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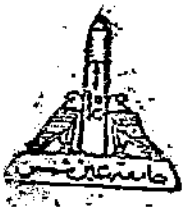

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Effect of Salicylic Acid and Gallic Acid on the Growth Responses and Physiological Changes of *Vicia faba* (cv. Giza 402) Seedlings.

I. Changes in growth responses, carbohydrate contents and activities of certain enzymes

By

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Abstract

The influence of two concentrations (1and 5mM) of salicylic acid (SA) and gallic acid (GA) on the changes of growth rate, carbohydrate contents and the activity levels of certain enzymes in *Vicia faba* (cv. Giza 402) seedlings were examined in the present study.

Treatment with SA and GA at 1 and 5mM inhibited the % of seed germination and supressed the rate of seedling growth (expressed as mean length of shoot and root and mean number and length of the lateral roots) except GA at 1mM which stimulated the seedling growth. GA at 1mM increased the activity level of catalase while it decreased AA-oxidase activity as compared with the untreated seedling. On the other hand, treatment with SA (1 and 5mM) and GA (5mM) declined catalase while they increased AA-oxidase activities below and over those of the untreated seedlings.

It was found that, the activities of polyphenol oxidase and invertase enzymes and the contents of reducing sugars (except at 1 and 2 days) polysaccharides and total carbohydrates were lower in seedlings treated with SA and GA than in the control ones. On the other hand, seedlings imposed to SA and GA at 1 and 5 mM, caused an elevation of both sucrose contents (except for seedlings grown with SA5) and α and β– amylase activity over those estimated in the control seedlings.

Key words: *Vicia faba*, Gallic acid at 1mM : GA1, Gallic acid at 5 mM: GA5, Salicylic acid at 1 mM: SA1, Salicylic acid at 5 mM: SA5.

Introduction

Many phenolic compounds, the most commonly identified as phytotoxins produced by higher plants (Rice, 1984) are widely spread in soil (Wang *et al.*, 1967). Whitehead

[illegible] found that benzoic and cinnamic acid derivatives are present in the soil solution at the concentration ranges of 0.01 to 0.1mM. These allelochemicals are released into the soil system by plant decomposition, leaching from leaves and root exudation (Guenzi and McCalla, 1966; Rovira, 1969 and Tukey, 1970).

Phenolic compounds have been shown to be of importance in the regulation of plant growth and metabolism and are no longer considered to be passive by-products (Jain and Srivastava, 1981). Ferulic acid (FA) alters seed germination (Leather and Einhellig, 1984), inhibits radicle growth (Blum *et al.*, 1984) and decreases leaf expansion, leaf production, dry weight accumulation (Patterson, 1981; Blum and Dalton, 1985 and Blum *et al.* 1985 a,b) photosynthetic rates (Patterson, 1981) and leaf chlorophyll content (Einhellig and Rasmussen, 1979 and Patterson, 1981). The magnitude of these responses varies with the concentration of ferulic acid (Einhellig and Eckrich, 1984).

Decreased leaf water potential (ψ_L), shoot turgor pressure (ψ_T) and osmotic potential (ψ_π) have been observed in soybean and sorghum seedlings after treatment with 250 to 1000uM FA for one day (Patterson, 1981 and Einhellig *et al.*, 1985). Transpiration and water utilization in cucumber seedlings decreased after 2 days of treatment with 500uM FA (Blum *et al.*, 1985 b). However Blum *et al.* (1985 b) noted that cucumber seedlings wilted within 2 h of treatment with 500uM FA but visibly recovered within 24 h. They suggested that FA temporarily modified water uptake by the roots, which reduced the inward diffusion of water needed for cell and leaf expansion.

Ahmed (1987) found that all doses of Na-salicylate caused significant decreases in reducing sugars , sucrose and polysaccharide contents at the early stages of leaf growth of wheat plant.

The present study deals with the effect of monophenolic acids (salicylic acid, SA) and polyphenolic acids (gallic acid, GA) on the growth, carbohydrate metabolism and activities of certain oxidative and hydrolytic enzymes during seed germination and early seedling growth of *Vicia faba* cv. Giza 402.

Materials and Methods

Growth of the plants.

Seeds of *Vicia faba* cv. Giza 402 were surface sterilized with 0.1 % $HgCl_2$ for 10 min and then washed thoroughly with sterile water. The seeds were germinated in plastic pots (15 cm in diameter and 11 cm in depth) on Whatman filter paper no. 45 under the following conditions: relative humidity of 60-65 %, day length of 12 h and day/night air temperature: 22/18°C. Ten seeds were planted in each pot and all pots were arranged into 5 groups each of 8 pots, then each group recieved 15ml of the following solutions.

1- Distilled water to represent the untreated plants.

2- Salicylic acid at the concentrations of 1 and 5mM.

3- Gallic acid at the concentrations of 1 and 5mM.

The solution of each treatment (pH7.8) was daily renewed and at intervals of 1,2,7 and 14 days, germinated seeds or seedlings of only 2 pots were harvested per treatment.

The percentage of germination and certain morphological measurements were taken and statistically analysed using the least significant difference (LSD) at 1% and 5% levels of probability (Snedecor and Cochran,1967).

Methods of analysis

Estimation of carbohydrates

The different carbohydrate fractions were determined in the oven dried plant tissues. The methods of extraction and clarification were similar to those adopted by Said and Naguib (1964). The direct reducing sugars were determined following the procedure of Somogyi (1937) as described by Younis (1963). The total reducing sugars were estimated after the hydrolysis of the sucrose by invertase. The sucrose content was calculated from the difference between the total reducing sugars and the direct reducing sugars. Polysaccharides were estimated in the dry residue left after extraction of soluble sugars by the method adopted by Naguib (1963).

Enzyme assays

Crude extracts were prepared by homogenizing 1-5g fresh matter with 30 cm^3 Tris-HCl buffer pH 7.4 (Guerrier and Strullu, 1990). The homogenates were centrifuged at 7000g for 30 min and the supernatants were directly used for the enzyme assays.

Activities of ascorbic acid oxidase (AA-oxidase), polyphenol oxidase and catalase were assayed according to the methods described by Mukherjee and Choudhuri (1981), Kar and Mishra (1976) and Biswas and Choudhuri (1976) respectively. Activities of AA-oxidase and polyphenol oxidase were expressed as the change in the optical density g^{-1} fresh weight h^{-1}. Catalase activity was expressed as u mole H_2O_2 destroyed g^{-1} fresh weight h^{-1}.

Activities of α and β amylases were assayed according to the methods described by Davis (1977) and Malik and Singh (1980) respectively- Activity of α- amylase was expressed as the decrease in optical density per unit time. The β-amylase activity, on the other hand, was expressed as ug maltose released from starch by the enzyme g^{-1} fresh weight h^{-1}. Invertase activity was assayed according to the method used by Russell and Jimmy (1980) with some modifications. The modifications were that the reducing sugars liberated from sucrose were determined by the method of Somogyi (1937). Invertase activity was expressed as mg reducing sugars released by the enzyme g^{-1} fresh weight h^{-1}.

Results

Effect of SA and GA on seed germination and seedling growth of *Vicia faba*.

Results presented in table 1 show that SA and GA at their used concentrations (1 and 5 mM) variably affected the % of seed germination of *Vicia faba* plants (24 h after treatment) so that high significant inhibition, complete inhibition, high significant stimulation and significant inhibition were observed at SA1, SA5, GA1, and GA5 respectively as compared to the control results. *Vicia faba* seeds imposed to SA1, SA5, GA1 and GA5 germinated at the % of 73.3, 60., 100 and 66.7. successively after 2 days which were highly significantly decreased (except GA1) than the contral results.

The protrusion of the plumule was completely inhibited in seeds imposed to while SA1, GA1 and GA5 highly significantly stimulated the plumule length at ag days above the untreated seeds. The radicle length was found to be significant highly significantly decreased at SA1 and SA5while GA1 and GA5 highly signif and significantly increased this criterion if being compared with that of the c seedlings after 7 days. All treatments adopted highly signilicantly depressed the length of both shoots and roots below the control seedlings after 14 days wi exception that GA1 highly significantly increased the same criteria. At the same a days), all treatments used (except SA1) either highly significantly decreased the n of lateral roots or highly significantly stimulated (except GA5) the mean length of roots below and above those of the untreated seedlings respectively.

Effect of SA and GA on the carbohydrate contents:

Reducing sugars: The contents of reducing sugars determined in *Vici* seedlings imposed to SA_1,SA5 GA1 and GA5 at 1 day and SA_1 and SA_5 at 2 day obviously elevated over that determined in the corresponding control. On the othe the same contents were found to be markedly declined in *Vicia faba* seedlings impo GA_1 and GA5 at 2 days and SA1, SA5, GA1 and GA5 at 7 and 14 days below th the controls. Among the used phenolic acid treatments, SA5 and GA1 gave the h and the lowest values of reducing sugars respectively throughout the experimental (table 2).

Sucrose: Table 2 shows that treatment with GA1, GA5 and SA1 obviously el the level of sucrose while SA5 treatment lowered this level above and below the st level determined in water - grown plants at all plant ages. The highest value of su was determined in plants imposed to 1mM gallic acid.

Polysaccharides: The polysaccharides contents determined in variously t and untreated *Vicia faba* seedlings were progressively and markedly decreased acr the plant age. Moreover this fraction was found to be lower in all treatments em than those of the untreated plants. The order of decreases were as follows: Co SA5> SA1> GA5> GA1.

Total carbohydrates. The values of total carbohydrates estimated in *Vicia faba* plants treated with SA1,SA5,GA1 and GA5 were found, in most cases, to be lower than those detected in the untreated plants. The sequence of decrease were shown to be: control> SA1> SA5> GA1> GA5 except in germinated seeds of 1 day-old.

Effect of SA and GA on the activities of invertase, ∝ and β- amylases:

The relative changes in the activities of invertase, ∝ and β-amylases are shown in table 3. As compared to the untreated plants the invertase activity of *Vicia faba* plants was obviously decreased in response to the phenolic acids treatments, at all ages. The sequence of decreases were found to be: control > SA1> SA5> GA1> GA5.

In contrast, the activities of ∝ and β-amylases were markedly elevated above the untreated plants throughout the experimental period. The order of increases were as follows: GA1> GA5> SA1> SA5> control.

Effect of SA and GA on the activities of certain oxidative enzymes.

Throughout the experimental period, GA1 markedly increased the activity of catalase enzyme above that of the control, whereas SA1, SA5 and GA5 markedly decreased the catalase activity below that of the control plants. The magnitude of decrease was most pronounced in plants imposed to SA5 (table 4). The sequence of increases in the catalase activity were: SA5> GA5> SA1> control .

Table 4 shows that SA1, SA5, GA1 and GA5 markedly declined the activity levels of polyphenol oxidase below those of the corresponding controls at the all plant ages. The sequence of decreases were as follows: control > SA1> SA5> GA1> GA5.

The activity levels of AA- oxidase detected in *Vicia faba* plants treated with SA1, SA5 and GA5 were found to be markedely elevated above those of the corresponding controls, while the activity of the same enzyme delected in plants imposed to GA1 was found to be lower than that detected in the control plants (table4).

Discussion

Phenolic acids at the concentrations used in this study significantly influen germination and growth of *Vicia faba* plants. SA and GA mostly inhibited se germination, SA significantly supressed the mean length of shoot and root ar significantly increased the number and mean length of lateral roots at the latest stage seedling growth. GA at the low concentration employed, generally increased the pla growth while its high concentration significantly suppressed the rate of the plant grow Phenolic compounds have been shown to be of importance in the regulation of pla gorwth and metabolism. It was noted that a low concentration of SA increased the grow of maize seedlings, while the higher concentration inhibited it (Jain and Srivastav 1981). Ferulic acid (FA) was found to alter seed germination (Leather and Einhell 1984), inhibits radicle growth (Blum *et al.*, 1984) and decreases leaf expansion and l production (Patterson, 1981; Blum and Dalton, 1985). The magnitude of these respons varies with concentration of FA (Einhellig and Eckrich, 1984). Several lines of eviden point to water stress as an important factor in the observed responses to FA. Transpiration and water utilization in cucumber seedlings decreased after 2 days treatment with 500uM FA and it modified water uptake by the roots which, in tur reduced the inward diffusion of water needed for cell and leaf expansion (Blum *et a* 1985 a,b and Booker *et al.*, 1992).

Although the role of phenolic substances as a plant morphogentic regulators is we known, there has been little study of the mechanism at the molecular level. The prese study proved that the phenolics at their high concentration used, decreased the activi levels of catalase and polyphenol oxidase while increased the activity level o AA-oxidase. In this connection, Hassanein *et al.* (1987 b) found that treating sorghu grains with coumarin resulted in decreased activities of catalase and peroxidase enzyme Positive correlation occurs between phenolic acids treatment and water stress in t treated plants (Patterson, 1981; Einhellig *et al.*, 1985 and Booker *et al.*, 1992). In th respect, Dwivedi *et al.* (1979), Mukherjee and Choudhuri (1981) and Hassanein a El-Telwany (1989) observed that water stress lowered the activity of catalase a AA-oxidase in the studied plants.

Thus the significant changes in the studied oxidative enzymes, in the present work, in response to seed treatment with phenolic acids used, may cause the accumulation of certain toxic substances that affect the metabolic pathways and consequently the rate of plant growth. The quinones formed by the oxidation of phenolic substances are quite toxic to the plant cell enzymes (Mayer and Harel, 1979), and the reduction in the activity of catalase may result in accumulated toxic levels of hydrogen peroxide which may be involved in the suppressed growth rate of *Vicia faba* plants imposed to phenolic acids in the present study.

Treating seeds and seedlings of *Vicia faba* with SA and GA gave rise, in general, to decreased levels of reducing sugars, polysaccharides and total carbohydrates while they caused general increases in the sucrose contents below and above those of the untreated plants respectively.

It may be stated that the effect of phenolic compounds on the metabolism varies with the plant, stage of plant growth, the organ of the plant and the doses of phenolics used. Ahmed (1987) found that all doses of Na-salicylate caused significant decreases in reducing sugars, sucrose, and polysaccharide contents at the early stages of leaf growth of wheat plant. However, Hassanein *et al.* (1987 a) found that no obvious changes in polysaccharides and reducing sugars contents and marked decreases in sucrose content were observed in coumarin treated sorghum grains as compared with those of the untreated ones.

