shells and immediately fixed in alcoholic Bouin's fluid. Tissues were then dehydrated, cleared, paraffin embedded and serial sections were cut at 6-8 microns in thickness. Sections were stained with Haematoxylin and eosin.

R E S U L T S

For the several days after exposure to miracidia, many snails appeared inactive and partly withdrawn. There after , most infected snails behaved normaly until about 5-6 weeks when cercariae began to emerge. Then snails again became relatively inactive, withdrawn and appeared weak .

Transverse sections through the soft parts of uninfected B.alexandrina showing the digestive gland tubules (Figs.1 and 2), ovotestis (Figs.3 and 4), the head-foot (Fig.5) are given to illustrate the architecture of uninfected specimens.

By 15 days post infection, the daughter sporocysts of S.mansoni concentrated in the region of the digestive gland (Fig. 6) and to some extent the region of the ovotestis (Fig.7) and also present in the various anteriorly situated organs as tentacles, head-foot and oesophagas Figs. 8 , 9 and 10). The digestive gland in which individual lobules are separated by a small amount of loss connective tissue (Figs. 1 and 2).

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Daughter sporocysts appeared in the interlobular connective tissue. When B.alexandrina digestive gland becomes filled with sporocysts , the enveloping tunica propria usually become ruptured, and the larval trematodes invade the adjacent ovotestis. It seems that this is usually the mechanism for invasion of the reproductive system (Fig. 10). S mansoni sporocyts invaded the connective tissue around and between the acini of the ovotestis but non of them were found inside any acini .

As infection progressed, daughter sprocysts increased in size and number by about 30 days post infection. Numerous daughter sporocysts occupied almost all of the interlobular connective tissue of the digestive gland and ovotestis (Fig. 11). Development of large numbers of daughter sporocysts in the digestive gland altered the hepatic architecture. As much as two thrids of the parenchymal tissue disappeared and the normal complex lobular branching structure was lost (Fig.12). The architecture of the ovotestis also a compound lobular gland was less altered than that of the digestive gland because few daughter sporocysts developed there. The reproductive cells within the ovotestis become very scarce.

By 45 days post infection, the uptake of nutrients by sporocysts takes place through the tegument , the effect causes toxic changes in the digestive gland cells, the epithelium distal wall of the cell often breaks , down (Fig.13), displacement of the tubules and loss of their branched

structure were observed (Fig.14). Ovotestis were most severly damaged (Fig. 15). The acini lost their regular shape and occupied mostly by the sporocysts. In some cases the reduction and often the complete obliteration of the acini were observed. As a result of infection heavy production and accumulation of excretory granules were observed accompanied by concurrent proliferation of interlobular connective tissue. It was observed that there are usually several cercariae in the region of ovotestis (Fig.16) digestive gland and in blood sinuses. Complete replacement of the digestive gland cells and the ovotestis acini were detected.

D I S C U S S I O N

By far the majority of digenetic trematodes larval stages utilize their molluscan intermediate host's digestive gland as a primary site of infection. Although if this organ becomes densely packed with sporocysts, some of these larvae may secondarily invade the adjacent ovotestis and other organs of the snail,Malek (1954, 1955, 1958) ; Cheng and Cooperman (1964) ; Pan (1965); Kamel (1979) and Kamel et.al.(1986).

In the present investigation, results indicated a great reduction in digestive gland and ovotestis, also some other organs as tentacles, head-foot and oesophagus as well as the connective tissues and muscular tissues. This destruction is of four types.

1- Mechanical destruction due to pressure exerted by the Parasite.

2- Extracorporeal digestion by sporocysts.

3- Lysis due to the parasite excreta.

4- Autolysis due to starvation .

Histologically, the present investigation indicated that the acinar cells affected by mechanical pressure appear extremeley compressed, and if ruptured the fragments reveal jugged edges. Furthermore, their nuclei are commonly not pycnotic, unless the nucleated fragments have persisted for some time . Cells were destroyed as the result of enzymes secreted from the tegument of sporo - cysts . The cells not in immediate contact with the parasite often show symptoms of destruction and/ or necrosis while in the case of mechanical damage, the compressed or cleanly ruptured cells usually occurred in the immediate proximity of the parasite.

In addition to the types of cell destruction, another histopathology feature of parasitized digestive gland is the occurance in large numbers of two types of globules of intracytoplasmic origin, especially if host parasite association is of relatively long duration. The first type of globules, known as excretory or ferment globule are yellowish brown even in stained sections. They are unucleated and represent accumulated metabolic wastes in hyper action excretory or ferment cells of the digestive gland . As the cells become ruptured, digested or lysed , the comparatively large excretory globules become dispersed -

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throughout the tissue. Another striking cytological feature commonly encountered in digestive gland cells of molluscs infected with larval trematodes is the reduction of the normal columnar epithelial cells of each acinus to cuboidal or even squamous epithelium . This phenomenon has been reported by Cheng and Snyder (1962) as has been confirmed by James (1965). This morphological change can be attributed to the starvation of cells as the result of the blockage haemolymph channels by the parasites (Rees, 1936; James 1965).

Considering the ovotestis, it has been noted earlier that a reduction in the fecundity of molluscs parasitized by larval trematodes is known . This information is the result of studies done by Hurst (1927) ; Rees (1936) ; Malek (1952) ; Coelho (1954); Pan (1965); Strock (1967); Kamel (1979) and Abdel Reheem (1985).

In the present study, it was observed that B.alexandrina infected with S.mansoni, sporocysts invaded the connective tissue around and between the acini of the ovotestis, but non of them found inside the gonad by this Schistosome having daughter sporocysts stage is not the result of mechanical influence of the parasite, but rather by induced physiological conditions that tend to disturb the metabolism of the snail host. The results run in full agreement with those described by Paust (1920), Agersborg (1924) and Malek (1980).

The present work is the first in a series dealing with the histopathological and immunological studies of Biomphalaria alexandrina infected with Schistosoma mansoni. It is hoped that the encouraging results obtained in the present study will stimulate further investigation in the field of Schistosomes control in Egypt.

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EXPLANATION OF FIGURES

Fig.1. Transverse section through the visceral hump of uninfected B.alexandrina showing the digestive gland tubules (X 33.3).

Fig.2. Transverse section through the digestive gland tubules of uninfected B.alexandrina showing the digestive gland tubules cell walls and the basal nucleus (X 66).

Fig.3. Transverse section through the ovotestis of uninfected B.alexandrina showing the manufun - tion of both ova and sperms (X66).

Fig.4. Magnified transverse section through the ovotestis acinus of uninfected B.alexandrina showing the germinal epithelium, oocytes , mature ovum and spermatozoa in the center of the acinus lumen (X 132).

Fig.5. Transverse section in the foot of uninfected B.alexandrina showing the foot epithelium , connective tissue and mucous cells (X 33).

Fig.6. Transverse section of 15 days post infected specimen of B.alexandrina showing the location of sporocysts between the tubules (X 66).

Fig.7. Transverse section of 15 days post infected specimen showing the location of the parasite between the ovotestis acini (X 132).

Fig.8. Different stages of developing sporocysts within the head-foot of 15 days post infected specimen of B.alexandrina (X 132).

Fig.9. Section through the mantle cavity of 15 days post infected B.alexandrina showing location of the parasites (X 33).

Fig.10. Section in 15 days post infected specimen showing infected foot and connective tissue (X 66).

Fig.11. Magnified transverse section of 30 days post infected specimen showing the sporocysts within the buccal cavity (X 66).

Fig.12. Transverse section of 30 days post infected specimen showing infected digestive gland and the ovotestis (X 66).

Fig.13. Transverse section of 45 days post infected specimen showing the break down of digestive gland tubules (X 33).

Fig.14. Transverse section in the ovotestis of 45 days post infection showing the displacement of the acini by sporocysts and cercariae (X 132).

Fig.15. Transverse section of 45 days post infected specimen showing the destruction of digestive gland cells (X 66).

Fig.16. Section of 45 days post infected ovotestis of B.alexandrina showing sporocysts and cercariae between the acini (X 66).

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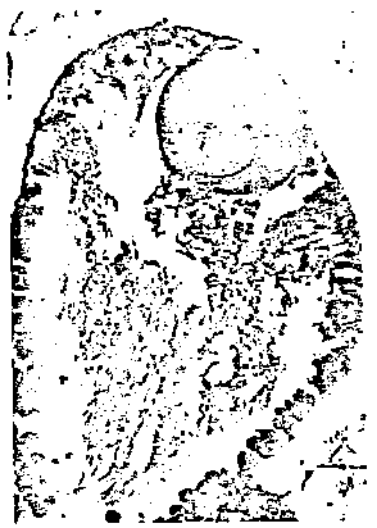

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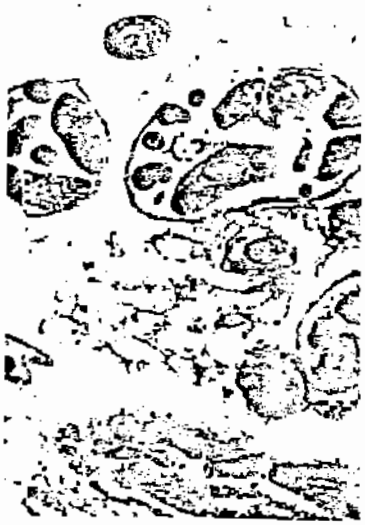

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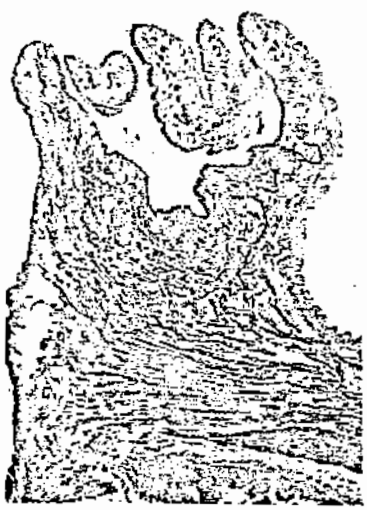

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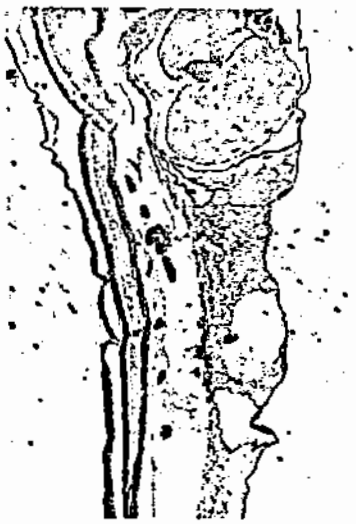

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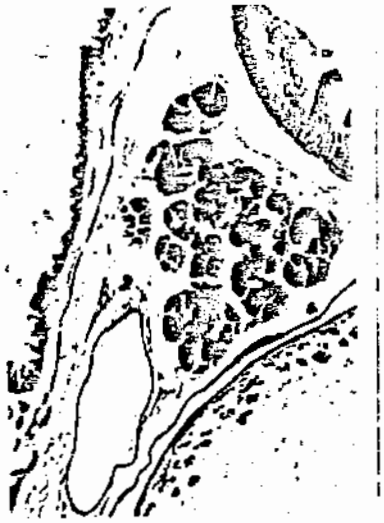

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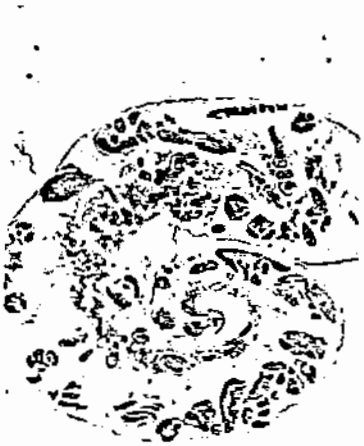

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EFFECT OF THE CHEMICAL INSECTICIDE "TAMARON" ON THE ACTIVITY OF CERTAIN DIGESTIVE ENZYMES IN THE WHITE RAT.

By

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Introduction

It is a well known fact that chemical insecticides have a hazardous effect on man and useful animals, when these chemical insecticides find their way into the body in amounts very far below the lethal doses and accumulated in the body. These hazardous effects are variable and do concern the different body systems and organs.

The present study is a contribution to what have been carried out by other workers in the field of the so called digestive toxicology. The early experiments of Webb (1948) showed a strong action of alkyl fluorophosphonate on milk lipase (77% inactivation), whereas it had no effect on both pancreatic amylase and lipase. In 1949, Jansen et al. found that a concentration of diisopropyl fluorophosphate of 4×10^{-4}M, caused a 80% inhibition of trypsin, while a concentration of 8×10^{-6}M caused 50% inhibition to chymotrypsin. Mounter et al. (1963) mentioned that diisopropyl phosphorof-

luoridate and other organophosphorous compounds react rapidly with the proteolytic enzymes causing inhibition which reached to 50 % for both trypsin and chymotrypsin. Dombrovskii et al., (1965) gave a dose of parathion which is 0.45 mg/rat/day for 60 days to albino rats; histochemical analysis revealed a sharply reduced phosphatase and esterase activities. Using frozen extracts of pig liver, Mendoza and Shields (1970) mentioned that esterase was sensitive to inhibition by the carbamates studied at the manogram to picogram levels. Gabr and Said (1972B) found that some chemical insecticides (DDT, Lindane and malathione) have a hazardous effect on the function of the peptic and oxyntic cells in mammals. In 1972, Gabr, et al. studied the effect of the previously mentioned insecticides on the activity of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic lipase) in the white rat Rattus rattus. They found that the activities of these enzymes showed a considerable reduction. Bunyan and Jennings (1976) found that liver and brain esterases were inhibited in the pheasant (Phasianius colchicus and pigeon (Columba livia) which fed with lethal and sublethal doses of 6 widely used carbamates (aldicarb, aminocarb, methiocarb, primicarb, propoxur and zectran).

The present work was carried out on the white rat (Rattus rattus) and dealt with the direct effect of the

chemical insecticide "tamaron" on the activity of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase) prepared from normal animals.

Materials and Methods

A- The animals used through this study were adult male white rats (Rattus rattus) weighing about 130 g each. Certain hydrolytic enzymes (salivary amylase, pepsin, trypsin, pancreatic amylase and pancreatic esterase) were prepared from these animals.

All the animals were kept on the same normal diet , during the experiment, to avoid the probable interference of the effect of food kind. This diet consisted of bread, milk (NIDO) and water. The animals were kept in the laboratory in cages about one week before use. For obtaining the digestive juices, free from food remains, animals were starved for at least two days. In each experiment not less than 30 animals were used after killing them by a blow on their heads.

Water extracts of fresh salivary glands(1:10), stomach mucosa extract (1:10) and pancreatic extract (1:10)were used. Enterokinase was prepared from duodenal mucosa extract according to the prescription of Waldschmidt-Lietz (1924) , treated with acetone and ether.

B- Buffers :

A number of different buffer solutions were used in the present work. The nature of each buffer used in the different experiments is indicated in the tables giving the experimental results.

The measurement of the pH values was done by the B.L.H. capillator. The error in this method is about 0.05 pH units.

C- Methods of measurement of the enzymes activity:

1. Peptidases :

The method of Willstätter and Waldschmidt-Leitz (1921) and Willstätter et al., (1926) was used (with some modification) for measuring peptic and tryptic activities.

For pepsin, the digestive mixture (total volume 5.0 c.c.)had the following composition:

2.5	c.c.	egg albumen 3 %
1.0	c.c.	citrate-HCl buffer (1N sod. citrate and 2.5 N HCl)
1.0	c.c.	distilled water
0.5	c.c.	enzyme solution
5.0	c.c.	Total volume.

The incubation time of 20 h was chosen since it was found to be the most suitable one.

For trypsin, the digestive mixture was made in the following way :

0.3 c.c. enzyme solution.

0.1 c.c. enterokinase solution.

0.35 c.c. buffer solution (0.2 N ammonia-ammonium chloride.

0.15 c.c. distilled water

The above mentioned solutions were mixed together (total volume 0.90 c.c.) and the pH value of the mixture was 8.0. The mixture was put in the thermostate at 37 °C for activation. After 30 minutes, the pH was adjusted to the desired value by the addition of 0.6 c.c. 0.1N veronal acetate buffer prewarmed to 37°C, after which 1.5 c.c. of 6% casein solution (Waldschmidt-Leitz, 1924) was added. The total volume of the digestive mixture was therefore 3.0 c.c. The buffer solutions were prepared according to Michaelis (1922 and 1931). The time of incubation was half an hour.

2- Carbohydrases :

Amylase:

The method of Hagedorn and Jensen (1922) was used in the present work.

For salivary amylase the digestive mixture had the following composition:

2.0	c.c.	2% starch solution.
0.2	c.c.	buffer solution (0.1 N veronal-acetate-HCl)
1.3	c.c.	distilled water (1.4 c.c. for pancreatic amylase).
0.5	c.c.	enzyme solution (0.4 c.c. for pancreatic amylase).
4.0	c.c.	Total volume

The digestive mixture was incubated at 37°C for exactly 10 minutes. The titration sample is of 0.05 c.c. It had been shown by Pucker and Finch (1938) and confirmed by Keddis (1952 and 1956) that each mg maltose had the same reducing action on potassium ferricyanide as 0.75 mg glucose. Using this factor, the extent of digestion was expressed as "increase in mg maltose" calculated by multiplying each figure of "increase in mg glucose" by the factor 100/75.

3- Esterases :

Pancreatic esterase:

The method of Willstatter, et al , (1923) was followed in the present work. The digestive mixture (total volume 5.0 c.c.) had the following composition :

1.00	c.c.	enzyme solution.
0.05	c.c.	ethyl acetate
1.00	c.c.	0.1N veronal-acetate buffer
1.00	c.c.	2% $CaCl_2$ and
1.95	c.c.	distilled water
5.00	c.c.	Total volume.

The digestive mixture was incubated at 37°C for 60 minutes. The titration sample was 0.5 c.c.

4- Estimation of the optimal pH values of the enzymes:

All the experiments of the present study were carried out at the optimal pH of the enzymes. The optimal activity of pepsin, trypsin, salivary amylase, pancreatic amylase and esterase were 2.1, 8.0, 7.1, 6.8 and 7.5, respectively.

5- Effect of the chemical insecticide on the digestive enzymes :

For studying the effect of the chemical insecticide "tamaron" on the activity of the hydrolytic enzymes (salivary and pancreatic amylases, pepsin, trypsin and pancreatic esterase), three doses (1/10, 1/100 and 1/1000 high dose) were testad. - The high dose was 30 mg/kg body weight/ day. This high dose is very near to the LD_{50} for the chemical insecticide (Hassan, 1983). The doses of the insecticide were added to the digestive mixture instead of a part of distilled water.

Results

a- Peptidases:

1. Pepsin :

Table I displays the data which concern the effect of tamaron on the activity of pepsin (in c.c. 0.1N KOH) prepared from normal white rats. The 1/10 high dose reduced the activity from 0.83 to 0.52 (=37.4 %) inactivation), the 1/100 high dose reduced the activity from 0.83 to 0.69 (=16.9% inactivation) and the 1/1000 high dose reduced the activity from 0.83 to 0.72 (= 13.3%) inactivation).

2- Trypsin:

Table 2 exhibits the data which concern the effect of tamaron on the activity of trypsin (in c.c. 0.02 N KOH) prepared from normal animals. The 1/10 high dose reduced the activity of trypsin from 0.97 to 0.90 (=7.2% inactivation), the 1/100 high dose reduced the activity from 0.97 to 0.91 (= 6.2% inactivation), and 1/1000 high dose reduced the activity from 0.97 to 0.95 (2.1% inactivation).

b. Carbohydrases:

1. Salivary amylase.

Table 3 displays the data which concern the effect of tamaron on the activity of salivary amylase (in mg maltose) prepared from normal animals. The 1/10 high dose reduced the activity of salivary amylase from 0.117 to 0.064(=45.3%

inactivation), the 1/100 high dose reduced the activity from 0.117 to 0.087 (=25.6% inactivation), and the 1/1000 high dose reduced the activity from 0.117 to 0.112(=4.3% inactivation).

2. Pancreatic amylase :

Table 4 shows the data which concern the effect of tamaron on the activity of pancreatic amylase (in mg maltose) prepared from normal white rats. The 1/10 high dose reduced the activity of pancreatic amylase from 0.159 to 0.081 (= 49.1% inactivation), the 1/100 high dose reduced the activity from 0.159 to 0.109 (=31.5% inactivation) and the 1/1000 high doss reduced the activity from 0.159 to 0.137 (=13.8% inactivation).

c- Esterases:

Pancreatic esterase:

Table 5 displays the data which concern the effect of tamaron on the activity of pancreatic esterase(in c.c. 0.01 N KOH) prepared from normal animals. The 1/10 high dose reduced the activity of pancreatic esterase from 0.38 to 0.20 (=47.4% inactivation), the 1/100 high dose reduced the activity from 0.38 to 0.27 (=29.0% inactivation) and the 1/1000 high dose reduced the activity from 0.38 to 0.34(=10.5% inactivation).

Discussion

The effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pepsin, trypsin, salivary amylase, pancreatic amylase and pancreatic esterase) of the white rat Rattus rattus; was carried out. The doses applied were 1/10, 1/100 and 1/1000 of the approximate lethal dose of tamaron. The approximate lethal dose of tamaron was 30 mg/kg body wt./day. The data in Table 6 indicate the following :-

Concerning the direct effect of tamaron on the activity of the digestive enzymes it could be noticed that:

a) The 1/10 high dose produced a considerable inactivation to all enzyme (pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase). Pancreatic amylase seems to be the most sensitive to the inactivation effect produced by the chemical insecticide, whereas trypsin seems to be the most resistible to such an inactivation.

b) The 1/100 high dose of tamaron produced a lower effect on pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase. Pancreatic amylase showed a considerable inactivation while trypsin seems to be the most resistible to the inactivation effect produced by this chemical insecticide. It is clear that the inactivation effects for the 1/100 high dose are lower than their correspondings for the 1/10 high dose.

c) The 1/1000 high dose of the chemical insecticide generally have a weak effect on pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase, yet pepsin and pancreatic amylase showed a considerable inactivation. It is clear that the inactivation effects for the 1/1000 high doses were lower than their correspondings for the 1/10 and 1/100 high doses.

The above mentioned results which concern the inactivation of the experimented on digestive enzymes, through the effect of the applied chemical insecticide "tamaron" agree with the results obtained by other workers dealing with the effect of certain chemical insecticides on the digestive enzymes (Webb, 1948; Jansen et al, 1949; Kounter et al.,1965; Dombrovskii et al.,1965; Mendoza and Shields, 1970; Gabr and Said, 1972B; Gabr et al;1972; Bunyan and Jennings,1976).

Summary

The effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase) of the white rat Rattus rattus was carried out. The doses applied were 1/10, 1/100 and 1/1000 of the approximate lethal dose of the chemical insecticide. The approximate lethal dose of tamaron for rats was 30 mg/kg body wt./day.

The 1/10 high dose produced a considerable inactivation to all the enzymes studied, Pancreatic amylase and pancreatic esterase seem to be the most sensitive to the inactivation effect, while trypsin seems to be the most resistible.

The 1/100 high dose produced an effect lower than that produced by the 1/10 high dose, in all the enzymes studied.

Pancreatic amylase and pancreatic esterase seem to be the most sensitive ones, while trypsin is the most resistible.

The 1/1000 high dose produced very slight effect on enzymes studied. Pancreatic amylase and pepsin showed a considerable inactivation.

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Table 1

Effect of tamaron on the activity of pepsin of normal rat.

Condition	Digestive mixture at 37°C, pH value 2.1, time of digestion 20 hours and titration sample 0.5 c.c.	Titration (c.c.0.1N KOH)		Activity
		After 0 hour	After 20 hours	c.c. 0.1N KOH
Normal (control)	2.5c.c 3% eggalbumen, 1.0 c.c. citrate-HCl buffer, 1.0 c.c. dist. H_2O and 0.5 c.c. water extract of stomach mucosa (1:10)	5.89	6.72	0.83
1/10 high dose	2.5 c.c. 3% eggalbumen, 1.0 c.c. citrate-HCl buffer, 0.07 c.c. tamaron in H_2O, 0.93 c.c. dist. H_2O and 0.5 c.c. water extract of stomach mucosa (1:10).	5.97	6.49	0.52
1/100 high dose	2.5 c.c. 3% eggalbumen, 1.0c.c. citrate-HCl buffer, 0.07c.c. tamaron in H_2O, 0.93 c.c. dist. H_2O and 0.5 c.c. water extract of stomach mucosa. (1:10)*	6.12	6.81	0.69
1/1000 high dose	2.5 c.c. 3% egg albumen, 1.0c.c. citrate-HCl buffer, 0.07** c.c. tamaron in H_2O, 0.93 c.c. dist. H_2O and 0.5 c.c. water extract of stomach mucosa (1:10).	6.65	7.37	0.72

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

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Table 1

Effect of tamaron on the activity of pancreatic trypsin of normal rat.

Condition	Digestive mixture at 37°C., pH value 8.0, time of digestion one hour and titration sample 0.5 c.c.	Titration(c.c.0.02N KOH)		Activity c.c. 0.02N KOH
		After 0 hour	After one hour	
Normal (control)	0.3 c.c. pancreatic extract (1:10),0.1 c.c.enterakinase solution, 0.35 c.c. 0.2N ammonium buffer,0.15c.c. dist. H_2O, 0.8 c.c. 0.1 N veronal-acetate buffer and 1.5c.c. 6% casein.	1.07	2.04	0.97
1/10 high dose	0.3c.c. pancreatic extract (1:10),0.1 c.c.enterokinase solution,0.35c.c. 0.2N ammonium buffer, 0.07 c.c.tamaron in dist. H_2O, 0.08 c.c. dist. H_2O, 0.6 c.c. 0.1 N veronal-acetate buffer and 1.5 c.c. 6% casein.	1.09	1.99	0.90
1/100 high dose	0.3c.c. pancreatic extract (1:10),0.1 c.c. enterokinase solution,0.35 c.c. 0.2N ammonium buffer,0.07 c.c. tamaron in dist. H_2O, 0.08 c.c. dist. H_2O, 0.6 c.c. 0.1 N veronal-acetate buffer and 1.5 c.c. 6% casein.	1.12	2.03	0.91
1/1000 high dose	0.3 c.c. pancreatic extract (1:10),0.1c.c. enterokinase solution,0.35 c.c. 0.2N ammonium buffer, 0.07c.c. tamaron in dist. H_2O, 0.08 c.c. dist. H_2O, 0.6 c.c. 0.1N veronal-acetate buffer and 1.5 c.c. 6% casein.	1.22	2.17	0.95

• The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

•• The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 3

Effect of tamaron on the activity of salivary amylase of normal rat.

Condition	Digestive mixture at 37°C., pH value 6.8, time of digestion 10 minutes and titration sample 0.05 c.c.	Digestion (mg glucose)		Activity	
		After 0 minute	After 10 minutes	mg glucose	mg maltose
Normal (control)	2.0c.c. 2% starch solution, 0.2c.c. 0.1 N veronal-acetate buffer, 1.3 c.c. dist. H_2O, and 0.5 c.c. salivary gland (parotid) extract[2] (1:10).	0.025	0.113	0.088	0.117
1/10 high dose	2.0c.c. 2% starch solution, 0.2c.c. 0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.23 c.c. dist. H_2O, and 0.5 c.c. salivary gland (parotid) extract[2] (1:10).	0.024	0.072	0.048	0.064
1/100 high dose	2.0 c.c. 2% starch solution, 0.2c.c. 0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.23 c.c. dist. H_2O, and 0.5 c.c. salivary gland (parotid) extract[2] (1:10).	0.027	0.092	0.065	0.087
1/1000 high dose	2.0 c.c. 2% starch solution, 0.2 c.c. 0.1N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.23 c.c. dist. H_2O, and 0.5 c.c. salivary gland (parotid) extract.[2]	0.027	0.111	0.084	0.112

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 4

Effect of tamaron on the activity of pancreatic amylase of normal white rat.

Condition	Digestive mixture at 37°C, pH value 7.1, time of digestion 10 minutes and titration sample 0.05 c.c.	Digestion (mg glucose)		Activity	
		After 0 minute	After 10 minutes	mg. glucose	mg maltose
Normal (control)	2.0 c.c. 2% starch solution, 0.2 c.c. 0.1N veronal-acetate buffer, 1.4 c.c. dist. H_2O, and 0.4 c.c. pancreatic extract (1:10).	0.041	0.150	0.119	0.159
1/10 high dose	2.0 c.c. 2% starch solution, 0.2c.c.0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.33 c.c. dist. H_2O, and 0.4 c.c. pancreatic extract (1:10)	0.031	0.092	0.061	0.081
1/100 high dose	2.0 c.c. 2% starch solution, 0.2c.c.0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.33 c.c. dist. H_2O, and 0.4 c.c. pancreatic extract (1:10).	0.038	0.120	0.082	0.109
1/1000 high dose	2.0c.c. 2% starch solution, 0.2 c.c.0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.33 c.c. dist. H_2O, and 0.4 c.c. pancreatic extract (1:10).	0.036	0.139	0.103	0.137

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 5

Effect of tamaron on the activity of pancreatic esterase of normal white rat.

