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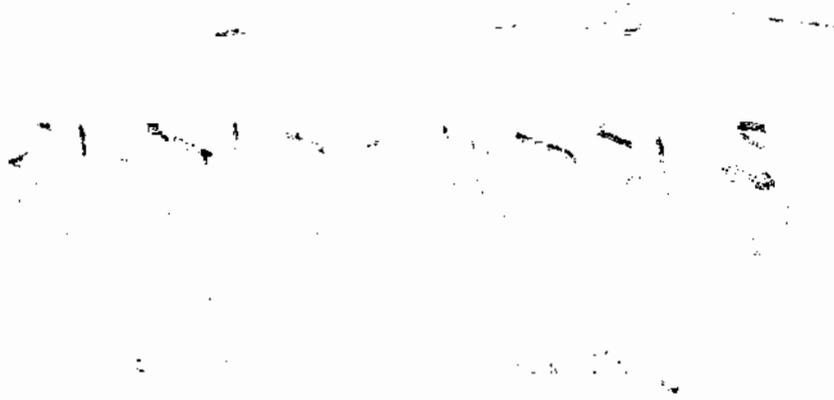
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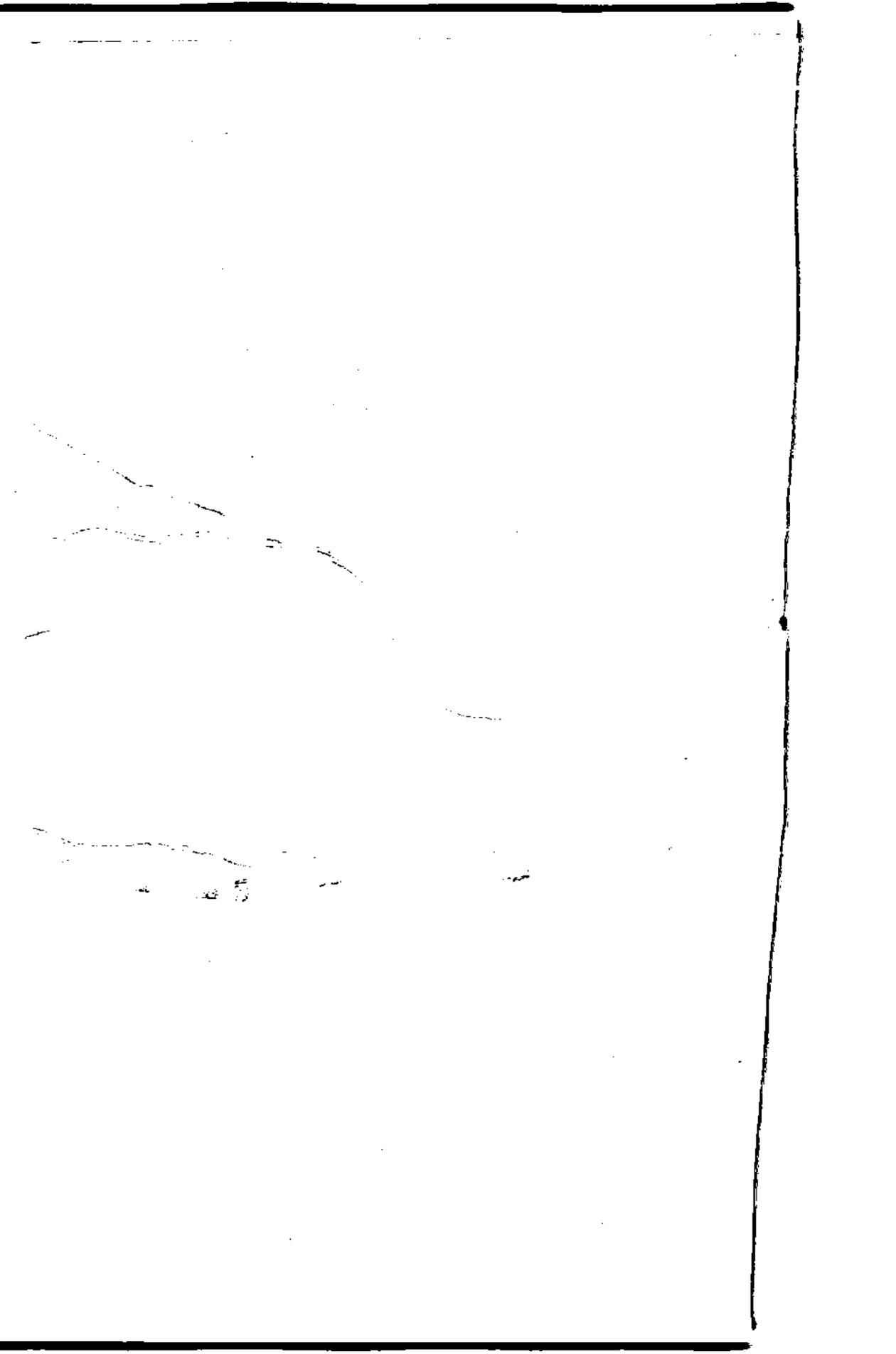
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CONTENTS

	page
Explicit Orthogonal Symmetrised Spin States for General Internal Cases, by N. G. El-Sharkawy	1
High Temperature Third Virial Coefficient for Square-Well Plus van Der Waals Potential, by M. Boghdady	17
The Steady Plane Couette Flow and Heat Transfer of a Rarefied Electron Gas, by M. A. Khidr and A. G. El-Sakka	31
Transistorized Gamma-Gamma Coincidence Spectrometer, by H. Abou-Leila and A. A. El-Kamhawy	43
Effect of External Magnetic Field on the Volatalization of Elements in the D.C. Arc, by A. A. Fakhry, and M. A. Eid	51
The Spectral Light Current of an Optical Resonator, by K. A. El-Dehmy and M. M. El-Necklawy	67
Effect of Humidity and Temperature on the Relative Efficiency of Streamer Counter, by H. M. Abu-Zeid and M. B. Osman	77
Characteristics of an Energy and Time Analysis System Using a Ge(Li) and a Fast Scintation Detectors, by H. Abou-Leila and S. M. Darwish	101

	page
of Polyamide Polycarboxylic Acids on the of Aluminum in Alkaline Medium, Mostafa and M. B. Hafez	129
and Meiotic Effects of Trifluralin on <u>Vicia</u> Kraem and A. Shehab	141
cal Effects of Contraceptives, Hakeem	153
with and Mitotic Effects of Nemagon and Nemacur <u>faba</u> , Shehab and H. Hakeem	167
on the Effect of Herbicides on Meiosis, Size of Grains and Pollen Viability, Kraem and A. Shehab	191
Comparative Study of Early Growth Stages and Cytological of Eight Herbicides on Plants, El-Moussa and H. Hakeem	203
Effect of the Spray of the Mineral Fertilizer "Schonchen" on Chlorophyll Content and Photosynthesis of Okra, El-Mowidar and M. El-S. Abdulla	221
Effect of Certain Pre-sowing Seed Treatments and Phosphorus Supplement on Mineral Composition of <u>a rays L.</u> Plants Grown under Soil Moisture Conditions, Gaber and M. T. El-Saidi	231

	page
Asparaginase Activity in Bacteria Isolated From the Soil of Kuwait. by S. A. Mustafa and M. Salama	247
Production of L-Asparaginase by <u>Streptomyces keratensis</u> and <u>Streptomyces venezuelae</u> . by S. A. Mustafa	259
The Interaction Between Dietary Protein and Alcon 1sm: Effect on Hepatic Lipids, by M. A. El-Ashry	277
Production of Lipas(s) by Some Yeasts: I. Effect of Temperature, pH and Triglycerides, by S. M. El-Gammal and M.I. Rizk	289
II. Effect on Some Carbohydrates and Nitrogenous Compounds, by S. M. El-Gammal and M. I. Rizk	305
Effect of Thiourea on the Germination, respiration and Growth of <u>Anaranthus chlorostachis</u> Seeds, by A.E. Dowidar and S. H. Rabie	321
Studies on Growth of <u>Rhizobium</u> of <u>Phaseolus sativum</u> under Stress Conditions, by F. A. Helemish	331



EXPLICIT ORTHOGONAL SYMMETRISED SPIN STATES : FOR GENERAL INTERNAL CASES (Letter)⁽⁶⁾

By

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ABSTRACT

In a previous work by the author (ref. 6) a simple explicit expansion is given for the states of n spin $1/2$ particles characterised by the Young tableaux for the function

$$\left| \begin{array}{cccc} 1 & \dots & n-1 & \\ P_1 P_2 & \dots & P_{m-1} & \bar{n} \end{array} \right| \quad \text{and} \quad \left| \begin{array}{cccc} & \dots & & n \\ P_1 P_2 & \dots & P_{m-1} & n-1 \end{array} \right|$$

the expansions given were proved to satisfy Young theorem for external symmetry and internal symmetry in three special cases.

In this paper the expansions given before (ref. 6) were proved to satisfy Young theorem for the general internal cases : $n'-1$ and n' in different rows and columns.

§ 1. The general internal case : in $n'-1$ and n' in different rows and columns.

The proof in this case is very similar to the general external case treated in sc. 4. (6) As before we have variables $x_1 x_2 \dots x_{h-1}$ where row

$$h' = n' - 2m' + 1 \quad (1)$$

and (with external type \uparrow_n for example)

$$\begin{array}{l} \uparrow_{(n')n} \left| \begin{array}{cccc} 1 & \boxed{h' - 1 = h' - 2m'} & n' - 1 & \dots & n - 1 \\ P_1 P_2 \dots P_{m'-1} n' & P_{m'+1} P_{m'+2} \dots P_{m'+k} & m & n & \end{array} \right| \quad \text{SS} \rangle, \\ \uparrow_{(n'-1)n} \left| \begin{array}{cccc} \boxed{1 \leftarrow h' - 1 = n' - 2m' \rightarrow n'} & \dots & n - 1 & \\ P_1 P_2 \dots P_{m'-1} n' - 1 & P_{m'+1} P_{m'+2} \dots P_{m'+k} & P_m & n \end{array} \right| \quad \text{SS} \rangle, \end{array} \quad (2)$$

there being $h' - 1 = n' - 2m'$

(3)

In full

$$\begin{aligned}
 \chi_{n'} &= \frac{a}{3 \cdot 2 (h'+1) h' 4 \cdot 3 \cdot 4 3} \sum_{q_1} \left| \begin{matrix} q_1 \\ 3 \end{matrix} \right| \left| \begin{matrix} q_2 \\ n'-1 \end{matrix} \right| \left| \begin{matrix} q_3 \\ 8 \end{matrix} \right| \left| \begin{matrix} q_4 \\ 10 \end{matrix} \right| a_1 a_2 \\
 \chi_{n'-1} &= \frac{1}{3 \cdot 2 h' (h'-1) 4 \cdot 3 \cdot 4 \cdot 3} \sum_{q_1} \left| \begin{matrix} q_1 \\ 3 \end{matrix} \right| \left| \begin{matrix} q_2 \\ n'-1 \end{matrix} \right| \left| \begin{matrix} q_3 \\ 8 \end{matrix} \right| \left| \begin{matrix} q_4 \\ 10 \end{matrix} \right| a_1 a_2 \\
 & \qquad \qquad \qquad a_1 a_2 \quad (11) \\
 & \qquad \qquad \qquad a_1 a_2 \quad (11)'
 \end{aligned}$$

The expansion of (11) contains 72 terms which in the abbreviated notation of the thesis of N.G. El-Sharkawy (p 267) may be written as

$$\begin{aligned}
 (12)^2 (45 (n'-1) 9) + (12) (45 (n'-1) (1245 (n'-1))) \\
 (1245 (n'-1) 9) \qquad \qquad \qquad (12)
 \end{aligned}$$

the expansion of (12)' contains 54 terms which may be written as

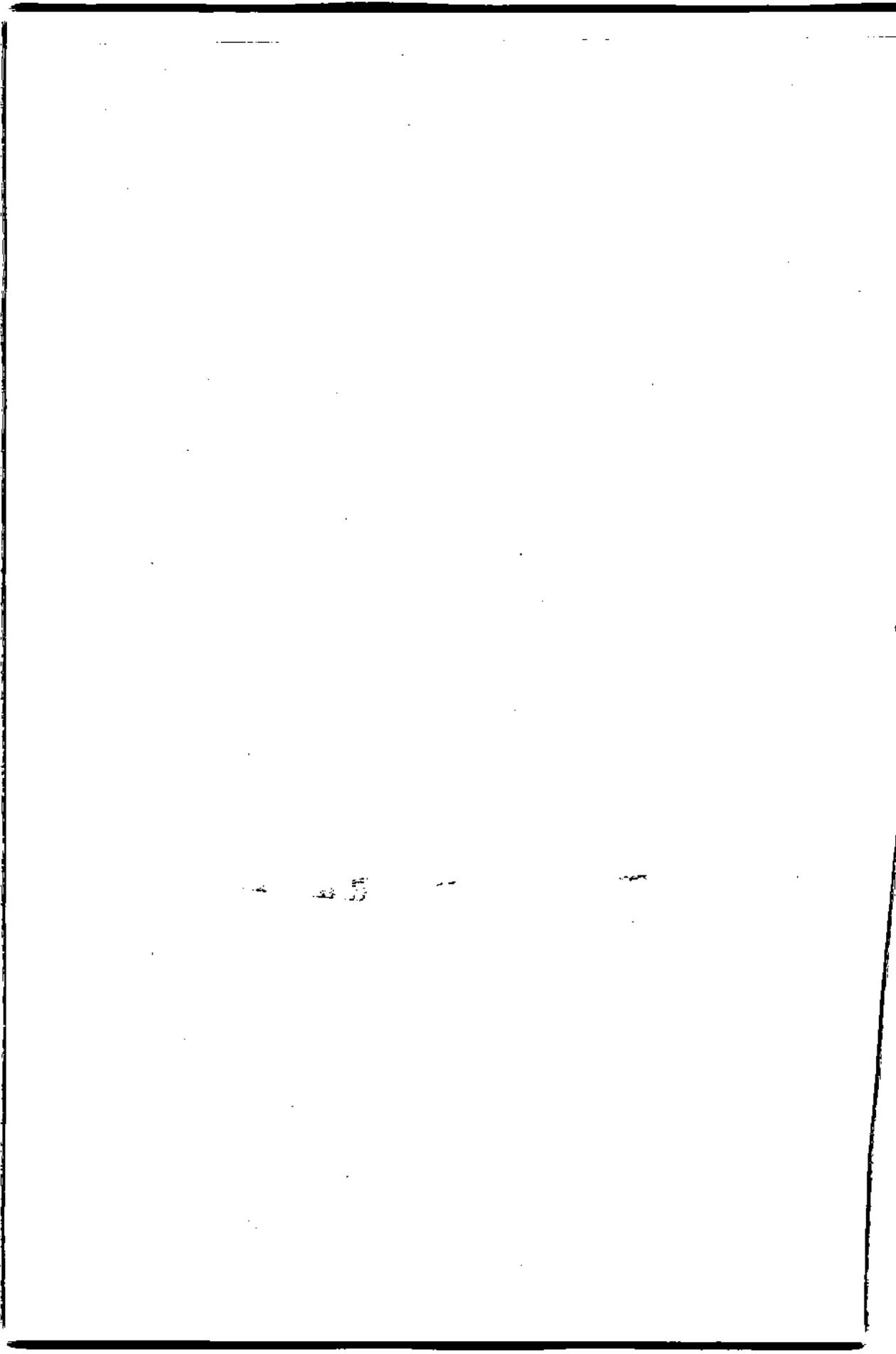
$$(12)^2 (45n') (45n'9) + (12) (45) (1245n') (12 45n'9) \qquad (12)'$$

The nature of this is such that all the allowed numerators in the product (in the form of two-particle determinants, with denominators 3, n', 8, 10 for n' and 3, n'-1, 8, 10 for n'-1) occur in the expansion by taking one number from each of the four reduced brackets avoiding repetition. Note that (12)² means (1) (2) + (2) (1).

n' we have thus 72 numerations.

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HOOK PRODUCT DECOMPOSITION OF YOUNG PATTERNS

By

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ABSTRACT

The Braunschweig-Hecht decomposition of Young patterns into linear combinations of either antisymmetric factor patterns (column outer products) or symmetric factor patterns (row outer products) is generalized to a decomposition into linear combinations of single hook outer products. The decomposition matrices, with their inverses, are tabulated for $n \leq 10$ and checked using the known outer product dimension formula. Applications to multiple hook Young operator expansions are being considered.

INTRODUCTION

Braunschweig and Hecht 1978 introduced decomposition of Young patterns into their completely symmetric or antisymmetric components.

In this paper the decomposition of Young patterns into linear combinations of either symmetric or an tisymmetric factor patterns is generalised to a decomposition into linear combinations of single hook outer products.

The decomposition matrices, with their inverses, are tabulated for $n \leq 10$ and checked using the known outer product dimension formula.

Applications to multiple hook Young operator expansions are being considered.

In the following tables the decomposition matrices with their inverses are presented.

Table 1 A

$f_{H'} = f_H$	$H' = H$	$f_{p'} = f_p$	f_p	1
1	H 1	$p' = p$	[1]	1

Table 1 B

$f_{H'} = f_H$	$H' = H$	$f_{p'} = f_p$	f_p	1
1	H 1	$p' = p$	H 1	1

Table 2 A

$f_{H'} = f_H$	$H' = H$	$f_{p'} = f_p$	f_p	1
1	H 1	$p' = p$	1^2 [2]	1

Table 2 B

$f_{H'} = f_H$	$H' = H$	$f_{p'} = f_p$	f_p	1
1	H 1	$p' = p$	H 1	1

Table 3 A

$f_{H'} = f_H$	H'	H	$f_{p'} = f_p$
1	$H1^3$	$H3$	$[1^3] \quad [3]$
2	$H21$		$[21]$

Table 3 B

$f_{p'} = f_p$	p'	p	$f_{H'} = f_H$
1	1^3	3	$H3 \quad 1$
2	$[21]$		$H21 \quad 2$

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Table 4 A

$f_{H'} = f_H$	H'	H	p'	p	$f_{p'} = f_p$
1	$H1^4$	$H4$	$[1^4]$	$[4]$	1
3	$H21^2$	$H31$	$[21^2]$	$[31]$	3
8	$H2^2$			$[2^2]$	2

Table 4 B

$f_{p'} = f_p$	p'	p	H'	H	$f_{H'} = f_H$
1	$[1^4]$	$[4]$	$H1^4$	$H4$	1
3	$[21^2]$	$[31]$	$H21^2$	$H31$	3
2	$[2^2]$			$H2^2$	8

Table 5 A

f_{H^1} $= f_H$	H^1	H / P^1	$f_{P^1} = f_P$			
			P	(5)	(41)	(32)
1	H15	H5				
4	H213	H41		1		
15	H2 ² 1	H32		1	1	1
6	H31 ²					1

Table 5 B

f_{P^1} $= f_P$	P^1	P^1 / H^1	$f_{H^1} = f_H$			
			H	H5 <th>H41 <th>H32 <th>H31²</th> </th></th>	H41 <th>H32 <th>H31²</th> </th>	H32 <th>H31²</th>
1	(15)	(5)	1			
4	(213)	(41)		1		
5	(221)	(32)		1	1	-1
6	(312)					1

Table 6 A

$f_{H^1} = f_H$	H^1	$H^1 \backslash P$	$f_P = f_{P^1}$							
			(1 ⁶)	(21 ⁴)	(2 ² 1 ²)	(31 ³)	(23)	(321)		
1	H1 ⁶	H6	1							
5	H21 ⁴	H51		1						
24	H2 ² 1 ²	H42		1	1	1				
10	H31 ³	H41 ²					1			
45	H23	H3 ²		1	1	1	1	1		
36	H321						1	1	1	

Table 6 B

$f_{P^1} = f_P$	P	$P \backslash H^1$	$f_{H^1} = f_H$							
			H1 ⁶	H21 ⁴	H2 ² 1 ²	H31 ³	H23	H321		
1	(1 ⁶)	(6)	1							
5	(21 ⁴)	(51)		1						
9	(2 ² 1 ²)	(42)		1	1	1				
10	(31 ³)	(41 ²)					1			
5	(23)	(3 ²)				1	1	1	1	
16	(321)						1	1	1	

Table 8 B

$f_p = p$	p^1	p	$f_H = f_H^1$														
			H	H ³	H/1	H62	H612	H53	H521	H513	H42	H431	H422	H4212	H3 ² 2		
1	(1 ⁸)	(8)															
7	(21)	(71)		1													
20	(2 ² 1 ⁴)	(62)		-1	1	-1											
21	(31 ⁵)	(61 ²)					1										
28	(2 ³ 1 ²)	(53)			-1	1	1	-1	1								
64	(321 ³)	(521)					-1	1	-1								
35	(41 ⁴)	(51 ³)							1								
14	(2 ⁴)	(42)						-1	1	-1	1	-1				1	
90	(3 ² 21)	(431)						-1	1	1		1				-1	
56	(3 ² 1 ²)	(42 ²)					1	-1	1				1			-1	
90	(421 ²)								1	1						1	
42	(3 ² 2)								1	1			-1	-1	2	1	

HIGH TEMPERATURE THIRD VIRIAL COEFFICIENT FOR SQUARE-WELL
PLUS VAN DER WAALS POTENTIAL

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ABSTRACT

As a function of the absolute temperature T , the third virial coefficient in the case of a square-well plus Van der Waals potential is evaluated up to terms in β^2 ($\beta = \frac{1}{kT}$). Suitable variables are used which simplify considerably the integrations involved. The result obtained is valid only at high temperatures and low densities.

INTRODUCTION

As is well known⁽¹⁾, the thermodynamic properties of a system modelled by a canonical ensemble are related to the interactions of particles in the system through the partition function

$$A = -kT \ln Z \quad (1)$$

where $Z = \frac{1}{N! \Lambda^{3N}} \int \dots \int e^{-U(\underline{r}_1, \dots, \underline{r}_N)/kT} d\underline{r}_1 \dots d\underline{r}_N \quad (2)$

$$\equiv \frac{Q_N}{\Lambda^{3N}}, \quad \Lambda^2 = \frac{h^2}{2\pi m kT} \quad (3)$$

and A is the Helmholtz free energy of the system composed of N particles. The integral Q_N is known as the configurational partition function. Having determined A as a function of T, V, N all thermodynamic functions can follow. For example, the pressure p of the system is given by

$$p = -\left(\frac{\partial A}{\partial V}\right)_T \quad \text{or} \quad pV = KT \left(\frac{\partial \ln Q_N}{\partial \ln V}\right)_T \quad (4)$$

It is possible⁽²⁾ to develop $\ln Q_N$ as a power series in the number density $\rho \equiv \frac{N}{V}$. This allows an equation of state to be written as the virial expansion

$$p = \rho KT [1 + B_2(T)\rho + B_3(T)\rho^2 + \dots] \quad (5)$$

where B_2, B_3, \dots are the second, third, ... virial coefficients which are expressed in terms of Mayer's f -bond functions⁽²⁾

$$B_m = -\frac{m}{m+1} \cdot \frac{1}{V^m} \int \dots \int \sum_{1 \leq i < j \leq m+1} f_{ij}(r_{ij}) dr_1 \dots dr_{m+1} \quad (6)$$

$$f_{ij}(r_{ij}) = e^{-\beta u(r_{ij})} - 1 \quad (7)$$

$u(r_{ij})$ is the pair-wise additive interaction potential between particles i and j , and $\beta = \frac{1}{KT}$. The virial expansion (5) with the virial coefficients given by (6) is valid only at low densities. Since this virial series was developed a considerable amount of attention was devoted to methods of evaluating the virial coefficients B_m . However, there are two difficulties in evaluating these virial coefficients. Firstly, the number of the f -functions in the integrand of (6) grows rapidly with

m which makes it rather difficult to evaluate the integrals for large m even for simplified potential functions. Secondly, the form of the pair potential $u(r_{ij})$ for (quasi)⁽³⁾ realistic potentials such as the Lennard-Jones (12-6) potential complicates the evaluation of B_m even for small m . As a consequence of the first difficulty, attention was focused on evaluating the first few manageable terms in the series (5). The second difficulty was treated in such a way as to deal with more tractable potentials such as the hard sphere^{(4), (5)} (HS), square-well^{(6), (7)} (SW) and other simplified forms for the potential. Though these are not real potentials, still important from the point of view of perturbation theory⁽⁸⁾ which is the most recent successful technique of equilibrium statistical mechanics. This theory is a mathematical means of expanding the configurational partition function Q_N of an original system around a relatively simple reference system whose properties are known. This reference system is usually chosen to be the (HS) one. In our paper, we choose for the pair potential energy a square-well plus a Van der Waals potential. This potential has all the characteristics of a real one, a hard repulsive core and a long range attractive tail. Suitable variables had been used which simplify the integrals considerably where the number of dimensionality of the integrals are reduced by one. As a consequence of expanding the f -functions in power series in β , the third virial coefficient is also obtained as a power series in β which is truncated after terms in β^2 because of the tedious integrals encountered in higher terms.

THE THIRD VIRIAL COEFFICIENT

The assumption of a pair-wise additive potential leads to the classical expression for the third virial coefficient

B_3 :

$$B_3 = -\frac{2}{3} \frac{1}{2V} \iiint f(r_{12}) f(r_{13}) f(r_{23}) d\mathbf{r}_1 d\mathbf{r}_2 d\mathbf{r}_3 \quad (8)$$

where the f -function is defined by (7). Our model is characterised by having the potential $u(r)$, defined as

$$\begin{aligned} u(r) &= \infty & r < \sigma \\ &= -\varepsilon & \sigma < r < \lambda\sigma \\ &= -\frac{\mu}{r^6} & r > \lambda\sigma \end{aligned} \quad (9)$$

σ is the rigid-core diameter of the molecules and ε is the depth of the well. The coefficient μ satisfies the relation

$$\frac{(\mu/\varepsilon)}{\sigma^6} = \lambda^6 \quad (10)$$

Expanding in powers of β , the function $f(r)$ will have the following forms:

$$\begin{aligned} f(r) &= -1 & r < \sigma \\ &= \beta\varepsilon + \frac{1}{2}\beta^2\varepsilon^2 + \dots & \sigma < r < \lambda\sigma \\ &= \frac{\beta\mu}{r^6} + \frac{1}{2} \frac{\beta^2\mu^2}{r^{12}} & r > \lambda\sigma \end{aligned} \quad (11)$$

Consider the integral

$$G(r_{13}) \equiv \int f(r_{12}) f(r_{23}) d\underline{r}_2 \quad (12)$$

We first assume that $\lambda \geq 3$. Consider the four different cases:

$$\begin{aligned} (\lambda+1)\sigma &\leq r_{13} \leq \infty \\ (\lambda-1)\sigma &\leq r_{13} \leq (\lambda+1)\sigma \\ 2\sigma &\leq r_{13} \leq (\lambda-1)\sigma \\ 0 &\leq r_{13} \leq 2\sigma \end{aligned}$$

In the first three cases we retain only terms linear in β for $G(r_{13})$ if the third virial coefficient is to be truncated after terms in β^2 since $f(r_{13})$ in these three cases contains terms at least linear in β . We introduce the variables ξ, η defined as

$$\frac{2}{r_{13}} r_{12} = \xi + \eta \quad ; \quad \frac{2}{r_{13}} r_{23} = \xi - \eta \quad (13)$$

$\xi = \text{constant}$ gives an ellipse and $\eta = \text{constant}$ gives a hyperbola. The element of volume $d\underline{r}_2$ in these variables is thus given by

$$d\underline{r}_2 = \frac{1}{4} \pi r_{13}^3 (\xi^2 - \eta^2) d\xi d\eta \quad (14)$$

and the integrals $G(r_{13})$ over the three-dimensional volume in each case are transformed into integrals over the corresponding areas in the $\xi-\eta$ plane. For example, considering the first of these cases we have (see Fig. I),

$$G(r_{13}) = -2 \int_{\text{dotted volume}} \frac{\beta u}{r_{12}^6} d\underline{r}_2$$

On using (13), the integral is now taken over the dotted area in the $\xi-\eta$ plane (see Fig.II)

$$G(r_{13}) = -2B\mu \times \frac{16}{r_{13}^3} \pi \int_{1-2\eta_0}^1 d\eta \int_1^{\eta+2\eta_0} \frac{(\xi-\eta)}{(\xi+\eta)^5} d\xi, \quad \eta_0 = \frac{\sigma}{r_{13}}$$

Carrying out the integral and setting $r_{13} \equiv R$, we get

$$G(R) = -2B\mu \times \frac{16}{R^3} \pi \left[\frac{1}{64} \left(\frac{R}{R+\sigma} \right)^2 - \frac{1}{64} \left(\frac{R}{R-\sigma} \right)^2 - \frac{1}{96} \left(\frac{R}{R+\sigma} \right)^3 + \frac{1}{96} \left(\frac{R}{R-\sigma} \right)^3 \right] \quad (\lambda+1)\sigma \leq R \leq \infty \quad (15)$$

Similarly, we have for the second case

$$G(R) = -2B\epsilon C_1(R) - 2B\mu C_2(R) \quad (\lambda-1)\sigma \leq R \leq (\lambda+1)\sigma \quad (16)$$

where

$$C_1(R) = \frac{1}{4} \pi R^3 \left[-(\lambda^2-1)^2 \left(\frac{\sigma}{R} \right)^4 + \frac{8}{3} (1+\lambda^3) \left(\frac{\sigma}{R} \right)^3 - 2(1+\lambda^2) \left(\frac{\sigma}{R} \right)^2 + \frac{1}{3} \right] \quad (17)$$

$$C_2(R) = \frac{16}{R^3} \pi \left[\frac{1}{64} \left(\frac{R}{R+\sigma} \right)^2 - \frac{1}{96} \left(\frac{R}{R+\sigma} \right)^3 - \frac{1}{64} \left(\frac{R}{\lambda\sigma} \right)^4 + \frac{1}{24} \left(\frac{R}{\lambda\sigma} \right)^3 - \frac{2\lambda^2-1}{64\lambda^2} \left(\frac{R}{\lambda\sigma} \right)^2 \right] \quad (18)$$

The third case is obvious

$$G(R) = -2B\epsilon \times \frac{4}{3} \pi \sigma^3 \quad 2\sigma \leq R \leq (\lambda-1)\sigma \quad (19)$$

In the fourth case we have to evaluate $G(R)$ up to terms in β^2 since $f(R)$ has the value -1 when $0 \leq R \leq \sigma$. Proceeding as in the first case we obtain the result

$$G(R) = C_3(R) - 2\beta\epsilon C_4(R) + \beta^2\epsilon^2 C_5(R) + 2\beta^2\epsilon\mu C_6(R) + 2\beta^2\mu^2 C_7(R) \quad 0 \leq R \leq 2\sigma \quad (20)$$

where

$$C_3(R) = \frac{4}{3}\pi\sigma^3 - \pi\sigma^2 R + \frac{\pi}{12} R^3 \quad (21)$$

$$C_4(R) = \pi\sigma^2 R - \frac{\pi}{12} R^3 \quad (22)$$

$$C_5(R) = \frac{4}{3}\pi(\lambda^3 - 1)\sigma^3 - \pi(\lambda^2 + 2)\sigma^2 R + \frac{\pi}{4} R^3 \quad (23)$$

$$C_6(R) = \frac{16\pi}{R^3} \left[\frac{1}{64} \left(\frac{R}{R+\lambda\sigma} \right)^2 - \frac{1}{96} \left(\frac{R}{R+\lambda\sigma} \right)^3 - \frac{1}{64} \left(\frac{R}{\lambda\sigma} \right)^2 + \frac{1}{24} \left(\frac{R}{\lambda\sigma} \right)^3 - \frac{1}{64} \left(\frac{R}{\lambda\sigma} \right)^4 \right] \quad (24)$$

$$C_7(R) = \frac{1024\pi}{R^3} \left[\frac{35(\lambda\sigma)^2 R}{2048(R+\lambda\sigma)^3} + \frac{525(\lambda\sigma)R^2}{12288(R+\lambda\sigma)^3} + \frac{385}{12288} \left(\frac{R}{R+\lambda\sigma} \right)^3 + \frac{35}{8192} \frac{R^4}{(\lambda\sigma)(R+\lambda\sigma)^3} - \frac{7}{8192} \frac{R^5}{(\lambda\sigma)^2(R+\lambda\sigma)^3} + \frac{7}{24576} \frac{R^6}{(\lambda\sigma)^3(R+\lambda\sigma)^3} - \frac{1}{8192} \frac{R^7}{(\lambda\sigma)^4(R+\lambda\sigma)^3} + \frac{1}{16384} \left(\frac{R}{\lambda\sigma} \right)^8 - \frac{35}{2048} \ln \left| \frac{R+\lambda\sigma}{\lambda\sigma} \right| \right] \quad (25)$$

The third virial coefficient is given by

$$B_3(T) = -\frac{1}{3} \int f(R) G(R) \cdot 4\pi R^2 dR \quad (26)$$

where $G(R)$ is readily obtained for the four different cases and $f(R)$ is to have the appropriate value in each case according to equation (11) and that all terms up to β^2 are to be considered in each case. Carrying out the integrations for each of the four cases and summing them up we finally obtain

$$\begin{aligned} B_3 = & \frac{5}{18}\pi^2\sigma^6 - \frac{17}{18}\pi^2\sigma^6\beta\epsilon + \frac{1}{36}\pi^2\sigma^6\beta^2\epsilon^2(64\lambda^3-36\lambda^2-113) \\ & + \frac{\pi^2\beta^2\epsilon\mu}{18\lambda^4(\lambda+1)^3}[-12\lambda^6-30\lambda^5-22\lambda^4+33\lambda^3+51\lambda^2+5\lambda-9] \\ & + \frac{2}{3}\pi^2\beta^2\epsilon\mu \ln\left(\frac{\lambda+1}{\lambda}\right) + \frac{\pi^2\beta^2\mu^2}{36\sigma^6\lambda^6(\lambda+1)^4(\lambda+2)^2}[-840\lambda^{13}-6300\lambda^{12} \\ & - 18760\lambda^{11} - 28070\lambda^{10} - 21728\lambda^9 - 7644\lambda^8 - 568\lambda^7 + 83\lambda^6 \\ & + 142\lambda^4 + 316\lambda^3 + 353\lambda^2 + 180\lambda + 36] \\ & + \frac{70\pi^2\beta^2\mu^2}{3\sigma^6} \ln\left(\frac{\lambda+1}{\lambda}\right), \quad \lambda \geq 3 \end{aligned} \quad (27)$$

We assume now $\lambda \leq 3$ and consider the four different cases

$$\begin{aligned} (\lambda+1)\sigma & \leq r_{13} \leq \infty \\ 2\sigma & \leq r_{13} \leq (\lambda+1)\sigma \\ (\lambda-1)\sigma & \leq r_{13} \leq 2\sigma \\ 0 & \leq r_{13} \leq (\lambda-1)\sigma \end{aligned}$$

The first two cases are exactly the same as those for $\lambda \geq 3$ which yield the same results for $G(R)$ equations (15), (16)-(18).

terms in β^2 which is obvious for the fourth case and yields the same expression (20) with $0 \leq R \leq (\lambda-1)\sigma$. For the third case, terms up to β^2 are needed when $\lambda \leq 2$ because of the form of the function $f(R)$ but for $2 \leq \lambda \leq 3$ terms linear in β are sufficient. The result for the third case is

$$G(R) = F_1(R) - 2\beta\epsilon F_2(R) - 2\beta\mu F_3(R) + \beta^2\epsilon^2 F_4(R) + 2\beta^2\epsilon\mu F_5(R) + 2\beta^2\mu^2 F_6(R) \quad (\lambda-1)\sigma \leq R \leq 2\sigma \quad (28)$$

where

$$F_1(R) = C_3(R) \quad (29)$$

$$F_2(R) = \pi[\frac{1}{2}(1-\lambda^2)\sigma^2 R + \frac{2}{3}(\lambda^3-1)\sigma^3 - \frac{1}{4}(\lambda^2-1)^2 \frac{\sigma^4}{R}] \quad (30)$$

$$F_3(R) = C_2(R) \quad (31)$$

$$F_4(R) = \pi[-\frac{1}{2}(1-\lambda^2)\sigma^2 R - \frac{2}{3}(\lambda^3-1)\sigma^3 + \frac{3}{4}(\lambda^2-1)^2 \frac{\sigma^4}{R}] \quad (32)$$

$$F_5(R) = \frac{16}{R^3} \pi [\frac{1}{64}(\frac{R}{R+\lambda\sigma})^2 - \frac{1}{64}(\frac{R}{R+\sigma})^2 - \frac{1}{96}(\frac{R}{R+\lambda\sigma})^3 + \frac{1}{96}(\frac{R}{R+\sigma})^3 + \frac{\lambda^2-1}{64\lambda^2}(\frac{R}{\lambda\sigma})^2] \quad (33)$$

$$F_6(R) = \frac{\pi}{720} [\frac{36R}{(\lambda\sigma)^{10}} - \frac{80}{(\lambda\sigma)^9} - \frac{8\sigma}{R(R+\sigma)^9} - \frac{36\sigma^2}{R(\lambda\sigma)^{10}} - \frac{1}{R(R+\sigma)^9} + \frac{45}{R(\lambda\sigma)^9}] + C_7(R) \quad (34)$$

With $G(R)$ in hand, the third virial coefficient $B_3(T)$ given by (26) can be obtained by choosing the appropriate form for the function $f(R)$ equation (11) and in such a way that all terms up to β^2 are considered and cases $\lambda \leq 2$, $2 \leq \lambda \leq 3$ are taken care of. The final results show that $B_3(T)$ has the same

expression (27) for $2 \leq \lambda \leq 3$, so that this expression stands for $\lambda \geq 2$.

For $\lambda \leq 2$, the result is given by

$$\begin{aligned}
 B_3(T) = & \frac{5}{18} \pi^2 \sigma^6 + \frac{\pi^2 \sigma^6 \beta \epsilon}{18} [-\lambda^6 + 18\lambda^4 - 32\lambda^3 + 15] \\
 & + \frac{\pi^2 \beta \mu}{18\lambda^3} [-5\lambda^3 + 36\lambda - 32] + \frac{\pi^2 \beta \mu}{3} \ln \left(\frac{\lambda}{2} \right) \\
 & + \frac{\pi^2 \sigma^6 \beta^2 \epsilon^2}{36} [-5\lambda^6 + 90\lambda^4 - 96\lambda^3 - 36\lambda^2 + 47] \\
 & + \frac{\pi^2 \beta^2 \epsilon \mu}{18\lambda^4 (\lambda+1)^4 (2\lambda-1)^2} [-40\lambda^{10} - 168\lambda^9 + 78\lambda^8 + 596\lambda^7 \\
 & \qquad \qquad \qquad + 150\lambda^6 - 624\lambda^5 - 287\lambda^4 + 228\lambda^3 \\
 & \qquad \qquad \qquad + 108\lambda^2 - 32\lambda - 9] \\
 & + \frac{2}{3} \pi^2 \beta^2 \epsilon \mu \ln \left(\frac{\lambda+1}{2} \right) \\
 & + \frac{\pi^2 \beta^2 \mu^2}{27648\lambda^9 (\lambda+1)^4 (\lambda+2)^2 \sigma^6} [\lambda^{15} - 645112\lambda^{14} - 4838374\lambda^{13} \\
 & \qquad \qquad \qquad - 14407636\lambda^{12} - 21557719\lambda^{11} - 16687084\lambda^{10} \\
 & \qquad \qquad \qquad - 5871356\lambda^9 - 442368\lambda^8 + 50688\lambda^7 + 13312\lambda^6 \\
 & \qquad \qquad \qquad + 191944\lambda^5 + 318464\lambda^4 + 190976\lambda^3 \\
 & \qquad \qquad \qquad - 59392\lambda^2 - 108544\lambda - 32768] \\
 & + \frac{70\pi^2 \beta^2 \mu^2}{9\sigma^6} [3 \ln (\lambda+1) - \ln (4\lambda)] \qquad \qquad \lambda \leq 2 \qquad (3)
 \end{aligned}$$

Expression (27) for $\lambda \geq 2$ coincides with expression (35) for $\lambda \leq 2$, at $\lambda = 2$ as it should be. As the third virial coefficient $B_3(T)$ is obtained only up to terms in β^2 , it is valid only at high temperatures. Our potential reduces to the

square-well one by putting $\mu = 0$. In this case, the third virial coefficient $B_3(T)$ equations (27) and (35) reduces to the well-known third virial coefficient for square-well potential⁽⁹⁾ considering only terms up to β^2 .

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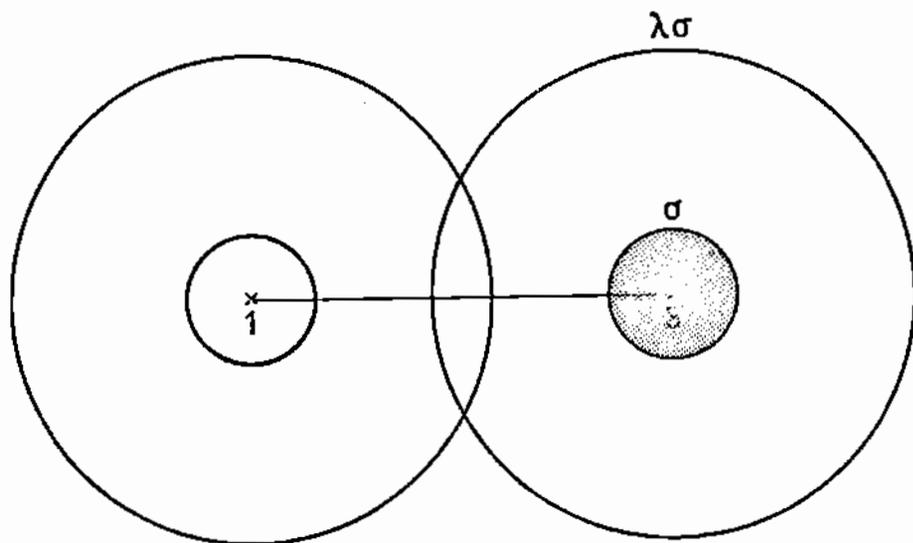


Fig. I

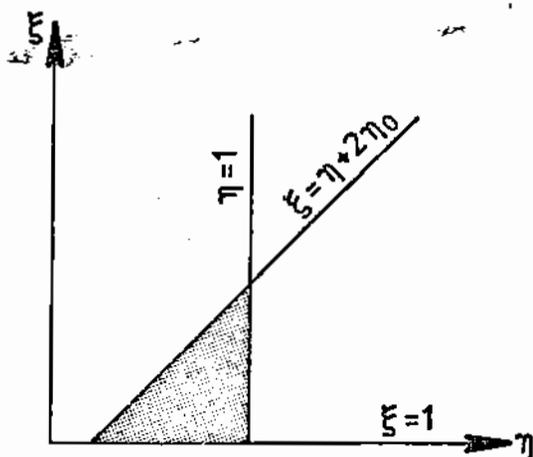


Fig. II

12 13 14 15

The Steady Plane Couette flow and heat transfer
of a rarefied electron gas

M.A. Khidr, A.G. El-Sakka and M.A. Hamdy.

Abstract:

Moments method has been used to replace a model kinetic equation for a rarefied electron gas by non-linear moments equations. These equations are solved by small parameter method to get insight into the behaviour of the flow for any degree of rarefaction.

Introduction and basic equations:

The electron gas is considered filling the space between two parallel impermeable non-heat conducting planes distance d apart. The upper plane is moving with a constant velocity $(+ U)$ in the x -direction, and is kept at a constant temperature T_0 .

The lower plane is moving with velocity $(- U)$ and is kept at a constant temperature T_0 . There is an external magnetic induction B_0 in the z -direction which is imposed on the system. The moments equations of the model kinetic equation [1] are:

$$\int \mathcal{L}_i(\vec{c}) \frac{\partial f}{\partial \vec{r}} + \frac{\vec{F}}{m} \frac{\partial f}{\partial \vec{c}}) d\vec{c} = \int \frac{n R T}{\mu} (f_0 - f) \mathcal{P}_i d\vec{c} \quad (1)$$

where f is the distribution function of the charges, f_0

the local Maxwell distribution, n the density, R is the gas constant, μ the coefficient of viscosity, T the temperature, φ_1 is a function of velocity, \vec{c} the velocity of the charges and \vec{r} its position vector. The moments equations from equations (1) are non-linear differential equations and they are solved by using of the small parameter method assuming a small relative temperature difference ($\chi = 1 + \chi^1$, $\chi^2 \ll 1$) between the two plates. We shall assume also that the flow occurs with a small Mach number and that:

$$\left(\delta^2 = \left(\frac{e B_0 U}{K T_0} \right)^2 \ll 1 \right)$$

From dimensional analysis it is clear that the induced magnetic field can be neglected relative to the induced electric field E . The latter is assumed to act in the y -direction. It can be considered composing of two fields E_1 emerging from electrons for which $c_y < 0$ and E_2 emerging from electrons for which $c_y > 0$. The flow velocity v is assumed also in the x -direction. As for the distribution function f we shall use two-stream local Maxwellian distribution function (2):

$$f = n_1 \left(\frac{m}{2\pi K T_1} \right)^{\frac{3}{2}} \exp \left\{ - m \frac{(c_x - v_1)^2 + c_y^2 + c_z^2}{K T_1} \right\}, c_y < 0$$

$$= n_2 \left(\frac{m}{2\pi K T_2} \right)^{\frac{3}{2}} \exp \left\{ - m \frac{(c_x - v_2)^2 + c_y^2 + c_z^2}{K T_2} \right\}, c_y > 0$$

where n_1, n_2, v_1, v_2, T_1 and T_2 are six unknowns to be determined from equations of type (1).

Boundary conditions:

Taking $\varphi_1 = 1, \varphi_2 = c_x, \varphi_3 = c_y, \varphi_4 = c_x^2 + c_y^2 + c_z^2,$
 $\varphi_5 = c_x c_y, \varphi_6 = c_x c_y^2,$ and taking f in

the form (2) substituting in equation (1) we get:

$$n_1 \sqrt{T_1} = n_2 \sqrt{T_2} \dots\dots\dots (3)$$

$$n_1 \sqrt{T_1} (v_2 - v_1) = \alpha_1 \dots\dots\dots (4)$$

$$\frac{d}{dy} (n_1 T_1 + n_2 T_2) + \frac{eE}{k} (n_1 + n_2) - \frac{eB_0}{k} (n_1 v_1 + n_2 v_2) = 0 \dots (5)$$

$$n_2 T_2^{\frac{3}{2}} - n_1 T_1^{\frac{3}{2}} + \frac{mn_1 \sqrt{T_1}}{4K} (v_2^2 - v_1^2) = \alpha_3 \dots\dots\dots (6)$$

$$\begin{aligned} \frac{d}{dy} (n_1 T_1 v_1 + n_2 T_2 v_2) + \frac{eE}{k} (n_1 v_1 + n_2 v_2) - \frac{eB_0}{k} (n_1 v_1^2 + n_2 v_2^2) = \\ = \frac{-nkT}{2m(1+\chi^2)^{3/4} \mu_0} \sqrt{\frac{k}{2\pi m}} \alpha_1 \dots\dots\dots (7) \end{aligned}$$

$$\begin{aligned} \frac{d}{dy} \left[(n_1 T_1^2 + n_2 T_2^2) + \frac{k}{5m} (n_1 T_1 v_1^2 + n_2 T_2 v_2^2) \right] + \frac{eE}{m} \left[\frac{k}{m} (n_1 T_1 + n_2 T_2) + \right. \\ \left. + \frac{1}{5} (m_1 v_1^2 + m_2 v_2^2) \right] - \frac{eB_0}{m} \left[\frac{k}{m} (n_1 T_1 v_1 + n_2 T_2 v_2) + \frac{1}{5} (v_1^3 + v_2^3) \right] = \\ = \frac{-8n T_0 T}{5(1+\chi^2)^{3/4} \mu_0} \cdot \frac{k^2}{m^2} \sqrt{\frac{k}{2\pi m}} \alpha_3 \dots\dots\dots (8) \end{aligned}$$

Supplemented with Poisson's equation

$$\frac{dE_1}{dy} = - \frac{e}{2\epsilon} n_1 \dots\dots\dots (9)$$

$$\frac{dE_2}{dy} = - \frac{e}{2\epsilon} n_2 \dots\dots\dots (10)$$

where e is the charge of the electron and ϵ the dielectric constant. Assuming complete momentum and energy accommodation at the two plates the boundary conditions will be:

$$\left. \begin{aligned} v_1 &= U, T_1 = T_0, E_1 = 0 \text{ at } y = \frac{d}{2} \\ v_2 &= -U, T_2 = \gamma T_0, E_2 = 0, n_2 = n_0 \text{ at } y = -\frac{d}{2}, \end{aligned} \right\} \quad (11)$$

assuming that n_2 is given at the lower plate.

Method of solution:

Let $y = y' d$, $n_i = n_i' n_0$, $T_i = T_i' T_0$, $v_i = v_i' U$,
 $E_i = E_i' B_0 U$; $i = 1, 2$ and neglecting terms of order M_0^2 ($M_0 = \frac{U}{\sqrt{\epsilon_0 m k T_0}}$) we get:

$$n_1' \sqrt{T_1'} = n_2' \sqrt{T_2'} \quad \dots\dots\dots (12)$$

$$n_1' \sqrt{T_1'} (v_2' - v_1') = \alpha_1' \quad \dots\dots\dots (13)$$

$$\frac{d}{dy'} (n_1' T_1' + n_2' T_2') + \gamma E' (n_1' + n_2') - \gamma (n_1' v_1' + n_2' v_2') = 0 \dots\dots (14)$$

$$n_1' \sqrt{T_1'} (T_2' - T_1') = \alpha_3' \quad \dots\dots\dots (15)$$

$$\begin{aligned} \frac{d}{dy'} (n_1' T_1' v_1' + n_2' T_2' v_2') + \gamma E' (n_1' v_1' + n_2' v_2') - \gamma (n_1' v_1'^2 + n_2' v_2'^2) = \\ = -\frac{1}{2} \gamma \alpha_1' (n_1' + n_2') \quad \dots\dots\dots (16) \end{aligned}$$

$$\begin{aligned} \frac{d}{dy'} (n_1' T_1'^2 + n_2' T_2'^2) + \gamma E' (n_1' T_1' + n_2' T_2') - \gamma (n_1' T_1' v_1' + n_2' T_2' v_2') \\ = -\frac{8}{5} \gamma \alpha_3' (n_1' + n_2') \quad \dots\dots\dots (17) \end{aligned}$$

$$\frac{dE'}{dy'} = -\gamma n_1' \quad \dots\dots\dots (18)$$

$$\frac{dE_2'}{dy'} = -\gamma' n_2' \dots\dots\dots (19)$$

where $\delta = \frac{d}{\mu_0(1+\chi)^{3/4}} \sqrt{\frac{mkT_0}{\pi}}$ is a measure of

rarefaction

$$\gamma = \frac{eB_0 U d}{k T_0} \text{ is a measure of magnetic field}$$

$$\gamma' = \frac{n_0 k T_0}{2 B_0 U^2} \text{ is a measure of the induced electric field, and } \alpha = c_p / \alpha_v$$

Together with the non-dimensional boundary conditions:

$$n_2' = T_2' = 1, v_2' = -1, E_2' = 0 \text{ for } y' = -\frac{1}{2} \dots\dots\dots (20)$$

$$T_1' = 1 + \chi, v_1' = 1, E_1' = 0 \text{ for } y' = \frac{1}{2} \dots\dots\dots (21)$$

To solve the system (12 - 19) we put in it:

$$n_i' = n_i'(0) + \gamma n_i'(1) \dots\dots\dots (22)$$

$$T_i' = T_i'(0) + \gamma T_i'(1) \dots\dots\dots (23)$$

$$v_i' = v_i'(0) + \gamma v_i'(1) \dots\dots\dots (24)$$

$$E_i' = E_i'(0) + \gamma E_i'(1) \dots\dots\dots (25)$$

$i = 1, 2$

Then, equating in both the sides of the equations of the system (12 - 19) termes free of γ gives:

$$n_1'(0) \sqrt{T_1'(0)} = n_2'(0) \sqrt{T_2'(0)} \dots\dots\dots (26)$$

$$n_1'(0) \sqrt{T_1'(0)} (v_2'(0) - v_1'(0)) = \mathcal{L}_1'(0) \dots\dots\dots (27)$$

$$n_1'(0) T_1'(0) + n_2'(0) T_2'(0) = \mathcal{L}_2'(0) \dots\dots\dots (28)$$

$$n_1'(0) \sqrt{T_1'(0)(T_2'(0) - T_1'(0))} = \alpha_2'(0) \alpha_3'(0) \dots \dots \dots (29)$$

$$\frac{d}{dy} (n_1'(0) T_1'(0) v_1'(0) + n_2'(0) T_2'(0) v_2'(0)) = -1/2 \delta \alpha_1'(0) (n_1'(0) + n_2'(0)) \dots \dots \dots (30)$$

$$\frac{d}{dy} (n_1'(0) T_1'(0)^2 + n_2'(0) T_2'(0)^2) = -\frac{8}{5} \delta \alpha_2'(0) \alpha_3'(0) (n_1'(0) + n_2'(0)) \dots \dots \dots (31)$$

$$\frac{dE_1'(0)}{dy} = -\delta n_1'(0) \dots \dots \dots (32)$$

$$\frac{dE_2'(0)}{dy} = -\delta n_2'(0) \dots \dots \dots (33)$$

which has a solution of the form:

$$n_1'(0) = 1 - \frac{\chi'}{2} + \frac{16}{5} \delta \alpha_3'(0) y \dots \dots \dots (34)$$

$$n_2'(0) = 1 - \frac{\chi'}{2} + \alpha_3'(0) + \frac{16}{5} \delta \alpha_3'(0) y \dots \dots \dots (35)$$

$$v_1'(0) = \frac{\delta \alpha_1'(0)}{2} \left[\left(1 - \frac{\chi'}{2} - \frac{8}{5} \delta \alpha_3'(0) y\right) y + \frac{8}{5} \delta \alpha_3'(0) y^2 \right] - \frac{\alpha_1'(0)}{2} \left(1 + \frac{\chi'}{4}\right) + \alpha_5'(0) \dots \dots \dots (36)$$

$$v_2'(0) = -\frac{\delta \alpha_1'(0)}{2} \left[\left(1 - \frac{\chi'}{2} + \frac{8}{5} \alpha_3'(0) y + \frac{8}{5} \delta \alpha_3'(0) y^2 \right) y + \frac{\alpha_1'(0)}{2} \left(1 + \frac{\chi'}{4} + \alpha_3'(0) y\right) + \alpha_5'(0) \dots \dots \dots (37)$$

$$T'(0) = \frac{n_1'(0) T_1'(0) + n_2'(0) T_2'(0)}{n_1'(0) + n_2'(0)} = 1 + \frac{\chi'}{2} - \frac{16}{5} \delta \alpha_3'(0) y \dots \dots \dots (38)$$

$$E_1'(0) = -\delta \left[\left(1 - \frac{\chi'}{2}\right) y + \frac{8}{5} \delta \alpha_3'(0) y^2 + \alpha_{61}'(0) \right] \dots \dots \dots (3)$$

$$E_2'(0) = -\delta \left[\left(1 - \frac{\chi'}{2} - \alpha_3'(0) y + \frac{8}{5} \delta \alpha_3'(0) y^2 + \alpha_{62}'(0) \right) \right] \dots \dots \dots (4)$$

where

$$\alpha_1'(0) = \frac{-8}{\delta(2 - \chi') + 4 + \chi' + 2\alpha_3'(0)} \dots\dots\dots (41)$$

$$\alpha_2'(0) = 2 - \alpha_3'(0) \dots\dots\dots (42)$$

$$\alpha_3'(0) = \frac{-5\chi'}{2(5+8\delta)} \dots\dots\dots (43)$$

$$\alpha_5'(0) = \frac{\alpha_1'(0)\alpha_3'(0)}{20} (4\delta^2 - 8\delta - 5) \dots\dots\dots (44)$$

$$\alpha_{61}'(0) = -1/2 (1 - \frac{\chi'}{2}) - \frac{2}{5}\delta\alpha_3'(0) \dots\dots\dots (45)$$

$$\alpha_{62}'(0) = 1/2(1 - \frac{\chi'}{2} - \alpha_3'(0)) - \frac{2}{5}\delta\alpha_3'(0) \dots\dots\dots (46)$$

The coefficients of δ in system (12 - 19) give the system of equations for first approximation:

$$2n_1^1(1) - 2n_2^1(1) + \Gamma_1^1(1) - \Gamma_2^1(1) = 0 \dots\dots\dots (47)$$

$$v_2^1(1) - v_1^1(1) = \alpha_1^1(1) - \alpha_1^1(0) (n_1^1(1) + 1/2 \Gamma_1^1(1)) \dots\dots (48)$$

$$n_1^1(1) + n_2^1(1) + \Gamma_1^1(1) + \Gamma_2^1(1) = \alpha_2^1(1) + y^2(2\delta - \frac{\delta\alpha_1^1(0)}{2}) \dots\dots (49)$$

$$\Gamma_2^1(1) - \Gamma_1^1(1) = \alpha_3^1(1) \dots\dots\dots (50)$$

$$\begin{aligned} \frac{d}{dy} [v_1^1(1) + v_2^1(1) + v_1^1(0)(n_1^1(1) + \Gamma_1^1(1)) + v_2^1(0)(n_2^1(1) + \Gamma_2^1(1))] + \\ + \delta\alpha_1^1(0)(2\delta - \frac{\delta\alpha_1^1(0)}{2})y^2 = \\ = \frac{\alpha_1^{1(0)2}}{2} - 1/2\delta[\alpha_1^1(0)(n_1^1(1) + n_2^1(1)) + 2\alpha_1^1(1)] \end{aligned} \dots\dots (51)$$

$$\begin{aligned} n_1^1(1) + n_2^1(1) + 2\Gamma_1^1(1) + 2\Gamma_2^1(1) = \alpha_4^1(1) - \frac{18}{5}\delta\alpha_3^1(0)y + \\ + (2\delta - \frac{\delta\alpha_1^1(0)}{2})y^2 \dots\dots\dots (52) \end{aligned}$$

$$\frac{dE_1^{(1)}}{dy} = -\delta n_1^{(1)} \dots \dots \dots (53)$$

$$\frac{dE_2^{(1)}}{dy} = -\delta n_2^{(1)} \dots \dots \dots (54)$$

with boundary conditions

$$n_2^{(1)} = T_2^{(1)} = v_2^{(1)} = E_2^{(1)} = 0 \text{ for } y = -1/2 \dots (55)$$

$$T_1^{(1)} = v_1^{(1)} = E_1^{(1)} = 0 \text{ for } y = 1/2 \dots (56)$$

The system of equations (47 - 54) has the solution:

$$n_1^{(1)} = n_2^{(1)} = 1/2(2\delta' - \frac{\delta \alpha_1^{(0)}}{2})(y^2 - 1/4) \dots \dots (57)$$

$$T_2^{(1)} = T_1^{(1)} = \alpha_3^{(1)} = \alpha_5^{(1)} = 0 \dots \dots \dots (58)$$

$$\alpha_2^{(1)} = \alpha_4^{(1)} = 1/4(2\delta' - \delta \alpha_1^{(0)}) \dots \dots \dots (59)$$

$$v_1^{(1)} = 1/2 \left\{ \alpha_1^{(1)}(1 - \delta y) + \alpha_1^{(0)^2} y - \alpha_1^{(0)}(2\delta' - \delta \alpha_1^{(0)}) \cdot \left[\frac{\delta}{3} y^3 + (y^2 - 1/4) \right] \right\} \dots \dots \dots (60)$$

$$\alpha_1^{(1)} = \frac{12 \alpha_1^{(0)} - \delta \alpha_1^{(0)}(4\delta' - \delta \alpha_1^{(0)})}{12(2 + \delta)} \dots \dots \dots (61)$$

$$E_1^{(1)} = -\delta \left[(2\delta' - \frac{\delta \alpha_1^{(0)}}{2})(\frac{y^3}{3} - \frac{y}{4}) + \alpha_6^{(1)} \right] = E_2^{(1)} \dots (62)$$

$$\alpha_6^{(1)} = -\frac{1}{12}(2\delta' - \frac{\delta \alpha_1^{(0)}}{2}) \dots \dots \dots (63)$$

Discussion and numerical results:

We have obtained the following analytical expressions for the density, temperature, velocity, and electric field

$$n' = 1 - \frac{\chi'}{2} - \frac{\alpha_3'(0)}{2} + \frac{16}{5} \delta \alpha_3'(0) y' + \gamma (2\gamma' - \frac{\delta \alpha_1'(0)}{2}) (y'^2 - 1/4)$$

$$T' = 1 + \frac{\chi'}{2} - \frac{16}{5} \delta \alpha_3'(0) y' + \gamma (2\gamma' - \frac{\delta \alpha_1'(0)}{2}) (y'^2 - 1/4)$$

$$v' = -\frac{\delta \alpha_1'(0)}{2} \left[\left(1 - \frac{\chi'}{2}\right) y' + \frac{8}{5} \delta \alpha_3'(0) y'^2 \right] + \frac{\alpha_1'(0)}{5} + \frac{\gamma}{2} \left[\left(\frac{\alpha_1'(0)}{2} - \delta \alpha_1'(1) \right) y' - \frac{\delta \alpha_1'(0)}{2} (2\gamma' - \frac{\delta \alpha_1'(0)}{2}) y'^3 \right]$$

$$E' = -\gamma \left[\left(2 - \alpha_3'(0) - \chi'\right) y' + \frac{16}{5} \delta \alpha_3'(0) y'^2 - \frac{4}{5} \delta \alpha_3'(0) + \gamma (4\gamma' - \delta \alpha_1'(0)) \left(\frac{y'^3}{3} - \frac{y'}{4} \right) \right]$$

We may note the following:

(I) For the number density $n'(y)$:

i) There is a density drop at the lower plate which equals to $\frac{-\chi'}{2(5+8\delta)}$.

It is independent of the fields and increases with δ

ii) For $\gamma = 0$, the density varies linearly with y , agreeing with the corresponding neutral case.

(II) For the temperature distribution we may note the following:

i) There is a temperature jump at the two plates

$$T_s(\pm 1/2) = \pm \frac{5\chi'}{2(5+8\delta)}$$

It is independent of the fields and decreases with δ

In the continuum case ($\delta \rightarrow \infty$) it vanishes.

- ii) For the neutral case T varies linearly with y .
For charged case T decreases with y to a minimum value then increases. It is larger at any point for the neutral case than that for the charged case.

(III) For the mean velocity we may note the following:

- i) There is a slip velocity at the two plates

$v_s(\pm 1/2) = \pm 1 - v(\pm 1/2)$, the magnitude of the slip velocity at the lower plate is larger in the neutral case than that for the charged case. For the charged case the slip velocity decreases with γ' , and for both cases it decreases with δ and vanishes as $\delta \rightarrow \infty$.

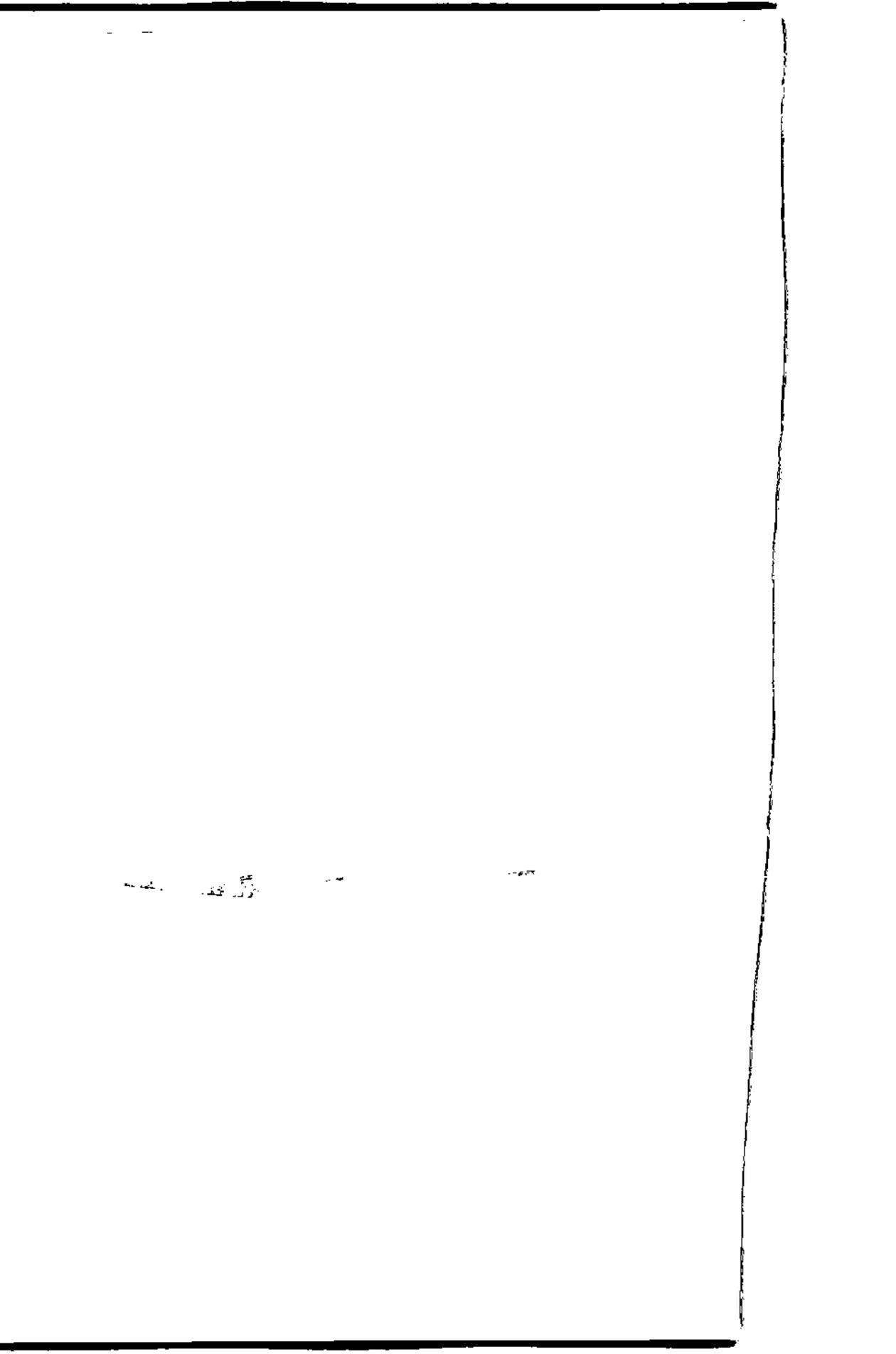
- ii) At any point the magnitude of velocity is larger for the neutral case.

(IV) The non-dimensional shear stress $p'_{xy} = \alpha'_1(0) + \gamma \alpha'_1(1)$ is constant and we may note that it decreases with δ and γ' .

(V) The heat flux vector $q'_y = \frac{-5\gamma'}{(5 + 8\delta)}$ is constant and is independent of the fields. It decreases with δ and vanishes in the continuum limit.