Our results also showed that, SA and GA decreased the activity of invertase while they increased the activities of α and β-amylases. In this respect, Hassanein *et al.* (1987 a) found that coumarin inhibited markedly the activities of amylases and invertase in sorghum grains. The relative amounts of sucrose in plants may be determined by the relative activities of both sucrose synthetase and invertase enzymes. On the other hand, there are more or less possitive relationship between the amounts of each of sucrose and reducing sugars and the activity of invertase since the reducing sugars may be used as respiratory substrates and/or in building the amino acids, amides and proteins (Abdalla *et al.*, under press). The changes in α and β-amylases activities of the treated and untreated

seed. and seedlings of *Vicia faba* are observed to coincide with the correspc changes in the polysaccharides level. These results are supported by the results ob by Hassanein *et al.* (1987 a). In addition, the decline in polysaccharides and conseq the total carbohydrate contents particularly in seedlings of 7 and 14 days old r attributed in part to the increased activities of α-and β-amylases and in part to the inh photosynthetic rate caused by phenolics treatments. In this respect, FA trea decreased leaf production and dry weight accumulation (Patterson, 1981; Blum Dalton, 1985 and Blum *et al.*, 1985 a,b), leaf chlorophyll content (Einhelli Rasmussen, 1979) and photosynthetic rates (Patterson, 1981).

Conclusion: It may be stated that treatment with SA and GA, decreased the p germination and the growth rate of *Vicia faba* seedlings except at 1mM gallic acid. increased the activity of catalase while it decreased AA-oxidase activity that may role in the obserbed stimulated plant growth. On the other hand, the declined activ catalase and the increased one of polyphenol oxidase especially at SA1, SA5 and may be participated in the inhibition of seed germination and seedling grow addition, treating seeds and seedlings of *Vicia faba* with the used phenolic acids i increased activities of α and β- amylases and sucrose content, while they decreased of invertase activity, reducing sugars, polysaccharides and total carbohydrate conten

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Table (1) Effect of salicylic acid and gallic acid on the % of germination, shoot and root length, mean number and length of lateral roots of *Vicia faba* seedlings. Each value is a mean of 8 replicates

Concentration (m M)	Days after treatment: 1	2	7	14			
	% of germination		Length of plumule	Length of radicle	M. L. of shoot	M. L. of root	M. No. of lateral roots	M. L. of lateral roots
Control (H_2O)	33.3	100	0.2	2.4	10.8	12.3	19	1.9
SA1	13.3-SH	73.3-SH	0.7 + SH	2.0 - S	7.0 - SH	5.3 - SH	21 + SH	4.8 + SH
SA5	0.0	60.0-SH	0.0	0.4 - SH	4.3 - SH	3.1 - SH	2 - SH	1.7 + SH
GA1	46.7+SH	100 NS	1.5 + SH	3.1 + SH	14.6 + SH	14.5 + SH	11 - SH	4.1 + SH
GA5	26.7- S	66.7-SH	0.5 + SH	2.8 + S	6.1 - SH	6.3 - SH	7 - SH	1.1 - SH
L. S. D at 5 %	4.8	5.1	0.03	0.4	0.75	0.86	0.93	0.12
L. S. D at 1 %	7.4	7.9	0.05	0.6	1.04	1.08	1.18	0.18

HS = high significant S = significant NS = non significant

ML = mean length M.No. = mean number

Table (2) Effect of salicylic acid and gallic acid on the carbohydrate contents during seed germination and early seedling growth of *Vicia faba* . Values listed are the average of triplicate determinations and expressed as mg glulcose equivalent g^{-1} dry weight (± sd) .

Age/ day	Concentration (m M)	Reducing sugars	Sucrose	Polysaccharides	Total carbohydrates
1	Control (H_2O)	13.1 ± 3.5	17.5 ± 2.6	88.7 ± 10.5	119.3 ± 20.3
	SA1	20.8 ± 3.8	30.1 ± 5.3	77.0 ± 8.7	127.9 ± 15.8
	SA5	26.9 ± 4.5	14.7 ± 2.5	80.6 ± 8.8	122.2 ± 15.8
	GA1	14.7 ± 3.3	35.6 ± 4.7	67.8 ± 5.9	118.1 ± 15.6
	GA5	16.8 ± 2.8	22.4 ± 3.6	73.3 ± 5.6	112.5 ± 10.5
2	Control (H_2O)	14.7 ± 4.1	11.9 ± 2.8	84.6 ± 8.3	111.2 ± 15.3
	SA1	16.8 ± 3.2	17.5 ± 2.2	71.8 ± 7.8	106.1 ± 10.4
	SA5	18.9 ± 3.5	8.8 ± 3.5	74.8 ± 6.3	102.5 ± 8.9
	GA1	11.2 ± 3.2	25.9 ± 5.4	61.2 ± 7.1	98.3 ± 12.1
	GA5	13.3 ± 2.2	15.2 ± 2.4	66.5 ± 6.2	95.0 ± 10.3
7	Control (H_2O)	20.3 ± 5.3	14.7 ± 3.3	79.2 ± 9.4	114.2 ± 18.6
	SA1	15.4 ± 4.4	19.3 ± 3.2	66.5 ± 5.8	101.2 ± 14.1
	SA5	16.8 ± 3.3	12.6 ± 1.8	69.6 ± 7.4	99.0 ± 8.6
	GA1	9.9 ± 4.5	27.3 ± 4.2	50.0 ± 5.5	87.2 ± 8.1
	GA5	12.6 ± 3.9	16.8 ± 2.0	55.8 ± 5.5	85.2 ± 6.4
14	Control (H_2O)	17.5 ± 5.6	17.5 ± 5.3	68.8 ± 10.3	103.8 ± 11.1
	-SA1	12.6 ± 3.6	21.4 ± 4.1	62.5 ± 7.5	96.5 ± 8.3
	SA5	14.7 ± 3.0	15.4 ± 2.6	65.2 ± 7.3	95.3 ± 6.8
	GA1	7.8 ± 3.1	30.1 ± 6.1	46.4 ± 3.8	84.3 ± 6.4
	GA5	10.9 ± 2.8	19.3 ± 3.6	50.0 ± 3.2	80.2 ± 5.9

Table (3) Effect of salicylic acid and gallic acid on the activities of invertase, -and - amylases during seed germination and early seedling growth of *Vicia faba* . Values listed are the average of triplicate determinations (± sd) .

Age/ day	Concentration (m M)	Invertase	- amylase as a decrease in optical density per unit time	- amylase as ug maltose released from starch by the enzyme g f.w.h
1	Control (H_2O)	6.6 ± 1.3	0.45 ± 0.11	10 ± 3
	SA1	3.7 ± 0.6	0.39 ± 0.12	20 ± 4
	SA5	2.8 ± 0.5	0.43 ± 0.10	15 ± 3
	GA1	2.0 ± 0.5	0.33 ± 0.15	30 ± 5
	GA5	1.1 ± 0.5	0.35 ± 0.13	25 ± 4
2	Control (H_2O)	4.5 ± 0.8	0.41 ± 0.16	15 ± 3
	SA1	3.3 ± 0.6	0.34 ± 0.11	25 ± 4
	SA5	2.4 ± 0.4	0.38 ± 0.06	20 ± 4
	GA1	1.5 ± 0.3	0.28 ± 0.05	40 ± 6
	GA5	0.5 ± 0.1	0.31 ± 0.08	30 ± 7
7	Control (H_2O)	8.3 ± 1.1	0.34 ± 0.10	30 ± 5
	SA1	7.5 ± 0.8	0.26 ± 0.03	45 ± 7
	SA5	6.3 ± 0.8	0.39 ± 0.05	35 ± 3
	GA1	4.8 ± 0.5	0.20 ± 0.03	70 ± 8
	GA5	3.4 ± 0.5	0.23 ± 0.03	55 ± 6
14	Control (H_2O)	11.8 ± 1.3	0.44 ± 0.10	40 ± 5
	SA1	9.2 ± 1.5	0.31 ± 0.06	50 ± 5
	SA5	8.3 ± 0.8	0.39 ± 0.05	45 ± 3
	GA1	5.0 ± 0.3	0.25 ± 0.03	80 ± 7
	GA5	4.2 ± 0.7	0.28 ± 0.03	75 ± 7

Table (4) Effect of salicylic acid and gallic acid on the activities of some oxidative enzymes during seed germination and early seedling growth of *Vicia faba*. Values listed are the average of triplicate determinations (± sd) .

Age/ day	Concentration m M	Catalase	Polyphenol-oxidase	AA - oxidase
1	Control (H_2O)	336 ± 33	58 ± 8	6.0 ± 1.3
	SA1	192 ± 10	29 ± 5	6.5 ± 1.0
	SA5	96 ± 10	15 ± 5	8.0 ± 1.4
	GA1	480 ± 25	12 ± 2	5.1 ± 0.6
	GA5	168 ± 15	9 ± 2	7.0 ± 0.8
2	Control (H_2O)	432 ± 20	25 ± 6	6.3 ± 0.9
	SA1	288 ± 20	12 ± 3	7.5 ± 0.6
	SA5	192 ± 15	10 ± 3	8.6 ± 0.8
	GA1	528 ± 26	8 ± 2	5.5 ± 0.6
	GA5	240 ± 28	5 ± 1	7.8 ± 1.2
7	Control (H_2O)	480 ± 26	48 ± 5	4.8 ± 0.3
	SA1	432 ± 22	42 ± 5	5.8 ± 0.3
	SA5	288 ± 16	39 ± 3	6.6 ± 0.8
	GA1	624 ± 19	31 ± 3	2.9 ± 0.2
	GA5	336 ± 18	25 ± 3	6.2 ± 0.5
14	Control (H_2O)	920 ± 36	60 ± 6	3.5 ± 1.1
	SA1	720 ± 33	48 ± 5	4.1 ± 0.7
	SA5	336 ± 19	43 ± 3	5.5 ± 0.7
	GA1	1392 ± 46	36 ± 4	2.3 ± 0.4
	GA5	528 ± 21	29 ± 4	4.8 ± 0.3

تأثير حمض السليسيليك وحمض الجاليك على النمو والتغيرات الفسيولوجية لبادرات الفول البلدى.

١ - التغيرات فى النمو ، المحتوى الكربوهيدراتى ونشاط بعض الانزيمات.

منى محمود عبدالله - شهيرة صالح رشدى - كمال احمد التلوانى

قسم النبات - كلية العلوم - جامعة عين شمس - العباسية - القاهرة - مصر .

١ - تبين من هذا البحث أن معاملة بذور الفول البلدى بحمض السليسيليك وحمض الجاليك بتركيز (1, 5 m M) أدى إلى خفض كل من نسبة الانبات ومعدل نمو البادرات (معبرا عنه بطول المجموع الخضرى والجذرى ، وعدد وطول الجذور الجانبية) ، ماعدا التركيز المنخفض (1 m M) لحمض الجاليك حيث نتج عنه زيادة فى معدلات النمو بالمقارنة بمعدلات الكنترول .

٢ - نتج عن استخدام التركيز المنخفض (1 m M) من حمض الجاليك زيادة لنشاط انزيم الكاتاليز ونقص نشاط انزيم إكسيديز حمض الأسكوربيك ولكن عند استخدام التركيز المرتفع (5 m M) من نفس الحمض الفينولى وتركيزات (1, 5 m M) من حمض السليسيليك نتج عنه خفض نشاط انزيم الكاتاليز وزيادة نشاط انزيم أكسيديز حمض الأسكوربيك مقارنة بنشاطها فى بذور وبادرات الفول غير المعاملة.

٣ - تبين أن لنشاط انزيمى أكسيديز بولى فينول والأنفرتيز وكذا محتوى كل من السكريات المختزلة (ماعدا بعد ١ ، ٢ يوم من المعاملة) والمواد مديده التسكر والكربوهيدرات الكلية انخفض فى بذور وبادرات الفول المعاملة بالأحماض الفينولية المستخدمة بالمقارنة بمثيلتها غير المعاملة . ومن ناحية أخرى ، نتج عن استخدام هذه الأحماض الفينولية بتركيزى (1 , 5 m M) زيادة محتوى السكروز (ماعدا البذور والبادرات المعاملة بالتركيز المرتفع من حمض السليسيليك) وكذا نشاط انزيمى ألفا وبيتا أميليز مقارنة بنفس المحتوى والنشاط فى الكنترول

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Effect of Salicylic Aicd and Gallic Acid on the Growth Responses and Physiological Changes of *Vicia faba* (cv. Giza 402) Seedlings.

II- Nucleic acid contents and nitrogen metabolism.

By

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Abstract

Treatment with SA and GA at 1 and 5mM, mostly elevated the values of nitrate-, nitrite-, ammonia- and amide- N in *Vicia faba* seeds and seedlings compared with those of the untreated ones, during the whole experimental period. *Vicia faba* seedlings imposed to 1 and 5mM SA and GA resulted in progressive increases in the contents of total free amino acids. Treatment with GA at 1mM, resulted in the highest level of total free amino acids throughout the experimental period. Conversely, using both types of phenolic acids at 1 and 5mM, mostly reduced the protein-N and total-N amounts during the different ages of germination and seedling growth as compared to the control ones, but unlikely, total soluble-N was variably changed due to these phenolic acid treatments.

The higher concentration (5mM) of phenolic acids employed comparably reduced the amounts of DNA and RNA than the control amounts while the low concentration. of these acids (1mM) increased them especially at the later ages of seedling growth (7 and 14 days). A completely reversed situation was realized regarding the activities of the nuclease anzymes DN- ase and RN - ase i.e raising the concentration of SA and GA from 1 to 5mM mised the activities of these hydrolytic enzymes.

Nitrate reductese activity (NRA) was markedly elevated above the control activity especially at the later ages of seedling growth (7 and 14 days) due to treatment with SA and GA. On the other hand, nitrite reductase activity (NiRA) was either generally reduced as a consequence of treatment with 5mM of SA and GA or highly accelerated due to application of 1mM of the same acids as compared to the control activities at all ages of germination and seedling growth, although 5mM GA increased this enzyme activity at the latest age of growth (2 weeks).

Key words: *Vicia faba*, Gallic acid at 1mM: GA1, Gallic acid at 5 mM: GA5, Salicylic acid at 1mM: SA1, Salicylic acid at 5mM: SA5, Nitrate reductase activity: NRA and Nitrite reductase activity: NiRA.

Introduction

Phenols and phenolic acids are of considerable importance in the regulation of plant growth and metabolism (Wain and Taylor, 1965 and Jain and Srivastava, 1981 a,b). Although their role as a plant morphogenetic regulator is well established (Asthana and Srivastava, 1978), there has been little study of the mechanism at the molecular level.

Phenolic derivatives of benzoic acid and cinnamic acid are commonly found in soils and many of these compounds are considered allelochemicals (Rice, 1984). Phenolic allelochemicals are released by plants into soils as leaf leachates, root exudates, and by plant tissne decomposition (Guenzi and McCalla, 1966; Tukey, 1970 and Rice, 1984).

The results of Glass (1974) and Glass and Dunlop (1974) showed that phenolics have nonspecific effects on root cell membranes and therefore on ion uptake, and that the degree of inhibition of ion uptake caused by a phenolic compound is correlated with its lipid solubility and its ability to depolarize the membrane potentials of root cells. Many phenolic compounds are known to possess chelating properties (Clemetson and Anderson, 1966). In fact, SA accelerates leaching of soluble nitrogen from maize endosperm (Jain and Srivastava, 1981 a). In addition, SA may induce NRA by interacting with the NR specific inhibitor which has been shown in many systems (Srivastava, 1980). It was found that incubation of both roots and shoots of maize with nitrate and low concentration of SA increased nitrate reductase activity (NRA) but did not affect nitrite reductase activity (NiRA), while using high concentration of SA decreased NR and NiR activities (Jain and Srivastava, 1981 a,b).