Condition	Digestive mixture at 37°C., pH value 7.5, time of digestion 60 minutes and titration sample 0.5 c.c.	Titration (0.01N KOH)		Activity c.c. 0.1 N KOH
		After 0 hour	After one hour	
Normal (control)	0.05 c.c. ethyl acetate, 1.0 c.c. 0.1N veronal-acetate buffer, 1.0 c.c. 2% $CaCl_2$ solution, 1.95c.c. dist. H_2O and 1.0c.c. pancreatic extract (1:10).	0.98	1.36	0.38
1/10 high dose	0.05 c.c. ethyl acetate, 1.0 c.c. 0.1N veronal-acetate buffer, 1.0 c.c. 2% $CaCl_2$ solution, 0.07 c.c. tamaron in dist. H_2O, 1.88c.c. dist. H_2O and 1.0 c.c. pancreatic extract (1:10).	0.98	1.18	0.20
1/100 high dose	0.05 c.c. ethyl acetate, 1.0 c.c. 0.1 N veronal-acetate buffer, 1.0 c.c. 2% $CaCl_2$ solution, 0.07c.c. tamaron in dist. H_2O, 1.88 c.c. dist. H_2O and 1.0 c.c. pancreatic extract (1:10).	0.98	1.25	0.27
1/1000 high dose	0.05 c.c. ethyl acetate, 1.0 c.c. 0.1N veronal-acetate buffer, 1.0 c.c. 2% $CaCl_2$ solution, 0.07 c.c. tamaron in dist. H_2O, 1.88c.c. dist. H_2O and 0.1 c.c. pancreatic extract (1:10).	0.98	1.32	0.34

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 6

Effect of the Chemical Insecticide "Tamaron" on the activity of the digestive enzymes.

Enzymes	Percentage of inactivation of the enzyme by the chemical insecticide.		
	1/10 high dose	1/100 high dose	1/1000 high dose
Pepsin	37.4	16.9	13.3
Trypsin	7.2	6.2	2.1
Salivary amylase	45.3	25.6	4.3
Pancreatic amylase	49.1	31.5	13.8
Pancreatic esterase	47.4	29.0	10.5

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TAXONOMIC STUDIES ON SOME ANTS (FORMICIDAE-HYMENOPTERA) OF SAUDI ARABIA

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ABSTRACT

This work deals with the taxonomy of ants belonging to three subfamilies: Ponerinae, Myrmicinae and Formicinae (Family: Formicidae, Order: Hymenoptera) collected from different areas in Saudi Arabia comprising 8 genera and 11 species and subspecies. Diagnosis, and keys accompanied with lillustrations are given to these species and subspecies.

INTRODUCTION

The faunistic approach of investigations in Saudi Arabia is new and the findings will make it possible to gain a better knowledge of the correlations between the various biotopes within Saudi Arabia.

The Arabian peninsula measures approximately 2.4 million Km^2, extending from about 32° 15′ N to Yemen and from the Red Sea to the Persian Gulf. The surface morphology is characterized by the plateau of 600 to 1,000 m. altitude east of the Hidjaz and Asir mountain range, it gradually declines to the east ends along the shores of the Gulf. The distance between the Red Sea and

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the Gulf is on the order of 1,200 km and between the Northern and Southern border some 1,500 km or more (Wittmer and Büttiker, 1979).

Saudi Arabia is one of the hottest, and driest countries in the world. The heat along the coastal belts is oppressive in summer with some relief in the winter months particularly along the Gulf during spells of cold northerly winds.

Ants occupy a unique position among all insects on account of their dominance as a group, and this dominance is shown in their high degree of variability as exhibited in the great number of their species and subspecies, in their wide geographical distribution and in their manifold relationships with plants and other animals-man included (Wilson, 1971).

The aim of the present work is to identify some species and subspecies of ants collected from Saudi Arabia during the period from February to April 1985. Ants have been discussed before by many authors, Emery (1908, 1909, 1912 and 1925); Santschi (1915); Crawley (1925); Finzi (1930); Menozzi (1930-1931) and (1931); Shalaby (1961); Wheeler (1960); Wheeler and Wheeler (1972); and Hassanein (1979).

MATERIALS AND METHODS

Sampling was done by means of direct collecting

from underneath stones, on the soil surface, and from different types of vegetations.

Specimens were collected from several regions in Saudi Arabia representing the Northern, Southern, Eastern, Western, and Middle regions (Fig. 1). These localities show different habitats, desertic, mountaneous, agricultural and vallies.

The specimens were examined and identified accurately from four main collections; the Ministry of Agriculture, Entomological Society, Cairo University and Ain Shams University collections, in addition to the Common Wealth Institute, British Museum, Entomology Department. Localities of each species are cited and number of specimens examined is written between brackets.

RESULTS

Ants show variation in several characters. The head varies enormously in shape, and the mandibles present an almost bewildering variety of form. The labrum is vestigial, the maxillary palps 1-6 segmented, the labial palps 1-4 segmented, the antennae 4-13 segments and usually the male has one more segment than the female or worker; compound eyes and three ocelli are well developed in the males, but in the females and specially the workers, the eyes are usually reduced or vestigial.

Specimens collected from Saudi Arabia is confined in three subfamilies: Ponerinae, Myrmicinae and Formicinae.

Key to subfamilies, genera, species and subspecies of ants from Saudi Arabia

1(8) Anal orifice terminal, circular (Fig. 2); sting vestigial; abdominal pedicel one segmented scale like (Fig. 3); no constriction between first and second gastral segments..... FORMICINAE

2(3) Insertion of antennae distant from the posterior margin of clypeus; wings long; ♂ gaster elongate... ..... CAMPONOTUS Mayr

—— Occiput divergent in the posterior border of head; first funicular segment longer than the second..... ..... Camponotus compressus thoracicus Fabricius .

3(2) Insertion of antennae close to the posterior margin of clypeus; wings short or moderately long; ♂ gaster short thick or oval.

4(5) Maxillary palps short, the 4th segment about equal to the 5th; funiculus elongate, antennal cavities separated from clypeus; abdomen covered with raised white hairs; scale depressed and very small .... PARATRECHINA Motschulsky.

—— Head finely striated longitudinally; thorax well striated; abdomen distinctly ponctuated .... Paratrechina longicornis (Latreille).

5(4) Maxillary palps very long, 4th segment about twice in length as the 5th; abdomen with a bronzy reflect; funiculus elongate, 2nd segment at least twice longer than large; female with thorax narrower than head; scale thick obtuseat apex..... .....CATAGLYPHIS Foerster

6(7) Glazy black with silvery hairs on the sides; abdomen globular; petiole flattened dorsally scale like;three spots present instead of ocelli; head as large as thorax..... Cataglyphis albicans livida (André).

7(6) Body reddish brown, gaster black; abdomen oval; petiole rounded; ocelli present in worker; head broader than thorax..... Cataglyphis bicolor nodus(Brullé).

8(1) Anal orifice ventral, slit shaped; sting well developed (Fig. 4) or vestigial; abdominal pedicel one or two segmented.

9(10) Abdominal pedicel consisting of a single segment; gaster with a distinct constriction between the first and second segment (Fig. 5). Frontal carinae separated or closed together, when closed dilated to form oblique or horizontal laminae partly covering insertions of antennae. Mandibles articulated near the middle of the ventral border of the head (Fig. 6). Sting well developed..... PONERINAE.

—— Eyes well developed; wings very broad; petiole with a squarish node.....Pachycondyla sennaarensis (Mayr)

10(9) Abdominal pedicel two segmented, nodiform or pedunculate with rounded node; postpetiole cup-shaped or bell-shaped (Fig. 7). Frontal carinae always well separated and covering antennal insertions; mandibles very long linear narrow and sharp pointed (Fig. 8)... .....MYRMICINAE

11(14) Clypeus with a longitudinal suture with the margin reaching the anterior border; antennae 12-jointed, with 3-segmented club; epinotum unarmed; major and

minor (1-2.5 mm) workers; color black or reddish....
..... MONOMORIUM Mayr.

12(13) Occiput straight; first flagellar segment equal to the second and third segments.....
..... Monomorium subopacum F. Smith.

13(12) Occiput not straight, first flagellar segment less than the second and third segments.....
..... Monomorium salomonis (Linnaeus)

14(11) Not as above

15(16) Antennae 11-jointed; eyes normal; major and minor workers, less than 3.5 mm.....
.....Tetramorium punicum sahlbergi Forel

16(15) Antennae 12-segmented; eyes rounded or oval; workers more than 3.5 mm.

17(20) Antennal club 3-segmented; major worker characterized by big head strongly incised at the base; epinotum dentate; big hairs in workers; border of mandibles with 2 teeth at the base and 2 stronger teeth at the extremity, unarmed in the middle, promesonotum rounded in the workers and soldiers.....
..... PHEIDOLE Westwood.

18(19) Head and occiput deeply incised medianly; head weakly striated...Pheidole pallidulla recticeps Forel.

19(18) Head and occiput not deeply incised medianly; head distinctly striated...Pheidole teneriffana Forel.

20(17) Antennal club little distinct, external borders of mandibles curved; workers (minor and major) with big

head; epinotum unarmed or slightly tuberculated; thoracic suture distinct; mandibles short and large. ..... Messor structor (Latreille).

Subfamily: Ponerinae

Subfamily Ponerinae is the most primitive one, ants are active and rapid moving. Social habits very weak, castes similar in size. Radial and median cells closed in the fore wing. Nests subterranean.

Distribution: Widely distributed, but reaches its highest development in the tropics and especially in Australia.

This subfamily is represented by one genus and one species:

Genus: Pachycondyla Smith

Pachycondyla Smith (1858) Cat. Hymen. Brit. Mus., 6(7):105.

Pachycondyla sennaarensis (Mayr)

Euponera sennaarensis Mayr (1862) Verh. Zool. Bot. Ges.Wien, Vol. 12, P. 721.

Pachycondyla sennaarensis Smith in Arnold (1915-1924) Ann.S. Africa Mus., 14: 53.

♂ : Head more or less transverse. Ocelli large; eyes very large, occupying nearly the whole of the sides of the head. Antennae long, scape shorter than the second joint of the flagellum. Node of peticle compressed laterally and smaller than in the ☿ .Wings very broad.

♀ and ☿ : Eyes situated in the anterior third of the sides of the head; promesonotal suture distinct; meso-epinotal suture obsolete above. Peticle generally with a thick,

squarish node. Claws simple.

Specimens examined: Kasseem (Brida); III, IV, 1985(22); Dwadmi, IV, 1985(17); Wadi Eldawaser, III, IV,1985(14).

Subfamily: Myrmicinae

Mandibles simple or toothed; eyes usually present; Wings with one or two closed cubital cells; gaster short; cerci present; sting well developed.

Injurious species, they are phytophagous and carnivorous or house-hold pests.

Distribution: It is widely distributed throughout the entire world.

Genus: Messor Forel

Messor Forel (1890) Ann. Soc. Ent. Belg., 34: IXX

Messor structor (Latreille)

Formica structor Latreille (1798) Ess. Hist. Fourm.France, VI: 46.

Aphaenogaster structor Roger(1863) Verz. Formicid. Gatt. Art., Berlin.

Messor barbarus structor Latreille in Alfieri (1931) Bull. Soc. Ent. Egypte, XV: 42-48.

Messor Structor Latreille in Pinzi (1936) Bull. Soc. Ent. Egypte, XX: 157.

Specimens examined: Wadi El Dawaser, III, IV, 1985 (8).

Genus: Monomorium Mayr

Monomorium Mayr (1855) Verh. Zool., Bot. Ges. Wien, V:452.

Monomorium salomonis (Linnaeus)

Formica salomonis Linnaeus (1758) Sys. Nat., I.

Monomorium Salomonis Roger (1862) Neut. Ent. Zeit. :253

Specimens examined: Riyadh, II, 1985(12); Jeddah, III, 1985 (12); Shagra, III, IV, 1985(17); Thadeg, IV, 1985(16); Wadi El Dawaser, III, IV, 1985(11); Kasseem (Brida), III, IV, 1985(19); Skaka, IV, 1985(16).

Monomorium Subopacum F.Smith

Monomorium subopaca F. Smith (1858) Cat. Hym. Brit. Mus. VI: 127.

Monomorium subopacum mediterraneum Emery (1881) Ann. Mus. Civic. Storia. Nat. Genov. XVI: 525.

Specimens examined: Riyadh, II, 1985(12); Jeddah, III, 1985 (19); Shagra, III, IV, 1985(22); Thadeg, IV, 1985(16); Wadi El Dawaser, III, IV, 1985(13); Kasseem (Brida), III, IV, 1985(12); Skaka, IV, 1985(11).

Genus: Pheidole Westwood

Pheidole Westwood (1841) Ann. Mag. Nat. Hist., VI: 87.

Pheidole pallidula recticeps Forel

Pheidole pallidula recticeps Forel (1909) Bull. Soc. Vaud. Sc. Nat., 45: 391.

Specimens examined: Riyadh, II, 1985(17); Shagra, III, IV, 1985(9); Thadeg, IV, 1985(11); Skaka, IV, 1985(7).

Pheidole teneriffana Forel

Pheidole teneriffana Forel (1892) Ann. Soc. Ent. Belg.; 463.

Specimens examined: Riyadh, II, 1985(5); Shagra, III, IV, 1985(9); Thadeg, IV, 1985(6); Skaka, IV, 1985(8).

Genus: Tetramorium Mayr

Tetramorium Mayr (1855) Verh. Zool. Bot. Ges. Wien, V:423.

Tetramorium punicum sahlbergi Forel

Tetramorium punicum sahlbergi Forel (1913) Rev. Suisse Zool., XXI: 431.

Specimens examined: Riyadh, II, 1985(7).

Subfamily: Formicinae

Mandibles broad and dentate or slender and pointed; clypeus separated by a suture in the frontal space; venation more or less reduced. Nests are constructed in diverse places, in the soil, in logs, in crevices of plants and even in houses.

Distribution: This subfamily is widely distributed and many genera are cosmopolitan.

Genus: Camponotus Mayr

Camponotus Mayr (1861) Europ. Formicid.: 35.

Camponotus compressus thoracicus Fabricius

Camponotus compressus thoracicus Fabricius(1804) Syst. Piez.: 397.

Specimens examined: Riyadh, II, 1985(27); Medina Munawara, III, 1985(15); Jeddah, III, 1985(12); Shagra, III, IV, 1985(16); Thadeg, IV, 1985(22); Wadi El Dawaser, III, IV, 1985(17).

Genus: Cataglyphis Foerster

Cataglyphis Foerster (1850) Verh. Naturh. Ver. Preuss. Rheinl., VII: 493.

Cataglyphis albicans livida (André)

Myrmecocystus albicans livida André (1881) Ann. Soc. Ent. Fr., 1:58.

Myrmecocystus albicans livida André (1882) Spec. Hym. Europe, 2:169.

Cataglyphis albicans livida Emery (1925) Gen. Insect. Formicidae: 262.

Specimens examined: Riyadh, II, 1985(13); Shagra, III,IV, 1985(9) ; Thadeg, IV, 1985(11); Kasseem (Brida), III, IV, 1985(16); Mekka, IV, 1985(7); Damman, IV, 1985(21); Dwadmi, IV, 1985(9); Wadi El Dawaser, III, IV, 1985(10).

Cataglyphis bicolor nodus (Brullé)

Formica nodus Brullé(1832) Exped. Sc. Moreé Zool., II:326.

Myrmecocystus viaticus André (1881) Ann. Soc. Ent. Fr., 1:56.

Myrmecocystus viaticus oreintalis Forel (1895) Mitt. Schweiz. Ent. Ges., IX: 288.

Cataglyphis bicolor nodus Emery (1926) Gen. Insect. Formicidae: 265.

Specimens examined: Riyadh, II, 1985(11); Abha, III, 1985(26); Shagra, III, IV, 1985(15); Thadeg, IV,1985(9); Kasseem (Brida), III, IV, 1985(16); Mekka, IV, 1985(13); Dwadmi, IV, 1985(16); Wadi El Dawaser, III,IV,1985(22); Skaka, IV, 1985(11).

Genus: Paratrechina Motschulsky

Paratrechina Motschulsky (1863) Spc. Nat. Moscow, Bull., 36:13.

Paratrechina Longicornis (Latreille)

Formica longicornis Latreille (1802) Hist. Nat. Fourm.: 113.

Formica vagans Jerdon (1851) Madras Jour. Litt. Sc., 17:124.

Prenolepis longicornis Roger (1863) erz. Formicid. :10

Paratrechina longicornis Emery (1925) Gen. Insect. Formicidae: 217.

Specimens examined: Riyadh, II, 1985(7).

DISCUSSION

It is obvious that zoogeographical studies of the Arabian peninsula and neighbouring regions will benefit from the results of this research. From this point of view, a knowledge of the fauna of Saudi Arabia is also of interest for zoologists studying the fauna of Africa, Europe and Asia.

The genera, species and subspecies reported in this work are confined to three zoogeographical regions, namely the Afrotropical (Ethiopian), Palaearctic and Oriental (Wittmer and Büttiker, 1979).

Specimens collected in this work from Saudi Arabia are more or less similar to those of Egypt (Hassanein, 1979), with the exception of Pachycondyla sennaarensis (Mayr) which is only represented in Saudi Arabia and not in Egypt.

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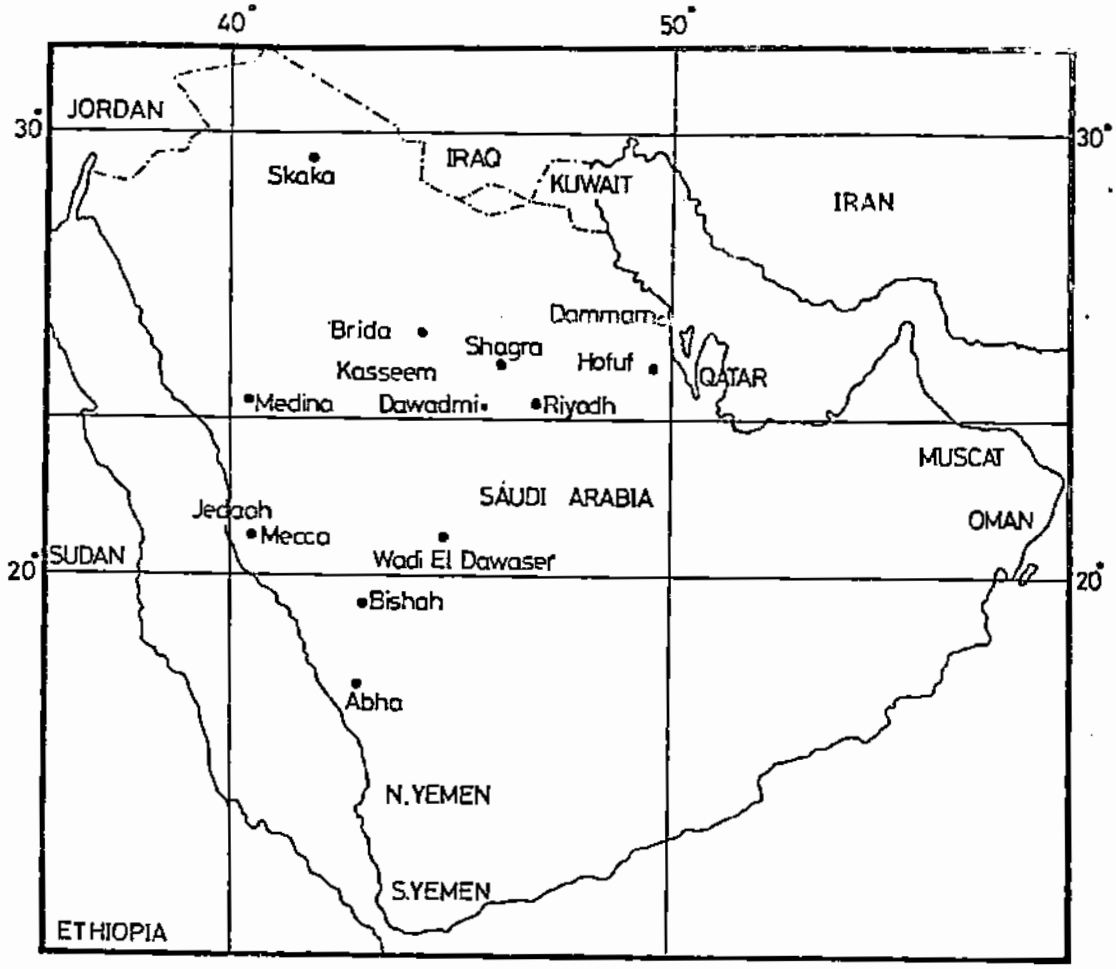


Fig.(1). Map of Saudi Arabia with main areas and locations of collecting activity

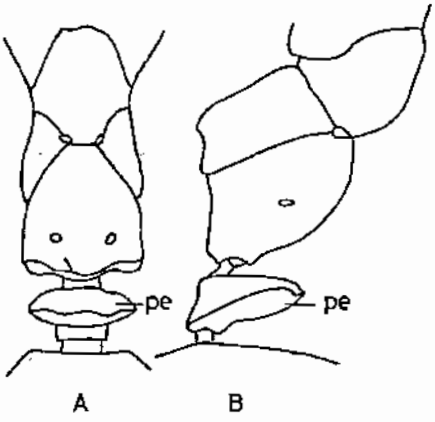


Fig. (3)

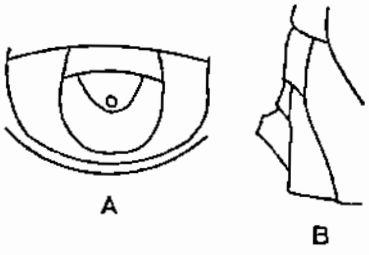


Fig. (2)

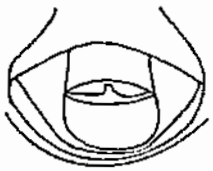
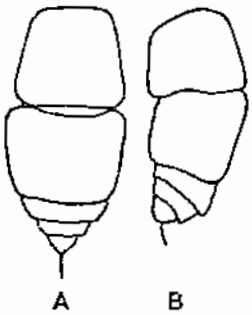


Fig (4)



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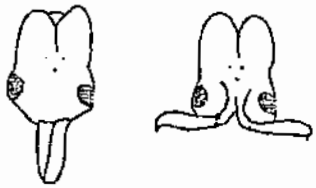

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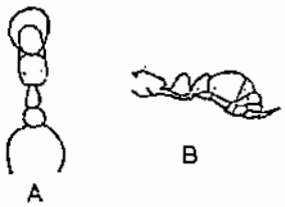

Fig. (7)

Fig. (8)

Fig. (2) Circular anal orifice, frontal(A) and side view(B).

Fig. (3) Abdominal pedicel (pe.), frontal(A) and side view(B).

Fig. (4) Slit-shaped anal orifice, frontal view.

Fig. (5) Gaster of Ponerinae, dorsal(A) and side view(B).

Fig. (6) Mandibles articulation in Ponerinae.

Fig. (7) Postpetiole of Myrmicinae, dorsal(A) and side view(B).

Fig. (8) Mandibles of Myrmicinae.

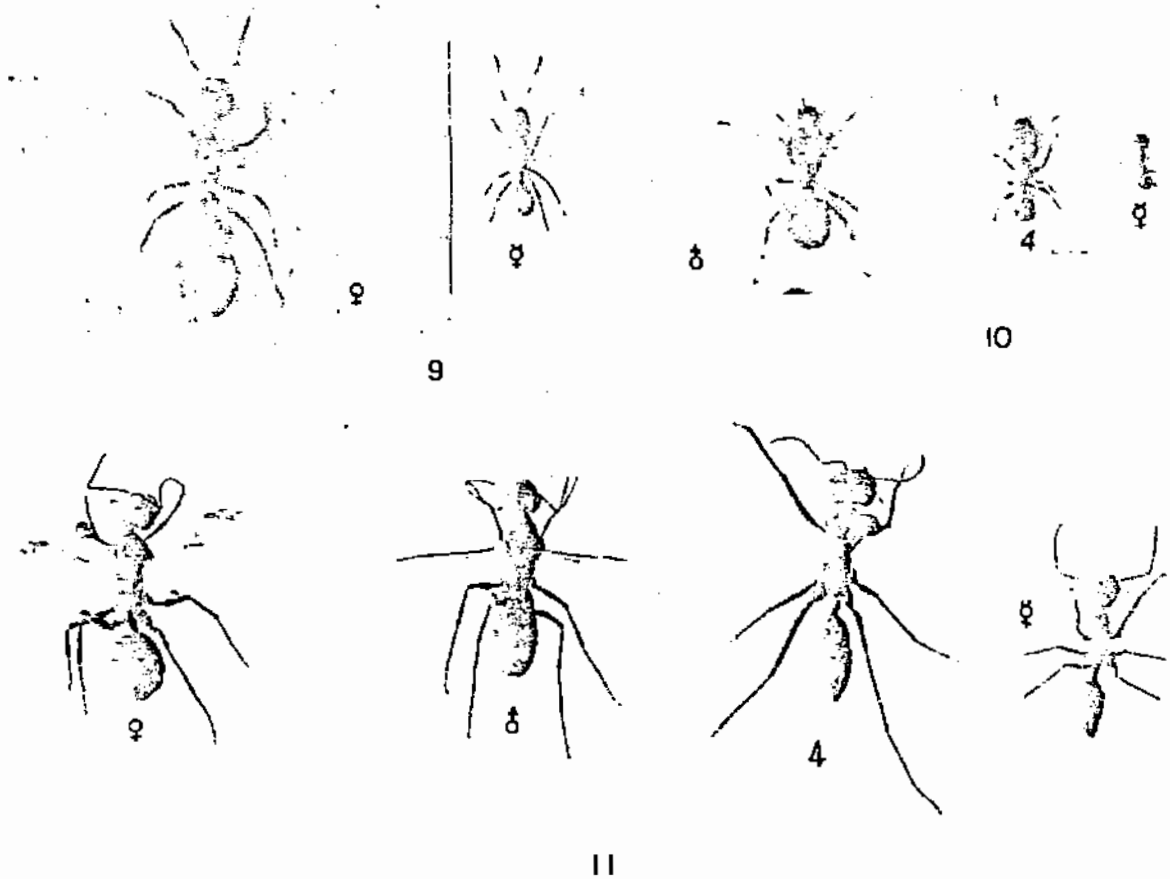

Fig. (9) Monomorium salomonis (Linnaeus)

Fig. (10) Pheidole pallidula recticeps Forel

Fig. (11) Cataglyphis bicolor nodus (Brullè)

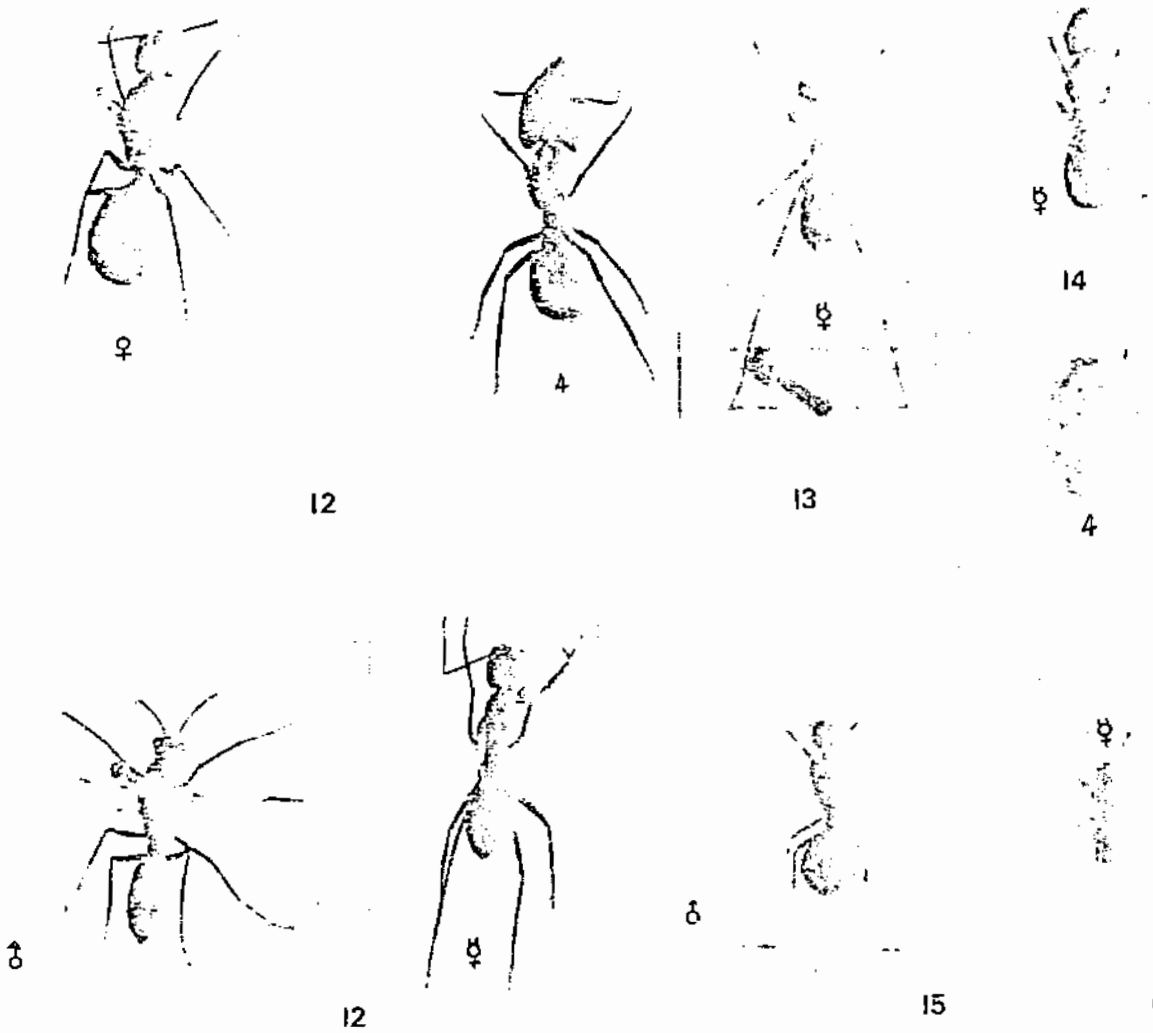

Fig. (12) Camponotus compressus thoracicus Fabricius

Fig. (13) Cataglyphis albicans livida (Andrè)

Fig. (14) Pheidole teneriffana Forel

Fig. (15) Tetramorium punicum sahlbergi Forel

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THE POTENCY OF CERTAIN DIGESTIVE ENZYMES PREPARED FROM THE WHITE RAT TREATED WITH THE CHEMICAL INSECTICIDE "TAMARON".