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A TRANSISTORIZED GAMMA-GAMMA COINCIDENCE SPECTROMETER

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Abstract. A completely transistorized coincidence spectrometer which could be used to study direct and coincidence gamma-gamma spectra is shown. The constructed electronic circuits, characteristics and performances of such spectrometer are presented. Direct and coincidence spectra for the decay of ^{152}Eu are also presented.

Introduction

Study of decay schemes of radioactive nuclei offers one of the most important methods to study the nuclear structure¹⁾. Direct spectra, coincidence spectra as well as angular correlation measurements supply very important knowledge about the decay scheme of the studied nucleus. A coincidence spectrometer which could be also used for angular correlation experiments is one of the most important equipments for any nuclear physics laboratory. Therefore, we have found it useful to construct a completely transistorized coincidence spectrometer. This spectrometer could be used to study direct gamma spectra, gamma-gamma coincidence spectra and moments of excited levels by angular correlation methods. It is also possible to measure beta-gamma or alpha-gamma coincidence by simply choosing suitable scintillators for beta- or alpha-particles. The spectrometer could be easily modified to study beta-gamma, alpha-gamma or gamma-gamma coincidences using high resolution detectors such as Ge(Li) detectors or surface barrier detectors (under construction) for alpha or beta particles.

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The purpose of this work is therefore to construct such a spectrometer. The spectrometer has been tested using ^{22}Na and ^{60}Co sources. For the completeness of the work ^{152}Eu has been simply investigated²⁾.

General Description

Fig. 1 shows a block diagram of the whole spectrometer. We have constructed two scintillation detectors³⁾ using two 153 AVP photomultipliers (PM) each of which is coupled to a 2" x 2" NaI(Tl) crystal. Pulses from the anode of each PM are fed to a variable resolving time fast coincidence circuit after suitable amplification and shaping. Part of the spectrum from one of the photomultiplier detectors PM(2) could be selected by a zero crossing single channel analyzer and then fed to one of the inputs of a slow coincidence circuit through a phase inverter and shaper. The other input of the slow coincidence circuit receives pulses from the output of the fast coincidence circuit after passing through a suitable delay circuit. The output pulses from the slow coincidence circuits are used to gate a 512 LABEN multichannel analyzer after being amplified, shaped and delayed by suitable circuits. The multichannel analyzer receives a direct spectrum from PM(1) through a suitable linear amplifier and a delay line.

The two detectors are mounted on an angular correlation table Fig. 2 with a movable source holder in the center of the table. Owing to the finite size of the detectors, the table permits coincidence measurements at angles varying from 60° to 240° .

Electronic Circuits

All the electronic circuits presented in fig. 1 except the linear amplifier and the zero crossing single channel analyzer in channel (2) have been constructed for the present work²⁾, however, only fast circuits will be presented.

(i) Fast Amplifiers:

Two identical fast amplifiers have been constructed. Fig. 3 shows the circuit of one of these amplifiers. It consists of a capacitive differentiator (200 pF) followed by an unsaturated differential amplifier (T_1 and T_2) and a gated ampli-

(T₃). T₄ and T₅ act as an amplifying stage followed by an emitter follower. The gain of this amplifier has been found to be in the order of 100. Its band width mounts to about 20 M Hz. The input pulses (from the photomultiplier) have about 25 ns rise-time and 5 μ s duration while the output pulses from the circuit have about 10 ns rise-time and 200 ns duration.

(ii) Fast Shapers:

Fig. 4 shows the circuit of one of the fast shapers used in the present work. It consists simply from a differentiating stage using a 25 pf capacitance followed by two amplifying stages (T₁ and T₂) and then an emitter follower T₃. The output pulses from such circuit are of about 7 volts amplitude and 20 ns duration.

(iii) Variable Resolving Time Fast Coincidences:

Fig. 5 shows the circuit used which consists of a fast coincidence stage, a fast discriminator stage and a fast shaper. The resolving time of the coincidence circuit could be changed by changing the discrimination level of the fast discriminator. The coincidence stage consists of two tunnel diodes whose biases are adjusted so that only negative pulses of amplitudes greater than 6 Volts can produce tunnel effects⁴). The output of the coincidence stage depends on the delay time between the two input pulses. For maximum overlap, the output pulses are of height ~ 0.5 volts and duration time ~ 40 ns. These pulses are fed to the discriminator through the emitter follower T₁. The fast discriminator used consists of two tunnel diodes followed by an amplifier T₂. The discriminator lower level and hence the resolution of the coincidence could be varied from 0 to 0.5 volt using two variable resistors 10 K Ω and 820 Ω .

Performance

Fig. 6 illustrates the resolving curves of the fast coincidence unit for ²²Na, ⁶⁰Co, and ¹⁵²Eu sources. For ²²Na curve the resolution (2 σ) was of the order of 36 ns while the slope of the curve has a T_{1/2} ~ 10 ns. The ratio of accidental to true coincidences is $\leq 2\%$. The spectrometer has been tested by performing direct and coincidence spectra in ²²Na and ⁶⁰Co sources.

In case of ^{22}Na source fig. 7 it is clear that the 1.275 MeV line is completely absent from the coincidence spectra, while for ^{60}Co curves fig. 8 the ratio between the intensities of the 1.18 and 1.33 MeV lines are greatly affected according to the position of the chosen window.

The spectrometer has been also used to study direct and coincidence spectra in the decay of $^{152}\text{Eu} \rightarrow ^{152}\text{Sm}$ and $^{152}\text{Eu} \rightarrow ^{152}\text{Gd}$. A simplified decay scheme including the investigated transitions is illustrated in fig. 9. The direct spectrum (I) obtained as well as coincidence spectra (II, III, IV) for different chosen windows are shown in fig. 10. When the multichannel analyzer was gated with the 122 KeV line in ^{152}Sm ($2^+ \rightarrow 0^+$ transition), the 0.245, 0.965, 1.09, 1.11 and 1.41 MeV lines are well observed while the 0.344 and 0.779 MeV lines due to ^{152}Gd , ~~are~~ are highly attenuated. When the multichannel analyzer was gated in the 0.344 MeV line in ^{152}Gd ($2^+ \rightarrow 0^+$ transition) the most pronounced line was the 0.779 MeV. When gating the multichannel analyzer by the 0.779 MeV in ^{152}Gd , the most pronounced line was the 0.344 MeV.

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Figure Caption

- Fig. (1) A block diagram of the apparatus.
- Fig. (2) Gamma-gamma angular correlation table.
- Fig. (3) A transistorized fast amplifier circuit.
- Fig. (4) A transistorized fast shaper circuit.
- Fig. (5) A transistorized variable resolving fast coincidence circuit.
- Fig. (6) Resolving time curves of the fast coincidence unit for ^{22}Na , ^{60}Co and ^{152}Eu sources.
- Fig. (7) Gamma-gamma coincidence spectrum using ^{22}Na source.
- Fig. (8) Gamma-gamma coincidence spectra using ^{60}Co source.
- Fig. (9) Simplified decay scheme of the decay of ^{152}Eu . Energies indicated by arrows are those observed by the system.
- Fig. (10) Gamma-gamma coincidence spectra using ^{152}Eu source.

مطياف جاما - جاما التطابقى

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البحث يتكون من وصف شامل لمطياف جاما - جاما تطابقى يمكن به دراسة الطيف الجاما المباشر والتطابقى كما يصلح أيضا لدراسة التوزيع الزاوى للأشعة المنبعثة من النواة . وقد تم بناء جميع دوائر المطياف من الترانزستور وكذلك كاشفين لأشعة جاما من النوع الومبضى . كما يمكن استعمال هذا المطياف باستخدام كواشف نصف موصلة تصلح لجسيمات بيتا وأشعة جاما . وقد ذكر فى البحث جميع خصائصه وامكانيات المطياف وقد تم تجريبه على بعض الأنوية العيارية مثل الصوديوم ٢٢ والكربلت ٦٠ . وشمل البحث أيضا عرضا مبسطا لبعض القياسات التى تمت بواسطة الجهاز لدراسة نواة الأورونيوم ١٥٢

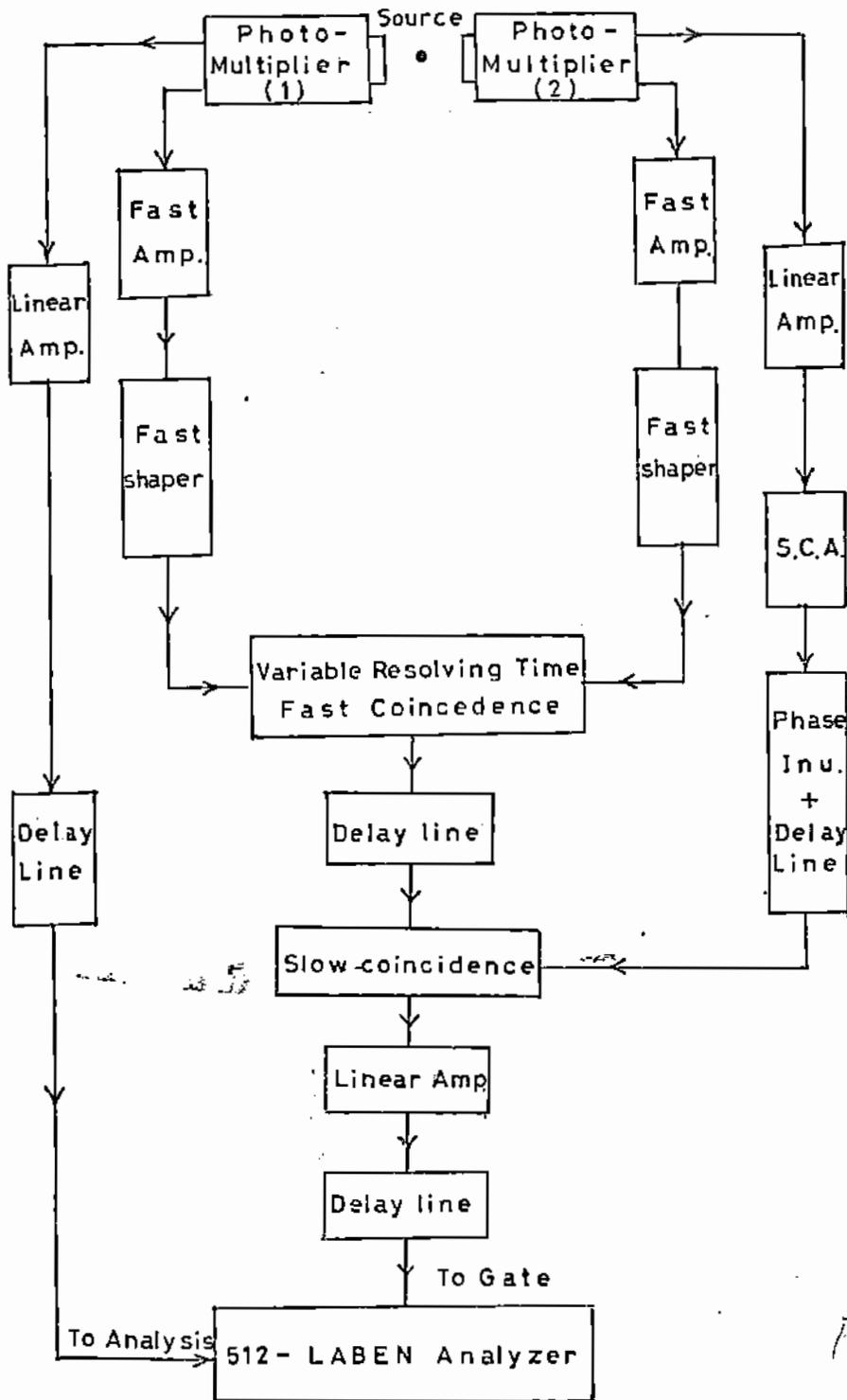


Fig 1

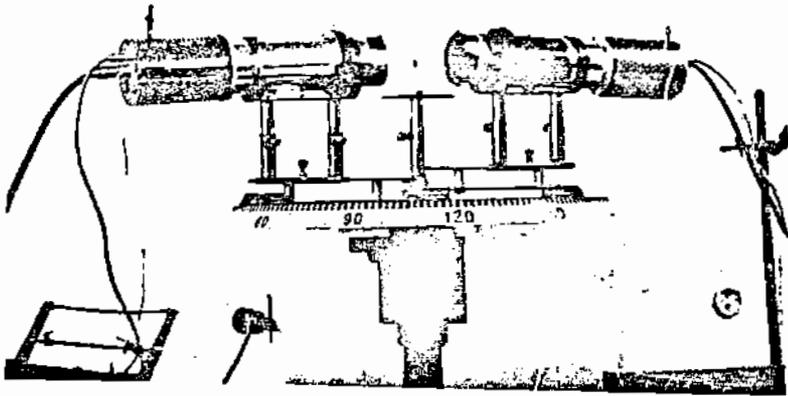
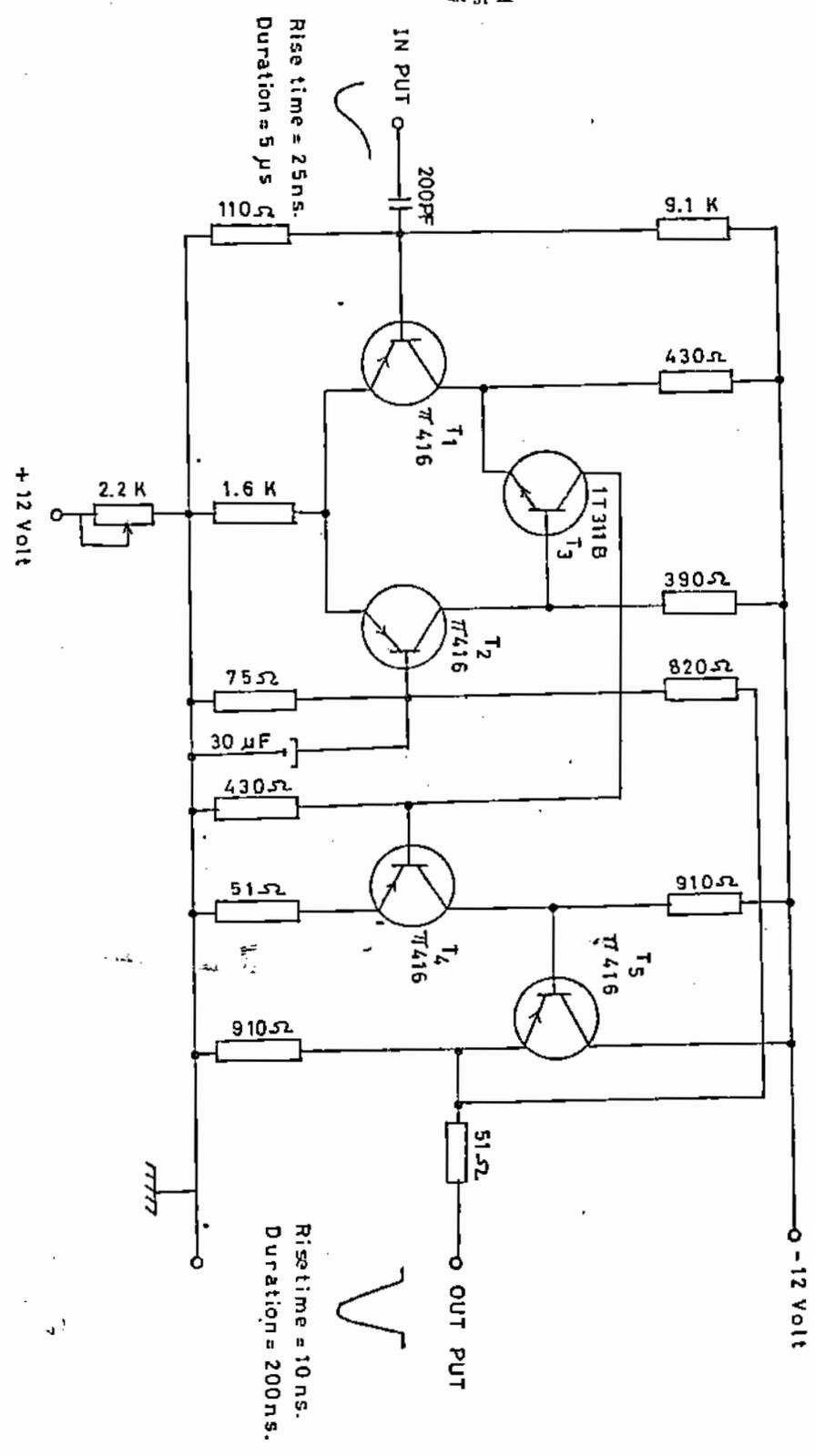


Fig. 2



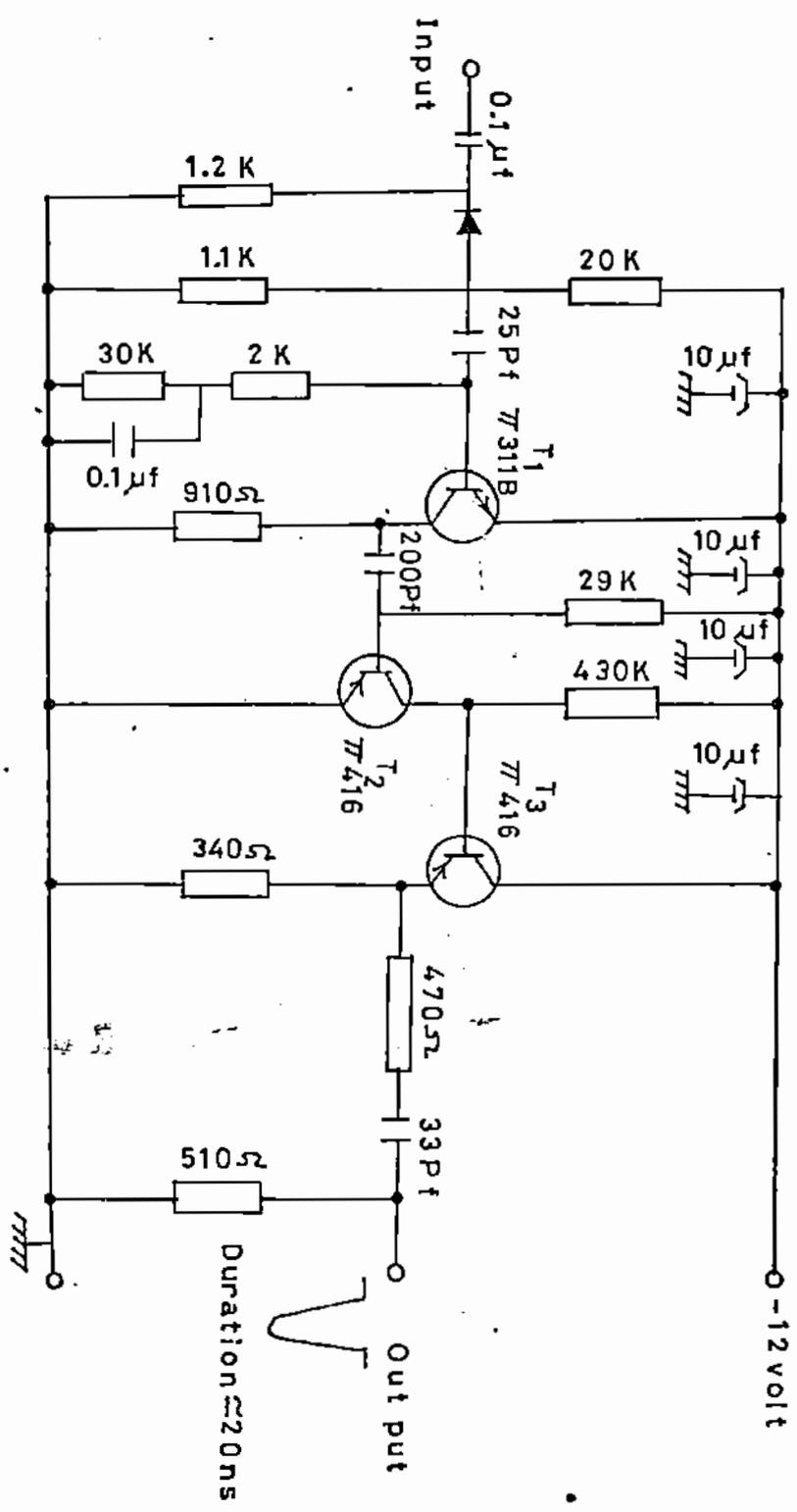
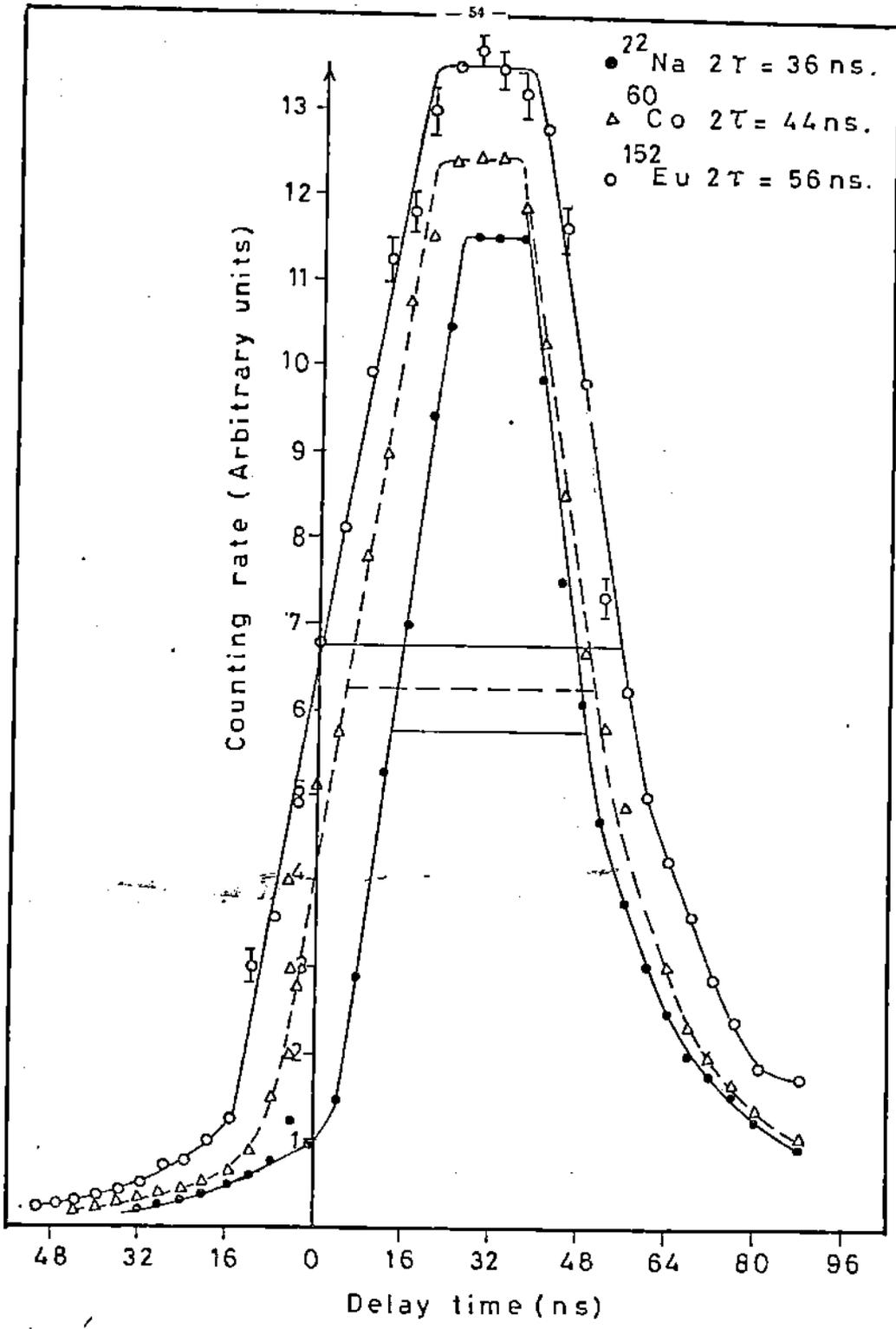
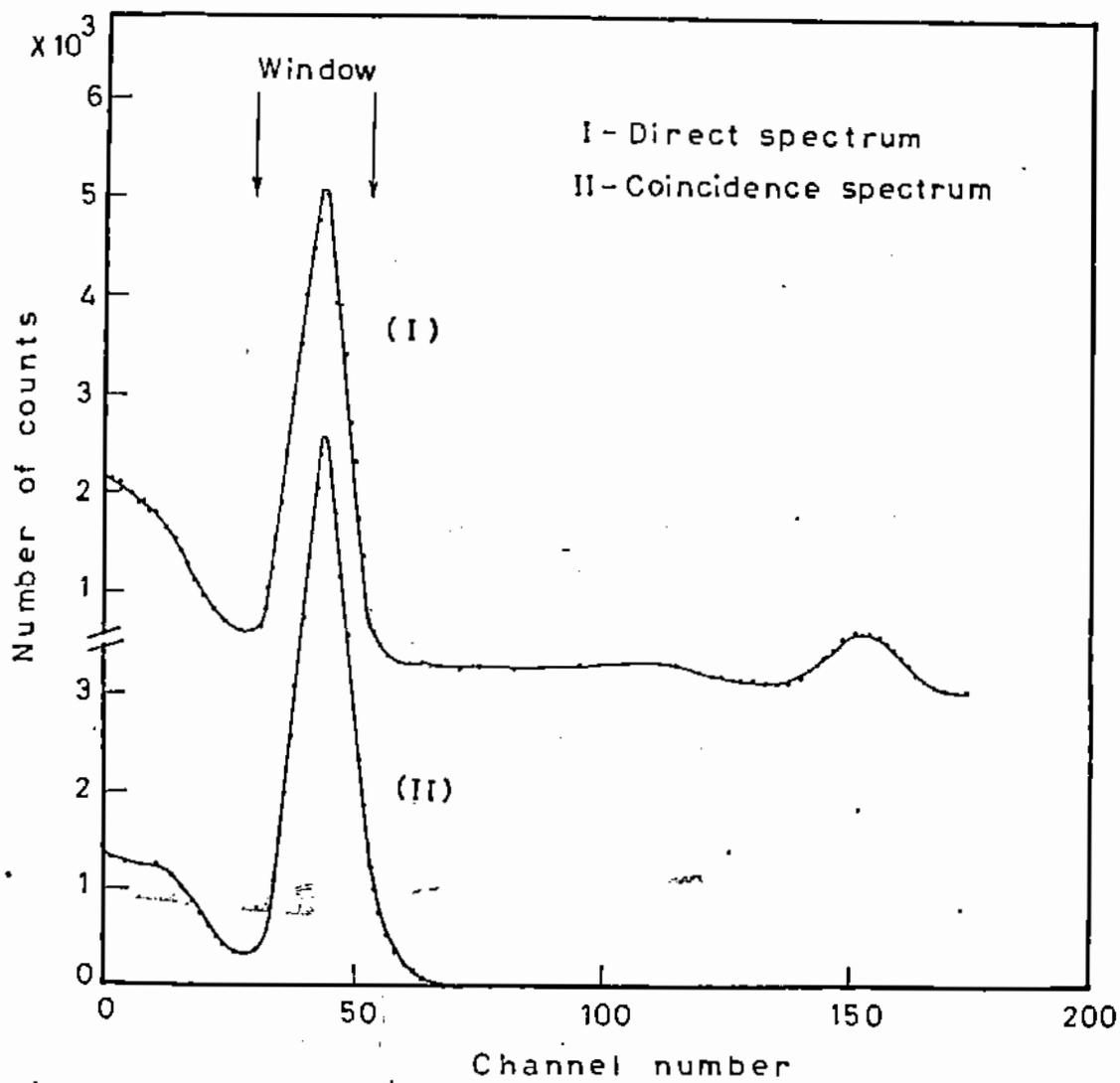


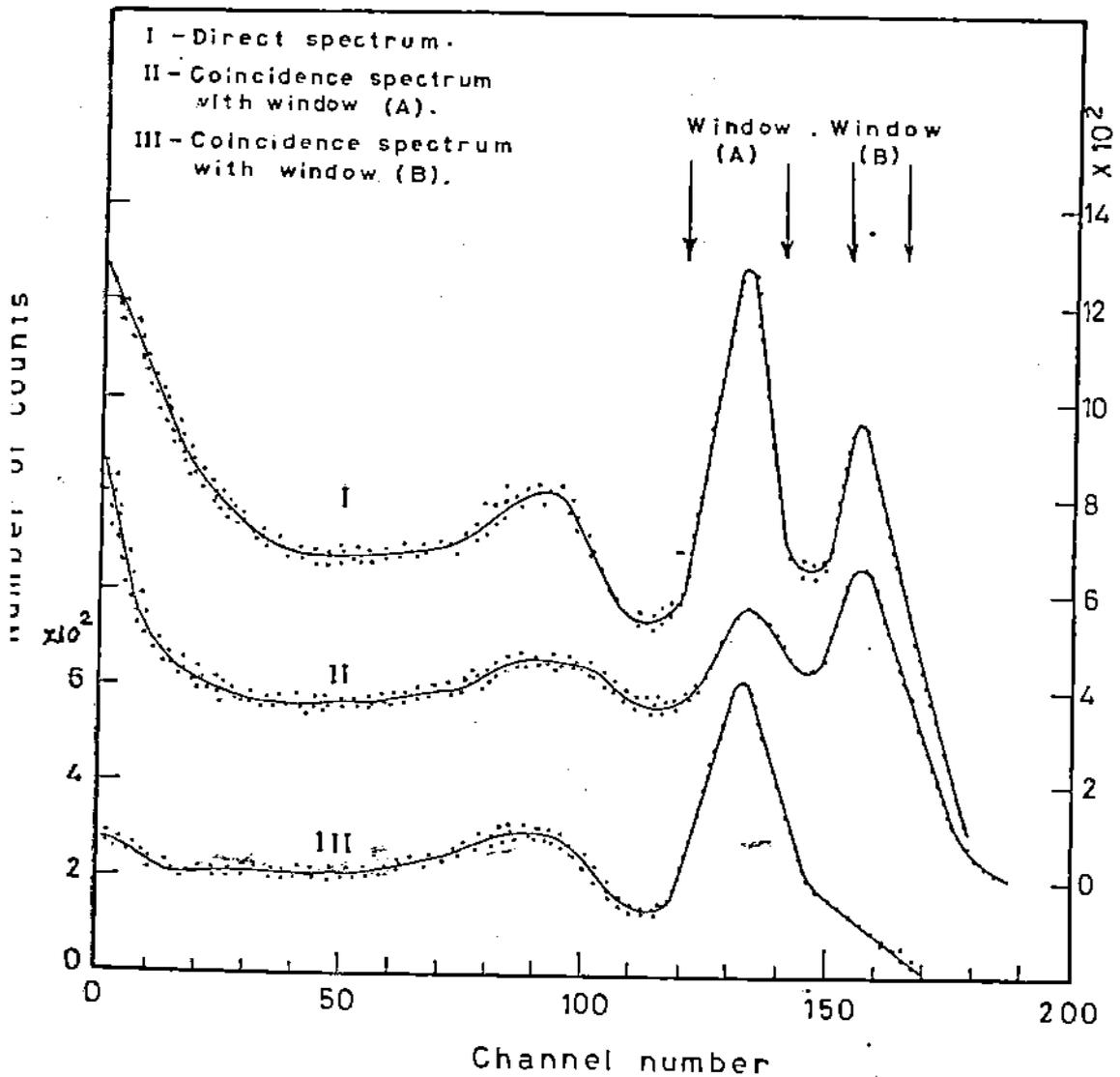
Fig 4

- ²²Na 2τ = 36 ns.
- △ ⁶⁰Co 2τ = 44 ns.
- ¹⁵²Eu 2τ = 56 ns.

Counting rate (Arbitrary units)

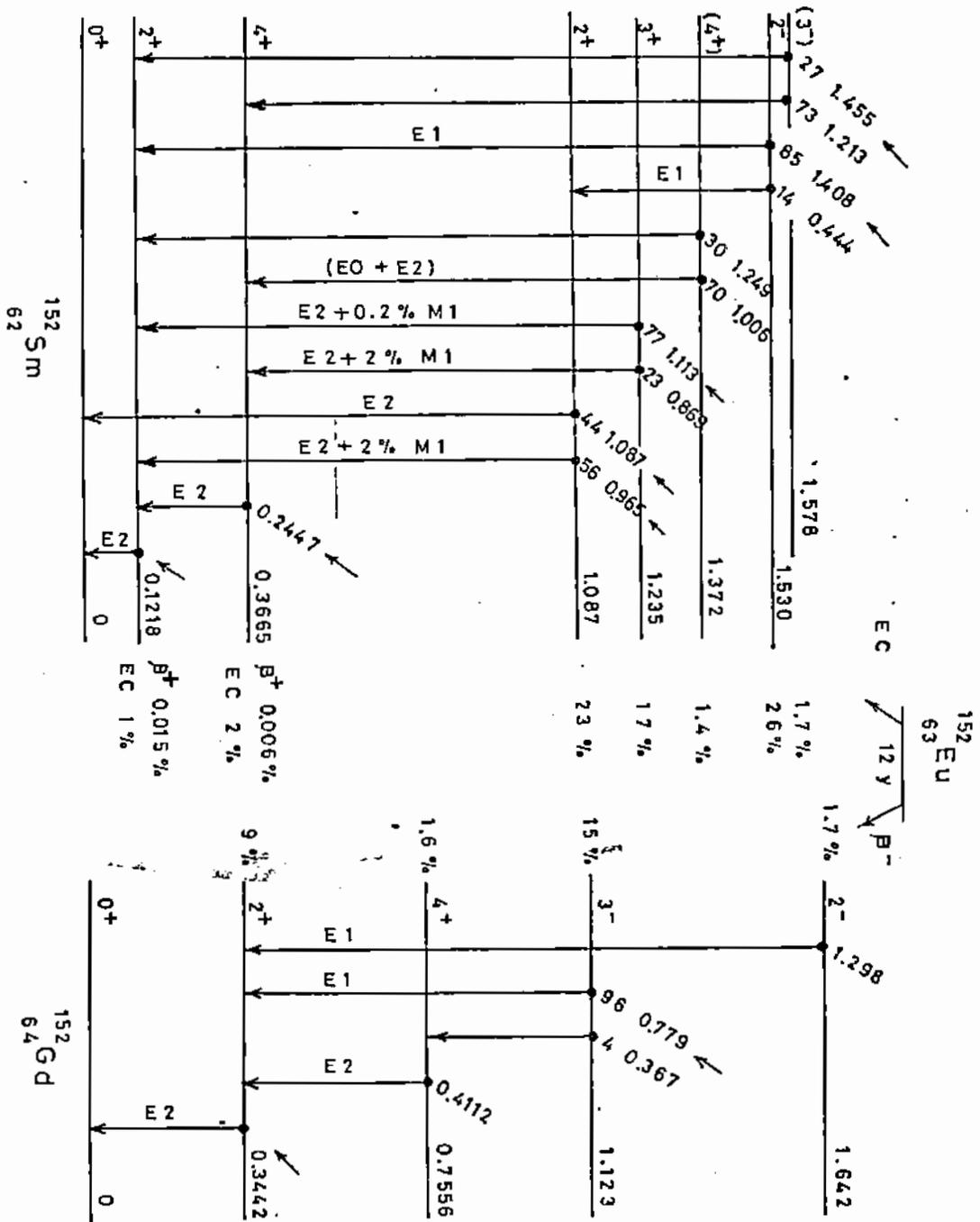


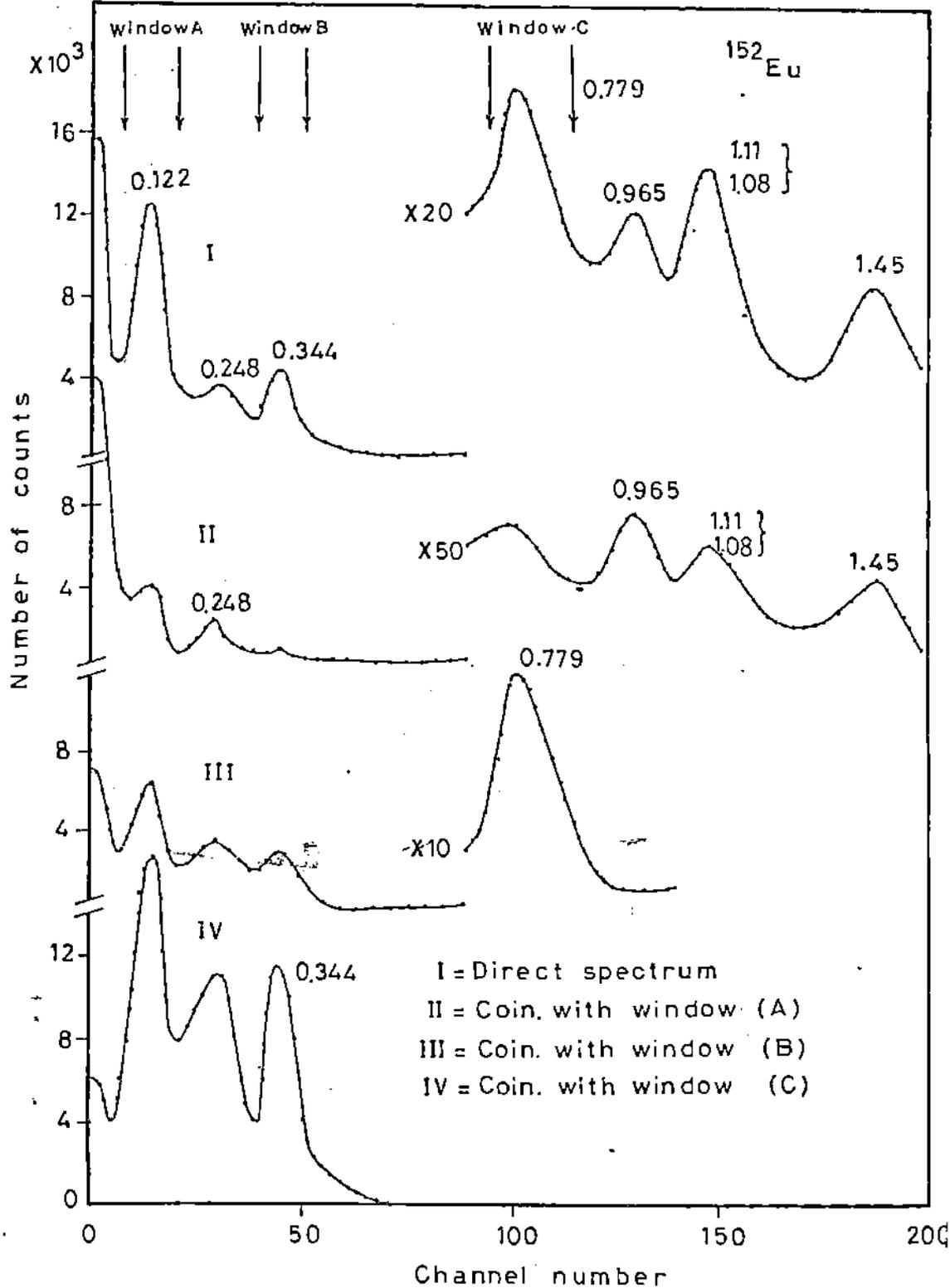




Vertical scale on the left represents spectrum III
Vertical scale on the right represents spectra II & I

1.48





EFFECT OF EXTERNAL MAGNETIC FIELD ON THE
VOLATILIZATION OF ELEMENTS IN THE D.C. ARC

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Abstract

In the present investigation opposite magnetic fields are applied to the free burning carbon arc, with a silicate sample (granodiorite) in its anode crater. The magnetic field promoted the selective volatilization of the elements present in the sample. As a result a depression in the spectral line intensities of Mn, Ti, Th and Fe as well as the background was observed. At the same time the intensity of the spectral lines of Ag, Ge, Pb, In and Cu is enhanced. The latter elements are of great significance since they are used as pathfinders for gold.

INTRODUCTION

Selective volatilization is a phenomenon observed in many light sources used in spectrochemical excitation specially in the d.c. arc. When a sample containing several elements is arced, it may happen that one or more elements distill almost completely before other elements begin to volatilize. It is generally attempted to eliminate or reduce the selective volatilization. On the other hand, there are cases in which it is desirable to improve it. In these cases it may be useful to suppress the emission of an element, which emits a complex spectrum, an intense background or both. This background as well as very complex spectra lower the sensitivity of detection and cause line interference.

Scribner and Mullin⁽¹⁾ described a method for the analysis of impurity elements in uranium oxide in which they added 2% Ga₂O₃ to suppress the volatilization of uranium, but not that of the more volatile elements. If the exposure is terminated before the main volatilization of uranium

starts, the spectra of the impurity elements can be examined in a relatively clean spectrum, resulting in a marked improvement of detection limits for a number of elements.

The application of stationary external magnetic fields to the d.c. arc in order to increase the spectral line intensity has been investigated by many researchers during the past decade. Vukanovic et al.^(2,3,4,5), Todrovic⁽⁶⁾, Lummerzheim and Nickel⁽⁷⁾, Leushacke and Nickel^(8,9) and others applied the external magnetic field to the d.c. arc to improve the sensitivity of detection of the elements under consideration.

Vukanovic⁽⁴⁾ compared the effect of the external magnetic field with that of the addition of Ga_2O_3 , in the analysis of ZrO_2 and found that both have the same tendency of lowering the detection limit of some impurity elements.

The aim of the present investigation is to explore the possible application of external opposite magnetic fields to the d.c. arc for improving selective volatilization, thus minimizing line interference of some major elements in the spectrum of a natural silicate sample. The sample under investigation is a granodiorite rock collected from the Fawakhir gold mine in Eastern Desert near red sea, Egypt. The elements Ag, Ge, Pb, In, and Cu are present in the sample in trace amounts. These elements are of great significance, since they are used as pathfinders for gold. On the other hand, the elements Mn, Ti, Th and Fe are present with concentrations higher than 1%.

EXPERIMENTAL AND RESULTS

The arc is placed between the two coils of an electromagnet, connected together to produce opposite magnetic fields with the neutral point at the center of the arc gap. The magnetic induction measured at the end of each coil could be varied between 0 and 200 gauss. Anode excitation technique has been used. In order to avoid globule formation, graphite powder was

maximum increase in spectral line intensities, for these elements, is achieved with magnetic induction values between 50 and 100 gauss. Copper is the only element whose magnetic response curve shows two maxima, the first one at 50 gauss and a second weaker one at 150 gauss.

For the elements Mn, Al, Th, and Fe, the magnetic field effect is to decrease the intensity of their spectral lines. Figure (2) shows the magnetic response curves for this group of elements.

volatilization behaviour in the magnetic field:

The effect of the magnetic field on the volatilization behaviour of the different elements in the sample was investigated. The anode craters were packed with samples and arced till completion with zero gauss and 75 gauss respectively, under the optimal conditions achieved. During the burning, the plate holder was racked every 10 seconds. From the obtained spectra, the time-intensity curves were plotted for the different elements under consideration. The results obtained are shown in figures 3 - 11.

From these figures it is obvious that the elements Ag, Ge, Pb and In show a similar behaviour. One can also see again that applying the magnetic field increases the spectral line intensity for these elements. In both cases, with and without magnetic field, the maximum of the time-intensity curve is reached almost after the same time. All curves show a tendency for complete evaporation in a slight shorter time by the application of the magnetic field. Without the magnetic field, the time-intensity curve for copper has two maxima; the first maximum after 10 seconds and another weaker one after 40 seconds. With the magnetic field applied, the intensity of the copper line reaches a maximum after 60 seconds then another maximum after 90 seconds. Copper is among those elements which show an increase in spectral line intensity in presence of the magnetic field.

For the elements Mn, Ti, Th, and Fe the magnetic field affects in delaying

added to the sample in ratio 1:5 . A medium quartz spectrograph Q 24 (Carl Zeiss Jena) was used with a slit width of 10 microns. The spectra were recorded on kodak spectral analysis plates No. 3 . The plate emulsion was calibrated using a platinum-on-quartz six step filter attached to the spectrograph slit.

The elements considered in this study are Ag, Ge, Pb, In, Cu, Mn, Ni, Zn and Fe. Preliminary experiments showed that the opposite magnetic fields applied to the arc caused a remarkable depression in the background of the whole spectrum and an increase in the line intensity of Ag, Ge, Pb, In and Cu. On the other hand the spectral lines of Mn, Ni, Zn and Fe showed decrease in intensity in the presence of the magnetic field.

The excitation conditions were chosen, which give the maximum increase in the intensity for the Pb line at 2833.07 \AA , taken as a test line. The change in spectral line intensity due to the magnetic field was measured in the intensity ratio (with background correction) I/I_0 with and without magnetic field. These conditions are as follows :

- a) Arc gap : 4 mm.
- b) Arc current : 4 amperes.
- c) Exposure time : 60 seconds.
- d) Anode : graphite rod 6.2 mm in diameter, with a cavity of 4.6 mm in diameter, wall thickness 0.8 mm and a central depth of 4.0 mm .
- e) Counter electrode : graphite rod of 4.5 mm in diameter pointed at its end to form a cone of solid angle of 28° .

Effect of the magnetic ^{field} on the intensity of the spectral lines :

Figure (1) shows the enhancement of the spectral line intensity I/I_0 as a function of the magnetic induction B, (as measured at the end of the coil) for those elements which show an increase in line intensity, in the presence of the magnetic field. From the figure one can see that the

their evaporation curves remarkably. Considering Mn, as an example, the maximum of the time-intensity curve is reached after 60 minutes without the magnetic field. Applying the magnetic field shifts the maximum about 220 seconds. Accordingly if the exposure is terminated after 60 seconds, the Mn spectral lines will hardly appear in the spectrum. This may give an explanation for the observed decrease in intensity of the spectral lines of these elements with the application of the magnetic field.

DISCUSSION

From the present study it is obvious that the external opposite magnetic fields applied to the d.c. arc have a remarkable effect on the volatilization behaviour of the elements present in the silicate sample under consideration. The elements investigated can be classified into two groups : The first group consists of those elements whose spectral line intensities increase with the application of the magnetic field. For these elements the magnetic field enhances the rate of volatilization and the evaporation is completed in slight shorter time. Fortunately these elements are mainly those used as pathfinders for gold.

For the second group of elements, which are mainly those constituting the major elements in the sample, applying the magnetic field results in delaying their evaporation with appreciable time intervals. Thus it can be concluded that the opposite magnetic fields, enhanced the selective volatilization of the elements present in the silicate sample. This observation can be useful, in the spectrochemical analysis of the natural silicates, in minimizing line interference.

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Figure Captions

- Fig. (1) Intensity ratio I/I_0 with and without magnetic field as a function of the magnetic induction B
- Fig (2) Intensity ratio I/I_0 with and without magnetic field as a function of the magnetic induction B
- Fig (3) Time-intensity curves for Ag.
- Fig.(4) Time-intensity curves for Pb.
- Fig. (5) Time-intensity curves for Ge.
- Fig. (6) Time-intensity curves for In
- Fig. (7) Time-intensity curves for Cu.
- Fig. (8) Time-intensity curves for Mn.
- Fig. (9) Time-intensity curves for Ni
- Fig. (10) Time-intensity curves for Ni.
- Fig. (11) Time-intensity curves for Fe.

تأثير المجال المغناطيسى العكسى على تيخر العناصر فى القوس الكهربي المستمر

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شعبة الفيزيكا - المركز القومى للبحوث

يتناول هذا البحث دراسة تأثير المجال المغناطيسى العكسى على تيخر العناصر الموجودة فى الخامات الطبيعية مثل خام الجرافوديريت ، عند تحليلها طيفيا باستخدام القوس الكهربي المستمر .

وقد وجد من الدراسة ان بعض هذه العناصر مثل المنجنيز والتيتانيوم والثاليم والحديد يتأخر تيخرها فى وجود المجال المغناطيسى . اما البعض الاخر مثل الفضة والجرمانيم والرصاص والاندسيوم والنحاس فانه يسهل تيخرها فى مدة زمنية اقل . والعناصر الاخرى لها اهمية كبيرة فى الكشف عن الذهب . ولذلك فانه يمكن الاستفادة من هذه الدراسة فى التحليل الطيفى لعنق هذه الخامات .

On The Spectral Light Current Of
An Optical Resonator

By

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and

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ABSTRACT

A theoretical derivation for the transmitted spectral light current distribution from an optical resonator is given taking into account the radiating solid angle of the point light source. From the energy law, the transmitted light current, in case of no interference, was calculated to test this derived spectral distribution.

(I) INTRODUCTION

Most of the previous work in the field of interference of light deals with the spectral light current density distribution of the resulting interference fringes. The calculations are based on the assumption that the incident wave of a certain amplitude⁽¹⁾ does not suffer any change due to the inverse-square law during its multi-reflections; i.e. the incident waves intensity is considered to be equal to its optical density. This assumption requires that the light source is illuminating the optical resonator with a unit solid angle.

From the experimental point of view, the optical set-up system illuminates the resonator in a given solid angle, which is not, by necessity, equal to a unit solid angle.

In the literature⁽²⁾ although it has been assumed that this angle is very small, but the optical resonator still

* Atomic Energy Authority, Cairo .

transmits within the solid angle a light current and not light current density. In the present work, the transmitted spectral light current from an optical resonator when illuminated within a given solid angle is theoretically calculated.

(II) THEORY

As seen in Fig. (1), we consider a deformed point light source illuminating an optical Fabry-Perot resonator with a current density (I_0) in a solid angle (ω) such that :

$$\omega > \frac{A}{[a + 2(N - 1)D]^2} ,$$

where : (A) is the area of the collimating lens,

(a) is the distance between the deformed point light source and the collimating lens.

(N) is the effective number of the transmitted light currents from the resonator (3)

The value of (N) can be calculated from :

$$N = 1/(1 - R^2) ,$$

where R is the reflection coefficient of the mirrors of the resonator.

For the given radiating solid angle (ω), the transmitted light current from the resonator can be calculated for two cases :

- 1) The optical path length (D) of the resonator is larger than the coherence length of the incident light. In this case, no interference occurs (4 & 5) and the detector will be affected by a light current (I_0) which can be derived from a sum of partial light currents of different radiating solid angles and of different light current densities; i.e. :

$$L_c = (1-R)^2 \left[\frac{I_0 A}{a^2} + \frac{I_0 A R^2}{(a + 2D)^2} + \frac{I_0 A R^4}{(a + 4D)^2} + \dots + \frac{I_0 A R^{2(n-1)}}{[a + 2(n-1)D]^2} \right]$$

or $L_c = I_0 (1-R)^2 \sum_{n=1}^{\infty} \frac{A R^{2(n-1)}}{[a + 2(n-1)D]^2}$ (1)

2) The optical path length (D) of the resonator is smaller than the coherence length of the light. In this case the condition for producing interference is satisfied and the detector will be affected by a light current of interference fringes which are produced from the interference of partial currents of wave amplitudes of different densities and of different solid angles, i.e. ,

$$L_c(\theta) = (1-R)^2 \left[\left(\frac{I_0 A}{a^2} \right)^{\frac{1}{2}} + \left(\frac{I_0 A}{(a + 2D)^2} \right)^{\frac{1}{2}} \cdot R \cdot e^{i\theta} + \left(\frac{I_0 A}{(a + 4D)^2} \right)^{\frac{1}{2}} \cdot R^2 \cdot e^{2i\theta} + \dots + \left(\frac{I_0 A}{[a + 2(n-1)D]^2} \right)^{\frac{1}{2}} \cdot R^{n-1} \cdot e^{(n-1)i\theta} \right]^2$$
 (2)

where $\theta/2$ is the phase shift of the wave per path. Eq. (2) can be rewritten as :

$$L_c(\theta) = (1-R)^2 I_0 \left[\left(\sum_{n=1}^{\infty} \frac{A^{\frac{1}{2}} R^{n-1} \cos (n-1) \theta}{a + 2(n-1)D} \right)^2 + \left(\sum_{n=1}^{\infty} \frac{A^{\frac{1}{2}} R^{n-1} \sin (n-1) \theta}{a + 2(n-1)D} \right)^2 \right]$$
 (3)

For testing eq. (3) we calculate the average energy quantity $\overline{L_c}(\theta)$ of the spectral light current $L_c(\theta)$ from the energy law. Integrating $L_c(\theta)$ over θ and calculating the average energy quantity, we get :

$$\overline{L_c}(\theta) = \frac{1}{2\pi} \int_0^{2\pi} L_c(\theta) d\theta = I_0 (1-R)^2 \sum_{n=1}^{\infty} \frac{A R^{2(n-1)}}{[a+2(n-1)D]^2} = L_c$$
 (4)

Thus the average energy quantity $\overline{L_c}(\theta)$ gives the same energy quantity of the light current (L_c) as in eq. (1) (6).

Let us now consider that the deformed point light source illuminates the resonator in a solid angle such that

$$w \leq \frac{A}{[a + 2(N-1)D]^2}$$

In this solid angle, all effective light currents transmitted from the resonator are collimated by the lens. As mentioned before, if we assume the case of no interference, then the detector will be affected by the sum of partial light currents of different current densities but of the same radiating solid angle (w) :

$$L_c = \frac{I_0 A (1-R)^2}{[a + 2(n-1)D]^2} \sum_{n=1}^{\infty} R^{2(n-1)}$$

or $L_c = \frac{I_0 A}{[a + 2(N-1)D]^2} \times \frac{(1-R)^2}{1-R^2}$ (5)

If now the condition of interference exists, the detector will be affected by the spectral light current arising from the sum of partial currents of wave amplitudes of different current densities but of the same solid angle (w) :

$$L_c(\theta) = \left[\sum_{n=1}^{\infty} I_0^{1/2} (1-R) \frac{A^{1/2} R^{n-1} e^{i(n-1)\theta}}{a + 2(N-1)D} \right]^2$$

$$= \frac{I_0 A (1-R)^2}{[a + 2(n-1)D]^2} \cdot \left[\sum_{n=1}^{\infty} R^{n-1} e^{i(n-1)\theta} \right]^2$$

or $L_c(\theta) = \frac{I_0 A}{[a + 2(N-1)D]^2} \times \frac{(1-R)^2}{1-2R \cos \theta + R^2}$ (6)

In this case, it is seen that the spectral light current has the same distribution of the well-known Airy-formula (1& 2) which deals with the spectral light current density distribution of the transmitted interference fringes of a Fabry - Perot resonator. In his calculations, Airy assumed that the light source is deformed to illuminate the optical resonator with a unit solid angles.

From the point of view of the energy law, we have :

$$\overline{L_c(\theta)} = \frac{1}{2\pi} \int_0^{2\pi} L_c(\theta) d\theta = \frac{1}{2\pi} \frac{I_0 A}{[a+2(N-1)D]^2} \int_0^{2\pi} \frac{(1-R)^2}{(1-2R\cos\theta+R^2)} d\theta$$

$$\text{or } \overline{L_c(\theta)} = \frac{I_0 A}{[a + 2 (n-1) D]^2} \times \frac{(1 - R)^2}{1 - R^2} \quad (7)$$

which is equal to L_c in eq. (5).

The spectral light current distributions for the two cases according to eqs. 3 & 6 are illustrated in Fig. (2).

Fig. (3) shows the variation of the average transmitted spectral light current with the distance (a) of the point source in the two cases when (w) is i) larger and ii) smaller or equal to $A / [a + 2 (N-1) D]^2$. It is seen that the average values of light current calculated from our theoretical derivation are in good agreement with that of Airy type at larger values of the distance (a). where the solid angle (w) is smaller.

III CONCLUSION

The transmitted spectral light current distribution from an optical resonator depends upon the radiating solid angle of the point light source. The transmitted light current is of Airy - distribution type, only when the deformed point light source illuminates the optical resonator with a solid angle (w) such that

$$w \leq A / [a + 2 (N-1) D]^2$$

Two different formula for the average transmitted light current are also found for the two cases when :

$$w > \text{ or } \leq A / [a + 2 (N-1) D]^2$$

Therefore it is important in the experimental measurements to take into account the radiating solid angle of the point light source which illuminates the optical resonator.

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ملخص بحث

التوزيع الطيفي لهدب التداخل المتكونة من رنان ضوئي

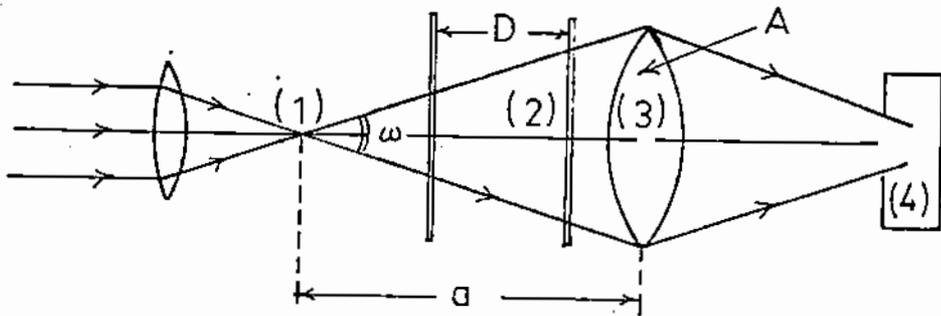
د • محمد منصور النكلاوي

د • كامل احمد الدهيمي

هيئة الطاقة الذرية

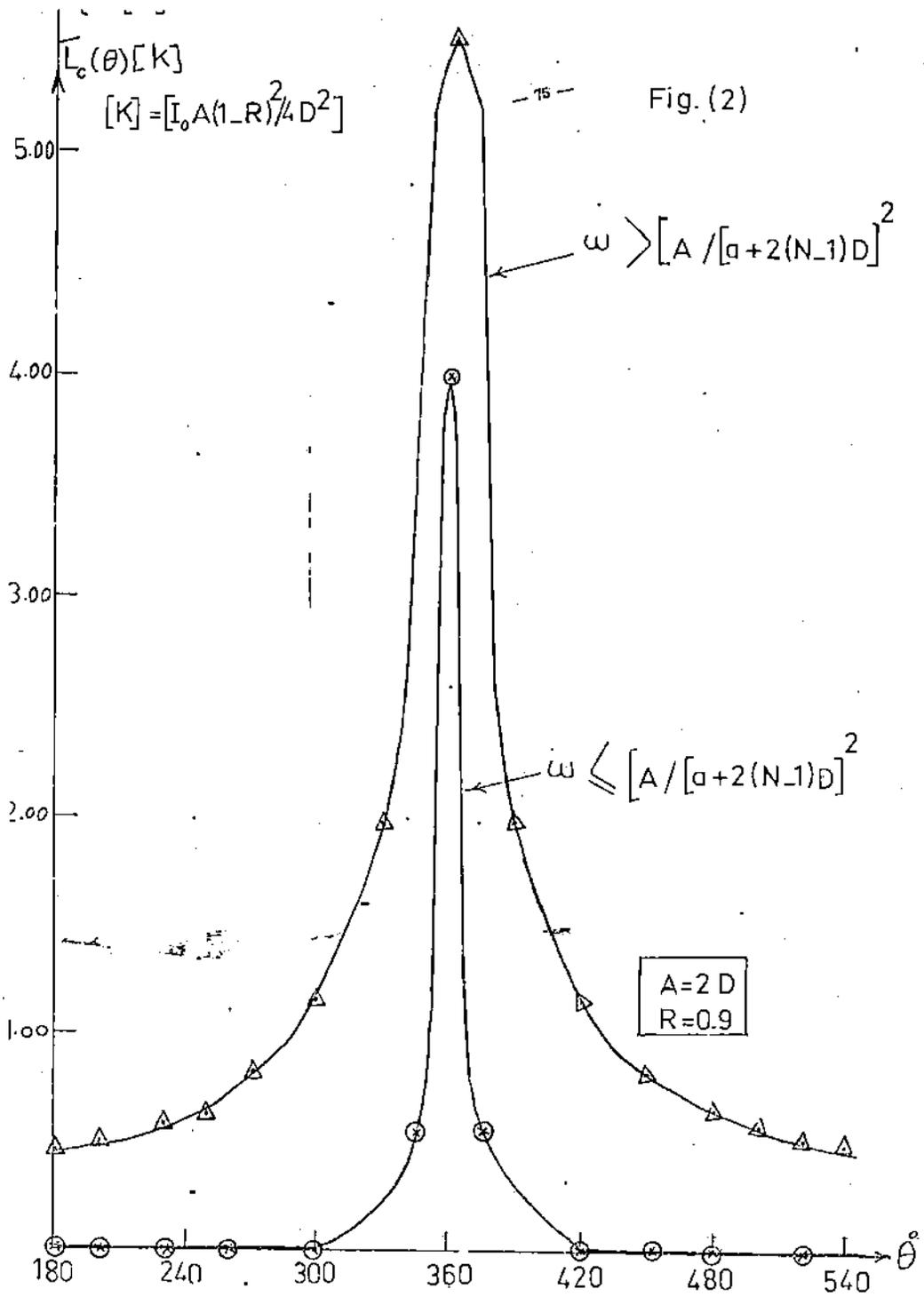
كلية التكنولوجيا - جامعة حلوان

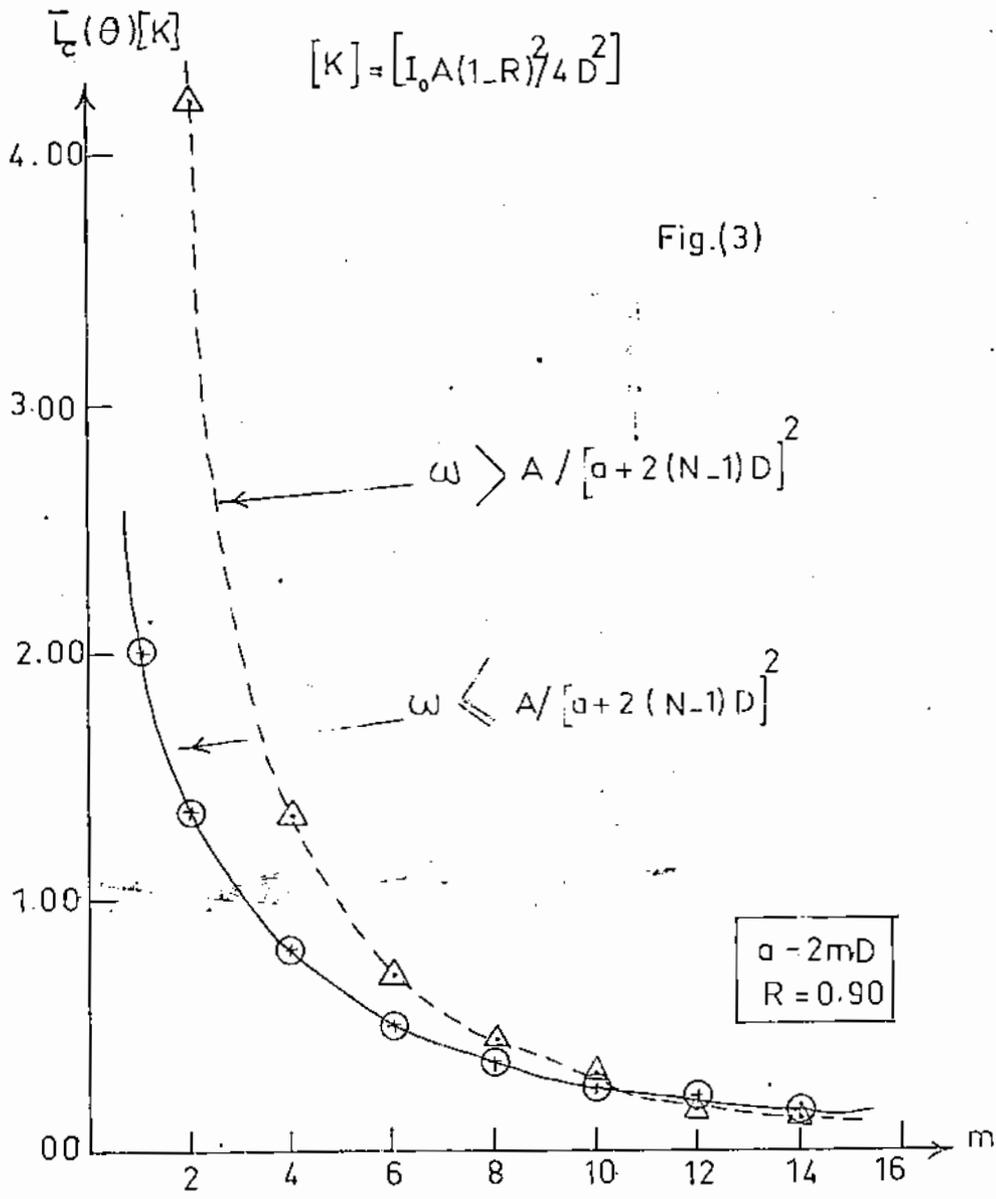
تعتمد الدراسات النظرية للتوزيع الطيفي للشدة الضوئية لهدب التداخل على اعتبار ان الاضاءة الساقطة على الرنان الضوئي تساوي كليا الكفاءة الضوئية للمصدر الضوئي نفسه أي أن المصدر الضوئي يضيء الرنان في زاوية مجسمة قيمتها الوحدة •
ومعالج هذا البحث نظريا هذه الدراسات باعتبار ان الاضاءة الساقطة تساوي تيارا ضوئيا اي ان الرنان يضيء بزاوية مجسمة لا تساوي بالضرورة الوحدة •
وقد وجد ان التوزيع الطيفي لهدب التداخل يأخذ صورة معادلة ايرى المعروفة اذا كانت الزاوية المجسمة اقل من قيمة معينة •
كذلك يتضمن البحث معادلات رياضية مستنتجة للتوزيع الطيفي عندما تكون الزاوية المجسمة اكبر او اقل عن هذه القيمة المعينة •



- 1-Point Light Source.
- 2-Resonator.
- 3-Collimating.Lens.
- 4-Detector.

Fig (1)





EFFECT OF HUMIDITY AND TEMPERATURE
ON THE RELATIVE EFFICIENCY OF STR-
REAMER COUNTER

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ABSTRACT

Investigations were made on the counting characteristics in relation to their dependence on the temperature and humidity. Curves connecting the fundamental parameters are given which are very useful for the design of any required streamer counter.

INTRODUCTION

The efficiency of a corona counter depends on the composition of the air filling. The water vapour concentration has the largest effect on the efficiency of the air filling spark counter⁽¹⁾. Recently more work⁽²⁾ has been done on its effect on the efficiency of streamer counters.

None of the earlier works⁽³⁻⁵⁾ have tried to study the temperature dependence of spark counters in detail. Counce⁽⁶⁾ has expressed the view that it is likely to be slight, but no considerable data on the temperature effect exist until now^(1,7).

The present work is intended to give a clear picture of the effect of both humidity and temperature on the operating characteristics of streamer counters.

APPARATUS

The streamer counter has been described elsewhere⁽⁸⁾ so that only a brief account may be given.

A highly polished stainless steel plate serves as cathode. Ankor anode wire of diameter 0.29 mm is held tightly parallel to the cathode at a distance of 7 mm. The effective length of the cathode is 72 mm and 10 M- Ω total resistance connecting anode to H.V.

EXPERIMENTAL RESULTS

(A) Effect of Humidity On the Relative Efficiency of Streamer Counters :

In The present work the dependence of the efficiency of the wire plane streamer counter on the concentration of the water-vapour in the filling air has been measured in the streamer region.