This study was carried out to investigate the changes in the nitrogen and nucleic acids (DNA and RNA) contents and DN ase, RN ase, NR and NiR activities in *Vicia faba* (cv. Giza 402) seedlings imposed to low and high concentrations (1 and 5mM) of salicylic acid (SA) and gallic acid (GA).

Materials and Methods

Plant material and growth conditions:

Detailed growth conditions for the germination of *Vicia faba* seeds (cv. Giza 402) and their treatments with phenolic acids (gallic acid and salicylic acid) have been previously described (Roushdy *et al* under press) and are only briefly outlined here.

Seeds of *Vicia faba* were germinated in 5 groups of plastic pots, each group consisted of 8 pots and each pot contained 15 equal seeds and recieved 15 cm^3 of the following solutions: The 1st group received distilled water to represent the control treatment, the 2nd group received salicylic acid (SA) solution at 1mM, the 3rd group received SA at 5mM, while the 4th and 5th groups received gallic acid (GA) at 1 and 5mM respectively. After 1,2,7 and 14 days, germinated seeds and seedlings were harvested and quickly oven - dried at 80°C for nitrogen estimation, air - dried for nucleic acid determination or kept frozen for the assay of enzyme activities. (During the experimental period, all pots were maintained under constant climatic conditions; day / night temperature 22-18 °C, relative air humidity 60-65%, 12 h day length).

Methods of analysis.

Nitrogen determination:

Total-N and total soluble-N were determined by micro- kjeldahl after digestion in sulphuric acid (Strauch, 1965). Subtracting the total soluble-N from the total-N gave the value of protein-N. Total free amino acids were estimated colourimetrically by ninhydrin method as adopted by Muting and Kaiser (1963). Estimations of NO_3-N, NO_2-N, NH_4-N and amide-N were carried out according to methods described by Snell and Snell, 1949; Snell and Snell, 1939; Delory, 1949 and Naguib, 1964 respectively.

Determination of DNA and RNA

DNA and RNA were extracted and determined according to the methods described by Schneider (1945) and Ogur and Rosen (1950).

Enzyme assays:

Enzymes studied in this work were estimated in the crude extracts that were prepared by homogenizing 1-5g fresh plant tissues with Tris-HCl buffer at pH 7.4 (Guerrier and Strullu, 1990). The homogenates were centrifuged at 7000g for 30min. and the supernatants were directly used for the enzyme assays.

Ribonuclease (RNase) and deoxyribonuclease (DN ase) were assayed by the method of Wilson (1968) using RNA and DNA as the substrates, respectively. Activities of nitrate reductase (NR) and of nitrite reductase (NiR) were assayed in vitro and NO_2 concentrations were measured colourimetrically (Stevens and Oaks, 1973 and Losada and Paneque, 1971).

The activity of each enzyme was expressed as (AxTV) / txv. where A is the absorbance of the sample after incubation minus the absorbance at zero time, TV is the total volume of the supernatant, t is the time (in minutes) of incubation with substrate and v is the total volume of the supernatant taken for incubation (Fick and Qualset, 1975).

Results and Discussion

Changes in DNA and RNA contents and DN ase and RN ase activities:

Results presented in table 1 show that there are marked and progressive increases in the relative contents of DNA and RNA correlated with the increase in seedling age from 1-14 days. Throughout the experimental period, *Vicia faba* seedlings imposed to GA1 contained the maximum level of nucleic acids while seedlings imposed to SA5 contained the minimum level. The order of increase in these contents in seedlings grown with SA1 and GA5 and untreated seedlings were as follows: control > GA5> SA1 after 1 and 2 days and SA1> control > GA5 after 7 and 14 days.

The pattern of changes in DN ase and RN ase activities, in response to the different treatments applied, was, more or less, the reverse of the DNA and RNA

behaviour. The rate of DNA and RNA hydrolysis in seedlings imposed to the low and high concentration of SA and GA were successively less and more than that in the control seedlings. These increases and decreases in the nucleic acid contents are parallel to the changes in the growth rates of *Vicia faba* seedlings grown with 1 and 5mM SA and GA (Abdalla, *et al*, underpress).

In this connection, Koves and Sirokman (1969), Kefeli (1978) and Hassanein *et al.* (1987) stated that, the natural phenolic inhibitors declined the nucleic acid (RNA and DNA) content in the treated plants. It is known that phenolic acids reduce plant water utilization (Blum and Dalton, 1985 and Booker *et al*, 1992), turgor and osmotic pressures and close stomata (Einhellig *et al.*, 1985 and Booker *et al.*, 1992). According to Kessler *et al.* (1964), Sheoran and Garg (1974) and Hassanein and El-Telwany (1989) water stress induced by phenolic treatments strongly suppressed the RNA and DNA content. A decrease in RNA was attributed to intensified activity of cytoplasmic RN ase, whereas reduced DNA content was attributed to impaired synthesis and / or due to enhanced DN ase activity under water stress condition. On the other hand, Rauser and Hanson (1966) showed that saline treatments caused leakage of divalent cations (Ca^{++} and Mg^{++}) which normally stabilize ribosomes against endogenous nucleases. Many phenolic compounds are known to possess chelating properties (Clemetson and Anderson, 1966). Chelation of some important elements of cellular and organellar membranes, thereby increases their permeability.

Changes in NR and NiR activities:

Table 2 shows that the activities of NR and NiR measured in *Vicia faba* seedlings exposed to the different treatments showed marked progressive increases towards the experimental end (except NRA of the control that sharply declined at 7 days). The pattern of increases in NRA was: GA5> SA1> control> SA5> GA1 at 1 and 2 days whereas it was: GA5> GA1> SA1> SA5> control at 7 and 14 days. On the other hand, NiRA was in the order of: SA1> GA1> control> SA5> GA5 after 1,2 and 7 days while it was: GA1> GA5> SA1> control > SA5, at the final [illegible] ages. The ratio of NRA (at earlier ages of germination) and NiRA by

5mM SA as observed in this experiment is consistent with the report of inhibition of NRA and NiRA by higher concentrations of phenolic acids (Schrader and Hageman, 1967 and Jain and Srivastava, 1981 a,b).

In addition, Jain and Srivastava (1981 a,b) demonstrated that in maize seedlings a low concentration of SA increased NRA while had no effect on nitrite reductase activity (NiRA). The increase in NRA in response to 5mM GA is supported by the data obtained by Knypl (1974) who found that 10mM chlorogenic acid induces NRA in cucumber cotyledons. The recorded influence of the used phenolic acids on the activities of both NR and NiR may be based on the following factors: a- Phenols may interact with specific inhibitors which has been shown in many systems (Srivastava, 1980). b-SA may allow the free access of metabolites involved in the induction of NRA (Jain and Srivastava, 1981 a). c- Phenols may affect the balance between synthesis / activation and degradation / inactivation processes of NR as reported by Jain and Srivastava (1981 a). d- Alternatively the effects of phenolic acids especially polyphenolic acids (GA) on the activities of NR and NiR may be mediated *via* phytohormones. Many phenolic compounds are known to provide protection to auxins against oxidation (Schneider and Whitman, 1974; Wilkins, 1979 and El-Telwany *et al.*, under press).

Changes in the nitrogen contents:

When comparing the values of nitrate-, nitrite-, ammonia - and amide-N detected in untreated *Vicia faba* seeds and seedlings during the whole experimental period, these fraction were remarkably elevated in SA and GA treated - plants (at concentrations of 1 and 5mM) although during the first 48 hours, slight reductions were determined in response to both GA treatments (1mM) in nitrate-N and (5mM) in amide-N and SA treatment (5mM) in amide-N (table3).

Treatment of *Vicia faba* seeds with 1 and 5mM, SA and GA resulted in progressive increases in the amount of total free amino acids. Its content was either increased (during the first 48 h) or decreased (at 7 and 14 days) above and below those determined in the control seedlings, whereas seedlings imposed to GA1 contained the highest level of total free amino acids throughout the experimental period.

Conversely, using both types of phenolic acids at 1 and 5mM, mostly reduced the protein-N and total-N amounts during the different ages of germination and seedling growth as compared to the control ones.

As regard to the total - soluble-N fraction estimated in both phenolic acids - treated plants, it either markedly increased due to 1mM GA (at 2,7 and 14 days), 5mM GA (at 7 and 14 days) and 1 and 5mM SA (at 7 days only) or mostly decreased due to 1 and 5mM SA (at 1,2 and 14 days), 1mM GA (at 1 day) and 5mM GA (at 1 and 2 days) above and below the contral.

Generally speaking, GA (1 and 5mM) applied to *Vicia faba* seeds, considerably increased both total-N and all soluble-N fractions as compared to SA.

Nitrogen is a crucial element in determing the rates of CO_2 fixation and plant growth (Winter, 1985 and Nobel, 1988). Several studies have found a lower mineral content, including nitrogen element, in plants treated with phenolic acids (Jain and Srivastava, 1981 a; Balke, 1985; Kobza and Einheilig, 1987, Mersie and Singh, 1988; Klein and Blum, 1990 and Booker *et al.*, 1992). The total nitrogen, organic nitrogen and protein contents of the maize embryonic axis (root + shoot) from seedlings raised with 10mM Ca $(NO_3)_2$ were substantially higher than those from the control when low concentration of SA was applied, whereas the nitrate contents at higher concentration of SA in both minus and plus nitrate seedlings were significantly lower than in their respective controls (Jain and Srivastava, 1981 a,b). In addition, Ahmed, (1987) found that Na-salicylate at 4000 and 8000ppm resulted in significant increases in grain protein content in wheat at beginning of maturation and non-significant changes later on.

The general decline in protein, DNA and RNA contents with concomitant marked and progressive increases in total free amino acids and amide-N contents in *Vicia faba* seedlings imposed to SA and GA may be attributed either directly to the increased activity of the proteolytic enzymes or/and the increased activities of DN

ase and RN ase or indirectly to the induced water deficiency caused by these phenolic acids at least in seedlings imposed to 5mM SA and GA (Kessler *et al.*, 1964; Sheoran and Garg, 1978 and Booker *et al.*, 1992). The general progressive increases in the nitrate and nitrite contents accompanied by a remarkable decline in protein and total-N observed in the present study are in accordance with results of both Kessler and Oesterheld (1970) who observed nitrate generation and NRA in N-starved algae and Funkhouser and Garay (1981) who suggested that reduced nitrogen compounds are oxidized to nitrate in seedlings of higher plants and algae. A correlation between NRA and NiRA and organic nitrogen as observed in several systems (Johnson *et. al.*, 1976 and Peuke and Tischner, 1991) suggests that these enzymes are operative in the assimilation of nitrate and nitrite. These conclusions support the results obtained in our work, where the progressive increases in nitrate and nitrite nitrogen contents were accompanied with increased NR and NiR activities.

The progressive decreases in the total-N contents in *Vicia faba* seedlings imposed to the used phenolic acids, as compared with the results of the untreated seedlings, may be attributed to the accelerated efflux of some soluble metabolies in response to the applied phenolic acids or/and to the increased demand of soluble N as a prerequsite for intensine growth rate at this period (Clemetson and Anderson, 1966; Jain and Srivastava, 1981 a ; Hassanein *et al* ., 1987; Bergmark, 1990 and Abdalla, *et al.*, under Press).

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Table (1) Effect of salicylic acid and gallic acid on the changes of DNA and RNA contents and DNase and RNase activities during seed germination and early seedling growth of *Vicia faba* . Each value is the average of three replicates (± sd).

Age/ day	Concenration mM	DNA	RNA	DN - ase	RN ase
		mg g^{-1} dry weight			
1	Control (H_2O)	0.62 ± 0.2	1.68 ± 0.3	19.8 ± 1.8	48.5± 4.5
	SA1	0.48 ± 0.1	1.03 ± 0.2	21.5 ± 1.5	51.3 ± 3.6
	SA5	0.41 ± 0.1	0.65 ± 0.2	25.3 ± 2.3	63.6 ± 3.1
	GA1	0.78 ± 0.2	2.11 ± 0.3	20.9 ± 2.2	43.6 ± 3.4
	GA5	0.58 ± 0.2	1.18 ± 0.3	23.6 ± 1.9	56.5 ± 2.8
2	Control (H_2O)	0.98 ± 0.2	2.31 ± 0.3	24.8 ± 2.0	59.2 ± 6.4
	SA1	0.72 ± 0.2	1.68 ± 0.1	23.6 ± 1.8	63.3 ± 3.5
	SA5	0.58 ± 0.1	1.37 ± 0.2	27.3 ± 1.8	66.5 ± 3.1
	GA1	1.10 ± 0.3	2.66 ± 0.2	22.6 ± 1.5	54.7 ± 3.7
	GA5	0.82 ± 0.3	1.98 ± 0.2	26.1 ± 1.6	64.8 ± 5.1
7	Control (H_2O)	1.16 ± 0.2	2.66 ± 0.3	23.2 ± 2.2	69.7±5.3
	SA1	1.37 ± 0.2	3.40 ± 0.3	21.3 ± 0.3	62.7±4.3
	SA5	0.83 ± 0.2	2.14 ± 0.2	25.6±1.7	76.8±3.8
	GA1	1.78 ± 0.3	3.87 ± 0.1	19.8±2.0	58.4±+2.6
	GA5	1.01 ± 0.3	2.33 ± 0.2	24.3±2.3	74.9±3.6
14	Control (H_2O)	1.60±0.3	3.84±0.2	19.3±0.9	48.5±6.3
	SA1	1.92±0.2	4.05±0.3	16.8±1.1	40.6±5.0
	SA5	1.30±0.2	3.23±0.2	24.4±1.5	54.6±4.4
	GA1	2.29±0.2	4.68±0.3	15.6±2.0	36,7±3.3
	GA5	1.48±0.1	3.63±0.3	23.1±1.6	52.4±4.1

Table (2) Effect of salicylic acid and gallic acid on the activities of NR and NiR enzymes during seed germination and early seedling growth of *Vicia faba*. Each value is the average of three replicates (±sd).

Age/ day	Concentration mM	NR mg NO_2 h^{-1} g^{-1} fr.wt.	NiR mg NO_2 h^{-1} g^{-1} fr.wt.
1	Control (H_2O)	60 ± 4	45 ± 3
	SA1	66 ± 4	54 ± 3
	SA5	50 ± 4	36 ± 3
	GA1	42 ± 3	50 ± 4
	GA5	83 ± 3	33 ± 4
2	Control (H_2O)	70 ± 5	60 ± 5
	SA1	78 ± 5	74 ± 5
	SA5	54 ± 2	54 ± 5
	GA1	48 ± 2	66 ± 4
	GA5	94 ± 5	45 ± 4
7	Control (H_2O)	33 ± 3	66 ± 5
	SA1	87 ± 8	94 ± 9
	SA5	78 ± 8	60 ± 4
	GA1	100 ± 10	81 ± 6
	GA5	124 ± 10	56 ± 6
14	Control (H_2O)	84 ± 6	78 ± 8
	SA1	114 ± 10	98 ± 8
	SA5	94 ± 9	74 ± 8
	GA1	125 ± 9	144 ± 10
	GA5	164 ± 11	110 ± 15

Table (3) Effect of salicylic acid and gallic acid on the changes in the nitrogenous constituents during seed germination and early seedling growth of *Vicia faba*. Each value is a mean of three replicates (± sd) and expressed as mg g^{-1} dry weight.