By

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INTRODUCTION

The present work is a part of a series of investigations which deal with the effect of the chemical insecticides on the physiological characterestics of mammals. One of these characterestics is the physiology of digestion. Gabr and Said (1972 A, B and C) found that some chemical insectioides (DDT, lindane and malathione) have a hazardous effect on the structure and function of the peptic and oxyntic cells. In 1972 Garb et al. studied the effect of the previously mentioned insecticides on the potency and activity of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic lipase in the white rat Rattus rattus. They found that the potencies and activities of these enzymes showed a considerable reduction. Said (1979), carried out experiments on the white rat

* The term potency designates the change in the activity of the enzyme prepared from insecticide treated animals.

Rattus rattus to study the effect of DDT, lindane and malathione on the potency of certain digestive enzymes (maltase and dipeptidase), which are secreted from the crypts of Lieberkühn. He found a reduction in the potencies of these enzymes. In 1981, Said experimented the previously mentioned insecticides on the potency of certain digestive enzymes (liver and ileum esterase and pancreatic lipase) prepared from the domestic pigeon Columba livia domestica. The enzymes of the 1/10 high dose (the approximately lethal dose) treated animals showed a considerable reduction in their potencies.

The present work aimed to study the effect of the chemical insecticide "tamaron" on the potency of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase) prepared from the white rat (Rattus rattus). This study might illuminate the way which leads to the clarification of one of the causes of the digestive troubles widely observed nowadays.

MATERIAL AND METHODS

A. Preparation of enzyme solution:

The animals used through this study were adult male white rats (Rattus rattus) weighing about 130 g each. All

the animals were kept on the same normal diet (bread, milk (NIDO) and water), during the experiment, to avoid the probable interference of the effect of food kind. The animals were kept in the laboratory in cages about one weak before use. In each experiment not less than 30 animals were used after killing them by a blow on their heads.

Certain hydrolytic enzymes (the salivary amylase, pepsin, trypsin, pancreatic amylase and pancreatic esterase) were prepared from these animals. Water extracts of fresh salivary (parotid) glands (1:10) stomach mucosa extract (1:10) and pancreatic extract (1:10) were used. Enterokinase was prepared from duodenal mucosa extract according to the prescription of Waldschmidt-Leitz (1924), treated with acetone and ether.

B. Buffers:

A number of different buffer solutions were used in the present work. The nature of each buffer used in the different experiments is indicated in the tables giving the experimental results. The measurement of the pH values was done by the B.D.H. capillator. The error in this method is about 0.05 pH units.

C. Methods of measurement of the enzymes activity:

1. Peptidases:

The method of Willstatter and Waldschmidt-Leitz(1921)

and Willstätter et al. (1926) was used (with some modifications), for measuring peptic and tryptic activities.

For pepsin, the digestive mixture (total volume 5.0 c.c.) had the following composition.

2.5 c.c. 3 % eggalbumen
1.0 c.c. citrate-HCl buffer
1.0 c.c. distilled water, and
0.5 c.c. enzyme solution

5.0 c.c. Total volume.

The incubation time of 20 h was chosen since it was found to be the most suitable one.

For trypsin, the digestive mixture was made in the following way:

0.3 c.c. enzyme solution
0.1 c.c. enterokinase solution
0.35 c.c. buffer solution (0.2 N ammonia-ammonium chloride).
0.15 c.c. distilled water.

The above mentioned solution were mixed together (total volume 0.90 c.c.) and the pH value of the mixture was 8.0 The mixture was put in the thermostate at 37°C for activation. After 30 minutes, the pH was adjusted to the desired value by the addition of 0.6 c.c. 0.1 N veronal

acetate buffer prewarmed to 37°C, after which 1.5 c.c. of 6 % casein solution were added. (Waldschmidt-Leitz, 1924). The total volume of the digestive mixture was therefore 3.0 c.c. The buffer solutions were prepared according to Michaelis (1922 and 1931). The time of incubation is half an hour.

2- Carbohydrases :

Amylase :

The method of Hagedorn and Jensen (1922) was used in the present work.

For salivary amylase, the digestive mixture had the following composition :

2.0 c.c. 2 % starch solution.
0.2 c.c. buffer solution (0.1N veronal-acetate-HCl)
1.3 c.c. distilled water (1.4 c.c. for pancreatic amylase).
0.5 c.c. enzyme solution (0.4 c.c. for pancreatic amylase).

4.0 c.c. Total volume.

The digestive mixture was incubated at 37°C for exactly 10 minutes. The titration sample is of 0.05 c.c. It has been shown by Pucker and Finch (1938) and confirmed by Keddis (1952 and 1956) that each mg maltose has the

same reducing action on potassium ferricyanide as 0.75 mg glucose. Using this factor, the extent of digestion was expressed as "increase in mg maltose" calculated by multiplying each figure of "increase in mg glucose" by the factor 100/75.

3- Esterases :

Esterase:

The method of Willstätter et al. (1923) was followed in the present work. The digestive mixture had the following composition :

1.00 c.c. enzyme solution.
0.05 c.c. ethyl acetate
1.00 c.c. 0.1N veronal-acetate buffer.
1.00 c.c. 2 % $CaCl_2$ and
1.95 c.c. distilled water.

5.00 c.c. Total volume.

The incubation time was one h at 37°C.

D- Estimation of the optimal pH value of the enzymes :

All the experiments of the present study were carried out at the optimal pH of the enzymes. The optimal activity of pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase were 2.1, 8.0, 6.8, 7.1 and 7.5 respectively (Hassan, 1983).

E- Effect of the chemical insecticide on the digestive enzymes :

For studying the effect of tamaron on the potency of the hydrolytic enzymes, 3 doses (1/10, 1/100 and 1/1000 high dose) were tested. The high dose in mg/kg. body weight/day was 30. This high dose is very near to the LD_{50} for tamaron (Hassan, 1983). The animals were given daily the 1/10 high dose, the 1/100 high dose or the 1/1000 high dose orally for 60 days.

RESULTS

a- Peptidases :

1- Pepsin :

Table I displays the data which concern the potency of pepsin prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 0.80 to 0.47 (=41.3 % reduction), the 1/100 high dose reduced the potency from 0.77 to 0.62 (=19.5 % reduction) and the 1/1000 high dose reduced the potency from 0.81 to 0.70 (=13.6 % reduction).

2- Trypsin:

Table 2 indicates the data which concern the potency of trypsin prepared from chemical insecticide treated animals as compared with those prepared from normal animals.

The 1/10 high dose reduced the potency from 0.97 to 0.78 (=19.6 % reduction) and the 1/100 high dose reduced the potency from 0.98 to 0.91 (=7.1 % reduction), while the 1/1000 high dose reduced the potency from 0.99 to 0.96 (=3.0 % reduction).

b- Carbohydrases:

1. Salivary amylase :

Table 3 shows the potency of the salivary amylase prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 0.120 to 0.061 (= 49.2 % reduction), the 1/100 high dose reduced the potency from 0.117 to 0.082 (=29.9 % reduction) and the 1/1000 high dose reduced the potency from 0.117 to 0.105 (=7.7 % reduction).

2. Pancreatic amylase :

Table 4 shows the potency of pancreatic amylase prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 0.133 to 0.061 (=54.2 % reduction), the 1/100 high dose reduced the potency from 0.131 to 0.083 (=36.6 % reduction) and the 1/1000 high dose reduced the potency from 0.133 to 0.112 (=15.8 % reduction).

c- Esterases :

Pancreatic esterase :

Table 5 exhibits the potency of pancreatic esterase prepared from tamaron treated animals as compared with those prepared from normal. The 1/10 high dose reduced the potency from 0.40 to 0.18 (=55 % reduction), the 1/100 high dose reduced the potency from 0.38 to 0.25 (=34.2 % reduction) and the 1/1000 high dose reduced the potency from 0.39 to 0.32 (=18.0 % reduction). Table 6 summarized all the previously mentioned results.

DISCUSSION

The results of the present investigation clearly show the reduction in the potencies of the digestive enzymes (pepsin, trypsin, salivary amylase, pancreatic amylase and pancreatic esterase) extracted from tamaron treated animals. These results could be explained as follows:

a) The enzymes of the 1/10 high dose treated animals, showed a considerable reduction in the potencies. It is clear that the pancreatic esterase was the most reduced by this chemical insecticide, while trypsin is the lowest reduced.

b) The enzymes of the 1/100 high dose treated animals, showed a lower reduction in their potencies as compared

with their correspondings for 1/10 high dose treated animals.

c) The enzymes of the 1/1000 high dose treated animals, showed a slight reduction in their potencies as compared with their correspondings for 1/10 and 1/100 high dose treated animals.

The previously mentioned results were in agreement with those obtained by other workers dealing with the effect of certain chemical insecticides on the potency of the digestive enzymes (Platonova, 1970; Gabr and Said A, Gabr et al. 1972; Said, 1979 and 1981).

SUMMARY

1. The enzymes of 1/10 high dosed treated animals with tamaron, showed a considerable reduction in their potencies. The potencies of pancreatic esterase and pancreatic amylase were the most reduced by tamaron, while that of trypsin is of the lowest reduction.
2. The enzymes of 1/100 high dosed treated animals, showed a lower reduction in their potencies than that of 1/10 high dosed treated animals. The potencies of pancreatic amylase and pancreatic esterase are the most reduced by the chemical insecticide, still that of trypsin is of the lowest reduction.
3. The enzymes of the 1/1000 high dosed treated animals, showed a lowest reduction in their potencies as compared

with their correspondings for 1/10 and 1.100 high dosed treated animals.

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Table 1

The potency of pepsin of tamaron treated white rat.

Digestive mixture 5.0 c.c. containing: 0.5 c.c. extract of stomach mucosa (1:10), 2.5 c.c. 3% eggalbumen, 1.0 c.c. citrate-HCl buffer and 1.0 c.c. distilled water.

Titration sample 0.5 c.c. Mean pH value 2.1 (initial pH 2.2 and final pH 2.0). Temperature 37°C . Time of digestion 20 hours.

Condition	Titration (c.c.0.1N KOH)		Potency c.c. 0.1 N KOH
	After 0 hour	After 20 hours	
Normal (control of 1/10 high dose)	5.88	6.68	0.80
Treated 1/10 high dose	6.87	7.34	0.47
Normal (control of 1/100 high dose)	5.91	6.68	0.77
Treated 1/100 high dose	6.69	7.31	0.62
Normal (control of 1/1000 high dose)	5.88	6.69	0.81
Treated 1/1000 high dose	6.66	7.36	0.70

Table 2

The potency of trypsin of tamaron treated white rat.

Digestive mixture 3.0 c.c. containing : 0.3 c.c. water extract of pancreas (1:10), 0.1* c.c. enterokinase solution, 0.35 c.c. 0.2 N ammonium buffer, 0.15 c.c. dist. H_2O, to which after 30 minutes were added 0.6 c.c. 0.1 N veronal-acetate buffer and 1.5 c.c. 6% casein.

Titration sample 0.5 c.c. pH value 8.0. Time of digestion one hour. Temperature 37°C.

Condition	Titration (c.c.0.02N KOH)		Potency c.c. 0.02N KOH
	After 0 hour	After one hour	
Normal (control of 1/10 high dose)	1.19	2.16	0.97
Treated 1/10 high dose	1.24	2.02	0.78
Normal (control of 1/100 high dose)	1.19	2.17	0.98
Treated 1/100 high dose	1.28	2.19	0.91
Normal (control of 1/1000 high dose)	1.18	2.17	0.99
Treated 1/1000 high dose	1.29	2.25	0.96

* Prepared from normal animals.

Table 3

The potency of salivary amylase of tamaron treated white rat.

Digestive mixture 4.0 c.c. containing: 0.5 c.c. patrotid glands extract (1:10), 2.0 c.c. 2% starch solution, 0.2 c.c. 0.1 N veronal-acetate buffer and 1.3 c.c. dist. H_2O.

Titration sample 0.05 c.c. Time of digestion 10 minutes. Temperature 37°C. pH value 6.8.

Condition	Digestion (mg glucose)		Potency	
	After 0 minute	After 10 minutes	mg glucose	mg maltose
Normal (control of 1/10 high dose)	0.032	0.122	0.090	0.120
Treated 1/10 high dose	0.029	0.075	0.046	0.061
Normal (control of 1/100 high dose)	0.032	0.120	0.088	0.117
Treated 1/100 high dose	0.031	0.093	0.062	0.082
Normal (control of 1/1000 high dose)	0.034	0.122	0.088	0.117
Treated 1/1000 high dose	0.034	0.113	0.079	0.105

Table 4

The potency of pancreatic amylase of tamaron treated white rat .

Digestive mixture 4.0 c.c. containing: 0.4 c.c. pancreatic extract (1:10), 2.0 c.c. 2% starch solution, 0.2 c.c. 0.1 N veronal-acetate buffer and 1.4 c.c. dist. H_2O.

Titration sample 0.05 c.c. Time of digestion 10 minutes. Temperature 37°C. pH value 7.1.

Condition	Digestion (mg glucose)		Potency	
	After 0 minute	After 10 minutes	mg glucose	mg maltose
Normal (control of 1/10 high dose)	0.124	0.224	0.100	0.133
Treated 1/10 high dose	0.129	0.175	0.046	0.061
Normal (control of 1/100 high dose)	0.124	0.222	0.098	0.131
Treated 1/100 high dose	0.129	0.191	0.062	0.083
Normal (control of 1/1000 high dose)	0.124	0.224	0.100	0.133
Treated 1/1000 high dose	0.127	0.211	0.084	0.112

Table 5

The potency of pancreatic esterase of tamaron treated white rat.

Digestive mixture 5.0 c.c. containing: 1.0 c.c. pancreatic extract, 0.05 c.c. ethyl acetate , 1.0 c.c. 0.1 N veronal acetate buffer, 1.0 c.c. 2% $CaCl_2$ and 1.95 c.c. dist. H_2O.

Titration sample 0.5 c.c.Time of digestion one hour. Temperature 37°C.pH value 7.5.

Condition	Titration (c.c.0.01N KOH)		Potency c.c. 0.01N KOH
	After 0 hour	After one hour	
Normal (control of 1/10 high dose)	1.14	1.54	0.40
Treated 1/10 high dose	1.18	1.36	0.18
Normal (control of 1/100 high dose)	1.20	1.58	0.38
Treated 1/100 high dose	1.19	1.44	0.25
Normal (control of 1/1000 high dose)	1.17	1.56	0.39
Treated 1/1000 high dose	1.19	1.51	0.32

Table 6

Effect of the chemical insecticide "tamaron" on the potency of the digestive enzymes.

Enzymes	Percentage of reduction of the potency of the extract of treated animals		
	1/10 high dose	1/100 high dose	1/1000 high dose
Pepsin	41.3	19.5	13.6
Trypsin	19.6	7.1	3.0
Salivary amylase	49.2	29.9	7.7
Pancreatic amylase	54.2	36.6	15.8
Pancreatic esterase	55.0	34.2	18.0

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Further studies on the effect of the chemical insecticide "Tamaron" on the activity of certain digestive enzymes in the White Rat.

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Introduction

It is a well known fact that chemical insecticides have a hazardous effect on man and useful animals, when these chemical insecticides find their way into the body in amounts very far before the lethal doses and accumulated in the body. These hazardous effects are variable and do concern the different body systems and organs.

The present study is a contribution to what have been carried out by other workers in the field of the so called digestive toxicology. Webb (1948); Jensen et al (1949); Hartley and Kilby (1950); Jensen and Balls (1952); Mounter et al (1957), Ooms (1961); Dombrovskii et al (1965), Gabr and Said (1972 B); Gabr et al (1972); Bunyan and Jennings (1976), Said (1979 & 1981), studied the effect of different chemical insecticides on the activity of some digestive enzymes. He found that the activity of these enzymes showed a considerable inactivation in vitro.

The present work was carried out on the white rat (Rattus rattus) and dealt with the direct effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase).

Material and Methods

A- The animals used through this study were adult male white rats (Rattus rattus) weighing about 130 g each. Certain hydrolytic enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase) were prepared from these animals.

All the animals were kept on the same normal diet, during the experiment, to avoid the probable interference of the effect of food kind. This diet consisted of bread, milk (NIDO) and water. The animals were kept in the laboratory in cages about one week before use. For obtaining the digestive juices, free from food remains, animals were starved for at least two days. In each experiment not less than 30 animals were used after killing them by a blow on their heads.

Water extracts of fresh pancreatic extract (1: 10) and intestinal mucosa extract (1:10) were used (Said,1979).

B- Buffers:

A number of different buffer solutions were used in the present work. The nature of each buffer used in the different experiments is indicated in the tables giving the experimental results. The measurement of the pH values was done by the B.D.H. capillator. The error in this method is about 0.05 pH units.

C- Methods of measurement of the enzyme activity:

1- Pancreatic lipase:

The method of Willstätter et al (1923) was followed in the present work. The digestive mixture (total volume 1.5 c.c.) had the following composition:

0.140	c.c.	olive oil
0.500	c.c.	0.2 N veronal-acetate buffer
0.025	c.c.	2 % $CaCl_2$
0.025	c.c.	3 % egg albumen
0.310	c.c.	dist. H_2O
0.500	c.c.	enzyme solution.
1.5	c.c.	Total volume.

The incubation time was one hour at 37°C.

2- Ileum dipeptidase:

The method of Willstätter and Waldschmidt-Leitz 1921, and Willstatter et al 1926, was used with

some modification for measuring dipeptidase activity The digestive mixture had the following composition:

1.0	c.c.	2 % glycylglycine solution
1.0	c.c.	0.1N veronal-acetate-HCl buffer
0.5	c.c.	dist. H_2O
1.0	c.c.	enzyme solution
3.5	c.c.	Total volume.

The incubation time was one hour at 37 °C.

3- Ileum maltase:

The method of Hagedorn and Jensen (1922), was used in the present work. The digestive mixture had the following composition:

2.0	c.c.	0.4 % maltose solution
0.8	c.c.	0.1 N veronal-acetate-HCl buffer
0.8	c.c.	dist. H_2O
0.4	c.c.	enzyme solution
4.0	c.c.	Total volume

The digestive mixture was incubated at 37 °C. for exactly 10 minutes. The titration sample was 0.1 c.c.

D- Estimation of the optimal pH values of the enzymes:

All the experiments of the present study were carried

out at the optimal pH of the enzymes. The optimal activity of pancreatic lipase, ileum dipeptidase and ileum maltase were 7.2, 8.1 and 7.3, respectively.

E- Effect of the chemical insecticide on the digestive enzymes:

For studying the effect of the chemical insecticide "tamaron" on the activity of the hydrolytic enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase), three doses (1/10, 1/100 and 1/1000 high dose) were tested.. The high dose was 30 mg/kg body weight/day. The high dose is very near the LD_{50} for the chemical insecticide (Hassan, 1983). The doses of the insecticide were added to the digestive mixture instead of a part of distilled water.

Results

1- Pancreatic lipase:

Table I displays the data which concern the effect of tamaron on the activity of pancreatic lipase (in c.c. 0.1 N KOH) prepared from normal white rats. The 1/10 high dose reduced the activity from 0.63 to 0.13 (= 79.4 % inactivation), the 1/100 high dose reduced the activity from 0.63 to 0.32 (= 50 % inactivation)

and the 1/1000 high dose reduced the activity from 0.63 to 0.43 (= 31.8 % inactivation).

2- Ileum dipeptidase:

Table 2 exhibits the data which concern the effect of tamaron on the activity of ileum dipeptidase (in c.c. 0.01 N KOH) prepared from normal animals. The 1/10 high dose reduced the activity of dipeptidase from 1.28 to 0.87 (= 32 % inactivation), the 1/100 high dose reduced the activity from 1.28 to 1.01 (=21 % inactivation) and the 1/1000 high dose reduced the activity from 1.28 to 1.13 (= 12 % inactivation).

3- Ileum maltase:

Table 3 displays the data which concern the effect of tamaron on the activity of maltase (in mg glucose) prepared from normal animals. The 1/10 high dose reduced the activity from 0.112 to 0.065 (= 42 inactivation), the 1/100 high dose reduced the activity from 0.112 to 0.084 (= 25 % inactivation) and the 1/1000 high dose reduced the activity from 0.112 to 0.092 (= 18 % inactivation).

Discussion

The effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pancreatic lipase,

ileum dipeptidase and ileum maltase) of the white rat Rattus rattus, was carried out. The doses applied were 1/10, 1/100 and 1/1000 of the approximate lethal dose of tamaron. The approximate lethal dose of tamaron was 30 mg/kg body wt./day. These data (Tables 1-3) indicate the following:

Concerning the direct effect of tamaron on the activity of the digestive enzymes it could be noticed that:

a) The 1/10 high dose produced a considerable inactivation to all enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase). Pancreatic lipase seems to be the most sensitive to the inactivation effect produced by the chemical insecticide, whereas ileum dipeptidase seems to be the most resistible to such an inactivation.

b) The 1/100 high dose of tamaron produced a lower effect on pancreatic lipase, ileum dipeptidase and ileum maltase. Pancreatic lipase showed a considerable inactivation while ileum dipeptidase seems to be the most resistible to the inactivation effect produced by this chemical insecticide. It is clear that the inactivation effects for the 1/100 high dose are lower than their correspondings for the 1/10 high dose.

c) The 1/1000 high dose of the chemical insecticide generally

have a week effect on pancreatic lipase, ileum dipeptidase and ileum maltase, yet pancreatic lipase and ileum maltase showed a considerable inactivation. It is clear that the inactivation effects for the 1/1000 high doses were lower than their correspondings for the 1/10 and 1/100 high doses.

The above mentioned results which concern the inactivation of the experimented on digestive enzymes, through the effect of the applied chemical insecticide "taamaron" agree with the results obtained by other workers dealing with the effect of certain chemical insecticides on the digestive enzymes (Webb, 1948; Jansen et al, 1949; Hartley and Kilby, 1950; Jansen and Balls, 1952; Mounter et al, 1957; Ooms,1961; Dombrovskii et al, 1965; Gabr and Said, 1972 B., Gabr et al, 1972; Bunyan and Jennings, 1976 and Said, 1979 & 1981.

Summary

The effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase) of the white rat Rattus rattus was carried out. The doses applied were 1/10, 1/100 and 1/1000 of the approximate lethal dose of the chemical insecticide. The approximate lethal dose of

tamaron for rats was 30 mg/kg body wt./day.

The high dose produced a considerable inactivation to all the enzymes studied. Pancreatic lipase and ileum maltase seem to be the most sensitive to the inactivation effect, while ileum dipeptidase seems to be the most resistable.

The 1/100 high dose produced an effect lower than that produced by the 1/10 high dose, in all the enzymes studied. Pancreatic lipase and ileum maltase seem to be the most sensitive ones while ileum dipeptidase is the most resistible.

The 1/1000 high dose produced a very slight effect on the enzymes studied. Pancreatic lipase showed a considerable inactivation.

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Table I

Effect of tamaron on the activity of pancreatic lipase of normal rat.

Condition	Digestive mixture at 37°C, pH value 7.2 and time of digestion one hour.	Titration (c.c. 0.1N KOH)		Activity c.c. 0.1N KOH
		After 0 hour	After one hour	
Normal (control)	0.14 c.c. olive oil, 0.5 c.c. 0.2 N veronal-acetate buffer, 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % egg albumen, 0.31 c.c. dist. H_2O and pancreatic extract (1 ; 10).	2.71	3.34	0.63
1/10 high doae	0.14 c.c. olive oil , 0.5 c.c. 0.2 N veronal-acetate buffer, 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % egg albumin , 0.07 c.c. tamaron in H_2O , 0.24 c.c. dist. H_2O and 0.5 c.c. pancreatic extract (1 : 10).	2.75	2.88	0.13
1/100 high dose	0.14 c.c. olive oil, 0.5 c.c. 0.2 N veronal-acetate buffer, 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % egg albumen, 0.07* c.c. tamaron in H_2O, 0.24 c.c. dist. H_2O and 0.5 c.c. pancreatic extract (1:10).	2.78	3.10	0.32
1/1000 high dose	0.14 c.c. olive oil, 0.5 c.c. 0.2 N veronal-acetate buffer , 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % egg albumen , 0.07** c.c. tamaron in H_2O , 0.24 c.c. dist. H_2O and 0.5 c.c. pancreatic extract (1:10).	2.74	3.17	0.43

* The concentration of this preparation is (1/10) of that used for the 1/10 high dose.
** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 2

Effect of tamaron on the activity of ileum dipepetidase of normal rat.

Condition	Digestive mixture at 37℃, pH value 8.1, time of digestion one hour and titration sample 0.5 c.c.	Titration (c.c. 0.01N KOH)		Activity c.c. 0.01N KOH
		After 0 hour	After one hour	
Normal (control)	1.0 c.c. 2 % glycylglycine solution, 1.0 c.c. 0.1 N veronal-acetate buffer, 0.5 c.c. dist. H_2O and 1.0 c.c. extract of ileum mucosa (1: 10).	1.91	3.19	1.28
1/10 high dose	1.0 c.c. 2 % glycylglycine solution, 1.0 c.c. 0.1N veronal-acetate buffer, 0.07 c.c. tamaron in H_2O, 0.43 c.c. dist. H_2O and 1.0 c.c. extract of ileum mucosa (1:10).	1.86	2.73	0.87
1/100 high dose	1.0 c.c. 2% glycylglycine solution, 1.0 c.c. 0.1N veronal-acetate buffer, 0.07* c.c. tamaron in H_2O, 0.43 c.c. dist. H_2O and 1.0 c.c. extract of ileum mucosa (1:10).	1.89	2.90	1.01
1/1000 high dose	1.0 c.c. 2% glycylglycine solution, 1.0 c.c. 0.1N veronal-acetate buffer, 0.07** c.c. tamaron in H_2O, 0.43 c.c. dist. H_2O and 1.0 c.c. extract of ileum mucosa (1:10).	1.90	3.03	1.13

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

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Table (3)

Effect of tamaron on the activity of ileum maltase of normal rat.