The counting characteristics are shown in ^{Fig.(1)} for various relative humidity extending from 30% to 100%.

The start of the corona streamer pulses V_{ac} increases with the increase of the amount of the water vapour in the counter, while the limiting voltage V_{as} remains nearly the same whatever may be the value of the relative humidity,

except for R.H. = 100%.

The observed dependence of the efficiency of counting on the moisture is a deficiency of the streamer counter, and in carrying out a number of measurements the counter must be placed in a closed enclosure, inside which the relative humidity has to be kept always constant during the running time of the experiments.

Fig. (2) represented the curve of the relative efficiency E_r $\left[E_r = N(x\%) / N(30\%) \right]$ i.e. the plateau counting rate relative to the counting rate of relative humidity 30% as function of the relative humidity.

From Fig. (2) we see that there is a rapid fall in the efficiency of counting on decreasing the humidity until R.H. is $\sim 40\%$ beyond which it decrease somewhat slowly with further decrease of the R.H. The increase in the efficiency of the streamer counter by increasing the humidity, apparently is linked with the formation of heavy negative ions. Increasing the humidity increases the probability of forming heavy negative ions and so as a result of this diffusion is decreased. This fact may bring about the development of streamer counter to operate under more favourable conditions and consequently counting alpha particles with greater efficiency. It is clearly seen from the current curve that the variation of humidity does not affect the corona current. But when V_a

increases the corona current I increases in a good agreement with the following empirical formula determined from the present experimental results applying the least - squares fits.

$$I = 1.5334 \times 10^{-12} V_a^2 + 0.0082 \times 10^{-6} V_a - 126.38 \quad (1)$$

Fig. (3) shows I vs. V_a given by equation (1). On the same figure are represented experimental values which show good agreement with the computed values.

This phenomenon is different from previous observed experimental results⁽²⁾, while in good agreement with that observed by Andreeschchev et al.⁽¹⁾, who had detected no change in the corona current for spark counters.

The internal resistance r_d of the anode-to-cathode discharge space has been determined as function of V_e which is determined by the relation

$$r_d = \frac{V_e}{I} \quad (2)$$

Fig. (4) shows the results. It is obvious that there is a gradual increase of r_d with decrease of V_e coming at the plateau threshold to a very steep rise.

Fig. (5) represents the a.c. resistance r_a vs. V_e which is given from the equation :

$$r_a = \frac{dV_e}{dI} \quad (3)$$

It is clear that r_a decreases with increasing V_e .

(B) Effect of Temperature On the Relative Efficiency of Streamer Counters.

To study the temperature dependence it is essential to allow for the apparent change in the range of particles caused by the change in the density of surrounding air as the temperature is changed.

This experimental work has investigated the effect of the variation of temperature on the streamer counter characteristics. For this purpose a counter with electrode separation 7 mm & anode wire of diameter $\phi = 0.29$ mm with 10 M-external resistance connecting anode to E.H.V. were used. The counting characteristics are shown in Fig. (6) for various temperature extending from 30°C to 80°C. The results indicate that the sensitivity of streamer counter is somewhat dependant on the temperature. Also it shows that the start of the corona streamer pulses increases with the increase of the temperature, in the same time a decrease in the length of the plateau is evident. However, a small positive plateau slope is noticed at 80°C.

Applying the least-square fits to experimental results of corona current which are represented in Fig. (7) by circles empirical formulas of the second order have been determined for I in terms of V_a corresponding to various values of temperature. These have been represented by the continuous curves in Fig. (7).

Fig. (8) indicates that the relative efficiency of the streamer detector increases gradually slow with the increase of temperature coming to a sharp rise at about 80°C.

The internal resistance r_d can be determined as function of V_e for different values of temperature by equation (2)

Fig. (9) shows the results • It is obvious that there is a gradual decrease in r_d with the increase of the temperature while at large values of V_e a very small variation in r_d is noticed.

Fig. (10) represents the a.c. resistance r_a given by eq. (3) vs. V_e for different values of temperature i.e. $T = 30, 40, 50, 60, 70, 80^\circ\text{C}$. It is clear that r_a decrease with the increase of the temperature and that a small variation in r_a is observed at high values of V_e .

Thus we conclude that the efficiency of streamer counter depends on the composition of the gas filling as well as its temperature. The water vapour concentration was the largest effect on the efficiency of the air filling streamer counter.

ACKNOWLEDGEMENT

The authors would like to express their thanks and gratitude to Prof. Dr. M. Mahrous Head of the Physics Department, Girls College, Ain Shams University for his

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تأثير الرطوبة ودرجة الكفاءة النسبية للعداد الحراري

د. حمنية محمد ابوزيد - السيدة / مرات بكر صديق عثمان
 قسم الطبيعة بكلية البنات جامعة عين شمس

ملخص البحث

درس تأثير كل من درجة الحرارة والرطوبة على خصائص العداد الفيزي
 ودرست المنحنيات التي تربط متغيرات العداد المختلفة . وقد وجد ان تأثير
 الرطوبة على كفاءة العداد النسبية كبيرا اذا تورن بتأثير درجة الحرارة عليها .

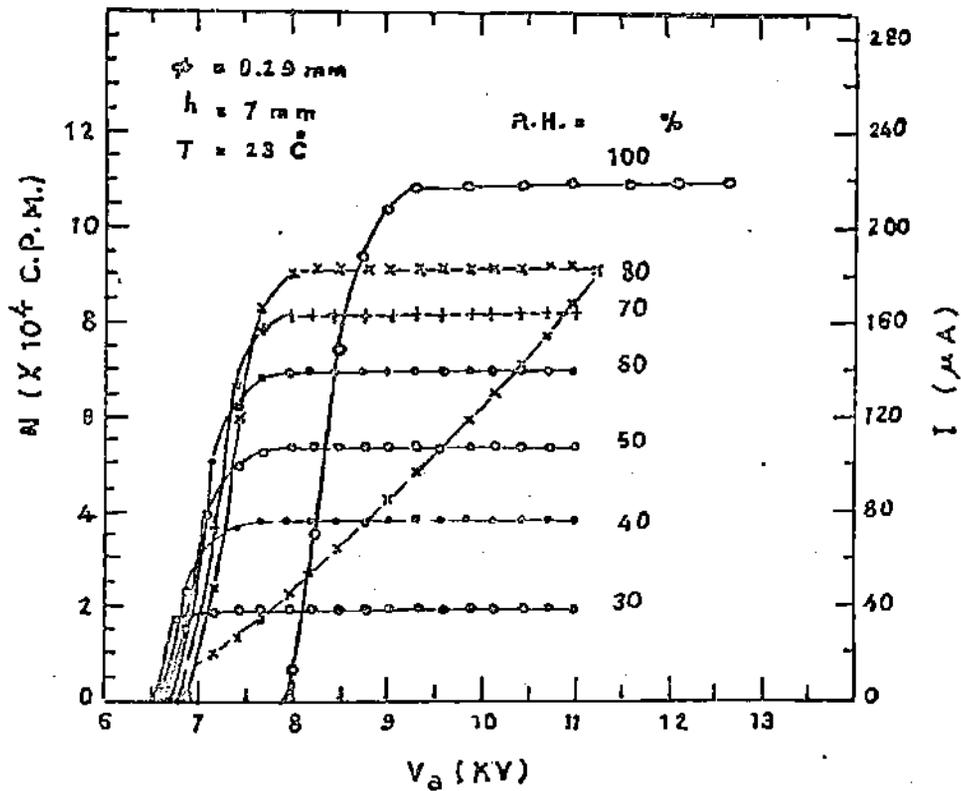


Fig. 1 Counting characteristics for different values of relative humidity.

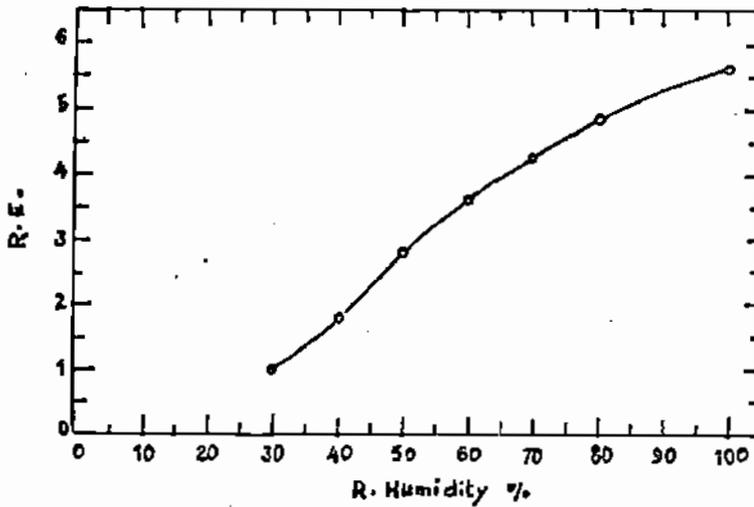


Fig. 2 Relative efficiency E_r VS relative humidity.

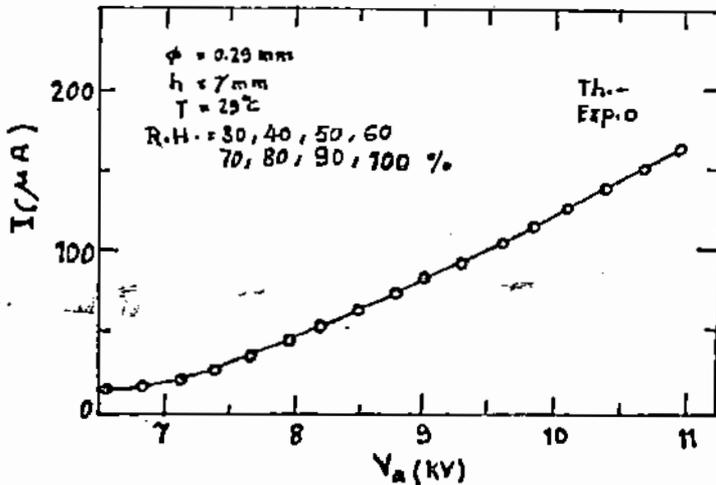


Fig. 3 Corona current for different values of R.H. full curve represented I VS. V_a from empirical formula, o , represent experimental results.

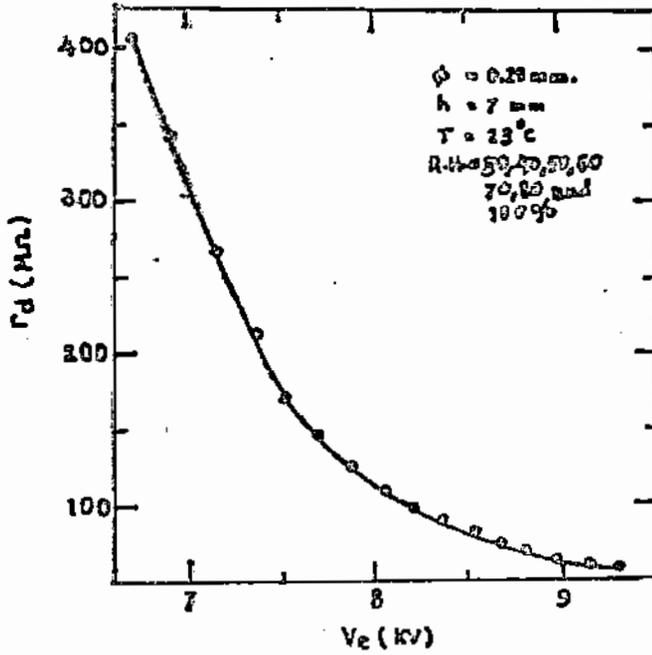


Fig. 4 r_d VS V_e for different values of R.H.

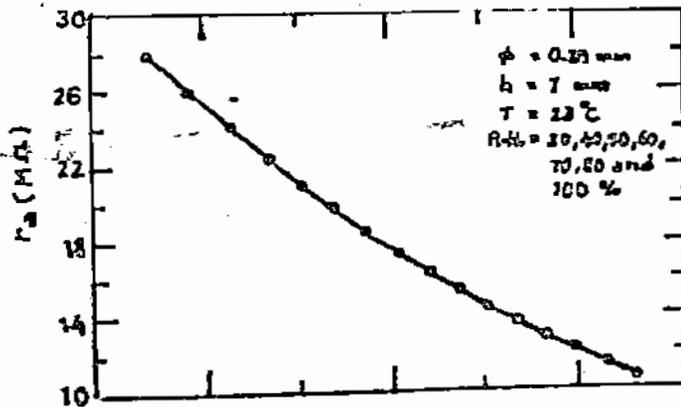


Fig. 5 r_a VS V_e for different values of R.H.

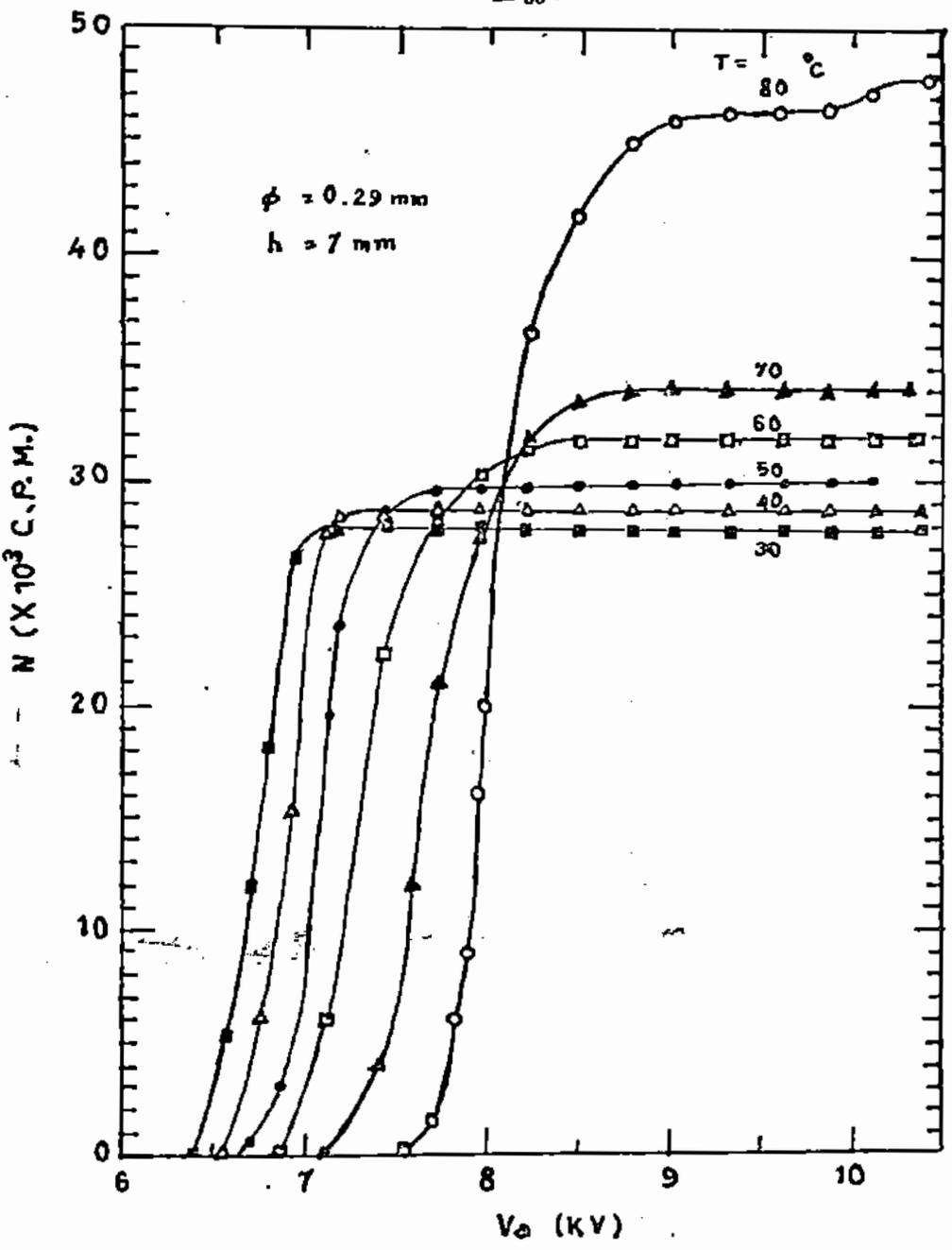


Fig. 6 The counting characteristics for different values of temperature.

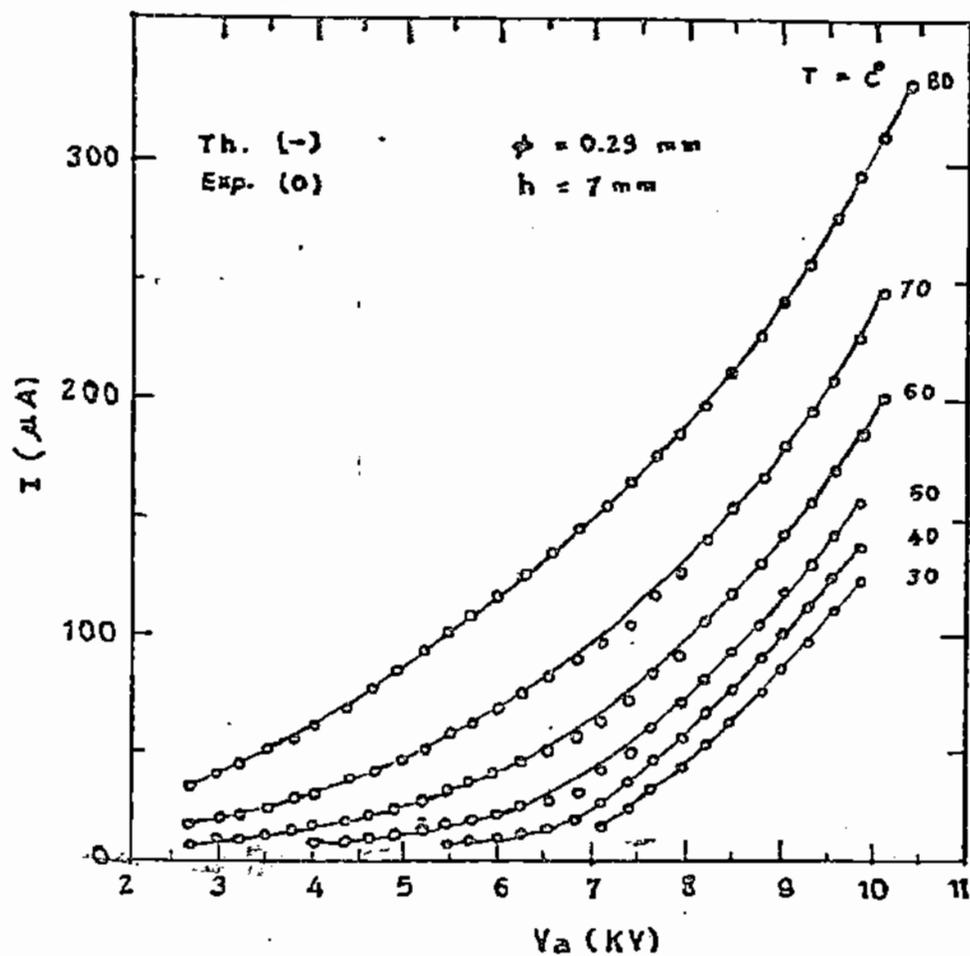


Fig. 7 Full curves represent I VS V_a from empirical formulas $\circ\circ\circ$ represent experimental results, for various values of temperature.

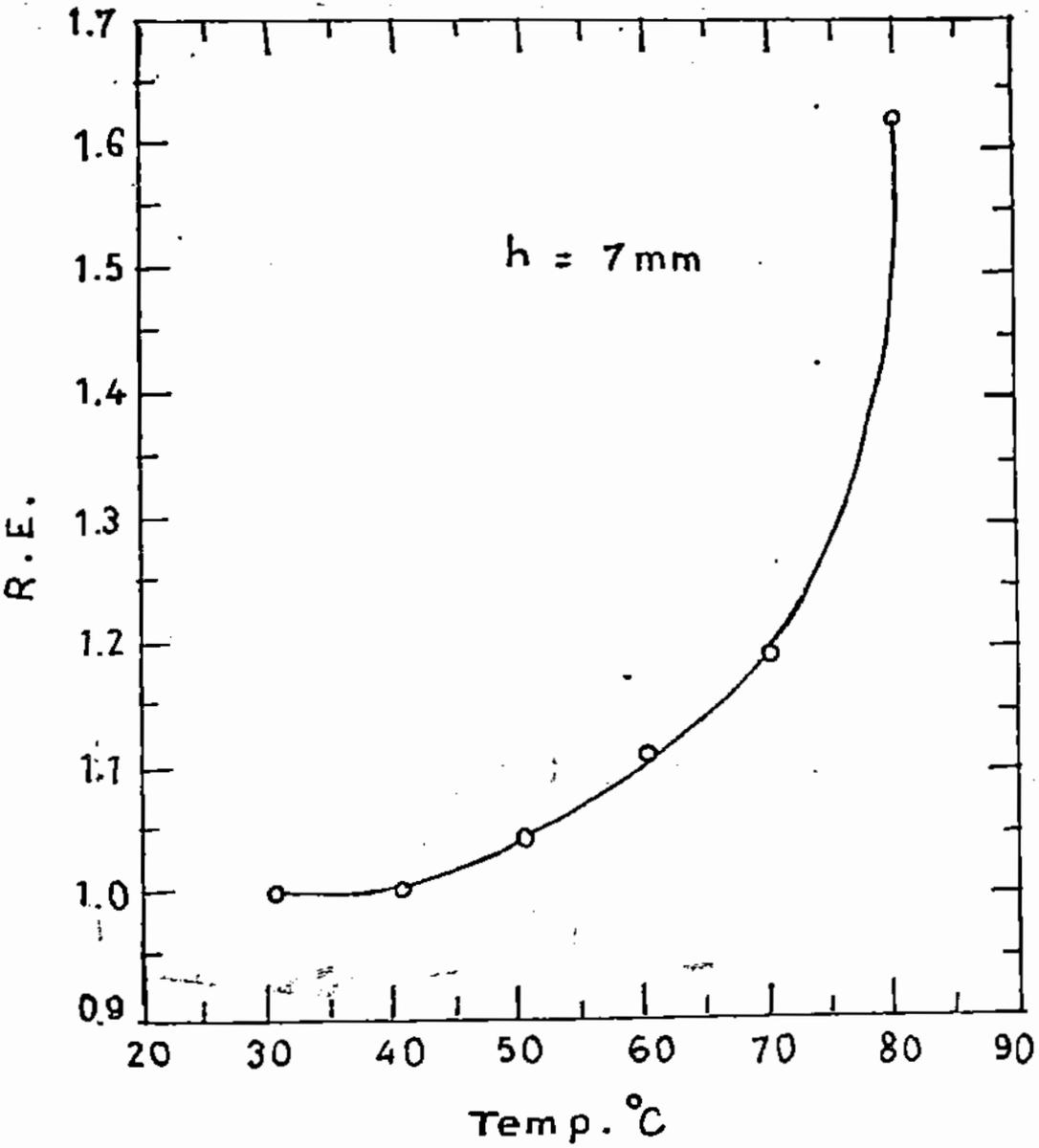


Fig. 8 Relative efficiency (R.E.) VS. temp.,

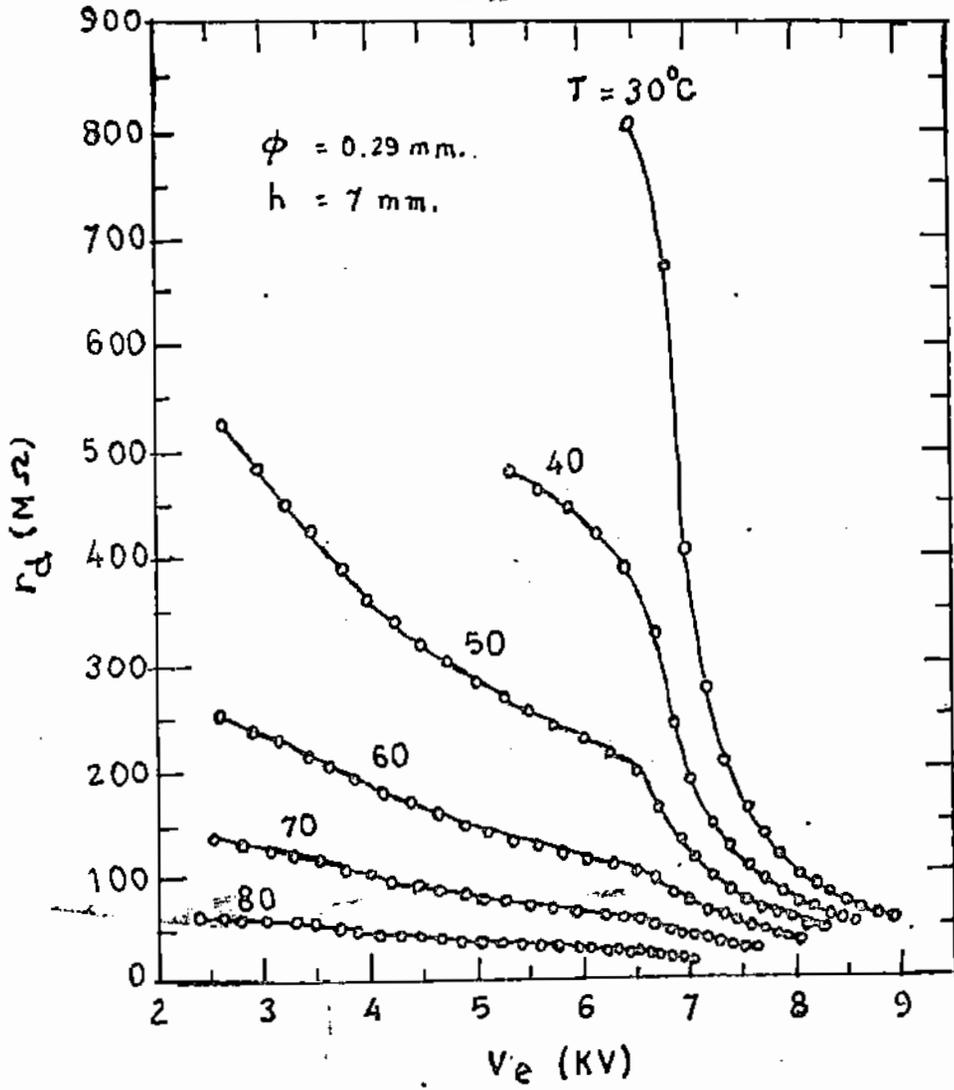


Fig. 9 r_d VS V_e for various values of temperature.

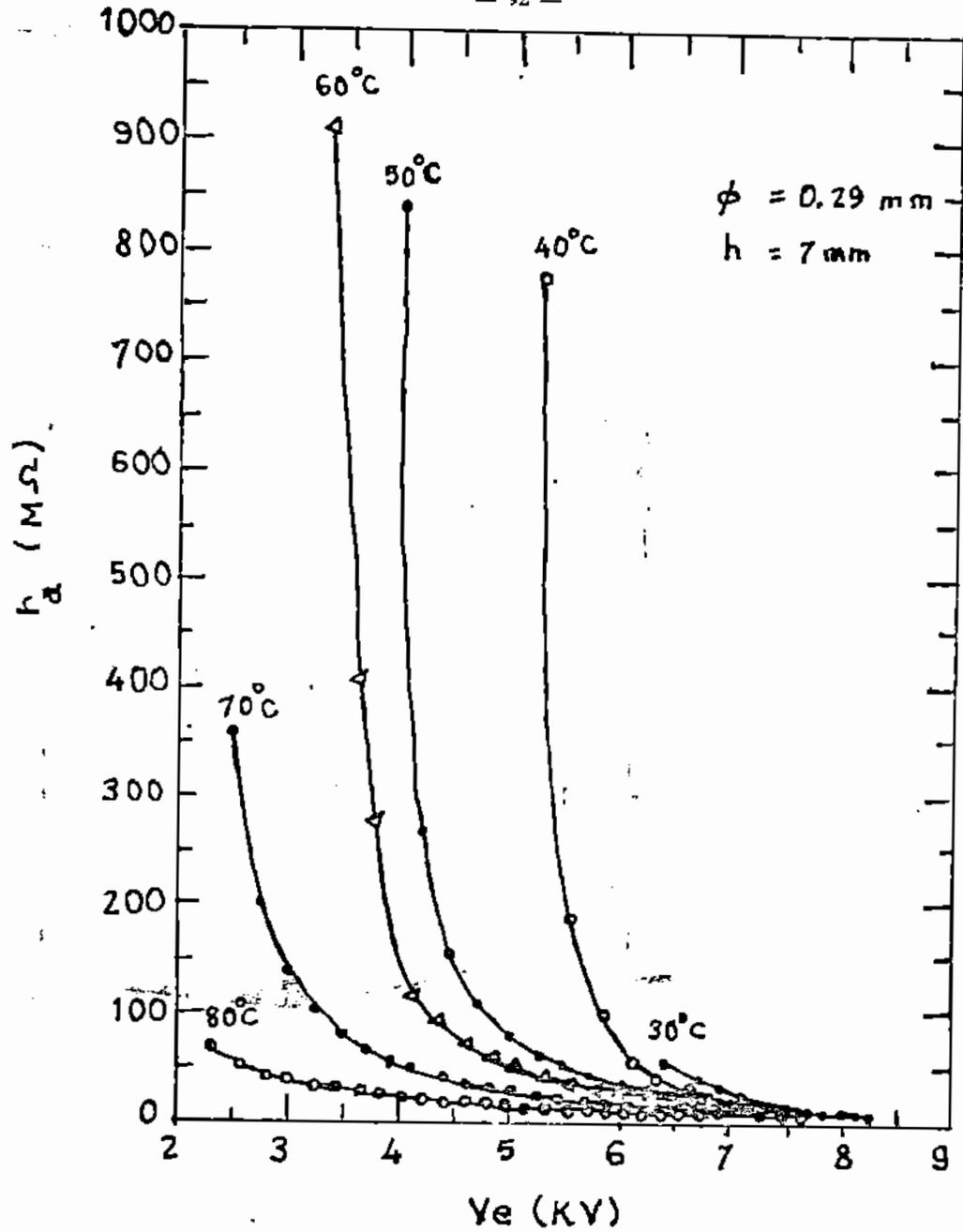


Fig. 10 r_a VS V_e for different values of temperature.

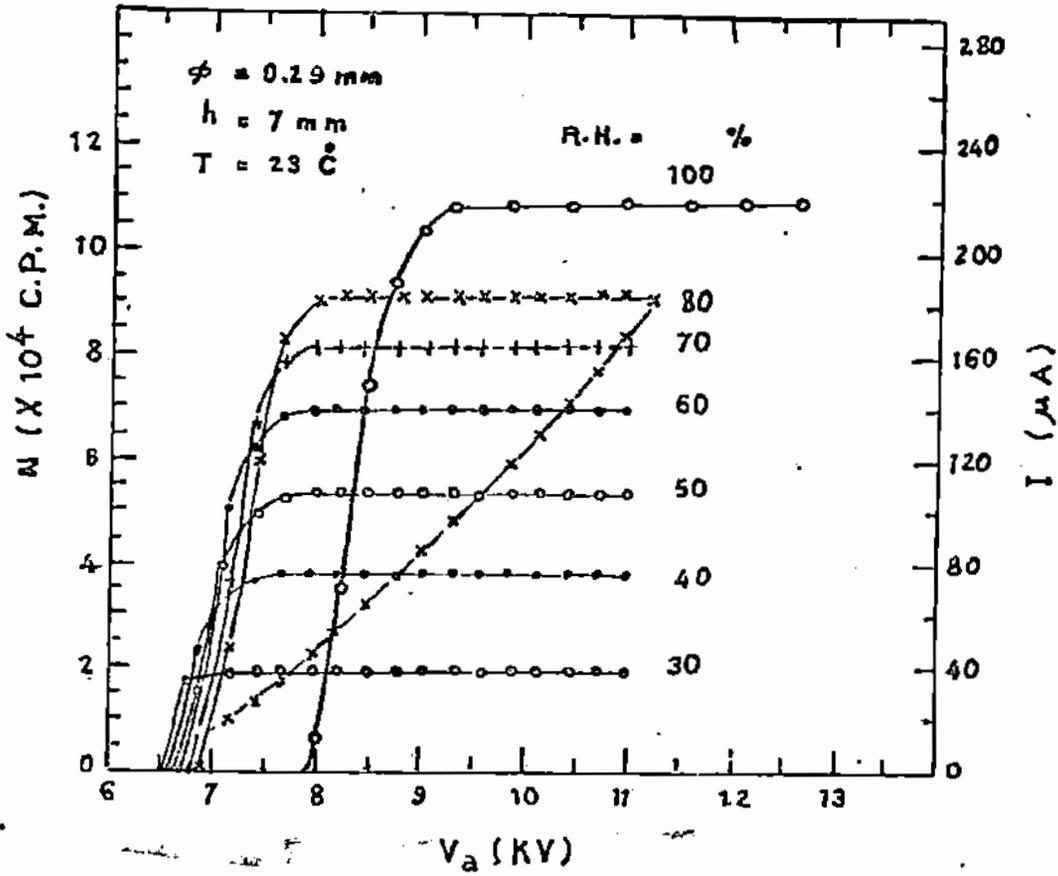


Fig. 1 Counting characteristics for different values of relative humidity.

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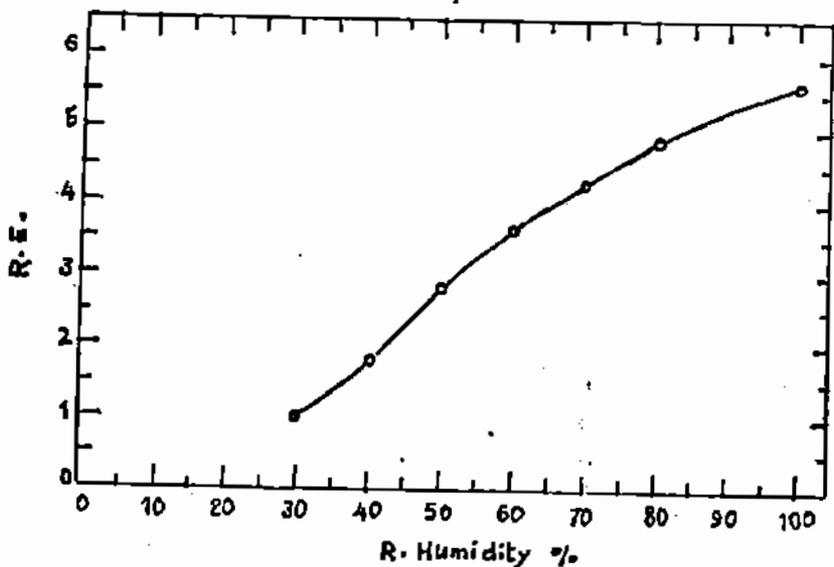


Fig. 2 Relative efficiency E_r VS relative humidity

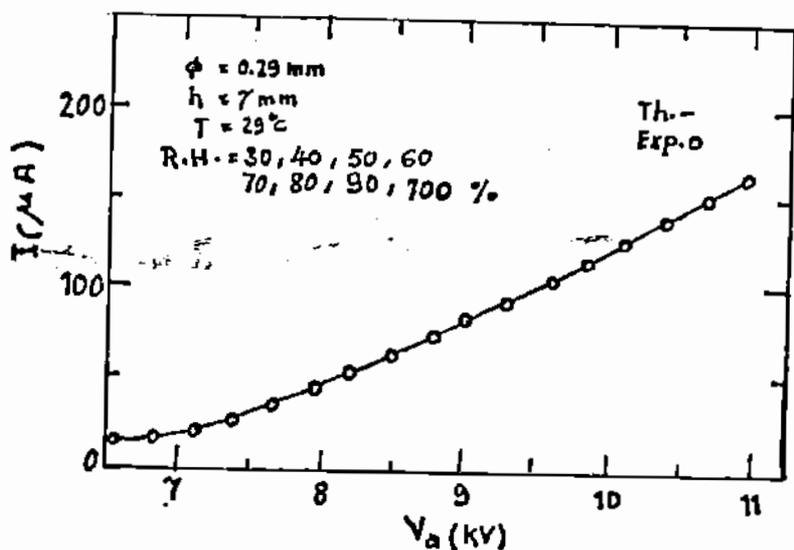


Fig. 3 Corona current for different values of R.H. full curve represented I VS. V_a from empirical formula, ooo, represent experimental results.

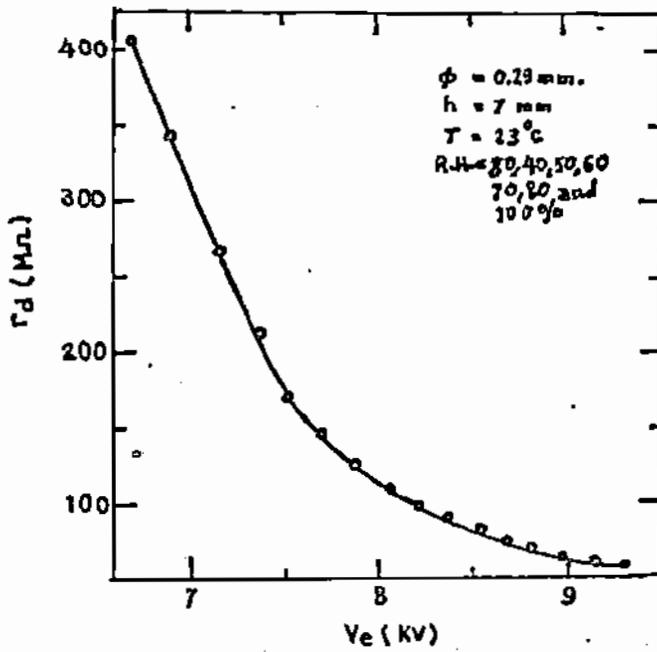


Fig. 4 r_d VS V_e for different values of R.H.

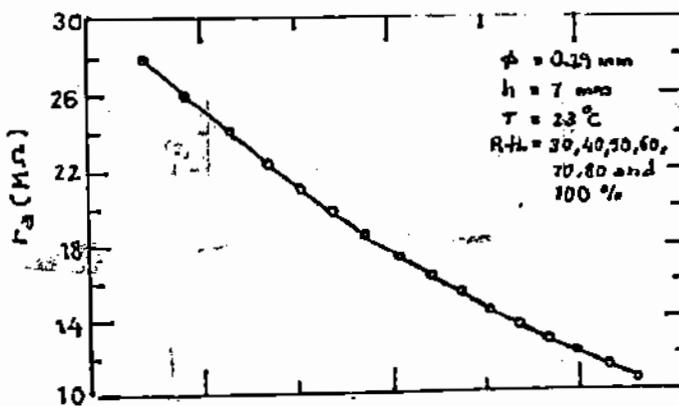


Fig. 5 r_a VS V_e for different values of R.H.

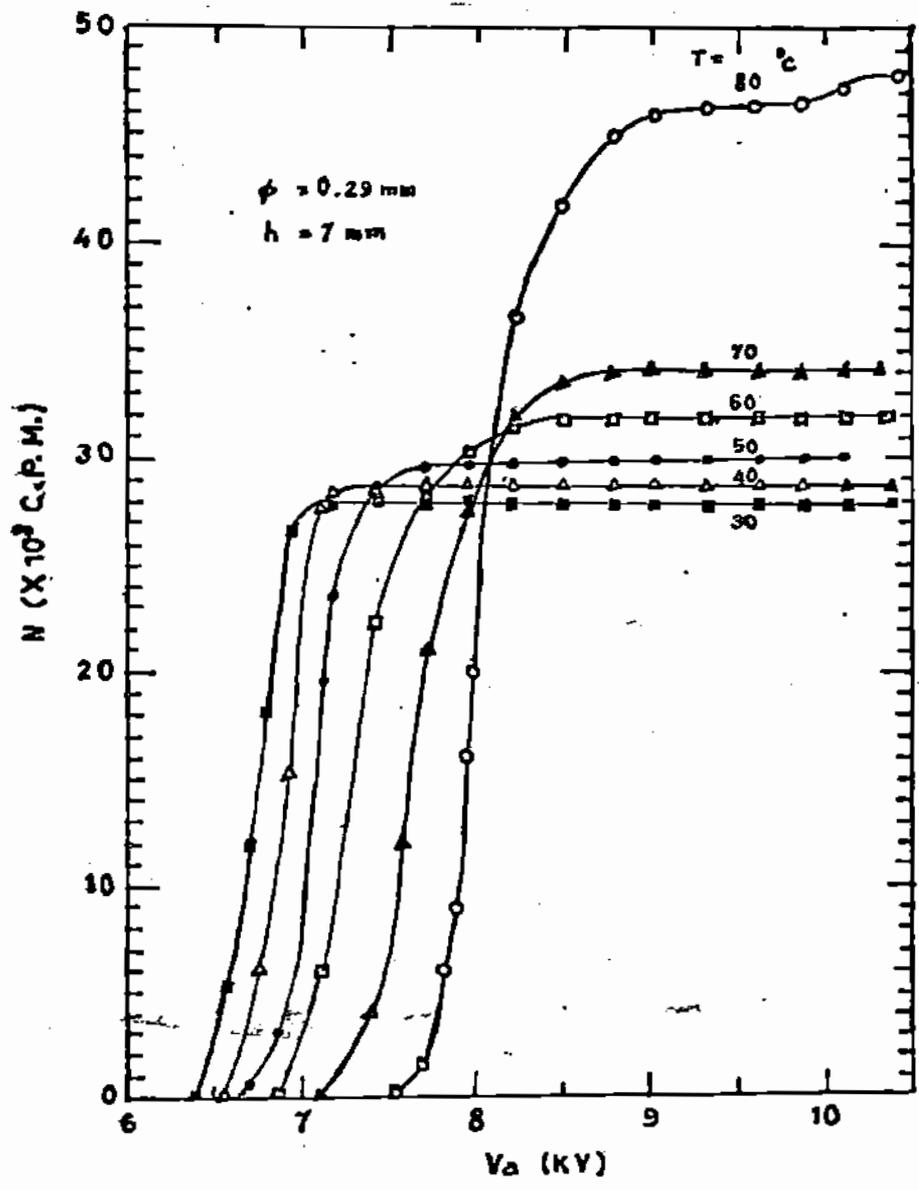


Fig. 6 The counting characteristics for different values of temperature.

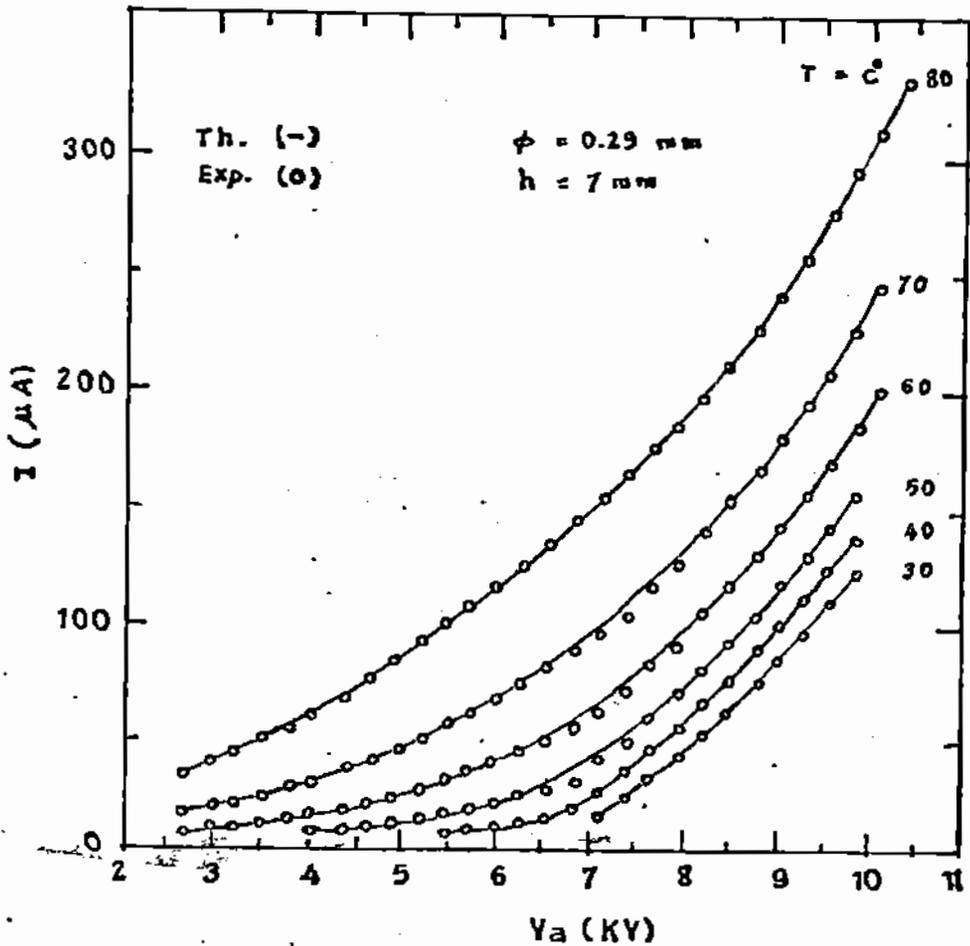


Fig. 7 Full curves represent I VS V_a from empirical formulas (—) represent experimental results, for various values of temperature.

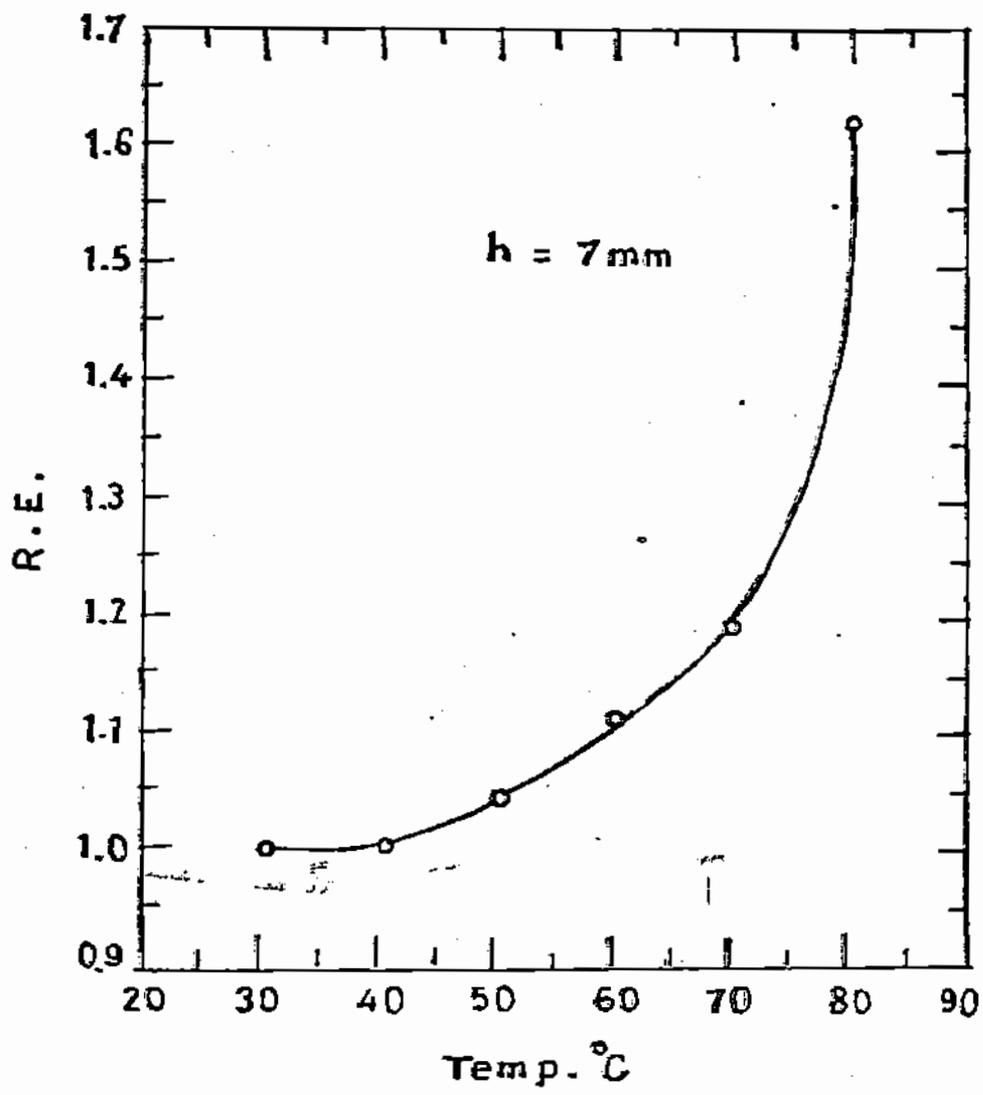


Fig. 8 Relative efficiency (R.E.) VS. temp.

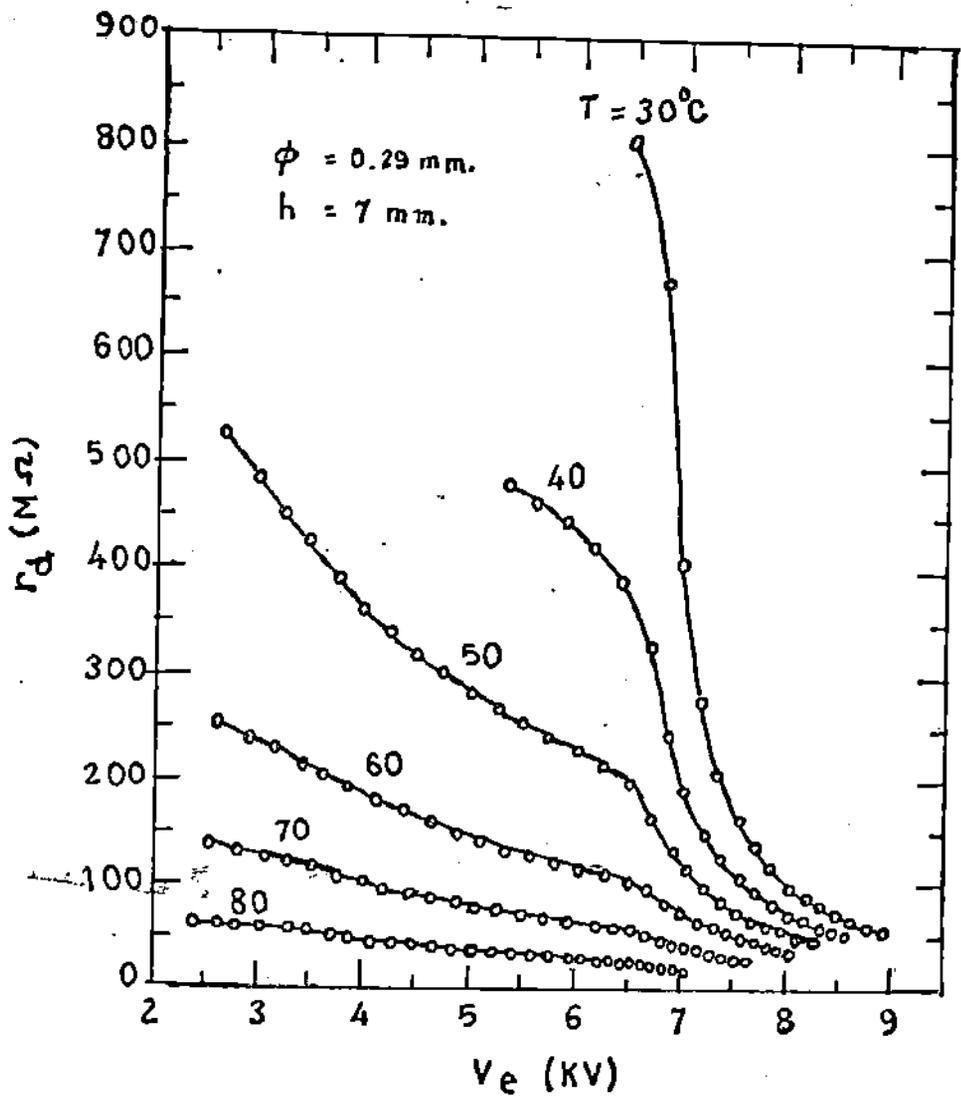


Fig. 9 r_d VS V_e for various values of temperature.

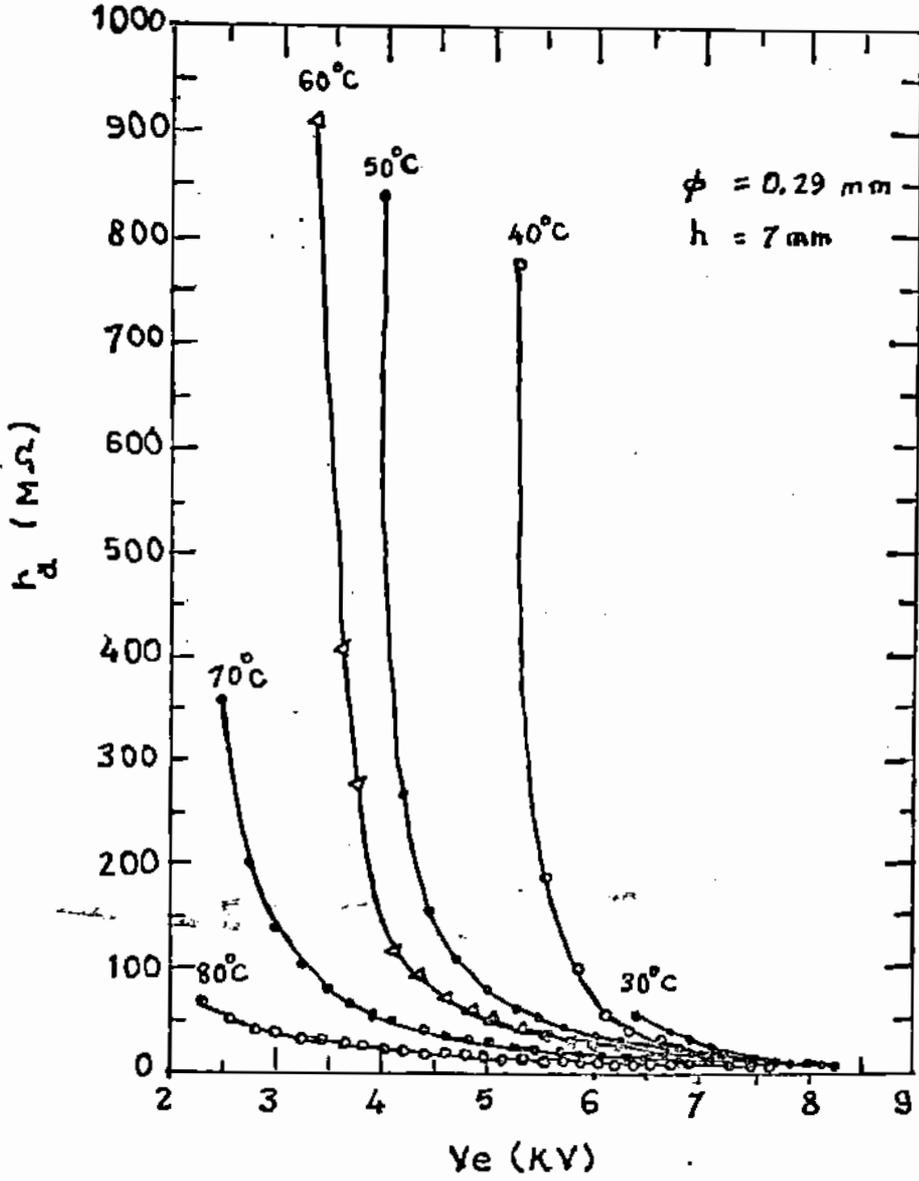


Fig-10 r_d VS V_e for different values of temperature.

CHARACTERISTICS OF AN ENERGY AND TIME ANALYSIS SYSTEM
USING A Ge(Li) AND A FAST SCINTILLATION DETECTORS

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Abstract

The characteristics, performances and capabilities of a system which can measure energies, intensities and lifetimes of gamma transitions in radioactive nuclei using a relatively large volume 76.1 cc Ge(Li) detector and a fast scintillation detector, was discussed. The system was used to measure the half-life of the 482 keV level in ^{181}Ta nucleus and was found to be 10.49 ± 0.09 ns. The obtained experimental transition probabilities of the different gamma transitions depopulating this level were compared with the theoretical estimates of the single particle model.

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1. Introduction

Scintillation detectors are still the fastest nuclear radiation detectors suitable for timing experiments. However, the excellent energy resolution of Ge(Li) detectors compared with scintillation detectors have encouraged many extensive¹⁻⁸⁾ work to study the timing properties of relatively small volume detectors (≤ 50 cc). Such extensive work concerning the timing properties of relatively high volume Ge(Li) detectors (≥ 50 cc) does not exist. In other words, the comparison of calculated time distribution with experimental time spectra⁹⁻¹³⁾ have not given complete agreement. In addition, relatively large high volume Ge(Li) detectors have better detection efficiency and thus they are more suitable for low activity measurements and detection of high energy gamma-rays.

The aim of this work is to investigate the characteristics, performances and capabilities of a system which can measure energies, intensities and lifetimes of gamma transitions in radioactive nuclei using a relatively large volume Ge(Li) detector (76.1 cc) and a fast scintillation detector. This system (together with a gamma-gamma coincidence system described before¹⁴⁾) is a part of a project in which the decay schemes of some radioactive nuclei will be studied¹⁵⁾. However, in this work also, the system was used to measure the half-life of the 482 keV level in ^{181}Ta nucleus. The obtained experimental transition probabilities of the different gamma transitions depopulating this level were compared with the theoretical estimates of the single particle model¹⁶⁾.

2. System Description

Fig. 1 shows a block diagram of the system. It consists of three distinct parts :

1) DETECTORS

— 103 —

The scintillation detectors constructed for use in this work, consists of a fast XP 1020 Philips photomultiplier tube and a suitable mechanical assembly which permit the housing of different crystals and the necessary electronics. The construction was made to allow the ability of using scintillators whose thicknesses may vary from few mm to about 60 mm. Considering the electronic part, a suitable voltage divider network with decoupling condensers with a chain current of 1.5×10^{-3} amp./ kilovolts and a focus controll was constructed. The fast negative output was taken directly from the anode of the photomultiplier while the linear positive output was taken from the ninth dynode. For this purpose, a low noise preamplifier having an emitter follower stage has been also constructed.

The Ge(Li) detector used was a coaxial ORTEC detector type VIP¹⁰ having a diameter of 4.45 cm and a height of 5.83 cm while its active volume is 76.1 cc. The preamplifier used was also an ORTEC 120B4 charge sensitive preamplifier mounted directly on the detector cryostat so that the input field effect transistor is kept at low temperature in order to provide good stability and low noise level.

ii) FAST LOGIC CHANNELS

Fast pulses from the anode of the photomultiplier are shaped by a constant fraction discriminator with an amplitude risetime compensation and then fed to the start input of a start-stop time to amplitude converter. Pulses from the timing output of the Ge(Li) detector preamplifier are amplified by a timing filter amplifier to optimize the signal to noise ratio, shaped by a second constant fraction discriminator and then fed to the stop input of the time to amplitude converter through suitable 50- Ω delay cables. One of the time to amplitude

converter outputs is fed to an Intertechnique 400 channel analyzer for time analysis while the other output is fed to the first input of a triple coincidence circuit through a suitable single channel analyzer and delay.

iii) SLOW LINEAR CHANNELS

The linear output from the photomultiplier was taken from the ninth dynode while that of the Ge(Li) detector was taken from the energy output of the detector preamplifier. Each of these outputs was amplified and then fed to a timing single channel analyzer. The outputs of these single channel analyzers are fed after suitable delays to the second and third inputs of the triple coincidence circuit whose output gates the multichannel analyzer for timing analysis.

Pulses from the Ge(Li) detector amplifier are fed to a biased amplifier which permit the division of the gamma-ray spectrum into several energy portions and expand each portion to cover the whole memory of a 512-LABEN multichannel analyzer and thus permit energy analysis down to 0.2 keV/channel. Each spectrum was thus recorded in series of overlapping segments. Both the detector amplifier and the biased amplifier were equipped with a base line restorer while a pile up rejector was present in the biased amplifier.

3. System Investigation

3.1) DETECTORS

a) Scintillation Detector :

The detector was found to have an energy resolution 12 % for the 661 keV line of ^{137}Cs source when using a 50 mm diameter x 50 mm

high NaI(Tl) crystal while the fast anode pulses were found to have a mean risetime ≈ 3 ns and a mean width at half maximum 200 ns when using 50 mm dia x 50 mm high NE102 plastic scintillator and a ^{137}Cs source .

b) Germanium Lithium Detector :

i) Energy Resolution

Although that this detector is of the high resolution type, yet, the best resolution obtained largely depends on the choice of the best working potential as well as the degree of matching and the way of coupling between the electronic equipments processing its signal and its output. Such conditions are specific for each detector and thus a systematic study of the dependence of the detector resolution on these conditions was performed. Regarding the dependence of the detector noise (preamplifier output) on the applied bias, the noise level at different bias voltages was measured by a 100 Mega cycles Tektronix oscilloscope model 465 after being amplified 40 times by an ORTEC low noise 47Z spectroscopy amplifier (Fig. 2a). At bias voltages greater than 500 volts, the noise level was largely reduced to about 250 microvolts owing to the decrease in the detector capacity. Upon increasing the bias, the detector noise remains nearly constant up to a bias voltage of the order of 3550 volts at which the detector noise began to increase again due to the increase in the leakage current through the detector. Therefore, we can conclude that the best working potential of the detector is ≈ 3400 volts at which, the detector has a minimum capacity and negligible leakage current.

However, the effect of the detector bias on the detector pulse height output was also measured using a ^{137}Cs source (fig. 2b) .

The pulse height output was analyzed on the 512-LABEN multichannel

analyzer and was found to be nearly constant (photo-peak position) over the range from ~ 2000 to 3500 volts above which the peak shape began to be distorted.

The energy resolution of the spectrometer (the full width at half maximum of the ^{photo}/peak) was then measured using a ^{60}Co source at different shaping time constants of the detector main amplifier ($0.5, 1, 2, 3$ and $6 \mu\text{s}$). In this investigation, a ^{60}Co source was put at about 25 cm distance from the detector and the integral counting rate was of the order of 1000 c/s. From this investigation, (fig. 3) a minimum resolution of 2.3 keV for the 1332 keV line in ^{60}Co was obtained for shaping time constant $\geq 3 \mu\text{s}$. Since large shaping time constants are not recommended for higher counting rates, a $3 \mu\text{s}$ shaping time constant was considered to be the most suitable for our detector. At this condition a photopeak to Compton ratio of $32/1$ was also obtained for the same ^{60}Co source.

Concerning the way of coupling between the detector main amplifier and the biased amplifier together with the effect of base line restoration in both amplifiers, the best settings to get the best resolution could only be done by experimental empirical means since there are many undefined variables for each particular system. For this reason, the energy resolution of the spectrometer (at $E_{\gamma} \approx 1332$ keV) was investigated at counting rates ranging from $\approx 3 \times 10^2$ c/s up to $\approx 3 \times 10^4$ c/s at three different levels of the discriminator used for base line restoration of the main detector amplifier (low, medium, and high) and at three different input circuit selection modes of the biased amplifier (D. C. coupled, A. C. coupled with low or high base line restoration). Fig. 4 shows the dependence of the spectrometer energy resolution for different settings. It is clear that as long

as the counting rate is $\leq 5 \times 10^3$ c/s, the base line restorer discriminator set at a medium level and a D. C. coupling between the main amplifier and the biased amplifier seems to be the most suitable settings. However, at counting rates $> 5 \times 10^3$ c/s, a high setting of the base line discriminator restorer level of the main amplifier and A. C. coupling with high level base line restoration of the biased amplifier are necessary to keep reasonable energy resolution. Otherwise a drastic deterioration of the spectrometer resolution is noticed.

In order to obtain the intrinsic resolution of the detector and the contributions of the electronic noise to the spectrometer resolution, the dependence of the spectrometer resolution on different gamma ray energies ranging from 150 to 2750 keV was studied. In this investigation, we have used the well known gamma-ray transitions in ^{24}Na , ^{131}Ba , ^{152}Eu , ^{182}Ta and ^{226}Ra sources. Fig. 5 shows a plot of the linear variations of the spectrometer resolution with the square root of the gamma ray energy. From this figure and using the least square fitting method, the following empirical formula was constructed to express the spectrometer resolution as a function of gamma-ray energy,

$$E \text{ (keV)} = (0.05 \pm 0.002)\sqrt{E} + (0.64 \pm 0.05)$$

From this deduced formula the intrinsic resolution of the detector was found to be $(0.05 \pm 0.002)\sqrt{E}$, while the contribution of the electronic noise was (0.6 ± 0.05) keV.

Comparing our experimental value of the intrinsic detector resolution to the theoretical value given by ¹⁷⁾ $2.35\sqrt{\omega F E_{\gamma}}$, where ω is the energy needed to produce one electron hole pair (2.8 eV) and F is the Fano factor, the Fano factor of our detector was found to be 0.16

ii) Detector Photopeak Efficiency

The detector efficiency of the spectrometer by the photoelectric effect varies rapidly with the energy of gamma-rays. However, in most of the experiments in nuclear spectroscopy, only the relative intensities of different gamma transitions emitted by radioactive nuclei are needed. Therefore, only the relative rather than the absolute photopeak detection efficiency of the spectrometer is all that required.

Since the shape of the photopeak efficiency curve of the detector was found to depend on the source to detector distance¹⁸⁾ and the counting rate¹⁹⁾, we have measured the relative photopeak efficiency of our detector at different gamma-ray energies in the energy range from 180 to 2400 keV. Using the well known gamma-ray transition intensities in ^{131}Ba , ^{152}Eu , ^{182}Ta and ^{226}Ra sources at a source to detector distance = 25 cm and at counting rate = 1000 c/s. In this investigation, the gains of both the detector main amplifier and the biased amplifier were adjusted in such a way to be able to expand part of the gamma spectrum on the analyzer to obtain ≈ 0.5 keV/channel. The complete spectrum of each source was thus obtained by taking a series of overlapped portions (from 6 to 8 portions) of the spectrum. Each portion was analyzed using the 512 multichannel analyzer and then normalized to each other to obtain the complete spectrum. The relative photopeak efficiency curves obtained for different radioactive sources overlap each other at a certain range of energy and thus could be normalized to each other to obtain the relative photopeak efficiency of the detector as a function of gamma-ray energy as shown in (fig. 6.) Taking into considerations, the statistical errors and the errors involved (average of 4 complete measurements of each spectrum) in

the intensities of the gamma-ray transitions used in this investigation, the errors in determining the values of the relative photopeak efficiency of the detector was found to be of the order of 4-5 % .