Age/ day	Concentration mM	Total free amino acids	Nitrate -N	Nitrite - N	Ammonia -N	Amide - N	Total Soluble - N	Total - N	Protein - N
1	Control (H_2O)	0.8 ± 0.15	0.38 ± 0.08	0.26 ± 0.03	0.09 ± 0.01	0.86 ± 0.2	30.0 ± 3.0	72.6 ± 5.1	42.6 ± 3.5
	SA1	4.1 ± 0.31	0.73 ± 0.10	0.27 ± 0.03	0.15 ± 0.02	0.98 ± 0.1	10.2 ± 1.2	59.7 ± 4.5	49.5 ± 3.3
	SA5	1.6 ± 0.18	0.51 ± 0.10	0.29 ± 0.03	0.28 ± 0.02	0.45 ± 0.1	7.6 ± 0.5	44.6 ± 4.2	37.0 ± 3.3
	GA1	5.1 ± 0.41	0.15 ± 0.01	0.28 ± 0.03	0.34 ± 0.02	1.18 ± 0.3	21.6 ± 1.9	66.9 ± 3.6	45.3 ± 4.1
	GA5	2.4 ± 0.41	1.20 ± 0.2	0.32 ± 0.02	0.46 ± 0.06	0.63 ± 0.2	20.1 ± 2.6	62.0 ± 3.0	41.9 ± 4.1
2	Control (H_2O)	1.7 ± 0.11	0.48 ± 0.05	0.24 ± 0.02	0.06 ± 0.01	0.81 ± 0.2	25.2 ± 5.0	66.4 ± 3.3	41.2 ± 4.5
	SA1	5.1 ± 0.33	0.90 ± 0.04	0.26 ± 0.02	0.11 ± 0.03	1.22 ± 0.2	16.8 ± 2.5	49.6 ± 3.1	32.8 ± 2.8
	SA5	2.4 ± 0.31	0.62 ± 0.03	0.28 ± 0.02	0.21 ± 0.03	0.63 ± 0.1	12.6 ± 2.5	36.8 ± 2.0	24.2 ± 2.5
	GA1	6.5 ± 0.42	0.90 ± 0.08	0.27 ± 0.02	0.26 ± 0.03	1.53 ± 0.3	30.2 ± 3.3	60.3 ± 4.5	30.1 ± 2.5
	GA5	3.8 ± 0.34	1.35 ± 0.1	0.30 ± 0.02	0.38 ± 0.03	1.00 ± 0.2	24.0 ± 3.3	52.2 ± 4.1	28.2 ± 3.2
7	Control (H_2O)	8.1 ± 0.44	0.23 ± 0.03	0.24 ± 0.02	0.12 ± 0.02	0.72 ± 0.1	19.4 ± 2.6	57.3 ± 5.3	37.9 ± 3.6
	SA1	6.3 ± 0.50	1.15 ± 0.09	0.25 ± 0.02	0.18 ± 0.02	1.29 ± 0.3	29.6 ± 3.1	39.8 ± 3.6	10.2 ± 1.6
	SA5	3.0 ± 0.36	0.90 ± 0.09	0.27 ± 0.02	0.18 ± 0.0 2	0.98 ± 0.2	27.6 ± 3.3	32.3 ± 3.6	4.7 ± 0.5
	GA1	12.0 ± 0.55	1.65 ± 0.11	0.26 ± 0.02	0.36 ± 0.03	1.62 ± 0.3	40.2 ± 4.0	49.3 ± 3.8	9.1 ± 0.4
	GA5	4.8 ± 0.37	2.33 ± 0.18	0.30 ± 0.02	0.44 ± 0.03	1.17 ± 0.2	33.6 ± 4.1	41.2 ± 4.0	7.6 ± 0.9
14	Control (H_2O)	16.0 ± 1.80	0.58 ± 0.15	0.27 ± 0.02	0.18 ± 0.01	1.41 ± 0.3	27.2 ± 2.4	54.6 ± 3.1	27.4 ± 2.3
	SA1	14.4 ± 1.31	1.80 ± 0.28	0.28 ± 0.02	0.28 ± 0.01	1.8 ± 0.3	24.0 ± 2.4	36.6 ± 3.0	12.6 ± 1.1
	SA5	10.5 ± 0.86	1.10 ± 0.14	0.30 ± 0.02	0.36 ± 0.01	1.53 ± 0.2	21.6 ± 1.8	28.4 ± 2.8	6.8 ± 1.1
	GA1	17.5 ± 1.45	2.10 ± 0.15	0.29 ± 0.02	0.56 ± 0.05	2.7 ± 0.5	33.8 ± 2.0	45.0 ± 2.5	11.2 ± 1.5
	GA5	11.9 ± 2.3	2.63 ± 0.35	0.33 ± 0.02	0.80 ± 0.08	1.62 ± 0.3	31.0 ± 3.3	40.3 ± 3.5	9.3 ± 0.8

تأثير حمض السليسيليك وحمض الجاليك علي النمو والتغيرات الفسيولوجية لبادرات الفول البلدي .

٢ ـ الأحماض النووية وأيض النيتروجين .

شهيرة صالح رشدى ، كمال احمد التلوانى ، منى محمود عبدالله.

قسم النبات ، كلية العلوم ، جامعة عين شمس ، العباسية ، القاهرة ، مصر .

١ – نتج عن معاملة بذور وبادرات الفول البلدى بالتركيز المنخفض والتركيز المرتفع (1 ,5 m M) من حمض السليسيليك وحمض الجاليك زيادة محتوى النترات ، النيترات ، الأمونيا ، الاميدات ، نقص فى البروتينات والنيتروجين الكلى ، تغيرات متابينه فى محتوى النيتروجين الكلى الذائب مقارنة بمحتواها فى الكنترول – وذلك خلال فترة التجربة .

٢ – تبين أن أعلى قيمة للأحماض الأمينية الحرة نتجت من معاملة بذور وبادرات الفول بالتركيز المنخفض (1 m M) من حمض الجاليك .

٣ – عند معاملة بذور وبادرات الفول بالتركيز المرتفع (5 m M) من حمض السليسيليك وحمض الجاليك نتج عنه نقص فى محتوى الأحماض النووية (RNA وDNA) أما التركيز المنخفض(1mM) ادى الى زيادة هذه الأحماض النووية خصوصا فى المرحلتين الأخرتين من التجربة ،كذلك تبين أن نشاط كل من RNase , DNase إزداد نتيجة المعاملة بتركيزى (1,5 m M) من حمض السليسيليك وحمض الجاليك مقارنة بالكنترول .

٤ – أدت المعاملة بحمض السليسيليك وحمض الجاليك بتركيزيهما إلى زيادة كبيرة في نشاط انزيم (NR)، أما نشاط إنزيم (NiR) فإنه نقص بصفة عامة نتيجة للتركيز المرتفع أو زاد زيادة كبيرة نتيجة للتركيز المنخفض لنفس المعاملة مقارنة بالكنترول .

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Protection Effect of N-acetyloxindolylidene-p-Chlorophenyl Butenolide on Corrosion of Zinc in Acid Solution

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Abstract:

The effect of N-acetyloxindolylidene -p- chlorophenyl butenolide[1] on the corrosion of zinc in 2M HNO_3 was studied by different methods including thermometry, weight loss and galvanostatic polarization. The results showed that this substance retards the dissolution of zinc in nitric acid solution. It was also shown that there is an apparent agreement between the inhibiting efficiencies concluded from the different employed methods. The inhibiting effect of this substance, most probably, functions through physical adsorption in accordance with the Langmiur isotherm.

Introduction:

The importance of the inhibition of zinc corrosion in acid media was reported previously by Desai, et al.[2] Several other reports discussed the inhibition in electrochemical energy generators having a reactive zinc anode, galvanized steel used in industry[3-8] and zinc metal itself in the case of atmospheric pollution.[3-8]

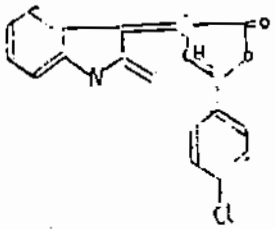
Organic compounds are widely used as corrosion inhibitors[3-15]. Most of these organic inhibitors are

compounds containing at least one polar functional group, having atoms of N, O, and/or S. In general, the polar function is regarded as the reaction center for establishment of chemisorption process. These organic inhibitors were used in aqueous corrosion of metals such as Fe, Al, Cu, Ni and Zn.[9-15] Thiourea and its derivatives were used to protect zinc in IN H_2SO_4[16,17] . Also, other workers used different ammonium derivatives to inhibit corrosion of zinc in IN HCl and IN H_2SO_4.[2-8, 16-18] and also the use of benzotriazole, benzothiazole derivatives and phosphonium salts in protection of zinc from corrosion in acid solutions.[19]

We thought that it is important and interesting to study the inhibiting effect of N-acetyloxindolylidene-p-chlorophenyl butenolide[1] on the corrosion of zinc in nitric acid solution, since this compound contains functional corrosion inhibiting polar groups, carbonyl and chloride, and was not tried before as corrosion inhibitor.

Experimental and Methods of Calculations:

The expected to be inhibitor used[1], N-acetyloxindoly-ledene-p-chorophenyl butenolide,



is a reddish black solid, very stable at high temperatures, checked for purity by elemental analysis and I.R. spectra.

The zinc used was spectroscopically pure (99.7%). All chemicals used were of A.R. grade. The solutions were prepared from bidistilled water. Zinc-test pieces measuring 2x5 cm^2 were used as electrodes in the weight loss method and in the thermometric method. The surface was mechanically polished on different grades of emery paper, degreased with acetone and then washed thoroughly with water, dried and then weighed. Corrosion tests were carried out in 50 ml beakers in which the specimen was suspended for 30 minutes in the test solution. The specimen was then removed, rinsed with conductivity water and finally dried and weighed. All corrosion tests were carried out in aerated unstirred solutions. Each experiment was carried out twice and the mean of the results was computed.

Temperature changes of the system involving zinc electrode in 2M HNO_3 solution were followed as a function of time in absence and presence of different concentrations of the inhibitor used. Each experiment was carried out with a newly polished electrode and with a fresh portion of the solution.

Galvanostatic anodic polarisation experiments were carried out under unstirred conditions with a fine luggen

capillary to avoid ohmic polarisation. Galvanostatic condition was maintained using a constant current. Zinc electrode was used in the form of a rod 5mm in diameter. A saturated calomel electrode and a platinum electrode were used as a reference and auxiliary electrodes, respectively.

Inhibition effeciencies were calculated as follows:

a) The calculation for the thermometric measurements were carried out by the Mylius method[20]

$$\% \text{ Inhibition} = \frac{(RN)\text{ free} - (RN)\text{in}}{(RN)\text{free}} \times 100 \quad \ldots\ldots\ldots \quad (1)$$

$$\text{where RN} = \frac{Tm - Ti}{t} \ ^{\circ}C \text{ min.}^{-1} \quad \ldots\ldots\ldots \quad (2)$$

where Tm and Ti are the maximum and initial temperatures, respectively, and t is the time in minutes taken to attain Tm.

b) Weight loss measurements

$$\% \text{ Inhibition} = \left(1 - \frac{W_2}{W_1}\right) \times 100 \quad \ldots\ldots\ldots \quad (3)$$

where W_1 and W_2 are the corrosion rate in absence and presence of a certain concentration of the inhibitor.

c) Galvanostatic polarisation measurements

$$\% \text{ Inhibition} = \frac{I - I^{-}}{I} \times 100 \quad \ldots\ldots\ldots \quad (4)$$

where I and I^{-} are the corrosion currents in absence and presence of the inhibitor respectively.

Results and Discussion:

The curves showing the change of temperature with time for zinc in 2M HNO_3 were followed in the absence and presence of the inhibitor[1]. The effect of gradually increasing the concentration of the added inhibitor on the thermometric curves is shown in Fig. (1).

These curves are characterised by a sharp rise and finally a decrease, after attaining a maximum value. The slope of the rising parts of the curves decreases in presence of the inhibitor. This indicates that the additives act as general inhibitors and adsorb on both anodic and cathodic sites[21]. The rising parts of the curve indicate direct attack of the corrosive medium, i.e. anodic dissolution of zinc. If these parts were exactly parallel and with constant slope, the process would be mainly anodically controlled.

A plot of % reduction in RN vs. log C is in fact similar to an adsorption isotherm, (Fig. 2). The curve obtained is invariably sigmoid in nature, and this behaviour is explainable on the basis of a single step adsorption process[22]. This curve consists of an initial ascending portion which passes to a region of constancy indicating the completion of a monolayer of the adsorbate.

Fig. (3) shows the variation of the protection efficiency, P of zinc metal as a function of the concentration of the inhibitor in 2M HNO_3 solution at different temperatures. The protection efficiency P of the inhibitor was calculated by the following equation:-

$$P = 100 \left(1 - \frac{W_2}{W_1} \right) \qquad (3)$$

where W_1 and W_2 are the corrosion rates in absence and presence of a certain concentration in the medium, approaching complete protection (84%) at 0.01M of the inhibitor.

Fig. (3) also shows the effect of the concentration of the inhibitor at various temperatures on the protection efficiency of zinc. It is clear that the percentage inhibition increases with increasing inhibitor concentration. In general, also, it can be seen from Fig. (3) that the protection efficiency increases with decreasing the temperature. Fig. (4) shows the variation of the corrosion rate of zinc in 2M HNO_3 as a function of the concentration of the used inhibitor at different temperatures. It can also be observed from Fig. (4) that, at constant temperature, the corrosion rate decreases as the concentration of the tested substance increases.

It can be seen that if the inhibitor functions via adsorption mechanism, i.e. the degree of coverage is directly

proportional to the protection efficiency, in accordance with the Langmiur isotherm relationship:-

$$\text{Log } \frac{P}{1-P} = \text{Log } [I] + \text{constant.} \qquad (5)$$

(where [I] is the inhibitor concentration), then this relation should result in a straight line with a slope of unity. Fig. (5) shows that such plots are in agreement with the Langmiur adsorption isotherm. Interaction of adsorbed species by mutual repulsion or attraction would cause the slope of the plot to deviate from unity.