Condition	Digestive mixture at 37°C , pH value 7. 3, time of digestion 10 minutes and titration sample 0. 1 c. c.	Digestion (mg glucose) After 0 minute	Digestion (mg glucose) After 10 minutes	Activity mg glucose
Normal (control)	2. 0 c. c. 0. 4% maltose solution, 0. 8 c. c. 0. 1 N veronal-acetate buffer, 0. 8 c. c. dist. H_2O and 0. 4 c. c. extract of ileum mucosa (1:10).	0. 158	0. 270	0. 112
1/10 high dose	2. 0 c. c. 0. 4 % maltose solution, 0. 8 c. c. 0. 1N veronal-acetate buffer, 0. 07 c. c. tamaron in dist. H_2O , 0. 73 c. c. dist. H_2O and 0. 4 c. c. extract of ileum mucosa (1:10).	0. 155	0. 220	0. 065
1/100 high dose	2. 0 c. c. 0. 4% maltose solution, 0. 8 c. c. 0. 1N veronal-acetate buffer, 0. 07* c. c. tamaron in dist. H_2O , 0. 73 c. c. dist. H_2O and 0. 4 c. c. extract of ileum mucosa (1:10).	0. 160	0. 244	0. 084
1/1000 high dose	2. 0 c. c. 0. 4 % maltose solution, 0. 8 c. c. 0. 1N veronal-acetate buffer, 0. 07** c. c. tamaron in dist. H_2O, 0. 73 c. c. dist. H_2O and 0. 4 c. c. extract of ileum mucosa (1:10)	0. 162	0. 254	0. 092

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.
** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

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TAXONOMIC STUDIES OF SOME ANTS (FORMICIDAE-HYMENOPTERA) FROM LIBYA

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ABSTRACT

This work deals with the taxonomy of four subfamilies, Ponerinae, Dorylinae, Myrmicinae and Formicinae(Family: Formicidae, Order: Hymenoptera)collected from different areas in Libya comprising 14 geners and 26 species and subspecies. Diagnosis and keys accompanied with illustrations are given to these species and subspecies.

INTRODUCTION

Libya is still one of the few countries where very little investigations on the general entomofauna have been carried out. A vast field of study is still open to the entomologists interested in many spheres of research. In particular, the taxonomist will find ample opportunities to collect specimens in biotopes of arid, semi-arid, mountaineous and coastal areas for morphological and taxonomical investigations.

The aim of the present work is to identify some species and subspecies collected from Libya for a period

of one year (1986). Several authors studied and revised ants from different regions as André (1881); Emery(1901, 1908, 1909, 1912 and 1925); Forel (1902); Santschi(1908, 1912, 1915, 1921, and 1929); Finzi (1930); Menozzi(1930-1931) and (1931); Donisthorpe (1947); Bernard (1956); and Hassanein (1979).

MATERIALS AND METHODS

Ants were collected by hand from gardens, houses, deserts and mountains (Fig. 1) and were reserved in alcohol for future identification. Different habitats were represented in the survey. The specimens were examined and identified accurately from different collections present in Egypt. Localities of each species are cited and the number of specimens examined is found between brackets.

RESULTS

Specimens collected from Libya is confined in four subfamilies: Ponerinae, Dorylinae, Myrmicinae and Formicinae.

Key to subfamilies, genera, species and subspecies of ants in Libya

1(2) Eyes absent or vestigial, pedicel usually of 2 segments in the worker (one in female and male); clypeus short; frontal carina short and vertical, not covering the antennal insertions; antennae usually short, epinotum usually unarmed (Fig. 2), promesonotal

suture weak or absent; palps of two or three segments ..... DORYLINAE

—— Male very long, about 40 mm. long; yellowish brown; female small blind; antennae 13 segments; workers strongly dimorphic .....Dorylus fulvus Westwood

2(1)Without this combination of characters.

3(12)Opening of posterior end of gaster (acidopore) terminal,circular and usually surrounded by a fringe of hairs (Fig. 3), sting vestigial; petiole usually scale like, one-segmented (Fig. 4)...FORMICINAE

4(5)Antennae 11-segmented in ☿ and ♀, and 12-segmented in males; size very small; ☿ 1-2 mm.; body brilliant yellowish brown .... Plagiolepis pallescens maura atlantis Santschi

5(4)Antennae 12-segmented in ☿ and ♀, and 13-segmented in males; size more than 3 mm. Reddish brown to black species.

6(9)Insertion of antennae distant from posterior margin of clypeus.

7(8) ☿ ☿ monomorphic; petiole, epinotum and pronotum always spinose or dentate; head broadly oval; clypeus with distinct medial vertical carina, anterior margin incised in the middle; antennal carina ? wide apart..... Polyrhachis simplex Mayr

8(7) ☿ ☿ Polymorphic; petiole, epinotum and pronotum unarmed; reddish black species; head broad ±

excised behind, narrower in front, very convex above, flattened beneath..... Camponotus sericeus(Fabricius)

9(6) Insertion of antennae close to the posterior margin of clypeus.

10(11) Funiculus long with the second segment twice longer than large; maxillary palps very long with the fourth segment twice the length of the fifth; thorax narrower than head; scale thick obtuse at apex.. .....Cataglyphis Foerster

a- Yellowish red species; body covered with fine white hairs; head rounded in the occipital region behind eyes..... C.albicans (Roger)

b- Reddish species; body coated with velvet silvery hairs; head straight in the occipital region behind eyes..... C.bombycina (Roger)

c- Blackish, mat species; head longer than broad; second funicular segment equal to the following segments; head reddish....C.bicolor(Fabricius)

11(10) Funiculus and maxillary palps shorter; the fourth maxillary palp segment about equal to the fifth; antennal cavities separated from clypeus; dark brown species..... ..... Paratrechina longicornis (Latreille)

12(3) Opening at posterior end of gaster (cloacal orifice) slit-like; sting well developed; petiole one or two segmented, if one segmented, a distinct constriction

exists between first and second gastral segments; mandibles very long, linear, narrow and sharp-pointed.

13(14) One segmented petiole; a distinct constriction exists between first and second gastral segments (Fig. 5). Pupa enclosed in cocoon... PONERINAE

—— Maxillary palp one or two segments; head without eyes or ocelli; epinotum rounded apically; without teeth; abdomen with the first 2 segments broad.... ..... Ponera ragusae santschii Emery

14(13) Petiole composed of two distinct segments; gaster with no constriction (Fig. 6). Pupa naked..... ..... MYRMICINAE

15(22) Epinotum armed; antennae 10-13 segments; club distinct more than 3 segments.

16(17) ♂ fore wing with 2 cubital cells, radial cell closed; discoid cell present; mesothorax with distinct Mayrian furrows; petiole very shortly pedunculate, first node wedge shaped or conical; border of mandible with 2 teeth at the base and 2 teeth at the extremity, unarmed in the middle; major workers with hig head strongly incised at the base; 3-4.mm., first abdominal segment larger than the rest ..... Pheidole Westwood

a- Head and occiput deeply incised medianly;head weakly striated.....P.pallidula recticeps Forel

b- Head and occiput not deeply incised medianly, head distinctly striated...P.teneriffana Forel

c- Head trapezoidal; occiput straight, sides rounded; head striated, the striae superficial in front, deep and large along the sides specially on the cheeks and around the eyes..... ..... P.sinaitica Mayr

17(16) ♂ fore wing with 1 cubital cell; mesonotum without or with distinct Mayrian furrows at least in the anterior third; petiole pedunculate; nodes of petiole variable; mandibles with normal teeth.

18(19) ♂ Mesonotum without Mayrian furrows, no radial or discoidal cells. ♀ , ☿ Antennae 12-segmented,club 4-5 segments; eyes rounded; length more than 3.5 mm.; petiole with a long peduncle... ..... Cardiocondyla nuda mauritanica Forel.

19(18) ♂ Mesonotum with distinct Mayrian furrows;one discoidal and closed radial cells present.
♀ , ☿ Antennae 11-jointed, club 3-segments; eyes normal; length less than 3.5 mm.

20(21) ♂ Antennae 10-segmented, second flagellar segment very long; shoulders angular; posterior margin of clypeus raised in a carina along the antennal fossettes..... Tetramorium simillimum (F.Smith)

21(20) ♂ Antennae 12-13 segmented, second flagellar segment short; shoulders rounded, clypeal margin not carinated....Leptothorax angulatus (Mayr)

22(15) Epinotum unarmed; antennae 10-12 jointed, club 2-3 segmented or indistinct.

23(24) Antennae 10-11 jointed with distinct club 2-segmented; workers very small, 3-5 mm., clear yellow ..... Solenopsis lou (Forel)

24(23) Antennae 11-12 segments, club distinct, 3-segmented or indistinct.

25(26) Antennae with distinct 3-segmented club; mandibles narrow with 3-4 acute teeth; clypeus with longitudinal suture with the margin reaching the anterior border; first joint of petiole shortly pedunculate; petiole pyramid shaped; postpetiole globular, smaller than petiole; minor and major workers (1-2.5 mm)..... Monomorium Mayr

a- Yellowish red; occiput straight; first flagellar segment less than the second and third segments; petiole cuneiform rounded..... ..... M.pharaonis (Linnaeus)

b- Reddish black; occiput straight; first flagellar segment equal to the second and third segments; petiole pyramid like..... ..... M. Subopacum phoenicia Emery

c- Reddish brown; occiput not straight, first flagellar segment less than the second and third segments; petiole somewhat raised than postpetiole ..... M.salomonis (Linnaeus).

—— Length more than 3 mm.; head and gaster black,head glazy, thorax mat, dark brown..... ..... M. salomonis sommieri Emery

—— Length 4.5 mm.; head and gaster brown or brown pitchy, head and thorax subbrilliant, thorax red testaceous to brown....M.salomonis obscurata Stitz

—— Length 3-3.4 mm.; head and gaster black glazy, thorax dark red.... M. Salomonis didonis Santschi

26(25) Antennal club little distinct; mandibles short, large with the two anterior teeth strong and blunt; clypeus narrow; workers minor and major with big head; first segment of petiole conical, pedunculate; second segment transversely globose, thoracic suture distinct; ☿ 4-13 mm. in length ..... Messor Forel

a- Yellowish red species; gula without hairs; promesonotum not gibbous..... ..... M. rufotestaceus (Foerster)

b- Black species with slightly reddish colour in the thorax; epinotum slightly tuberculate with very short teeth.....M.aegyptiacus Emery

c- Body black, legs blackish; head larger than long; occiput more or less emarginate .... M.aegyptiacus foreli Santschi

d- Head and thorax red, gaster usually reddish black to black; epinotum slightly tuberculate

with pointed teeth....M.arenarius (Fabricius)

Subfamily: Ponerinae

The colonies are mostly small, ants are all predaceous and carnivorous, their pupae are always enclosed in cocoons, and a well-developed sting is always present in the worker and female.

Distribution: Widely distributed, especially in the tropics and Australia.

This subfamily is represented by one genus and one species:

Genus: Ponera Latreille

Ponera Latreille (1805) Nouv. Dict. d'Hist. Nat., 24:178.

Ponera ragusae santschii Emery

Ponera ragusae Santschii Emery (1909) Deutsch. Ent. Zeit.: 371.

Specimens examined: Beni Gazi, IX ,1986(5); El Beida, IX, 1986(7); Musrata, IX, 1986(10).

Subfamily: Dorylinae

The males of the drivers are winged and with eyes, they are frequently taken at lights, the workers are blind, and the females are both blind and wingless. Members of genus Dorylus are almost entirely subterranean, rarely coming to the surface except in dull, cloudy weather. They do not make permanent nests. The three castes are entirely different from one another. The queens are excessively rare and known only in a few

species, and the males have been taken in company with their workers.

Distribution: Small subfamily of mostly tropical species, some of which occur in the temperate regions of Africa and North and South America.

This subfamily is represented by one genus and one species:

Genus: Dorylus Fabricius

Dorylus Fabricius (1793) Ent. Syst., II: 194.

Dorylus fulvus Westwood

Dorylus fulvus Westwood (1840) Intr. Class. Ins. II:219.

Dorylus fulvus punicus Santschi (1926) Bull. Soc. Hist. Nat. Afr. N., XVII: 230.

Dorylus fulvus ruficeps Santschi (1926) Bull. Soc.Hist. Nat. Afr. N., XVII: 232.

Specimens examined: Tarhuna, VI, 1986 (11).

Subfamily: Myrmicinae

This subfamily is divided into a large number of genera, the majority of which are cosmopolitan. All workers possess a sting, and the pupae are always naked. Petiole 2-jointed in all the sexes, the second joint very rarely nearly as wide as the first abdominal segment.

This large and important subfamily contains many annoying and injurious species, and with numerous numbers of individuals. The larger species are called bulldog

ants, they bite their victims severely; mandibles simple or toothed, small or wide. Compound eyes present, rarely vestigial or absent, clypeus may or may not extend between frontal carinae. Reproductives usually winged;fore wing with one or two closed cubital cells. Male genitalia partly concealed or exerted.

Distribution: Widely distributed throughout the entire world.

Genus: Cardiocondyla Emery

Cardiocondyla Emery (1869) Ann. Acad. Nat. Napoli, II:20

Cardiocondyla nuda mauritanica Forel

Cardiocondyla nuda mauritanica Forel (1890) Ann. Soc.Ent. Belg. LXXV.

Specimens examined: El Gouf, IV, 1986(9); Tarhuna, V, 1986(6); Gagbub, V, 1986(7); Elaziziya, X, 1986(5).

Genus: Leptothorax Mayr

Leptothorax Mayr (1855) Verh. Zool. Bot. Ver. Wien, 5 : 431.

Leptothorax angulatus Mayr

Leptothorax angulatus Mayr (1862) Zool. Bot. Gesell. Wien : 739.

Specimens examined: ElGouf, IV, 1986(7); El Khoms, IV, 1986(7); Tripoli, IV, 1986(7); El Kofra, IV, 1986; Musrata, X, 1986(5); Sabha, XI, 1986(7).

Genus: Messor Forel

Messor Forel (1890) Ann. Soc. Ent. Belg., 34 : IXX.

Messor aegyptiacus Emery

Messor aegyptiacus Emery (1908) Deut. Ent. Zeit.: 451.

Specimens examined: Tripoli, III, 1986 (12); Tarhuna, VI, 1986 (9); Marzouk, V, 1986(9). Elbeida, IX, 1986 (12); Sert, IX, 1986 (9); Musrata, X,1986 (11).

Messor aegyptiacus foreli Santschi

Messor aegyptiacus foreli Santschi (1923) Rev. Suiss. Zool., 30: 323.

Specimens examined: Tarhuna, IV, 1986(7); Gagbub, V, 1986 (6); Marzouk, V, 1986 (6);Beni Gazi, IX, 1986(8); Sert, IX, 1986 (4); Sabha, XI, 1986 (9).

Messor arenarius (Fabricius)

Formica arenarius Fabricius (1787) Mant. Insect., I:307-311.

Aphaenogaster arenaria Roger (1863) Verz. Formicid.Gatt. Art., Berlin.

Messor arenarius Fabricius in Alfieri (1931) Bull. Soc. Ent. Egypte, XV.

Specimens examined: Tarhuna IV, 1986 (11); Elbeida, IX, 1986 (9); Sert, IX, 1986 (12); Musrata, X, 1986 (10); Elaziziya, X, 1986 (13).

Messor rufotestaceus (Foerster)

Myrmica rufotestacea Foerster (1850) Verh. Natur. Ver. Preuss. Rheinl., VII: 489.

Aphaenogaster rufotestacea Roger (1863) Verz. Formicid: 30.

Anhaenogaster gracilinodis Emery (1878) Ann. Mus. Stor. Nat. Genova, XII: 55.

Messor rufotestaceus Emery (1908) Deutsch Ent. Zeit.: 437.

Specimens examined: Tripoli, II, 1986 (6); Beni Walid, IV, 1986 (6); El Gouf, IV, 1986 (8); El Kofra, IV, 1986 (9); Marzouk, V, 1986 (12); Tarhuna, V, 1986 (10); Sabha, VI, 1986 (12); Beni Gazi, IX, 1986 (12); Elbeida, IX, 1986 (15); Sert, IX, 1986 (12).

Genus: Monomorium Mayr

Monomorium Mayr (1855) Verh. Zool. Bot. Ges. Wien, V:452.

Monomorium pharaonis (Linnaeus)

Formica pharaonis Linnaeus (1758) Syst. Nat., I

Myrmica domestica Shuckard (1838) Mag. Nat. Hist. II:226.

Monomorium pharaonis Mayr (1862) Verh. Zool. Bot., Wien, II: 649.

Specimens examined: El Khoms, III, 1986(11): Tripoli, III, 1985 (17); El Kofra, IV, 1986 (12); Beni Walid, IV, 1986 (15); El Gouf, IV, 1986 (9); Marzouk, V, 1986 (11); Tarhuna, VI, 1986 (22); Elbeida ., IX, 1986 (9); Sert, IX, 1986 (10); Beni Gazi, IX, 1986 (13); Sabha, IX, 1986 (16); Elaziziya, X, 1986 (17); Musrata, X, 1986 (17).

Monomorium salomonis (Linnaeus)

Formica salomonis Linnaeus (1758) Sys. Nat., I.

Monomorium salomonis Roger (1862) Deut. Ent. Zeit., VI:283.

Specimens examined: Tripoli, IV, 1986 (19); El Kofra, IV, 1986 (16); El Gouf, IV, 1986 (12); Beni Walid, V, 1986 (15); Tarhuna, V, 1986 (20); Beni Gazi, IX, 1986 (12) ;

Sert, IX, 1986 (15); Musrata, X, 1986 (13).

Monomorium salomonis didonis Santschi

Monomorium salomonis didonis Santschi (1921) Mem. R. Soc. Esp. Hist. Nat. : 425.

Specimens examined: El Khoms, III, 1986 (6); Tarhuna, III, 1986 (5); Marzouk, V, 1986 (6); Elbeida, IX, 1986 (7); Musrata, X, 1986 (12).

Monomorium salomonis obscurata Stitz

Monomorium salomonis obscurata Stitz (1916) Mitth. Zool. Mus. Berlin: 346.

Specimens examined: El Khoms, IV, 1986 (8); Beni Walid, IV, 1986 (6); Gagbub, V, 1986 (4); Beni Gazi, IX, 1986 (7); Sert, IX, 1986 (9); Elaziziya, X, 1986 (10).

Monomorium salomonis sommieri Emery

Monomorium salomonis sommieri Emery (1908) Deut. Ent. Zeit., : 676.

Specimens examined: El Khoms, IV, 1986 (4); El Kofra, IV, 1986 (6); El Gouf, IV, 1986 (7); Gagbub, V, 1986 (7); Sabha, XI, 1986 (6).

Monomorium subopacum phoenicia Emery

Monomorium salomonis subopacum phoenicia Emery (1908) Deut. Ent. Zeit. : 677.

Monomorium subopacum phoenicia Santschi (1927) Bull., Ann. Soc. Ent. Belg., 67: 242.

Specimens examined: El Kofra, IV, 1986 (7); El Gouf, IV, 1986 (5); Tripoli, IV, 1986 (6); Tarhuna,VI, 1986 (8); Elbeida, IX, 1986 (7); Sert, IX, 1986 (9); Musrata, X,

1986 (6); Elaziziya, X, 1986 (5).

Genus: Pheidole Westwood

Pheidole Westwood (1841) Ann. Mag. Nat. Hist., VI: 87.

Pheidole Pallidula recticeps Forel

Pheidole pallidula recticeps Forel (1909) Bull. Soc. Vaud. Sc. Nat., 45:391.

Specimens examined: Tropoli, III, 1985 (5); El Khoms, III, 1986 (9); Tarhuna, V, 1985 (7); Musrata, X, 1986(11).

Pheidole sinaitica Mayr

Pheidole sinaitica Mayr (1862) Verh. Zool. Bot. Gesell. Wien. : 745.

Specimens examined: Tripoli, IV, 1986 (12); El Gouf, IV, 1985 (8); El Khoms, IV, 1985 (6); Beni Walid, IV, 1986 (9); Gagoub, V, 1985 (7); Tarhuna, V, 1985 (9).

Pheidole teneriffana Forel

Pheidole teneriffana Forel (1892) Ann. Soc. Ent. Belg.: 465.

Specimens examined: Tripoli, II, 1985 (5); Tarhuna, V, 1986 (8); Beni Gazi, IX, 1985 (11); Sert, IX, 1985 (7); Elbeida, IX, 1986 (10).

Genus: Solenopsis Westwood

Solenopsis Westwood (1841) Ann. Mag. Nat. Hist. VI: 86.

Solenopsis lou (Forel)

Solenopsis lou Forel (1902) Ann. Soc. Ent. Belg. XLVI: 152.

Specimens examined: Elbeida, IX, 1986 (6); Elaziziya, X, 1985 (4).

Genus: Tetramorium Mayr

Tetramorium Mayr (1855) Verh. Zool.-Bot. Ges. Wien, V.423.

Tetremorium simillimum (F.Smith)

Myrmica simillima F. Smith (1854) Trans. Ent. Soc. Lond., III: 95.

Tetramorium simillimum Mayr (1861) Europ. Formicid. Wien,I.

Specimens examined: Tripoli III, 1986 (9); El Khoms, IV, 1986 (9); El Kofra, IV, 1986 (8); Marzouk, V, 1986 (7); Tarhuna, VI, 1986 (7); Elaziziya, X, 1986 (6); Musrata, X, 1986 (11).

Subfamily : Formicinae

It is the largest and most important subfamily. The members are variable in size, ranging from 2-20 mm. in length. Their habits, mental and social behaviour, and morphologically they are considered the most highly developed of all ants. They are vegetarian and feed on sugary substances, which are stored by the workers. Certain species of Polyrhachis and Camponotus build silk nests in leaves.

Distribution: This subfamily is widely distributed and many genera are cosmopolitan.

Genus: Camponotus Mayr

Camponotus Mayr (1861) Europ. Formicid. : 35.

Camponotus sericeus (Fabricius)

Formica sericea Fabricius (1798) Ent. Sys. Supplement:279.

Camponotus sericeus Mayr (1862) Verh. Zool. Bot., Wien, XII : 649.

Specimens examined: Tripoli, III, 1986 (11); El Gouf, IV, 1986 (9); El Khoms, IV, 1986 (12); El Kofra, IV, 1986 (7); Marzouk, V, 1986 (11); Tarhuna, V, 1986 (7); Beni Gazi, IX, 1986 (14); Elbeida IX, 1986 (13); Sert, IX, 1986 (11); Elaziziya, X, 1986 (13); Musrata, X, 1986 (6).

Genus: Catsglyphis Foerster

Cataglyphis Foerster (1850) Verh. Naturh. Ver. Preuss. Rheinl., VII: 493.

Cataglyphis albicans (Roger)

Formica albicans Roger (1859) Deut. Ent. Zeit., 3:225-259.

Cataglyphis albicans Roger (1863) Verz. Formicid.Gatt. Und Arten, Berlin.

Specimens examined: El Khoms, IV, 1986 (11); Sert, IX, 1986 (9); Elaziziya, X, 1986 (6); Musrata, X, 1986 (12); Sabha, XI, 1986 (13).

Cataglyphis bicolor (Fabricius)

Formica bicolor Fabricius (1787) Mantissa Insectorum, I:307-311.

Cataglyphis bicolor Fabricius in Alfieri (1931) Bull. Soc. Ent. Egypte, XV: 42.

Specimens examined: Beni Walid, IV, 1986 (11); El Gouf, IV, 1986 (12); El Khoms, IV, 1986 (11); Tripoli, IV, 1986 (14); Tarhuna, V, 1986 (16); Elbeida , IX, 1986 (12); Sabha, XI, 1986 (9).

Cataglyphis bombycina (Roger)

Formica bombycina Roger (1859) Deut: Ent. Zeit., III:225-259.

Cataglyphis bombycina Roger in Santschi (1929) Rev. Suiss. Zool. : 60.

Specimens examined: Tripoli, II, III, 1986 (17); El Khoms, III, IV, 1986 (16); Beni Walid, IV, 1986 (11); El Gouf, IV, 1986 (9); El Kofra, IV, 1986 (9); Gagbub, V, 1986 (13); Marzouk, V, 1986 (15); Tarhuna, V, VI, 1986 (11); Beni Gazi, IX, 1986 (16); Elbeida ., IX, 1986 (7); Sert, IX, 1986 (11); Musrata, X, 1986 (15); Sabha, XI, 1986 (13).

Genus: Paratrechina Motschulsky

Paratrechina Motschulsky (1863) Spc. Nat. Moscou, Bull., 36:13.

Paratrechina longicornis (Latreille)

Formica longicornis Latreille (1802) Hist. Nat. Fourm.:113.

Formica vagans Jerdon (1851) Madras Jour. Litt. Sc. , 17: 124.

Prenolepis longicornis Roger (1863) erz.Formicid.:10.

Paratrechina longicornis Emery (1925) Gen. Insect.Formicidae: 217.

Specimens examined:Elbeida, IX, 1986 (11); Beni Gazi,IX, 1986 (7); Sert, IX, 1986 (10); Elaziziya, X, 1986 (9).

Genus: Plagiolepis Mayr

Plagiolepis Mayr (1861) Europ. Formicid.: 42.

Plagiolepis pallescens maura atlantis Santschi

Plagiolepis pallescens maura atlantis Santschi (1920) Bull. Soc. Vaud. : 171.

Specimens examined: El Kofra, IV, 1986 (11); Beni Walid, IV, 1986 (9); El Gouf, IV, 1986 (13); Gagbub, V, 1986 (12); Marzouk, V, 1986 (4).

Genus: Polyrhachis Smith

Polyrhachis Smith (1858) Jour. Proc. Linn. Soc., XI:58.

Polyrhachis simplex Mayr

Polyrhachis simplex Mayr (1862) Verh. Zool. Bot. Ges. Wien. XII: 682.

Specimens examined: Tripoli, II, 1986 (7); El Khoms, III, 1986 (8); Tarhuna, VI, 1986 (6); Musrata, X, 1986 (9).

DISCUSSION

The species and subspecies of Libya mentioned in this work were collected for a period of one year. According to the present situation, the myrmecological Libyan fauna has not circum mediterranean character; is mostly composed of oriental elements; undoubtedly belongs to the eremic region; includes some ethiopic oriental ants, and includes only few elements of the coastal african minor. For a great extent it seems that the fauna of Libya is more or less similar to that of Egypt.

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دراسات تصنيفية لبعض النمل (فصيلة فورميسيدى ــ رتبة غشائية الاجنحة) فى ليبيا

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قسم العلوم البيولوجية والجيولوجيا ــ كلية التربية ــ جامعة عين شمس

تناولت هذه الدراسة تصنيف ست وعشرون نوعا وتحت نوع تندرج تحت أربعة عشر جنسا وتحت فصائل أربع هى بونيرينى ، دوريلينى ، ميرميسينى وفورميسينى (فصيلة فورميسيدى ــ رتبة غشائية الاجنحة) والتى جمعت من مناطق مختلفة فى ليبيا ، تم تشخيص (تحليل) وعمل مفاتيح تصنيفية مصحوبة ببعض الرسومات التوضيحية لهذه الأنواع وتحت الأنواع .

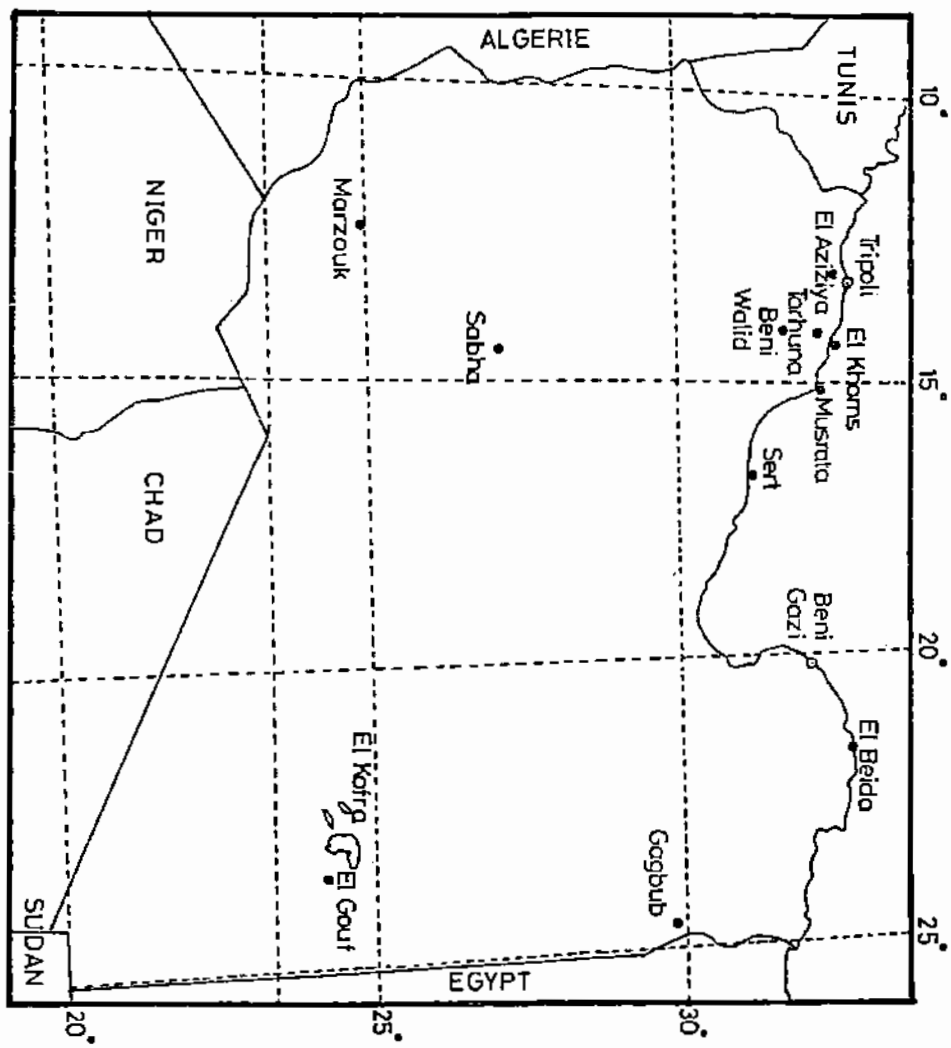
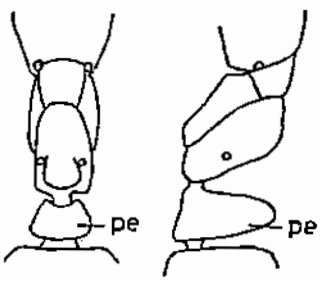


Fig.(1). Map of LIBYA with main areas and locations of collecting activity

Fig.(2)

Fig. (3)

Fig. (4)



(A) (B)

Fig.(5)

(C)

Fig. (6)

Fig. (2) Epinotum of Dorylinae.