3.2 TIME RESOLUTION

When Ge(Li) detectors are applied for lifetime measurements by the delayed coincidence techniques, one can obtain a time resolution of the order of nanoseconds. However, the most important source of time spread is the dependence of the detector pulse rise time on the place of the interaction of gamma-rays inside the detector. For relatively high volume detectors (good detection efficiency), this effect will certainly deteriorate the time resolution of the system. However, in the present work the use of the constant fraction pulse height triggers largely compensate the effects of walk and rise time variation . In this work, the time resolution of the system was measured by performing delayed coincidences between the 1174 keV and the 1332 keV gamma lines populating and depopulating the 1332 keV level in ^{60}Co source . The 1332 keV line was selected in the scintillation detector channel while the 1174 keV line was selected in the Ge(Li) detector channel. Fig.7-a shows the prompt resolution curve obtained when the 1332 keV line was detected by a 50 mm diameter x 50 mm high NaI(Tl) crystal. In this case, the time resolution curve obtained was found to have a full width at half maximum = 2.61 ± 0.1 ns , a full width at tenth of the maximum = 6.71 ± 0.1 ns while slopes of 1.03 ± 0.06 and 0.64 ± 0.06 ns for the two exponential decays of the prompt curve were obtained. When the 1332 keV line was detected by a 50 mm diameter x 50 mm high NE 102 plastic scintillator, the prompt resolution curve obtained (fig.7-b) was found to have a full width at half maximum = 2.42 ± 0.1 ns , a full width at tenth of the maximum

$= 6.11 \pm 0.1$ ns, while the slopes of the two exponential decays of the prompt curve were found to be 0.88 ± 0.06 and 0.70 ± 0.06 ns.

4. Lifetime of The 482 keV Level in ^{181}Ta

The half-life of the 482 keV level in ^{181}Ta has been measured by several authors using several techniques²⁰⁻²⁴). However, no measurements of the lifetime of such level using Ge(Li) detectors have been reported before. Therefore, we have found it useful to measure the half-life of this level using our Ge(Li) detector-scintillation detector timing system.

i) SOURCE PREPARATION

The excited levels of ^{181}Ta is obtained from the decay of ^{181}Hf to ^{181}Ta . The ^{181}Hf source was obtained by irradiating a sample of natural Hf oxide in the RAER at Inshass for a period of 48 hours at a neutron flux of the order of 10^{12} n/cm²/s. Since natural hafnium contains a mixture of ^{174}Hf (0.163%), ^{176}Hf (5.21%), ^{177}Hf (18.56%), ^{178}Hf (27.1%), ^{179}Hf (13.75%) and ^{180}Hf (35.22%), the obtained activity only contained, ^{175}Hf ($T_{1/2} = 70$ days), $^{180\text{m}}\text{Hf}$ ($T_{1/2} = 5.5$ hours) and ^{181}Hf ($T_{1/2} = 42.5$ days). The source was used 20 days after irradiation and thus the main activity were due to ^{175}Hf and ^{181}Hf sources.

ii) MEASUREMENTS

Fig. 8 shows a partial decay scheme of the ^{181}Hf nucleus²⁶) in which only the most intense transitions are indicated.

In order to measure the lifetime of the 482 keV level in ^{181}Ta , we have measured the single gamma-ray spectrum using both Ge(Li) detector and NaI(Tl) scintillation detector. According to the partial level scheme of ^{181}Ta and the single gamma-ray spectra obtained, the lifetime of the 482 keV level in ^{181}Ta was measured in two ways.

First, we performed delayed coincidences between the 133 keV

gamma-ray transition populating this level in the start channel (selected with the help of NaI(Tl) detector) and the 482 keV gamma-ray transition depopulating this level in the stop channel (selected with the help of Ge(Li) detector). The time spectrum thus obtained is given in figure-9 . The value obtained for the half-life of the 482 keV level in ^{181}Ta is 10.38 ± 0.12 ns . This value was deduced after the subtraction of the chance coincidences obtained with a prompt spectrum using ^{22}Na source under the same experimental condition. The data were least-squares fitted .

Secondly, we have performed delayed coincidences between the 133 keV gamma-ray transition populating this level in the start channel (selected with the help of NaI(Tl) detector) and the 345 keV gamma-ray transitions depopulating this level in the stop channel (selected with the help of Ge(Li) detector). The time distribution spectrum obtained is given in fig. 10 . The prompt contribution observed in this spectrum was due to the presence (in the stop channel) of part of the 343.4 keV transition depopulating the fast 343.4 keV level in the $^{175}\text{Hf} \rightarrow ^{175}\text{Lu}$ decay . In fact, this contribution could be reduced by choosing narrower window in the slow Ge(Li) detector channel. This solution was not used since narrower window will cause the elimination of part of the gamma-rays depopulating the 482 keV level under investigation and a reduction in the delayed coincidence counting rate will be obtained. From the obtained spectrum, the value deduced for the half-life of the 482 keV level in ^{181}Ta is 10.50 ± 0.13 ns. This value was deduced by least-squares fitting after the subtraction of the chance coincidences obtained with the prompt spectrum of a ^{22}Na source, under the same energy settings.

From the results obtained from these two measurements, no significant differences in the values of the measured half-lives are noticed .

Taking into consideration the statistical and systematic errors due to time calibration and electronic instability of the apparatus, a mean value for the half-life of the 482 keV level deduced from the slopes of the time distribution curves was found to be ,

$$T_{\frac{1}{2}} (482 \text{ keV level}) = 10.49 \pm 0.09 \text{ ns}$$

5. Discussion

The investigation done in this work have permitted us to study in details the characteristics and performance of the described system. Such study is very useful in order to obtain the best operating conditions as well as the maximum capabilities of the system. The system described here can be used to measure accurately gamma-ray energies with a resolution ranging from ≈ 1 keV at gamma-ray energy of the order of 100 keV to a resolution ≈ 2 keV at gamma-ray energy of the order of 1 MeV. In addition the relative gamma transitions could be also obtained in the energy range from ≈ 60 to ≈ 2400 keV. However, below ≈ 200 keV the detection efficiency decreases rapidly due to the absorption of gamma radiations in the dead layer of the detector and in its packing materials. Concerning the time resolution of the system, the prompt resolution curves obtained showed that lifetimes in the nano-second range could be easily measured by relatively large volume Ge(Li) detectors. In addition, the value of the half-life of the 482 keV level in ^{181}Ta nucleus obtained in this work 10.49 ± 0.09 ns is in excellent agreement with the most recent value (10.81 ± 0.05) ns obtained by Lowe et al. ²⁴⁾ in 1973 and with the values (10.56 ± 0.15) , (10.40 ± 0.2) , (11.0 ± 0.2) ns and (11.02 ± 0.35) ns reported by references 20, 23 respectively. However, using the theoretical values of the internal conversion coefficients tabulated by Sliv and Band ²⁵⁾ and the branching ratios of the different gamma-ray lines depopulating this level,

the experimental partial gamma-ray half lives $T_{\frac{1}{2}\text{exp}}$ for all transitions have been calculated using the formula ,

$$T_{\frac{1}{2}\text{exp}} = \frac{T_{\frac{1}{2}\text{obs.}} (1 + \alpha)}{\alpha}$$

These values are compared with the theoretical single particle Weisskopf estimates¹⁶⁾ given by ,

$$T_{\frac{1}{2}W} (M1) = 2.24 E_{\gamma}^{-3} 10^{-5} \text{ ns ,}$$

$$T_{\frac{1}{2}W} (E2) = 9.37 A^{-4/3} E_{\gamma}^{-5} \text{ ns and}$$

$$T_{\frac{1}{2}W} (M2) = 11.9 A^{-2/3} E_{\gamma}^{-5} \text{ ns.}$$

where E_{γ} is the gamma-ray transition energy in MeV.

To obtain the Weisskopf estimate a nuclear radius constant of 1.2 fm and a statistical factor $S = 1$ were used¹⁶⁾.

Table 1 shows the hindrance factors calculated relative to the theoretical single-particle Weisskopf estimate of the partial half-lives. The retardation factors for the 482 keV and the 345 keV E2 transitions are similar. It is difficult to understand why the 482 keV M1 transition is more than 10^3 times more retarded than is the other 476 keV M2 transition.

Table 1

Hindrance factor for gamma-ray transitions of the 482 keV level in ^{181}Ta nucleus

E_{γ} transition (KeV)	Multipolarity	$T_{\frac{1}{2}\gamma}^{\text{exp}}$ (ns)	$T_{\frac{1}{2}\gamma}^{\text{exp}} / T_{\frac{1}{2}W}$
482	E 2	12.80	36.57
	2 % M 1	13.25	6.62×10^4
476	pure M2	624.60	16.02
345	pure E2	77.39	42.99

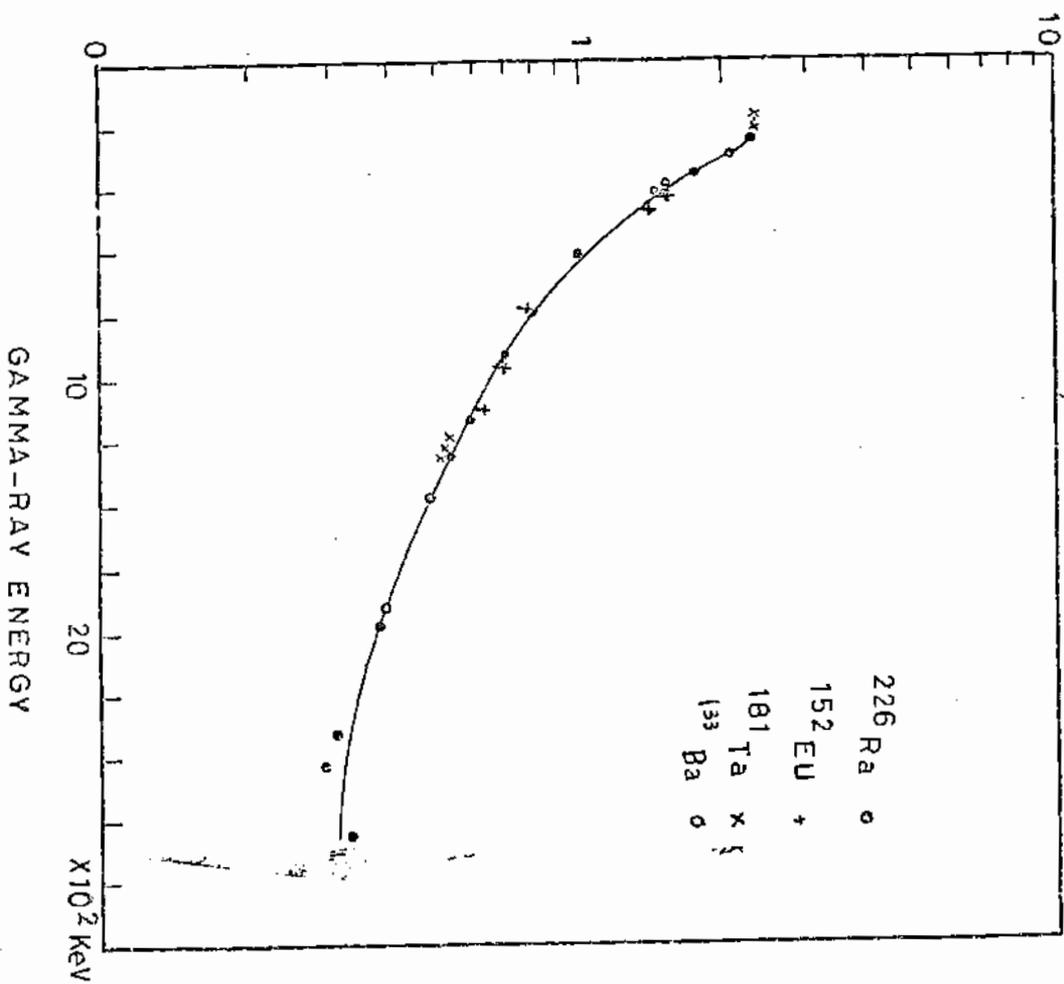
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RELATIVE EFFICIENCY



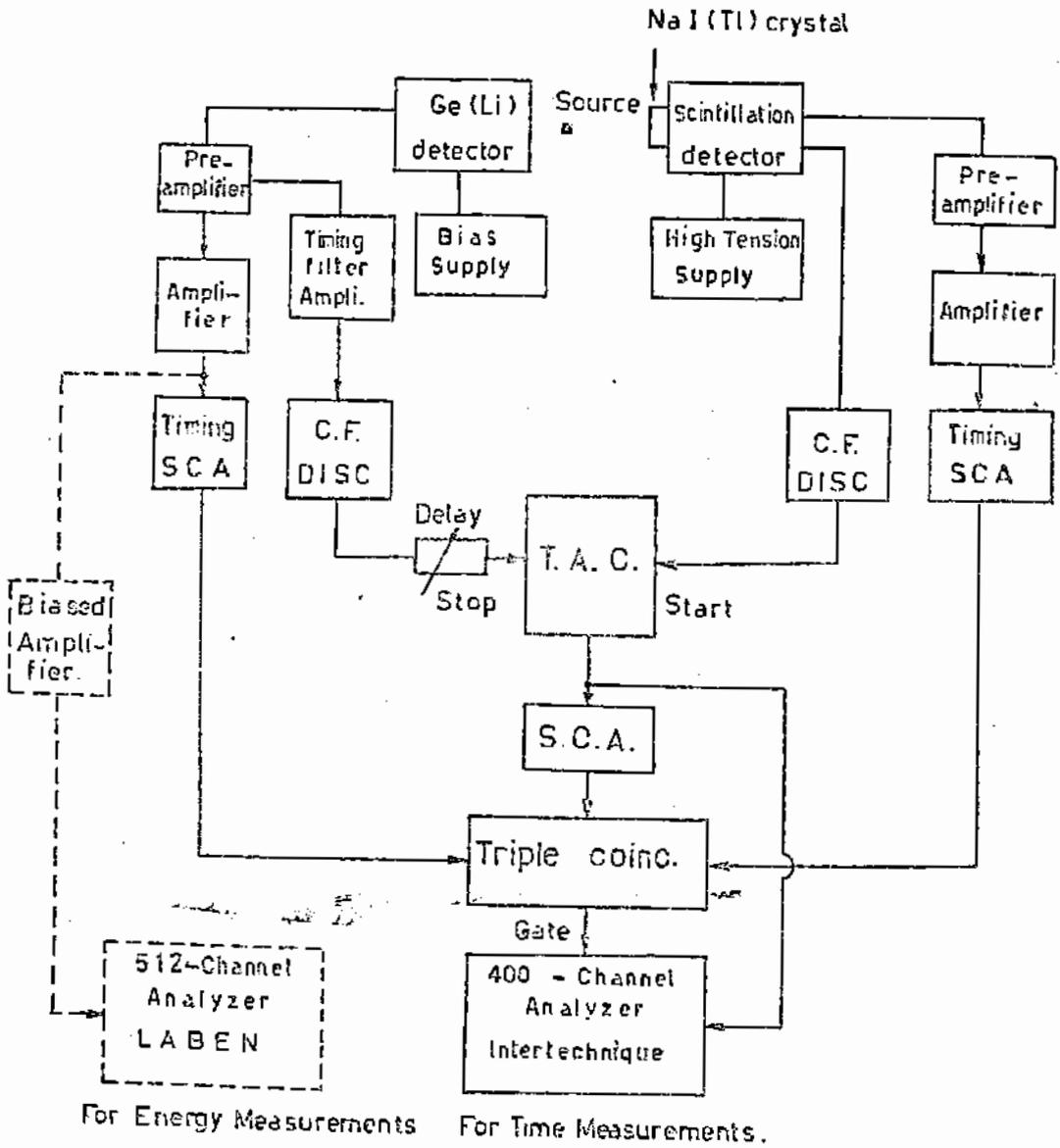


Fig. 1

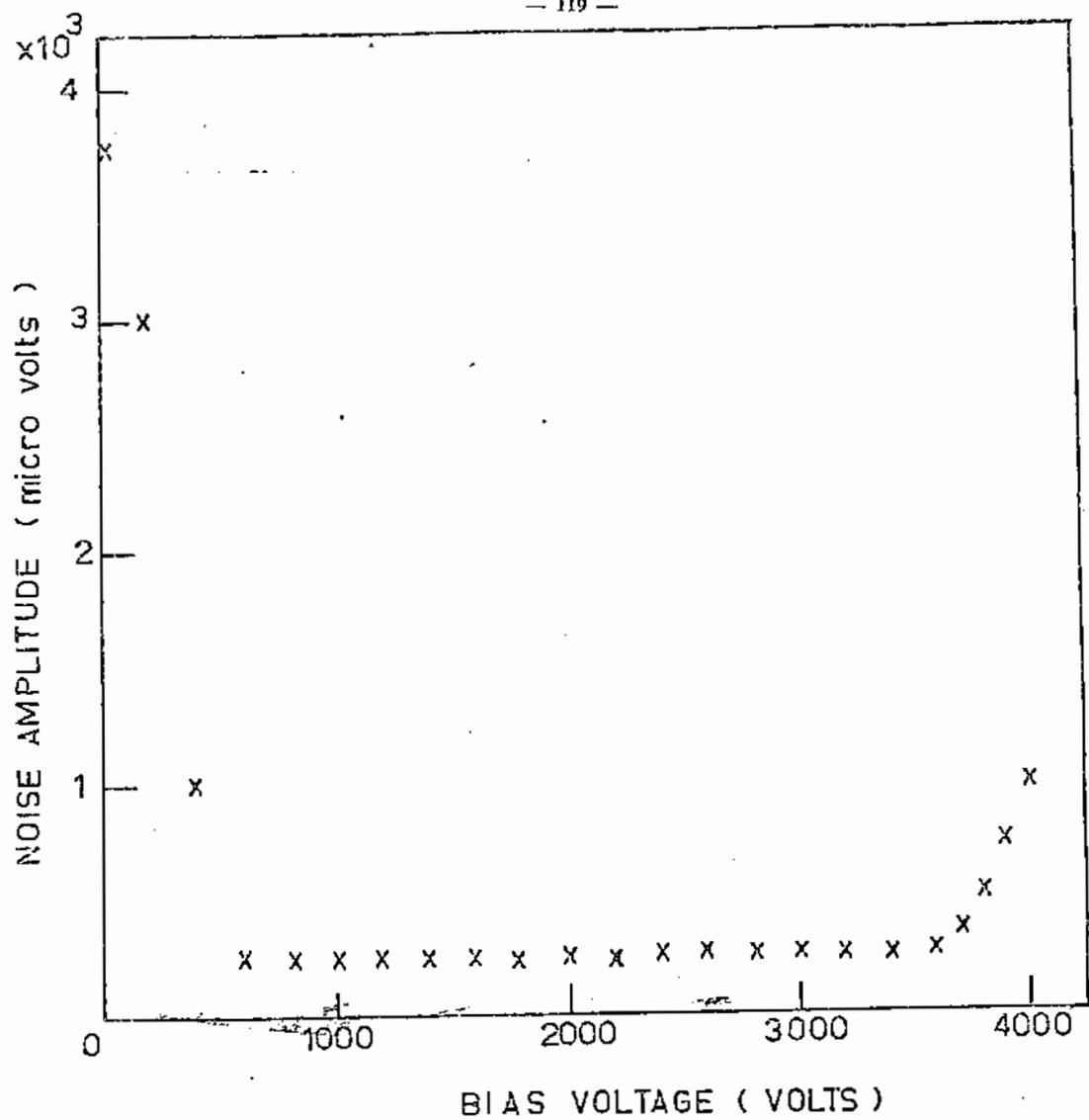
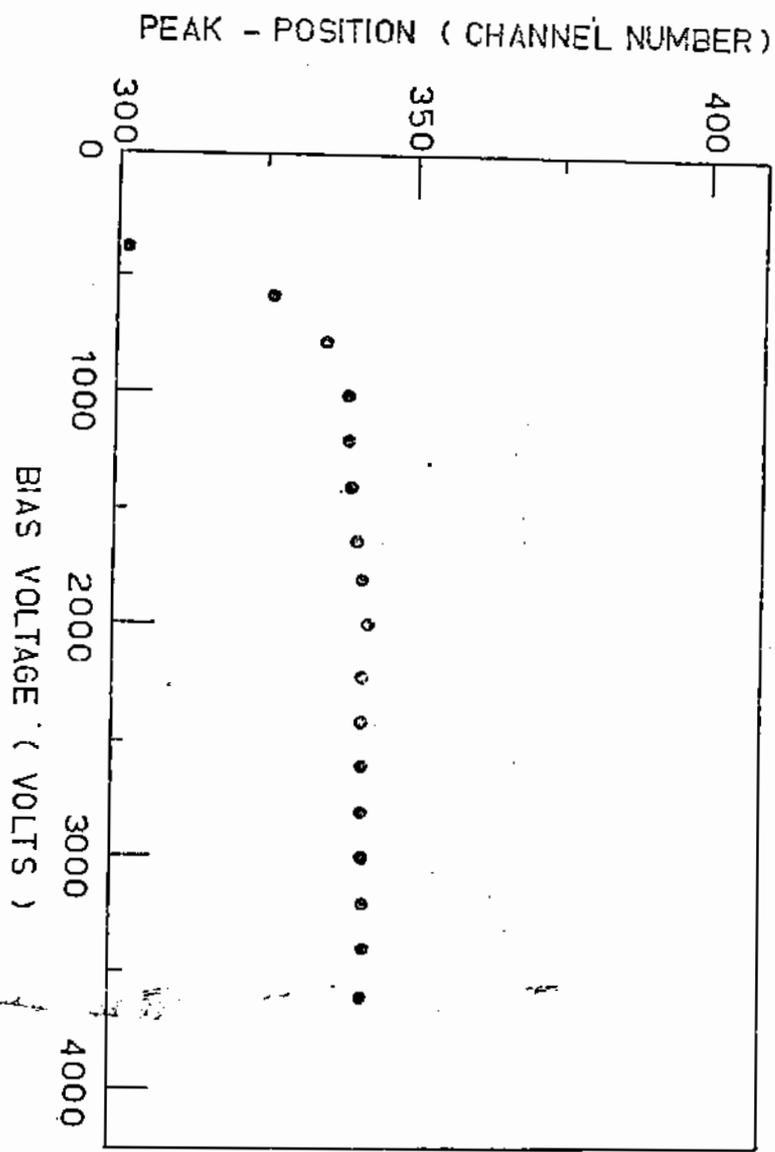
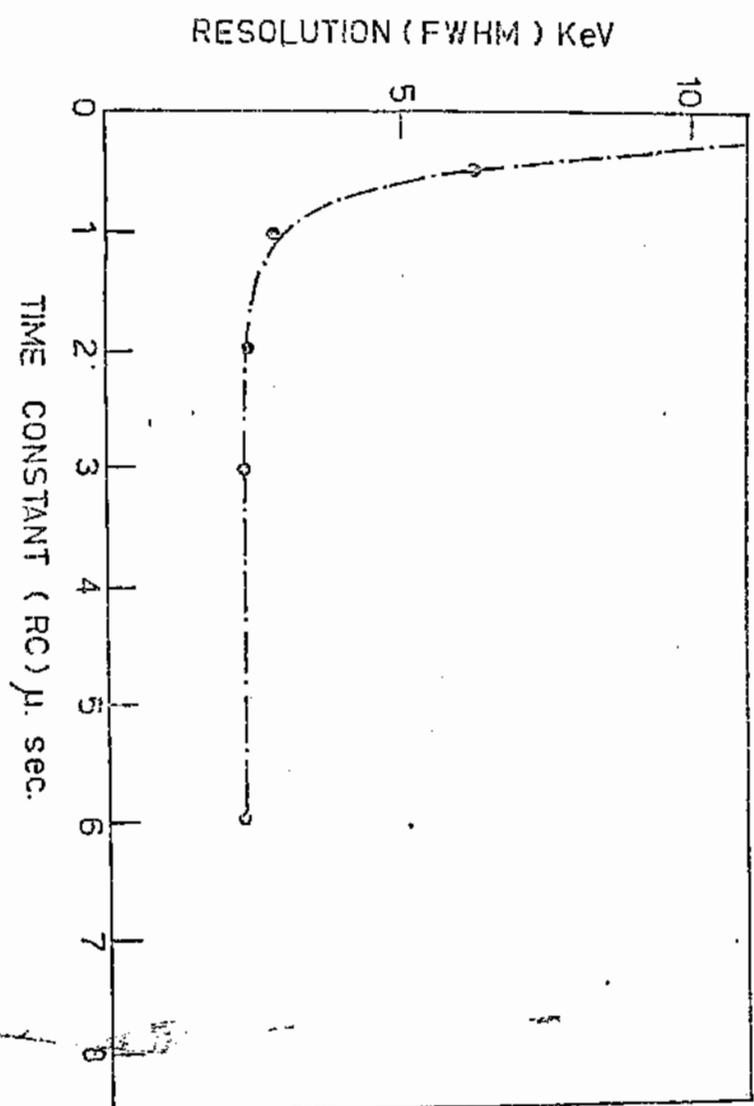


Fig. 2c



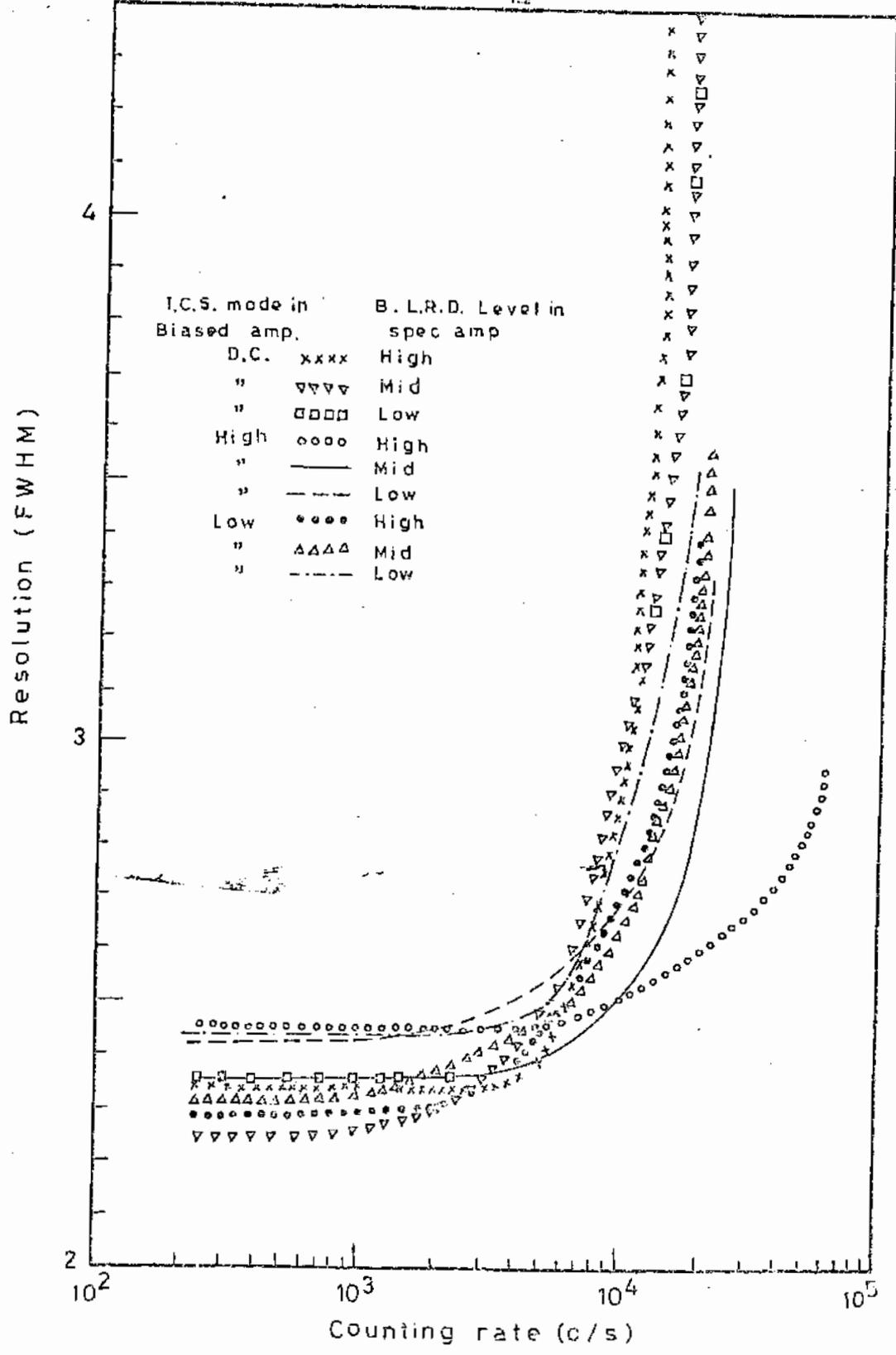
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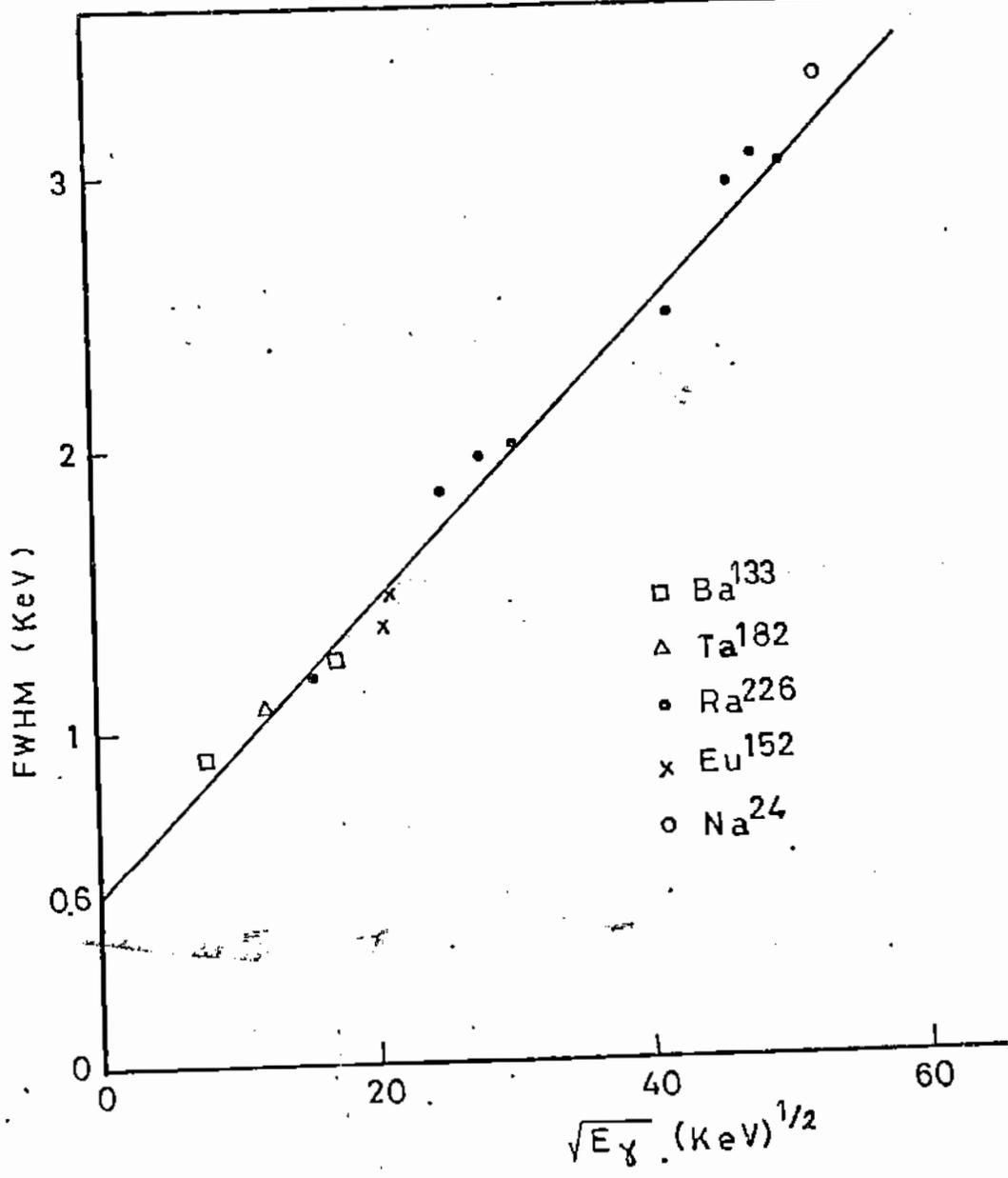
Fig. 2 (B)



- 121 -

Fig. 3





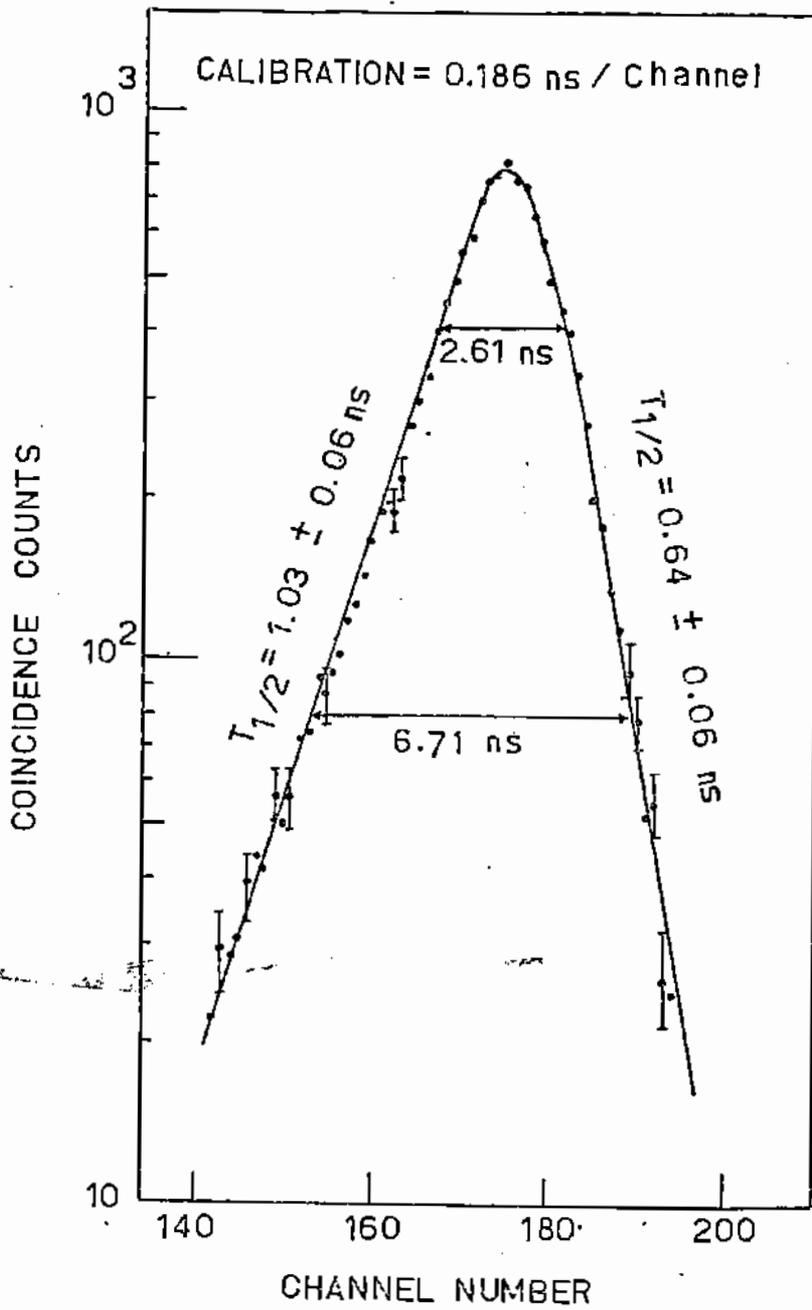


Fig. 7(a)

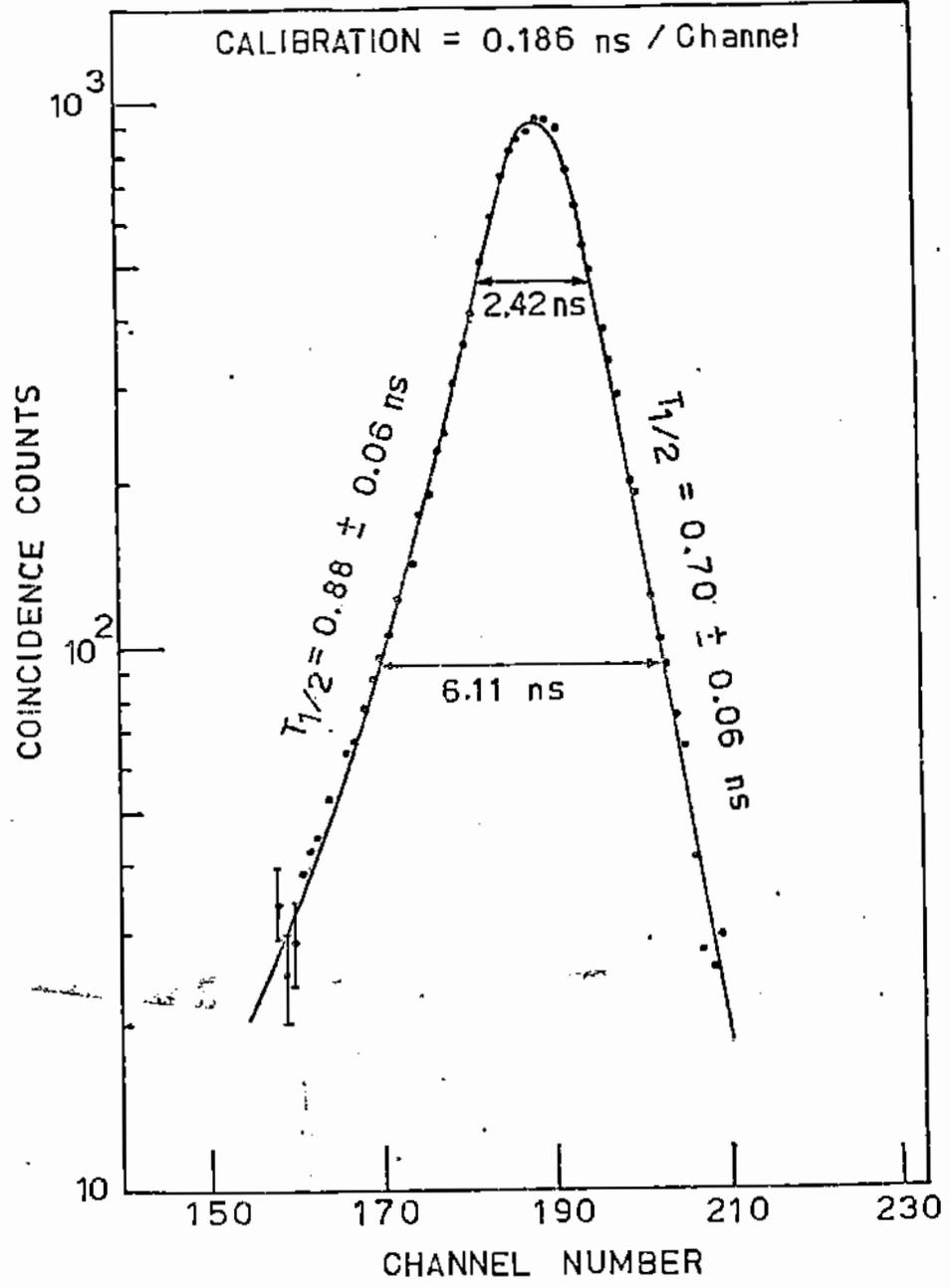


Fig. 7

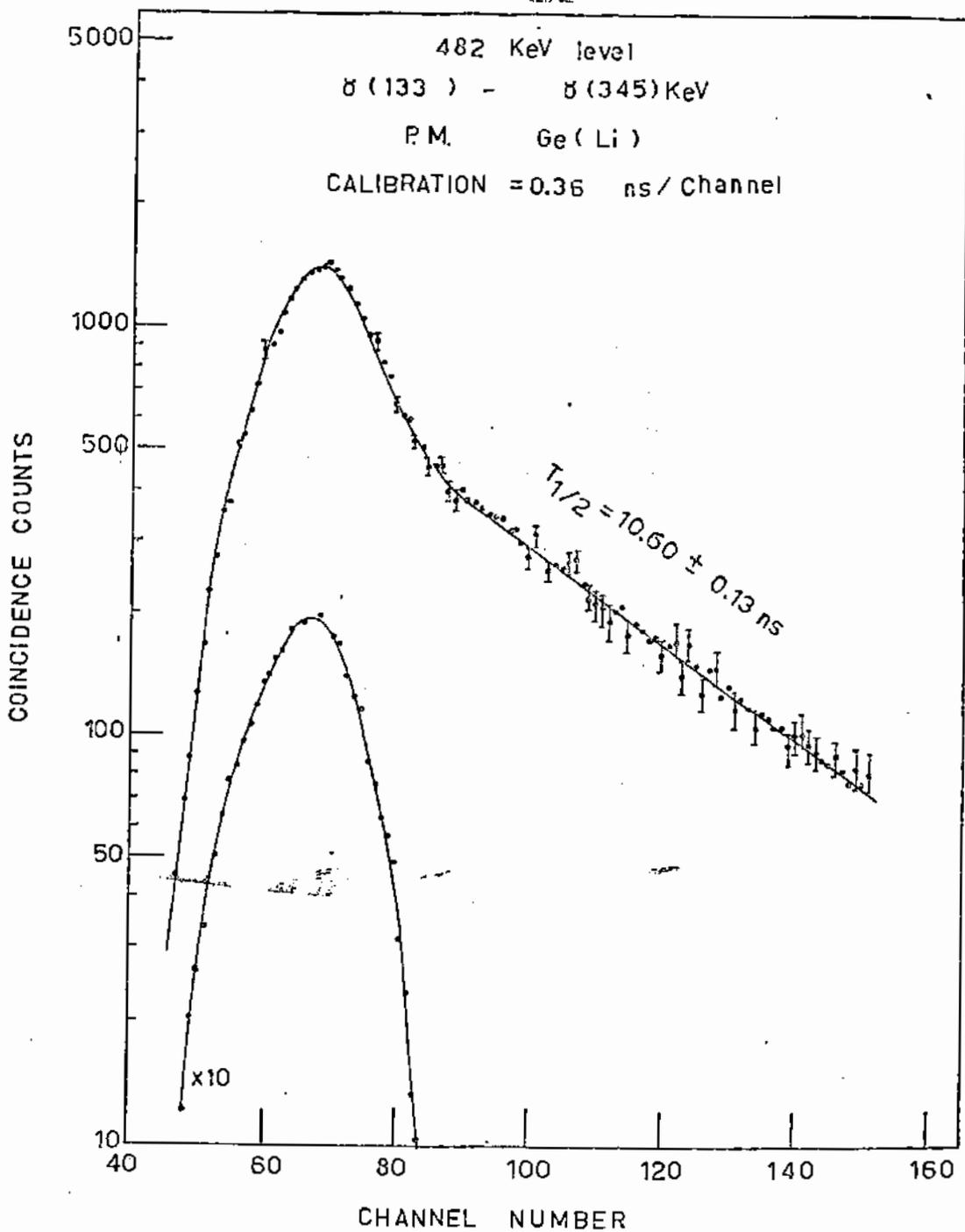


Fig. 3

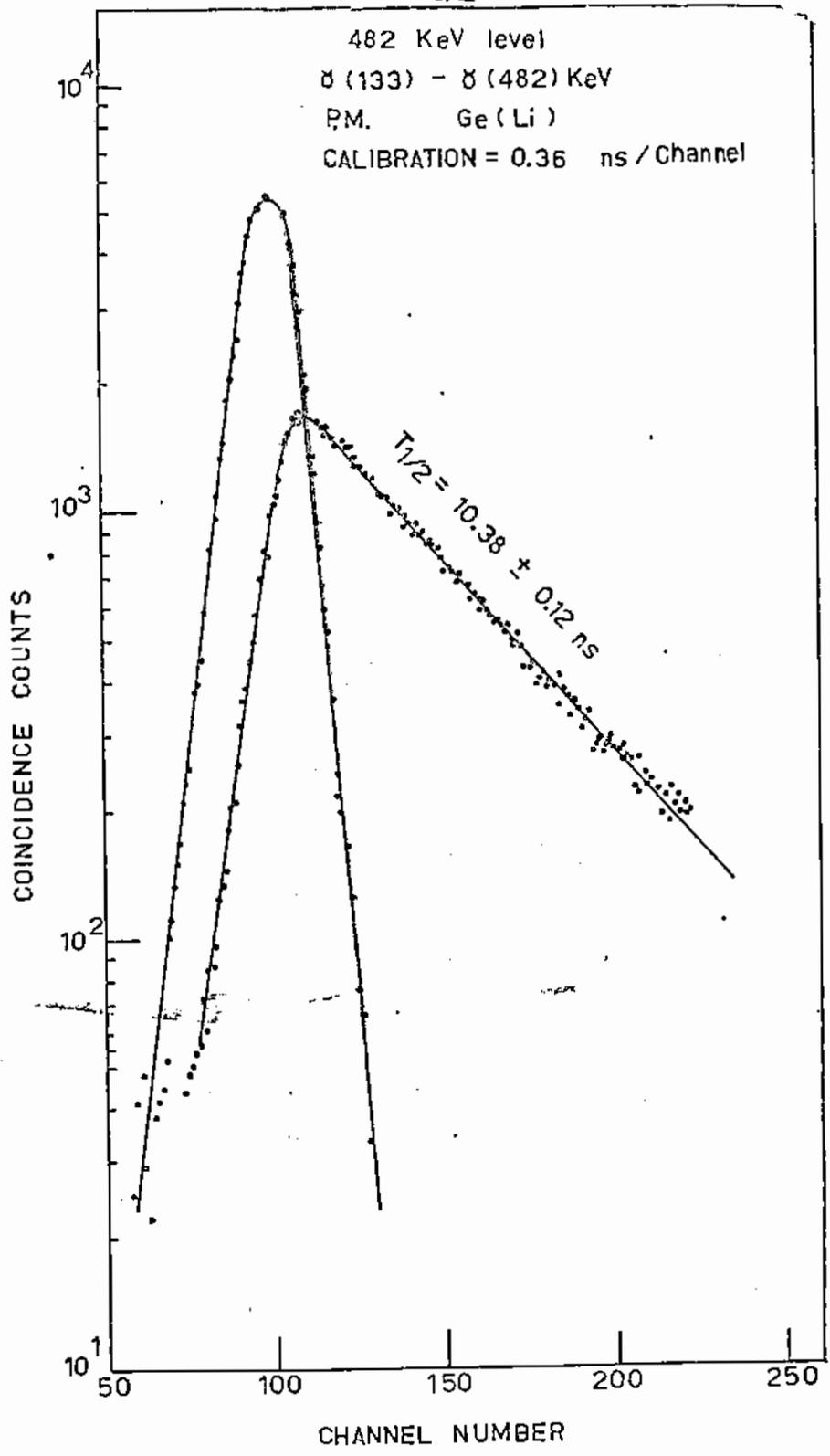


Fig. 8

Figure Captions

- Fig. 1 : Block diagram of the system.
- Fig. 2 a) The dependence of the detector noise (pre-amplifier output) on the applied bias at different bias voltages.
- b) The effect of the detector bias on the detector pulse height output using a ^{137}Cs source.
- Fig. 3 : Gamma-ray energy resolution of the Ge (Li) spectrometer as a function of amplifier shaping time constant
- Fig. 4 : Effect of input circuit selection (I.C.S.) mode in spectroscopy amplifier and base line restoration discriminator level (B.L.R.D.) in biased amplifier on energy resolution.
- Fig. 5 : Linear variations of the spectrometer energy resolution with the square root of the gamma-ray energy.
- Fig. 6 : Relative photo peak efficiency for different gamma-ray energies of the Ge (Li) detector.
- Fig. 7 : Prompt time resolution curve for the 1332 keV level of ^{60}Co .
- a) detected by 50 mm dia x 50 mm high NaI (Tl) scintillation detector in the start channel and Ge(Li) detector in the stop channel,
- b) detected by 50 mm dia x 50 mm height NE 102 plastic scintillation detector in the start ^{channel} and a Ge(Li) detector in the stop channel.
- Fig. 8 : A partial decay scheme of the ^{181}Hf nucleus (Ref. 26).
- Fig. 9 : Delayed time distribution coincidence spectrum for the 482 keV level in ^{181}Ta , (133 keV) gamma-ray line in the start channel and (482 keV) gamma-ray line in the stop channel.
- Fig. 10 : Delayed time distribution coincidence spectrum for the 482 keV in ^{181}Ta (133 keV) gamma-ray line in the start channel and (345 keV) gamma-ray line in the stop channel.

EFFECT OF POLYAMINO POLYCARBOXYLIC ACIDS ON
THE CORROSION OF ALUMINIUM IN ALKALINE MEDIUM

BY

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ABSTRACT

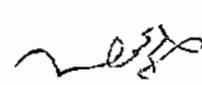
The corrosion rate of aluminium, in 0.15 N NaOH solution, in presence of some polyamino polycarboxylic acids (chelating agents) was studied by weight loss techniques. The corrosion rate of aluminium was found to be initiated in presence of such chelating agents. The dissolution of aluminium increased as the donor atoms increased. A proposed mechanism was given to explain this phenomenon.

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INTRODUCTION

The inhibition of the corrosion of some metals such as aluminium and zinc, in aqueous solutions of alkalis, whose hydroxides are amphoteric have been thoroughly investigated^{1,2}. The behaviour of the inhibitors in this case is strictly related to the solubility of the surface oxides or hydroxides at various pHs. The action of the inhibitors is explained by assuming that they have an effect in repairing the pores in oxide and hydroxide films. The inhibitors can no longer exercise any effective action in a clearly alkaline medium in which the oxides and hydroxides of aluminium and zinc are soluble.

The efficiency of inhibitors depends on their molecular structures³, their molecular area⁴ and their molecular weights^{5,6}. Therefore, it is of great interest to study the effect of some polyamino polycarboxylic acids having different functional groups as well as various molecular weights on the corrosion of aluminium in alkaline medium. The polyamino polycarboxylic acids used in the present investigation are: ammonia triacetic acid (TTN), ethylene glycol - bis - (β -amino ethyl ether) - N,N - tetra acetic acid (EGTA), ethylene diamine tetraacetic acid (EDTA) and diethylene triamine pentaacetic acid (DTPA).



EXPERIMENTAL

Polyamino polycarboxylic acids (TTN, EGTA, EDTA and DTPA) were of the highest purity available. Their structures and dissociation constant values⁷ are given in Table 1. The concentration of the chelating agent studied varied between 0.38×10^{-4} and 0.54×10^{-2} mole. Aluminium sheets of spectroscopic purity (Johnson and Matthey, London) were used in these investigations. The metallic sheets had an apparent surface area of 100 cm^2 each. Determination of the corrosion rate was performed by weight loss techniques. Measurements were made in 0.15 N sodium hydroxide free carbonates at $32 \pm 0.5^\circ$ using bidistilled water. The results were obtained in duplicate and no significant discrepancies were observed.

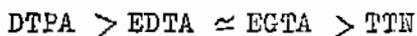
RESULTS AND DISCUSSION

Figs. 1,2,3 and 4 show the corrosion rate of aluminium at different concentrations of TTN, EGTA, EDTA and DTPA in 0.15 N sodium hydroxide solution, respectively. It was observed that corrosion of aluminium increases in presence of polyamino polycarboxylic acids. Also as the concentration of the chelating agent increases the corrosion rate increases. These results indicate that the studied chelating agents act as accelerators for the corrosion of aluminium in alkaline medium.

Polyamino polycarboxylic acids form complexes with metal ions such as aluminium, iron, cobalt, nickel and copper^{8,9}. The formation of such complexes depends on the acidity as well as the alkalinity of the medium. The normal complex is stable in the pH range 4—10. At pH >10 hydroxo complex is formed, while at pH < 3.5 protonated complex is formed which decomposes at pH < 1.5 to free metal and chelating agent. Since the experimental work was done at pH >10, it may be expected that hydroxo aluminium complex is formed at such medium.

Therefore, one can suggest the effect of the studied polyamino polycarboxylic acids on the alkaline corrosion of aluminium. The hydroxyl ions react with aluminium forming sodium aluminate. Simultaneously, polyamino polycarboxylic acids react with the aluminium surface forming hydroxo complex. This leads to an increase in the corrosion rate of aluminium.

Also, it was experimentally observed that, the corrosion rate of aluminium in alkaline medium increases in the order:



This can be explained by the increasing number of the donor atoms of the chelating agents which increase the probability of forming the soluble hydroxo compound.

It can be concluded that TTN, EGTA, EDTA and DTPA act as accelerators for the alkaline corrosion of aluminium.

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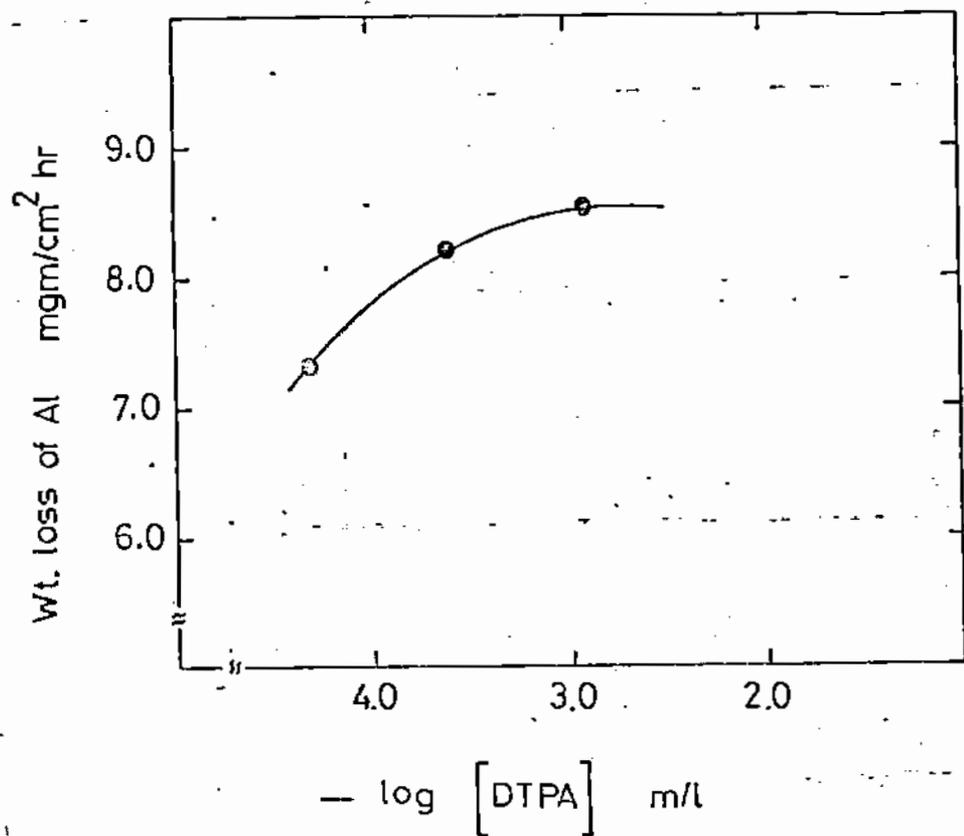


Fig. 4

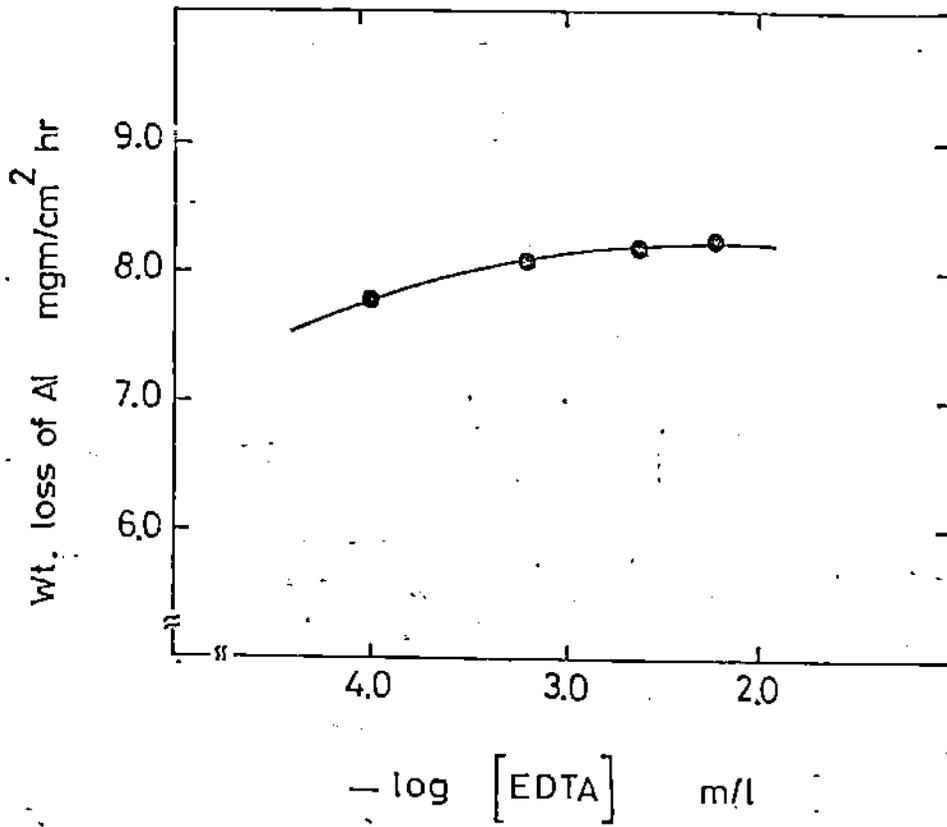


Fig. 3

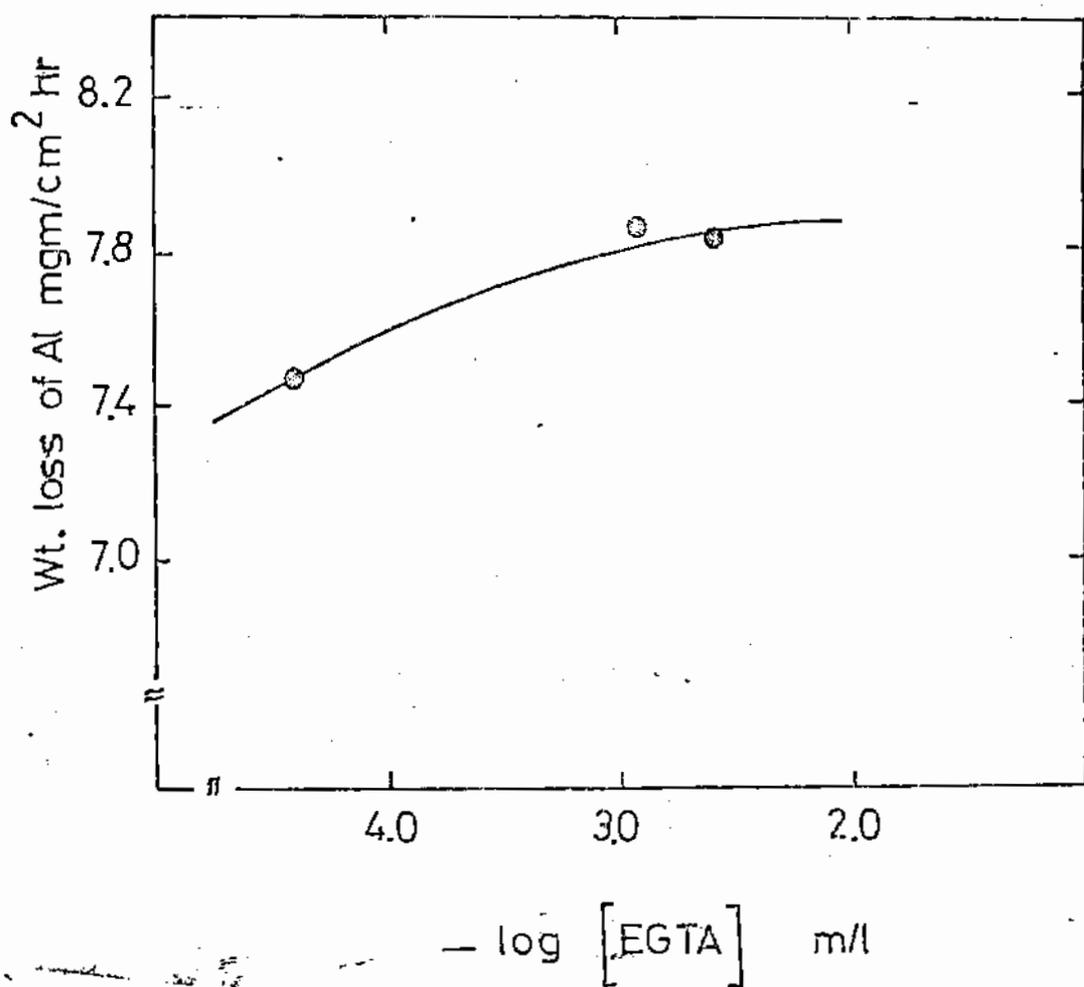


Fig. 2

Table. 1 : Dissociation Constants of some Polyamino polycarboxylic acids

Name and formula	Dissociation constants ¹				
	pK ₁	pK ₂	pK ₃	pK ₄	pK ₅
Ammonia triacetic acid (TTH). $\begin{array}{c} \text{CH}_2\text{COOH} \\ \diagdown \\ \text{N} \\ \diagup \\ \text{CH}_2\text{COOH} \\ \diagdown \\ \text{CH}_2\text{COOH} \end{array}$					
Ethylene glycol -bis-(B-amino ethyl ether)- N,N tetra acetic acid (EGTA). $\begin{array}{c} \text{HOOC-CH}_2 \\ \diagdown \\ \text{N} \\ \diagup \\ \text{HOOC-CH}_2 \end{array} \left[(\text{CH}_2)_2\text{-O} \right]_2 \text{-} (\text{CH}_2)_2 \text{-} \begin{array}{c} \text{CH}_2\text{-COOH} \\ \diagdown \\ \text{N} \\ \diagup \\ \text{CH}_2\text{-COOH} \end{array}$	2.00	2.68	8.85	9.43	
Ethylene diamine tetraacetic acid (EDTA) $\begin{array}{c} \text{HOOC-CH}_2 \\ \diagdown \\ \text{N} \\ \diagup \\ \text{HOOC-CH}_2 \end{array} \text{-CH}_2\text{-CH}_2\text{-N} \begin{array}{c} \text{CH}_2\text{-COOH} \\ \diagdown \\ \text{N} \\ \diagup \\ \text{CH}_2\text{-COOH} \end{array}$	1.99	2.67	6.16	10.26	
Diethylene triamine pentaacetic acid (DTPA). $\begin{array}{c} \text{HOOC-CH}_2 \\ \diagdown \\ \text{N} \\ \diagup \\ \text{HOOC-CH}_2 \end{array} \text{-} (\text{CH}_2)_2 \text{-N-} (\text{CH}_2)_2 \text{-N} \begin{array}{c} \text{CH}_2\text{COOH} \\ \diagdown \\ \text{N} \\ \diagup \\ \text{CH}_2\text{COOH} \end{array}$	1.80	2.55	4.33	8.60	10.58

FIGURES CAPTIONS

- Fig. 1 : Effect of the concentration of ammonia triacetic acid on the corrosion of aluminium in 0.15 N NaOH at 32°.
- Fig. 2 : Effect of the concentration of ethylene glycol - bis -(β - amino ethyl ether) - N,N - tetra acetic acid on the corrosion rate of aluminium in 0.15 N NaOH at 32°.
- Fig. 3 : Effect of the concentration of ethylene diamine tetraacetic acid on the corrosion rate of aluminium in 0.15 N NaOH at 32°.
- Fig. 4 : Effect of the concentration of diethylene triamine pentaacetic acid on the corrosion of aluminium in 0.15 N NaOH at 32°.

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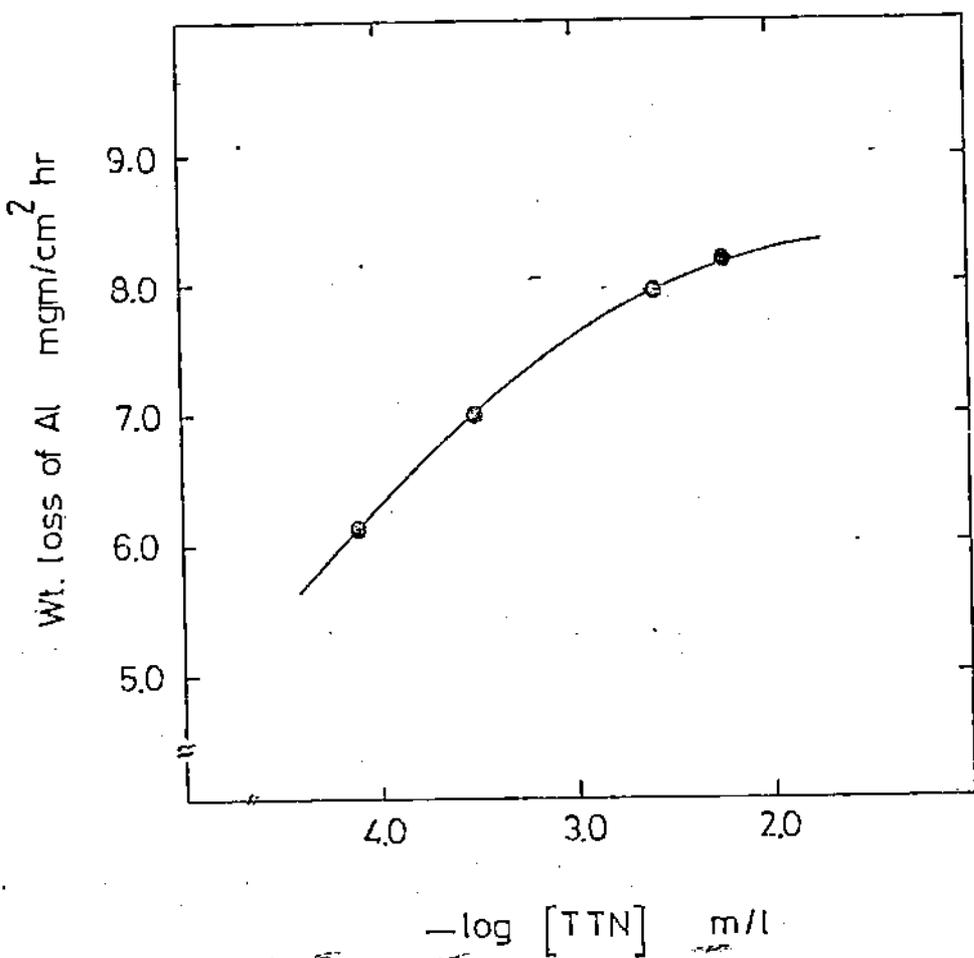


Fig.1

Mitotic and Meiotic Effects of
Trifluralin on *Vicia faba*

By

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C A I R O

(141)

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I N T R O D U C T I O N

The herbicide trifluralin (a, a, a, trifluoro -2, b - dinitro - N - N - dipropyl toluidine) is used as a weed control in cotton. It is a soil active compound. Growth and physiological effects of the herbicide were studied by Kempen (1965), Standifer and Thomas (1965), Ne^eg^e et al (1968) and Schultz^e et al (1968). Mitotic effects were studied by Bayer et al (1967) on cotton, and Hackaylo and Amato (1968), on corn and cotton.

In this study mitotic and meiotic effects of trifluralin on Vicia faba were experimented.