It was pointed out[23] that the logarithm of the corrosion rate is a linear function of $\frac{1}{T}$ (Arrhenius equation), where T is the absolute temperature.

$$\text{Log corrosion rate} = - \frac{Ea}{(RT)} + B \qquad (6)$$

where Ea is the apparent activation energy, R is the universal gas contant (1.98 Cal/mole. degree) and B is a constant. In Fig. (6) the logarithm of the corrosion rates of zinc are plotted as function of ($\frac{1}{T}$) in absence and presence of the studied inhibitor, respectively. From Fig. (6) the calculated value of the apparent activation energy is 9.21 Kcal/mole, a value that agrees with that reported previously[10]. This value is also of the order of the activation energies encountered for the hydrogen evolution reaction[24]. This is in accordance with the fact that

the hydrogen evolution reaction in the absence of an inhibitor is the rate determining step for the overall corrosion reaction. For 10^{-4} to 10^{-2}M inhibitor solutions, the calculated value of the activation energies are 9.67 and 11.98 Kcal/mole, respectively (Fig. 6). These differences are not considered to be significant. Therefore, the presence of the studied inhibitor does not affect the activation energy of the corrosion process. These results indicate that the tested substance does not change the mechanism of the rate-determining step of the corrosion process, although it, significantly, reduces the rate of corrosion itself.

Pig. (7) shows the anodic polarisation curves of zinc in 2M HNO_3 at different concentrations of the studied substance at 30°C. The anodic polarisation curves shifted to more positive values as the inhibitor concentration increases. These results confirm the assumption that the substance used acts as a powerful type of inhibitor, and so affects hydrogen evolution and anodic metal dissolution.

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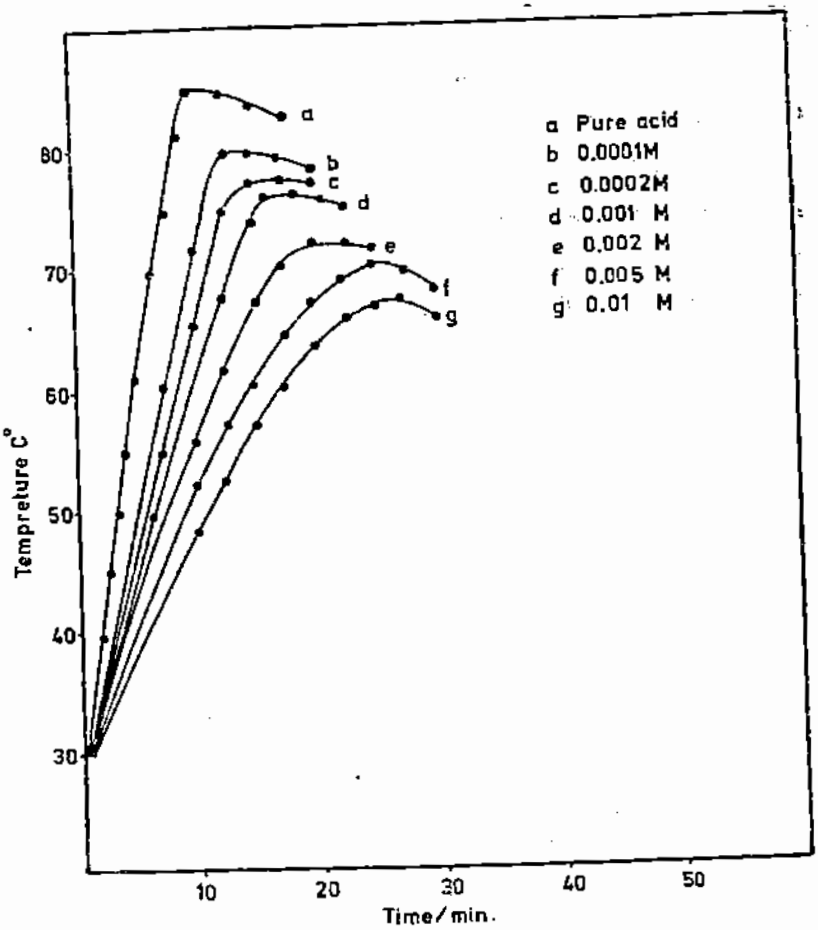

Fig.(1) : Effect of addition of the used substance on the thermometric behaviour of Zn in 2M HNO_3

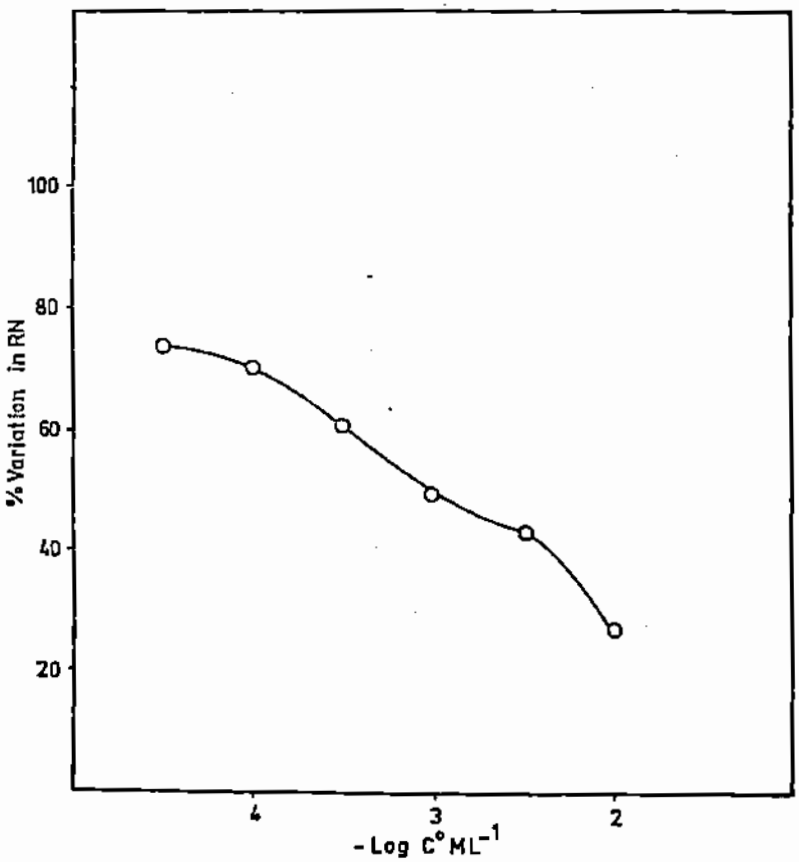

Fig.(2): Percent variation of RN with concentration of the used substance .

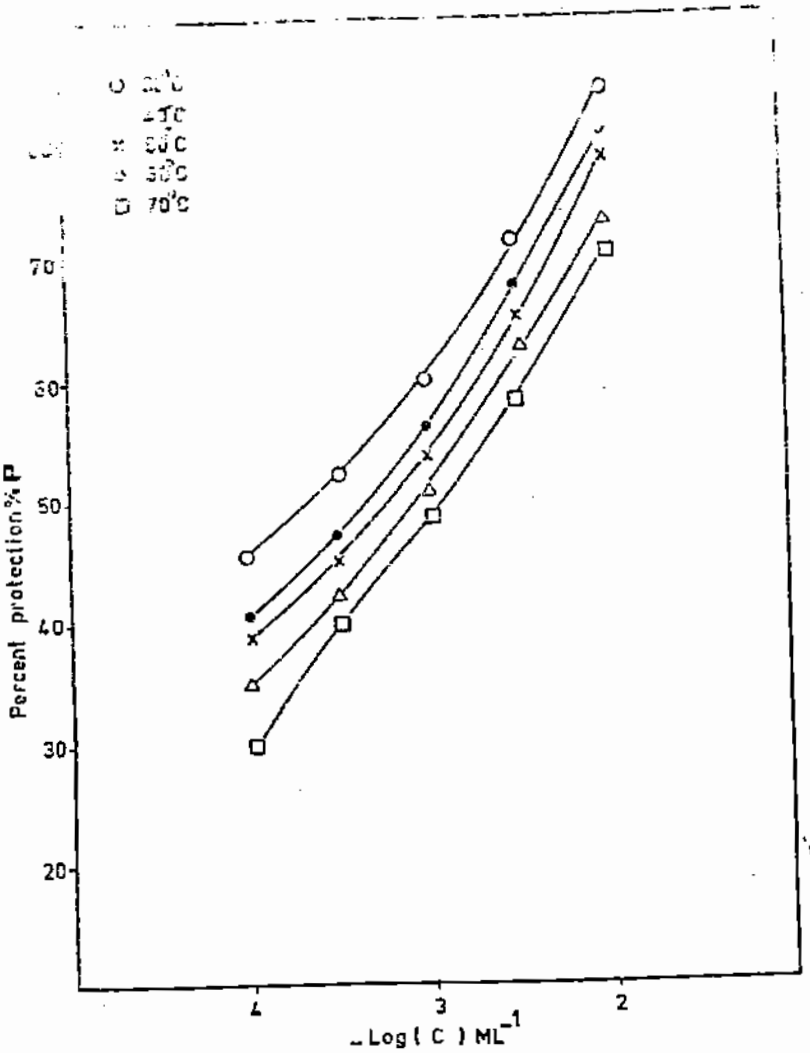

Fig.(3): Effect of concentration of inhibitor on the protection efficiency of Zn in 2M HNO_3 at various tenperatures.

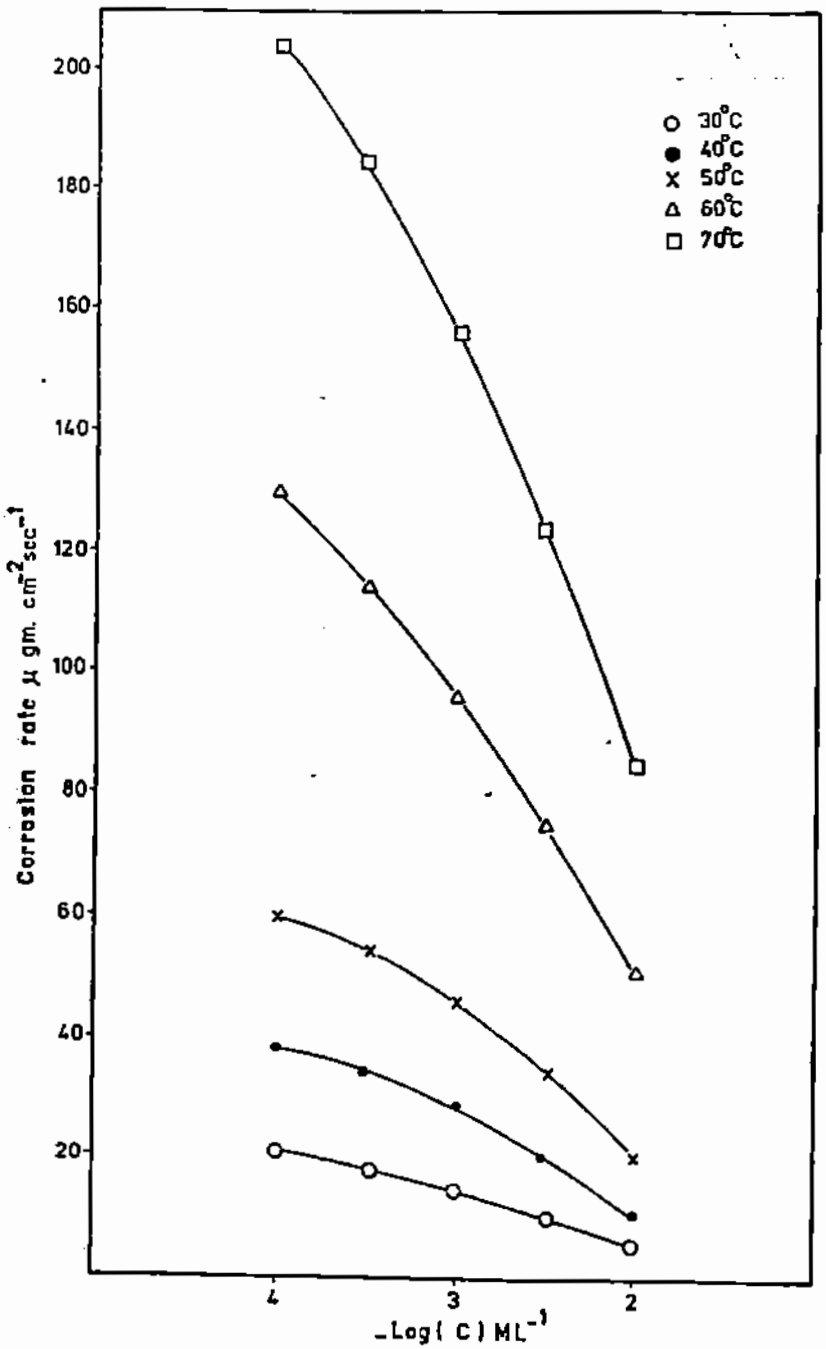

Fig.(4): Effect of the concentration of inhibitor on the corrosion rate of Zn in 2M HNO_3 at different temperatures.

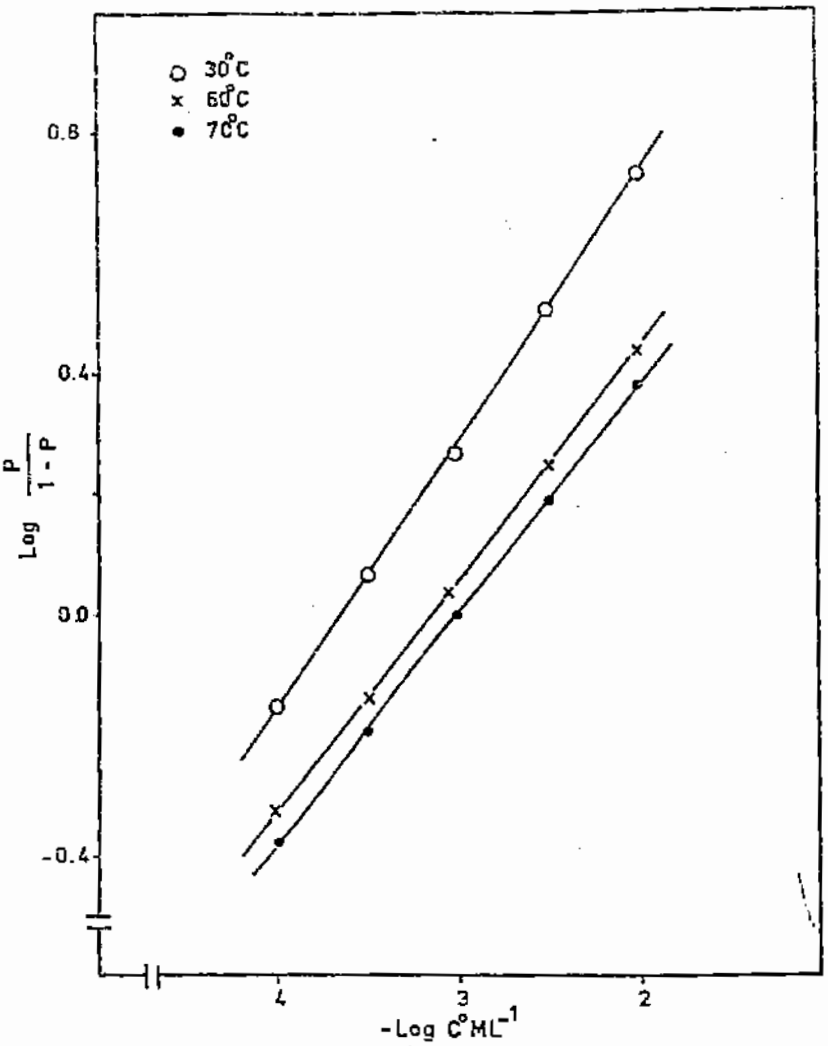

Fig.(5): Plot of $\text{Log}\frac{P}{1-P}$ vs. log concentration of inhibitor for Zn in 2M HNO_3 at different temperatres.