Fig. (3) Terminal, circular acidopore.

Fig. (4) Petiole of Formicinae.

Fig. (5) Petiole, dorsal (A)and side view (B),and gaster (C)of Ponerinae.

Fig. (6) Petiole and gaster of Myrmicinae.

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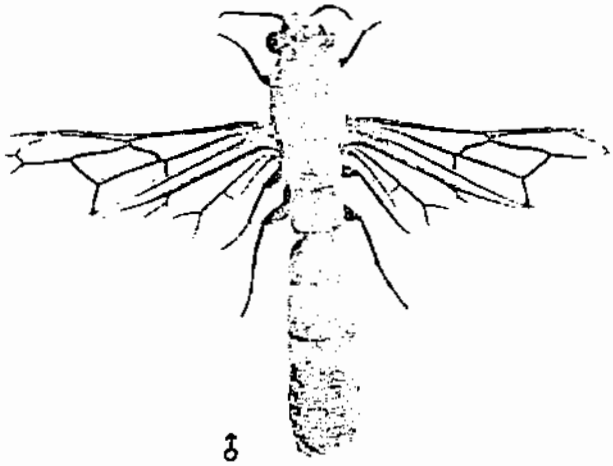

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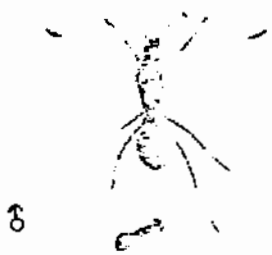

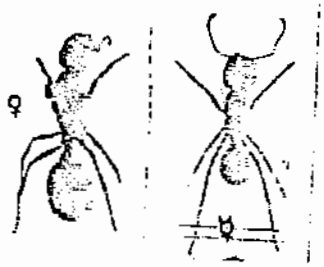

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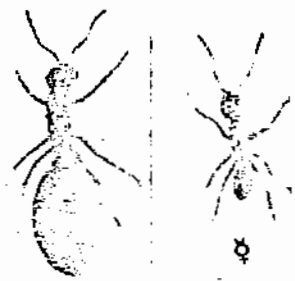

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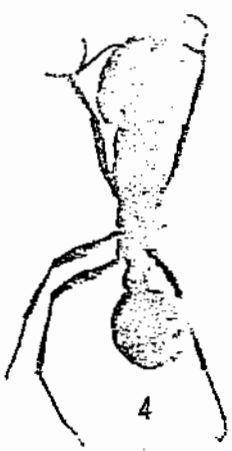

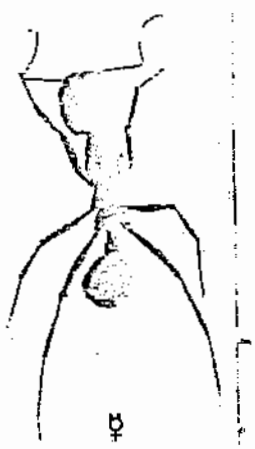

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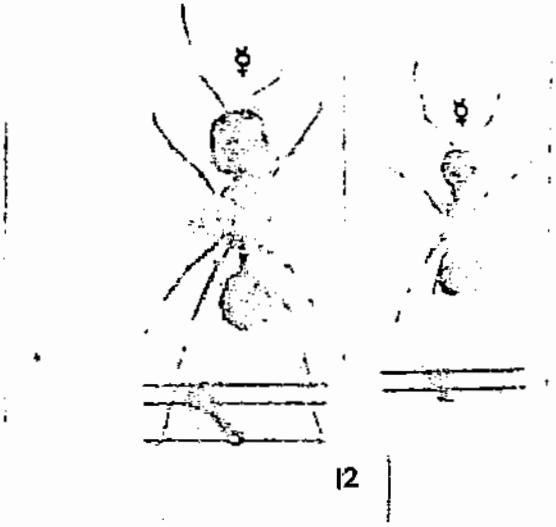
Fig. (7) Ponera ragusae santschii Emery

Fig. (8) Dorylus fulvus Westwood

Fig. (9) Messor aegyptiacus Emery

Fig. (10) Monomorium salomonis (Linnaeus)

Fig. (11) Messor arenarius (Fabricius)



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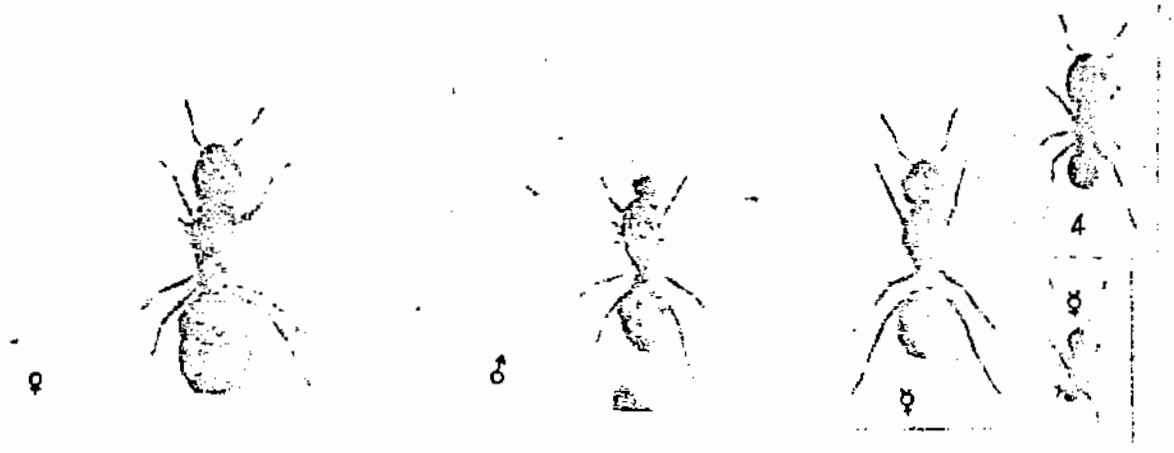

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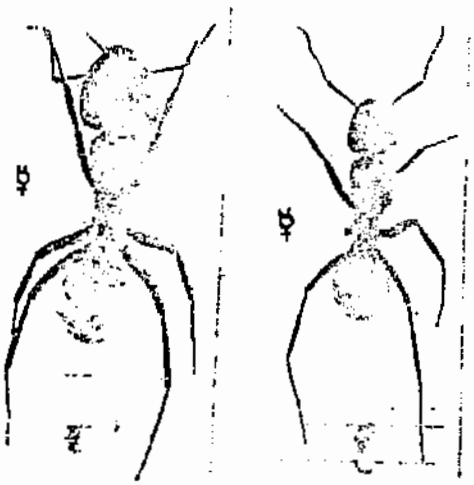

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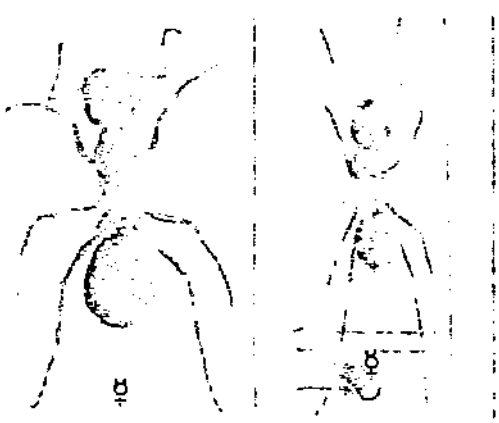
Fig. (12) Messor rufotestaceus (Foerster)

Fig. (13) Pheidole pallidula recticeps Forel

Fig. (14) Pheidole teneriffana Forel

Fig. (15) Camponotus sericeus (Fabricius)



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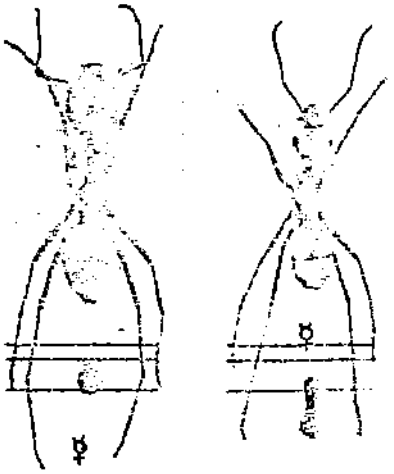

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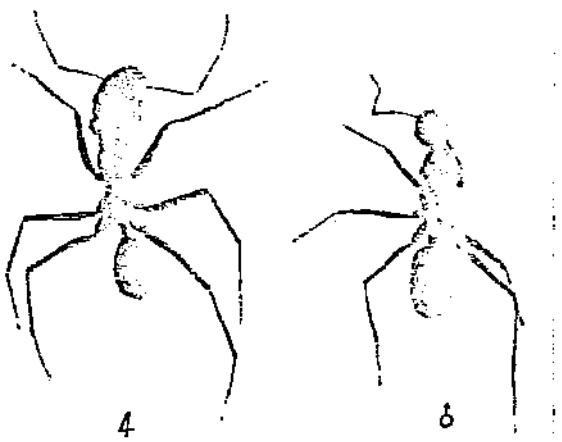

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Fig. (16) Gataglyphis albicans (Roger)

Fig. (17) Gataglyphis bicolor (Fabricius)

Fig. (18) Gataglyphis bombycina (Roger)

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SELECTIVE DETERMINATION OF VERAPAMIL IN SOME PHARMACEUTICAL PREPARATIONS USING SIMPLE POTENTIOMETRIC SENSOR

By

-27-

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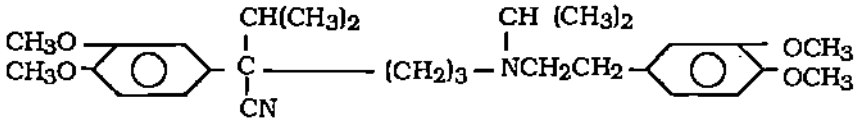
SUMMARY

A PVC membrane electrode for verapamil has been prepared for the direct potentiometric determination of verapamil in pharmaceutical preparations. It exhibits a near Nernstian response in the range $7x10^{-5}$-10^{-2}M verapamil hydrochloride with a slope of 55 mv per concentration decade. The electrode has a reasonably wide working pH range (2-7.6) in 0.1M NaCl, a fast response time (less than 60 S) and is stable for at least three months. It shows a good selectivity for verapamil in the presence of various excipients commonly present in a number of pharmaceutical preparations. The mean relative standard deviation for verapamil determination in 40 $\mu g\ ml^{-1}$-$1mgml^{-1}$ aqueous verapamil hydrochloride solutions is 1.2%.

INTRODUCTION

Verapamil 5-[3,4-dimethoxyphenethyl methyl-amino]-2-(3,4-dimethoxyphenyl)-2- isopropylvaleronitrile (I), is being intensively studied because of its ability to suppress inward calcium currents in cardiac and other excitable tissues.[1-3] Such "slow" calcium currents may be involved in fatal arrhythmias occurring during myocardial ischemia or infraction and associated with sudden

cardiac death[4]. The calcium antagonist verapamil reduces pathologically raised blood pressure by dilating the peripheral blood vessels peripheral resistance diminishes and the load on the heart is thus relieved.



I

Verapamil hydrochloride in pharmaceutical preparations is extracted from powdered tablets and the supernatent solution is analysed by h.p.l.c.[5-8]. The high-performance liquid-chromatographic system is also recommended for the determination of the drug in blood corpuscles[9], blood plasma or serum[10], plasma[11-13], human serum[14] and in postmortem specimens[15]. Analysis of verapamil and its metabolites in human plasma[16], post mortem specimens[17], liver tissue or stomach content in fatal cases[18] and serum[19] has been performed by the gas-chromatography. Verapamil determination in biological material[20], stomach content and liver[21] were assigned by thin layer chromatography. Fluorometric determination of verapamil in blood, urine or tissue homogenates has been discribed[22]. Verapamil hydrochloride in pharmaceutical preparations was also determined spectrophotometrically[23].

The present study was made for preparing a PVC membrane electrode responsive for verapamil and using it for selective potentiometric measurments of verapamil in pharmaceutical preparations.

EXPERIMENTAL

Apparatus

The potentiometric measurments were made at 25±1°C using an Orion digital-pH-millivoltmeter (Model-720) with the PVC verapamil-TPB membrane electrode using an Orion (90-02) double junction Ag/AgCl reference electrode using 10% m/v potassium nitrate in the outer compartment and an Orion (90-00-02) solution in the inner compartment. pH measurments were made by an Orion combination glass electrode Model(91-01). Standardization of TPB was made by an Orion Ag-Ag_2S membrane elecrode (Model 94-02) versus the previously mentioned double junction reference electrode.

Reagents and Materials

Materials and reagents were obtained as follows:-

Sodium tetraphenyl borate from fluka, dioctylphthalate (DOP) and tetra hydrofuran from Aldrich, the PVC used was (Breon III Ep from UK). All other chemicals were of analytical reagent grade. A standard verapamil hydrochloride sample was a gift from Elnasr Pharm. Co, Egypt. Other verapamil drugs were obtained from local drug stores.

Solutions were prepared with deionized water. Sodium tetra phenyl borate (NaTPB) was prepared, filtered twice and standardized potentiometrically with standard 10^{-2} M $AgNO_3$ solution. 10^{-1}M verapamil hydrochloride standard was prepared by dissolving 4.91g of pure verapamil hydrochloride in 100 ml of 0.1M NaCl solution. A series of standard solutions were also prepared by appropriate dilution with NaCl solution from 10^{-1}-10^{-6} M.

Electrode Preparation

The sensor was prepared by mixing 100ml aliquot of 10^{-2} M aqueous verapamil solution and 100 ml aliquot of 10^{-2} M aqueous sodium tetraphenylborate solution. The white ion-pair precipitate was filtered off, washed several times with deionized water and dried at room temperature. The infrared spectrum of the complex displayed almost all absorption bands that present in the individual spectra of both verapamil HCl and NaTPB.

Master membranes were cast from PVC (190mg), plasticising solvent mediator (350mg) and sensor (10mg). The previous cocktail was dissolved in 5 ml of freshly distilled tetra hydrofuran (THF), then poured into a 5 cm diameter petri-dish. The solvent was allowed to evaporate slowly over a period of 48 h, whereby a transparent disc, approximately 0.2mm thick was formed, A disk was cut from the master membrane, and mounted in an electrode configuration according to the procedure of Moody et al[24].

The electrode internal solution was prepared from a mixture of equal volumes of $5x10^{-2}$ M of verapamil hydrochloride and $5x10^{-2}$ M of sodium chloride.

The membranes were stored at room temperature in closed containers. Before calibration, a fresh electrode was preconditioned in 10^{-2} M verapamil hydrochloride solution for at least 24h. The electrode was Kept in 10^{-2} M verapamil hydrochloride after each measurment . A steady state dynamic response was normally obtained in less than 60 S.

Electrode Calibration

Standard solutions of verapamil hydrochloride in the concentration range 10^{-1}-10^{-6} M were prepared. Aliquots of 10 ml of the standard solutions were transferred into 50-ml beakers and the PVC verapamil-TPB membrane electrode in conjunction with an Orion double-junction Ag-AgCl reference electrode (Model 90-02) was immersed in the solutions starting from 10^{-6} to 10^{-1}. The measured potentials were followed for a period of 15 min for each concentration, and plotted against the logarithm of the verapamil concentration. A steady state response has been reached in 90 S and the calibration curve show a slope of 55 mv/conc. decade Fig (1).

Determination of Verapamil

For direct potentiometric assay of verapamil in drugs, a 80 mg portion of the tablet was finelly powdered and transferred quantitatively with 0.1M NaCl solution into a 250ml calibration flask. The solution was then diluted to the mark with 0.1M NaCl solution and shaken for 5 min. Aliquots (10ml) of these drug solutions were transferred into 50 ml beakers, the PVC verapamil-TPB membrane electrode and Orion double junction Ag-AgCl reference electrode were immersed in the solutions. The electrode system was allowed to equilibrate with stirring and the e.m.f. was recorded and compared with the calibration graph. Alternatively, the standard addition (Spiking)[25] technique was used by recording the e.m.f. before and after the addition of 1.0 ml of standard 10^{-2} M verapamil hydrochloride solution to the obove solutions. The change in the potential readings was recorded and used to calculate the verapamil content in various pharmaceutical preparations.

Potentiometric Titration

Aliquots (10-30 ml) of verapamil hydrochloride test solutions (10^{-3}M) were pipetted into a 100-ml beaker. The verapamil selective electrode and the double junction reference electrode (Orion 92-02) were immersed into the solution and the stirred solution was titrated slowly with a standard $6.5x10^{-3}$ M sodium tetra phenylborate (NaTPB) solution. The inflection point of the titration curve corresponds to 1:1 verapamil: TPB reaction. 1.00 ml of $6.5x10^{-3}$ M NaTPB ≡ 3.195 mg of verapamil hydrochloride.

RESULTS AND DISCUSSION

Electrode Characteristics

The specific response characteristics of a PVC verapamil-TPB membrane electrode are shown in Table (1) and Fig (1). Calibration were made at a constant pH and ionic strength using 0.1 M NaCl solution. Table (1) shows that the electrode displays a linear response for aqeous verapamil hydrochloride solutions over the concentration range $7x10^{-5}$-10^{-2}M. The potential readings were stable and consistent to ± 2 mV day-to day for at least 4 weeks . The calibration slope is 55± 0.2 mV per concentration decade, the detection limit, as defined by an e.m.f. difference of 18 mV between the calibration graph and the extrapolated linear section, is better than $9x10^{-5}$ M (Ca 44.0 µg/ml). This indicates the feasibility of using this electrode to determine verapamil in pharmaceutical preparations.

Effect of pH and Time

The pH dependence of the electrode potential was measured as described previously[25]. Although the potential readings

displayed by the PVC verapamil-TPB membrane electrode are reasonably stable, measurments in NaCl solution provides stable potential readings for up to 10 min. The static response time of PVC verapamil-TPB membrane electrode is fast Ca 90S for 10^{-3} M verapamil, but the dynamic response is even better. All potentiometric measurments were made at 25± 1°C. The potential response of the electrode for 10^{-6}-10^{-1}M aqueous verapamil hydrochloride solutions was followed.

Effect of Interferents

The response of PVC verapamil TPB membrane electrode was checked in the presence of a number of organic and inorganic cations and other organic species. The resulting selectivity coefficients are shown in Table (2). The verapamil pharmaceutical drugs commonly contain excipients and diluents, such as talc powder, starch and lactose. None ef these diluents in concentration level as high as 100-fold excess affect the response of the electrode.

Determination of Verapamil

Results for measurments of pure aqueous verapamil hydrochloride solutions at concentrations of 44μg/ml-0.74μg/ml using the PVC verapamil -TPB membrane electrode (DOP plasticiser) and the standard addition spiking technique[25] are given in Table(3). The mean relative standard deviation is 1.2%. Potentiometric titration of 4.9-14.7 mg verapamil hydrochloride v.s standard $6.5x10^{-3}$M aqueous NaTPB solution using the PVC verapamil-TPB membrane electrode as an indicator electrode Fig.(2) shows potential break of Ca 100-150 mv at the stoicheiometric 1:1 molar reaction.

Determination of Verapamil in Pharmaceutical Preparations

Table (3) presents results obtained by direct potentiometry of verapamil in pharmaceutical tablets using th PVC matrix membrane electrode. Anaveragerecovery of 99.0% (SD 0.1% n=5) of the nominal values is obtained, for direct potentiometry and standard addition method.

In conclusion, the proposed membrane electrode system offers a simple, rapid and accurate, screening technique for the determination of verapamil in pharmaceuticals.

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Table (1)

Response characteristics of the PVC Verapamil-TPB membrane electrode with DOP plasticiser.

Parameter	DOP
Slope/mV decade-1	55.0
Intercept /mv	140 mV
Lower Limit of Linear ran ge/M	$7X10^{-5}$
Detection Limit/M	$9X10^{-6}$
Dynamic response time for 10^{-3}M Verapamil, s	60

Table (2)

Selectivity Coefficient of Some Organic and Inorganic Interferents

Interferent	$K^{pot}_{ver.\ B}$
Glutamic	$1.1x10^{-2}$
Urea	$7.3x10^{-3}$
K^+	$6.83x10^{-3}$
Mg^{++}	$6.72x10^{-3}$
Na^+	$6.66x10^{-3}$
Glucose	$1.4x10^{-2}$
Alanine	$3.99x10^{-3}$
Ca^{++}	$6.72x10^{-3}$
Maltose	$1.3x10^{-2}$
Amm. chloide	$7.5x10^{-3}$
m. aminophenol	$7.2x10^{-3}$
P-aminophnol	$9.9x10^{-3}$
Glycine	$1.42x10^{-3}$
ethylamine	$1.8x10^{-3}$
Butylamine	$1.2x10^{-4}$

Table (3)

Determination of Verapamil in Some Pharmaceutical Preparations

Pharmaccutical product and source	Recovery %	
	Direct Potentiometry	Standard addition
80 mg, Elnasr Pharm. Co, Egypt.	98.5%	99.2%
40 mg, Elnasr Pharm. Co, Egypt.	100%	98.5%

* Average of five measurments

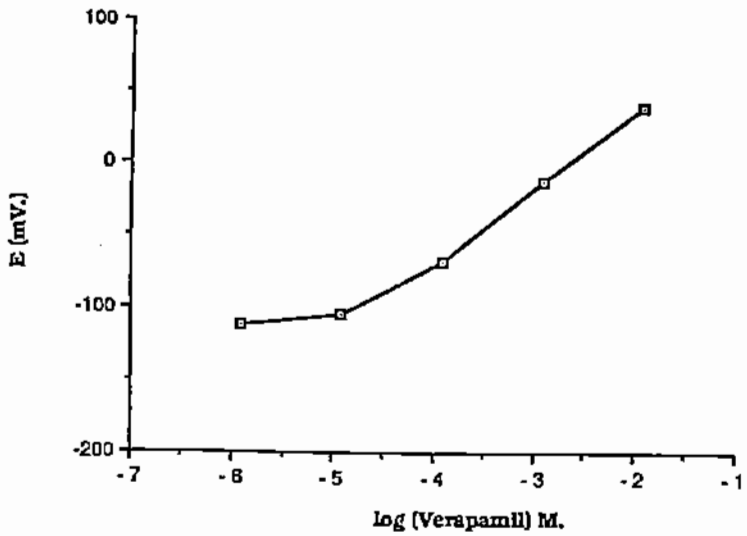

Fig (1) CALIBRATION CURVE

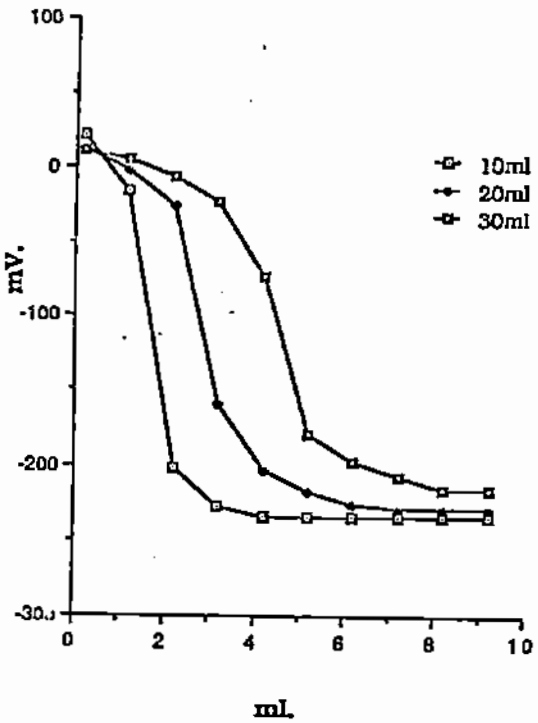

Fig (2) Potentiometric Titration of Verapamil 10^{-3} M v.s. Standard 6.5×10^{-3} M Na. TPB.

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التقدير المنتخب للفراباميل فى بعض المستحضرات الدوائية بأستخدام قطب بسيط نو غشاء من كلوريد البولى فينيل

منى عبد العزيز أحمد
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القاهرة – مصر

يتناول هذا البحث طريقة لتحضير قطب منتخب لمادة الفراباميل نو غشاء سائل محمل على مادة كلوريد البولى فينيل واستخدامه للقياس الجهدى المباشر للفراباميل فى بعض المستحضرات الدوائية . وقد دلت الدراسة على أن هذا القطب يستجيب استجابة تكاد تتطابق مع معادلة نرنست فى مدى تركيز من $١٠^{-٢}$–٧×١٠^{-٥} مولارى من الفراباميل مع ميل يقدر بـ ٥٥ ملى فولت لكل وحدة تركيز .

ولقد اظهر هذا القطب صلاحية للاستخدام فى مدى واسع من الأس الايدروچينى يتراوح بين ٢ و ٧.٦ فى محلول تركيزه ١٠ . مولارى من كلوريد الصوديوم حيث كان وقت الاستجابة سريع (أقل من ٦٠ ثانية) . ويعمل هذا القطب بكفاءة عالية على مدى ثلاثة اشهر على الأقل وهذا القطب له اختيارية عالية للفراباميل واظهرت النتائج دقة عالية تقدر بـ ٩٩٪ ، ومتوسط حيود ١.٢٪ عند تقدير كميات من الفراباميل تتراوح بين ٤٠ ميكروجرام و١ ملى جرام/ملى لتر .

وتم تقدير الفراباميل فى بعض المستحضرات الدوائية المستخدمة فى حالات ارتفاع ضغط الدم . حيث كانت النتائج مطابقة لتلك التى حصل عليها باستخدام الطرق المعتمدة فى دستور الأدوية الأمريكى .

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STRUCTURAL EFFECTS ON THE ACID-CATALYZED HYDROLYSIS OF (E)-3-CARBOXY-4-ARYL-BUT-3-ENOIC ANHYDRIDES

_28_

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The rates of acid-catalyzed hydrolysis of (E)-3-carboxy-4-phenyl(1),4-(1-naphthyl)(2),4,4-diphenyl(3) and 4-methyl, 4-phenyl(4)-but-3-enoic- anhydrides are compared in dioxane-water mixtures at different temperatures and pH values and are in the sequence 1 > 2 > 3 > 4. The rate decreases with increase of pH, reaches a minimum at about pH 3.1 then increases gradually till pH 5.7.

INTRODUCTION

Hydrolysis of carboxylic anhydrides in initially neutral solution have been studied extensively. Reaction is retarded by the addition of non-polar solvents to the water and values of energies of activation are lower, and the entropies more negative, than those observed for nucleophilic substitution at a saturated carbon atom[1,2]. Bunton and co-workers[3] have reported that bond breaking is not kinetically important in the hydrolysis of

some carboxylic anhydrides and the reaction is slower in the less aqueous solvents.The reactivities of symmetrical anhydrides of both aliphatic and aromatic carboxylic acids are increased by electron-attracting substitutents[4,5]. However,although some work on the kinetics of the hydrolysis of anhydrides has been reported, the pH-rate profile and solvent effect on the hydrolysis of unsymmetrical α,β-unsaturated carboxylic anhydrides have not been much studied. Thus it was of great interest to investigate the rate of hydrolysis of unsymmetrical anhydrides 1-4 which contain an olefinic linkage conjugated with only one carbonyl group and extended conjugation with one or two aromatic rings.