MATERIAL and METHODS

Seeds of Vicia faba (Var. Giza I) were used in this test. For mitotic studies roots 4 days old were dipped in well aerated experimental solutions (0.1, 1, 10, 100 and 500 ppm.) for 24 and 72 hours. Tap water was used in control experiments. All observations were made from Feulgon permanent root tip squash preparations. For meiotic studies two types of treatments were carried on:-

1. Flower buds were directly treated with an aqueous solution of trifluralin (500 ppm.) using moisted piece of cotton for 3 hours, buds were gathered 24 hours after treatment. Moisted piece of cotton with tap water was used for controls.
2. Soil treatment, seeds were sown in pots and as shoot sprouts, they were irrigated regularly with the herbicide solution (10 ppm.) until flowering when flower buds were collected. Tap water was used for irrigating control plants. Pollen mother cells were examined from permanent preparations using aceto-carmin

smear method.

Stainability of pollen grains with aceto-carmin was taken as an index for determining pollen sterility.

Length of pollen grains was accurately measured by ocular micrometer.

RESULTS and DISCUSSION

The inhibiting mitotic effects of trifluralin (Table I) agree with those observed by Tablert (1965), Bayer et al (1967) and Hačlavý and Amato (1968). 1966

After 24 hours treatments the percentage of metaphases increased gradually on the expense of prophase, while after 72 hours treatments the percentage of metaphases increased markedly on the expense of both prophase and ana-telophases (table I). Generally the total percentage of abnormal cells increased with increase of time of treatment and concentration (table I). Stickiness was a common abnormality in 72 hours treatment. Accumulation of metaphases may be attributed to different degrees of spindle disturbances shown in the presence of prophase-

metaphases, ball and star-shaped metaphases, lagging chromosomes and scattering of all the chromosomes all over the cell (Fig. 1-2-3-4). Prophase-metaphases has been observed by many others after treatment of colchicine (barber and callan 1942), ethylene glycol (Damato 1948) chromosomes remain nearly in their arrangement as they were during prophase due to inhibition of spindle formation. Amer (1965) considered star-metaphase type as a fore-step of complete disturbance of the spindle. Barthelmeas (1977) attributed the phenomenon of lagging chromosomes to the adhesion of the centromeres of one or more chromosomes to the outer layer of plasma while other chromosomes continue moving to the equatorial plate.

Anaphase abnormalities were: sticky anaphases with a bridge, c-anaphases and tetripolar anaphases (Fig. 6, 7) which ensure evidences of spindle disturbances. The polyploidising action of the herbicide is indicated by the presence of tetraploid and polyploid cells resulting in giant nuclei and restitution nuclei (Fig. 7, 8). Multipolarity resulted in polynucleate cells, (Fig. 5) Most of the above observations agree with those of Bayer et al (1967) on the effect of trifluralin on cotton. These abnormalities ensured that

116/16
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multipolarity represented in multipolar anaphases
multinucleate cells are found together with c-meta
anaphases, polyploid cells and restitution nuclei.

The most common meiotic abnormalities were stick-
and sticky bridges (Fig. 9). After direct
treatment these abnormalities are obviously higher in
first division than the second division, Table II.
Temporary effect of trifluralin on meiosis agrees
the assumption of Amer and Ali (1968) that meiotic
abnormalities induced by external agents seems to be temporary.
Table II shows also that in case of soil treatment the
percentage of abnormalities in the first and second
division did not show much variation. This may lead
one to believe that the induced abnormalities after soil
treatment may be the result of changes in the ionic
environment and metabolic activities thus leading to
secondary effects such as those observed.

Table II shows also that direct treatment of buds
(0 ppm.) had no effect on either fertility or length
of pollen grains, while soil treatment (10 ppm) induced
sterility and ^{abnormal} ~~abnormal~~ effect on pollen grains

22/2

shown in the increase in their length. These results strengthen the assumption that meiotic treatments with external agents exert temporary effects.

Comparing mitotic and meiotic effects of influralin it is observed that most of the mero-statokinetic abnormalities of the herbicide in mitosis disappeared during plant growth and were not traced during meiosis.

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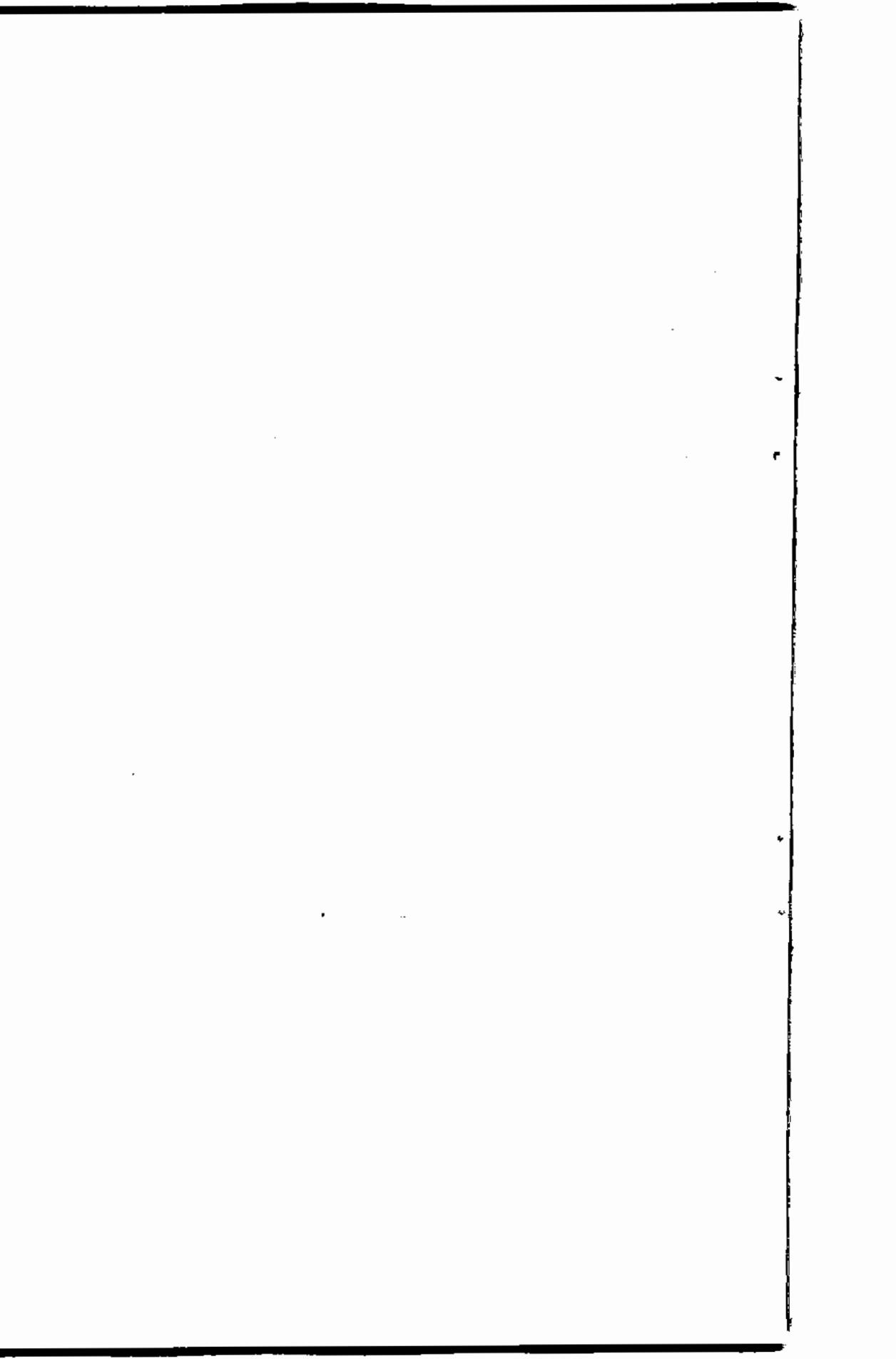


TABLE (I)

MITOTIC INDEX, TOTAL PERCENTAGE OF ABNORMALITIES AND PERCENTAGE OF DIFFERENT PHASES IN TREATED VICIA ROOT CELLS

Concentration	Mitotic Index		Total % of abnor.	Prophase %		Metaphase %		Ana-telophase %	
	24 h	72 h		24 h	72h	24h	72h	24h	72h
Control	82±5.3	79±5.1	-	32.8	32.9	24.4	25.2	42.6	41.7
0.1 ppm.	64±12	64±11.2	4.7	25	35.9	31.1	31.2	42.2	34.3
1 ppm.	64±9.4	65±4.4	12.3	23.4	21.6	39.1	53.9	37.3	24.6
10 ppm.	66±4.8	61±15.2	10.6	19.7	19.6	39.4	65.5	40.9	14.7
100 ppm.	65±13.2	54±15.5	12.2	24.6	24.1	30.7	53.7	41.5	22.2
500 ppm.	48±9	Toxic	29.1	22.9	Tox.	39.5	Tox.	37.5	Tox.

TABLE (II)
 PERCENTAGE OF ABNORMALITIES, POLLEN STERILITY AND LENGTH OF POLLEN
 GRAINS, OF TREATED VICIA PLANTS AFTER SOIL AND DIRECT TREATMENT

Type of	No. of counted cells	Total% abnorm	1st Division		2nd Division			Pollen Steri- lity	Length of p.-g. in micr.
			Blak- met.	Ara- teloph.	Total% of abnorm.	met.	ana-telo phase		
Control	2129	3.8	3.5	2.5	2.9	2.2	3.01	2.4	38.5
Soil	1101	19.6	38.5	15.1	26.1	25.7	11.5	19.3	55.65
Direct	1574	20.2	24.8	28.4	29.1	13.4	5.9	8.01	39.60

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13

Cytological Effects of Some Contraceptives

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Introduction

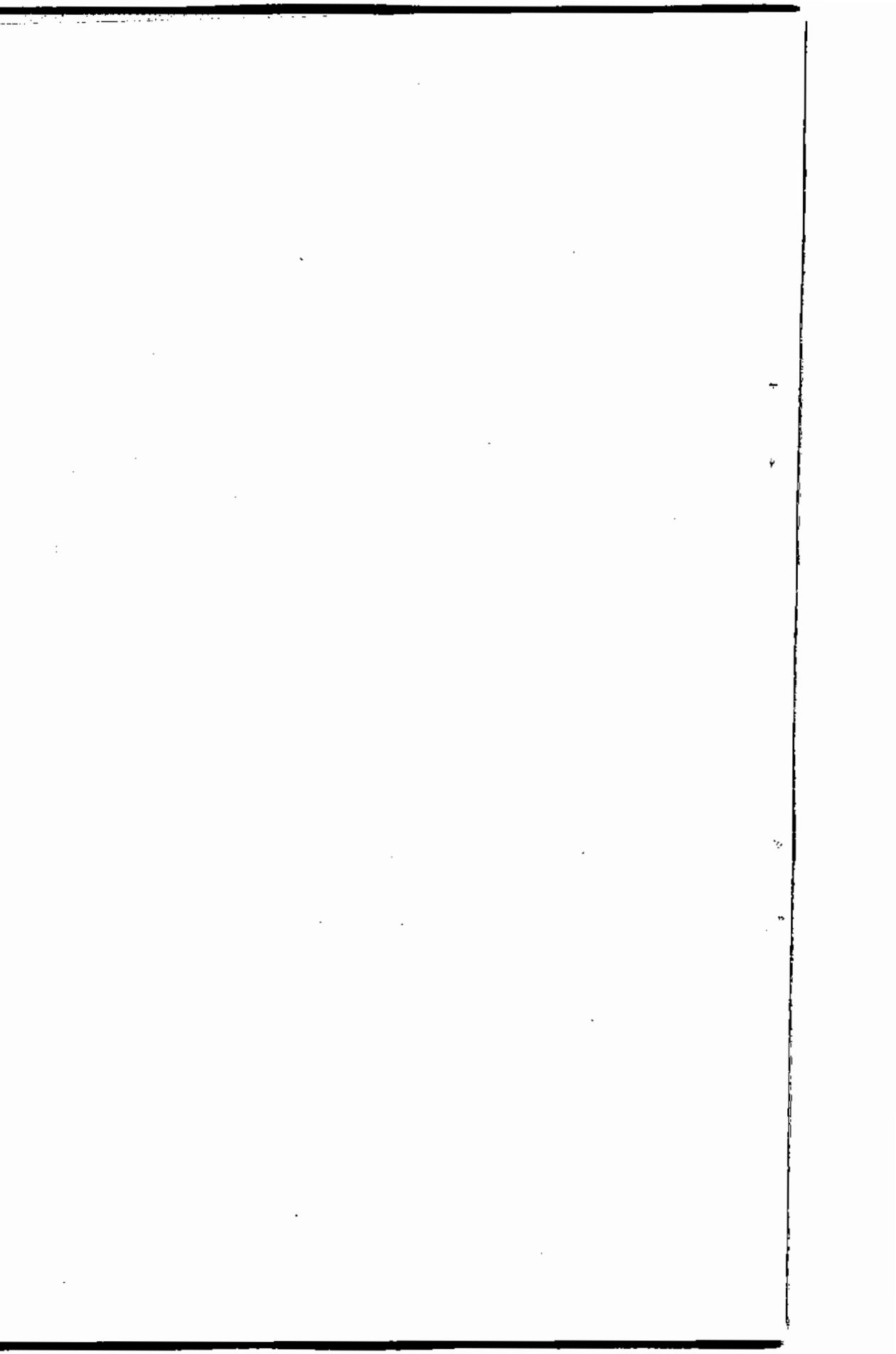
The number of chemical compounds used as contraceptives has been continually increasing in recent years. The cytological effects of some of these drugs are now being of interest; Hakeem and Amer (1966), Kabarity and Khodary (1967). Nowadays other contraceptives are at hand, it seems therefore necessary to continue investigation of their cytological effects. Five new types of contraceptive tablets are now available and widely used in Egypt. Ovulen (Kahera c.) Ovral (Nile. C.) Gyne anovlar, Anovlar I and Primovlar, (Cid.C.).

The present work represents a comparative study on the effect of these five contraceptives on the meiosis of Vicia faba

Material and Methods

Vicia faba plants (var. Giza I) were treated with aqueous solutions of contraceptive tablets. Two types of treatment were conducted :

- a) Direct treatment of flower buds for 3 hours using a moistened piece of cotton.



The percentage of abnormalities decreased gradually as we approached the stages of the 2nd division, (Table II). Spraying with Ovålen and Ovrål did not follow this rule.

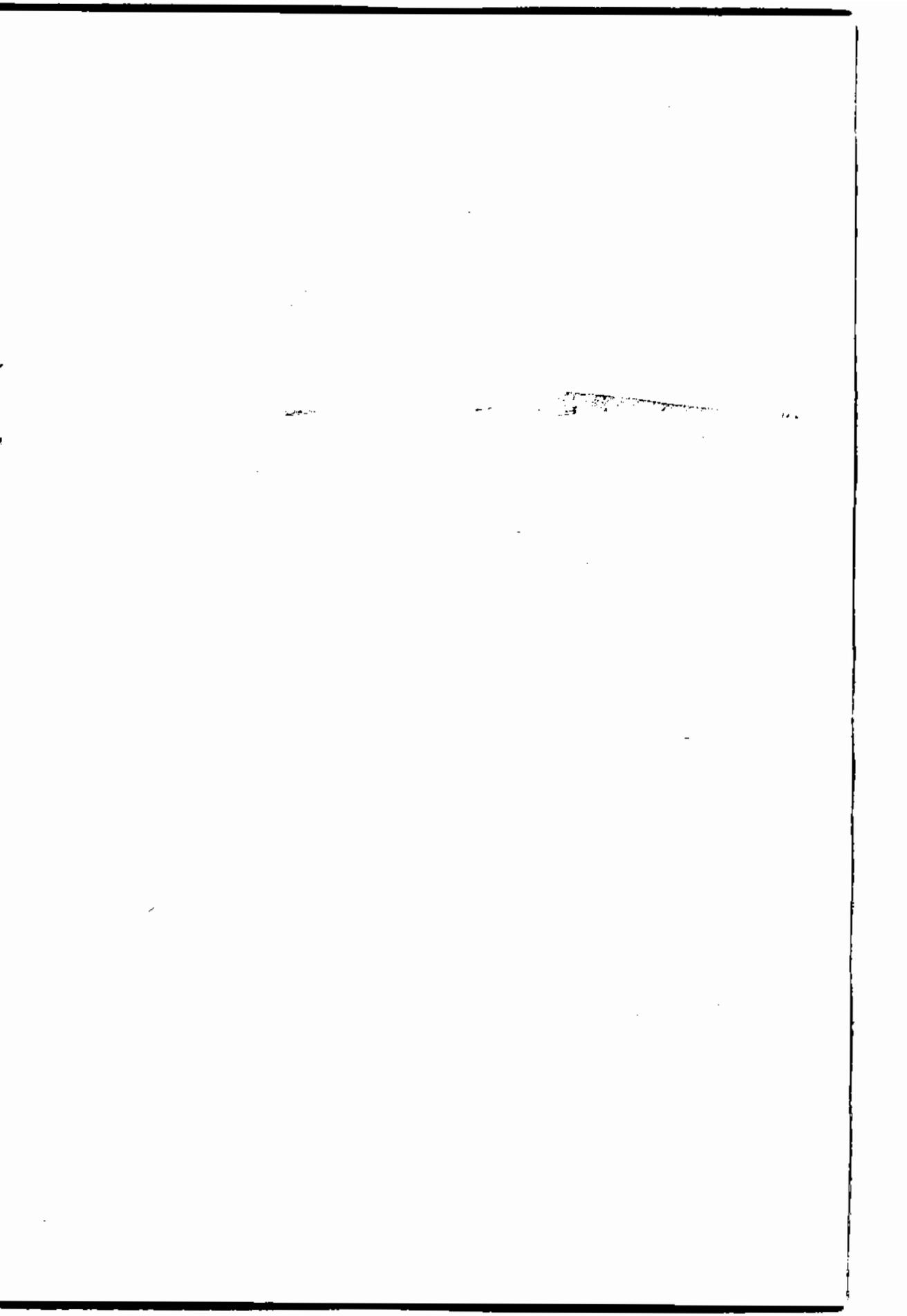
All the five contraceptives gave nearly the same types of abnormalities. Stickiness, sticky bridges and lagging chromosomes were the most common configurations (Table I). The highest degrees of stickiness resulted in a clumped mass in the middle of the cell (Fig 6). In lighter degrees of stickiness the sticky ends of bivalents resulted in various configurations such as chains and rings of bivalents (Fig 1,2,3,4,5) Sax (1941) attributed stickiness of x-rayed chromosomes to excess charge of nucleic acid on the chromosomes. The phenomenon of stickiness which is usually induced by many chemical and physical agents in meiotic chromosomes was observed by many authors. Ohno (1960) reported sticky meiotic chromosomes after treating *Allium* with extracts from noxious plants, Hakeem and Amer (1966) treating *Vicia* buds with saturated contraceptive solutions (Anovlar, Conovid, and Lyndiol) reported also a high degree at sticky meiotic chromosomes.

Sticky bridges and bridge fragment configurations were observed in both types of treatments (Fig2). All applied contraceptives gave nearly the same percentage of sticky bridges

which are the result of stickiness, such configurations were few in second division phases after treatment with Anovlar I, Gyne Anovlar and prymovlar they were common after Ovulen and Ovlar treatment.

Lagging chromosomes or bivalents (Fig 2) were also more common in the first phases of divisions than the second ones. This type of ^baberration was induced by all contraceptives in the two types of treatments with nearly the same percentages (Table I) such ^baberration may be attributed to the hinderance of separation of bivalents at the end of metaphases due to stickiness. Lagging bivalents appear to be a result of clumping of the other bivalents together. The phenomenon of lagging was reported by many authors, Ohno and Tanihuzi (1960), Hussein and Hakeem (1960) and Hakeem and Shehab (1970). Barthelmess (1957) attributed such phenomenon, in mitosis, to hinderance of prometaphase movement of chromosomes accompanied by adhesion of the centromeres to the adjacent inner surface of the plasma.

Other observed phenomenon of less importance such as fragments and cytomixes were recorded. Fragments and paired fragments (Fig 7) were few and only observed in Prymovalar, Ovlar and Ovulen. Cytomixes was reported only in Gyne Anovlar,



158

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— 158 —

Summary

Meiotic effects of five contraceptives; namely ^AChovlar I, Gyne Anovlar I, Gyne anovlar, Prymovlar, Ovulen and Ovral were studied on *Vicia faba*. The most common abnormalities were, stickiness, sticky bridges and lagging chromosomes. Spraying with ovral and Ovulen resulted in more toxicity giving higher percentages of abnormalities and affecting pollen viability. No traces of spindle disturbances were reported.

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151

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Table I
Number and percentages of total abnormalities
and different types of abnormalities

Exp. Agent	Type of treat.	No. P.M.Cs	abn. P.M.Cs	%	Percentage of different types of abn.					
					Stalk	Long	Brd	Frags	Spidi	Cyrom
Control	Direct Spray	1853	37	1.9	100	-	-	-	-	-
		1981	41	2.09	100	-	-	-	-	-
Anovlar I	D S	1920	148	7.7	76	14	10	-	-	-
		1725	214	12.4	71	16	11	-	-	-
Gyne anovlar	D S	2045	157	7.6	78	13	9	-	-	-
		1888	182	10.6	75	13	10	2	-	-
Prymovlar	D S	1966	167	8.4	73	13	11	-	-	3
		2120	224	10.5	76	14	8	-	-	2
Ovral	D S	1870	194	10.3	65	16	12	5	-	2
		1737	289	16.6	70	12	10	4	-	5
Ovulen	D S	1590	139	8.7	69	17	8	3	-	3
		1913	219	11.4	70	11	10	5	1	3

Table II
 Number and percentage of abnormalities in different meiotic phases and percentage of pollen viability after direct treatment and spraying flower buds with contraceptives

Exp. agent	Type of treat.	1st ana-metaphase			2nd metaphase			2nd ana-metaphase			% pollen viabil.			
		No. P.M. Cs.	abn. P.M. Cs	%	No. P.M. Cs	abn. P.M. Cs	%	No. P.M. Cs	abn. P.M. Cs	%				
Control	Direct Spray	608 650	17 21	2.7 3.2	493 533	- -	- -	513 548	11 23	2.1 4.2	242 250	- -	- -	98.1 97.3
Amovlar	D S	809 750	78 121	9.6 16.1	317 241	33 39	10.3 13.06	559 432	34 35	6.09 8.1	235 262	10 19	4.2 7.2	97.6 96.7
Gyne Amovlar	D S	911 711	93 94	10.2 13.2	450 381	42 46	9.3 12.06	314 519	25 31	7.9 5.9	370 277	12 11	3.2 3.9	95.9 95.4
Prymovlar	D S	853 927	87 111	10.1 11.9	395 471	39 67	9.8 14.2	416 583	27 38	6.7 6.5	302 139	14 8	4.6 5.7	96.2 95.9
Ovral	D S	822 631	102 135	12.4 22.6	501 411	56 63	11.1 15.3	310 573	27 76	8.7 13.2	237 122	9 15	3.7 12.2	94.3 91.5
Ovulen	D S	740 644	76 109	10.2 12.9	219 523	16 58	7.2 11.04	464 399	31 35	6.7 8.7	167 147	16 17	9.5 11.5	94.9 93.4

163
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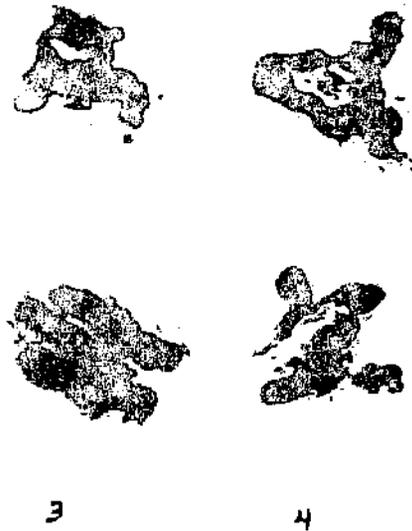


Fig. 1-4 Photo-micrographs of meiosis in Vicia faba as a result of direct treatment with Anovlar Fig 1, Sticky metaphase; Fig 2, Sticky metaphase with an attached fragment; Fig 3, Chains of bivalents; Fig 4, Early metaphase showing sticky bivalents with stretched ends. X.

164
11

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Fig. 5. Group of F.M.C. representing normal 1st metaphase-polar view (right), 1st metaphase with chain of bivalents (left), and different types of sticky late second metaphase (middle), as a result of spraying with ovlar.

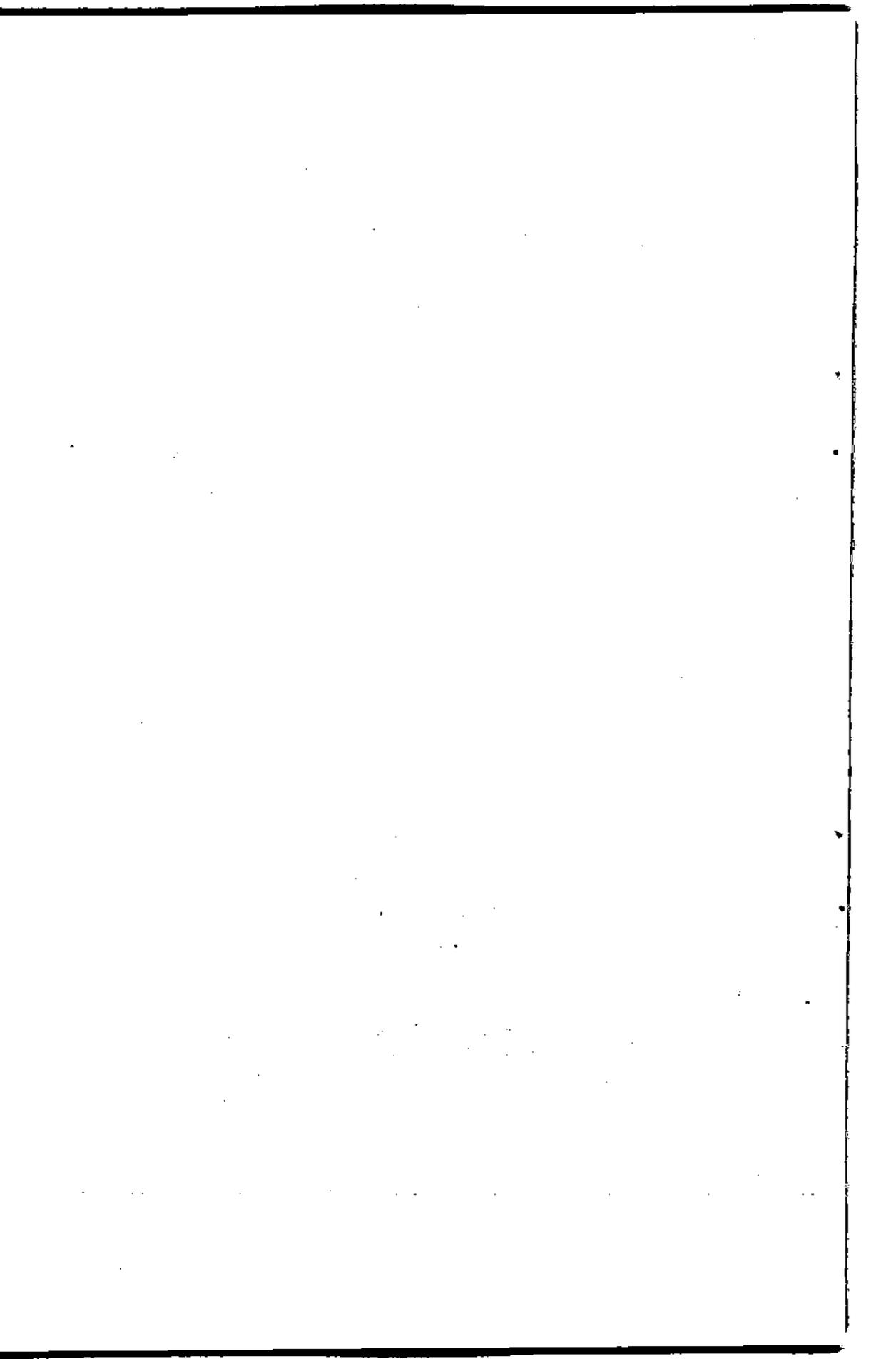
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Fig. 6.

Fig. 7.

Clumped 2nds metaphase (Fig.6) and diakinesis with paired fragment (Fig 7) resulting from treatment with ovulen.



Root growth and Mitotic effect of
nemagon and nemacur on Vicia faba

By

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INTRODUCTION

The progress in the methods of chemical control of nematodes has made its way during the past years. However, most of the research works have been conducted to study the effect of nematicides on crop yield and nematode population, (Bindar and Chabra (1972), Smith et al. (1973), Ranjit et al. (1974), Singh (1974) and Miller and Saul Rich (1974).).

In the present work the effect of two nematicides, nemagon (halogenated aliphatic compound) and nemacur (phosphorus aromatic compound) on root growth and mitosis of Vicia faba was studied.

Material and Methods

Vicia faba seeds (Var. Rebaya 40) were subjected to two types of experiments:-

Field experiments:-

In these experiments the actual field doses used in bean fields were applied. For nemagon, 7.5, 6.2 and 5 ppm concentrations were prepared from the original solution and for nemacur 3.8, 2.8 and 1.9 ppm concentrations were prepared from the original solid.

Seeds were incubated in water for 24 hours then sown in pots containing 5 kgm. sow dust. The sow dust was mixed thoroughly with the appropriate concentrations of the nematocides used prior to sowing. Twenty pots were used for each treatment, each containing 5 seeds. Tap water was used for irrigation. For control, 20 pots were used in which tap water was used instead of the experimental solutions.

The study comprises measurement of the root length after 4, 6, 8, 10, 12 and 14 days after sowing taking the cotyledons as a starting point.

For mitotic study, the secondary roots of treated plants 12 days old were used. The roots were cut, fixed in Carnoy's fixative (1-3 acetic alcohol) for 24 hours then stored under refrigeration in 70% alcohol.

II. Laboratory experiments:

Vicia faba roots 4-days old (1.5 cm. in length) were dipped in varying concentrations of nemagon and nematicur (1, 10, 100 and 500 ppm) for 3, 6, 12 and 24 hours. After the fixed time, the roots were cut, fixed and stored as previously mentioned.

For recovery tests, another group of seedlings were taken out of the solutions after every time of treatment (3, 6, 12 and 24 hours), washed with running tap water then dipped in tap water for 24 hours. The recovered roots were cut, fixed as previously mentioned.

Observations were made from permanent Feulgen stained root tip squash preparations.

Mitotic index was calculated as the mean of dividing cells from 10 different root tips, for every treatment 10000 cell were counted.

Nuclear volume was estimated from a random sample of nuclei for each treatment and was calculated as

$$\frac{4}{3} \pi \left(\frac{\text{Length} + \text{Breadth}}{4} \right)^3 \quad \text{Bennet 1970}$$

Results

Root growth

Statistical analysis showed that the stimulatory effect of the two nematocides was not significant except for the last reading of the median nematocid dose, Figs. (1 and 2).

Cytological studies

Tables 1 and 2 show that the two nematocides affected the percentage of mitotic phases. Accumulation of prophase was obvious after treatment with field doses of the two nematocides. In the direct treatment, accumulation of prophase was observed after 6 hours treatment with 1 ppm nemagon.

Recovery experiments of nemagon 1 and 10 ppm tends to regain the normal percentage of the mitotic phases as

the control, while that of nemacur deviates the percentage of phases away from their normal ones, Table (2).

Toxicity appeared after recovery in nearly all time intervals of 100 and 500 ppm nemagon and nemacur. Nemacur was more toxic than nemagon, toxicity starting earlier (after 6 hours 100 ppm).

Inhibition of mitotic index was observed after treatment with both field and laboratory doses of the two nematocides. After field doses, inhibition of mitotic index was more obvious after nemagon treatments, while after direct laboratory treatments nemacur affected the mitotic index severely.

After recovery, the mito-depressive effect of nemacur decreased except after the longer periods of treatment.

Table 3 shows that maximum field doses of nemagon and nemacur decreased the average volume of the nucleus.

An obvious decrease in nuclear volume was observed after 24 hours treatment with 1 and 10 ppm nemagon and nemacur direct and recovery treatments. A marked increase in nuclear volume was observed after nemacur treatment for 3 hours with 1 ppm direct and after 6 hours with 1 ppm and 3 hours with 10 ppm recovery.

After field doses, the total percentage of abnormalities increased with increase of concentration after nemagon treatment. On the other hand, it was not correlated with concentration after nemacur treatments.

After direct treatment with the two nematocides, the total percentage of abnormalities was not correlated with concentration. Table (5) shows that a maximum percentage of abnormal cells (57.1) was observed after recovery of 10 ppm 24 hours treatment.

Most of the abnormalities were restricted to metaphases except with minimum nemacur doses. Prophase has the least percentage of abnormalities with the two nematocides.

Abnormal prophases, Fig. (3) were observed in Vicia roots after treatment with nemagon field doses, Table (6) and were more pronounced after laboratory treatment reaching 42.2% after 6 hours with 1 ppm.

Stickiness was a prominent abnormality after treatment with the two nematocides with both field and laboratory doses. After field doses the percentage of stickiness was inversely proportional to the concentration.

Stickiness was more obvious after direct treatment with nemagon than nema-cur. Fig. (4) shows sticky meta-phase chromosomes.

The percentage of bridges was higher in nema-cur than nemagon after treatment with field doses. On the other hand, nemagon brought about a higher percentage of bridges than nema-cur after laboratory treatment, Tables (6 and 7), Figs. (5 and 6).

Laggards were a less dominant abnormality after both field and laboratory doses of the two nematocides, Tables (6 and 7), Fig. (7).

The disturbed type was the other dominant abnormality observed after treatment with the two nematocides. After nemagon field doses the percentage of types of spindle disturbances increased with increase of doses. Yet with nema-cur maximum percentage of spindle disturbance was reached after the median dose. With laboratory treatment, nema-cur induced a high percentage of disrupted spindles compared with nemagon, Tables (6 and 7), Fig. (8).

Fragmentation was a less common abnormality after both field and laboratory doses of the two nematocides.

Recovery treatment with 3h ppm nemaour showed that fragmentation was the only abnormality scored (100%), Table(7), Fig. (9) .

micro and multinucleate cells, Figs.(10 and 11) were not a common abnormality in this investigation.

Discussion

The present results indicate that the two nematocides had no significant effect on the root growth of Vicia faba i.e. Vicia roots tolerate the effect of the nematocides.

Mitotic observations proved that nemaour was more toxic than nemagon. Prophase accumulation which was more pronounced after nemagon field and laboratory doses than nemaour indicates delay in the spindle formation. However, recovery after nemagon treatment changed those percentages to the normal level.

Applying field doses of nemagon proved to be a mitotic poison decreasing the mitotic index more than nemaour, while after nemaour laboratory doses mitotic poisoning starts earlier and more severely. However, this mitodepressive effect of nemagon increased after recovery

experiments while it decreased in case of nemacur recovery experiments.

Maximum nuclear volume reduction being induced 24 hours after treatment with both nematocides indicated that they might play a role in DNA synthesis (Kihlman 1971).

Most of the abnormalities scored after nematocidal treatment were at metaphase stage, ensuring that the two nematocides showed a stathmokinetic tendencies, yet they did not affect an increase in the mitotic index due to accumulation of cells at prophase and metaphase.

Prophase poisoning might be due to different changes in the readily dividing cells and this leads to a critical retardation of the course of mitosis. This abnormality was similar to that reported by Levan and Tjio (1948) after treatment with phenols, D'Amato (1950) using natural compounds, and Barthelmeß and El-Kabarity (1966) using acetanilide.

Nemacur induced higher percentage of stickiness than nemagon with field doses, while after laboratory direct and recovery experiments nemagon brought about a higher percentage of stickiness.

After treatment with the higher concentrations of the two nematocides sticky bridges were observed. While after treatment with the lower concentrations the bridges formed might be due to either adhesion of chromosomes or exchanges and reunion of chromatid or subchromatid. Bose and Bose (1970) found bridges with or without fragments treating Tomato with colchicine, diethyl sulphate and triethylamine.

Lagging chromosomes were more common after nemagon treatment than nemacur field and laboratory doses. Laggards and fragments might give rise to micronuclei.

After short periods treatment, 24 hours recovery was sufficient for the total percentage of abnormalities and mitotic phases to regain their normal behaviour in both nematocides.

Nemacur harmful effects were repaired by recovery treatments than nemagon.

Summary

Vicia faba has been subjected to two types of treatments using different doses of nemagon and nemacur. Field

(14/7)

- 177 -

doses used in bean fields were tested and laboratory doses where the concentrations used were 1, 10, 100 and 500 ppm for 3, 6, 12 and 24 hours. For both nematocides recovery tests for 24 hours in tap water were carried out.

The two nematocides showed no significant effect on root growth of Vicia except for the median nemacur dose, where stimulation in root length took place.

Under field conditions, nemagon proved to be a mitotic poison more than nemacur. While under laboratory conditions the mito-depressive effect as well as induction of spindle disruption was more pronounced in nemacur.

A reduction of nuclear volume was recorded after longer hours of treatment of nearly all laboratory doses of both nematocides.

The most dominating abnormalities were :- stickiness, bridges, laggards, spindle disturbance, fragments and micronuclei.

The two nematocides used proved to be stathmokinetic agents.

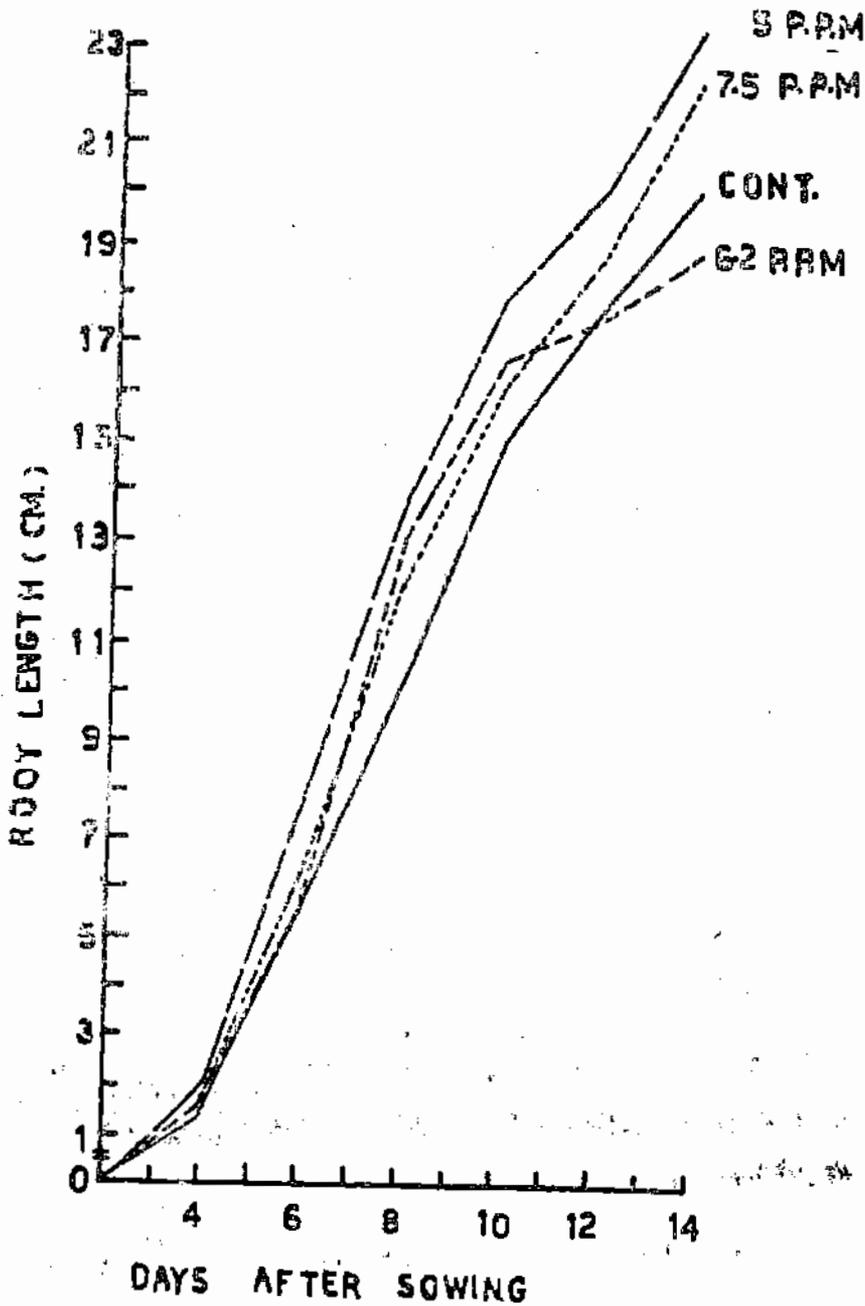


FIG. 1: ROOT LENGTH AS AFFECTED BY NEMAGON

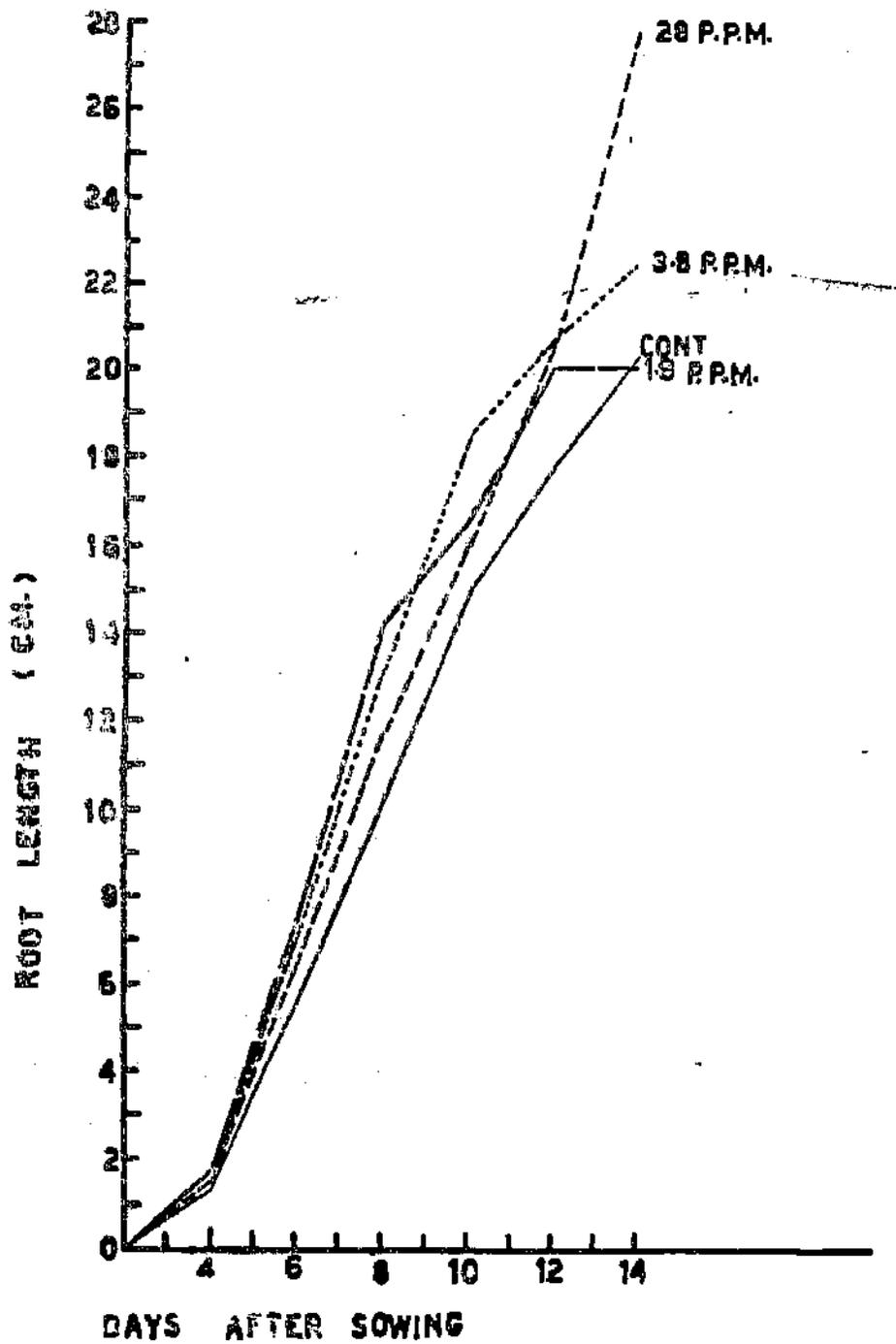


FIG. 2: ROOT LENGTH AS AFFECTED BY NEMACUR.

Table (1)

Percentage of Phases and mitotic index as effected by nemagon and Nemacur field doses

Type of treatment	Prophase	Metaphase	Ana-telophase	M
Control	43.8	16.50	40.0	1
Nemagon 5.0	64.5	6.45	28.0	0
6.2	66.6	10.40	22.9	4
7.5	70.4	13.60	15.9	1
Nemacur 1.9	52.9	19.60	27.4	1
2.8	47.2	15.20	37.5	1
3.8	55.8	14.70	29.4	1

Table (2)

Percentages of phases and mitotic index after direct and recovery laboratory Hemagon and Nemour treatments.

Type of	Prophase				Metaphase				Anaphase - telophase				Mitotic index			
	Hemagon		Nemour		Hemagon		Nemour		Hemagon		Nemour		Hemagon		Nemour	
	Direct	Recovery	D	R	D	R	D	R	D	R	D	R	D	R	D	R
Control	44.0	31.0	44.0	31.0	20.3	21.0	21.0	20.3	34.2	41.6	34.2	41.6	61	72	61	72
1 ppm 3h	41.5	26.1	41.0	45.2	20.4	20.4	20.4	20.4	27.7	38.8	40.9	38.8	64	37	64	37
12h	70	35.3	44.0	18.5	11.5	11.5	11.5	11.5	25.2	30.9	26.9	30.9	61	43	61	43
24h	32.5	27.7	25.0	12.8	23.4	23.4	23.4	23.4	35.5	31.6	38.7	31.6	65	54	65	54
24h	17.6	27.4	25.9	36.7	27.4	27.4	27.4	27.4	36.7	33.3	41.6	33.3	63.7	51	63.7	51
10 ppm 3h	20.2	22.0	47.3	41.8	14.1	14.1	14.1	14.1	14.4	28.2	27.9	28.2	52	39	52	39
6h	51.1	34.3	41.3	24.5	21.3	21.3	21.3	21.3	25.2	35.2	35.2	35.2	36	41	36	41
12h	30.4	14.7	24.2	28.6	26.6	26.6	26.6	26.6	32.1	42.6	43.6	42.6	58	69	58	69
24h	18.4	32.7	24.1	30.0	27.2	27.2	27.2	27.2	30.1	16.3	38.1	16.3	50	61	50	61
100 ppm 3h	39.0	16.1	27.8	62.7	32.7	32.7	32.7	32.7	19.6	9.8	24.8	39.6	64	53	64	53
6h	56.3	Toxic	Toxic	Toxic	16.4	16.4	16.4	16.4	Toxic	Toxic	Toxic	Toxic	94	Toxic	94	Toxic
12h	60.5	25.3	Toxic	Toxic	18.2	18.2	18.2	18.2	Toxic	Toxic	19.2	38.0	26	63	26	63
24h	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	toxic	toxic	toxic	toxic	toxic	toxic	toxic
500 ppm 3h	57.6	Toxic	Toxic	Toxic	24.7	24.7	24.7	24.7	Toxic	Toxic	Toxic	17.5	Toxic	36	Toxic	Toxic
6h	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
12h	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
24h	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic

181
181

Table (3)
Nuclear volume (u³) as affected by Nemagon and Nemacur

Field	Laboratory					
	Nemagon			Nemacur		
	Direct	Recovery	Direct	Recovery	Direct	Recovery
Control	3580.00	3580.00	3580.00	3580.00	3580.00	3580.00
Nemagon P.P.M.						
5	1981.5	3h	3580.00	3930.0	6061.1	4411.5
6.2	2475.1	6h	4697.5	4055.5	2855.9	5260.5
7.5	1371.8	12h	4973.6	3694.5	3580.0	3356.7
		24h	1494.5	1146.7	1761.4	1692.1
Nemacur P.P.M.		3h	3580.00	4834.1	1761.4	5711.5
1.9	2219.1	6h	4431.5	4302.5	3811.0	3352.7
2.8	1981.5	12h	4176.0	3146.6	2845.5	2943.6
3.6	1632.1	24h	1624.4	2343.6	1200.6	814.7
		100 P.P.M. 3h	3811.01	3100.7	3930.0	4697.5
		6h	3811.03	2653.9	2268.8	Toxic
		12h	4302.5	2146.6	1905.3	Toxic
		24h	Toxic	Toxic	Toxic	Toxic

Table (14)

Total percentage of abnormalities and distribution of percentages of abnormalities in the different mitotic phases after treatment with field doses of Nemagon and Nemacur.

Type of treatment	Total percentage of abnormalities.	Prophase	Metaphase	Ana-telophase
Control	2.2	-	-	-
Nemagon ppm. 5	7.6	0	57.0	43.0
6.2	6.7	9.5	52.3	38.0
7.5	11.9	11.5	69.2	19.2
Nemacur ppm. 1.9	20.3	0	66.6	33.9
2.8	14.8	0	70.4	29.6
3.8	16.5	0	42.8	57.1

Table (6)

Percentages of different types of abnormalities after treatment with field doses of Nomeson and Nemour

Type of treatment	Types of abnormalities					
	Abn. proph.	Stick.	Bridges	Loggings	Sp. dist.	frag.
Nomeson ppm 5.0	0.0	17.3	34.7	0	47.5	0.0
6.2	9.5	4.7	9.5	19.0	57.0	0.0
7.5	11.5	0.0	11.5	3.8	72.9	0.0
Nemour ppm 1.9	0.0	42.5	32.3	0.0	24.0	0.0
2.8	0.0	16.6	18.5	11.1	49.9	3.7
3.0	0.0	5.3	50.0	3.5	37.4	3.5

Table (7)

Percentages of different types of abnormalities after laboratory
Direct and Recovery treatment with nemagon and nemsaur.

Type of treatment	Types of abnormalities																							
	Abn. propth.				Stickiness				Bridges				Lagging				Sp. dist				Frag.			
	Nemagon		Nemsaour		Nemagon		Nemsaour		Nemagon		Nemsaour		Nemagon		Nemsaour		Nemagon		Nemsaour		Nemagon		Nemsaour	
1 p.p.m 3h	6.5	9.7	5.0	-	39.1	58.5	5	-	12.1	-	19.5	-	10.7	4.8	15	-	21.9	7.3	700	-	2.1	12.1	5	100
6h	42.2	-	5.7	-	33.3	20.0	-	15.7	25.7	-	4.4	-	4.4	80.0	-	10.5	11.0	-	31.3578	4.0	-	17.1	15.7	-
12h	3.0	3.0	-	11.1	21.2	15.1	45.6	27.7	18.1	3	6.5	22.2	24.1	2.1	-	3.63	51.4	56.327.7	-	3.0	8.6	11.1	-	-
24h	-	-	7.1	6.2	7.1	56.8	21.4	-	2.2	1.4	32.1	-	3.5	4.5	7.1	-	50.0	36.228.575.0	7.1	-	14.2	18.7	-	-
10 p.p.m 3h	-	27.7	2.3	-	44.4	22.2	23.0	9.0	11.1	11.0	11.4	-	11.1	5.5	7.1	18.1	22.2	27.7	30.8	18.0	11.1	5.5	35.6	5.5
6h	5.2	7.1	-	-	5.26	39.2	-	15.6	15.7	-	-	-	7.8	31.5	10.0	-	18.3	33.1	40.0	4.2	-	-	-	-
12 h	2.3	8.1	-	-	30.2	24.3	29.5	14.6	-	8.1	16.3	9.5	11.6	8.1	1.6	4.7	44.1	48.6	50.8	71.4	4.6	3.7	1.6	-
24 h	-	1.4	-	-	4.7	29.4	5	86.3	40.4	4.4	16.5	9.0	21.3	5.8	-	-	26.1	32.9	38.8	4.5	7.1	5.8	38.8	-
100 p.p.m 3h	-	-	23.7	4.7	2.3	56.0	25.0	9.5	4.6	12.0	-	-	6.8	4.0	-	-	84.8	34.0	44.1	35.7	1.1	4.0	7.6	-
6h	21.4	Toxic	-	-	23.8	Toxic	Toxic	9.5	Toxic	9.5	Toxic	-	11.8	Toxic	-	-	23.4	-	-	-	4.7	-	-	-
12h	-	1.7	Toxic	-	45.4	64.2	Toxic	Toxic	40.9	5.3	Toxic	-	45	7.1	Toxic	-	9.0	19.6	Toxic	-	1.7	Toxic	-	-
24h	Toxic	Toxic	-	-	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	-	Toxic	Toxic	-	-	Toxic	Toxic	Toxic	-	Toxic	Toxic	-	-

187

- 187 -



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Plate I

- Fig. 3 : Irregular prophase after treatment with nemagon 10 ppm 24 hours and recovery.
- Fig. 4 : Sticky metaphase after treatment with 100 ppm 6 hours nemagon.
- Fig. 5 : Sticky bridge at anaphase after treatment with maximum nemacur field dose 3.8 ppm.
- Fig. 6 : Chromatid bridge after treatment with maximum nemacur dose 3.8 ppm.
- Fig. 7 : Lagging chromosome (two chromatids) after treatment with nemagon 10 ppm for 24 hours and recovery.
- Fig. 8 : Disturbed metaphase after treatment with maximum nemagon.
- Fig. 9 : Metaphase breakage after treatment with nemagon 10 ppm 24 hours and recovery.
- Fig. 10 : Multinucleate after treatment with nemagon 10 ppm 24 hours and recovery.

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II. Studies on the effect of herbicides
on meiosis, size of pollen grains
and pollen viability.

By

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The present study aimed to compare the effect of eight organic herbicides on percentage of meiotic abnormalities, size of pollen grains as well as pollen viability of *Vicia faba* plants. The herbicides used are : 2,4 - D amine, Dalapon, Eptam, Cotoran, Linuron, Simazine, Treflan and Gramoxone.

Material and Methods

Vicia faba plants (Var Giza¹) were subjected to two types of treatments :

1. Direct treatment :

Flower buds were directly treated with herbicide solution. For water soluble herbicides as treflan, dalapon, gramoxone and 2,4-D-amine solutions 500ppm were used. For slightly soluble herbicides concentrations used were : 375 ppm for eptam, 90 ppm for cotoran, 75 ppm for linuron.

and 5 ppm for simazine. Treatment was applied on buds by a moisted piece of cotton for 3 hours during that time buds were protected by sulphane bags. For control experiments tap water was used.

2. Soil treatment:

(For herbicides used as soil sterilants.)

Seeds were sown in pots and as shoot sprouts they were irrigated with the herbicide solution, until the total holding capacity of the soil. Concentrations applied were: 10 ppm for treflan, eptam, linuron, and cotoran. For simazine 5 ppm was used. For controls tap water was used. Such low concentrations were chosen for soil treatment as the herbicide solution will remain for a long time in contact with plant roots until flowering when buds were collected. Examination of P.M.Cs and P.Gs. was carried out using permanent aceto-Carmine smear method. Stainability of P.Gs was taken as an index for pollen viability. P.Gs. that were shrunk, and could not stain were calculated as abortive. Size of P.Gs. was recorded by an accurately standardized ocular micrometer.

Results and Discussion

Comparing the effects of direct and soil treatment on meiotic abnormalities (Table I), it is obvious that soil treatment affected the percentage of abnormalities more than direct treatments. Simazine 4I.I, eptam 33.9, Treflen gave nearly the same percentage of anomalies in the two treatments (20.2, 19.6).

Stickiness and sticky bridges were the most dominating abnormalities (plate I). Other abnormalities such as multipolarity and multinucleate P.M.Cs were also common.

Table I.

Percentage of meiotic abnormalities, average length of pollen grains and percentage of abortivness in treated Vicia after direct and soil treatment

Herbicide	No. of examined P.M.Gs.	% of abr.	No. of examined P. Gs.	Mean size of P. Gs. in microns	% of abortivness
<u>Direct treatment</u>					
Control	I997	2.6	2500	38.50	2.3
2,4-D amine	I355	9.2	1532	46.20	5.1
Dalapon	II68	1.9	2709	42.35	4.3
Eptam	2I27	7.6	2483	46.15	2.9
Linuron	I4I3	10.4	1796	44.00	6.1
Cotoran	I526	6.09	2152	42.90	0.97
Simazine	II68	14.9	1838	46.75	7.3
Treflan	I554	20.2	2650	39.60	2.7
Gramoxone	I630	3.4	2050	40.15	5.4
<u>Soil treatment</u>					
Control	2I29	3.8	2500	38.0	2.3
Eptam	653	33.9	1787	50.60	11.5
Linuron	II96	5.4	1896	44.00	59.9
Simazine	505	11.1	2147	36.00	5.6
Treflan	II01	19.6	2013	55.65	4.1
Cotoran	toxic	toxic	toxic	toxic	toxic

Examination of size of pollen grains proved also that soil treatment had a stronger effect on pollen grains than direct treatment. Soil treatment with Treflan and simazine (55.65, 50.60) showed marked increase in size of pollen grains. Soil treatment with simazine gave beside the enlarged P.M.Cs and P.Gs. a number of small sized ones (Plat I), this phenomenon was accompanied by contraction of chromosomes.

Said and El-Hakim (1955) attributed the increased size of pollen grains after colchicine treatment of Luffa plants to the storage or accumulation of a greater amount of hormone or its precursor in such grains than in controls. Such hormonal accumulation might induce increase in size of P.Gs.. probably by increasing the plasticity of the wall. Hakeem (1968) after studying the effect of Podophyllin suspensions on P.Gs. of Vicia Faba and Luffa Cylindrica found increase in size of P.Gs.

Generally speaking the eight herbicides were not so effective on pollen viability in direct treatments. In soil treatments linuron gave marked decrease in pollen viability (59.5), with many small sized, non storable. P.Gs. Eptam could be considered as pollen sterilizer. Simazine and treflan are nearly non-effective in either treatments (Table I).

It is noteworthy to report here that Linuron which proved to be the most effective pollen sterilizer in soil treatment nearly gave no change in percentage of abnormalities or size of pollen grains. Simazine which gave a considerable percentage of abnormal P.M.Cs. in soil treatment was not effective on pollen viability.

Plate I

Shows 18 normal and treated P.M.Cs with different herbicides having the same (X). Figs 6-II showed enlarged P.M.Cs., Figs 12-18 show contracted P.M.Cs.

- 1,2 Normal P.M.Cs of Vicia plant.
 3 chains of bivalents in treated P.M.C with simazine (5 ppm) after soil treatment.
 4,5 Sticky bridges with fragments in treated P.M.Cs. with treflan (500 ppm) after direct treatment.
 6,7 Disturbed on a phases in treated P.M.Cs. with 2,4-D amine (500 ppm) after direct treatment.
 8,9 lagging chromosomes at 2nd meta and telophase in treated P.M.Cs. with 2,4-D amine (500 ppm) after direct treatment.

197

- 197 -

(7)

- I0 Disturbed 2nd telophase in treated P.M.Cs. with 2,4-D amine (500 ppm) after direct treatment.
- II Penta-nucleate P.M.C. treated with 2,4-D amine (500 ppm)
- I2,I3,I4 Severe stickiness in 1st metaphases with contracted chromosomes and P.M.Cs. treated with simazine (5 ppm) after soil treatment.
- I5,I6,I7 Severe stickiness in 2nd metaphases with fragments in treated P.M.Cs. with simazine (5 ppm) after soil treatment.
- I8 Penta-nucleate and small size of pollen mother cell treated with simazine (5 ppm) after soil treatment.

X = 900

123

- 198 -

(B)



Plate I

Thus there is no correlation between ability of herbicide to produce meiotic abnormalities and its effect on pollen viability.

Thus although the eight herbicides were nearly identical in their action on types of meiotic abnormalities yet they differed from each other in their end effect on P.M.Cs. and only eptam and linuron were effective on pollen viability.

Kuratie (1969) reported that the primary mode of action of linuron is probably inhibition of Hill's reaction. Craft (1966) showed that eptam is an effective fumigate and inhibits the release of oxygen in the process of photosynthesis.

There may be some internal physiological disturbances, such as those found by the above authors which are beyond these cytological aspects which may have caused pollen sterility.

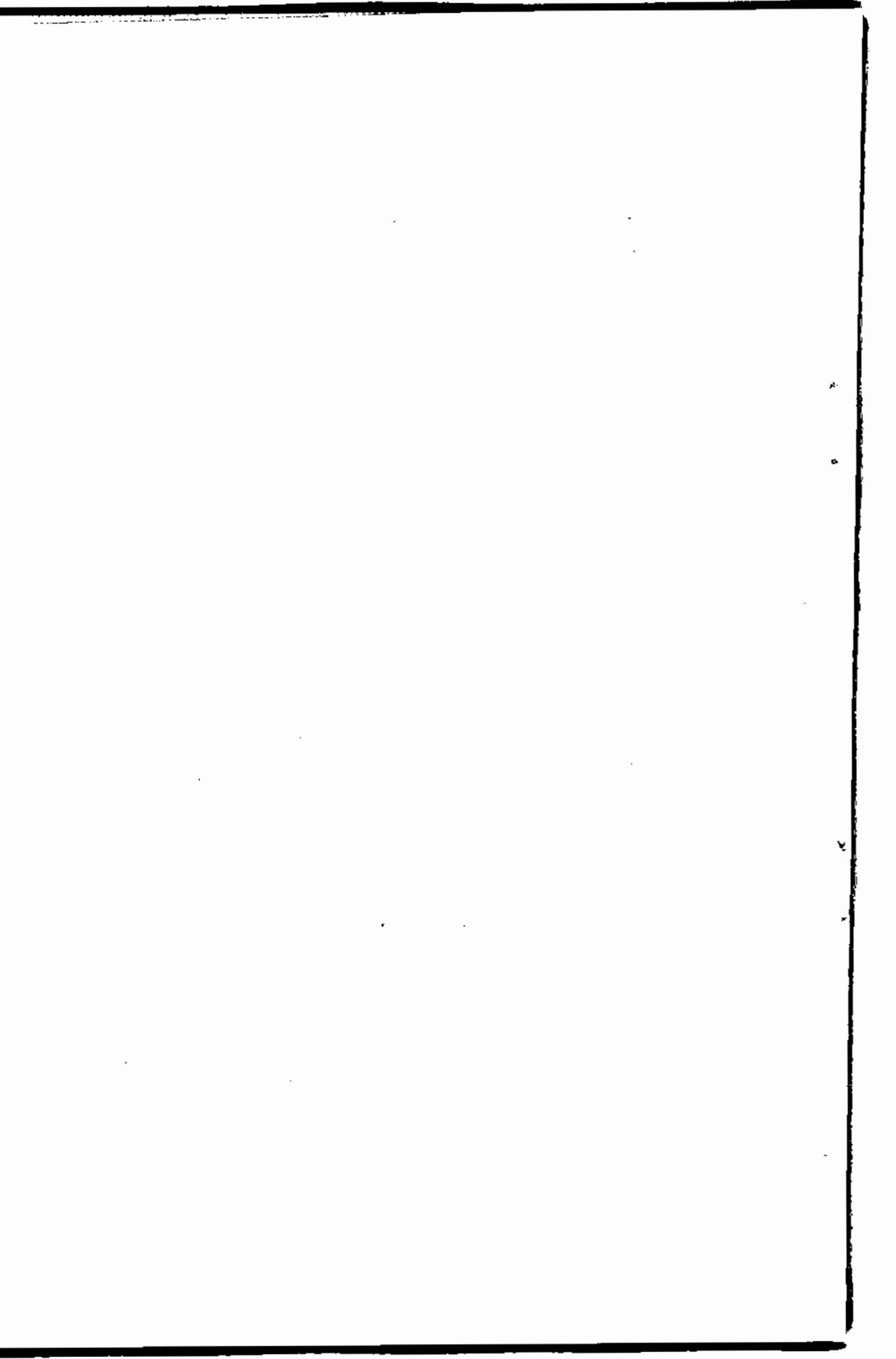
Summary

- I. The effects of 8 herbicides : 2,4-D amine, dolocon, eptam, coteran, linuron, simazine, treflan and gromoxone were recorded on; meiotic abnormalities, size of pollen grains, and pollen viability of Vicia Faba .
2. Two types of treatments were carried; direct treatment of buds and soil treatment with experimental solutions.

3. The induced types of abnormalities were stickiness and sticky bridges.
4. Soil treatment was more effective than direct treatment.
- 5? There was no correlation between pollen sterility and percentage of abnormalities (exception eptam), Linuron which in soil treatment was the most effective pollen sterilizer (59.9) was less effective on percentage of abnormalities .
6. Simazine which gave the highest percentage of abnormal P.M.Cs (41.1) could not affect pollen viability.
7. In the two types of treatment treflan affect the percentage of anomalies (20.2, 19.6) , but proved to be the most effective herbicide on size of P.Gs (55.65)/

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Comparative Study of early growth Stages
and cytological effects of eight herbicides
on plants

By

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Introduction

The rapid progress in the use of herbicides in agriculture has nowadays attracted the attention of botanists. This study is basically assigned to growth and cytological criteria. Eight different organic herbicides belonging to different groups have been tested on three plants namely; Vicia, Faba, Zea mays and Vicia sativa. The herbicides are :

1. 2,4-D amine : amine salt of chlorophenoxy acetic acid.
2. Dalapon : Sodium salt of 2,2-dichloropionic acid.
3. Eptam : Ethyl N.N-Di-n-propyl thiocarbamate.
4. Linuron : 3- (3,4-dichlorophenyl) - 1-methyl methoxy
-1- methyl urea.
5. Cotoran : N-(3- trifluoromethyl phenyl) -N-N
dimethyl urea.
6. Simazine : 2- Chloro-4-6, bis (ethyl amino) -S-
triazine.

7. Treflan : : ,a,a,a - trifluoro -2,6- dinitro- N,N-
dipropyl-P- toluidine.
8. Gramoxone : I-I- dimethyl -4-4- dipyridylum dihydrochlorides.

Material and Methods

The present study was carried out on Vicia faba (var. Giza 1), Zea mays (Shami, hybrid 17) and Vicia sativa (vetch). Concentrations of the herbicides used were 0.1, 1, 10, 100, 500 ppm. for treflan, dalapon, gramoxone, and 2,4-D amine (water soluble). For slightly soluble herbicides, concentrations used were 0.1, 1, 10, 100 and 375 ppm for eptam, 0, 1, 1, 10 and 90 ppm. for cotoran, 0.1, 1, 10 and 75 ppm. for linuron and 0.1, 1, and 5 ppm, for simazine.

For early growth stage studies, 20 seedlings with roots 2 cm. in length were planted in jars containing the herbicide solutions. Tap water was used for controls. Root length was measured every 24 hours. Observations on seedlings with roots immersed in well aerated solutions were continued for 10 days, the fresh weights of the plants were recorded and also mean increase in root length. The results were taken of the mean of 2 replicates.