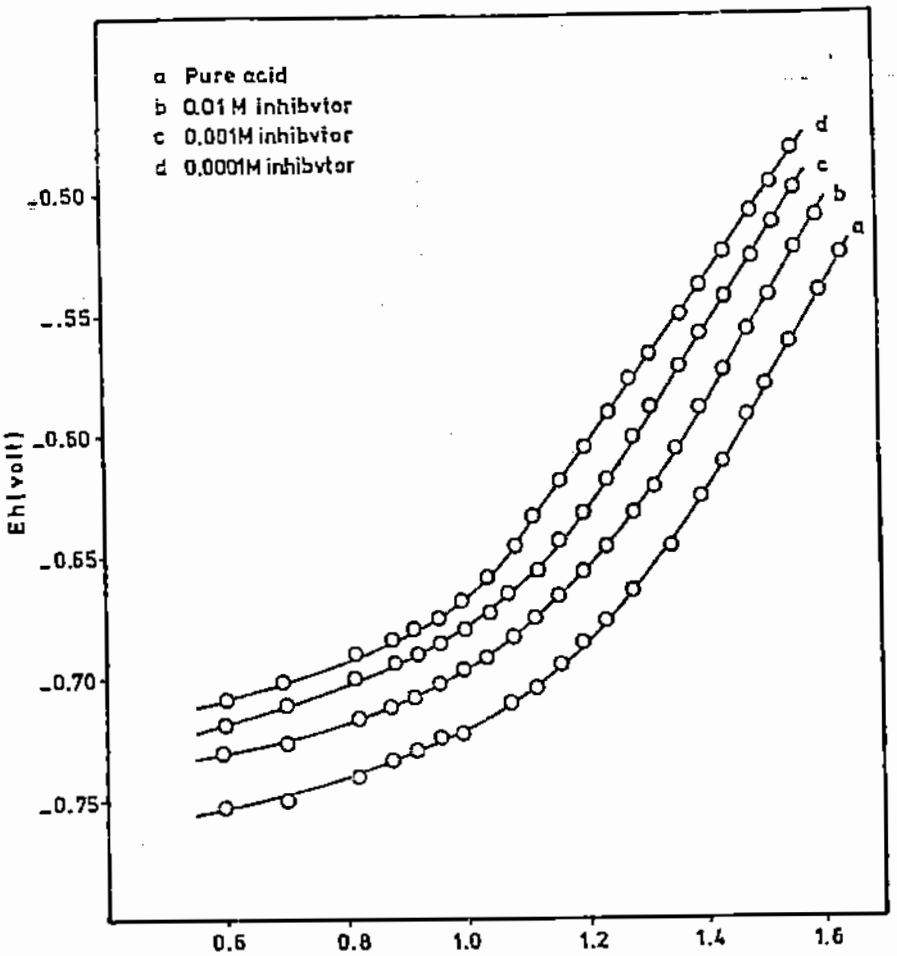

Fig. (7): Anodie Tafel plots for Zn in 2M HNO_3 at different concentrations of substance[1]

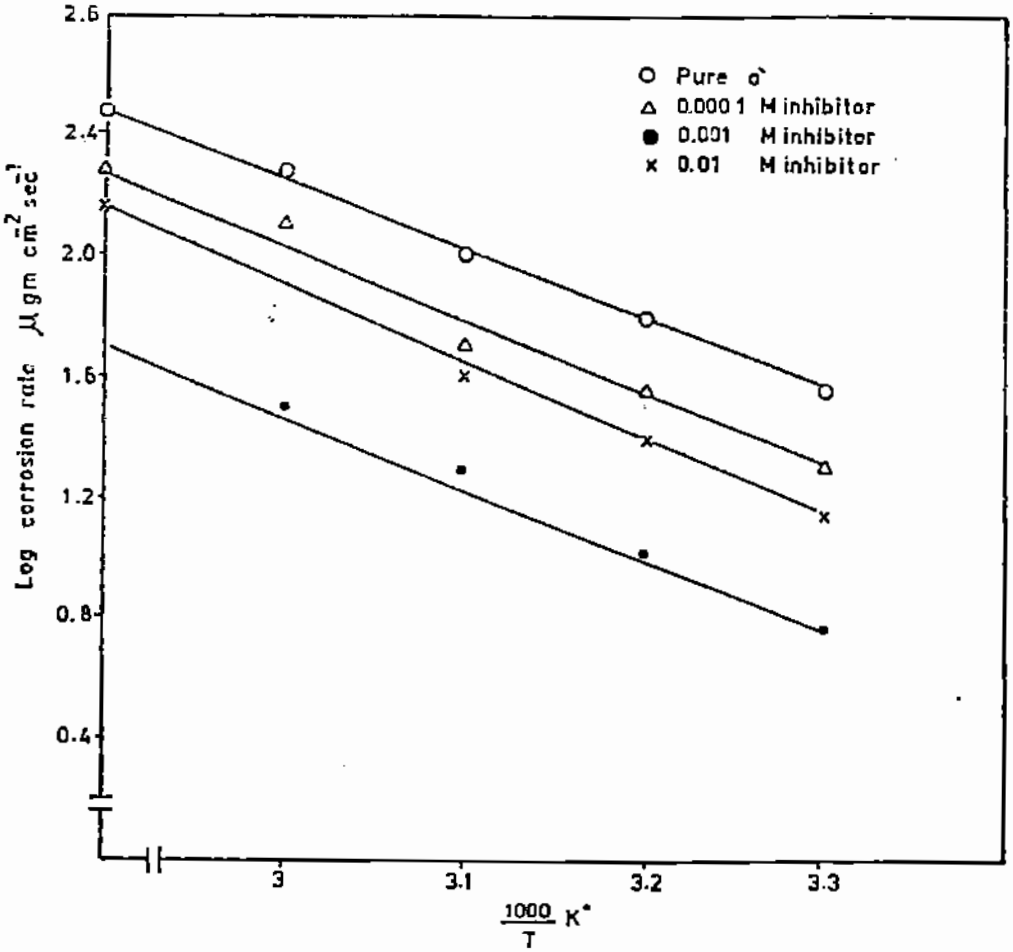

Fig.(6):Arrhenius plot of the corrosion rate of Zn in 2 M HNO_3 in absence and presence of inhibitor.

Growth response of Medicago sativa to phosphorus fertilization, cycocel and Rhizobium as affected by water regime

By

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Summary:

A pot experiment was conducted to evaluate the growth response of Medicago sativa to super-phospbate nitrate fertilizer alone or in combination with cycocel and/or Rhizobium when subjected to water regime. Particular emphasis was given to growth parameters, yield and nutrient content as well as sugars, carbohydrates and water use efficiency.

The water regime undertaken for this experiment was to keep the soil moisture always at the field capacity, one irrigation interval (4 days) was tested in a factorial randomised design with three replications.

Application of superphosphate fertilizer alone or combination with cycocel and/or Rhizobium as biofertilizer greatly affected fresh and dry weight of the stressed plant, root-shoot ratio and leaf area as well, in addition water use efficiency and nutrient concentration in plant tissues.

The stimulation effect induced by different treatments was generally more evident in superphosphate fertilizer plus cycocel, while progressive inhibition associated with superphosphate plus cycocel and/or Rhizobium was shown.

Water use efficiency and sugar content were the only parameters recorded noticeable increase with superphosphate fertilizer plus cycocel and/or Rhizobium application.

Introduction:

Water is one of the most limiting factors affecting crop production in semiarid regions, where irrigation has supplemented rainfall for crop production, competition from urban water users has created a need for better understanding of crop water requirement and yield relationships (Labanauskas et al., 1981). Moreover very close relationship has been found to exist between continuous availability of soil moisture and response of plant to fertilizer application. If the soil moisture becomes a limiting factor during any stage of the growing season, the addition of fertilizer under such condition may even adversely affect the yield (Reddy et al., 1980). Those authors also found that on a well fertilized plant, the corn roots developed up to 105 cm in the soil and utilized 8.75 cm^3 more water than utilized by the unfertilized plant.

The adequate supply of fertilizer with suitable irrigation intervals was reported by many authors (Sekhon et al., 1981; Verma and Kalrs 1981, 1983 and Sharma et al., 1984). The last authors reported that the highest seed yield of Lentil with higher nitrogen and phosphorus content were obtained by using of 20 Kg N and 60 Kg P with two irrigation intervals. On the other hand Mohamed et al., (1981) stated that soybean plant responded markedly to N & P application and the maximum yield was produced when N and P were applied together. Furthermore, Saraf and Baitha (1982) found an increase in both dry weight as well as nodule numbers of Lentil plant by the application of phosphorus fertilizer. Hussein et al., (1984) mentioned that 32 Kg P/Fed=4200 m^2 and inoculation of Lentil seeds with specific rhizobia, increased the numbers of pods per plant and nitrogen content. However, rhizobial inoculation seemed to give higher seed yield than the un-inoculated treatments.

The aim of this study was to show that if Medicago sativa plant could establish good growth when subjected to water regime by the application of chemical fertilizer alone or in combination with cycocel and/or Rhizobium as biofertilizer. On these basis, this experiment was conducted to explain these aspects.

Materials and Methods:

Seeds of Medicago sativa cv. Esna were obtained from Agriculture Centre, Ministry of Agriculture, Giza. The experiment was conducted in the experimental garden of the Botany Department, Women's College, Ain Shams University.

Cylindrical plastic pots, 21 cm. diameter and 18 cm. depth were chosen for cultivation. In each pot, 4 Kg of loamy soil was placed. Some physical and chemical soil characters were determined as presented in Table (1), following the methods described by Jackson (1973). Chemical fertilizer, mainly containing supper phosphate-nitrate-potassium, as this type of fertilizer was recommended for optimal results of growth and yield. 0.2 g/pot was applied throughout this experiment, twenty seeds of Medicago sativa plant were sown per pot, after two weeks the seedlings were subjected to the irrigation regime interval (4 days) keeping 5 replicate pots for each treatment, which consist of pots containing cycocel (40 ppm) or Rhizobium (2 gm/pot dried cells). Each of them were applied with superphosphate fertilizer, another treatment containing superphosphate fertilizer plus cycocel and/or Rhizobium was applied. After 90 days from sowing, plants were uprooted, washed and dried at 70°C to constant weight. Growth parameters including, fresh and dry weight of plant, leaf area and root-shoot ratio were recorded.

Total carbohydrates and total soluble sugar contents were estimated as g/100 g dry weight of shoot including the leaves. The method used was described hy (Dubois et al., 1956). Nitrogen content was determined in dried and finally pulverised plant shoots using H_2SO_4 and $HClO_4$ 1:1 (Jackson, 1973). Mineral determinations in the plants were carried out after digestion of the dried ground material following the method described by Champann and Pratt, (1978).

The obtained data were subjected to analysis of variance (Snedecor and Cochran, 1980).

Results and Discussion:

Growth parameters:

Previous investigation by Abou-Bakr et al., (1993) had found that Medicago-sativa growth was greatly affected when subjected to water stress conditions at different irrigation intervals. An attempt was performed to clarify if stressed Medicago-sativa (4 days interval) could establish good growth by application of chemical fertilizer alone or in combination with cycocel and/or Rhizobium as biofertilizer. Particular emphasis was undertaken to growth behaviour, water use efficiency and nutrient concentration in plant tissue as well. The obtained results revealed that fresh and dry weight of stressed plant (4 days) irrigation interval were enhanced by different treatment application (Figs. 1 & 2). Enhancement of the plant growth was more evident by the application of superphosphate fertilizer plus cycocel as compared to superphosphate alone (control), while noticeable decrease associated with superpbosphate fertilizer in combination with cycocel and/or Rhizobium was shown. Similarly leaf area was affected by different treatments (Fig. 1), being increased by superphosphate fertilizer plus cycocel as compared to the fertilizer alone (control) and decreased by superphosphate fertilizer plus cycocel and/or Rhizobium. Reduction in root-shoot ratio by dry weight may be attributed to reduced root growth which resulted from moisture stress. These findings were in full agreement with that obtained by Mbagwa and Osuigwe (1984) who showed that moisture stress generally reduced root growth of maize and cowpea and thereby decreased root-shoot ratio. On the other hand Reddy et al., (1980) found that if the soil moisture becomes a limiting factor during any stage of the growing season, addition of fertilizer under such conditions may even adversely affect the yield. Furthermore a great reduction on the dry wight of plant which grown under water stress condition was previously studied by many authors (Mbagwa and Osuigwe, 1984; Shouse et al., 1981; Sinclair et al., 1975 and Summerfield et al., 1976), while other workers (Babalola, 1980 and Denmead and Show, 1960) attributed decreased dry weight associated with reduced amounts of water to water stress conditions which invariably resulted to stomatal closure. This leading to the over all effect of reducing the photosynthetic efficiency of the leaves with consequent reduction in yield.

Yield production:

Different treatments had a major effect on yield production. The most severe reduction in yield was found in the treatment of phosphorus fertilizer plus cycocel and/or Rhizobium in which the yield reduction was about 53% of treatment supplying phosphorus fertilizer alone (control). These findings are in complete agreement with (Turk et al., 1980; Shouse et al., 1981 and Hiller et al., 1972), but in disagreement with Summerfield et al., (1976) and Wein et al., (1979). Discrepancies in the experimental findings may be due to the in-determine reproduction nature of certain plant varieties, differing varietal response to water stress at the different growth stage, possible differences in degree of treatment application or other environmental factors not taken into account.

Water use efficiency:

The water use efficiency of the plant (Fig. 5) showed that the treatment for dry matter yield was achieved by the application of phosphorus fertilizer plus cycocel and/or Rhizobium, Other treatment gave poor water use efficiency specially the treatment which supplied with phosphorus fertilizer plus cycocel compared to the treatment receiving phosphorus fertilizer alone. Water use efficiency of stressed plants was relatively low and application of chemical fertilizer (superphosphate) growth promoters (cycocel) and biofertilizer (Rhizobium) had improved it. Kowall and Kassan (1973) reported reduce water use efficiency for maize when soil moisture decreased below its field capacity. Other workers observed that during silking stage for (maize) and flowering and filling stage for (cowpea) water use efficiency was decreased as a result of induced moisture stress.