EXPERIMENTAL

The (E) anhydrides 1, m.p., 166-7°C[6]; 2, m.p., 165-6°C[7]; 3, m.p., 149-51°C[8], and 4, m.p. 112°C[9], were prepared and their structures confirmed.

The ^{1}HNMR were determined in $CDCl_3$ with a Bucker WH-400 instrument (TMS as internal standard).

Anhydride 2: ^{1}HNMR.- δ = 3.81 (d, J = 2.5 Hz; CH_2), 7.60 (dd, J = 8, 1.5 Hz; 7-H), 7.63 (mc; 2-, 3-H), 7.66 (dd, J = 8, 1.5 Hz, 4-H), 7.93, 8.11 (AA'BB' system, J = 9 Hz; 5-, 6-H), 7.99 (dd, J = 8, 1.5 Hz; 1-H), 8.57 (t, J = 2.5 Hz; 8-H).

Anhydride 4: ^{1}HNMR.- δ = 3.49 (q, J = 1 Hz; CH_2), 2.68 (t, J = 1 Hz; Me), 7.25 (dd, J = 8, 1.5 Hz; 1-H), 7.44 (mc; 2-,3-H).

Kinetic Measurements

The rates of hydrolysis of dicarboxylic anhydrides(1-4) were studied in different mixtures of redistilled CO_2-free water and pure dioxane[10] containing different buffers[11] at pH, s 1.2-5.7 and at 25-50°C. The kinetic measurements were made using 0.015mol of anhydride in 100 ml buffer. The general procedure used for the hydrolysis of anhyrides by addition of aniline[12] could not be applied to the anhydrides 1-4, because the reaction with aniline was too slow. Fortunately reaction with hydroxide is also relatively slow at low temperature, so that the acids produced could be titrated directly with alkali[13].A titrimetric procedure involving the estimation of the liberated dicarboxylic acid in the presence of diphenol purple as indicator[11] was followed. The structures of the dicarboxylic acid produced after complete hydrolysis were identical with that stated in the literature[6-9].

A thermostat was used so that the temperature could be adjusted to ± 0.02°C. The rate constants k_1 were calculated graphically by using the integrated form of the first order rate equation and were reproducible within ± 1%.

RESULTS AND DISCUSSION

The specific rates of the acid-catalyzed hydrolysis of the dicarboxylic anhydrides1-4 in different buffer solutions at pH,s 1.2-5.7 in 50% aqueous dioxane (v/v) were presented in Table 1 at the given temperatures. Fig 1 shows the inverted bell-shaped rate-pH profile with a minimum at about pH 3.5. This indicates

that acid catalysis is considerably less effective than base catalysis.At the minimum in the pH-rate profile $k_b/k_a=10^7$, where k_a and k_b are the catalytic coeffecients of acid and base, respectively[14].

A statistical least squares treatment[15] of the Arrhenius equation was used to calculate the activation energy $E^{\neq}$ based on the rate constant k_1 at pH values 1.2 and 5.7 in 50% aqueous dioxane.The enthalpies ($\triangle H^{\neq}$),entropies ($\triangle S^{\neq}$) and free energies ($\triangle G^{\neq}$) of activation and also the Arrhenius frequency factor (A) were calculated using the absolute rate equation[16] and are given in Table 2 . However, although the values of $\triangle H^{\neq}$ and $\triangle S^{\neq}$ for anhydrides 1-4 somewhat change as the structures are changed, the compensation effect between $\triangle H^{\neq}$ and $T\triangle S^{\neq}$ plays a part in keeping $\triangle G^{\neq}$ more or less constant. This is ascertained by the linear relationship between them with slope unity.

Enhancement of the rate of hydrolysis with the increase of the dielectric constant of the medium (D) can be attributed to the high polarity and greater solvation of the transition state. The linear relationship betweem log k_1 and $D-1/2D+1$ with positive slope indicates the dipolar interaction[17]. The dielectric constants of the dioxane-water mixtures were obtained from data of Akerlof[18]. The results are presented in Table 3 . The linear relationship between log k_1 and log $[H_2O]$ with slope equals to unity at pH,s 1.2 and 5.7 indicate that a water molecule acts as a strong nucleophile and is involved in the rate-determining step[19]. Thus, from the given results and rate profile the

hydrolysis seems to take place by $A_{Ac}2$,and the reaction is catalyzed by both acid and by base but catalysis by base is rather more effective since it becomes dominant while the solution is still acidic[14]. The bimolecular mechanism is substantiated by the values of $E^{\neq}$, $\triangle S^{\neq}$ and the Arrhenius frequency factor log A. The large negative value of entropy of activation indicates that initial protonation is followed by synchronous displacement of the carboxylate group (A2 or S_N2) with no discrete tetrahedral intermediate[20.21].

The low sensitivity of the reaction to substituent effect is in accord with the $A_{Ac}2$ mechanism.

However, in the usymmetrical anhydrides, 1-4, the only reactive centre of nucleophilic attack is the nonconjugated carbonyl group where conjugation of the other carbonyl group with the olefinic linkage and its extention to aryl group lowers its susceptibility toward nucleophilic attack (Scheme1). The sequence of reactivity 1>2>3>4 reveals that the important effect which influences hydrolysis, similar to cyclization of acids to anhydrides, is steric in origin[21]. It appears that the larger the substituent, the lower is the rate of hydrolysis. Table 2 shows that both enthalpy and enteropy changes become less favorable to hydrolysis.

Acknowledgement: The author thanks Prof. Dr. P. Weyerstahl, Institute für Organische Chemie, Technische Universität Berlin, for recording the ^{1}HNMR.

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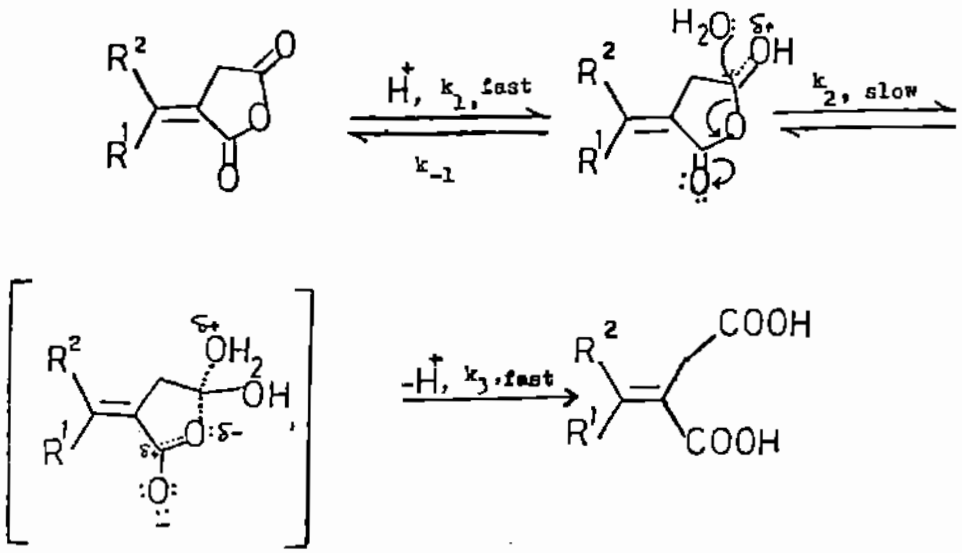
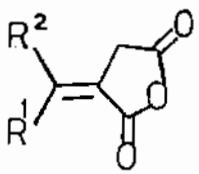


Scheme I

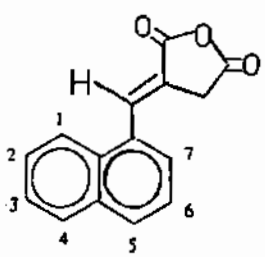


1 , R^1 = Ph , R^2= H

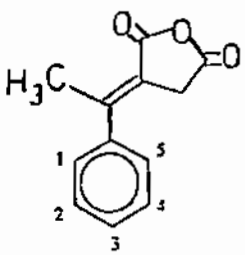
2 , R^1= 1-Naphthyl , R^2 = H

3 , R^1= R^2 = Ph

4 , R^1 = Ph , R^2 = Me



2



4

TABLE 1

RELATION BETWEEN pH VALUES AND VELOCITY CONSTANTS AT DIFFERENT TEMPERATURES IN 50% AQUEOUS DIOXANE

pH*	t°C	$10^4 \times k_1$ sec^{-1}			
		Anhydride			
		1	2	3	4
1.2	25	3.327	2.851	1.203	0.770
	30	5.246	4.467	1.919	1.279
	35	7.805	6.998	3.071	2.047
	40	11.643	10.619	4.734	3.199
	45	17.912	16.032	7.423	5.118
	50	25.845	24.266	11.515	7.933
2.1	40	7.293	6.397	2.687	2.175
3.1	40	5.374	4.989	2.303	1.791
4.8	40	8.700	8.316	4.094	2.303
5.7	25	4.222	3.838	2.047	0.870
	30	6.525	5.757	3.071	1.407
	35	9.852	8.700	4.862	2.239
	40	14.840	12.794	7.293	3.455
	45	22.134	18.936	10.747	5.374
	50	31.986	27.764	15.737	8.316

*The buffers used are reported in literature[11]. The buffer solutions were prepared in large batches in order to give reproducible media.

TABLE 2

ACTIVATION PARAMETERS FOR THE HYDROLYSIS OF ANHYDRIDES (1-4) IN 50% AQUEOUS DIOXANE

Anhydride	pH value	$E^{\neq}$ K cal mol^{-1}	$\Delta H^{\neq}_{40°}$ K cal mol^{-1}	$-\Delta S^{\neq}_{40°}$ cal deg^{-1} mol^{-1}	$\Delta G^{\neq}_{40°}$ K. cal mol^{-1}	log $A_{40°}$
1	1.2	16.581	15.955	21.562	22.704	8.5693
	5.7	15.670	15.044	23.987	22.552	8.0427
2	1.2	17.042	16.416	20.273	22.761	8.8491
	5.7	16.121	15.495	22.843	22.645	8.2911
3	1.2	17.963	17.337	18.947	23.267	9.1373
	5.7	17.042	16.416	21.065	22.997	8.6859
4	1.2	18.424	17.798	18.259	23.513	9.2869
	5.7	17.503	16.877	21.047	23.465	8.6814

TABLE 3

RELATION BETWEEN VELOCITY CONSTANTS AND DIELECTRIC CONSTANTS OF THE MEDIUM AT pH 1.2 AND 5.7

Anhydride	D-W mixture (v/v)	$D_{40°}$	4 + log k_1	
			pH 1.2	pH 5.7
1	60:40	35.39	0.9763	1.0847
	50:50	37.41	1.0661	1.1715
	40:60	39.45	1.1444	1.2500
	30:70	41.80	1.2108	1.3056
	20:80	44.19	1.2625	1.3671
2	60:40	35.39	0.9132	1.0266
	50:50	37.41	1.0261	1.1070
	40:60	39.45	1.0937	1.1714
	30:70	41.80	1.1562	1.2406
	20:80	44.19	1.2108	1.2970
3	60:40	35.39	0.5840	0.7602
	50:50	37.41	0.6752	0.8629
	40:60	39.45	0.7405	0.9395
	30:70	41.80	0.8059	0.9991
	20:80	44.19	0.8552	1.0612
4	60:40	35.39	0.4081	0.4494
	50:50	37.41	0.5049	0.5383
	40:60	39.45	0.5840	0.6122
	30:70	41.80	0.6511	0.6868
	20:80	44.19	0.7198	0.7504

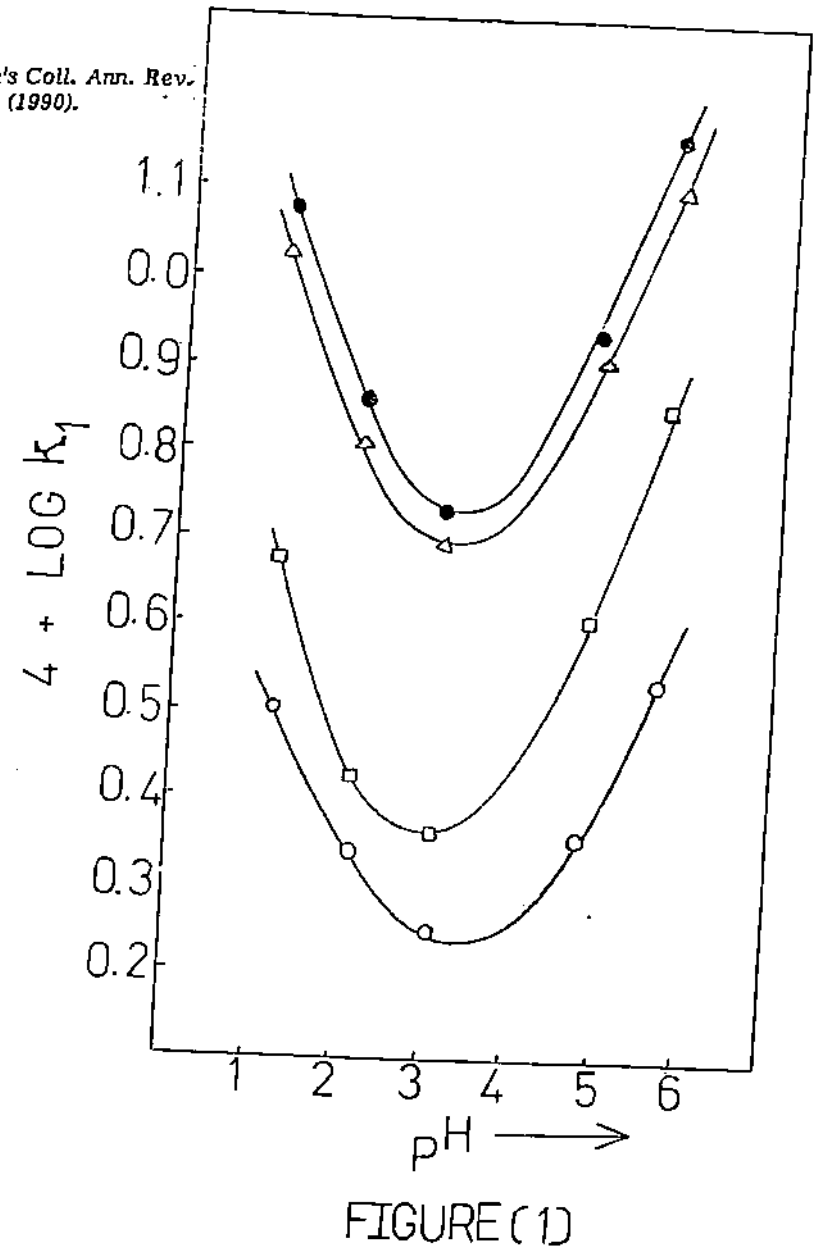


FIGURE (1)

Fig. 1: pH-rate profile for the hydrolysis of anhydride 1 •—•, 2 △—△, 3 □—□ and 4 ○—○ at 40°C in 50% aqueous dioxane.

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ملخص عربى

تاثير التركيب البنائى على معدل التحلل المائى فى وسط حمضى لانهيدرات (E)-٣- كربوكسى -٤-اريل - بيوت -٣-إينوك

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القاهرة

تم مقارنة معدلات التحلل المائى فى وسط حمضى لانهيدرات (E) -٣- كربوكسى -٤-فينيل (١) ، ٤-(١-نفثيل) (٢) ، ٤و٤-داى فينيل (٣) و ٤-ميثيل-٤- فينيل (٤) - بيوت -٣-إينوك فى مخاليط من الديوكسان والماءعند درجات حراره مختلفه وأس هيدروجينى مختلف .

وقد وجد ان هذه المعدلات تتبع التوالى الآتى ١ > ٢ > ٣ > ٤ .

وقد وجد ايضا أن معدل التحلل المائى ينخفض بزياده الأس الهيدروجينى ليصل ادناه عند أس هيدروجينى ٣ر٢ ثم يزداد تدريجياًحتى أس هيدروجينى ٧ر٥ .

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CHARGE TRANSFER COMPLEXES BETWEEN SOME POLAR SOLVENTS AND π-ACCEPTORS

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ABSTRACT

The charge-transfer complexes formed by a number of π-acceptors with dimethylsulphoxide, dimethylformamide and tetrahydrofuran have been measured in methylene chloride at 25 °C. Transition energies of these complexes as well as ionization potentials of the π-donors have been determined. All CT complexes studied have a 1:1 stoichiometry. Solvent effect on the electronic transition of the CT have been presented and discussed.

INTRODUCTION

Solvent effect studies on the position of the 1-propanamine-3-triethoxysilyl:chloranil complex bands, in organic solvents of different dielectric constants, have shown that the transition energies decrease as the polarity of the solvent increases[1]. However, complexation in oxygen-containing solvents such as dimethylsulphoxide (DMSO) exhibits no correlation between the polarity of the solvent and the energy of the charge- transfer transition. We proposed that this might be due to the formation of complexes with these solvents.

According to M. A. Slifkin[2], there is an interaction between chloranil and polar solvents DMSO, tetrahydrofuran, (THF), and dimethylformamide (DMF), probably the formation of a 1:1 charge-transfer complex.

The objective of the present study is to determine the stoichiometry of the charge-transfer (CT) complexes between DMSO, THF and DMF with chloranil (CHL), 2,3-dichloro 5,6-dicyanobenzoquinone (DDQ) and iodine (I_2) in methylene chloride. In addition, we wish to apply the tool of CT complexation to estimate the ionization potentials of the three organic solvents.

EXPERIMENTAL

The purification of DMSO has been described earlier[3], other organic solvents used were purified ac-

cording to standard methods[4]. CHL (Merk) was crystallized from benzene, DDQ (Aldrich) was crystallized from benzene:chloroform (2:3).

For the purpose of determining the stoichiometry utilizing the conventional continuous variation method (Job's method[5]), stock solutions (10^{-2}M) of donor and acceptors were prepared. The electronic spectra of the complexes were measured in 3 ml stoppered silica cells after mixing the donor and acceptor solutions at 25°C using a Perkin-Elmer spectrophotometer model λ_4. All measurements were made immediately after mixing the two components. The electronic spectra of all molecular complexes formed were recorded within the wavelength range 200 - 750 n.m., using methylene chloride as a solvent.

RESULTS AND DISCUSSION

It was observed that spectra of the π-acceptors DDQ, CHL and I_2 in the polar solvents DMSO I, DMF II and THF III are not very different from that of the acceptor in an inert solvent[6]. However, by studying the difference spectra between polar solvent + π-acceptor versus π-acceptor solution of the same concentration (as illustrated in fig.1) the electronic spectra of I, II, and III with various electron acceptors under investigation in methylene chloride solution show an extra absorption band(s) not due to either component alone. These bands have been attributed to the formation of donor-acceptor molecular complexes in these solutions. The

values for the positions of absorption maxima, molar extinction coefficients, transition energies of the molecular complexes of the electron donors I, II and III with the different electron acceptors are listed in table (I).

Table (I) Maximum absorption wavelength λ_{max}(nm), molar extinction coefficient ε_{max}($mol^{-1}cm^2$) and transition energies E(K cal mol^{-1}) of the charge-transfer complexes formed between polar organic solvents (DMSO, DMF and THF) and π-acceptors in methylene chloride at 25°C as well as the ionization potentials i.p. (e.v.) of the donors.

Electron Donor	Acceptor	λ_{max}	ε_{max}	Transition Energy	i.e.
	DDQ	368	46.0	77.7	9.89
DMSO	CHL	377	16.0	75.9	9.82
	I_2	276	28.0	103.6	9.82
	DDQ	426	32.0	67.1	9.30
DMF	CHL	372	12.2	76.9	9.10
	I_2	307	9.4	93.1	9.06
	DDQ	440	24.0	65.0	9.20
THF	CHL	387	10.0	73.9	8.95
	I_2	314	11.0	91.1	8.90

The ionization potential (i.p.) values listed in table (I) were calculated from the energies of the charge-transfer bands applying the following empirical equations [7,8] which were used in substituted benzene and polynuclear hydrocarbons as donors:

(A) i.p. (e.v.) = 5.00 + 1.53 10^{-4}, $\bar{\nu}_{CHL}$ (cm^{-1})

(B) i.p. (e.v.) = 5.76 + 1.52 10^{-4}, $\bar{\nu}_{DDQ}$ (cm^{-1})

(C) i.p. (e.v.) = 2.90 + 1.89 10^{-4}, $\bar{\nu}_{I_2}$ (cm^{-1})

where, $\bar{\nu}$ is the wavenumber corresponding to the charge-transfer band. These equations have also been used in estimation of i.p. of heterocyclic compounds [9]. This method provides a simple means of estimating ionization potentials which may be difficult to determine by other methods, such as Rydberg series, photoionization and electron impact, because of practical details such as the low vapour pressure of some of these substances [8].

A 1:1 composition of the complexes between acceptors and donors is observed, by employing the Job's continuous variation method [5] (Fig. 2, 3 and 4), except in the case of the complex between DMSO and DDQ (Fig. 2) the absorption maxima at 0.4 indicates that the ratio of D:A is 2:1. DDQ relatively higher electron affinity (1.9 e.v.)[7] might account of the 2:1 D:A ratio.

Solvent effect studies on the position of CHL-DMSO complex bands, in organic solvents of different dielectric

constants, have shown that the transition energy generally increases with the polarity of the solvent (Table II). A similar increase in transition energies with the polarity of the solvent has been reported by Kosower[10]. The decrease of the energy of the transition as the polarity of the solvent increases might be due to the high stabilization of the excited state in which the charge is probably more separated than in the ground state.

In the case of oxygenated solvents no correlation appears between polarity of the solvent and the energy of the transition.

The stability of the CT complexes is proportional to the electron affinity of the electron acceptor compounds and on the ionization potential of the electron donor components and increases with the following order:

THF ≈ DMF > DMSO

DDQ > CHL > I_2 [7]

Table (II) Effect of solvent on position of the maximum absorption wavelength band of CHL-DMSO complex in different organic solvents at 25°C.

Solvent	λ_{max}	ε_{max}	Transition Energy	Dielectric Constant at 25°C
Ethyl acetate	405	6.6	70.6	6.0
Chloroform	400	15.3	71.5	4.8
Methylene chloride	377	16.0	76.0	9.1
Benzene	408	12.0	70.1	2.3
Cyclohexane	415	8.0	68.9	2.0
Acetone	375.5	4.5	76.3	21.0
Acetonitrile	325	11.6	88.0	37.5

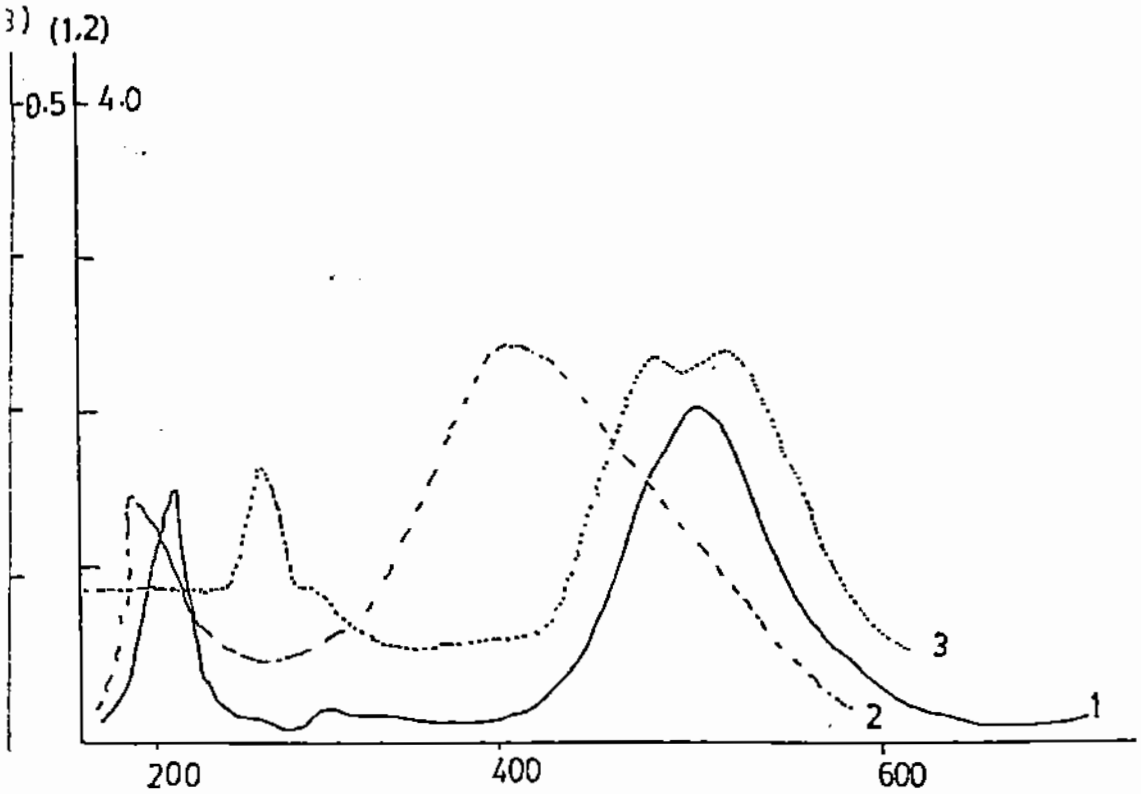

Fig.1.Absorption spectra.
0.1 M iodine in methylene chloride ——— 1
0.1 M dimethylsulphoxide in methylene chloride - - - - 2
0.1 M iodine vs. 0.1 M iodine + 0.1 M dimethylsulphoxide in methylene chloride 3

Fig. (1)

"DDQ MOL. FRA. vs DONOR ABS"

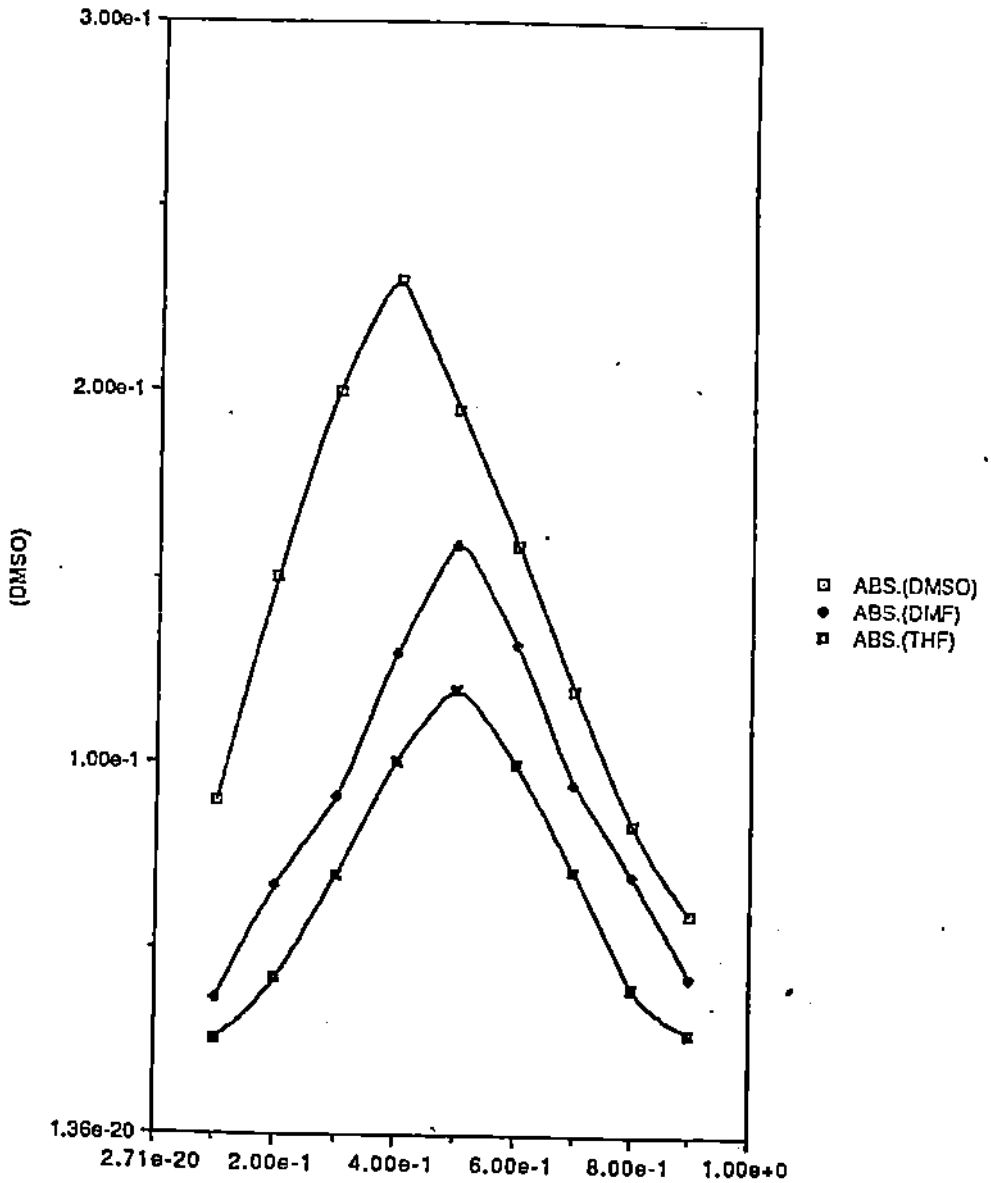

Fig. (2)