For mitotic study broad beans (Vicia faba) was chosen as an example. Four days old roots were dipped in the different concentrations of the herbicide solutions for 24 and 72 hours. Tap water was used for control experiments. Counts were made from permanent root tip squash preparations stained with Feulgen.

Results and Discussion

1. Early growth effects :

The effects of different concentrations of the used herbicides on early growth stages of broad beans, maize and vetch have revealed that nearly all concentrations inhibited root growth, (Figs 1-3). However, higher concentrations inhibited lateral root initiation and growth as well. The daily growth rates of the treated plants were usually less than the controls in nearly all herbicides used, (Figs 4-II); the degree of inhibition increased with increase of concentration. Some higher concentrations such as 500 ppm 2,4-D amine, and trellan, 375 ppm optam and 100 ppm gramoxone were toxic. Diminution in the fresh weight of the treated plants occurred in nearly all herbicides used and specially at higher concentrations.

Tolerance of the tested plants could be measured by determining the strength of the herbicide solution which causes 50%

diminution of fresh weight. These concentrations are reported in Table I(a). Tolerances of the tested plants towards various experimental herbicides could be arranged in ascending order as follows :

2,4-D amine, eptam, cotoran and Simazine :

1- Vetch 2- broad beans 3- maize.

Dalapon, and linuron ;

1- Vetch 2- Maize 3- broad beans

Treflan ;

1- Vetch 2- maize and broad beans.

Gramoxone :

1- nearly equal tolerance of the three plants.

Table I (a)

Herbicide concentration causing 50 % diminution of plant fresh weight

Agent	Tested plants		
	Vetch	Broad beans	Maize
1. 2,4-D amine	0.1 ppm	1 ppm	100 ppm
2. Dalapon	0.1 "	100 "	1 "
3. Eptam	0.1 "	1 "	100 "
4. Cotoran	0.1 "	1 "	90 "
5. Linuron	1 "	-	10 "
6. Simazine	1 "	-	-
7. Treflan	0.1 "	10 "	10 "
8. Gramoxone	1 "	1 "	1 "

Fig (I) shows root failure to elongate normally and club-shaped roots in corn as a results of treatment with treflan.

A similar observation was reported by Haçskaylo and Amato (1968).

Kuratle (1969) found that linuron decreased cell activity, root length, dry weight and increased root width in cucumber.

Table I (b)

Herbicide causing 50 % diminution in fresh weight of broad beans in 10 days arranged in descending order effect.

- 1- 2,4-D amine, gramoxone, eptan, cotoran.
- 2- Simazine, treflan, linuron.
- 3- Dalapon.

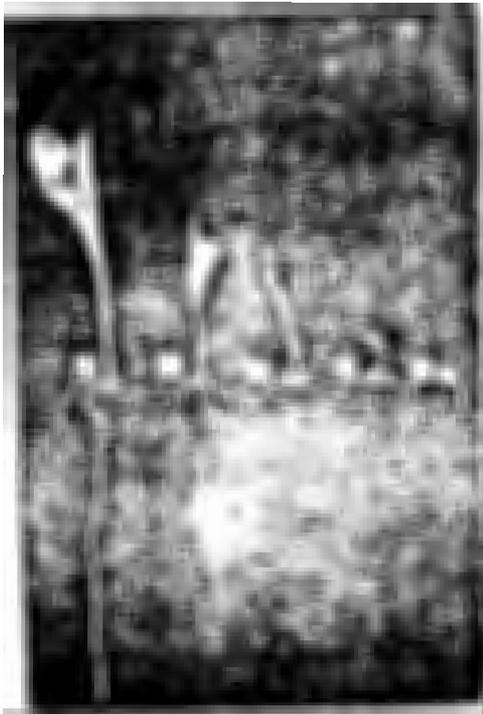


Fig (I)

Control maize plant (16 days old).

2,3,4,5 Treated maize plants with O.I. I. IC. 100 ppm treflan.

Note swelling of the root tips.

Z-9

- 209 -

(7)

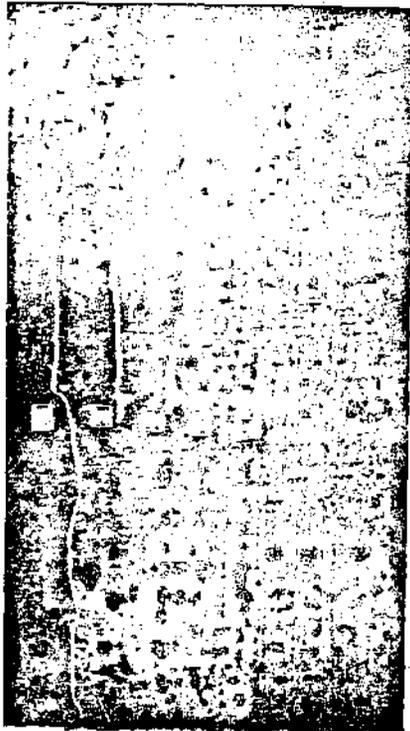


Fig (2)

- I Control Vetch plants (14 days old)
2,3,4,5 Treated Vetch plants with 0.1, 1, 10, 100 ppm.
2,4-D amine.

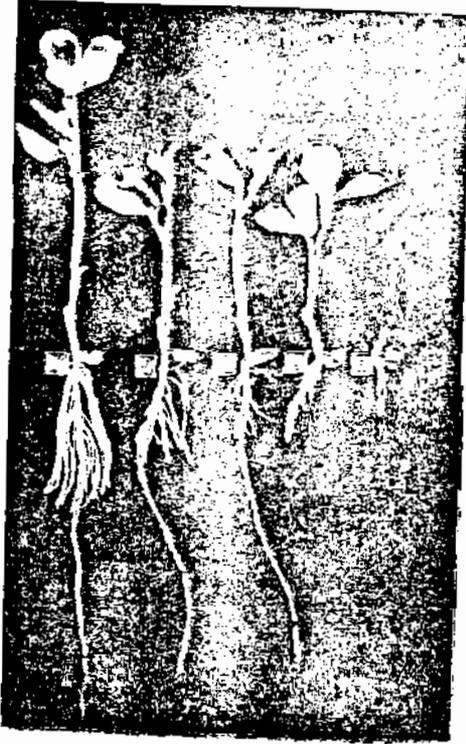


Fig (3)

- I. Control Vicia plant (14 days old.)
2,3,4,5 Treated Vicia plants with 0.1, 1, 10, 90 ppm. cotoran.

Note: chlorosis of 10 ppm treated plants

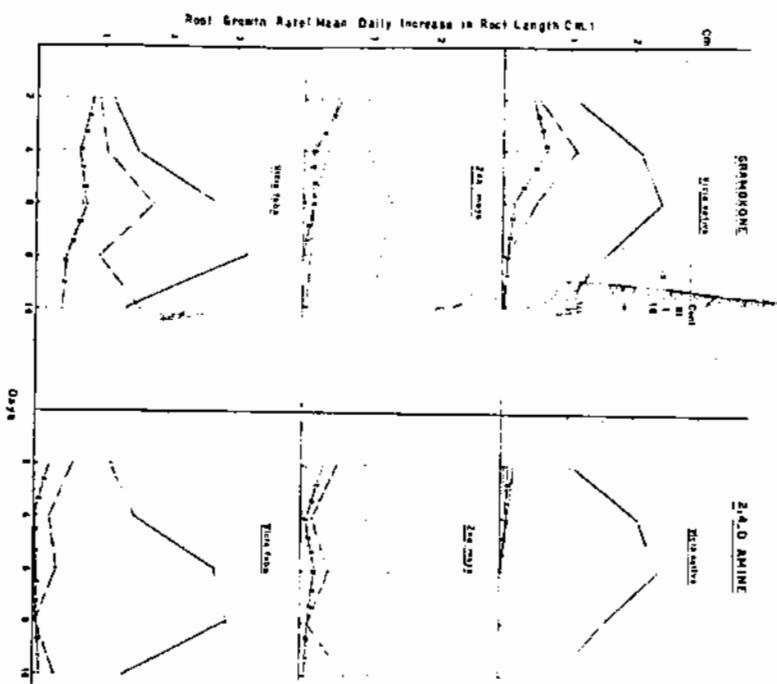


FIG (4) : Fresh weight/plant and root growth (length) of beans, maize and vetch as affected by 2,4-D amine and Gramoxone.

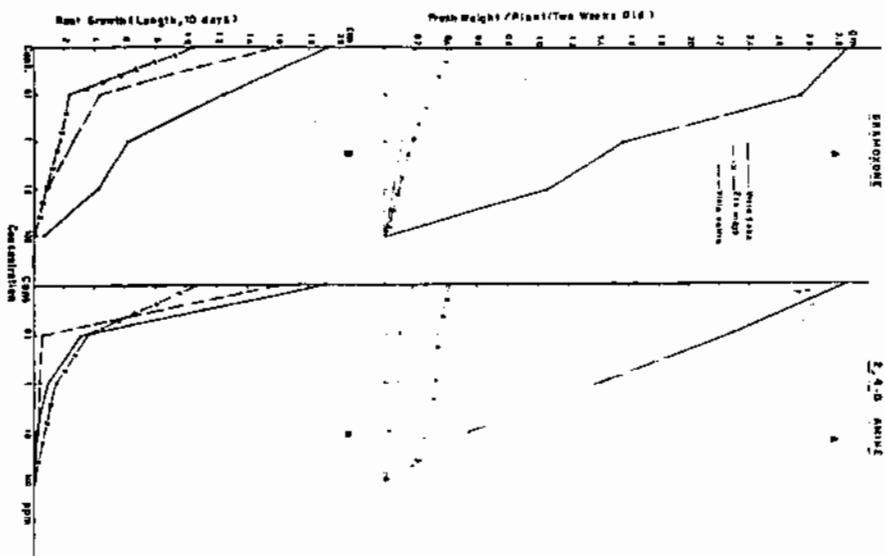


FIG (5) : Root Growth rate of beans, maize and vetch as affected by 2,4-D amine

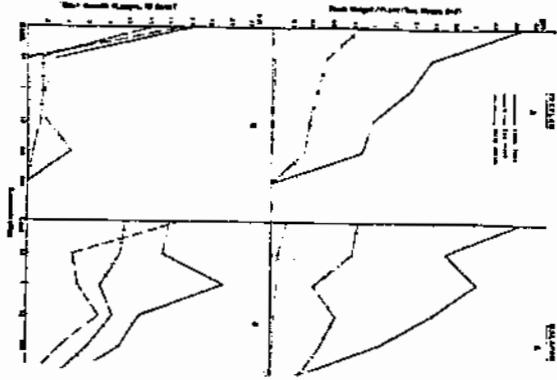


Fig (6) : Fresh weight/plant and root growth (length) of beans, maize and vetch as affected by trellis and delpon.

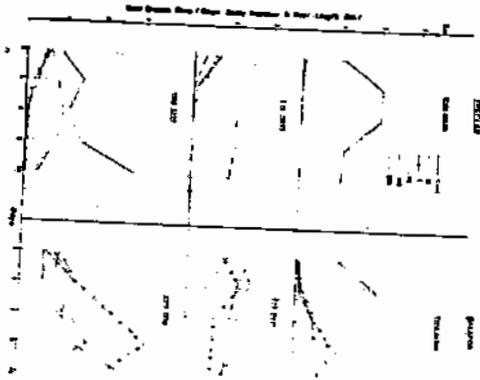


Fig (7) : Root growth rate of beans, maize and vetch as affected by trellis and delpon.

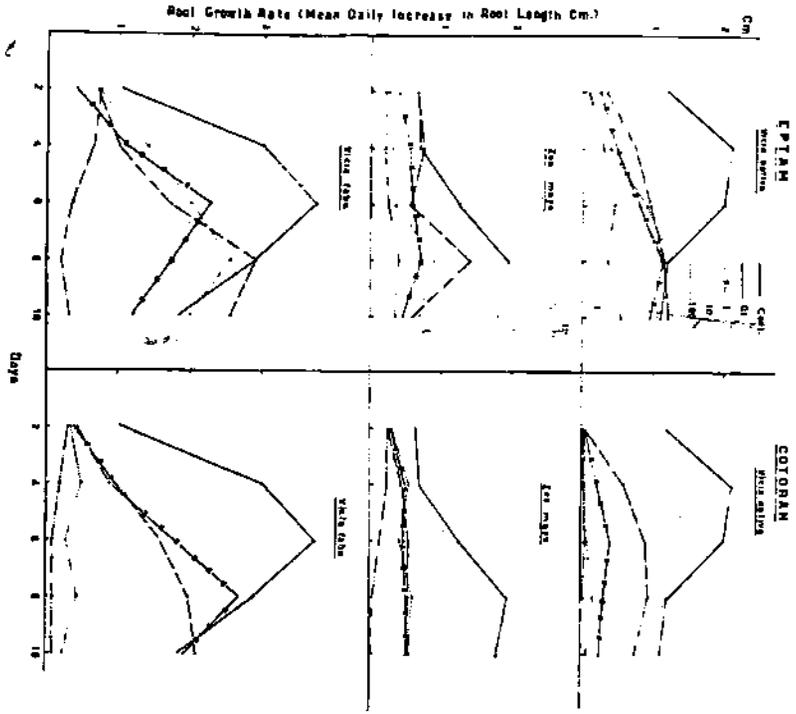


Fig (8) : Fresh weight/plant and root growth (length) of beans, maize and vetch as affected by eptam and Cotoran.

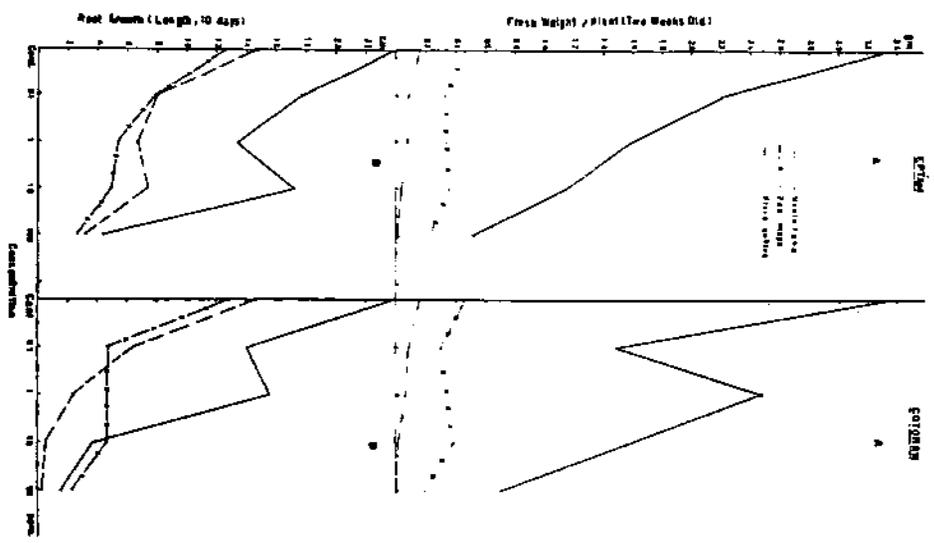


Fig (9) : Root growth rate of beans, maize and vetch as affected by eptam and Cotoran.

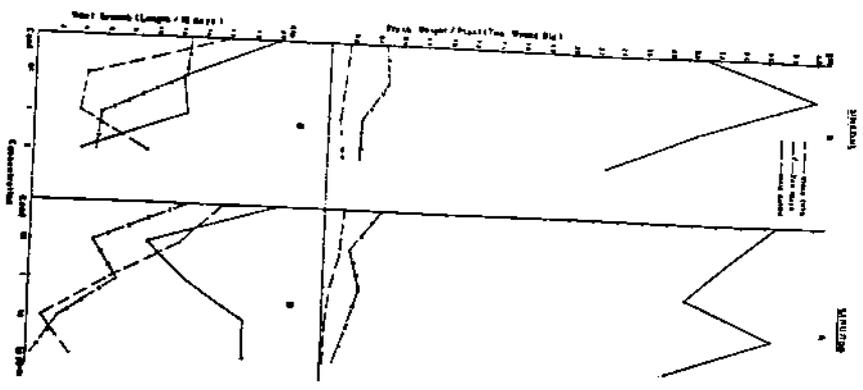


FIG (10) : Fresh weight/plant and root growth (length) of beans, maize and vetch as affected by Slinazine and Ilnurin.

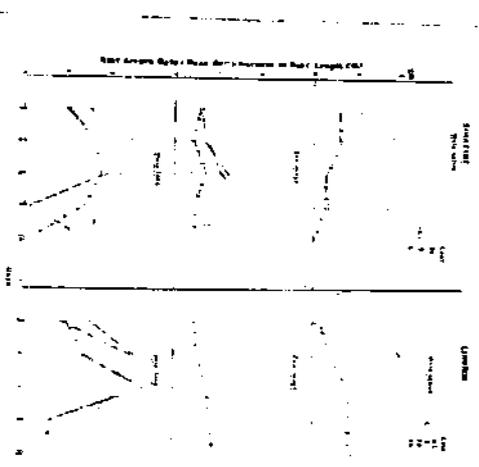


FIG (11) : Root growth rate of beans, maize and vetch as affected by Slinazine and Ilnurin.

(13)

Cytological effects :

Comparing the effects of herbicides on mitotic index; it is observed that simazine, eptam, linuron, dalapon and cotoran have slight inhibiting action on mitotic index in 24 hours treatment, the degree of inhibition rises with increase of concentration and time of treatment (Table 2). Gramoxone, treflan, 2,4-D and eptam caused either toxic effect in higher concentration, or increase in rate of inhibition of mitosis.

Thus most of the herbicides used in this study can be considered as mitotic poisons, though at different degrees. This is apparent from the obtained figures of root growth rates and their simulates in mitotic indices. Nearly 50 % inhibition of mitosis was induced by 0.1 ppm and 1 ppm 2,4-D amine for 24 and 72 hours treatment, 5 ppm simazine and 10 ppm linuron for 72 hours treatment (Table 3). It is noticed that a concentration of 0.1 ppm 2,4-D amine was effective in causing 50% diminution in mitotic index as compared with 500 ppm for dalapon. Reference to Table 1 (b) shows concordance of results with 2,4-D as the strongest root growth inhibitor and dalapon as the weakest.

Table 2

(24)

Mitotic index of *Vicia faba* as affected by the used herbicides

Experimental agent	Mitotic index after treatment for		Experimental agent	Mitotic index after treatment for	
	24 h.	72 h.		24 h.	72 h.
<u>2,4-D acine</u>			<u>Cotoren</u>		
Cont.	82 ± 5.3	79 ± 5.1	Cont.	82 ± 5.3	79 ± 5.1
O.I ppm	50 ± 8.4	38 ± 12.5	O.I ppm	72 ± 6.3	66 ± 14.1
I "	40 ± 11.2	28 ± 4.9	I ppm	64 ± 4.0	67 ± 8.4
IO "	35 ± 13.4	27 ± 13.2	IO ppm	65 ± 8.1	61 ± 3.1
IOO "	32 ± 8.4	17 ± 9.2	90 "	56 ± 7.8	52 ± 2.6
500 "	30 ± 11.1	toxic			
<u>Dalapon</u>			<u>Sinazine</u>		
Cont.	82 ± 5.3	79 ± 5.1	Cont.	82 ± 5.3	79 ± 5.1
O.I ppm	76 ± 9.3	68 ± 4.0	O.I ppm	72 ± 5.3	56 ± 15.7
I "	76 ± 13.2	67 ± 6.7	I "	70 ± 3.4	53 ± 16.2
IO "	77 ± 4.4	64 ± 4.4	5 "	68 ± 4.4	40 ± 6.2
IOO "	59 ± 8.4	57 ± 11.2			
500 "	58 ± 13.2	53 ± 13.6			
<u>Eptam</u>			<u>ureflan</u>		
Cont.	82 ± 5.3	79 ± 5.1	Cont.	82 ± 5.3	79 ± 5.1
O.I ppm	71 ± 7.6	63 ± 9.6	O.I ppm	64 ± 12.0	64 ± 11.2
I "	72 ± 6.7	55 ± 11.5	I "	64 ± 9.4	65 ± 4.9
IO "	72 ± 11.8	62 ± 12.2	IO "	66 ± 4.8	61 ± 15.2
IOO "	71 ± 12.4	58 ± 4.0	IOO "	63 ± 13.2	54 ± 15.3
375 "	toxic		500 "	48 ± 9.0	toxic
<u>Linuron</u>			<u>Gromoxone</u>		
Cont.	82 ± 5.3	79 ± 5.1	Cont.	82 ± 5.3	79 ± 5.1
O.I ppm	76 ± 6.7	51 ± 14.2	O.I ppm	72 ± 8.0	63 ± 14.1
I "	77 ± 9.3	50 ± 12.8	I "	66 ± 13.7	62 ± 14.9
IO "	71 ± 8.4	42 ± 14.7	IO "	57 ± 14.2	55 ± 4.0
75 "	69 ± 12.2	46 ± 9.4	IOO "	toxic	toxic

(15)

Table (3) : The concentration of herbicide causing about 50 % diminution in M.I in broad beans for 72 h. soak treatment in descending order :

Agent	Concentration	Agent	Concentration
1. 2,4-D acine	0.1 pps	5. Eptan	100 pps
2. Simazine	5 "	6. Treflan	100 "
3. Linuron	10 "	7. Cotoran	100 "
4. Gramoxone	10 "	8. Dalapon	500 "

Some other workers noticed similar results. Talbert (1965) and Bayer et. al. (1967) reported that treflan was a mitotic poison in treated plants. Hacskeylo and Amato (1968) found that cell division was markedly inhibited in treflan treated plants.

Schultz et al. (1958) found as a result of treatment of maize roots with treflan solution, radial enlargement of cortical cells and multinucleate cells in the meristematic regions; synthesis of DNA and RNA and protein was suppressed.

Chkanikov et al (1968) reported that the main reason for the resistance of graminaceous species to 2,4-D is that most of the chemical was found bound to subcellular structures and cytoplasmic protein. In dicotyledonous species, on the other hand most 2,4-D was found as free acid in both tolerant and sensitive species. All plants contained a non protein 2,4-D

derivative which released 2,4-D on acid hydrolysis, no correlation was found between quantity of this substance and the degrees of plant resistance to 2,4-D.

Summary

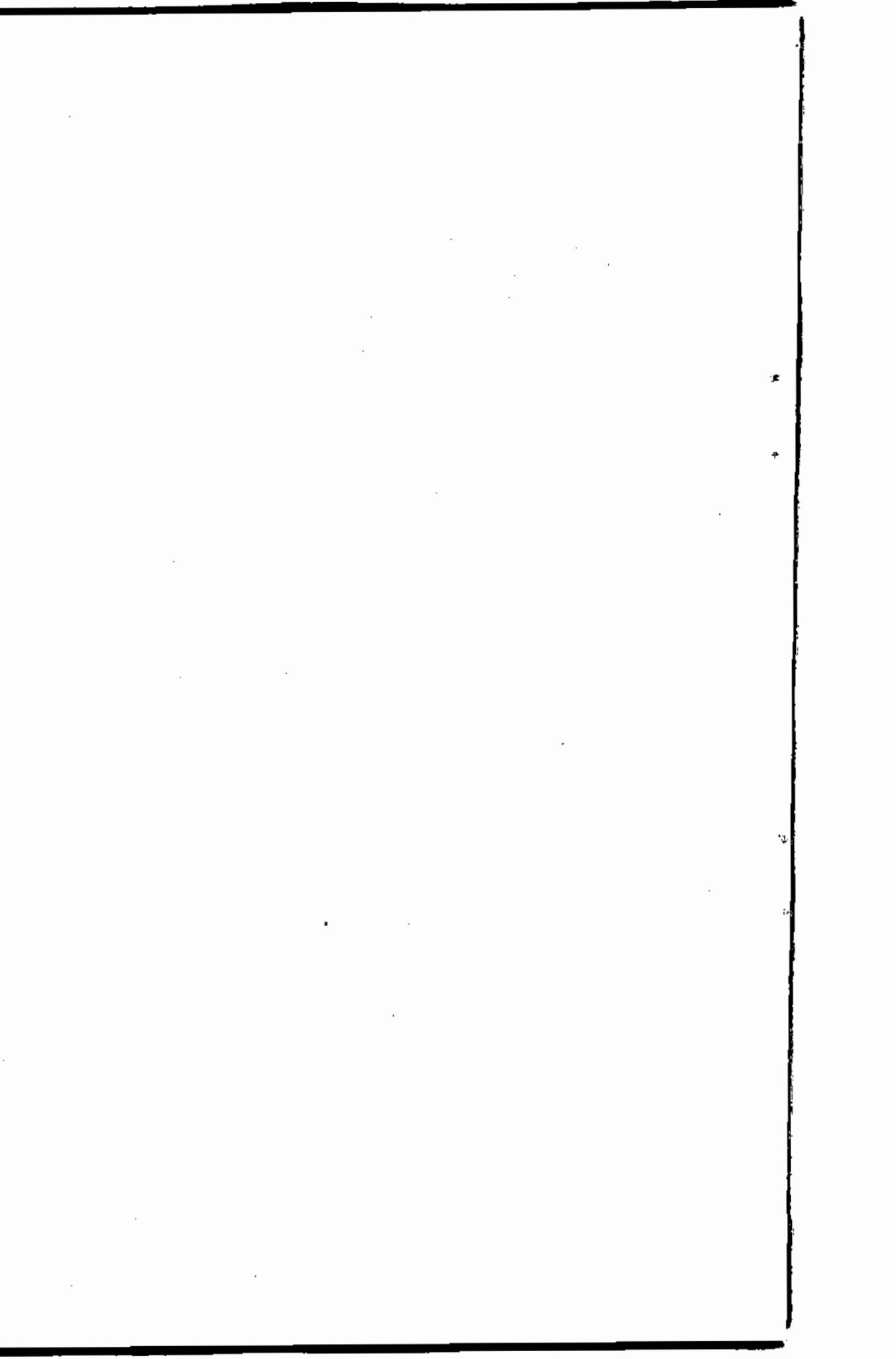
The present study aimed to compare the effect of eight herbicides, namely : 2,4-D amine, dalapon, eptam, cotoren, linuron, simazine, triflan and gramoxone on early growth stages of three plants, namely; Vicia faba, Zea mays and Vicia sativa. For mitotic studies Vicia faba was chosen as an example.

The daily growth rates of treated plants decreased than that of controls. Tolerance of tested species was measured by the strength of herbicide solution causing 50 % diminution of fresh weight. Vicia faba was the most tolerant plant, followed by Zea mays.

Inhibition of root growth was accompanied by decrease in mitotic index. Inhibition of mitosis increased with increase of concentration and time of treatment.

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19

Effect of the Spray of the Mineral Fertilizer
"Schouchan" on the Chlorophyll Content and
Photosynthesis of Okra Leaves.

By

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Abstract. Schouchan, in general, increased chlorophyll content and the rate of photosynthesis of okra leaves. The maximum effect was observed after 30 hours of application. It started to decrease afterwards but remained above the control.

"Schouchan" is a spray fertilizer manufactured by El-Nasr Chemical Co. Afifi and Abdulla (in press) reported that spraying okra plants variety "Gold Coast" with this fertilizer resulted in a decrease of the growth vigour of the plant. Analysis of the shoot-system of the treated plants showed a general decrease in ammonia-N and organic phosphorus.

In the present paper, the effect of "Schouchan" on the chlorophyll content and photosynthesis of Okra leaves will be presented.

221

Material and Methods

were

Seeds of okra, variety "Gold Coast" cultivated in 25 cm-diameter pots containing clay soil. Ten pots were used for each treatment. The pots were irrigated every other day with equal amounts of tap water. Forty days old plants were sprayed with half, one and double field dose of the fertilizer suspension. The concentration of the recommended field dose equals 1 gm/300 ml distilled water. The mineral fertilizer "Schouchan" was reported to contain N₂, P, Ca, Mg, Fe, Cu, S, B, Zn and Mn.

After different hours of application, leaves of control and treated plants were homogenized in 50% acetone and their chlorophyll content was calculated according to Vernon (1960). At the same time, the rate of photosynthesis of leaf discs was carried out in a Warburg apparatus using the method adopted by Goren (1969). A constant concentration of 2% atmospheric CO₂ was maintained in the Warburg vessels by carbonate buffer (Bladergroen, 1960). The experiments were carried out at 20°C and under light intensity of 3000 footcandle.

Results

After thirty hours of "Schouchan" treatment, the total chlorophyll content of okra leaves (fig.1) was found to be 100%, 130% and 132% of the control when half, one and double field dose were used respectively. Chlorophyll a (fig.2) was found to be 98%, 120% and 143% respectively, and chlorophyll b

(fig.3) was found to be 114 %, 150 % and 114 % respectively.

After one hundred hours of application, the total chlorophyll content of okra leaves (fig.1) was found to be 103 %, 103 %, and 108 % of the control at the above mentioned concentrations respectively. Chlorophyll a (fig.2) was found to be 101 %, 102 % and 100 % of the control and chlorophyll b (fig.3) was found to be 106 %, 110 % and 100 % respectively.

The rate of photosynthesis of excised okra leaves (fig.4) was found to be 111 %, 115% and 135% of the control in the first hour after application with half, one and double field dose respectively. After thirty hours of application, the rate of photosynthesis was found to be 110 %, 118 % and 163% of the control. After one hundred hours of application, it was found to be 110 %, 113 % and 125 % of the control.

Discussion

The possibility of supplying nutrient elements to the plants as foliar spray (Boynton, 1954; Shereverya, 1959 and Narula et al.; 1967) is undoubtedly of great importance. Ashour (1972) studied the effect of spraying wheat plants with the micro-elements; B, Zn, Mn, Cu and Mo on yield components and chemical constituents of grain.

Although "Schouchar" is used as a spray fertilizer, Afifi and Abdulla (in press) reported that spraying okra plants with this chemical resulted in a significant decrease in the root length, fresh and dry weight of the plants. Analysis of the treated plants showed that the total carbohydrate and the nitrogen fractions decreased with the exception of the ammonia-N fraction which increased. We thought that the effect

of "Schouchan" on the growth vigour and the carbohydrate content of okra plants may be attributed to the effect of the fertilizer on the chlorophyll content and consequently the rate of photosynthesis of the treated plants.

However, the results in this investigation showed that there is a transient increase in the chlorophyll content of okra leaves in the first thirty hours after application. This increase in the chlorophyll content started to decrease with time. After one month's course of application chlorophyll content - the important pigment in photosynthesis - was almost the same as that of the control.

It was also observed that the rate of photosynthesis was increased in the first thirty hours of application and started to decrease with time, but remained above the control.

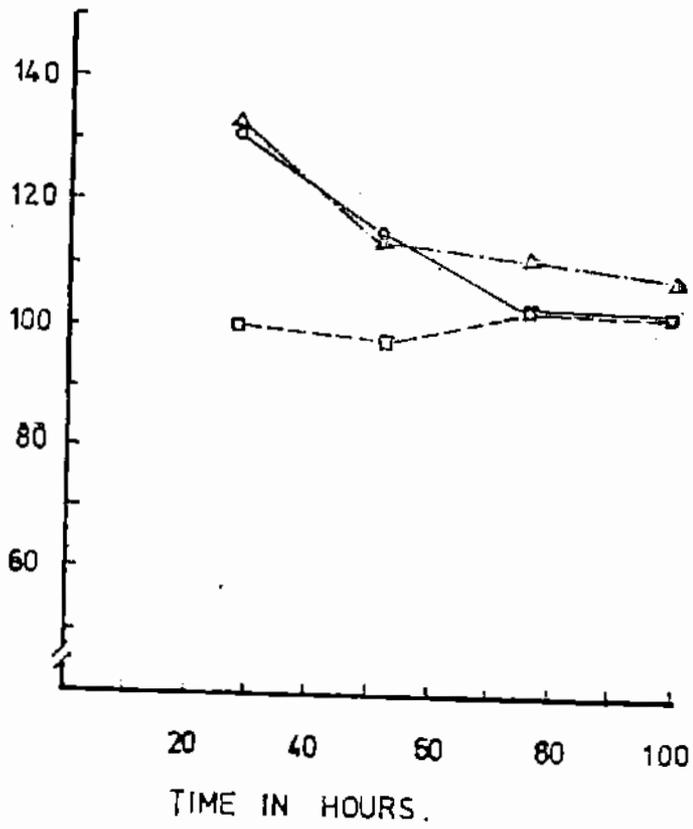
Fabry et al. (1973) studied the effect of trace elements on *Spinacia oleracea* and found that boron and manganese activated chlorophyll biosynthesis in the plants. Thus the increase in chlorophyll content and the consequent increase in the rate of photosynthesis due to "Schouchan" application reported in the present investigation may be attributed to the presence of boron and manganese. However, the effect of "Schouchan" on the growth vigour, carbohydrate and nitrogen content of okra plants, reported by Afifi and Abdulla (in press), could not be attributed to the effect of the fertilizer on the chlorophyll content and photosynthesis of okra plants.

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WATER SOLUBILITY COEFFICIENT, % OF CONTROL.



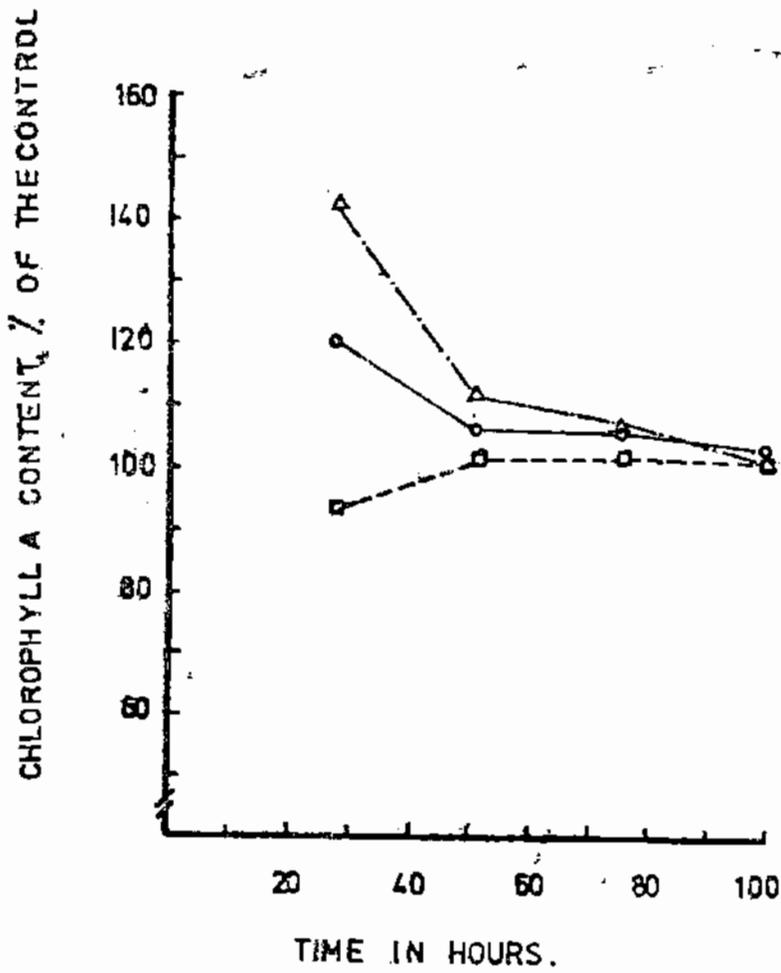


Fig. 2. Effect of "Schouchan" on chlorophyll

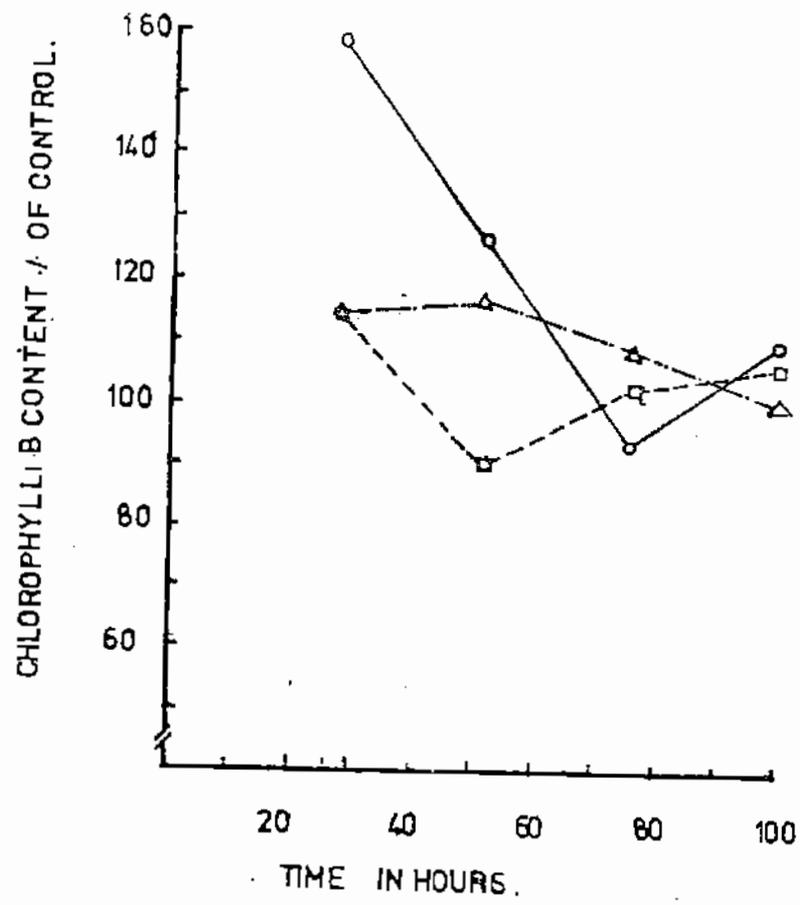


Fig. 3. Effect of 'Sneuchan' on chlorophyll b content

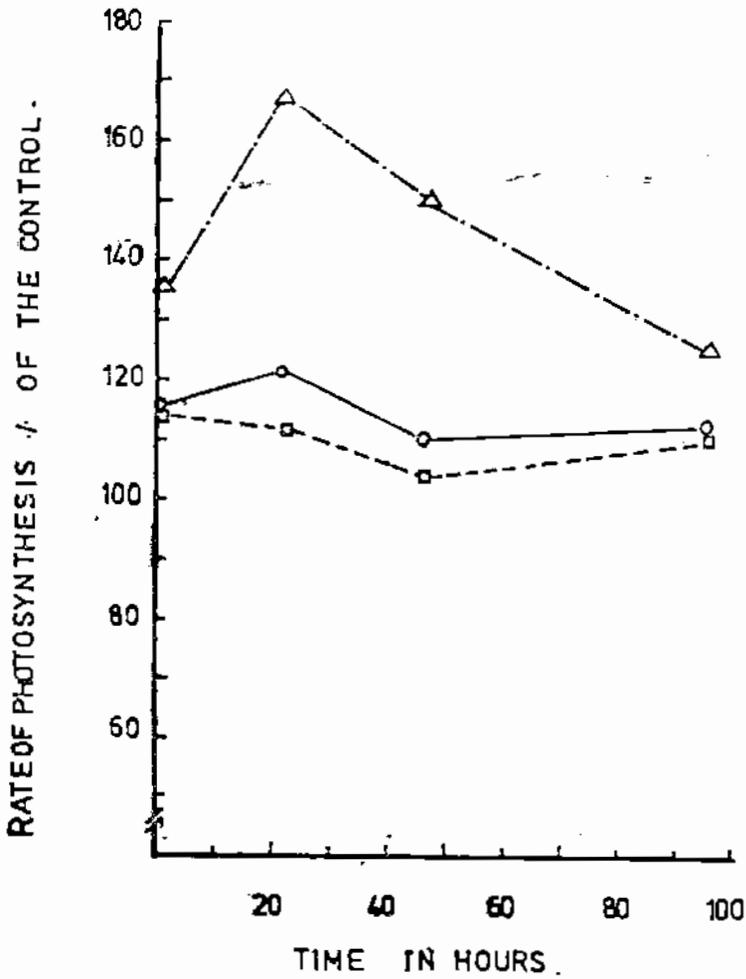
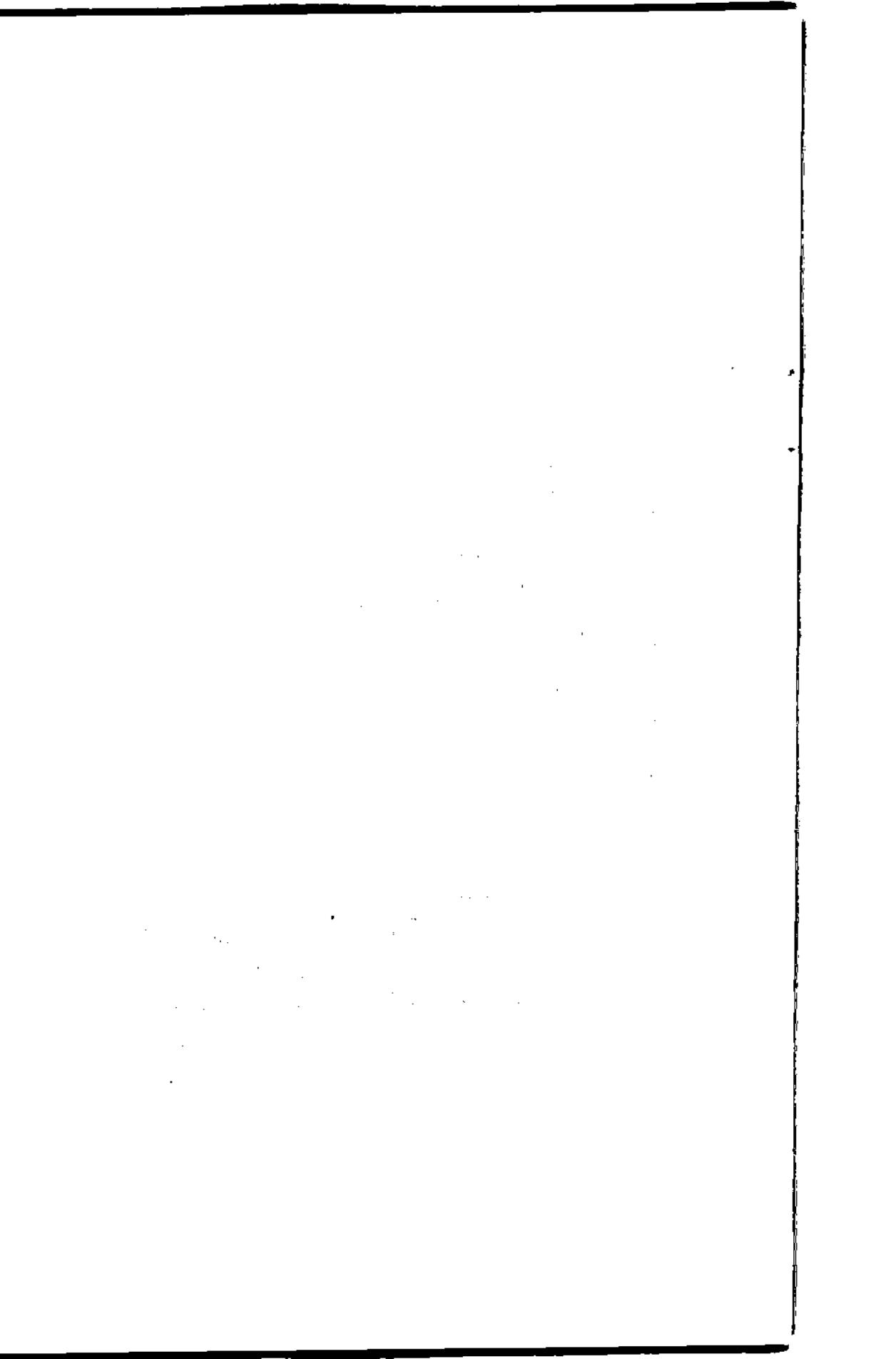


Fig 4: Effect of "Schouchan" on the rate of photosynthesis of okra leaves (□ - 1/2 Field dose, ○ - Field dose, ▲ - 2 Field dose)



THE EFFECT OF CERTAIN PRE-SOWING SEED TREATMENTS AND EARLY
PHOSPHORUS SUPPLEMENT ON MINERAL COMPOSITION OF CORN

(Zea mays L.) PLANTS GROWN UNDER SOIL

MOISTURE STRESS CONDITIONS

By

A.I.Gabr*, M.T.El-Saidi**, M. El-Kadi*, and H. A. El-Zeiny**

I. INTRODUCTION

Certain trials aiming to raise the drought resistance of plants are met with in literature. In this area of research, the effective use of such methods as Henckel's seed treatment (e.g. Henckel, 1963), early fertilization with phosphorus supplements (Samuilov and Maslova, 1963), seed soaking in salt solution of Zn (Matukhin *et al.*, 1962) or Mn (e.g. Novitskaya, 1958) has been reported. All these methods, together with seed-soaking in a Ca salt solution alone or followed by a hardening treatment according to Henckel, were generally shown by El-Zeiny (1972) to be of benefit in raising the drought resistance of corn plants through increasing the grain yield.

It seems necessary to get information about the metabolic

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Plants treated in such ways, the data thus obtained helpful in accounting for the raised drought resistance. the metabolic aspects that could be concerned, is the composition of plant tissues, particularly that related to nitrogen, phosphorus and potassium.

should be recognized that, in literature, data dealing influence exerted upon the content of any of these minerals in corn plants due to treatments used to increase drought resistance are conspicuously deficient. The present accordingly carried out to throw some light upon the shown under such conditions. The soil moisture stress and either to be maintained throughout the growth or to be restricted to the period starting from tasselling. moisture deficit during such period was revealed to be influencing in decreasing the yield of corn plants (see et al., 1963 and El-Saidi, 1969).

II. MATERIAL AND METHODS

The present work was conducted during the 1968 season in my Laboratory, National Research Centre, Cairo, using a male D., variety Giza hybrid 67. Plants were grown "banks from tin", measuring 30 cm in diameter x 50 cm. t. The inner surface of pots was coated with three of bitumen to prevent direct contact between soil and

metal. Every pot contained 30 kg. of air-dry Nile-clay soil, and a special drainage system was employed, so that water movement was from the base upwards.

The scheme of experiment is presented in Table (1). The maize grains were subjected before sowing to different treatments, then the plants were grown at any of three soil moisture levels, an additional dose of P being supplied to seedlings in certain cases. For each soil moisture level in any given treatment, 9 replicates were used. The pre-sowing soaking of grains was maintained for 24 hours. CaCl_2 solution was 0.25 M; whereas each of the ZnSO_4 and MnSO_4 solutions was 0.05 %. In both series II and III, the soaked grains were exposed to a hardening process according to Henckel (1964). In this process, the soaked grains were kept in Petri-dishes in an incubator at 32°C for 24 hours.

Sowing was always carried out at the rate of 5 grains per pot, and the seedlings were thinned to two after one week. Phosphorus and potassium fertilizers were added to soil before sowing at the rate of 5 g. P_2O_5 per pot in the form of calcium superphosphate for the former and 2 g. K_2O in the form of potassium sulphate for the latter. Nitrogen fertilizer was added at the rate of 5 g. N per pot in the form of ammonium nitrate at three portions : 2, 2 and 1 g. during seedling stage, at the

: Types of treatments before and after the sowing of corn grains.

ts	Type of pre-sowing seed treatments	Sub-treatments	Soil moisture (% of water holding capacity)	
			Seedling-tasseling stage	Tasseling-maturity stage
	Distilled water	A	65	65
		B	30	30
		C	65	30
I	Distilled water ²	A	65	65
		B	30	30
		C	65	30
II	Distilled water + hardening	A	65	65
		B	30	30
		C	65	30
I	CaCl ₂ + hardening	A	65	65
		B	30	30
		C	65	30
V	CaCl ₂	A	65	65
		B	30	30
		C	65	30
V	ZnSO ₄	A	65	65
		B	30	30
		C	65	30
C	MnSO ₄	A	65	65
		B	30	30
		C	65	30

In this series were soaked, as in control, in distilled water but the plants were supplied at the seedling stage with additional dose of superphosphate; thus the total phosphorus was not reached (total phosphorus ...)

appearance of 10th leaf, and at the start of tasseling respectively.

Sampling was carried out four dates : at the appearance of 10th leaf (after 23 days from sowing), almost at the time between the appearance of the 10th leaf and beginning of tasseling, at the beginning of tasseling, and at the start of milky stage. Each sample consisted of three plants from three pots; rethinning being carried out after the first sample to leave one plant per pot. Such sampled plants were separated into different organs. Both leaves and stems are dried at 70°C for 48 hours, then the dry materials of the three replicates were finely ground, mixed together and kept for the following analysis:

- 1) Total nitrogen content : being determined coulourimetrically as described by Yuen and Pollard (using Nessler reagent.
- 2) Total phosphorus content as P_2O_5 : being determined coulourimetrically by the hydroquinone method, as described by Snell and Snell (1954).
- 3) Potassium content as K_2O : being determined using Beckman flame photometer, as described by Brown and Lilleland (1946).

III. RESULTS

Effect of presowing seed treatments and early phosphorus supplement on:

- 1) Total nitrogen content of stem and leaves of corn plant:

the relative content of nitrogen in corn plants (mg./g. dry wt.) at different developmental stages as influenced by pre-soaking seed treatments followed by a special water regime.

Sub-treatments	Sample 1		Sample 2		Sample 3		Sample 4	
	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves
A	2.15	2.52	1.94	2.23	1.55	1.97	1.55	1.72
B	2.38	2.91	2.24	2.75	1.83	2.37	1.87	2.15
C	-	-	-	-	-	-	1.55	1.67
Mean	2.25	2.72	2.09	2.49	1.70	2.17	1.47	1.91
A	2.35	2.80	2.10	2.51	1.75	2.29	1.61	2.11
B	2.68	3.20	2.41	2.97	2.03	2.55	1.80	2.27
C	-	-	-	-	-	-	1.70	2.14
Mean	2.52	3.10	2.28	2.79	1.88	2.42	1.70	2.17
A	2.32	2.75	2.06	2.55	1.59	2.25	1.54	2.10
B	2.64	3.14	2.56	2.80	2.01	2.45	1.72	2.17
C	-	-	-	-	-	-	1.55	2.12
Mean	2.48	2.95	2.21	2.75	1.85	2.35	1.64	2.13
A	2.50	2.75	2.01	2.70	1.65	2.21	1.58	2.12
B	2.58	2.95	2.55	2.79	1.95	2.38	1.71	2.17
C	-	-	-	-	-	-	1.62	2.13
Mean	2.44	2.84	2.18	2.75	1.81	2.30	1.64	2.14
A	2.33	2.74	2.07	2.68	1.71	2.20	1.59	2.09
B	2.55	3.12	2.29	2.85	1.97	2.45	1.66	2.18
C	-	-	-	-	-	-	1.64	2.11
Mean	2.50	2.93	2.18	2.76	1.84	2.35	1.64	2.15
A	2.27	2.71	2.15	2.68	1.65	2.20	1.55	2.09
B	2.54	3.05	2.25	2.81	1.90	2.41	1.72	2.16
C	-	-	-	-	-	-	1.65	2.10
Mean	2.41	2.89	2.20	2.75	1.78	2.31	1.63	2.12
A	2.25	2.69	1.97	2.44	1.60	2.05	1.45	2.00
B	2.50	2.97	2.50	2.63	1.86	2.47	1.70	2.21
C	-	-	-	-	-	-	1.60	2.07
Mean	2.38	2.83	2.14	2.64	1.74	2.26	1.59	2.09
A	2.28	2.71	2.04	2.57	1.66	2.17	1.53	2.05
B	2.57	3.05	2.36	2.63	1.94	2.44	1.71	2.19
C	-	-	-	-	-	-	1.60	2.08

stress led to an increase in the nitrogen percentage in both stems and leaves, comparing with the case of normal irrigation. Such a trend was found to be consistent in all treatments and for the whole growth season. The extent of increase shown in this concern for a given plant organ was a function of the type of treatment used, the time of sampling and the duration of drought.

The data presented in Table (2) revealed further that the employment of any of the studied treatments led to higher values, comparing with control, for the relative content of nitrogen in either stems or leaves, irrespective of the soil moisture level used, and the stage of development concerned. The treatment of early phosphorus supplement appeared generally to be the most effective in this concern; whereas the converse tendency was shown in most cases when soaking the seeds in $MnSO_4$ solution.

2) Total phosphorus content of stem and leaves :

From Table (3), it could be shown that the exposure of plants to drought led generally to a rise in phosphorus percentage in both stems and leaves, comparing with the case of normal irrigation. Such a trend appeared to be consistent,

P₂₅

the relative content of phosphorus in corn plants (mg./g. dry
 .) at different developmental stages as influenced by pre-
 sowing seed treatments followed by a special water regime.

Sub- treat- ments	Sample 1		Sample 2		Sample 3		Sample 4	
	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves
1	1.20	1.10	0.82	0.98	0.73	0.91	0.57	0.83
2	1.42	1.26	0.92	1.19	0.68	1.01	0.75	0.96
3	-	-	-	-	-	-	0.71	0.87
mean	1.51	1.18	0.87	1.09	0.62	0.95	0.71	0.89
1	1.54	1.27	0.95	1.14	0.84	1.07	0.75	0.97
2	1.61	1.40	1.14	1.23	0.96	1.17	0.85	1.04
3	-	-	-	-	-	-	0.80	0.99
mean	1.48	1.34	1.05	1.19	0.90	1.12	0.79	1.00
1	1.29	1.25	0.92	1.17	0.82	1.03	0.71	0.95
2	1.50	1.35	1.03	1.21	0.90	1.11	0.79	0.98
3	-	-	-	-	-	-	0.74	0.98
mean	1.40	1.30	0.98	1.19	0.85	1.07	0.75	0.98
1	1.22	1.26	0.85	1.17	0.78	1.01	0.68	0.95
2	1.48	1.51	0.95	1.20	0.90	1.08	0.77	0.97
3	-	-	-	-	-	-	0.72	0.95
mean	1.35	1.29	0.90	1.19	0.84	1.05	0.72	0.96
1	1.27	1.24	0.92	1.19	0.81	1.02	0.69	0.94
2	1.53	1.36	0.97	1.22	0.91	1.12	0.75	0.98
3	-	-	-	-	-	-	0.73	0.95
mean	1.40	1.30	0.95	1.21	0.86	1.07	0.72	0.96
1	1.27	1.23	0.90	1.18	0.80	1.02	0.69	0.95
2	1.48	1.29	0.94	1.21	0.88	1.08	0.76	0.98
3	-	-	-	-	-	-	0.74	0.95
mean	1.38	1.26	0.92	1.20	0.84	1.05	0.73	0.96
1	1.26	1.21	0.87	1.06	0.78	0.99	0.68	0.91
2	1.45	1.29	0.93	1.21	0.89	1.06	0.76	0.99
3	-	-	-	-	-	-	0.73	0.93
mean	1.36	1.25	0.90	1.14	0.84	1.03	0.72	0.94
1	1.26	1.22	0.89	1.13	0.81	1.01	0.69	0.93
2	1.49	1.32	0.98	1.21	0.90	1.09	0.78	0.99
3	-	-	-	-	-	-	0.74	0.94

duration of drought, appeared to determine the extent of rise obtained in this regard for a given organ. Furthermore, it was revealed that the phosphorus percentage in either organs was generally increased, comparing with control, whenever the studied treatments, particularly that of early phosphorus supplement, were employed. Such picture was noticed throughout the growth season irrespective of the soil moisture level used.

3) Potassium content of stem and leaves :

From Table (4). it appeared that the potassium percentage in stems and leaves of corn plants was raised due to the exposure to drought, comparing with the case of normal irrigation. Such a trend was consistent throughout the growth season for each of the treatments employed. As in case of the relative contents of either nitrogen or phosphorus, the extent of rise in potassium percentage in a given organ due to exposure to soil moisture stress was shown to be dependent upon the kind of treatment used, the stage of development and the duration of drought. In addition, it appeared that the employment of any of the studied treatments led to higher values, comparing with control, for the potassium percentage in either stems or leaves irrespective of the soil moisture level used.

The relative content of potassium in corn plants (dry wt.) at different developmental stages as influenced by pre-sowing seed treatment followed by a special water regime.

Sub-treatments	Sample 1		Sample 2		Sample 3		Sample 4	
	30th Leaves							
A	5.51	3.24	4.71	3.24	2.35	2.47	1.87	1.99
B	5.73	3.60	5.33	3.42	2.57	2.71	2.09	2.13
C	-	-	-	-	-	-	1.92	2.05
Mean	5.52	3.37	5.03	3.18	2.46	2.69	1.96	2.06
A	5.55	3.41	4.95	3.32	2.52	2.58	1.89	1.99
B	5.61	3.43	5.48	3.31	2.68	2.95	2.11	2.11
C	-	-	-	-	-	-	2.00	2.05
Mean	5.68	3.62	5.21	3.42	2.60	2.82	2.00	2.05
A	5.75	3.37	4.91	3.27	2.45	2.39	1.89	1.99
B	5.83	3.75	5.33	3.45	2.63	2.54	2.09	2.13
C	-	-	-	-	-	-	1.92	2.05
Mean	5.81	3.56	5.11	3.36	2.54	2.71	2.01	2.06
A	5.65	3.39	4.73	3.31	2.40	2.31	1.89	1.99
B	5.87	3.72	5.37	3.44	2.61	2.71	2.10	2.13
C	-	-	-	-	-	-	1.93	2.05
Mean	5.75	3.53	5.03	3.38	2.51	2.71	1.97	2.06
A	5.76	3.35	4.82	3.29	2.41	2.62	1.89	1.99
B	5.80	3.76	5.39	3.48	2.64	2.64	2.11	2.13
C	-	-	-	-	-	-	2.00	2.05
Mean	5.81	3.56	5.21	3.37	2.53	2.73	2.00	2.06
A	5.84	3.38	5.20	3.28	2.41	2.60	1.89	2.04
B	5.88	3.68	5.34	3.44	2.61	2.78	2.09	2.09
C	-	-	-	-	-	-	1.98	2.05
Mean	5.86	3.53	5.27	3.37	2.51	2.69	1.99	2.06
A	5.67	3.34	4.77	3.30	2.38	2.57	1.87	1.99
B	5.81	3.65	5.35	3.46	2.58	2.80	2.09	2.13
C	-	-	-	-	-	-	1.92	2.05
Mean	5.74	3.50	5.03	3.38	2.48	2.69	1.96	2.06
A	5.72	3.34	4.86	3.25	2.42	2.60	1.89	1.99
B	5.84	3.71	5.38	3.45	2.62	2.62	2.11	2.11
C	-	-	-	-	-	-	1.98	2.03

IV. DISCUSSION

Our results showed that soil moisture stress led generally to increased values for the relative contents of N, P or K in corn stems and leaves. Such type of observations could agree with the findings of, amongst others, El-Zeiny, (1972) on corn and Gates (1957) on tomato. It might be recognized that most of the data available in literature in this area of research dealt with the response of the content of minerals to drought whenever the seeds were normally treated. According to our results, the type of treatment appeared not to alter the manner of such response.

On the other hand, the relative contents of N, P and K in either stems or leaves appeared in our experiments, in general, to be raised due to the employment of the different treatments used for improvement of drought resistance. It might be thought that the uptake of the concerned minerals was enhanced under these conditions; such enhancement might even have exceeded that in growth. Henckel (1954) reported that hardening increases respiration, and this in turn might be considered as an indirect proof for the increased rate of mineral accumulation under these conditions, probably through the elevation of energy level. On the other hand, the stimulation of dry matter accumulation in both stems and leaves of corn

plants in certain treatments used for the improvement of drought resistance was shown by El-Zeiny (1972) to be of frequent occurrence under drought conditions. It seems that the growth of roots of our experimental plants has been similarly stimulated due to the employment of such treatments; this in turn might contribute to the possible enhanced mineral uptake. In this regard, Curtis and Clark (1950) cited that the increase in phosphorus is likely to increase root growth as well as the $\frac{\text{root}}{\text{shoot}}$ ratio in certain cases. In addition, Henkel (1964) reported that hardened plants have a greater total and absorbing surface in the root system; and also they bear more primary roots. According to the same author, the stimulative effect of seed treating with CaCl_2 on the development and growth of the root system has been met with in literature.

7. SUMMARY

The present investigation aimed mainly to study the effect of certain methods used for increasing the drought resistance on the relative contents of nitrogen, phosphorus and potassium in both stems and leaves of corn plants. The following results were obtained :

- 1) Whatever was the type of treatment used, the relative content of nitrogen, phosphorus or potassium in either corn stems or leaves appeared generally at all developmental stages to be higher under drought conditions, comparing with the case of normal irrigation.

whatever was the level of irrigation, the relative content of nitrogen, phosphorus or potassium, in either corn stems or leaves appeared generally at all developmental stages to be raised, comparing with control, due to the employment of special treatments, particularly that of early phosphorus supplement.

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تأثير بعض معاملات البذرة قبل الزراعة والامداد الفوسفورى المبكر على محتوى
العناصر المعدنية فى نباتات الذرة الشامية النامية تحت ظروف نحر الماء فى التربة

د . عبد الرحمن ابراهيم جبر * د . محمد طلعت الصعيدى * د . محمد القاضى *

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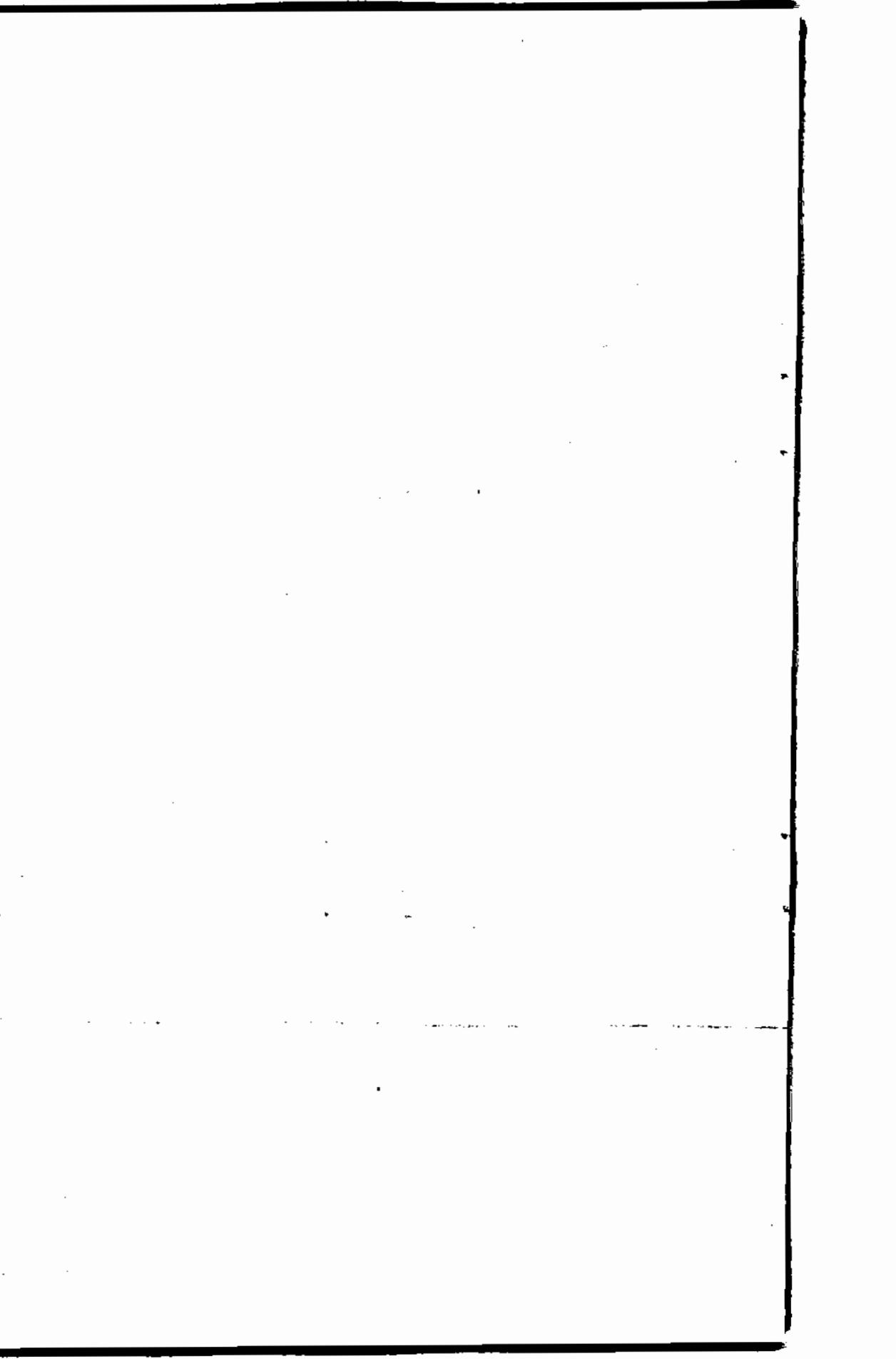
اجرى هذا البحث بهدف دراسة تأثير بعض الطرق المستخدمة لزيادة مقاومة
الجفاف على المحتوى النسبى لكل من النيتروجين والفوسفور والبوتاسيوم فى سوق
وارراق نباتات الذرة الشامية .

وقد اوضحت النتائج ما يلى :

مهما كان نوع المعاملة المستخدمة ، فقد ادى الجفاف عموما خلال جميع
مراحل النمو الى زيادة فى المحتوى النسبى لكل من النيتروجين والفوسفور
والبوتاسيوم فى سوق وارراق نباتات الذرة الشامية (بالمقارنة بالمستوى العادى
للرطوبة الارضية) .

مهما كان مستوى الرى فى اثناء الطرق المستخدمة فى هذا البحث لمقاومة الجفاف ،
خصوصا المعاملة التى استخدمت فيها جرعة اضافية مبكرة من الفوسفور ، فقد
ادت عموما خلال جميع مراحل النمو الى زيادة فى المحتوى النسبى للنيتروجين
والفوسفور والبوتاسيوم فى سوق وارراق نباتات الذرة الشامية (بالنسبة لنباتات
المقارنة) .

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ASPARIGINASE ACTIVITY IN BACTERIA ISOLATED FROM

THE SOIL OF KUWAIT

by

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INTRODUCTION

Amidases especially L-asparaginase and L-glutaminase has received considerable attention due to their practical importance in cancer treatment (CAPPIZZI et al 1970; EL-ASMAR and GREENBERG, 1966). Some neoplastic cells are unable to survive in the absence of L-asparagine yet they lack adequate L-asparagine synthetase activity. Removal of L-asparagine from these cells due to the action of L-asparaginase results in their death (COONEY and HANDSCHUMACHER, 1970). MASHBURN and WRISTON (1964) used E.coli to obtain an L-asparaginase which proved effective against experimental tumors and upon clinical evaluation revealed its special value in the treatment of acute leukaemia. Screening procedures of many bacteria from American type culture collection; International Collection of phytopathogenic bacteria; National Collection of dairy organism; National Collection of Marine Bacteria; National Collection of plant Pathogenic Bacteria revealed the widespread occurrence of L-asparaginase, however, few of them produced substantial amounts of this enzyme (WADE et al. 1971).

Most, if not all, published results on screening procedures for L-asparaginase producers were carried on identified bacteria obtained from various culture collections (WADE et al. 1971). The present investigation aims at the isolation and identification of potent L-asparaginase producer by direct isolation from enriched and non-enriched soil samples.