Nitrogen uptake of the plant:

Nitrogen uptake by the stressed plant shown in Fig. (6) indicated that there were variation in nitrogen percent taken up by the plant. The main concentration of N in the plant ranged from 3.45 to 4.7%. The lowest concentration of N was found in plant treatment receiving fertilizer plus Rhizobium and the highest one was treatment

supplying with fertilizer plus cycocel. Generally, N uptake was improved hy phosphorus fertilizer application plus cycocel more than the other treatments. These results were expected since Rhizobium could not fix nitrogen under water regime or drought condition. These findings might be attributed to the inability of Rhizobium to survive these condition or the inactivation of such strain under water regime. It is known that water stress decreases symbiotic N_2-fixation and growth of legumes (Finn and Brun, 1980 and Dejong and Phillips 1982).

Some data indicated that water stress disrupts interaction between Rhizobium and bost plant directly by altering nodule fine structure which leads to change in either nodule membrane permeability or enzyme activity (Sprent, 1976).

Other evidence suggests that root nodules are affecting indirectly after a decrease in photosynthesis (Finn and Brun, 1980). It is also known that water stress decreases nitrate-reductase activity (Srivastava, 1980) and negatively affects various other aspects of nitrogen metabolism (Hasiao, 1973).

Nutrient uptake of the plant:

Different treatment affected phosphorus concentration in plant tissues (Fig. 7), being high in treatment supplied with phosphorus fertilizer plus Rhizobium and cycocel and low in treatment supplied either with phosphorus alone or in combination with cycocel. Its value was ranged from 0.28% to 0.38%. These concentration were substantial and clearly suggested that the plant was not suffering phosphorus deficiency. Data obtained by other investigators on the effect of phosphorus nutrition in wheat grain and cowpea clearly showed that the values of phosphorus found in Medicago sativa were adequate (Labanauskas et al., 1978 and Labanauskas et al., 1981). These data were contradictory with the obtained data of nitrogen. This manifested that water regime affected nitrogen fixation process but not phosphorus concentration in the plant tissues.

Water regime did not significantly influence the concentration of K in the plant, but there was some variations in K concentration particularly in treatment supplied with phosphorus fertilizer plus cycocel and/or Rhizobium. Concentration of K in plant tissues ranged from 5.8% to 6.8%. The values of K in the plant tissues were substantially

higher than found in the wheat seeds in hydroponic or soil studies (Labanauskas et al., 1975, 1978) and cowpea (Labanauskas et al., 1981).

The concentration of Ca were not influenced by water regime. All the treatments were nearly similar except those receiving fertilizer plus cycocel, slight increase was clearly shown. Calcium concentration ranged from 1.19-1.39% (Fig. 9). These values were found higher comparable to those obtained by Labanauskas et al., (1981) on cowpea.

Magnesium concentration in the Medicago sativa ranged from 0.53-0.59% and the concentration levels were not significantly affected by the treatments or by water regime. The concentration levels of Mg in the cowpea seeds in Labanauskas et al., (1981) experiment ranged from 0.18 to 0.19% under different irrigation treatments. The present data were found to be higher than those obtained either by Labanauskas et al., (1976) on wheat or Labanauskas et al., (1981) on cowpea.

Those authors found that concentration levels of Mg in the cowpea seeds were about the same as found in wheat grain from the hydroponic and soil media. Loneragan et al., (1976) had classified N, P and K as mobile elements, Ca as immobile and Mg as intermediate. According to Scott and Brewer (1980), water stress soybean plants do not transpire as much water as well watered plants, therefore lower nutrient element transport occurs in the plant.

Sodium concentration in the plants was not affected by different treatments or by moisture regime. The Na concentrations of about 0.1-0.18% (Fig. 10). In Labanauskas et al., (1981) investigation, Na concentration was lower (0.02%) in cowpea plant subjected to different irrigation treatments. The present data were found high when compared with obtained data by those authors.

The concentration of Fe in Medicago sativa plant were affected by the irrigation treatments and by water regime (Fig. 11). Fe concentration ranged from 0.28% to 0.65%. The higher value obtained from treatment supplied with superphosphate fertilization plus cycocel and/or Rhizobium and the lowest value obtained from treatment supplied with superphosphate only. Although there were statistically differences in Fe concentration due to treatments, the plant was well supplied with Fe

and no deficiency symptoms appeared at any stage.

Total carbohydrate content %:

Water regime (4 days) did not affect total carbohydrate content. It recorded 3.2% in treatment supplied with phosphate fertilizer alone which acted as control, on the other hand application of other treatments clearly showed decline in carbohydrate content revealed progressive decline with increase in soil moisture stress associated with the elongation of irrigation intervals (Abd El-Rahman et al., 1993; Hodges and Heatherly, 1983; Krizek et al., 1985 and Batanouny et al., 1988). Abou El-Seud (1987) showed that decreasing the applied available water resulted in a continuous decline in total carbohydrate content during the different stress periods. Depletion of total carbohydrate content in treatment supplied with phosphate fertilizer plus cycocel and/or Rhizobium was attributed to the depletion of N_2-fixation process which took place by the bacteria. Some evidence suggested that carbohydrate contents in leguminous plants were affected indirectly after a decrease in photosynthesis (Finn and Brun, 1980). Furthermore Abd El-Rahman et al., (1993) stated that the reduction in carbohydrate accumulation under drought conditions might be referred to decrease in photosynthetic activity, moreover the obtained results may be due to higher resistance of CO_2 diffusion due to narrower or closure of stomatal opening and hence lower chlorophyll and carbohydrate content.

Sugars content %:

Four days water regime affected sugar content in Medicago sativa plant. These findings more found in the treatment supplied with phosphorus fertilizer (control). Contrary, treatments supplied with phosphorus fertilizer plus cycocel and/or Rhizobium greatly promoted sugar content by more than 50%. Abd El-Rahman et al., (1993) mentioned that soluble sugar content demonstrated a gradual and remarkable increase with increase moisture stress associated with increase in irrigation intervals. These findings were noticed only with treatments supplied with fertilizer plus cycocel and/or Rhizobium which were also recorded lower carbohydrate content. This might be

attributed to the effect of N_2-fixation process which decrease total carbohydrate content and consequently increased sugar content. It might also be attributed to the presence of cycocel promoters which enhanced sugar content and delayed carbohydrate content. Decrease in carbohydrate content and increase in sugar content in plant is refereed to osmotic adjustment when plants are subjected to increase in moisture stress (Abd El-Rahman et al., 1993). The same authors found also that accumulation of the osmotically active substances such as soluble sugars raises the capacity of roots for water absorption. Furthermore, Hussein et al., (1988) reported that elongation of irrigation intervals resulted in a reduction of total carbohydrate content and a significant increase in soluble sugars content.

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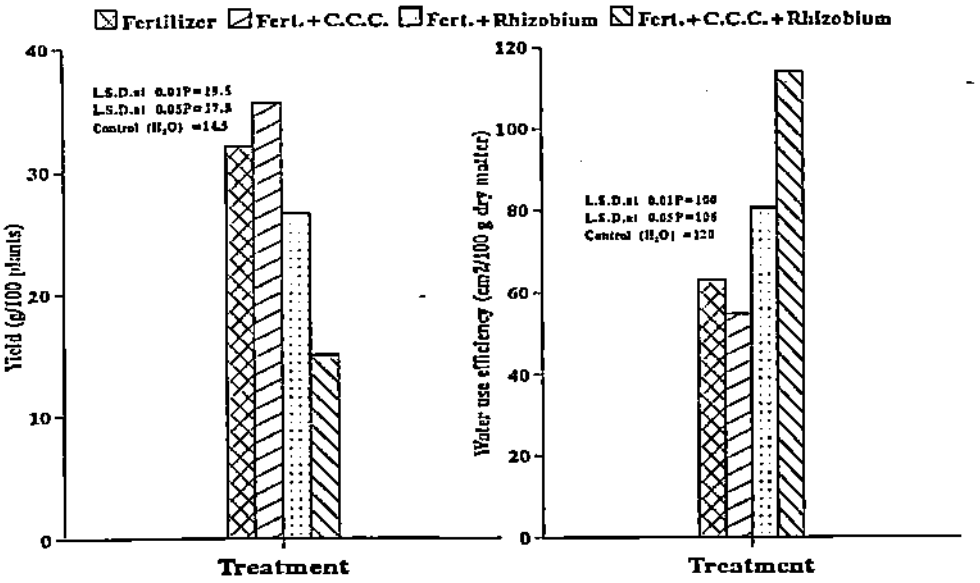

Fig. (2): Effect of fertilizer, cycocel, Rhizobium and mixture treatments on yield (g/100 pl.) and water use efficiency (cm^2/ 100 g dry matter) of Medicago sativa, grown at (4-day) irrigation intervals (plant age 90 days)

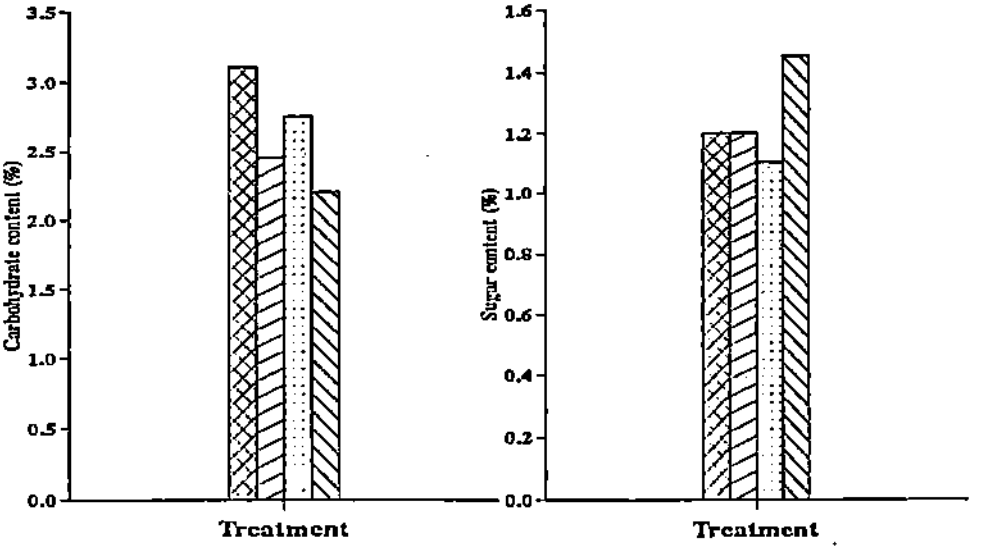

Fig. (4): Effect of fertilizer, cycocel, Rhizobium and mixture treatments on carbohydrate (%) and sugar (%) of Medicago sativa, grown at (4-day) irrigation intervals (plant age 90 days)

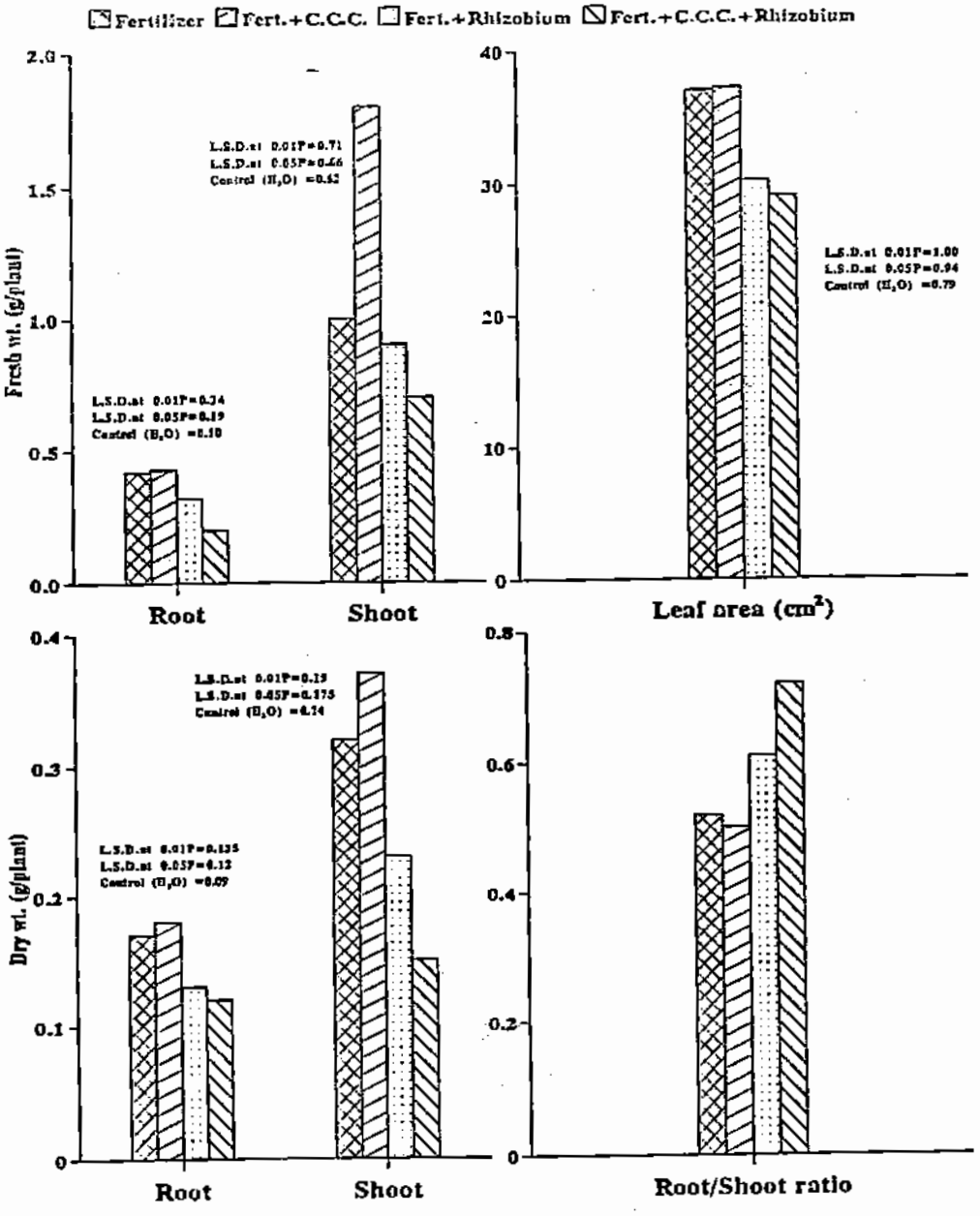

Fig. (1): Effect of fertilizer, cycocel, Rhizobium and mixture treatments on fresh and dry weight (g/pl.), leaf area (cm^2) and root-shoot ratio of Medicago sativa, grown at (4-day) irrigation intervals (plant age 90 days)