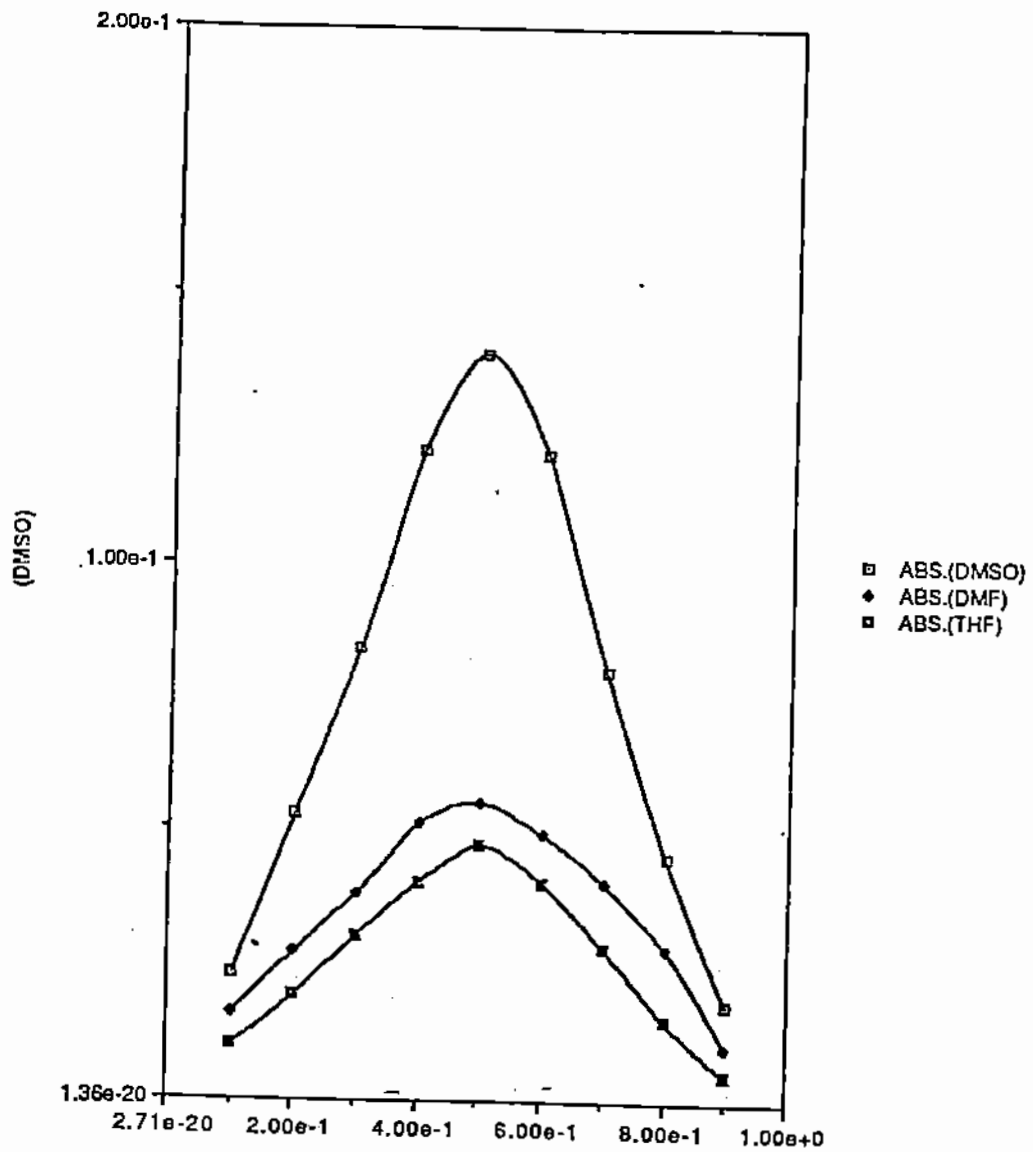
"IODENE MOL.FRA. vs DONOR ABS."
2.00e-1
1.00e-1
1.36e-20
(DMSO)
2.71e-20
2.00e-1
4.00e-1
6.00e-1
8.00e-1
1.00e+0
ACC.MOL.FRA.
ABS.(DMSO)
ABS.(DMF)
ABS.(THF)

Fig. (1)

"CHL. MOL. FRA. vs. DONOR ABS."

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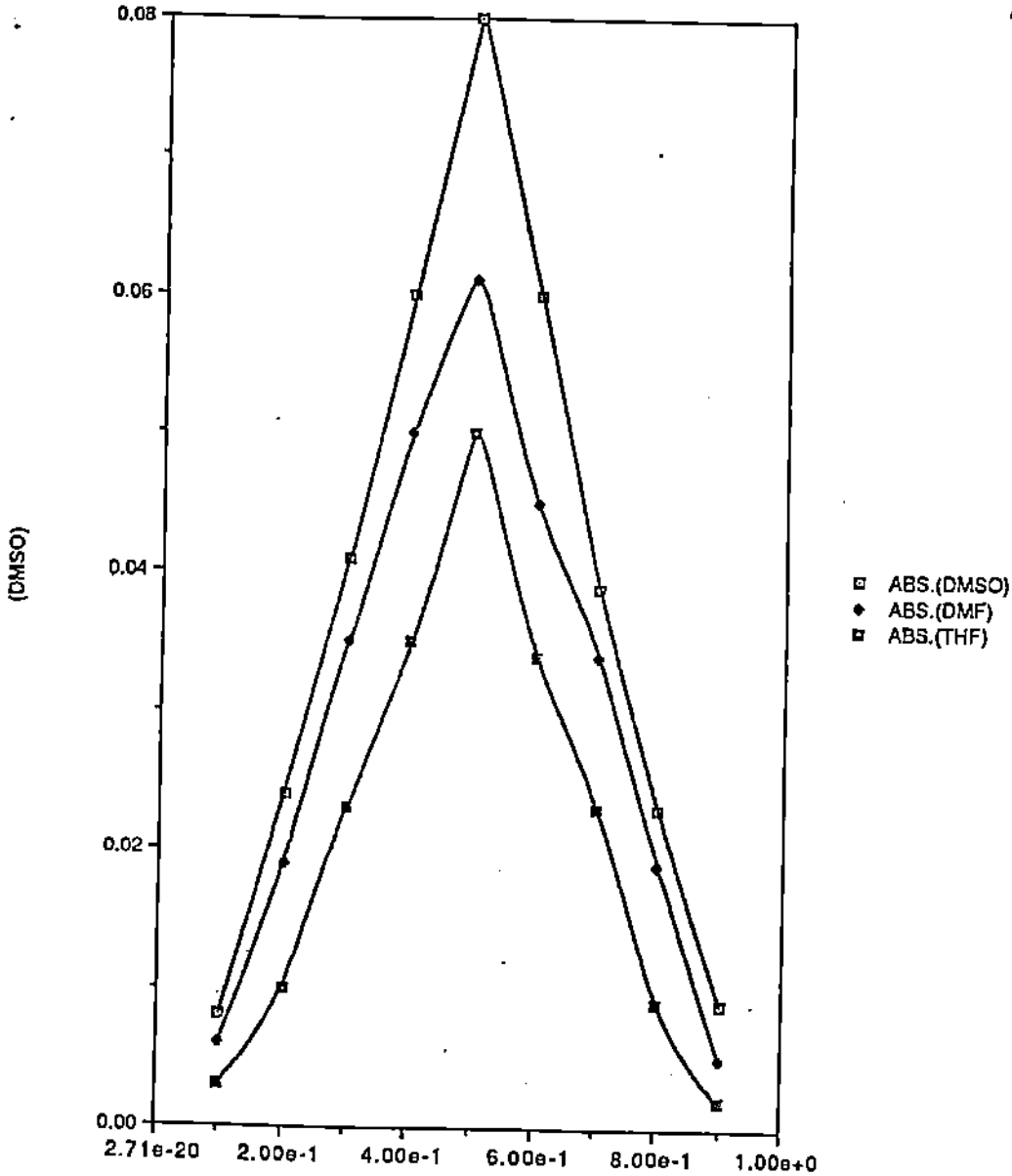

Fig. (4)

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-30- CORRELATION OF THE VISIBLE INDUCED CENTERS IN SODA-SILICA GLASS AND SMOKY QUARTZ.

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Abstract

A similarity in position's, shape's and width's at half-maxima of the visible induced bands in oxidized soda-silica glass and smoky quartz has supported the view that the bands at about 1.9 eV and 2.58 eV in smoky quartz are not related to substitutional aluminium in the quartz structure as it was reported earlier. This has been further substantiated by the development of a band at about 3.0 eV in smoky quartz whose intensity correlates with the visually observed smoky color. It is believed that the two bands H_3^+ and H_2^+ in irradiated soda-silica glass as well as the two bands A_1 and A_2 in smoky quartz have the same origin related to paramagnetic oxygens in the silica structure.

Introduction

Several investigators have studied the optical absorption caused by x-irradiation in a large number of simple soda-silica glasses. Smith and Cohen (1964) studied the coloration produced by x-irradiation in highly pure oxidized soda-silica glasses. Gaussian resolution of the induced absorption spectra showed three absorption maxima at about 1.96 eV (632nm), 2.7 eV (460nm) and 4.1 eV (302nm). The bands were attributed to sites introduced to the glass network where electrons or holes could be trapped.

By studing the effects of Ce^{3+} and Ce^{4+} in the oxidized soda-silica glasses, Stroud et. al. (1966) were able to show that the visible bands were due to trapped holes, while the other bands in the near ultra-violet (at about 4.0 and 5.27 eV) were assigned to trapped electrons. The same conclusions were reached by Mackey et. al. (1966, 1970), who assigned the two centers at 1.99 eV and 2.7 eV to two distinct trapped holes H_3^+ and H_2^+ which proved to have a common structural precursor.

Color centers were also observed and reported in natural smoky quartz and irradiated colorless quartz by many investigators. Two optical absorption bands, designated A_1 and A_2 about 2.0 eV (620nm) and 2.7 eV (460 nm) respectively, were usually assigned to the smoky color produced by x-irradiation of naturally colorless quartz. The A_1 and A_2 bands were attributed to an aluminium atom replacing a silicon atom in the quartz structure with an alkali or hydrogen in a nearby intersitial location providing charge balance (Griffiths et.al, 1955; Mackey (1963).

Nassau and Persoctt (1975, 1977) performed a series of irradiation and heat treatment experiments on a large number of natural and synthetic quartz. In some of the studied samples, the A_1 and A_2 optical absorption bands

were present without any smoky color. The smoky color, however developed upon further irradiation and was accompanied by a new absorption band A_3 at about 2.9 eV (427nm) whose intensity correlated with the substitutional aluminium content, and an ESR signal due to substitutional aluminium. Accordingly, the authers suggested that the smoky color in quartz due to substitutional aluminium was associated with A_3 optical band at 2.9 eV rather than the A_1 and A_2 bands proposed earlier.

The purpose of the present study is to investigate the correlation if any, between the two induced hole centers H_2^+ and H_3^+ in irradiated soda-silica glass and the visible induced centers in crystalline silica, and to assign models for the various present bands.

Experimental

Soda-silica glasses were prepared using ultra-pure sodium carbonate (Alpha grade) for Na_2O and corning purified fused silica for SiO_2.

Batches were prepared to yield glasses of the composition Na_2O. 3.0 SiO_2.

The batch constituents were then throughly mixed and melted in a Pt-Rh crucible in a high temperature pot furnace with Kanthal elements. To reduce the

volatilzation losses, the furnace temperature was slowly raised and then maintained at 1200°C for the hour, after which the melt was allowed to cool gradually to the room temperature. The glass samples were then ground and carfully polished, cerium oxide being used to obtain the final highly polished surfaces for optical measurements.

The natural smoky quartz sample was a Brazilian crystal.

Optical spectra were taken at room temperature by means of a recording spectrophotometer in the range 1 to 6.0 eV.

The specific absorbance in cm^{-1}, $\alpha = (\frac{1}{t}) \log_{10} (I_o/I)$, where t is the sample thickness in cm., was calculated without reflectance correction. X-irradiation experiments were carried out at room temperature at tube settings of 45 KV and 35 mA.

A curve resolver, a modified analog computer, was used to resolve the bands. Band parameters, viz., width at half maximum with absorption maximum (α_m) and energy at maximum absorption band positions were obtained from the resolved absorption curves.

Results and Discussion

Figure (1) shows the optical absorption spectra of Na_2O, $3.0SiO_2$ glass melted under oxidizing conditions prior to and after 30 mins, of x-ray irradiation. The unirradiated glass was colorless and showed no absorption bands in the visible region of the spectrum. The effect of x-irradiation on the absorption spectrum is best illustrated by a difference spectrum. This spectrum is obtained by subtracting the spectrum taken before irradiation from the one measured after irradiation and is referred to as the induced spectrum.

Figure (2) shows the induced spectrum of the oxidized soda-silica glass together with Gaussian resolution for the absorption bands. The absorption is resolved into three bands at about 1.98, 2.68 and 4.0 eV (widths at half-maxima are 0.5, 1.08 and 1.16 eV respectively) being characteristic of oxidized soda-silica glasses. The absorption band positions are in proper agreement with those reported by Mackey et.al. (1966, 1970). As was mentioned earlier, the bands H_3^+ and H_2^+ (1.98 and 2.68 eV) were assigned to two distinct hole centers, while the band E_3^- (4.0eV) was assigned to a trapped electron.

Figure (3) illustrates an approximate gaussian resolution for an irradiated natural Brazilian smoky

quartz. A striking similarity in band positions and shapes was observed between the A_1 and A_2 bands at 1.9 and 2.58 induced in irradiated natural smoky quartz and the bands at about 1.98 and 2.68 eV induced in irradiated sodium silicate glass. The slight differences in positions and widths at half-maximum between the two spectra as presented in Table (1) were not entirely unexpected. These differences can be interpreted in view of the regular arrangement of the SiO_4 tetrahedron in the case of quartz compared to the random network on glass.

Table 1

Comparison of Band Parameters in Smoky Quartz to those in Soda-Silica Glass

Absorption Band	Smoky Quartz		$Na_2O.\ 30SiO_2$ Glass	
	Peak position eV	Width at half-max. eV	Peak position eV	Width at half-max. eV
A_1 or H_3^+	1.9	0.7	1.98	0.5
A_2 or H_2^+	2.58	0.8	2.68	1.08

The band at about 3.0 eV in smoky quartz resembles the A_3 band observed by Nassau and Prescott (1978) with peak position varying between 2.58 and 3.1 eV, and width at half-maximum varying between 1.3 and 1.7 eV. They

attributed the half-maximum varying between 1.3 and 1.7 eV. They attributed the changes in the band position and width to non-equivalent of silicon sites by aluminium. The positions and widths at half-maxima of the gaussian resolved bands that provided satisfactory fit in smoky quartz together with those given by Nassau and Prescott are given in table 2.

Table (2)

Positions and width at Half-maxima ($\frac{W_1}{2}$) of Appproximate Gaussian Resolved Bands in Smoky Quartz as Compared to those given by Nassau and Prescott (1975, 1977 and 1978).

Absorption band	The study band Position	$W_{\frac{1}{2}}$	Nassau and Prescott band Position	$W_{\frac{1}{2}}$
A_1	1.9	0.7	1.8	0.7
A_2	2.58	0.8	2.5	0.8
A_3	3.05	1.1	2.85-3.1	1.3-1.7
B	3.98	1.28	3.9	1.2
C	5.0	--	.0	--

The observed similarity of the A_1 and A_2 bands in smoky quartz and the H_3^+ and H_2^+ bands in soda-silica glass strongly supports Nassau's and Prescott's conclusion

regarding the A_1 and A_2 bands and their non-correlation to the substitutional aluminium hole centers in the quartz structure. This is further substantiated by the development of the band at 3.05 eV in the absorption spectra of smoky quartz. The intensity of this band increased in accordance with the intensity of the smoky color.

Conclusion

The H_3 and H_2 absorption bands in x-irradiated soda-silica glasses and the A_1 and A_2 bands in smoky quartz may be associated with a defect in the silica structure which persists even when the long range order is destroyed on transition from the crystalline lattice to the more random vitreous state. It is well known that the introduction of an alkali into the silica network breaks up the siliconoxygen bond forming one non-bridging oxygen for each alkali ion. During irradiation this defect losses an electron and results in a trapped hole center, with the hole restricted to the non-bridging oxygen. The released electron can be trapped in a nearby precursor defect which comprises an electron trap, or the Na^+ ion can trap the released electron and become a neutral atom. Since the sodium atom is sufficiently small, it is free to migrate through the network to an electron-trapped defect. This defect may accept the charge and the sodium atom becomes an ion again. However, that the two centers

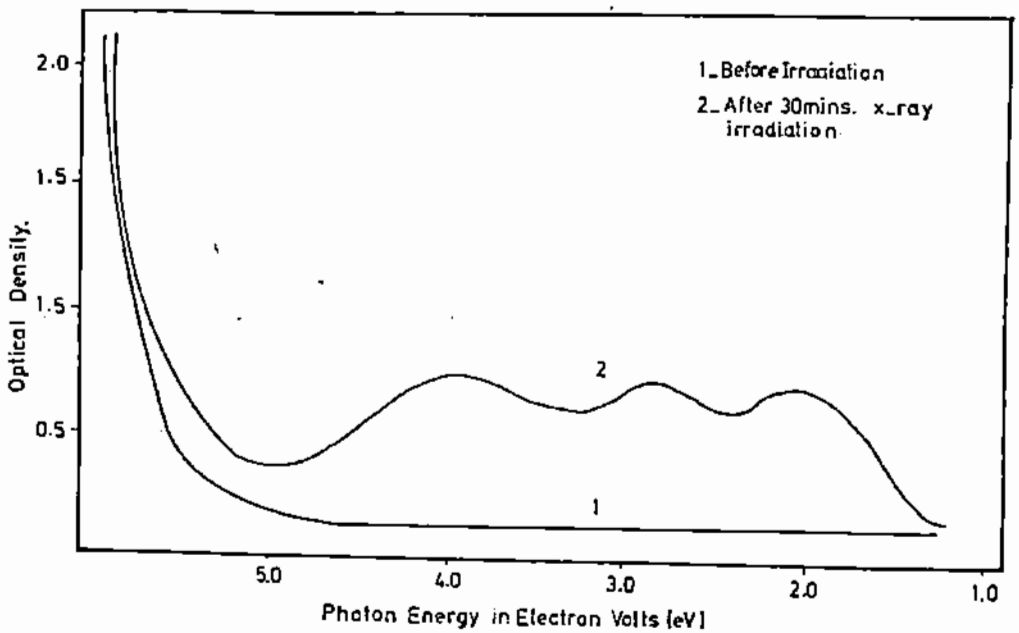

Fig.(1): The optical absorption spectra of sodium silicate glass melted under oxidizing conditions before and after 30 mintes x_ray irradiation.(sample thickness 0.09 cm).

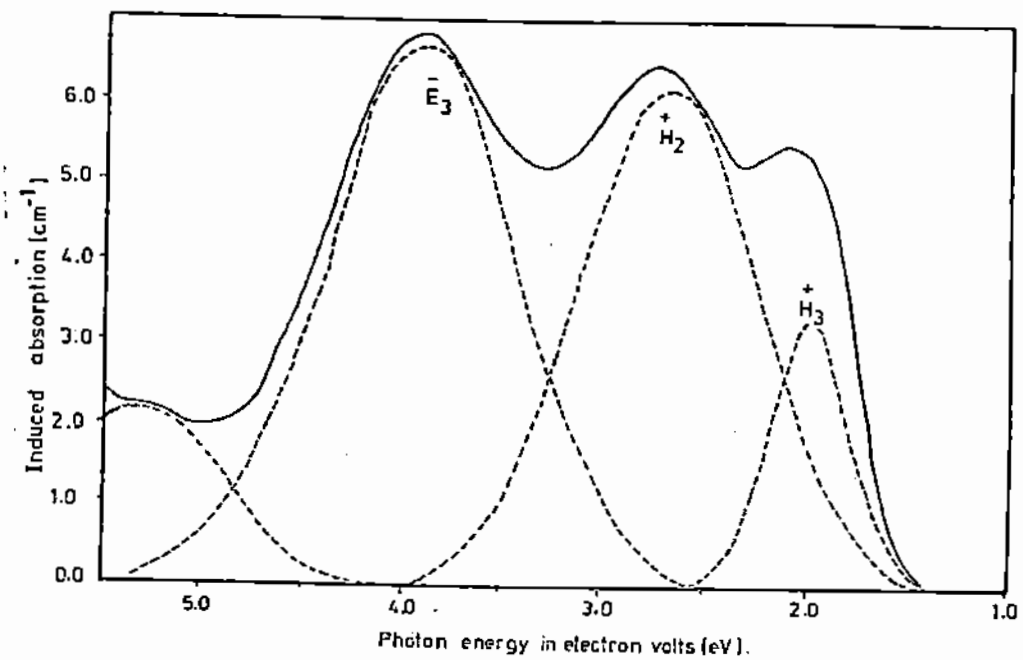

Fig.(2): Approximate gaussian resolution of the induced absorption spectrum of sodium silicate glass.

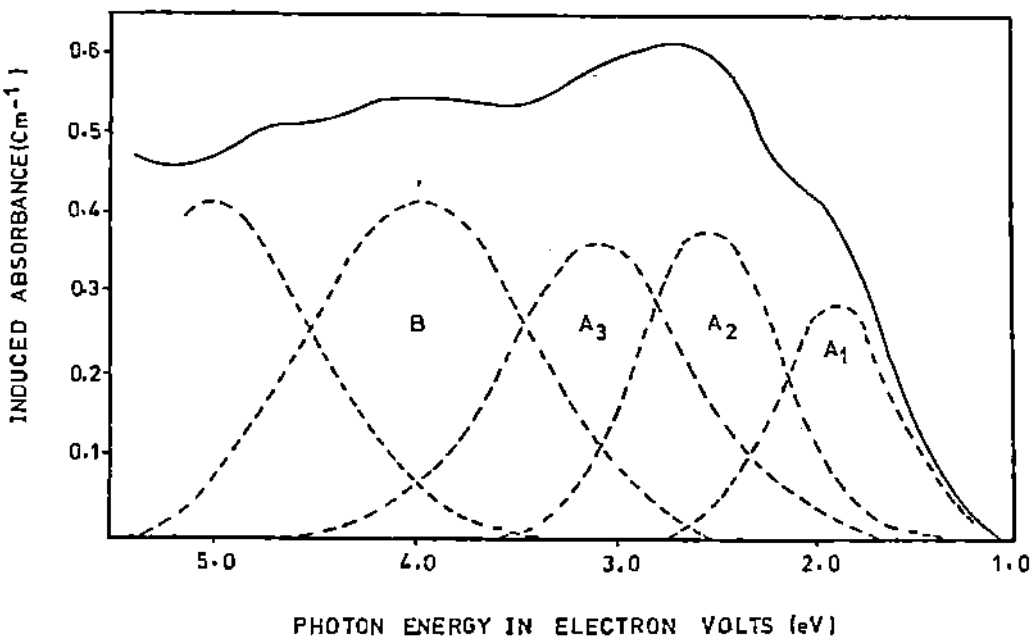

Fig.(3). Approximate gaussian resolution of the induced absorption spectrum of natural smoky quartz.

H_3^+ and H_2^+ are transitions of the same center is unlikely in the light of earlier observation by Mackey et. al. (1970) to the effect that their intire ratio varies strongly during the coloring and bleaching processes, and that their rate of annihilation varies with increasing the titanium concetration in the glass.

We can, therefore, consider the idea that the two centers differ in the number of non-bridging oxygens as suggested by Schreurs (1967). We may also suspect that the origin of the two bands in smoky quartz is related to paramagnetic oxygens similar to their origin in glass. It is obvious, however, that further studies must be carried out to clarify this problem. It would be helpful in investigating the nature of these bands in quartz, to illuminate the samples at low temperature with light that falls mainly in the 1.98 eV region, and study the variations in the intensity ratio of the two hands with further x-irradiation and/or bleaching.

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EFFECT OF CATHODE LENGTH ON THE CHARACTERISTICS OF THE GRIDDED CORONA VOLTAGE STABILIZER DEVICE

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ABSTRACT

The present work is intended to investigate the properties of a gridded wire-plate corona device when used as corona voltage stabelizer (GCVS). In particular it is devoted to the investigation of the dependence of the stabelization on the cathode length (L_c).

INTRODUCTION

The need for voltage stablization has been so common and important that certain preferred circuits and standard practices have been developed. The corona voltage stabelizer at normal atmospheric pressure was investigated[1]. The basic mechanism of various corona modes and corona stabelization have been studied by Loeb[2]. More work has been done on the wire-plane [3,4,5] and gridded devices[6] for CVS devices.

The study of the cathode length variation is found to be more complicated as it is necessary to take into consideration the fact that the discharge of electricity is confined to that portion of the anode wire covered hy luminous glow. If the length of the cathode is varied the operating length of the wire increases and therefore the corona current increases. The present work is devoted to study the effect of cathode length on the characteristics of GCVS device.

EXPERIMENTAL SET UP:

The arrangement of the GCVS device is shown in Fig.(1) which consists of a highly polished plate serves as cathode of area 14.2 x 6.2 cm^2. Ankor wires of diameter 0.33 mm were used as anode and grid wires. The distance between the single anode wire and the cathode plane h_a is 2 mm, the distance between the double wires grid and the cathode plane h_g is 5 mm and the separation between the grid wires is 3 mm The limiting resistance on both anode R_a and grid R_g is each 20 MΩ .

Two equal mica strips have been used to insulate the two rounded ends of the plane cathode from the anode wire and also to vary the effective cathode length L_c from 1.2 to 13.2 cm which is fixed by the separation distance between the two mica strips.The GCVS device is placed in a closed enclosure inside which the relative humidity R.H. and temperature T are kept constant during the running time of the experiment i.e. R.H. = 30% and T = 25°C.

RESULTS AND DISCUSSION

The variations of the corona current I_a with the anode applied votlage V_a was determined and shown in Fig.(2). It has been found from this figure that for a given applied voltage, the corona current I_a increases as L_c expands,and for a given L_c the steady corona current starts by a very small region where it increases nonlinearly with increasing the applied voltage beyond which it almost increases linearly

according to the relation

$$I_a = m V_a + I_o \qquad (1)$$

where m and I_o are constants.

Applying the least squares fit to the present experimental results in these linear region of the corona current curves the constant values of m and I_o corresponding to different values of L_c were determined and represented in table (1). Applying the least-squares fit for m and I_o represented in Fig. (3), empirical formulas of the second order have been determined for m and I_o in terms of L_c . Thus an empirical formula was determined for I_a interms of V_a for different values of L_c in the form.

$$I_a = \left[(-196.3 - 8.1 L_c + L_c + 0.47 L_c^2) + (39.53 + 1.83 L_c - 0.084 L_c^2) \times 10^{-3} V_a\right] \quad 10^{-6} \text{ amp.} \qquad (2)$$

where L_c in mm and V_a in volts.

Table (1) The computer values of m and I_o

L_c (cm)	$I_0 \times 10^{-6}$ (Amp.)	m x 10^{-6} (ohm^{-1})
13.2	-221.3 ± 0.0239	0.0490 ± 0.0041
12.2	-225.6 ± 0.0188	0.0494 ± 0.0043
11.2	-228.1 ± 0.0389	0.0495 ± 0.0039
10.2	-230.0 ± 0.0267	0.0495 ± 0.0039
9.2	-231.0 ± 0.0340	0.0493 ± 0.0040
8.2	-231.0 ± 0.0340	0.0489 ± 0.0069
7.2	-230.3 ± 0.0180	0.0481 ± 0.0063
6.2	-228.5 ± 0.0630	0.0476 ± 0.0013
5.2	-225.7 ± 0.0188	0.0468 ± 0.0013
4.2	-222.0 ± 0.0113	0.0457 ± 0.0067
3.2	-217.4 ± 0.0134	0.0445 ± 0.0067
2.2	-211.8 ± 0.0860	0.0431 ± 0.0013
1.2	-205.3 ± 0.0794	0.0416 ± 0.0025

In Fig.(2) the computed values (full curves) of I_a vs. V_a are plotted. On the same figure the experimental values (circles) are represented, it show good agreement.