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Materials and Methods

Isolation, purification and identification of L-asparaginase producing bacteria.

The organisms used in the present investigation were isolated from fertile soil (collected from the agricultural experimental station, Kuwait), either directly or after enrichment. Soil enrichment with L-asparaginase producers was achieved by mixing 1gm L-asparagine with 10gm finely powdered soil and 10 ml sterile water. This was followed by incubation at 28 - 30°C for two weeks. Suitable dilutions of the enriched and non-enriched soil samples were plated on a modified Dox agar medium in which L-asparagine was the only added nitrogen source. This medium contained g/L : Sucrose, 7.5; Asparagine, 2.0; K_2HPO_4 , 0.5; $MgSO_4 \cdot 7H_2O$, 0.25; $FeSO_4$, 0.005; Agar 20. Incubation at 28-30°C continued for 10-12 days, after which all the bacterial colonies that developed were purified by streaking several times on agar plates of the same medium.

Purified bacterial isolates were subjected to morphological examination as well as physiological and biochemical reactions according to the methods recommended in the Manual of Microbiological methods (1957). Tentative identifications were carried out using BERGEY (1974).

Production and assay of L-asparaginase:

The procedure of WADE et al. 1971 was used after applying some modification. This modified procedure could be summarized in the following: A reaction mixture which consisted of 20 μ -moles L-asparagine; 250 μ -moles of tris buffer pH 7.3; 0.5 ml of a 2-6 day old shaken culture of the specified organism (as a source of enzyme) in a total volume of 2 ml, was incubated at 37°C for 30 minutes, after

which the reaction was terminated by the addition of 0.5ml of 1.5 M trichloroacetic acid (TCA). The reaction mixture was then centrifuged at 6000 r.p.m. for 10 minutes after which the amount of ammonia liberated (due to the enzyme activity) was measured spectrophotometrically as follows: 0.2 ml of the supernatant was mixed with 1 ml of 1N-NaOH and after two minutes 1 ml of Nessler's reagent (BDH laboratory reagents). The colour was allowed to develop for 20 minutes at room temperature before its intensity was measured at 450 nm. Two controls were always included, a substrate free control and an enzyme control in which TCA denatured enzyme was used.

The bacterial cultures which were used as a source of enzyme were prepared by inoculating the specified isolate in 50 ml (in a 250 ml conical flask) of modified Dox liquid medium. Incubation at 28 - 30°C on a rotary shaker (120 r.p.m.) continued for 2 - 6 days.

In addition to the modified Dox medium described before, another medium similar to Dox (but with starch instead of sucrose as a carbon source and asparagine as a nitrogen source) and nutrient broth were also tested in an attempt to improve the enzyme production.

The activity of L-asparaginase in whole bacterial cultures was compared with that in the culture filtrate and that in washed bacterial cells. This was carried out by centrifuging the bacterial culture at 6000 r.p.m. for 10 minutes after which the supernatant was removed and the pellet was washed three times in distilled water. The washed cells were resuspended in .04M tris buffer pH 7.5 to restore the volume to the volume of the original culture. The culture filtrate

and the bacterial suspension were used separately as a source of enzyme in the L-asparaginase reaction mixture described before.

Results

Isolation, purification and identification of L-asparaginase producers.

Twenty-four and fifteen bacterial colonies developed on agar plates inoculated with the enriched and non-enriched soil respectively. Upon purification by streaking, on modified Dox agar plates only twenty one isolates most of them obtained from the enriched soil were able to survive Gram stain, morphological and physiological studies of these isolates suggested that they represent six genera, namely Bacillus, Pseudomonas, Lactobacillus, Staphylococcus, Micrococcus, Corynebacterium.

Enzyme activity of the various bacterial isolates.

The results (of Table 1) indicate that all the isolates but one produced detectable amounts of L-asparaginase. Among these isolates the most potent enzyme producers were three strains of Bacillus sphaericus (isolates 2, 30 and 36) followed by a Corynebacterium (isolate 10).

The potent isolates were used to examine enzyme production after different incubation periods on nutrient broth and Dox liquid media.

The results which are shown in table 2 suggest that enzyme production was generally higher on Dox liquid medium than on nutrient broth. Moreover on the former medium all the isolates consistently produced more enzyme at a culture age of one week. It was also found that sucrose as a carbon

source in Dox liquid medium stimulated more enzyme production than starch did.

The results of Table 3 indicate that the majority of the enzyme activity is intracellular or associated with the cells, a negligible part was present in the culture filtrate.

Discussion

The results of the present investigation support the general belief of the wide spread occurrence of L-asparaginase in microorganisms. The enrichment technique however, increased the number of L-asparaginase producing isolates by sixty per cent. Isolates which failed to utilize asparagine as a nitrogen source were unable to survive as pure cultures on the medium which contained asparagine as the only added nitrogen source.

The enzyme activity is fully expressed in the intact organisms. This agrees with the findings of CEDAR and SCHWARTZ, 1967 who were able to conclude that the antitumor L-asparaginase obtained from E. coli is located in the periplasm. It should be pointed out that by using whole cells in the enzyme assay, any cytoplasmic asparaginases that are present will be overlooked. The cytoplasmic asparaginase are unlikely to have a high affinity for asparagine (in competition with enzymes of protein synthesis) and unlikely therefore to be of much practical importance (BROOME 1965).

One of the first bacterial species that received special attention with view to the therapeutic use of their amidases was Pseudomonas aeruginosa (GREENBERG et al 1964). In the present investigation however, Pseudomonas aeruginosa was not among the most potent enzyme producer. This points out to strain variation among members of the same species and also the environmental and nutritional condition should not be ignored.

SUMMARY

Twenty-one L-asparaginase producing bacteria were isolated and identified from the soil. Soil enrichment increased the number of enzyme producers by 60%. The isolates represented six different genera and the most potent enzyme producers were 3 strains of *Bacillus sphaericus* and one *Corynebacterium* sp. All the potent isolates had their enzyme associated with the cells and negligible amounts were found in the culture filtrate. More enzyme was produced on a synthetic liquid medium in which asparagine was the only added nitrogen source than on nutrient broth.

253

— 253 —

Table 3

Isolate number and identity.	Relative enzyme activity O.D. ₄₅₀		
	Whole culture	culture filtrate	washed cells
2 <u>Bacillus sphaericus</u>	.27	.017	.25
10 <u>Corynebacterium sp.</u>	.54	.05	.48
30 <u>Bacillus sphaericus</u>	.18	0	.15

L-asparaginase activity in whole cultures, culture filtrate and washed cells of two isolates of *B. Sphaericus* and one isolate of *Corynebacterium*,.

Table 1

Isolate number	Identity	Relative enzyme activity O.D. ₄₅₀
1	<u>Bacillus sphaericus</u>	0.27
2	<u>Bacillus sphaericus</u>	0.54
3	<u>Pseudomonas aeruginosa</u>	0.08
4	<u>Lactobacillus coryneformis</u>	0.02
5	<u>Staphylococcus aureus</u>	0.15
6	<u>Lactobacillus coryneformis</u>	0.17
10	<u>Corynebacterium Sp.</u>	0.34
11	<u>Micrococcus varians</u>	0.01
12	<u>Pseudomonas aeruginosa</u>	0.08
13	<u>Bacillus brevis</u>	0.13
14	<u>Lactobacillus coryneformis</u>	0.01
15	<u>Staphylococcus aureus</u>	0.0
30	<u>Bacillus sphaericus</u>	0.42
31	<u>Bacillus sphaericus</u>	0.28
32	<u>Bacillus firmus</u>	0.17
33	<u>Bacillus macerans</u>	0.31
34	<u>Bacillus sphaericus</u>	0.06
35	<u>Micrococcus luteus</u>	0.25
36	<u>Bacillus sphaericus</u>	0.52
37	<u>Bacillus firmus</u>	0.23
38	<u>Bacillus alvei</u>	0.14

Table 2

Isolate	Culture age (hrs)	Relative enzyme activity O.D. ₄₅₀	
		Dox	Nutrient Broth
2 (<u>Bacillus sphaericus</u>)	48	.185	.26
	168	.270	.1
	216	.06	-
10 (<u>Corynebacterium</u>)	48	0	.13
	168	.54	.03
	216	-	-

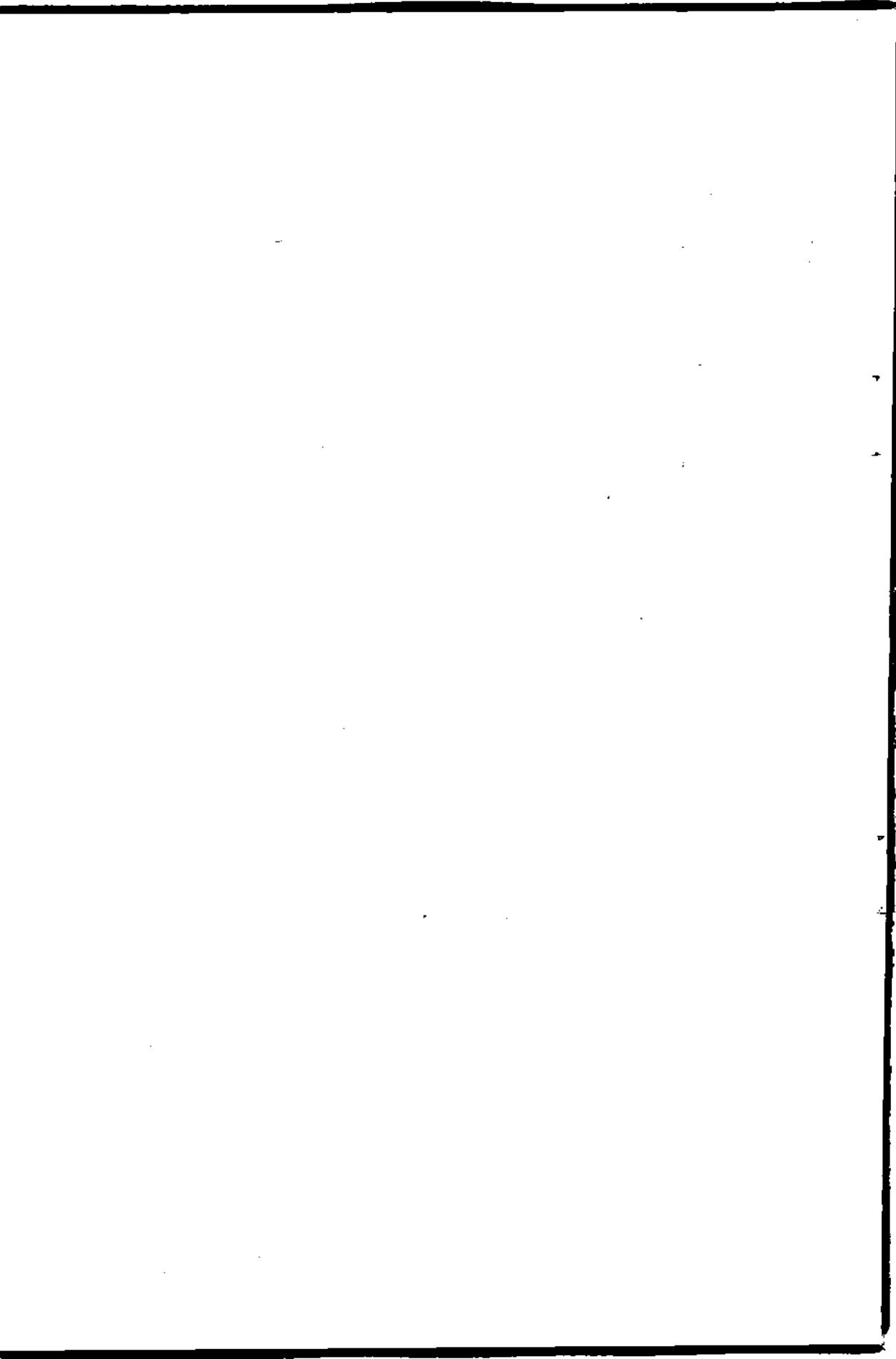
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"Production of L-asparaginase by Streptomyces karatakensis
and Streptomyces venezuelae"

by

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INTRODUCTION

Considerable attention to the enzyme L-asparaginase (L-asparagine amino hydrolase) has resulted from the discovery of its ability to inhibit growth of tumors in the mouse, rat and dog and to suppress human leukemias in clinical trials (CAPIZZI et al 1970). It has been suggested that the antitumor activity of this enzyme is due to the fact that a number of tumor cells responding to it lack adequate L-asparagine synthetase activity and require an exogenous supply of L-asparagine. Depletion of this amino acid by L-asparaginase in vitro or in vivo results in the death of these tumor cells.

A tumor inhibitory asparaginase, designated EC-2 and obtained from strains of E. coli was prepared on a large scale and has been used extensively for clinical trials and other investigations. Factors affecting the biosynthesis in flask cultures has been studied (ROBERTS et al 1968). A partially purified asparaginase from S. marcencens ATCC 60 was described (ROWLEY and WRISTON 1967). and fermentation conditions which produce high yields of asparaginase in shaken cultures was investigated (HEINEMANN and HOWARD 1969).

Actinomycetes has been neglected as a potential source of L-asparaginase. Recently a number of Streptomyces isolates obtained from the soil produced detectable amounts of this enzyme. (MOSTAFA 1977). The present investigation is carried to determine the growth conditions for two different streptomyces which produce high yield of asparaginase in surface cultures.

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Materials and Methods.

1. Microorganisms.

The two microorganisms used in this study were isolated from fertile Egyptian soil and one of them has been previously identified as a strain of Streptomyces karnatakensis (MOSTAFA 1977). The second (Streptomyces - 9) will be identified in this report. All the methods used for morphological and physiological studies were those of the International Streptomyces Project (I.S.P.) as described by SHIRLING and GOTTLEIB (1966). Direct mount of spores on collodium films was examined by a transmission electron microscope (EM9S-2), The Electron Microscope Unit, Kuwait University) and electron photomicrograph was taken. Tentative identification was made mainly by using the key suggested by KUSTER (1972), however, BERGEY (1974) and SZABO (1975) were consulted.

L-asparaginase assay:

Mycellium matt obtained from a 6 - 8 days old culture and washed three times with distilled water was used as a source of enzyme. The standard L-asparaginase test system contained in a total volume of 2 ml : 20 μ moles. L-asparagine; 500 μ moles tris buffer pH 8.6; 100 mg (fresh wt) mycellium. The reaction was initiated by the addition of the substrate and the reaction mixture was incubated at 37°C for 1 hour after which the reaction was terminated by the addition of 0.5 ml of 1.5 M trichloroacetic acid. This was followed by filtration on Whatman no. 1 filter paper. The amount of ammonia liberated was then measured spectrophotometrically at 450 nm by Nesslerization. For each enzyme assay two controls were always included, one with TCA denatured enzyme and the other without substrate. All enzyme assays were carried in triplicate and the average was recorded. The concentrations of ammonia in the test solutions were determined from a standard curve with ammonium sulphate as the source of dissolved ammonia.

The rate of the assay reaction was determined to be linear with respect to enzyme concentration under the conditions and over the range of the enzyme assay. One international (I.U.) L-asparaginase unit is defined as the amount of enzyme which liberates 1 μ mole of ammonia / minute at 37°C.

L-asparaginase production and the factors affecting it.

A number of liquid media were examined for their effect on L-asparaginase production these included nutrient broth, inorganic salts starch as described by KUSTER (1959), Czapek, and ISA liquid medium. The latter contained (g/L): K_2HPO_4 , 1; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 1; trace salts solution (as described by KUSTER (1959), 1 ml; L-asparagine, 2.0; soluble starch 10.

The above ISA liquid medium was used as the basal salt medium for investigating various environmental and nutritional factors affecting L-asparaginase production. Thus the effect of various carbon sources was examined by substituting starch in the above medium with one of the tested carbon sources namely, glycerol, glucose, fructose, sucrose, lactose and maltose. Various nitrogen sources were also investigated by substituting L-asparagine in the ISA medium with an equimolar amount of one of the following amino acids: isoleucine, leucine, arginine, Aspartic, Cystine, Methionine, histidine and glutamine. Moreover the effect of histidine, glutamine and aspartic when each of them was used in the ISA medium additional to asparagine was examined. Various starch and L-asparagine concentrations in the ISA medium were also investigated.

L-asparaginase produced during incubation at various temperatures was assessed by incubating inoculated ISA medium for 7 days at the specified temperature.

Static and shaken cultures were examined for their growth and L-asparaginase productivity at various ages. Growth was followed by dry weight determination.

In all cases a standard inoculum was used. The standard inoculum was prepared by suspending the sporulating growth on an agar slant of inorganic salts starch agar medium (SSA) in 3 ml of sterile water, this was transferred aseptically to a small screw cap sterile bottle containing few glass beads. The spore suspension is shaken vigorously for a few minutes with the glass beads to form homogenous suspension. This suspension was used for inoculation at a final concentration of 2%.

Results.

Identification of Streptomyces - 9.

Streptomyces-9 is a member of the grey series, is melanin positive, does not produce soluble pigments, mature spores were carried in straight or wavy chains (Plate 1) i.e. section rectiflexibles. Photoelectron micrograph revealed smooth spore surface (Plate 1). Spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on oatmeal agar, salts starch agar and glycerol asparagine agar. Fragmentation of the substrate mycellium was sometimes noticed on glycerol asparagine agar medium. On agar media containing glucose the substrate mycellium showed a distinct reddish brown colour. Streptomyces-9 produced an antibiotic which was active against Gram positive (*Bacillus subtilis*) and Gram negative (*Escherichia coli*) organisms. Streptomyces-9 was able to utilize any of glucose, xylose, rhamnose and arabinose. Fructose was utilized poorly while inositol, sucrose, mannitol and raffinose were not utilized by this organism.

Comparing the above morphological and physiological characteristics with those used in the working key suggested by KUSTER 1976, Streptomyces-9 was identified as Streptomyces venezuelae. Furthermore, comparing all the present isolate characteristics with those of *S. venezuelae* as described by SHIRLING and GOTTLIEB, they were found identical.

Production of L-asparaginase by *S. karnatakensis* and *S. venezuelae*

Among all the tested media for L-asparaginase production both *S. karnatakensis* and *S. venezuelae* produced more enzymes when they were grown on the ISA liquid medium. Consequently this medium was used as the growth medium for investigating the various factors affecting L-asparaginase production.

Factors affecting L-asparaginase production:

Growth and enzyme production were greatly reduced to various degree when starch in the ISA liquid medium was substituted with any of the carbon sources examined. Consequently starch was used as the carbon source in the ISA medium for further investigation. Increasing concentrations (up to a certain extent) of starch in the ISA medium stimulated growth and enzyme productivity of both organisms (Table 1).

Both organisms produced more L-asparaginase when L-asparagine was used as the only added nitrogen source in the ISA medium than when equimolar amounts of any of the tested amino acids was used. Few of the tested amino acids e.g. aspartic, glutamic, cystine and tryptophan supported neither growth nor enzyme production (untabulated). Leucine and isoleucine gave poor growth while arginine supported good growth and the enzyme activity in that growth was about 50% that obtained when L-asparagine was used. The two organisms responded differently to the presence of histidine and glutamine in the growth

medium. Thus while histidine inhibited growth and enzyme production by S. karnatakensis, it stimulated S. venezuelae growth and enzyme production when it was present additional to asparagine. Glutamine stimulated enzyme productivity of both organisms when it was present additional to asparagine (Table 2).

Optimum L-asparaginase production by S. karnatakensis and S. venezuelae occurred when the L-asparagine concentration in the ISA medium was 1 and 0.5% respectively (Fig.1). Optimum growth did not always coincide with optimum enzyme production (Fig.1).

28°C was found optimum for growth and enzyme production by both organisms. At temperature above 40°C both organisms were unable to show detectable growth or enzyme activity. At 15°C S. venezuelae but not S. karnatakensis showed some growth.

Both organisms showed their maximum enzyme production in shaken cultures after 48 hours of incubation at 28°C, older shaken cultures showed increased growth but declined enzyme productivity. Static cultures of S. karnatakensis and S. venezuelae showed their optimum enzyme productivity after 5 and 2 days respectively. (Table 3). Although S. venezuelae was able to grow and produce asparaginase at the tested pH values, optimum growth and enzyme production occurred at pH 8.5 and 6.5 respectively (Fig. 2) S. karnatakensis showed its optimum growth and enzyme productivity at pH 7.4. Higher and lower pH values inhibited growth and enzyme production.

Discussion.

The results of this study indicate that the identity of the organism as well as the environmental factors are important for the biosynthesis of L-asparaginase. As with a number of other enzymes, formation of L-asparaginase is inhibited by the addition

of sugars particularly glucose (HEINEMANN and HOWARD 1969). The mechanism of this depressive effect is thought to result from the presence of glucose metabolic products (MOSES and PREVOST 1966). In the case of L-asparaginase biosynthesis the depressive effect of carbohydrates may be a function of their ability to lower the pH value of the growth medium (HEINEMANN and HOWARD, 1969). It is interesting to point out that starch proved to be the best carbon source for L-asparaginase production. The use of starch as a carbon source and consequently the production of α amylase has been linked with the biosynthesis of other enzymes e.g. lipase (ELWAN et al 1977). The results of the present investigation might add another evidence to the possible association of starch metabolism with the biosynthesis of other enzymes namely L-asparaginase. The depressive effect of high starch concentrations is probably due to an excess carbohydrate and/or of a low pH (WADE et al 1971). The latter is more likely in the present investigation. Thus S. Venezuelae which exhibited its optimum enzyme productivity at pH 6.5 was also able to produce more enzyme at higher starch concentration (4%), and lower asparagine concentration (0.5%). On the other hand S. karnatakensis which showed an optimum pH of 8.5 required lower starch concentration (2%) and higher asparagine concentration (1%) for optimum enzyme productivity. The substrate of L-asparaginase stimulated its biosynthesis while L-aspartic which is the reaction product, inhibited the enzyme biosynthesis. This might be another example of feed-back mechanism, however, the effect of aspartic in lowering the pH of the medium and consequently inhibiting the enzyme biosynthesis and/or activity could not be ignored. The feed back mechanism might explain the depression of enzyme productivity of both organisms in static as well as in shaken cultures after a certain enzyme level has been attained. This enzyme level is reached after 48 hours of incubation of S. Venezuelae in static or shaken cultures. S. karnatakensis on the other hand reaches this enzyme level after 48 and 120 hours in shaken and static cultures

respectively. The high enzyme level in the medium results in the accumulation of aspartic which inhibits the biosynthesis of more enzyme upon further incubation. Aeration of the culture medium is stimulatory for the growth of both S. karnatakensis and S. venezuelae but not for the production of L-asparaginase. This is in accordance with the results of HEINEMANN and HOWARD (1969) who noticed a decreased enzyme synthesis by S. marcescens upon aeration. Moreover enhanced biosynthesis of L-asparaginase by E. coli under anaerobic conditions has been reported by CEDAR and SCHWARTZ (1968).

The information gained from the present investigation will make it possible to prepare uniform growth from S. karnatakensis and S. venezuelae which could be disrupted and the L-asparaginase can be purified and studied in the cell free extract.

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Summary

Production of L-asparaginase by two soil isolates identified as S. karnatakensis and S. venezuelae under different environmental and nutritional conditions was investigated. The presence of carbon sources other than starch in the growth medium or amino acids, other than L-asparagine inhibited the enzyme biosynthesis. L-aspartic inhibited growth and enzyme production due to a feedback mechanism, and/or lowering the pH value. Both organisms were stimulated to produce more enzyme with increasing concentrations of starch and L-asparagine, however, the optimum starch and L-asparagine concentration depended on the tolerance of the organism to low and high pH respectively. Aeration stimulated growth but not enzyme production and both organisms produced more enzyme in static cultures than in shaken cultures.

Table 1

Starch Conc. %	Streptomyces karnatakensis			Streptomyces venezuelae		
	Dry wt mg /100 ml medium	Final pH	Enzyme conc. I.U. / gm dry wt.	Dry wt. mg /100 ml medium	Final pH	Enzyme conc. I.U. / gm dry wt.
0.1	060	8.0	61.6	020	8.6	10
0.5	165	7.9	62.5	080	8.3	20.83
2.0	200	7.5	70.83	240	5.7	38.83
4.0	120	7.2	0.66	220	5.7	55.83

Effect of starch conc. on L-asparaginase production by S.karnatakensis and S.venezuelae.

Medium was ISA liquid medium with starch concentration as specified in the table.

Incubation in 50 ml medium in 250 ml at 28°C for 6 days in static cultures. Enzyme assay was carried out as described in the text.

Table 2

Amino acid used	Streptomyces karnatakensis			Streptomyces venezuelae		
	Dry wt. mgm / 100 ml medium	Enzyme conc I.U. / gm dry wt.	Final pH	Dry wt. mgm / 100ml medium	Enzyme conc. I.U. / gm dry wt.	Final pH
Asparagine	190	40.8	7.45	240	20.83	8.0
Histidine	-	-	5.8	251	8.33	6.8
Histidine + asparagine	-	-	5.8	271	35.00	8.14
Glutamine	138	20	6.18	021	5.00	7.0
Glutamine + asparagine	237	116.6	8.22	208	33.33	7.9
Aspartic	-	-	3.60	-	-	3.6
Aspartic + asparagine	-	-	3.70	-	-	3.7

(-) not carried out due to poor growth

L-asparaginase productivity of S. karnatakensis and S. venezuelae in the presence of different amino acids.

Medium was ISA liquid medium with .015 M of the specified amino acid as the nitrogen source. Incubation at 28°C for 6 days in static cultures. Enzyme assay was carried out as described in the text.

Table 3

Culture age (hrs)	Streptomyces karnatakensis						Streptomyces venezuelae					
	Static cultures			shaken cultures			static cultures			shaken cultures		
	dry wt. mgm /100 ml medium	enzyme conc. I.U./ gm dry wt.	Final pH	dry wt. mgm /100 ml medium	enzyme conc. I.U./ gm dry wt.	Final pH	dry wt. mgm /100 ml medium	enzyme conc. I.U./ gm dry wt.	Final pH	dry wt. mgm /100 ml medium	enzyme conc. I.U./ gm dry wt.	Final pH
48	050	4.5	7.0	75	16.66	7.0	090	67.5	7.0	225	11.66	7.0
96	27	5.83	7.5	247	12.58	7.6	220	43.33	7.5	615	6.33	7.4
120	150	17.5	7.9	180	9.16	7.9	240	21.16	7.7	517	4.16	7.7
144	220	17.5	8.1	247	4.16	8.0	270	5.00	8.0	498	4.16	8.0
192	310	11.66	8.5	330	4.16	8.5	190	2.5	8.3	187	4.1	8.6

L-asparaginase productivity of *S. karnatakensis* and *S. venezuelae* in static and shaken cultures at different culture ages. Medium was ISA liquid medium, incubation was at 28°C for the specified period of time in static or shaken culture as indicated. Enzyme assay was carried out as described in the text.

243

273

Figure 1.

Effect of L-asparagine concentration in the growth medium, on L-asparaginase productivity of S. karnatakensis and S. venezuelae. Medium was ISA liquid medium with L-asparagine conc. as specified on the figure, incubation was at 28°C for 7 days in static cultures. Enzyme assay was carried out as described in the text.

Figure 2.

Effect of the medium initial pH on L-asparaginase productivity of S. venezuelae. Medium was ISA liquid medium with its pH adjusted to the values indicated in the tables. Incubation for 7 days at 28°C in static cultures. Enzyme assay was carried out as described in the text.



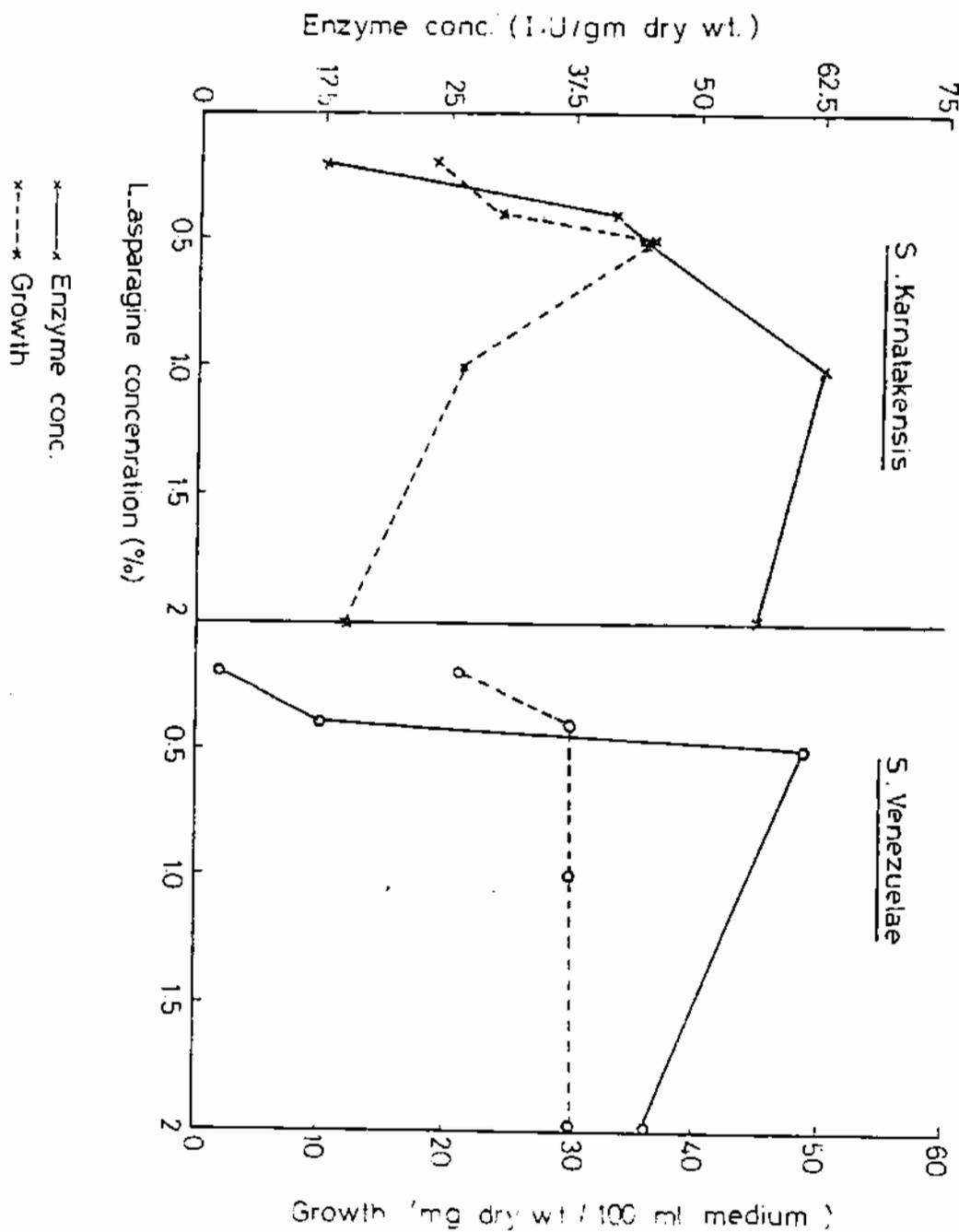


Fig. 1

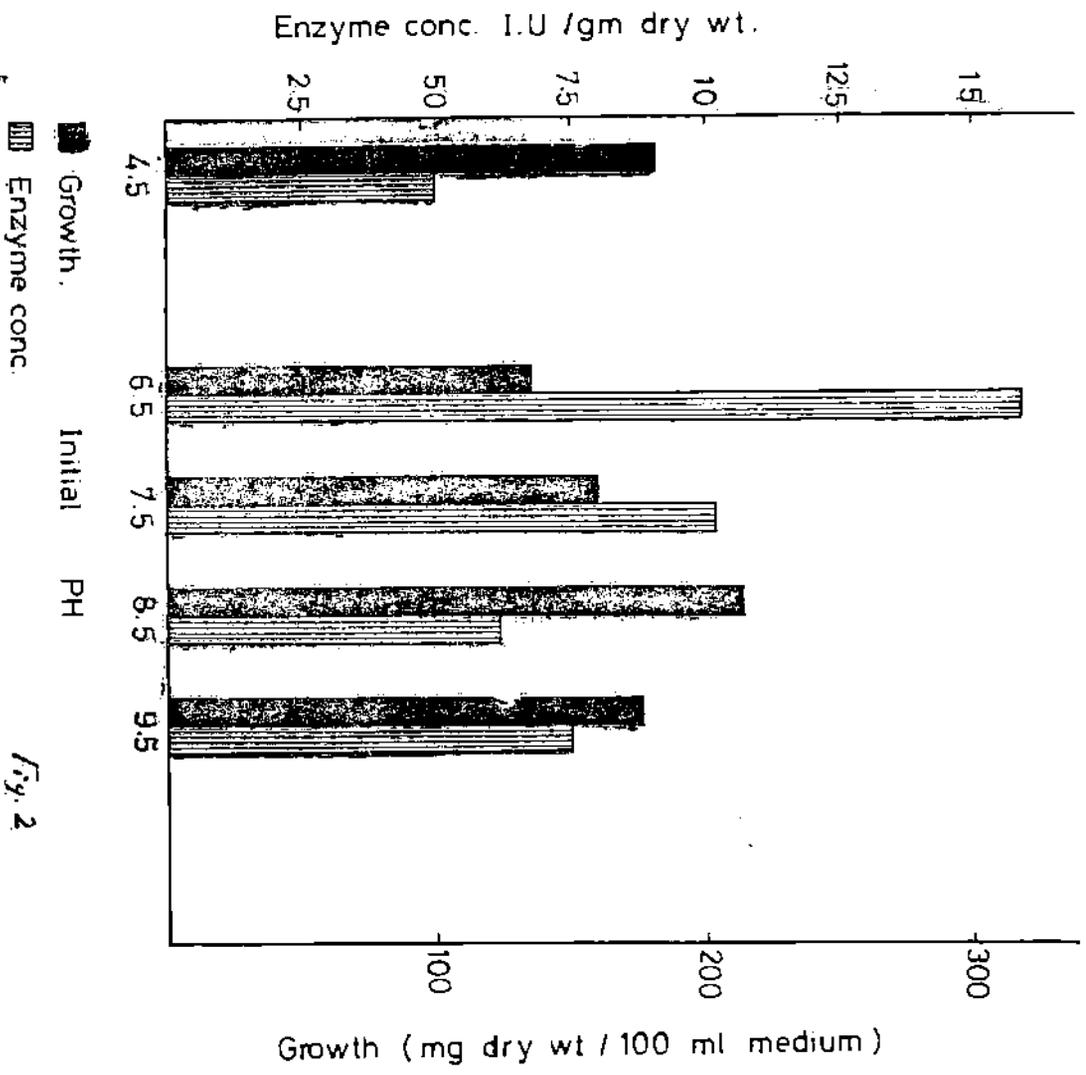


Fig. 2



Plate 1.

Electron photomicrograph of S. venezuelae showing spore chains of the RF type and smooth spore surface.

24

THE INTERACTION BETWEEN BILIARY
PROTEIN AND ALCOHOLISM:

I. Effects On Hepatic Lipids.

By

MADHA, A. ASHRY

INTRODUCTION

It has been established that protein malnutrition produces fatty liver both in human subjects and in experimental animals (Thomas, 1974). On the other hand, fatty liver has been considered one of the characteristic changes associated with human alcoholism despite adequate diet (Leiber et al., 1965). This change has also been reproduced in experimental animals.

Controversy continues, however, of whether this lesion is the result of a direct hepatotoxic effect of alcohol (Reubner et al., 1972) and (Nair et al., 1975); or that ethanol could alter nutrition by interfering with intestinal absorption of protein (Barboriak and Meade, 1969) and (Forte et al., 1970).

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In the present investigation, an attempt has been made to characterize the role of dietary protein and alcoholism as Co-factors in the development of fatty liver.

MATERIAL AND METHODS

Male Albino guinea pigs, of the strain bred and brought up in the serum and antigen Laboratory farms-Helwan, averaging 530 grams of body weight at the commencement of the experiment were used. They were divided into four groups according to the scheme in table (1).

Table (1)

Experimental design and group distribution

Group N°.	Experimental Design
Group I	High Protein diet & Alcohol administration
Control I	High Protein diet.

Group II	Low Protein diet & Alcohol administration
Control II	Low Protein diet.

The experimental diets, which were fed ad-libitum, consisted of:

Table (2)

Composition of diets

Ingredient	% Composition	
	High Protein diet	Low Protein diet
Casein	25%	5%
Corn Starch	62%	82%
Cotton Seed Oil	7%	7%
Mineral Mixture (Jones and Foster, 1942)	4%	4%
Vitamin Mixture (Morcos, 1967)	2%	2%

Alcohol was administered orally to groups I and II daily; for six days per week, at dosage level of 0.6 ml./Kg./day.

Animals from each group were autopsied at the end of the second and fourth weeks. Fresh liver weights were determined. The total lipid content was estimated from 5 gram liver samples using a Soxhlet apparatus, following the method of Frazer (1949). The lipid was then dissolved in benzene and the free fatty acids (FFA) were estimated by the method of Varley, (1969).

For histological examination, liver samples were fixed in 10% formaline. Frozen sections were cut at 10 microns, and stained with Sudan IV for lipids.

RESULTS

The effect of different treatments on hepatic weights, total lipids, and free fatty acids is shown in table (3).

When liver weight data were expressed in terms of percentage per body weight, the diet showed no significant effect on liver weight. Alcohol administration reflected marked increase in liver weight. This was rather aggravated by the protein deficient diet.

Analysis of the livers for total lipids revealed marked elevation in both groups receiving ethanol. The low protein diet alone increased the hepatic lipids by only 2 : 3 fold; compared with that of the high protein diet.

The increase in free fatty acids in guinea pigs administering alcohol was marked at two weeks. A similar increase was brought up later at four weeks by protein deficiency. When ethanol was administered with low protein diet, FFA showed drastic rise, approximating three times of the normal.

Histological Examination:

The livers of guinea pigs fed low protein diet revealed moderate to marked increase in intra-cellular lipids. (Figure 1) This was most marked in the central

Table (3)

Mean Values of liver weight, total lipids and

free fatty acids:

Experimental design	Duration		Liver weight		Total lipid		Free fatty acids	
	weeks		% per body wt.	% per fresh liver	% per fresh liver	mg./1 gm. Liver		
High Protein diet + Alcohol (Group I)	2		3.47%	5.44%		31.4		
	4		3.19%	7.74%		39.1		
High Protein diet (Control I)	2		2.67%	1.97%		21.7		
	4		2.27%	1.70%		21.5		
Low Protein diet + Alcohol (Group II)	2		3.66%	9.07%		39.3		
	4		2.80%	9.81%		52.0		
Low Protein diet (Control II)	2		2.62%	3.70%		21.0		
	4		3.16%	5.76%		34.8		

portions of the liver lobules.

Alcohol administration when associated with a high protein diet developed severe fatty metamorphosis of peripheral and mid lobular hepatocytes. About 40 to 50 per cent of the cells composing hepatic lobules were affected by small intracellular particles and large extracellular fatty cysts (Figure 2).

When alcoholism was super-imposed on low protein diet, the livers from animals of this group demonstrated extensive fatty metamorphosis. Large extracellular lipid particles occupied 75 to 80 per cent of the hepatocytes throughout the liver lobules (Figure 3).

DISCUSSION

The present investigation indicated that ethanol administration developed acute fatty liver without a quantitatively important malabsorption of fat, and despite adequate diet. Inhibition of intestinal transport of nutrients by ethanol (Leiber et al. 1969) did not seem to increase losses of fat from the intestine during the development of alcohol - induced fatty liver.

On the basis of the amount and distributional pattern of hepatic lipids in protein deficiency, it is suggested that the lesion is in its early stage. Severe fatty metamorphosis similar to that produced by alcohol administration seem to require prolonged periods of protein depletion (Kosterlitz, 1947).

In comparing the dietary effect with ethanol administration, it was evident that 80% decrease in protein intake doubled hepatic lipid, whereas a moderate dose of ethanol increased it 2 : 3 fold in a similar duration. Consequently, it is proposed that hepatic response to ethanol is ~~immediate~~ and severe. In connection with this finding, several investigators have reported that a single dose of ethanol can produce fatty liver in experimental animals, (Di Luzio et al., 1958), Strubelt, 1972) and (Kair et al., 1973). This has either been attributed to an enhanced mobilisation of FFA from the adipose tissues through an adrenal hypophyseal pathway (Maling et al., 1960). Other studies have emphasized the direct hepatotoxic effect of alcohol leading to hepatic steatosis (Klatskin, 1961). The possibility that alcohol may induce a relative lipotropic

deficiency is suggested by Auebner et al., (1972). If this is so, then it might account for the present finding that hepatic steatosis in alcoholism was accentuated by additional protein deficiency.

ABSTRACT

Albino guinea pigs were given ethanol orally, and hepatic lipid response was determined biochemically and histochemically in the presence of high or low protein diets.

The investigation indicated the following:

1. Analysis of the livers for total lipids and free fatty acids revealed marked elevation in both groups receiving ethanol. The low protein diet alone reflected moderate increase.
2. Histochemical study of rat demonstrated generalized fatty metamorphosis of extracellular lipid when ethanol was administered with a low protein diet. With a high protein diet, peripheral and midlobular fatty infiltration developed, while the low protein diet reflected a moderate increase in intracellular lipids in a similar duration.

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Figure (1)

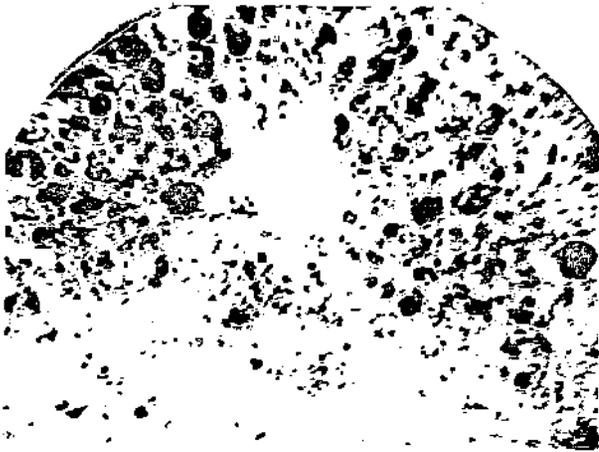
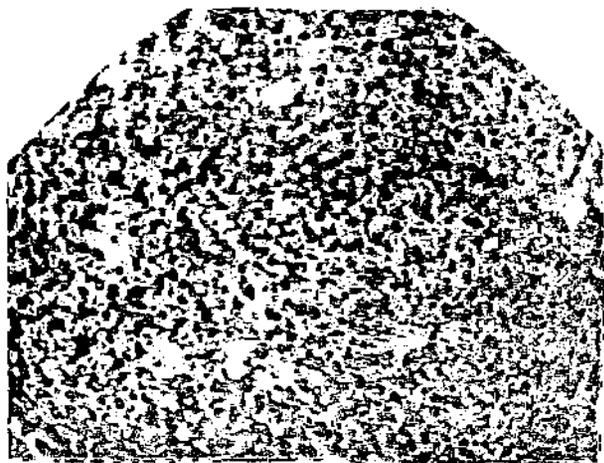


Figure (2)

Figure (3)



Captions of Figures

(Frozen Sections, Sudan IV, X 125)

- Figure (1): 4 weeks on a low protein diet. Intracellular lipid particles.
- Figure (2): 4 weeks of alcohol administration and a high protein diet. Large lipid particles in the mid-lobular hepatocytes.
- Figure (3): 4 weeks of alcohol administration and a low protein diet. Generalized fatty metamorphosis.

PRODUCTION OF LIPASE (S) BY SOME YEASTS

I. EFFECT OF TEMPERATURE, pH AND TRIGLYCERIDES

By

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ABSTRACT

An investigation was carried out to evaluate lipolytic activities of three anascosporogenous yeasts viz. Rhodotorula rubra, Candida utilis, C. guilliermondii and an ascosporogenous yeast Debaryomyces hansenii. Lipase (s) was synthesized under the influence of different incubation time, pH, temperature and triglycerides. R. rubra gave the highest level of enzyme after 6 days at 30°C, on sugar - free peptone yeast extract-corn oil medium, of pH 4.

Both C. utilis and C. guilliermondii exhibited optimum conditions for maximum lipase biosynthesis in presence of olive and coconut oil respectively. Corn oil induced enzyme biosynthesis in R. rubra, D. hansenii, C. utilis and C. guilliermondii in presence of 0.2%, 0.6%, 0.8% and 1% respectively.

However, the ascosporogenous yeast D. hansenii which was grown on the same medium showed optimum lipase biosynthesis at 20°C after 48 hours of growth.

INTRODUCTION

Recently, microbial lipase (s) witnessed a tremendous progress and development regarding their production for industrial and clinical application. Production of yeast extracellular lipase (s) is confined largely to

anascosporogenous species, particularly members of the genus Candida (Werner, 1960).

Among the active producers of extracellular lipase (s) that have been studied are Candida lipolytica (Vickery, 1936), Mycotorula lipolytica (Peters and Nelson, 1948), C. cylindrica (Yamada and Machida, 1962), C. paralipolytica (Ota and Yamada, 1966), C. humicola (Bours and Mossel, 1969) and Torulopsis ermobii (Motai et al., 1966). It is well known that production of lipase (s) by different microorganisms is affected by many environmental and nutritional requirements.

The present work shows the effect of temperature, pH and different triglycerides on the biosynthesis of lipase by three anascosporogenous yeasts, Rhodotorula rubra, Candida utilis and C. guilliermondii and the ascosporogenous yeast Debaryomyces hansenii.

MATERIALS AND METHODS

Test organisms:

Candida utilis was kindly provided by the Department of Microbial Chemistry, National Research Centre, Cairo. However, Rhodotorula rubra (Domme), Lodder as well as Candida guilliermondii (Cast.), Iangeron et guerra had already been isolated from apricot and pears respectively and identified by Mahmoud et al., (1980). Also,

Debaryomyces hansenii was isolated from a sample of an Egyptian cheese brine called Mesh, and identified by Mahmoud (1978).

Growth medium:

The nutrient medium used throughout these investigations is constructed as follows (g/L): peptone, 5.0; yeast extract, 5.0, KH_2PO_4 , 2.0, MgSO_4 , 7 H_2O , 0.5, corn oil, 10 initial pH 6.5.

Lipase assay:

Lipase (s) in the growth filterates of the yeasts under study were assayed by the tributyrin cup plate clear zone technique (TCZ) as described by Elwan et al., (1977), devised from the technique of Lawrence et al., (1967). Standard curve showing linear relation between (Fulka AG, Buchs SG pancreatic lipase) concentrations and mean clearing zone diameters (mm) as determined by TCZ assay at pH 8 and after 24 hours incubation at 30°C was used for lipase assay in growth filtrates.

Factors affecting lipase biosynthesis:

The organisms were grown in 25 ml portions of the liquid growth nutrient medium in 100 ml capacity Erlenmeyer flasks. The liquid nutrient medium was inoculated with the yeasts under study.

Four levels of incubation temperatures were tested namely, 20, 25, 30 and 35°C. The initial pH of the nutrient medium was adjusted in the range from 4 to 8.

The effect of different triglycerides and corn oil concentration on lipase (s) biosynthesis was also investigated. Medium supplemented with 1% of each of corn oil, coconut oil, cotton seed oil and olive oil at pH 6.5 were inoculated, incubated at 30°C and lipase enzyme was assessed after 2, 4 and 6 days.

RESULTS AND DISCUSSION

Incubation temperature in relation to lipase biosynthesis

Table (1) represents the levels of lipase biosynthesis by the organisms under study as they were grown at 20, 25, 30 and 35°C. From the results it is obvious that 20°C is the optimum temperature for lipase biosynthesis by D. hansenii through the incubation periods, where the maximum lipase yield (6.310 ug/ml) was attained after 2 days incubation.

Optimum temperature for lipase production by R. rubra, C. utilis and C. guilliermondii was 30°C. No lipolytic activity was detected in case of C. guilliermondii after 4 or 6 days at 35°C. This difference in optimum temperature for lipase biosynthesis could be correlated with the optimum growth temperature for the different

yeasts. Phaff et al. (1966) reported that D. hansenii multiply at or close 0°C and have maximum growth temperature at 35°C, while C. utilis can grow only at minimum temperature of 5 - 10°C, Rose and Evison (1965). The production of enzymes are related to the internal biosynthetic processes inside the organism, these processes are quite complicated.

Previous investigations revealed that a fixed period of incubation could not be considered optimum for the production of an enzyme even by members of the same genus or species. Johnson and Snygg, (1974) claimed that lipase (s) production was attained after 2 - 7 days incubation by the four lipolytic microorganisms: Saccharomyces lipolytica, Micrococcus caseolyticus, Bacillus licheniformis and Staphylococcus sp. It is worthy to mention that the optimal temperature for the production of lipase (s) enzyme varied with different yeast strains. Thus, optimal incubation temperature of 33°C was recorded for Torulopsis ernobii (Yoshida et al. 1968), 30°C for Mycotorula lipolytica (Peters and Nelson, 1948), C. paralipolytica (Ota and Yamada (1966) and Saccharomyces lipolytica (Johnson and Snygg, 1974).

Effect of pH on lipase productivity:

Table (2) represents the levels of lipase activity at different pH ranges from 4 to 8 of the yeasts

under investigation. Maximum lipase (s) biosynthesis was attained at pH 4, followed by pH 7. Incubation time of 6 days at pH 8 was found inhibitory to lipase biosynthesis by all the yeasts under test though this pH was optimal for maximum enzyme activity (unpublished data). Lipolytic activity could not be detected also after 6 days incubation at pH 6 or 7 by both C. utilis and D. hansenii. Several reports indicate different optimum pH values for yeast lipase (s) optimum of pH 5 was recorded for Torulopsis ernobii (Yoshida et al. 1968), pH 5.8 for Torulopsis sp. (Dessouky, 1976), pH 9.5 for Saccharomycopsis lipolytica (Johnson and Snygg, 1974) and pH 4.5 for Mycotorula lipolytica (Peters and Nelson, 1948).

Effect of triglycerides on lipase (s) production:

The effect of different oils as inductive substrates on the biosynthesis of lipase (s) by the yeasts under study was investigated (Table 3). One noticed that lipase enzyme could be suggested to be constitutive in case of both ascosporogenous and anascosporogenous tested yeasts.

Corn oil induced lipolytic activity in all the tested yeasts. Olive oil retarded lipase productivity in case of C. guilliermondii and D. hansenii. Lipase productivity by D. hansenii was also retarded in presence of either coconut oil or cotton seed oil. Also C. guil-

guilliermondii failed to produce lipase when grown on cotton seed oil throughout the tested period. Maximal induction of lipase (s) production by R. rubra and/or D. hansenii were obtained when cotton seed oil was used as a substrate. Both olive oil and cotton seed oil induced maximal lipase production in case of C. utilis, while C. guilliermondii produced maximum lipolytic activity only when grown on olive oil.

Table (4) represents lipase productivity by the different yeasts in presence of different concentrations of corn oil. Maximum lipase (s) activity was detected in presence of 0.2% corn oil by R. rubra, 0.6 - 1% by D. hansenii and C. guilliermondii and 0.6 - 0.8% by C. utilis. It has been already found that the preferential oil and its optimum concentration varied greatly with oil structure and type of microbe. Yoshida et al. (1968) reported that the maximum lipase production by Torulopsis ernobii was at 0.2 - 0.6% (w/v) olive oil while Dessouky, 1976 recorded 0.2 - 0.3% (w/v) corn oil for Torulopsis sp. The maximum production at 0.2 - 0.3% (w/v) of corn oil for Torulopsis sp.

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Table (1)

Effect of incubation temperature on lipase (s) Produced by G. uttara , G. guilliermondii , R. rubra and D. hansenii
 Data are expressed as UG/ml (ug lipase/one ml cell free filtrates)

Temp. (°C)	Organisms											
	<u>G. uttara</u>			<u>G. guilliermondii</u>			<u>R. rubra</u>			<u>D. hansenii</u>		
	48 h.	96 h.	144 h.	48 h.	96 h.	144 h.	48 h.	96 h.	144 h.	48 h.	96 h.	144 h.
20 °C	5.495	5.495	3.476	2.570	3.467	2.951	3.467	3.15	2.951	6.310	3.467	3.981
25 °C	2.570	3.981	3.981	1.905	2.239	2.239	3.467	3.981	3.476	3.467	3.981	3.476
30 °C	5.971	5.813	6.344	2.237	3.040	3.914	4.677	5.044	6.822	4.677	3.467	3.467
35 °C	4.677	3.981	3.467	---	---	---	4.677	3.467	2.951	3.467	2.570	2.239

Table (2)

Effect of pH on Lipase (a) produced by C. utilis, C. guilliermondii
R. rubra and D. hansenii

Data are expressed as $\mu\text{p/ml}$ (μg Lipase/one ml cell free filtrates)

pH	Organisms					
	<u>C. utilis</u>			<u>C. guilliermondii</u>		
	48 h.	96 h.	144 h.	48 h.	96 h.	144 h.
4	7.586	7.586	7.586	6.310	7.586	7.586
5	2.951	3.467	3.467	7.604	3.467	4.677
6	5.455	3.981	---	3.467	2.570	---
7	5.495	5.495	---	7.586	4.677	2.570
8	4.677	3.981	---	6.310	3.467	---

Table (3)

Effect of different triglycerides on Lipase (a) produced by C. utilis, C. guilliermondii, R. rubra and D. hansenii
 (Data are expressed as ug/ml (as lipase / one ml cell free filtrates))

Triglyceride	C. utilis			C. guilliermondii			R. rubra			D. hansenii		
	48 h.	96 h.	144 h.	48 h.	96 h.	144 h.	48 h.	96 h.	144 h.	48 h.	96 h.	144 h.
Corn Oil	5.971	5.813	5.344	2.237	3.040	3.167	4.677	5.044	6.822	4.877	3.467	3.981
Coconut Oil	5.495	7.604	7.604	7.506	7.586	3.901	2.570	3.467	4.677	---	3.981	3.981
Cotton seed Oil	7.604	7.604	7.604	---	---	---	4.677	5.495	5.129	---	2.570	7.604
Olive Oil	7.586	7.604	7.962	---	---	3.981	3.467	3.981	4.677	---	2.951	3.467
NO	7.604	7.962	8.730	2.239	2.570	2.570	3.981	6.110	6.310	2.570	5.495	5.495

Table (4)

Effect of corn oil concentration on lipase (a) produced by S. utilla, C. zulligerensis, R. rubra and D. hansenii
 Data are expressed as $\mu\text{g/ml}$ (0.15 ml of one ml cell free filtrates)

Oil concn %	Organisms							
	<u>S. utilla</u>		<u>C. zulligerensis</u>		<u>R. rubra</u>		<u>D. hansenii</u>	
	96 h.	144 h.	96 h.	144 h.	96 h.	144 h.	96 h.	144 h.
0.2	4.677	3.981	3.467	3.981	5.495	7.604	2.570	3.467
0.4	4.677	3.981	2.570	3.981	3.467	7.604	2.951	3.981
0.6	7.566	3.981	3.981	3.981	3.467	3.981	3.467	3.981
0.8	7.604	3.981	3.981	3.981	3.467	3.981	3.467	3.981
1.0	5.003	6.344	3.040	3.981	5.044	6.822	3.467	3.981

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

انتاج انزيم الليبوز ببعض الخنافس
١- تأثير درجة الحرارة - الرقم الايدروجيني والجلسيدات

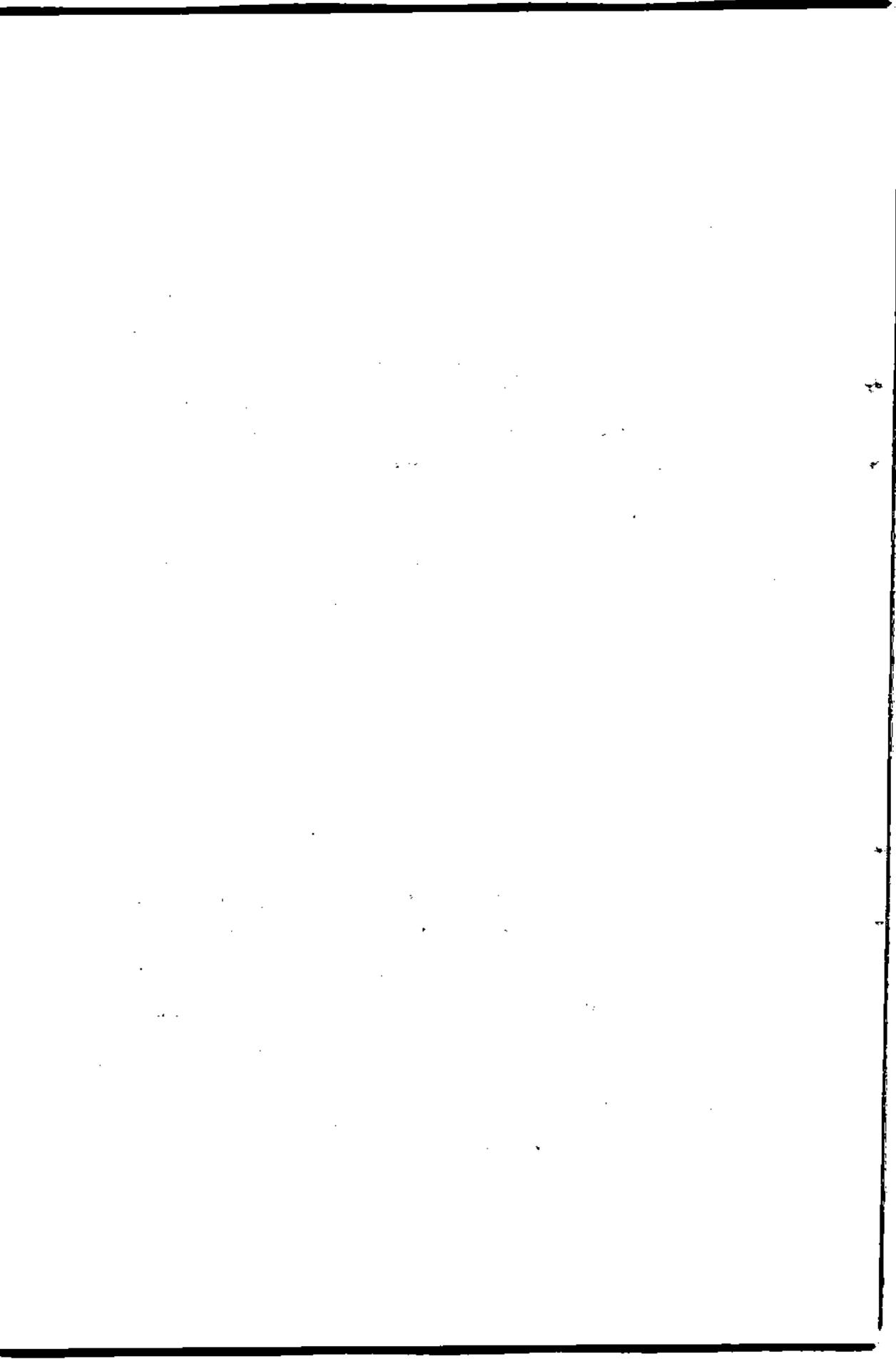
سومى محمد أحمد الجبال و منى اسحق رزق
قسم النبات - كلية النبات - جامعة عين شمس

عملت محاولة لتقدير النشاط الليبوزى لى ثلاثة خمائر غير متكيفة وهى
رودوتورولا روبرا ، كانديدا يوتلس و كانديدا جويليروموندى واحسيد
انواع الخمائر المتكيفة وهى دياروسيس هاتسنى .

وقد انتج انزيم الليبوز تحت مؤثرات مختلفة عنها فترات التحضين
الرقم الايدروجينى ، ودرجات الحرارة وكذلك انواع مختلفة من
الجلسيدات .

وقد اعطت رودوتورولا روبرا اعلى نشاط انزيمى بعد فترة تحضين
سنة ايام عند ٢٠م على بيئة خالية من السكر وتحتوى على زيت المينوره
عدد رقم ايدروجينى ٠٤ . وقد اعطت كل من كانديدا يوتلس و كانديدا
جويليروموندى اعلى نشاط انزيمى عند نفس الظروف السابقة ولكن لى
وزن زيت الدره بالنسبة للكائن الاول وزيت جوز الهند للثانى ، زيت
الدره احسن على انتاج الانزيم لكل من رودوتورولا روبرا ، دياروسيس
هاتسنى ، كانديدا يوتلس و كانديدا جويليروموندى عند تركيبات
٦ ، ٦ ، ٦ ، ٨ ، ١ % بالترتيب .

ولكن بالنسبة للخميرة المتكيفة التى نمت على نفس البيئة اعطت
احسن نشاط انزيمى عند ٢٠م بعد ٤٨ ساعة نمو .



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Production of lipase (S) by Some Yeasts :

II. Effect of Some Carbohydrates and Nitrogenous
Compounds

Sawaan M.A.El-Gammal and Mona I. Rizk
with 2 Figures

Maximum lipase production by all experimented yeasts was obtained in presence of 0.5 % yeast extract only. All tested nitrogenous compounds failed to do so. When the medium was amended with xylose or starch, maximum production of lipase per unit of medium was achieved by Rhodotorula rubra, Candida utilis and C. guilliermondii, while Sucrose and maltose showed the same effect on using Debaryomyces hansenii.

Introduction :

It has been suggested that certain lipases are adaptive in nature and their production is stimulated by the presence of specific substrates. In other words, the elaboration of the enzymes depends upon preferential utilization of certain substrates by the organisms (Cutchins et al., 1952).

Sometimes, lipase production depends essentially on the organism itself as mentioned by Naylor et al., 1930.

Chopra & Chander (1983) observed that lipase production by Syncephalastrum racemosum was subjected to catabolite repression when certain nitrogen and carbon sources were used in the growth medium.

Ammonium ions increased lipase production when used as nitrogen source by Torulopsis sp. (Dasouki, 1976), while they completely inhibited the synthesis of the same enzyme by Pseudomonas fluorescens (Cutchins et al., 1952). On the other hand a small amount of lipase was obtained by Thermoactinomyces vulgaris (Elwan et al., 1978).

Different carbon sources were found to be optimal for lipase biosynthesis. Yasuhida et al. (1973, 1978) used 2 % glucose and 2 % olive oil for the production of lipase by Candida parailipolytica. Soybean meal and bran stimulated lipase formation when added separately to C. parailipolytica 739 cultures as sole carbon source (Vecozola & Luka, 1979).

The present investigation aimed to study the effect of some carbon and nitrogen sources on lipase production by certain yeasts.

Material and Methods :

Test organisms and medium:-

The same medium as well as the four yeasts, Candida utilis, C. guilliermondii, Rhodotorula rubra and

Debaryomyces hansenii used in a previous investigation (El Gammal & Rhizk, 1984) were tested.

Preparation of cell-Free culture filtrates and estimation of growth :

Five ml of the "growth culture filtrates six day old were centrifuged at 3000-4000 rpm for 10 minutes and the supernatant was stored at 0°C.

The enzyme was assayed as previously described by El-Gammal & Rhizk (1984). Yeast cells were washed three times with distilled water, dried at 90°C in an electric oven for 16 h and then reweighed. Growth was expressed in terms of mg dry wt/ml broth.

Effect of nutrients :

The effect of the following groups of nutrients was examined. Various sugars (sucrose, lactose, maltose, arabinose and xylose), starch and glycerol were separately incorporated in the growth medium to study their effect on enzyme production. They were added in such amounts that provide the carbon weight located in 0.5 % of glucose.

As nitrogen sources, peptone was separately replaced by the following nitrogen compounds, NaNO_3 , casein hydrolysate, beef extract and urea in 0.5 % concentration(W/V).

Results and Discussion :

Results recorded in Table (1) and represented in Fig.(1) show that lipase production by the four different yeasts was greatly affected by the kind of carbon source. For D. hansenii, sucrose was the most favourable for lipase production; maltose showed the same effect followed by lactose and arabinose.

However, xylose, starch and glycerol slightly enhanced lipase production. In case of the anascosporogenous yeasts, C.utilis, C.guilliermondii and R.rubra either xylose or starch induced lipase productivity. All the other used sugars decreased lipase production. These results are in agreement with those obtained by Mohaved (1984) who stated that the most suitable sugars for the induction of lipase production by Aspergillus sydowi were sucrose and D(+) xylose since their presence in the mineral salts production medium gave the highest yield of lipase. The noticed result that D.hansenii favoured sucrose as the best carbon source for lipase production is in accordance with what has been noticed by Ammar & McDaniel (1979). They found that 0.5% sucrose was the best sugar and concentration for inducing lipase formation by Bacillus stearothermophilus.

Interestingly, there was a relatively limited stimulation when 1 % starch was added to the cultural medium of D.hansenii and C.utilis. However, for the other yeasts; C.guilliermondii and R.rubra 100% and 30% induction respectively was observed. Although starch inhibited lipase production when it was supplied to Aspergillus sydowi either separately or in combination with oil in the mineral salts medium (Mohawed 1983), yet starch was used in the production medium of certain yeast lipase e.g Torulopsis ernobii (Yoshida et al., 1968).

The failure of glycerol to induce lipase production by the yeasts under investigations may be explained by the suggestion of Temmisto (1933) that certain lipolytic species are unable to hydrolyze fat when they are grown on glycerol - fat agar due to the inactivation of the lipase by acid developed in glycerol fermentation .

Table (2) and Fig(2) represent the effect of different nitrogen sources on lipase production by the four experimented yeasts. One notes that omission of peptone from the growth medium, using yeast extract only stimulated maximum lipase production by all the tested yeasts, though a concentration of 2 % peptone gave maximum lipase production by many microorganisms (Tsujisaka

et al., 1973, Akhtar, 1979; Chander et al., 1980).

Our results are also contradictory to those obtained by Hosono & Tokita (1970) who found enhancement of lipase production by Candida mycoderma on using peptone.

However, the present results agree with those obtained by Vecozola & Luka (1979) who used 1-2 % yeast autolysate for the production of lipase by Candida paralytolytica. Also, Chander et al., (1980) found that addition of 0.5% (W/V) yeast extract to the medium had a stimulatory effect on the synthesis of lipase by Aspergillus wentii.

The present results show that incorporation of yeast extract in the medium was essential for the optimum production of the enzyme. Peptone gave increased growth than all other nitrogenous tested compounds, but there was a corresponding marked reduction in lipase synthesis when expressed per unit of growth. Also lipase reduction was obtained with casein in case of C.utilis, casein and urea in case of C.guilliermondii and all other tested nitrogenous sources in case of R.rubra and D.hansenii.

The data in Table (2) represent good evidence that lipase production by C.utilis, C.guilliermondii, R.rubra and D.hansenii was subjected to catabolite repression when certain nitrogenous compounds were incorporated in the

growth medium. This is in agreement with Chopra et al. (1983) who experimented with Syncephalastrum racemosum using groundnut protein, Soybean meal, milk casein and wheat bran as sources of nitrogen.

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Table (1) : Effect of some sugars and other carbon compounds on growth and lipase production by *Candida utilis*, *G. Sullitermondii*, *Rhodotorula rubra* and *Debaryomyces hansenii*.