In the steady corona region the effective voltage V_{ae} between the anode and the cathode is given by

$$V_{ae} = (1 - m R_a) V_a - I_o R_a \qquad (3)$$

Substituting for R_a = 20 MΩ and (m, I_o) from table (1) the relation between V_{ae} and V_a is determined and represented in Fig. (4) for various values of L_c. From this figure it is clear that the slopes of the plateau regions are positive and increase with decreasing L_c.

From Eq.(3)

$$\frac{dV_{ae}}{dV_a} = 1 - mR_a \qquad (4)$$

The values of dV_{ae}/dV_a vs. L_c are illustrated in Fig. (5) which are constructed from the basic data of the corona current with the help of eq. (4). It is clear that the value of dV_{ae}/dv_a decreases with decreasing L_c from L_c = 13.2 to 10.2 cm after which it increases with decreasing L_c.

The values of the internal resistance r_d of the device are calculated by the equation.

$$r_d = \frac{V_{ae}}{I_a} \qquad (5)$$

Fig.(6) gives the relation between (r_d) and the applied voltage V_a for different values of L_c. From this figure it is clear that the value of r_d increases gradually with the decrease of V_a comming to steep rise.

Fig.(7) shows the relation between L_c and the a.c. resistance r_a of the device which is denoted by eq.(6).

$$r_a = \frac{dV_{ae}}{dI_a} \qquad (6)$$

From Fig.(7) it is clear that r_a decreases with increasing of L_c until L_c = 11.2 cm while it increases with increasing L_c for L_c = 12.2 cm and 13.2 cm.

Most often stabilizer devices are used in circuits where the load resistance is constant and the source voltage is subject to variations. Then as the source voltage rises, the current through the device increases, and nearly all voltage change is absorbed across R_a, so that the voltage across the device remains practically constant (or increases insignificantly) provided the device current remains within the normal glow region of the characteristic.

Nevertheless, the cathode length L_c was found to be an interesting parameter which affects the working conditions. Thus it is necessary to select the suitable L_c for any experiment and fix this value during the time of the results.

ACKNOELEDGEMENT

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تأثير طول المهبط على خصائص جهاز مثبت الجهد الهالى الشبكى

دكتوره/ اعتماد عبدالغنى جاد

قسم الطبيعة بكلية البنات ـ جامعة عين شمس

ملخص البحث

يتناول هذا البحث تأثير تغير طول المهبط على خصائص جهاز مثبت الجهد الهالى الشبكى الذى يتكون مهبطه من لوح معدنى مستوى وشبكى المصعد والشبكه .

وقدوجد من خلال هذا البحث أن طول المهبط له تأثير فعال على خصائص التشغيل للجهاز ، ولذلك من الضرورى عند تصميم جهاز مثبت للجهد الهالى اختيار طول المهبط المناسب للحصول على معامل تثبيت جهد جيد.

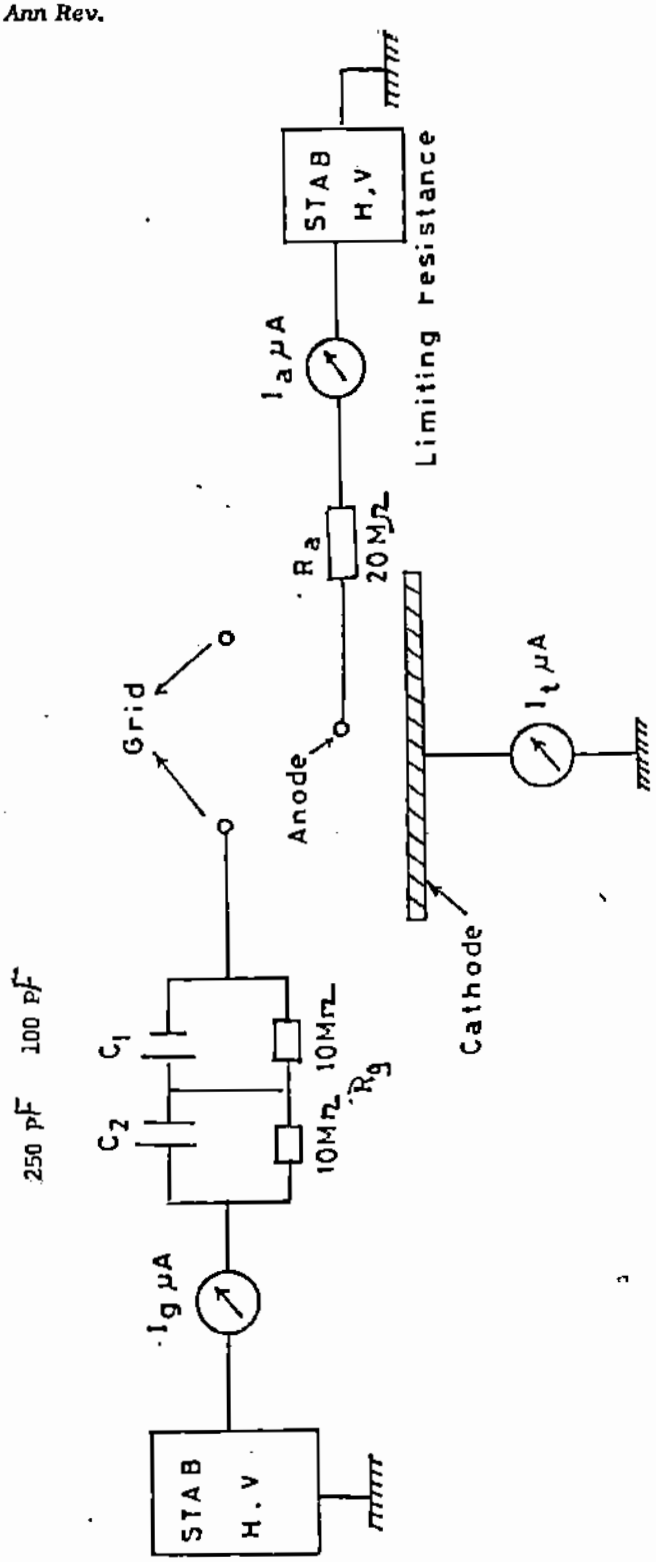


Fig. (1) The arrangement of the corona voltage stabilizer.

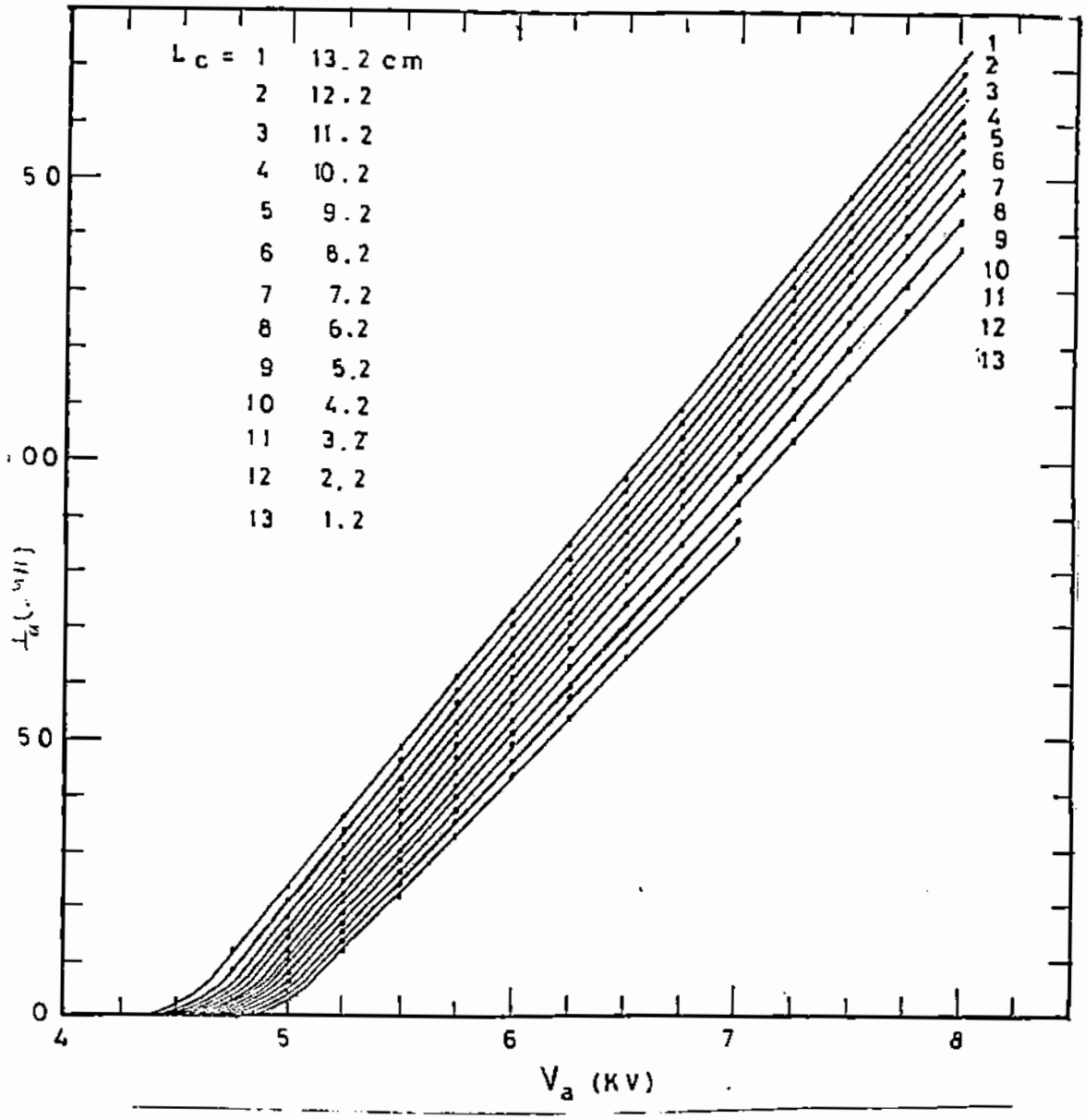


Fig. (2) I_a Vs. V_a for different values of L_c.

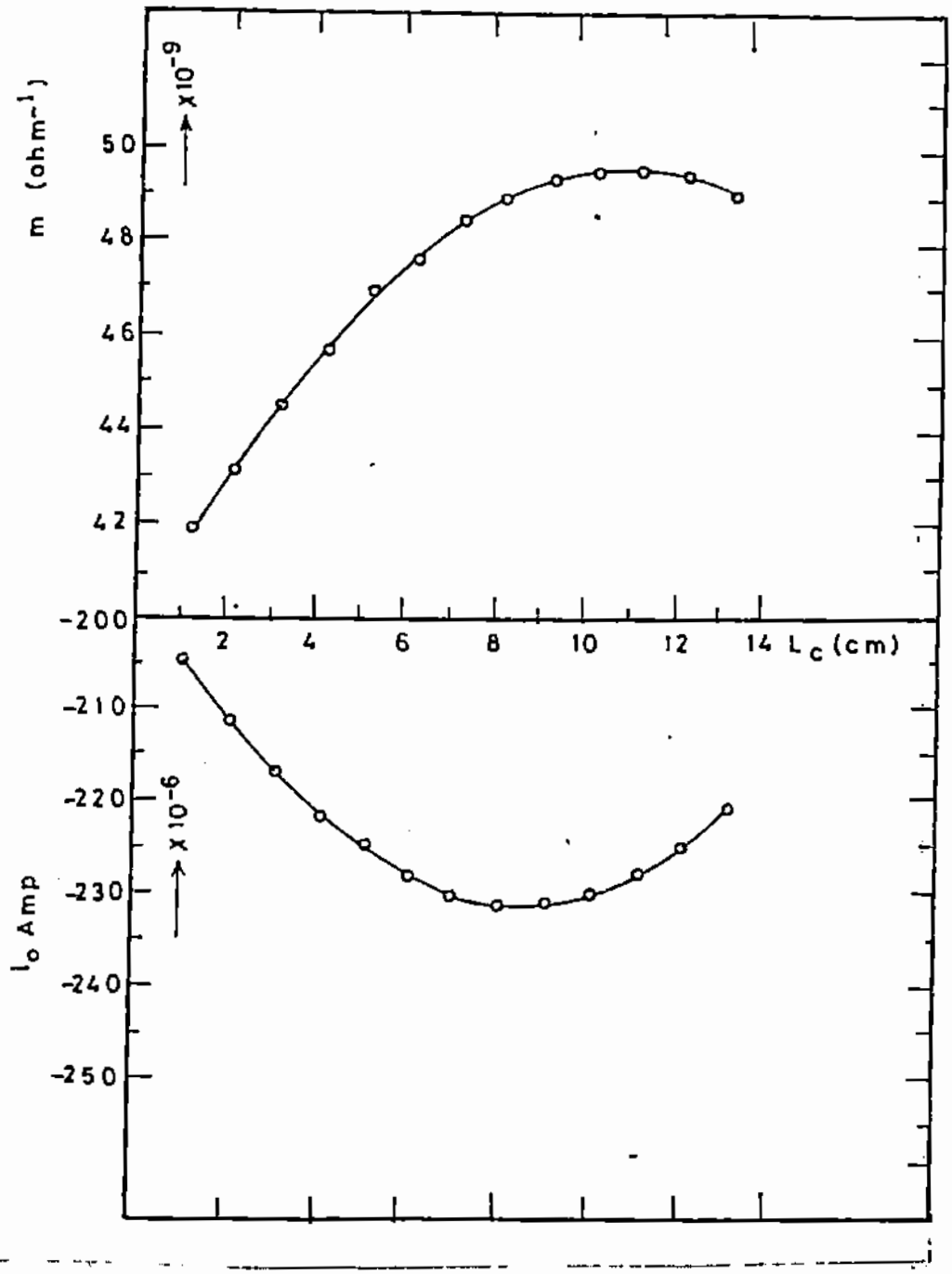
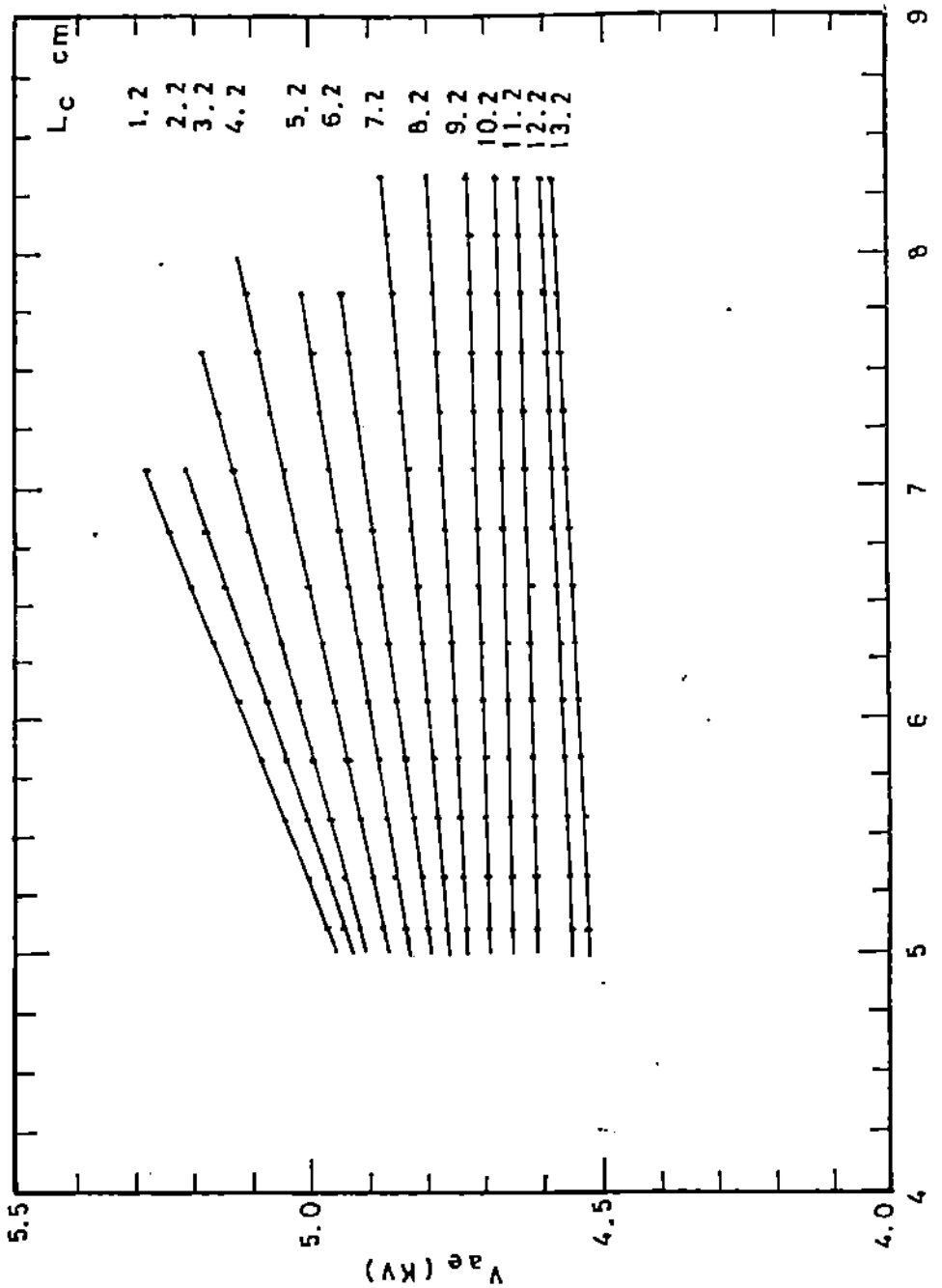


Fig. (3)m & I_o vs. L_c for various values of L_c.


Lc cm
1.2
2.2
3.2
4.2
5.2
6.2
7.2
8.2
9.2
10.2
11.2
12.2
13.2
5.5
5.0
4.5
4.0
V_{ae} (KV)
4
5
6
7
8
9
V_a (KV)

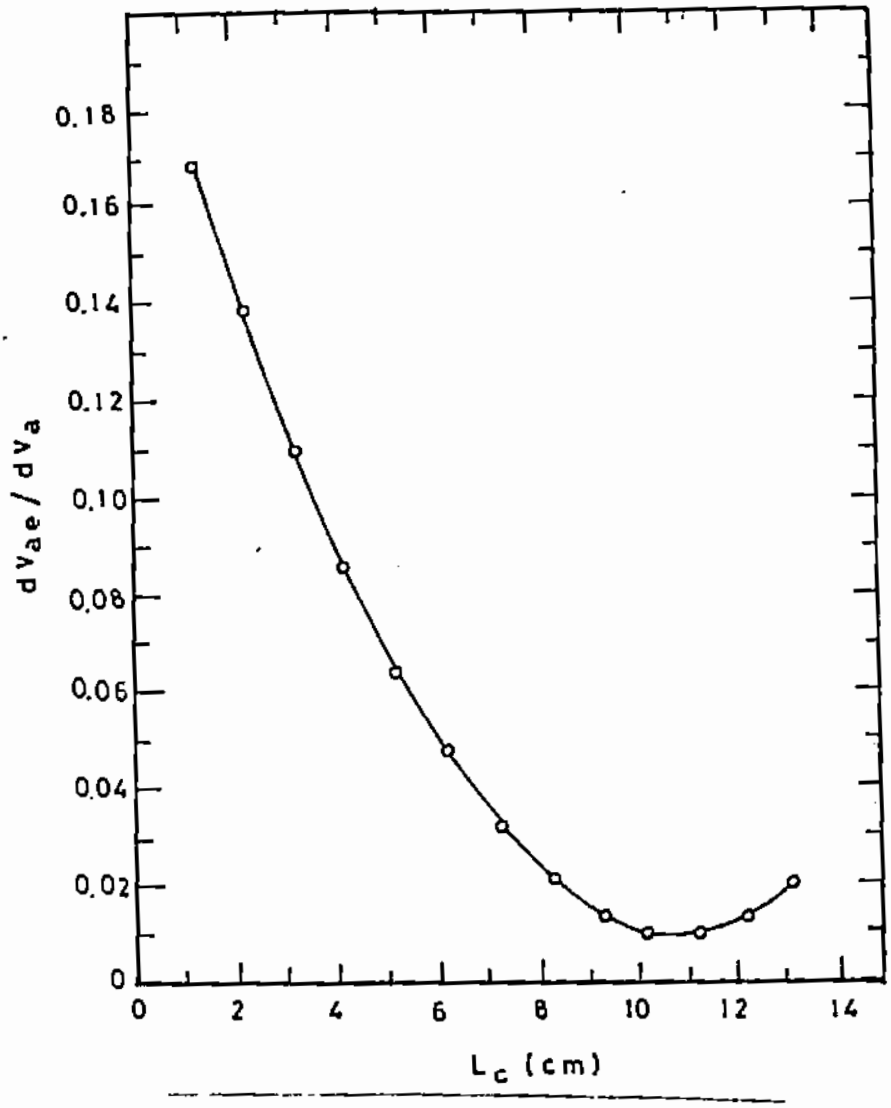


Fig. (5) $\frac{dV_{ae}}{dV_a}$ Vs. L_c.

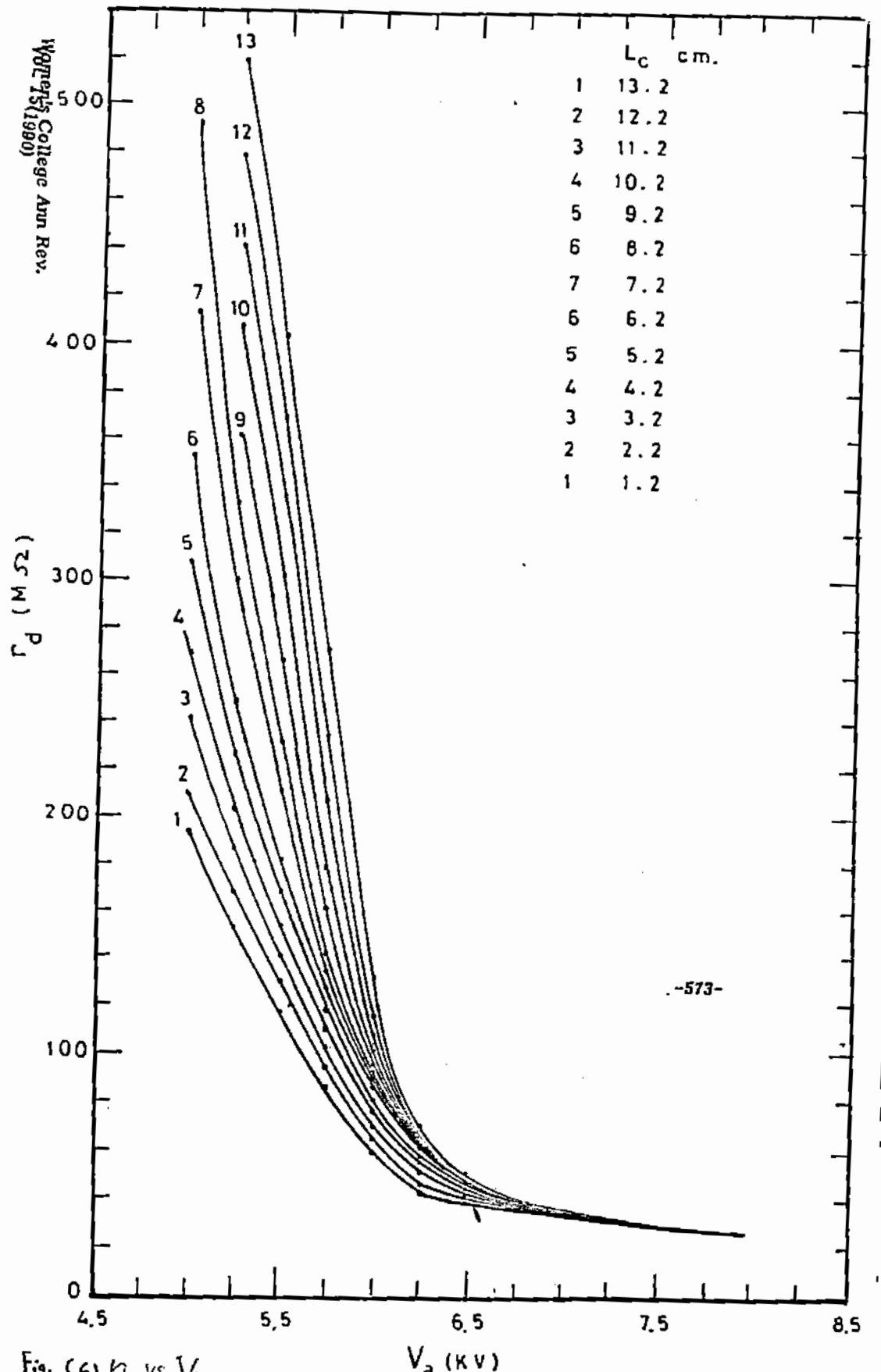


Fig. (6) η vs. V_a

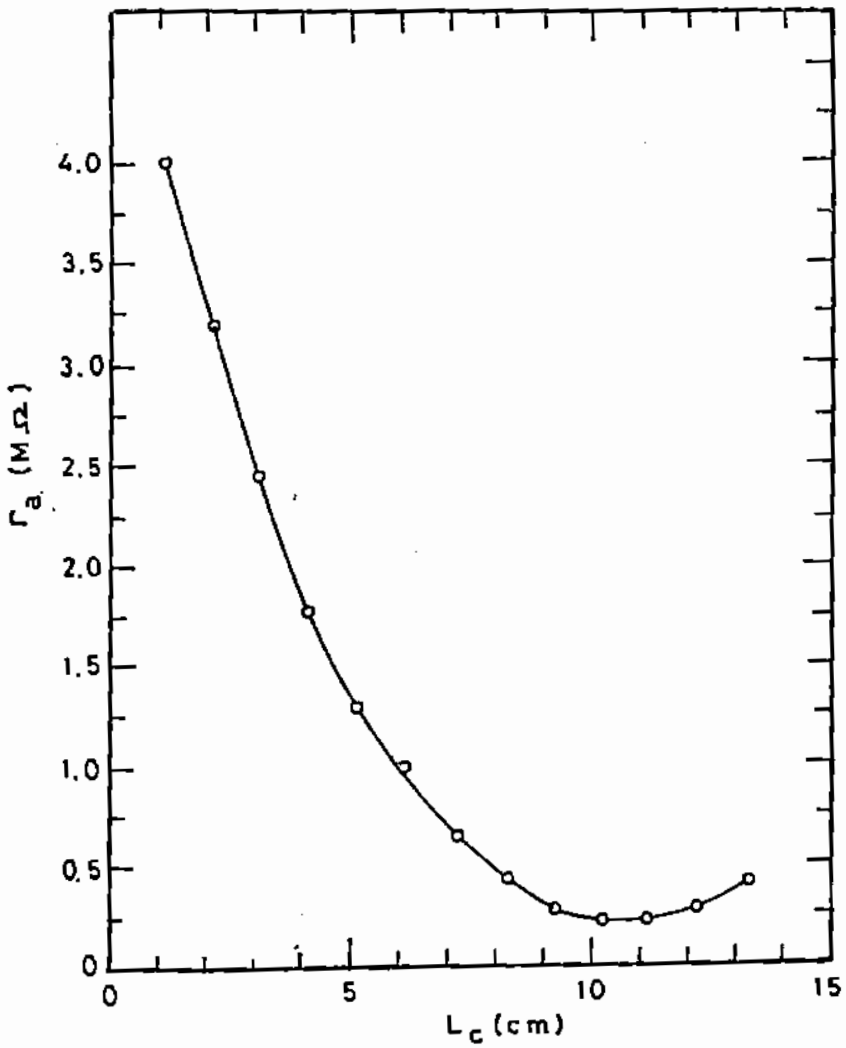


Fig. (7) r_a vs. L_c.

حـــوليـــــة كليـــــة البنـــات

بجـامعـة عيـــن شـــمـس

تصـــدرهـا كليـــة البنـــات ـ جـامعـة عيـن شـمـس

رؤســـاء التحـريـر:

أ.د/ أحمـد عبـد الرحيـــم طـه — عميـــد الكليـــة

أ.د/ نفيســه محمـد عبد السلام عليـش — وكيلة الكليـة للدراســات العليا والبحـوث

ســكرتير التحـريـر:

أ.د/ محمـد ابراهيم حســن محمـود