Carbon Source	<i>C. utilis</i>			<i>G. Sullitermondii</i>			<i>R. rubra</i>			<i>D. hansenii</i>		
	biomass mg dry wt/ml broth	lipase/ ml broth	lipase/mg dry wt.	biomass mg dry wt/ml broth	lipase/ ml broth	lipase/mg dry wt.	biomass mg dry wt/ml broth	lipase/ ml broth	lipase/mg dry wt.	biomass mg dry wt/ml broth	lipase/ ml broth	lipase/mg dry wt.
Lactose	0.90	3.9	22.1	0.70	2.2	15.9	0.88	3.9	29.2	0.69	4.6	34.3
Sucrose	0.92	3.9	21.6	0.96	2.2	11.6	0.90	3.9	22.1	0.69	7.5	39.5
Arabinose	0.89	3.4	19.4	0.90	2.2	12.4	0.90	3.4	19.2	0.98	4.6	23.8
Maltose	0.94	2.2	11.9	0.62	2.2	18.0	0.90	3.4	19.2	0.90	7.5	42.2
Xylose	1.85	7.6	20.5	1.89	7.6	20.1	1.91	10.2	26.8	1.83	3.9	10.8
Sterch	1.85	6.6	17.2	1.97	7.6	19.2	1.90	8.7	22.9	1.92	3.9	10.3
Glycerol	0.96	3.4	18.0	1.11	2.2	10.0	0.89	3.9	22.3	0.84	3.9	23.6
No sugar	1.86	5.8	23.4	1.84	3.0	9.4	1.89	5.0	20.0	1.85	3.4	9.3

Table (2): Effect of some nitrogen sources on growth and lipase production by Candida utilis, C. Guilliermondii, Rhodotorula rubra and Debryomyces hansenii.

Nitrogen Source	C. utilis			C. Guilliermondii			R. rubra			D. hansenii		
	biomass mg dry wt./ml broth	US lipase/ mg dry wt.	US lipase/ mg dry wt.	biomass mg dry wt./ml broth	US lipase/ ml broth	US lipase/ mg dry wt./ml broth	biomass mg dry wt./ml broth	US lipase/ ml broth	US lipase/ mg dry wt./ml broth	biomass mg dry wt./ml broth	US lipase/ ml broth	US lipase/ mg dry wt.
Yeast extract	0.348	10.9	31.5	0.220	5.4	24.9	0.208	8.7	41.9	0.208	3.9	19.1
Na NO ₃	0.200	10.9	54.7	0.218	4.6	21.4	0.356	3.9	11.1	0.352	3.4	9.8
Casein	0.366	10.9	29.8	0.214	3.9	18.6	0.352	6.3	17.9	0.962	-	-
Beef extract	0.204	10.9	53.6	0.212	4.6	22.0	0.374	7.9	21.2	0.358	3.9	11.1
Urea	0.240	10.9	45.5	0.204	2.9	14.4	0.356	5.4	15.4	0.208	3.4	16.6
Peptone	0.372	6.3	23.4	0.368	3.4	9.4	0.378	6.8	20.0	0.370	3.4	9.3

mg lipase / ml cell free filtrates

D. hansenii 
 R. rubra 
 C. utilis 
 C. guilliermondii 

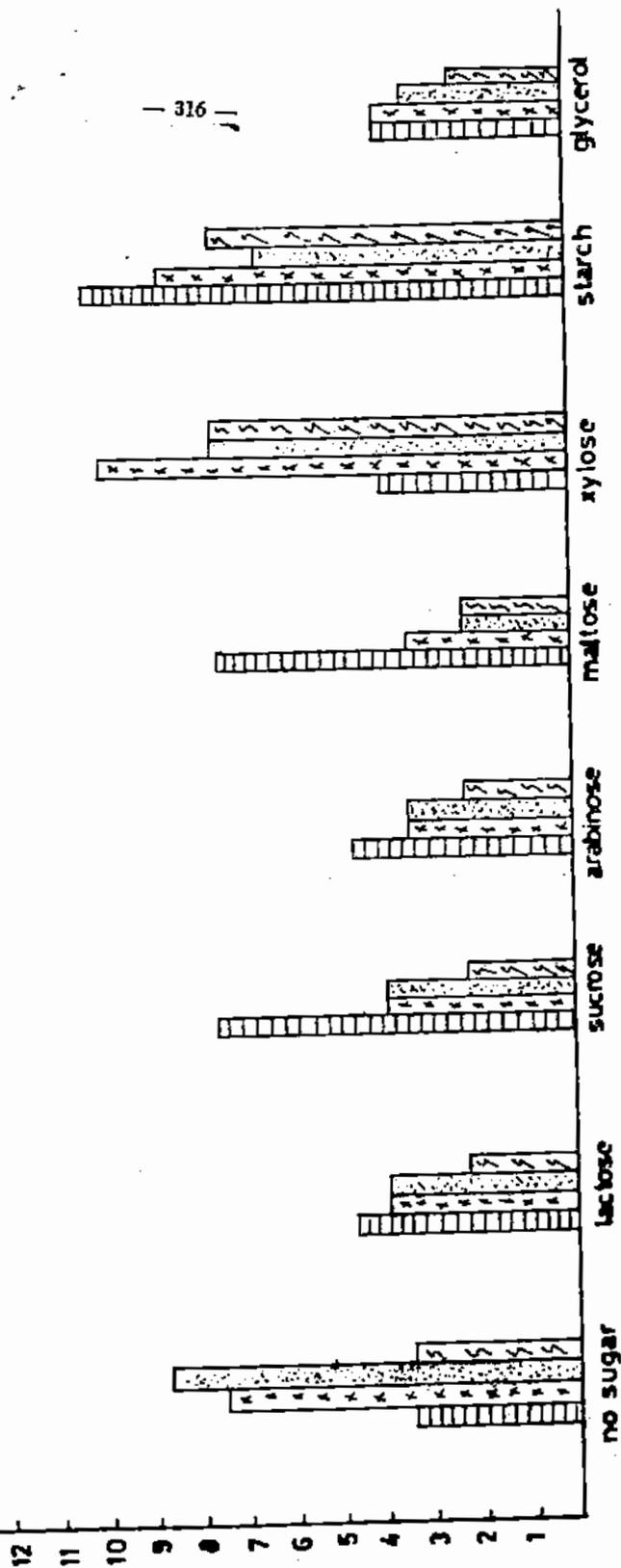


Fig. (1) : Effect of Some Sugars on Lipase Production by *C. utilis*, *C. guilliermondii*, *R. rubra* and *D. hansenii*.

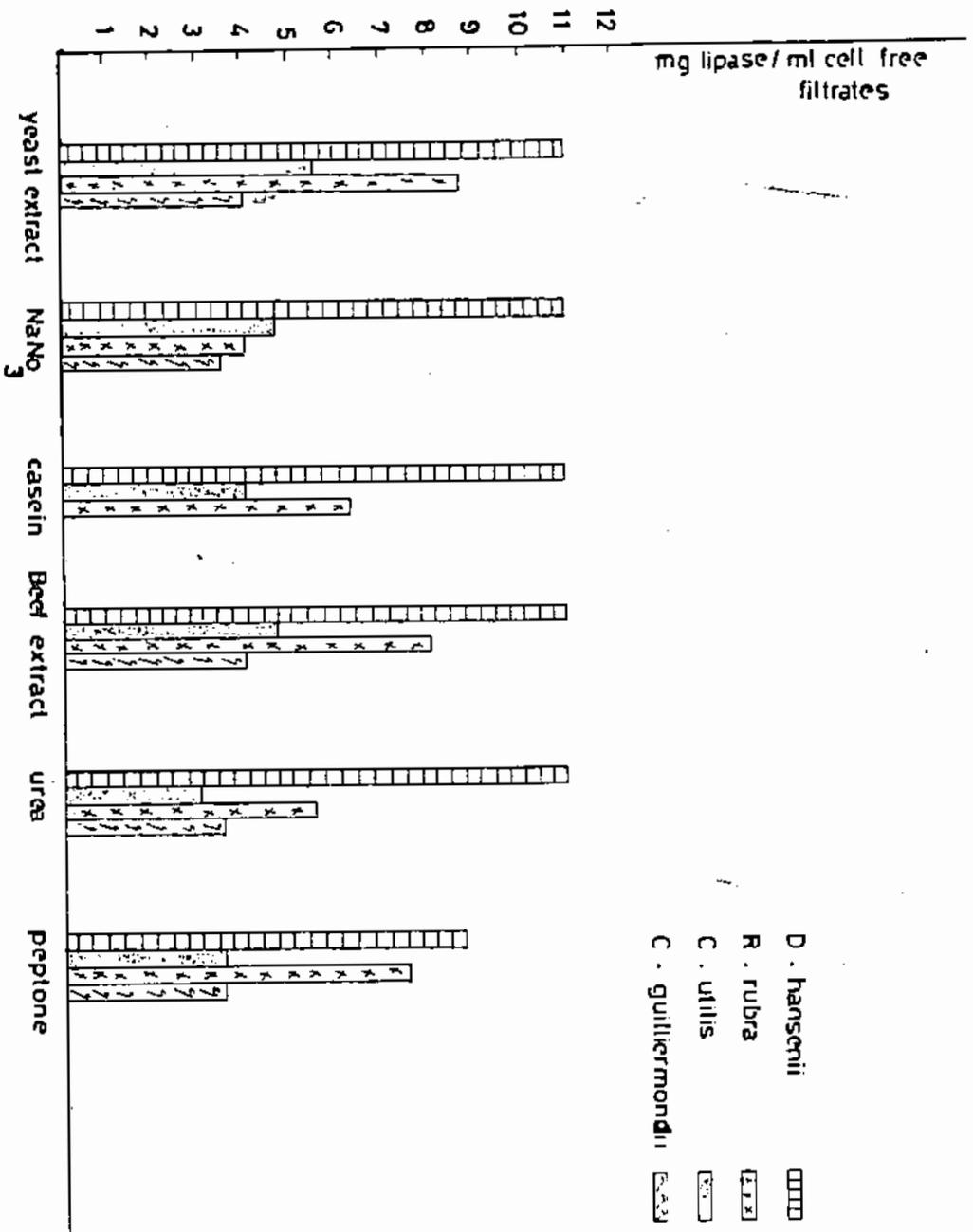


Fig. (2) : Effect of Some Nitrogenous supplements on Lipase production by *C. utilis*, *C. guilliermondii*, *R. rubra* and *D. hansenii*.

قسم النبات - كلية النبات - جامعة عين شمس

انتاجية

انزيم اللييز بواسطة بعض الخائمر

٢- اثر بعض المعادن الكربوهيدراتية والنتروجينية

موسم محمد عبد العزيز الجمال ، م م احمق رزق

اظهرت الخائمر المختبرة كلها اعلی انتاجية لانزيم اللييسيز

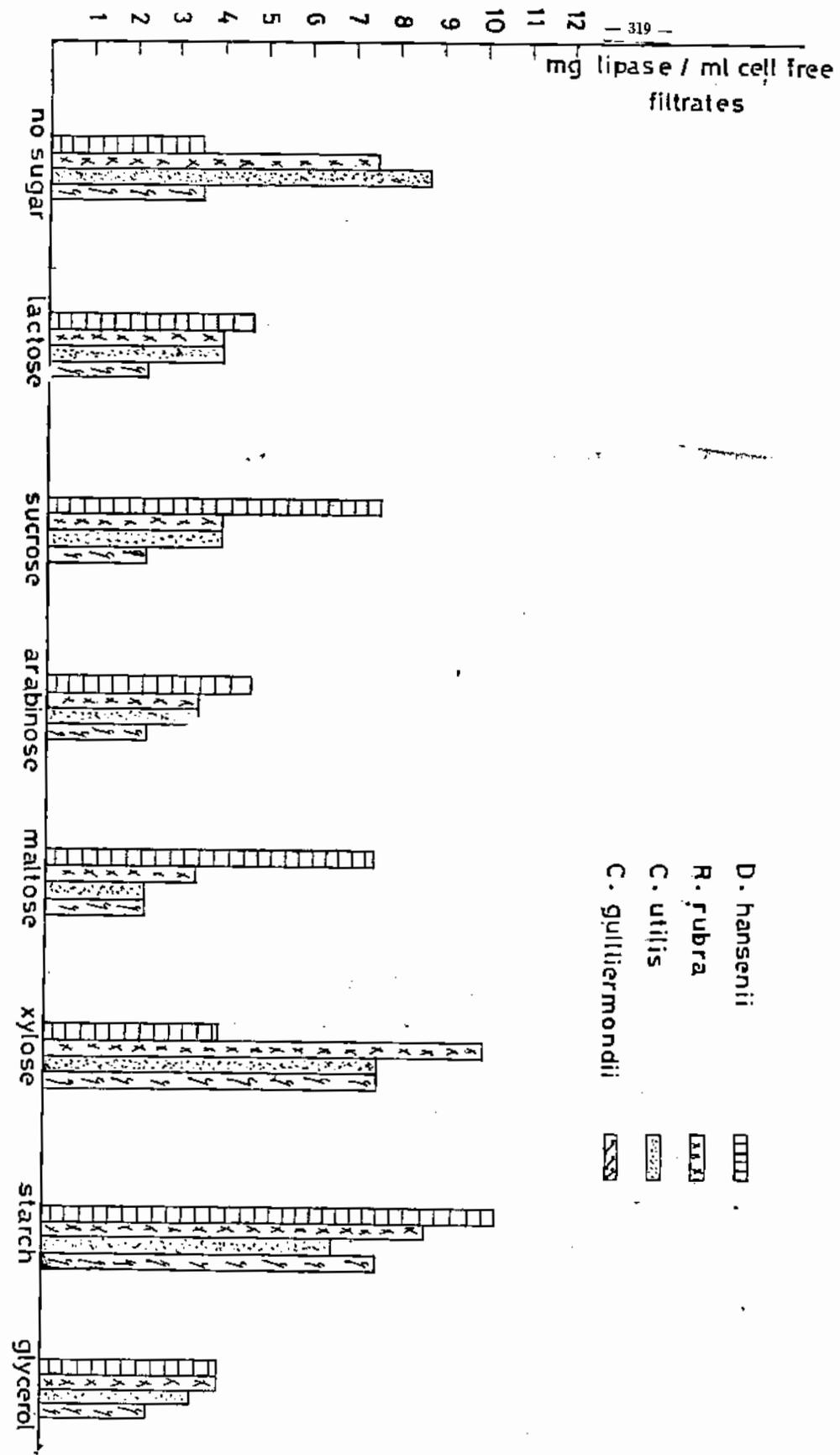
باضافة ٥ ٪ مستخلص الخميرة لبيئة النمو بينما اختلفت جميع المواد

النتروجينية الاخرى في اعطاء اعلی انتاجية . ونتج عن اضافة سكر

الزيلوز او النشا الى بيئة النمو اكبر كمية من الانزيم بواسطة كل من

خميرتي رودوثوريولا روسا وكانديدا ييثيلس بينما نتج نفس التأثير نفس

حالة دياروسيس هانغياى باضافة السكر او المالتوز .



D. hansenii

R. rubra

C. utilis

C. guilliermondii

Fig. (1) : Effect of some Sugars on Lipase Production by *C. utilis*, *C. guilliermondii*, *R. rubra* and *D. hansenii*.

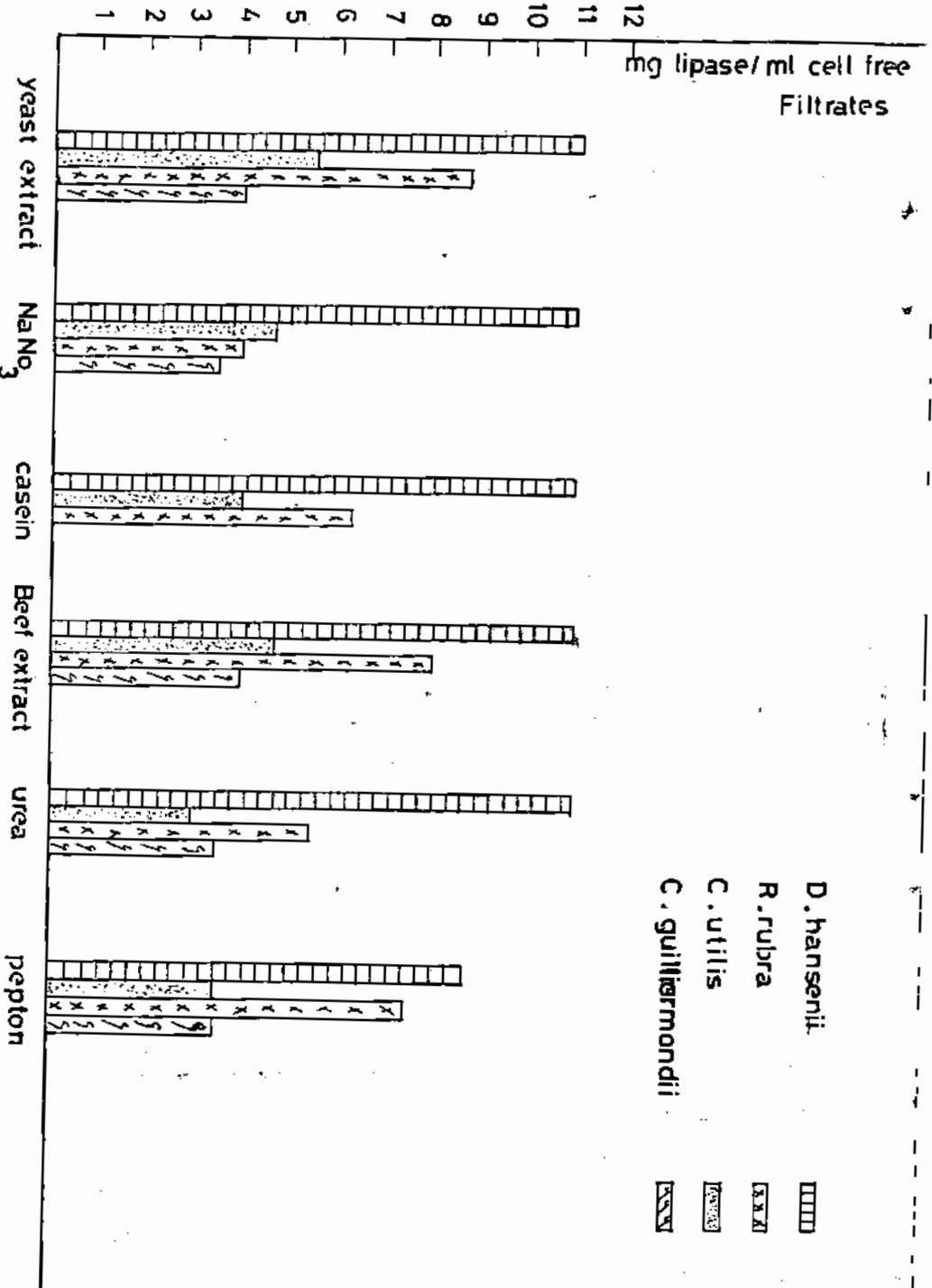


Fig. (2) : Effect of Some Nitrogenous supplements on Lipase

modifications by addition of nutrients

Effect of Thiourea on the Germination, Respiration and Growth
of Amaranthus chlorostachys Seeds.

By

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Abstract:- The effect of thiourea on the germination, respiration and growth of Amaranthus chlorostachys seeds is presented. It is suggested that thiourea may act on the seeds by changing the nature and amount of their growth regulators. This change may channel respiration in the direction of energy yielding processes.

Amaranthus chlorostachys is a widely distributed weed in Egypt. The percentage of germination of the freshly harvested seeds was found to be about 57%. Thompson and Kesar (1939) studied the effect of six sulphur compounds on the germination of lettuce seeds. They reported that thiourea is the most generally effective compound in promoting germination of lettuce seeds. Since that time, thiourea has been used by many investigators to stimulate the germination of many seeds (Delvia, 1969; Mayer and Plojloff-Mayber, 1975).

In this paper, the effect of thiourea on the germination, respiration and growth regulators of Amaranthus chlorostachys seeds will be represented.

Material and Methods

Amaranthus chlorostachys seeds were collected from the Botanical Garden of the Faculty of Education, Ain Shams University, Cairo. The seeds were either soaked in distilled water or in different concentrations of thiourea for 48 hours. After thoroughly washing the seeds, they allowed to germinate in petri-dishes lined with filter paper at 35°C. At least one hundred seeds were used in each treatment. After 72 hours incubation, the percentage of germination of the seeds was estimated.

Conventional manometric technique (Umbreit et al .,1959) was used in the respiratory studies. 0.05M potassium buffer at pH 6.0 was used as it was found to be the optimum pH for the respiration of Amaranthus chlorostachys seedlings. The Warburg water bath was at 35°C, the optimum temperature for the germination, growth and respiration of the seeds.

For the extraction and detect of the growth regulators, the seeds were soaked either in distilled water or 0.2M thiourea for 48 hours and washed thoroughly. The methods used for the extraction and assay for promoters and inhibitors were that adopted by Foda and Radwan,(1962).The seed extract was fractionated by descending paper chromatography using 80% isopropyl alcohol:20% distilled water was the running solvent.The method used to assay the gibberellins and gibberellin-like substances was the lettuce hypocotyl test(Frankland and Wareing,1960 and Crozier et al .,1970).

The air dried chromatograms were subjected to some chemical tests, where a longitudinal strip from chromatograms was sprayed with certain reagents to test for indole compounds(Kefford,1955; Powell,1959), hydroxyl groups(Swain,1953),reducing substances and amino acids(Hunt,1959; Smith,1960),Gibberellins(Jones et al .,1963; Kagawa et al .,1963) and unsaturated lactones(Swain,1953).

Results

Increasing thiourea concentrations resulted in a change in the percentage of germination of Amaranthus chlorostachys seeds(fig.1.) The percentage of germination of the seeds reached a maximum of 99% at a concentration of 0.2M thiourea. Increasing thiourea concentrations above that level resulted in a decrease in the percentage of germination of the seeds to a very low value of about 20% with 0.617 concentration.

There was a gradual increase in the rate of the endogenous oxygen uptake of amaranthus chlorostachys seeds during the first four hours of soaking the seeds in water(fig.2). After that, the rate of oxygen uptake remained constant until the 52nd hour. After that time, the rate of oxygen uptake started to increase again with time. When the seeds were treated with 0.2M thiourea(fig.2), the rate of the oxygen uptake increased by increasing time. Beside the

disappearance of the first constant rate which appeared in the untreated seeds between the fourth and the fifty second hour due thiourea treatment, the rate of oxygen uptake of the treated seeds was usually higher than the untreated seeds. The rate of carbon dioxide evolution followed almost the same trend as that of oxygen uptake (fig.3), but the respiratory quotient was not affected as it was about 0.7 in the treated and untreated seeds.

The extract of the untreated seeds showed in both the coleoptile and hypocotyl tests growth inhibitor zones only (fig.4 and 5). The coleoptile tests showed seven growth inhibitor zones (R_p 0.1-0.2, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, 0.7-0.8 and 0.9-1.0) and the hypocotyl tests showed nine growth inhibitor zones (R_p 0.0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.6-0.7, 0.7-0.8, 0.8-0.9 and 0.9-1.0). All of these growth inhibitor zones gave a positive colour reaction with diazotized p-nitro alanine indicating that these growth inhibitor zones may contain unsaturated lactones. When Amaranthus chlorostachys seeds were treated with 0.2M thiourea, the growth inhibitor zones were reduced in both the coleoptile and the hypocotyl tests (fig.4 and 5). Besides, three promoter zones (R_p 0.1-0.2, 0.3-0.4, and 0.5-0.6) appeared in the coleoptile tests. The second and third zones gave a positive colour reaction with indole reagents whereas the first zone gave a positive colour reaction in the test for the hydroxyl groups. In the hypocotyl tests, the growth inhibitor zones were decreased, and one promoter zone (R_p 0.5-0.6) appeared. This zone gave a positive colour reaction in the test for hydroxyl groups.

Discussion

It was found that soaking Amaranthus chlorostachys seeds in 0.2M thiourea for 48 hours resulted in an increase in the percentage of germination of the seeds from 57% to 99% (fig.1).

The rate of respiration of Amaranthus chlorostachys seeds was found to pass through several phases during germination (fig.2 and 3). An initial rapid increase during the first four hours of soaking the seeds, a plateau when it remains constant until the 52nd hour and a second increase. This trend of the rates of oxygen uptake and carbon dioxide evolution of Amaranthus chlorostachys seeds during germination is similar to that reported by Spragg and Yean

for pea seeds. The first rise in the rate of respiration is not necessarily related to germination as such, and occurs equally in seeds which will germinate and those which will not (Mayer and Poljakoff-Mayber, 1975). The rise after the plateau occurs only in seeds which germinate and is closely associated with seedling growth. If germination is prevented in some way, the second rise in respiration is usually also prevented.

When Amaranthus chlorostachya seeds were treated with 0.2M thiourea the rate of respiration increased with time, (fig. 2&3). The plateau which appeared in the untreated seeds between the fourth and the 52nd hour of germination disappeared. Beside the disappearance of this plateau, the rate of respiration of thiourea treated seeds was higher than the untreated ones.

The fractionated extract of Amaranthus chlorostachya seeds showed growth inhibitor zones in both the coleoptile and the hypocotyl tests (fig. 4&5). When the seeds were treated with 0.2M thiourea, the growth inhibitor zones were decreased and growth promoter zones appeared.

Germination is an energy requiring process and is therefore dependent on the respiration of the seeds. Degey and others (1965) reported that thiourea is not known to have any direct effect on the respiratory enzyme system of a particular fraction similar to mitochondria isolated from lettuce seedlings. In 1960, Whitman and Poljakoff-Mayber studied the effect of thiourea on the phosphorylating activity of lettuce seed mitochondria. They reported that thiourea stimulates the P/O ratio.

It is suggested that thiourea may act on Amaranthus chlorostachya seeds by changing the nature and amount of the growth regulators present in the seeds. This change may affect the respiratory mechanism possibly by rapidly channelling all respiration in the direction of energy yielding processes.

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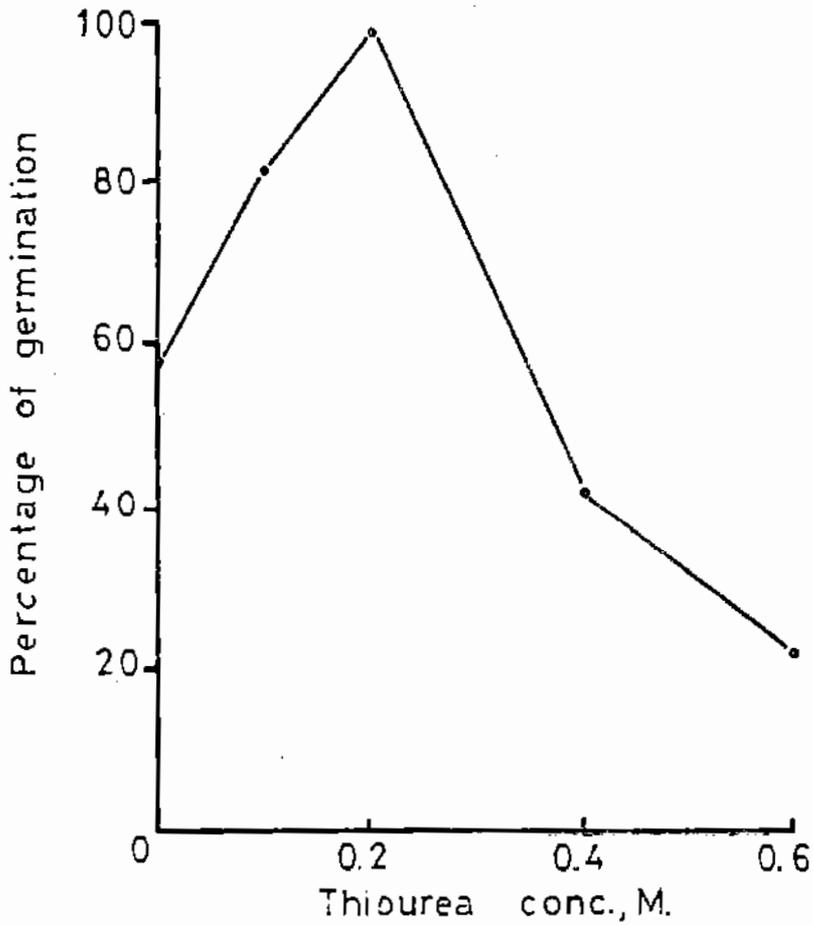


Fig.(1): Effect of thiourea on the percentage of germination of Amaranthus chlorostachys seeds

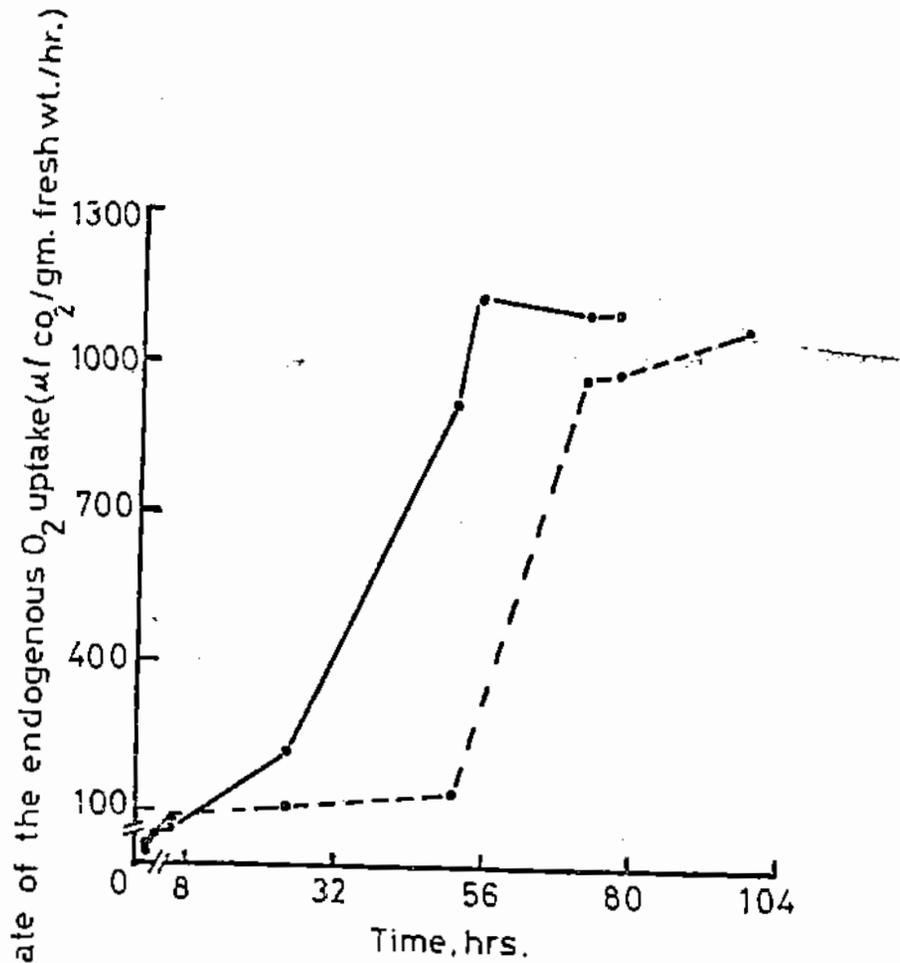


Fig. (2): Effect of thiourea on the endogenous oxygen uptake of Amaranthus chlorostachys seeds during germination.

(treated —●—, untreated - - - ● - -)

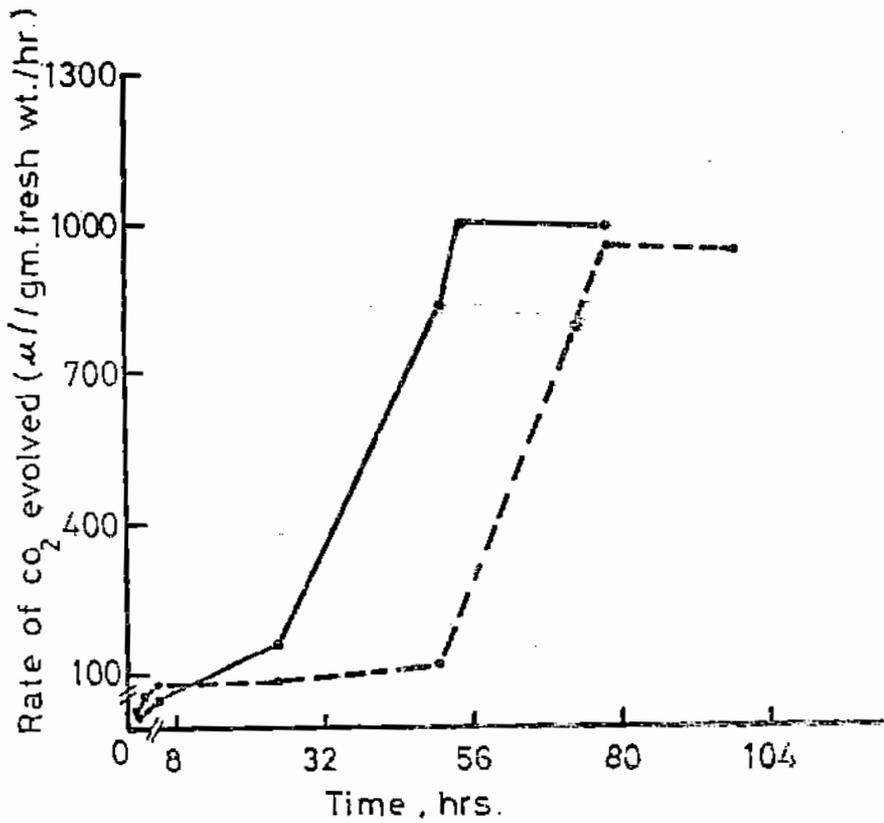


Fig.(13): Co₂ evolution of untreated and treated *Amaranthus chlorostachys* seeds with thiourea during germination.
(●—● treated, ●---● untreated.)

Change in length of coleoptile sections as % of control.

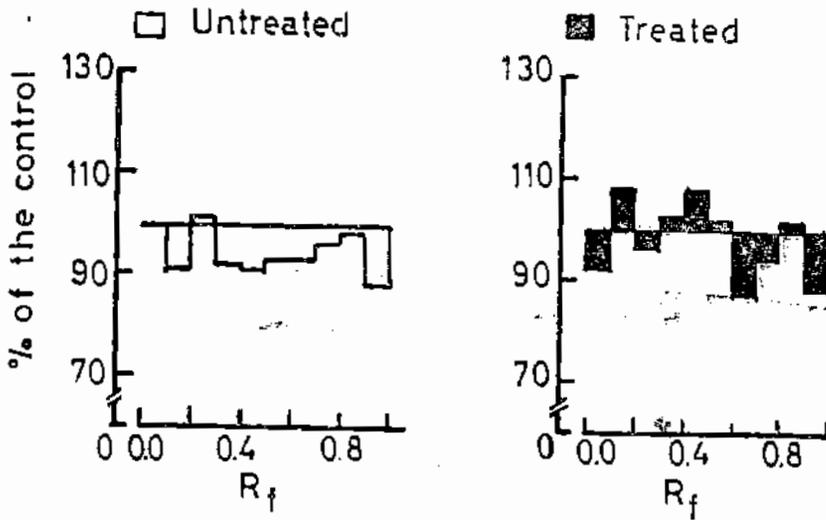


Fig.(4): Coleoptile test for fractionated extracts of thiourea treated and untreated Amaranthus chlorostachys seeds.

Change in length of hypocotyl sections as % of control.

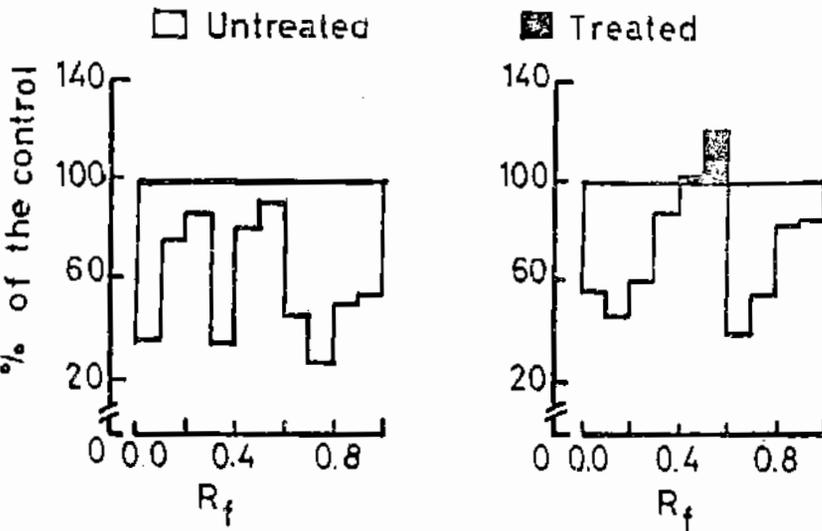
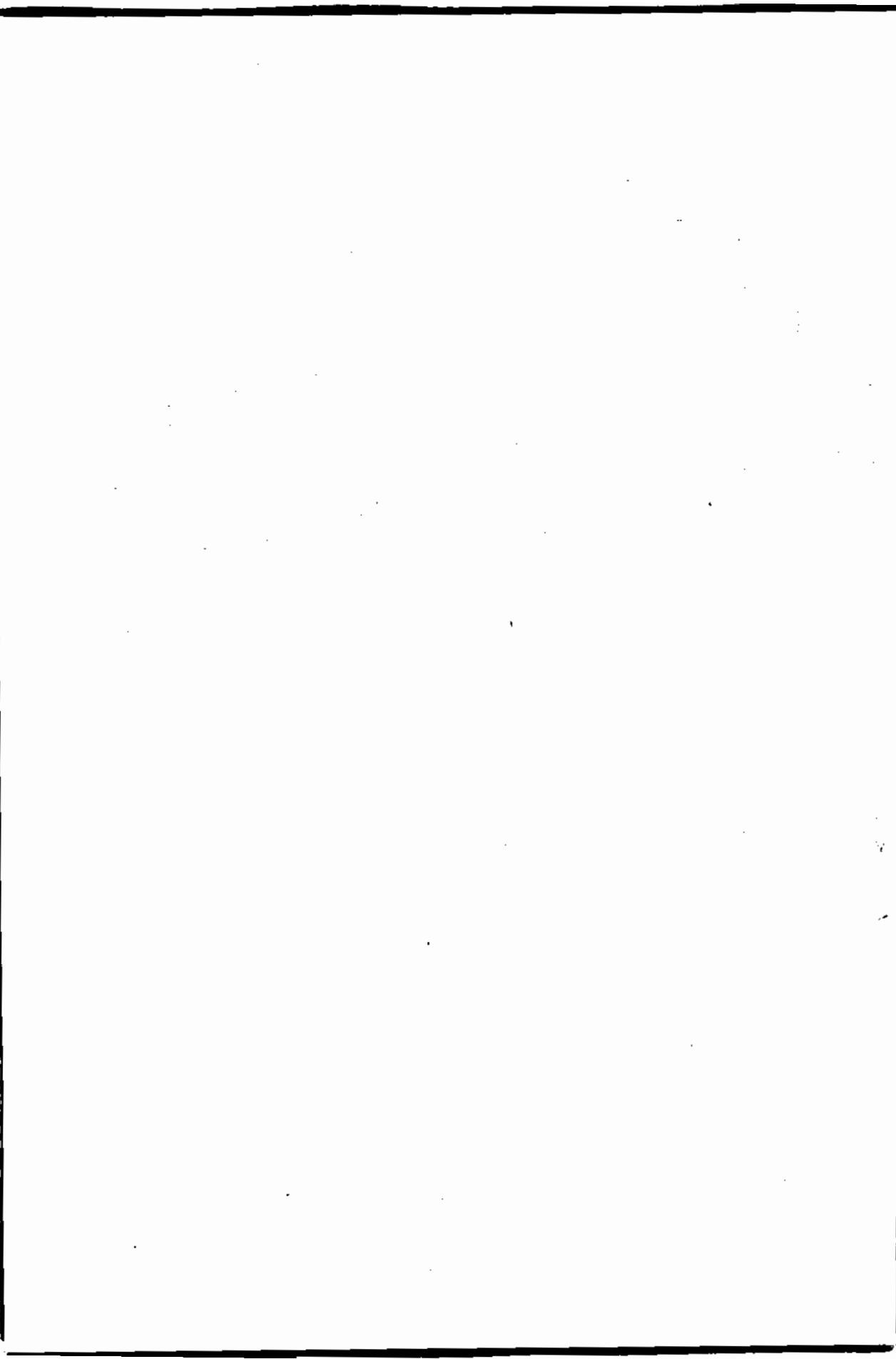


Fig.(5): Hypocotyl test for fractionated extracts of thiourea treated and untreated Amaranthus chlorostachys seeds.



Studies on growth of Rhizobium of Pisum sativum under stress conditions.

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SUMMARY

One fast growing acid producer Rhizobium strain (local strain) of Pisum sativum was screened for growth behaviour in acid, saline and alkaline broth culture. It grew in yeast extract mannitol broth wide pH range (3.5-10) at varying concentrations of NaCl, Na₂CO₃, Na HCO₃ and CaCl₂.

The growth of this strain increases when NaCl concentrations were raised from 0.2-0.8%, and decreases when CaCl₂ concentrations were raised from 0.2-3.5% and Na₂CO₃ and Na₂HCO₃ from 0.1-1%. The results indicate that growth of Rhizobium infecting Pisum sativum is adversely affected by saline and alkaline conditions. The effect of alkalinity is more drastic than salinity. This in-turn suggests that the ability of Rhizobium to establish a successful symbiotic relationship with Pisum sativum is inhibited by these conditions.

Introduction

Pea, Pisum sativum is an important food in tropical regions of Egypt and Africa. Lands of Egypt are characterised by the presence of excessive soluble salts (about 6% water soluble salts in saline soils). The dominant ions are sodium, calcium, chloride, carbonate and bicarbonate. Most common are sodium and calcium chloride type of salinity.

To obtain satisfactory yields of Pisum sativum in such saline and alkaline soils, varieties of Rhizobium and host genotype

that are tolerant to the stresses associated with these soils are desired. These stresses include high pH and high levels of sodium chloride, calcium chloride, sodium carbonate and bicarbonate.

Some authors studied the effect of salinity on the growth of Rhizobium spp. in saline soils and in broth culture (Upchurch and Elkan 1977, Steinborn and Roughley 1975, Abdel Wahab and Wahran 1979 and Bhardwaj 1972), while others studied the effect of alkalinity on the growth of Rhizobium spp. in alkaline soils and in broth culture (Helemish and El-Gammal 1985, Yadav and Vyas 1971 and Singh et al., 1973). Some strains have been found to be salt sensitive to even 0.1% (Helemish 1985, Abdel Wahab and Zahran 1979), while others have been found to be salt resistance to even 3.0% NaCl (Pallai and Sen 1966, Yadav and Vyas 1973 and Subba Rao et al. 1972).

Optimum pH for rhizobia was neutral or slightly alkaline (Yadav and Vyas 1971) and they were sensitive to acidity (Allen and Allen 1950). According to Pandher and Kahlon (1978), Rhizobium leguminosorum isolated from Pea (Pisum sativum) failed to grow at pH 3.0, maximum growth was attained at pH^{6.5-8.0}. On the other hand pH's above . . . 8.5 was not lethal but did not support growth.

One should aim to find Rhizobium strains host variety combinations which yield well in particular soils and environments in order to exploit the full potential of the symbiotic system.

MATERIAL AND METHODS

Isolation of Rhizobium of Pisum sativum: Pure culture of Rhizobium of Pisum sativum was isolated according to the method described by Allen and Allen (1950), from the roots of Pisum sativum. A big red effective nodule was selected, carefully cleaned with running water from adhering soil particles, the surface was then sterilized in 1:100 HgCl₂ solution for 3-6 minutes and in 95% ethanol for a similar period of time. The nodule was then consecutively transferred to sterile petridishes containing sterile water and continuously agitated to secure sufficient rinsing. The nodule was then removed to a sterile petridish and crushed in one ml sterile distilled water. Streaking using a loopful of the concentrated crushed nodule exudate on the surface of each of 5 plates was carried-out. Yeast extract-mannitol-agar medium was used for such isolation.

The petri-dishes were then incubated at 30°C for one week after gram staining (-ve) and microscopic testing of some colonies under strict sterilized conditions for each purity, loopfuls from a selected colony were inoculated into slopes of yeast extract-mannitol agar the composition of which is as follows: Mannitol, 10 gm; NaCl, 0.1 gm; MgSO₄ · 7H₂O, 0.2 gm; K₂HPO₄, 0.5 gm; yeast extract 0.5 gm; CaCl₂ 0.1 gm, -ph was adjusted at 7.2, sterilization was carried out at 1.5 atm. for 15 min, 2% agar was added when necessary.

Flasks containing 50 ml liquid medium were inoculated with 2 ml bacterial suspension (about 10⁶) prepared from scraping of

2-day old agar cultures, flasks were shaken at 120 rpm./min. at 30°C growth in liquid medium was assessed turbidimetrically using Bausch and Lomb Spectronic 20 at 540 um. every 24 hours. All treatments were replicated twice.

Effect of pH: The effect of pH on the growth of the organism was assessed in liquid medium with the post-sterilisation pH values Viz: 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5 and 10. The pH was adjusted by Tacussel pH meter.

Effect of different salts: The salts tested were NaCl, CaCl₂, sodium carbonate and bicarbonate. NaCl and CaCl₂ were supplied at contrations of 0.0 (control), 0.2, 0.5, 0.8, 1.5, 2.5 and 3.5% (W/V) respectively. Carbonate and bicarbonatge were supplied at concentra-tions of 0.0 (control), 0.1, 0.2, 0.5, 0.8 and 1% (W/V) respectively. All other growth conditions were performed as previously mentioned.

RESULTS

Effect of pH: The results presented in table (I) on growth and change in pH of the medium after 72 hours and after one week incubation clearly show that this strain could be grown at a wide pH range (3.5-10). Subsequently, the pH variations from the initial levels were also noted. This strain was classified as a fast growing acid producer as when it was grown in broth of pH 4.5 and 5.5 decreased to 3.8 and 4.6 within 72 hours.

The decrease in pH was more acute at high initial broth pH 8.5, 9.5 and 10.

Effect of NaCl: Data in Fig. (1) on the growth of Rhizobium of Pisum sativum in broth containing different concentrations of NaCl show that the strain can grow in presence of various concentrations of NaCl. The growth was high at concentration from 0.2-0.8% and low at concentrations from 1.5-3.5% NaCl relative to control.

Effect of CaCl₂: Effect of different concentrations of CaCl₂ on the growth of Rhizobium of Pisum sativum Fig. (2) shows that the strain can grow slowly at concentrations varying from 0.2 to 3.5% CaCl₂, maximum growth was recorded in absence of CaCl₂. At all concentrations the growth decreases and it was lower than control even at 0.2% CaCl₂.

Effect of carbonate and bicarbonate: Effect of different concentrations of sodium carbonate and bicarbonate on the growth of Rhizobium of Pisum sativum Fig. (3) and Fig. (4) indicates that the strain is nontolerant to these salts and even 0.1% was inhibitory to the growth.

Effect of carbonate was much higher than bicarbonate. The growth decreased with the increase of salt concentrations.

DISCUSSION

One of the means of solving salinity and alkalinity problems in saline/alkaline soils is to screen rhizobia and hosts for tolerance to these stress conditions.

The aim of this investigation is to test the behaviour of Rhizobium of Pisum sativum in acid, saline and alkaline broth culture.

The Rhizobium strain of Pisum sativum was classified as fast growing acid producer, since the pH values of 4.5 and 5.5 were decreased to 3.8 and 4.6 respectively within 72 hours of incubation. It grows at a wide range of pH values (3.5-10) with maximum growth at pH values from (4.5-5.5). The results obtained in this investigation were in full agreement with the results obtained by many authors (Rai and Prasad 1984, Helemish and El-Gamma! 1985, Okafor and Alexander 1975 and Yadav and Vyas 1973). Contrary to these results Graham and Parker (1964) found that pH 10 was critical for all rhizobia spp.

Rhizobium strain of Pisum sativum could be grown in presence of various concentrations of NaCl with maximum growth at 0.2-0.5% NaCl although growth was low at extreme higher concentrations.

This is in harmony with the results obtained by Rai and Prasad (1984). However Pillai and Sen (1973) reported that the growth rate of Rhizobium spp. increased with 1% NaCl added to broth media. calcium chloride was inhibitory even at 0.2% the growth was reduced. Steinborn and Roughley (1975) have shown that the growth of both R. trifolii and R. meliloti were decreased by the addition of salts, and CaCl_2 was more toxic than NaCl in broth and peat culture. While Helemish and El-Gamma! (1985) found that Rhizobium leguminosarum TAL 271 strain could tolerate NaCl levels up to 3%, while CaCl_2 was found more toxic.

Sodium carbonate and bicarbonate reduce the growth of Rhizobium of Pisum sativum even at 0.1% concentration. The inhibitory effect of carbonate and bicarbonate was much higher than in chlorides. These results are in harmony with the results obtained by Helemish and El-Gammal (1985) who found that the threshold of tolerance for carbonate and bicarbonate was much lower than in case of chlorides in Rhizobium leguminosarum TAL 271. However, Singh et al., (1973) reported that even 0.1% of either Na_2CO_3 or NaHCO_3 significantly reduced the number of nodules formed by Medicago sativa.

Addition of structural compounds which enable rhizobia to overcome salinity is another way to solve salinity problem. Sawage et al. (1983) found that addition of 10 mM glycine betaine improve the salt tolerance of R. meliloti. However research for new compounds that improve the salts tolerance of rhizobia needs further study.

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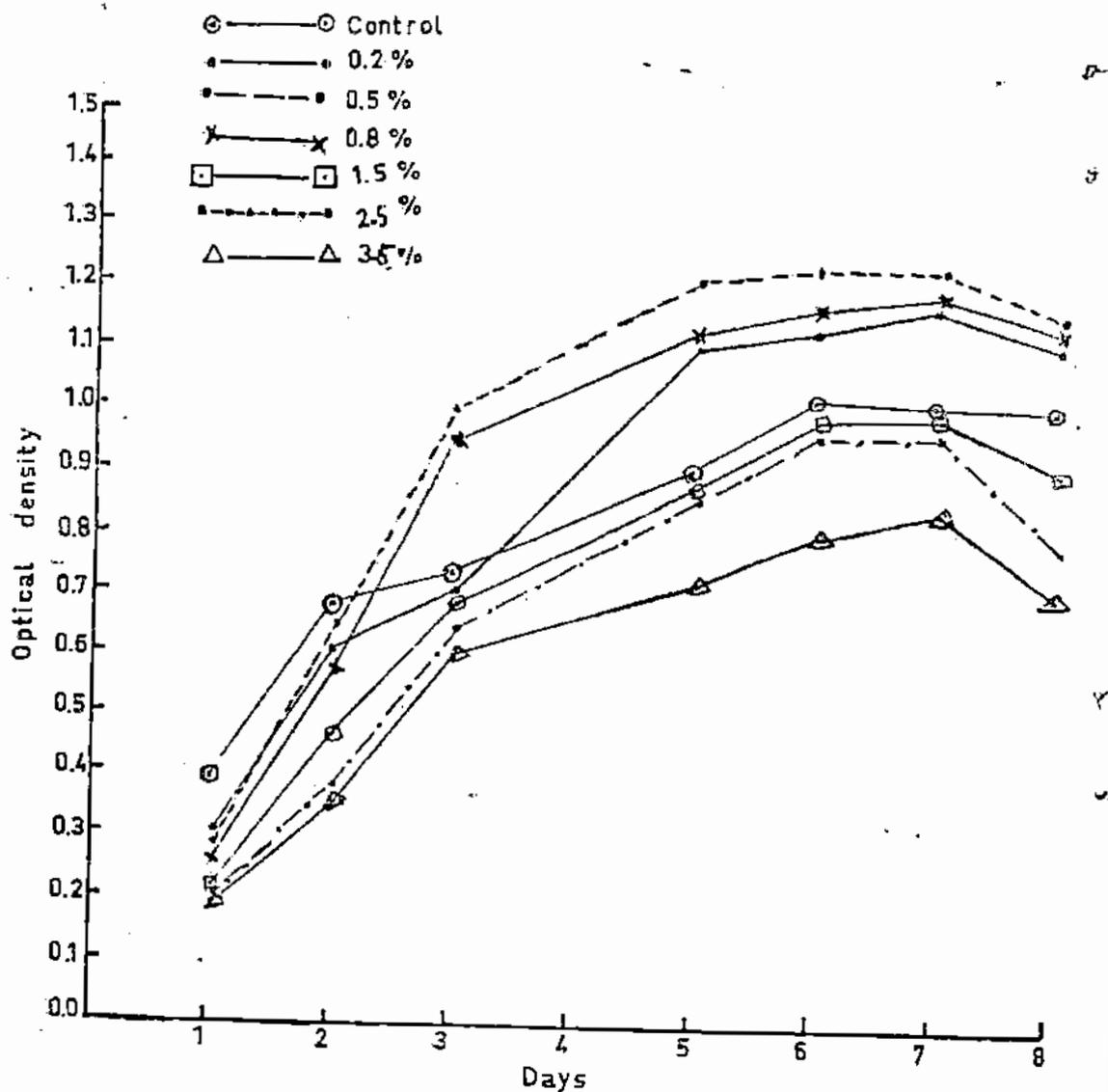


Fig. (1). Growth of *Rhizobium* under different level of NaCl.

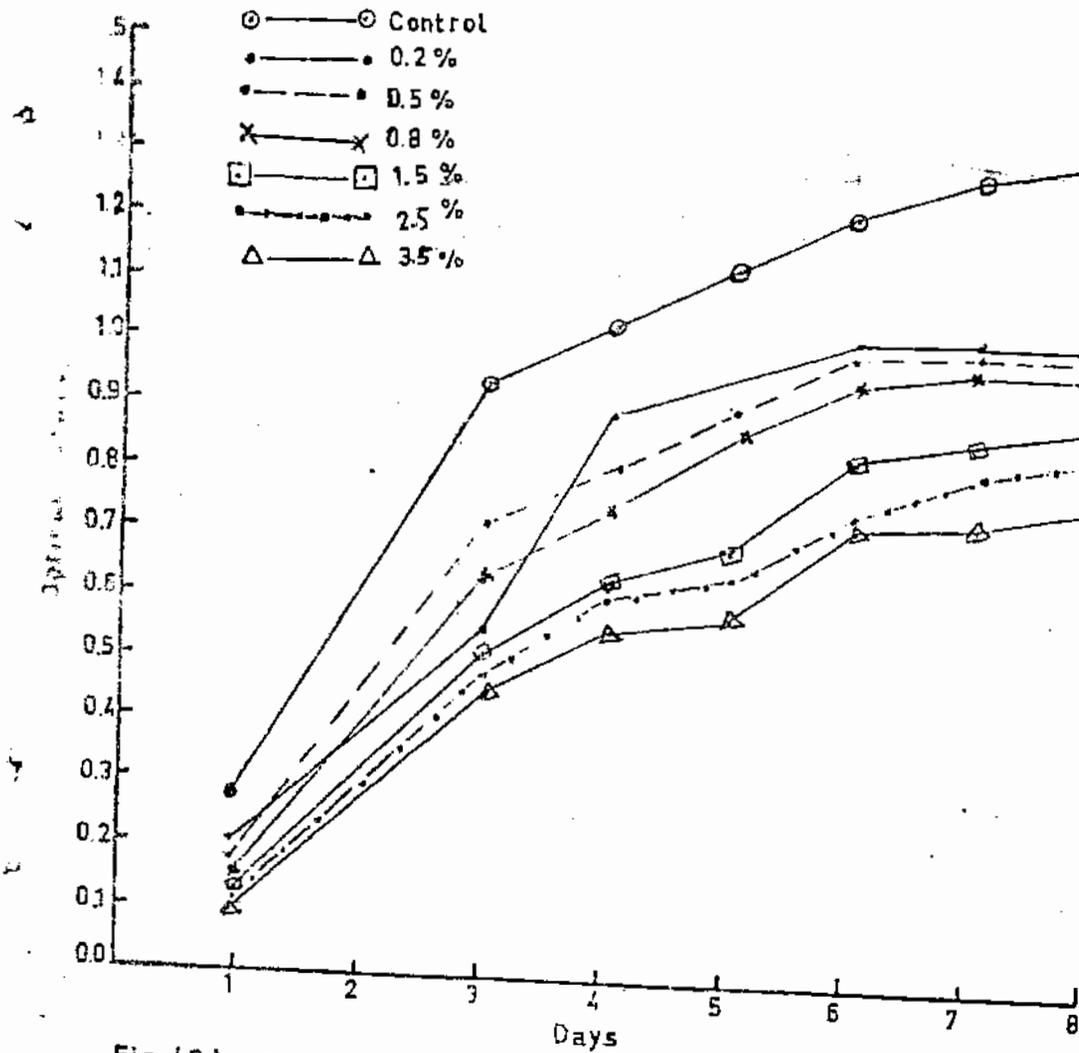


Fig. (2): Growth of Rhizobium under different level of $CaCl_2$

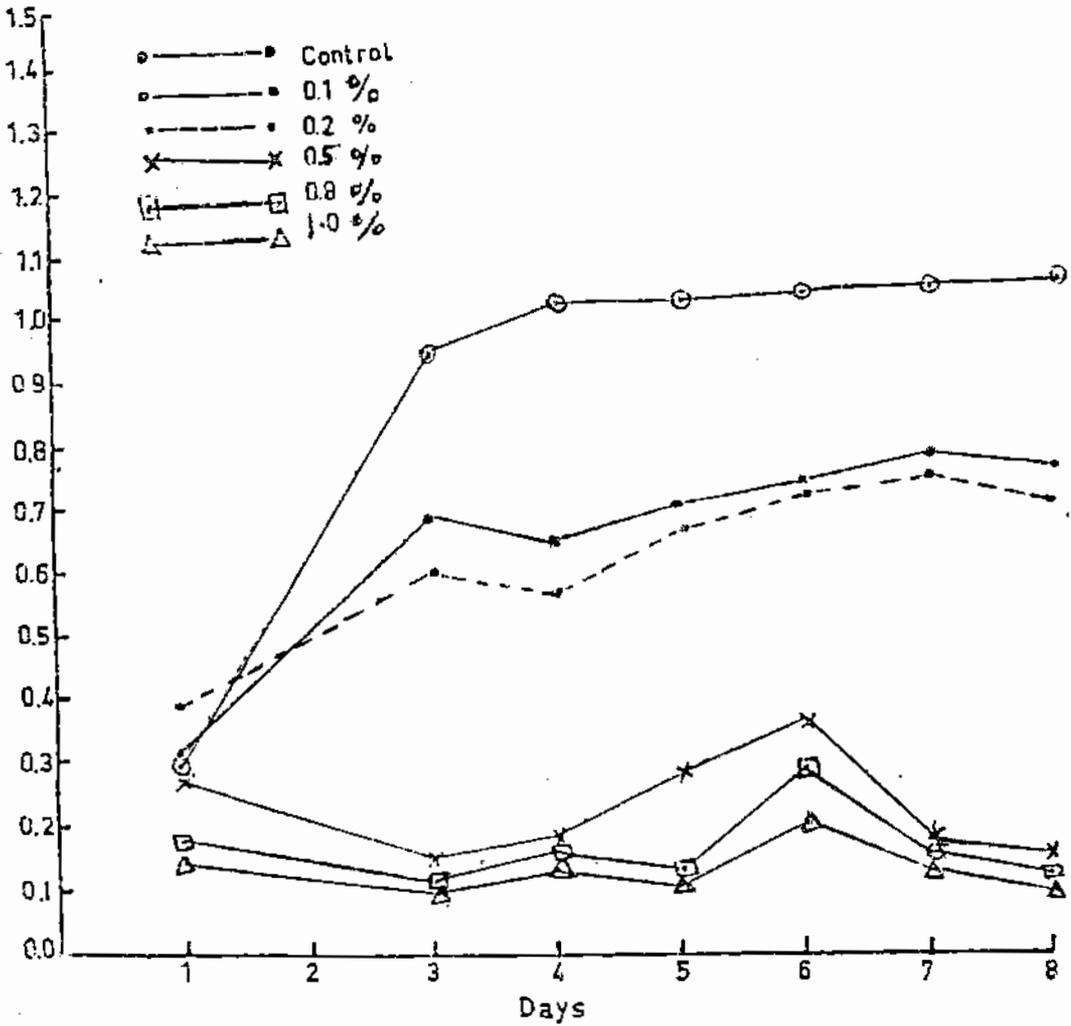


Fig. (3): Growth of Rhizobium under different level of carbonate.

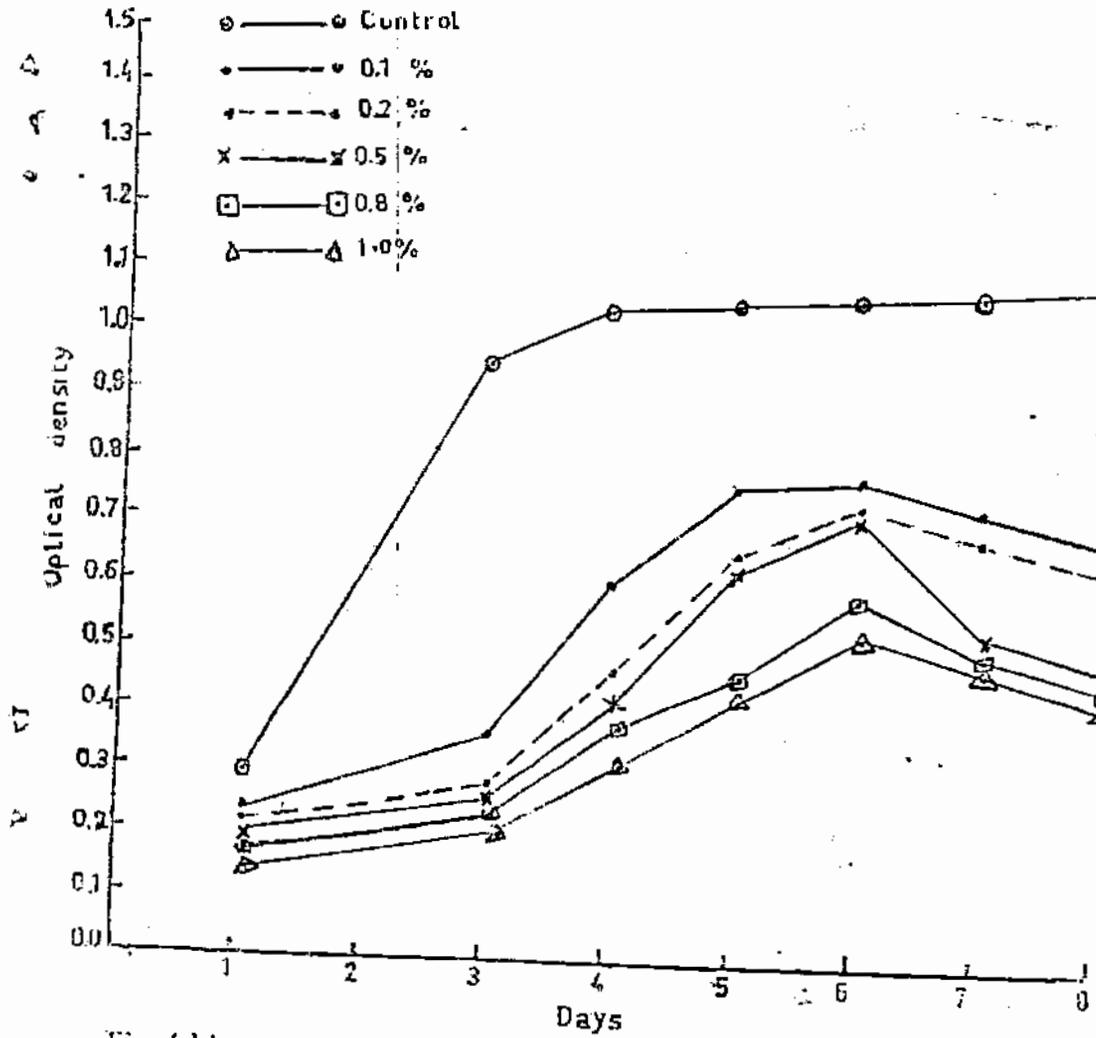


Fig (4). Growth of Rhizobium under different level of bicarbonate.

Table I: Effect of pH on growth of Rhizobium and change in initial broth pH after 72 hrs and after one week of incubation.

Initial pH value	O.D. after 72 hours	pH after 72 hrs.	O.D after one week	pH after one week
2.5	0.07	2.2	0.07	2.2
3.5	0.40	3.2	0.58	3.2
4.5	0.80	3.8	0.80	3.8
5.5	0.96	4.6	0.84	4.6
6.5	0.68	5.6	0.68	5.6
7.5	0.59	5.8	0.68	5.8
8.5	0.47	6.8	0.52	6.8
9.5	0.45	6.2	0.52	6.2
10	0.42	6.2	0.43	6.2

دراسات على نمو ريزويم البسلة تحت ظروف غير ملائمة

فأما عبد الهادي حليش

قسم النبات - كلية البنات - جامعة عين شمس - جمهورية مصر العربية - القاهرة

مخلص

يهدف هذا البحث الى دراسة نمو ريزويم البسلة تحت ظروف غير ملائمة من

الحموضة والملوحة والقلوية في بيئه العرق السائلة بفرض الحصول على سلالات تستطيع

النمو في الاجرء الحاضيه والملحيه والقلويه وذلك لانتشار هذه الاراضى في جمهوريه

مصر العربيه وخصوصا في المناطق المستصلحة التى تتميز بوجود نسبة عاليه من الاملاح

القابله للذوبان في الماء . هذا وقد اوضحت النتائج ما يأتى /

١ - استطاع ريزويم البسلة النمو على مدى واسع من الرقم الايدروجينى (٣.٥ - ١١.٥)

في بيئه العرق السائله وكان اكثر نمه عند رقم ايدروجينى ٥.٥ .

٢ - اعتبرت هذه السلاله سريعه النمو ومنتجه للحامض لان الرقم الايدروجينى تسد

نقص بعد ٢٢ ساعه من النمو وبعد ان كان ٤.٥ - ٥.٥ اصبح ٣.٨ - ٤.٦ على

التوالى وكان النقص اكثر ما يمكن عند الرقم الايدروجينى المرتفع و (٥.٨ - ٥.٩ ،

١٠) .

٣ - استطاع ريزويم البسلة النمو على بيئه العرق السائله المحتويه على تركيبات شدرجه

من كلوريد الصوديوم من ٢ر٠ - ٨ر٠ % وكان اكثر نمو عند تركيبات تراوحت بين

٢ر٠ - ٥ر٠ % بينما كانت التركيزات العاليه شبطه للنمو .

٤ - لم تستطع هذه السلاله النمو على بيئه العرق السائله المحتويه على تركيبات شدرجه

من كلوريد الكالسيوم حيث كان اقل تركيز ٢ر٠ % له تاثير شبطه .

٥ - اعتبرت كربونات وبيكربونات الصوديوم ذات تاثير شبطه على نمو ريزويم البسلة وذلك

لنقص النمو حتى عند تركيز ١ر٠ % .

MS. B. 1. 1

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