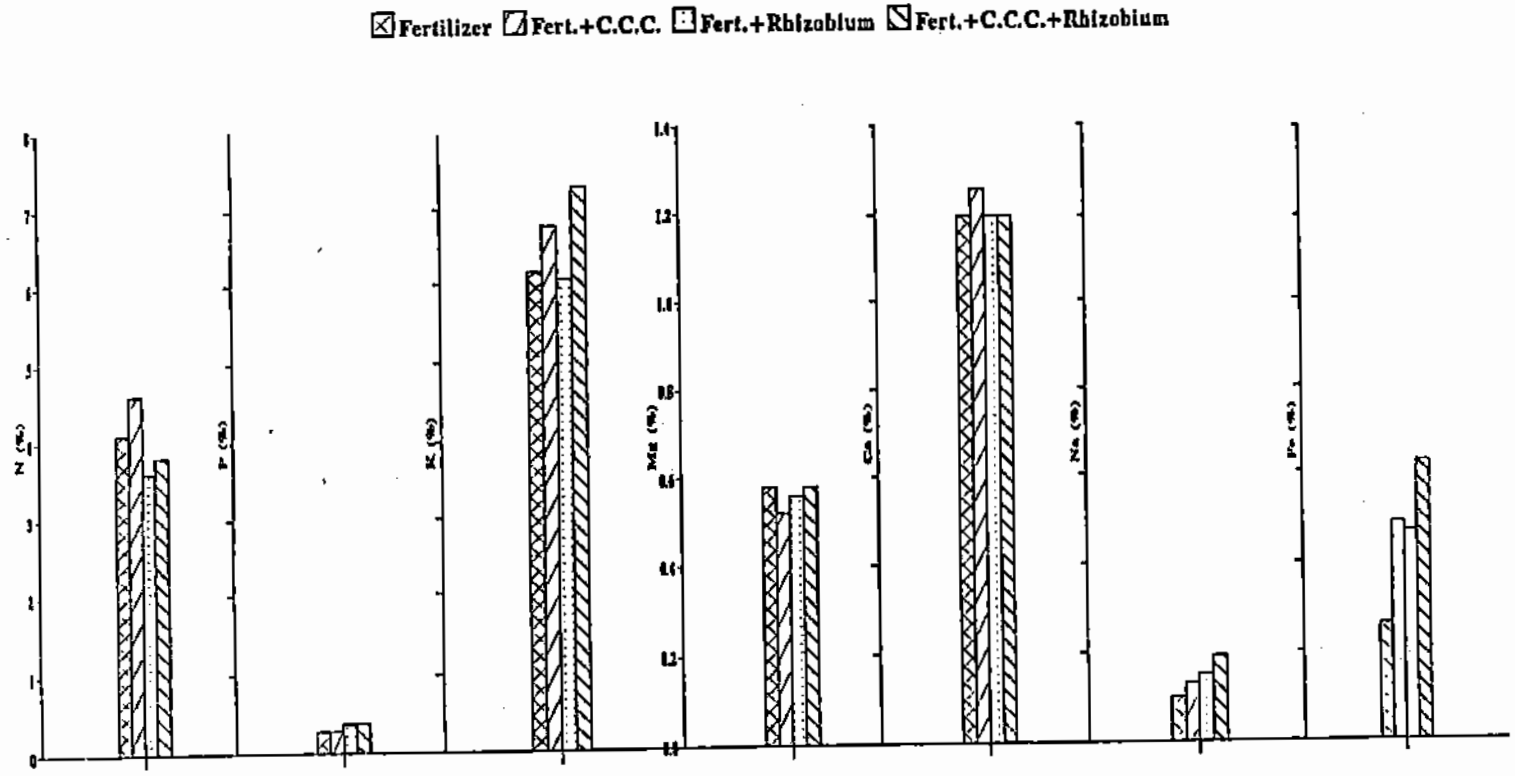

Fig. (3): Effect of fertilizer, cycocel, Rhizobium and mixture treatment on nutrient uptake (%) of Medicago satlva, grown at (4-day) irrigation intervals (plant age 90 days)

Table (1) : Mechanical and chemical properties of the soil used in the experiments.

Mechanical Analysis:		
Coarse and fine sand	%	36.4
Silt	%	30.0
Clay	%	31.6
Soil texture		Loamy soil
Field capacity	%	21.00
Chemical Analysis:		
$CaCO_3$	%	2.46
pH	1:2.5	8.31
E.C.	mmhos/cm	0.66
Organic matter	%	1.05
Extraction	(mg/100g soil)	
N	%	1.05
P		3.51
K^+		41.48
Na^+		48.60
Ca^{++}		62.0
Mg^{++}		32.55
$CO_3^=$		—
HCO_3^-		68.32
CL^-		91.0
$SO_4^=$	ppm	22.14
Available microelements	ppm	
Fe		7.54
Mn		38.85
An		3.5
Cu		3.36

Table (2): Climatic conditions during the growth period.

	Air temperature °C		Relative humidity %
	Mean minimum	Mean maximum	
January	10.0	18.8	62
February	10.6	20.0	56
March	12.5	23.2	54
April	16.5	28.5	46
May	18.4	31.8	48
June	20.8	34.4	52
july	23.5	35.7	56

بسم الله الرحمن الرحيم

الملخص العربى

إستجابه نمو البرسيم الحجازى للتسميد الفوسفورى، السيكوسيل والريزوبيم عندما يتأثر بالرجيم المائى

فاطمة عبد الوهاب حليمش، منى عبد الواحد نعيم وزينب يوسف محمد أبوبكر

قسم النبات - كليةالبنات - جامعة عين شمس - القاهرة - مصر

أجرى بحث فى أصيص لتوضيح مدى إستجابه نمو البرسيم الحجازى للتسميد بنترات السوبرفوسفات منفرد أو مجتمع مع السيكوسيل و/أو الريزوبيم عندما يتعرض النبات للرجيم المائى، مع الأخذ فى الإعتبار معدلات النمو فى المحصول والمحتوى الغذائى وكذلك المحتوى الكربوهيدراتى والسكرى وفاعليه الماء المستعمل. وقد كان الرجيم المائى المستخدم لهذه التجربه هو بقاء المحتوى المائى للتربه دائما عند السعة الحقلية مع إختبار فتره رى واحدة (٤ أيام)، هذا وقد أوضحت النتائج مايلى:-

تأثر الوزن الرطب والجاف كثيرا للنباتات المجهده ونسبه الجذر إلى الساق وكذلك مساحة الورقة بالإضافة إلى فاعلية الماء المستعمل وتوكيز المواد الغذائية فى أنسجة النبات عند إستعمال سماد نترات السوبر فوسفات منفرد أو مجتمع مع السيكوسيل و/أو الريزوبيم كسماد طبيعى، وعلى العموم فقد كان التأثير المحفز للمعاملات المختلفة أوضح ما يكون فى سماد نترات السوبر فوسفات والسيكوسيل، بينما شوهد تثبيط شديد للسوبرفوسفات زائد السيكوسيل و/أو الريزوبيم. فى حين كان فاعلية الماء المستعمل والمحتوى السكرى هما المعاملات الوحيدة التى سجلت زيادة ملموسة مع سماد نترات السوبر فوسفات زائد السيكوسيل و/أو الريزوبيم.

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Physicochemical studies on ZnO- Silica gel system

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Abstract:

Several mixed ZnO-Silica gel systems were prepared by the impregnation method and calcined for 5 h at 115, 300, 600 and 1000°C. The crystal structure, surface acidity, surface basicity, surface area, catalytic decomposition of H_2O_2 and the electrical conductivity of the samples prepared were studied. It was found that the decomposition of H_2O_2 is catalysed by each of the acidic and basic sites formed on the catalyst surface.

$ZnSiO_4$ spinel was found to be formed at temperatures $\geq$ 760°C. The results obtained were correlated together and the effect of composition on the physical properties of oxides investigated have been discussed.

Introduction:

The binary oxides are widely used in catalysis[1,2]. The main class of reactions that lead to the formation of active oxide catalysts is essentially thermal reactions of suitable compounds. The chemical composition of the

starting materials and the methods of preparation play an effective role in altering the properties of the final products.

Several studies[3-7] on the catalytic and the acidic properties of some inorganic solids showed the presence of a correlation between the acidic and catalytic properties of many inorganic solids. Also, the acid and the basic properties of solid surfaces are interesting aspects of surface structure and important in the fields of ion exchange.

The present work was designed to measure the acidity and the basicity of binary oxides, Zinc oxide and silica gel, in order to test how the catalytic activity correlates with the acid strength of the catalyst. The catalytic decomposition of H_2O_2 by the pure and mixed oxides as well as the electrical conductivity of these oxides have been also studied.

Experimental:

The binary oxides $ZnO-SiO_2$ were prepared in the composition 0,15,30,50,70,85 and 100 mol % ZnO from BDH quality chemical by impregnation technique. The impregnated oxides were dried at 115°C for 5 h. The samples thus obtained were powdered and only such samples were collected

between 100 and 150 mesh sieves were used. The powders thus obtained were calcined at 115, 300, 600 and 1000°C for 5 h. at each one of these temperatures. The samples after calcination were cooled in a desicator and preserved in covered glass tubes under vacuum.

DTA and TGA studies for pure and mixed oxides were carried out using Shimadzu model-30, at a heating rate of 10°K mim^{-1} in air atmosphere, using a 20 mg sample. The surface area of the binary mixed was determined with a surface area measurer B.E.T. micrometer p 2200.

I.R. spectra of the samples were recorded in the range of 200-4000 Cm^{-1} using a Beckman infrared spectra-photometric unit. The KBr disc technique was used in this study. X-ray diffraction patterns of the samples were obtained by using Shimadzu X-ray Diffraction Unit with the aid of Phillips unit type PW2103/00, using copper target and nickel filter. The acidity of the oxides investigated were measured by using the amine titration method developed by Johnson[8]. So 0.2g. of the mixed oxides suspended in benzene was titrated with a solution of 0.1N n-butylamine in benzene using Benzene-azodiphenylamine (pK_a = 1.5), P-dimethylamineazo-benzene (pK_a = 3.3). Benzalnaphthylamine (pK_a = 4.0) and neutral red (pK_a = 6.8) as indicators. The surface acidity of

n-butylamine used in the titration of 1 gm of the oxides investigated is expressed as m-mol.

The surface basicities of oxides were determined by titration method using 0.1N benzoic acid in benzene and bromothymol blue of (pK_a = 7.1) and phenolphthaline of (pK_a = 9.3) as indicators. The value given for each of the surface acidity and basicity is the mean of three experiments.

The decomposition of H_2O_2 was selected for the study on the catalytic reactivity of the catalysts and was conducted as described by Keating[9] in a temperature range of 45 to 65°C.

The electric conductivity of the oxides investigated has been measured by a method was reported elsewhere[10].

Results and discussion:

The DTA and TGA diagrams of ZnO and silica gel SiO_2xH_2O are given in fig.(1). The TGA diagrams for $SiO_2.H_2O$ showed a decrease in the weight at a temperature range of 30-1200°C for all calcined samples. Two endothermic peaks could be characterized by DTA for the silica gel samples. The first small one at about 70°C is attributed to the elimination of water molecules adsorbed on the surface,

while the second peak at ~600°C can be explained on the basis of the transformation of silica gel to SiO_2. This transformation was also observed by others[11,12].

The DTA and TGA diagrams of ZnO fig.(1), show an endothermic peak at 200 to 300°C with a decreasing in the weight (~10%) due to the elimination of water molecules adsorbed on the surface. This loss in the weight decreases with increasing the calcination temperature.

The diagram of $ZnO-SiO_2.xH_2O$ (~1:1 molar ratio) mixtures shows in addition to the peaks obtained in each of ZnO and silica gel a new endothermic peaks at 760°C. This new peak is attributed to a solid state reaction occuring between ZnO and SiO_2 to form Zn_2SiO_4 spinl. However Wolf[13] reported that Zn_2SiO_4 is formed at higher temperature than that found here.

Fig.2. illustrates the X-ray diffraction patterns of the pure and the product of thermal treatment of ZnO and silica gel at different calcination temperatures. It can be seen an amorphous nature for silica gel at all calcined temperatures. Also, the intensive lines of Zn_2SiO_4 spinel (d-values of 3.52, 2.82, and 2.67) could only be dectected for calcined mixtures at 1000°C. This confirm the formation of the spinel at 1000°C.

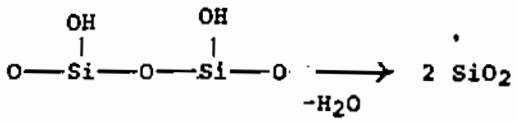
Fig.(3) shows the IR spectra of ZnO, $SiO_2.xH_2O$ and their mixtures ($\sim$1:1 molar ratio) calcined at different temperatures. The figure shows for each sample a band at $\sim$3410 cm^{-1} whose intensity decrease with increasing the calcination temperature. This band was assigned to -OH group[14] present in each of the hydrated ZnO, $SiO_2.xH_2O$ and their mixture. While IR spectra of calcined mixtures (at temperature 125, 300 and 600°C) show the same peaks appeared for each of the individual compounds, the spectra of calcined mixed oxides at 1000°C show new bands in the range 850-950 cm^{-1}. This new bands confirm also the formation of Zn_2SiO_4 at 1000°C as obtained from X-ray results.

The results of surface acidity are shown in fig.(4). It shows a non linear behaviour between the acidity and the composition of the oxides with maxima at 30 and 85 mol % ZnO.

The acidity was found to be increasing with increasing the calcination temperature and reaches a maximum at 600°C before its decreasing again. The decrease in the acidity surface at 1000°C is due to the decrease in the number of SiOH groups present on the surface at 1000°C and/or due to the formation of Zn_2SiO_4 at this temperature as revealed by X-ray analysis.

The surface basicities of $ZnO-SiO_2$ at various compositions are given in fig.(5). It shows a maximum basicities sites at 85 mol. % ZnO. The number of basicities sites increases in the manner observed for acidities i.e. at 600>300>1000°C.

The acidic and basic sites on the surface of SiO_2 can be explained according to the assumption that the acid sites of silica gel which has been dehydrated at high temperatures may be formed by lattice distortions.



As the calcination temperature is raised, a water molecule is removed from two hydroxyl groups attached to silicon atoms at the Si-O-Si link. The Si-O-Si link is readily formed between neighbouring Si-OH groups in the early stages of dehydration, but the distortion between them becomes progressively greater with further dehydration of Si-O-Si link (which is responsible for the acid strength). The increase in the acidic and basic sites by adding ZnO to silica gel is due to substitution of divalent zinc for tetravalent silicon in the silicon lattice. This creates a negative charge at the substituted silicon point on the solid surface -Si-O-Zn-O-Si, which causes an increase in the basicity. On the other hand

due to electrical neutrality, vacancy in oxygen sites may be formed which acts here as acidic sites. This causes also an increase in the acidity which is the case in our results.

The results of surface area measurements are given in table (1). It shows a change in the surface area with changing the oxide composition in a manner similar to that obtained in the results of surface acidity and basicity (except in pure silica gel.).

The results of decomposition of H_2O_2 over $ZnO-SiO_2.xH_2O$ system are shown in fig.(6) and summarized in table (2). The decomposition rate varies with each of the change in the composition and the calcination temperature, higher reactivity is found at 30 and 85 moles % ZnO (which showed also maximum acidity and basicity). It means that the rate of H_2O_2 decomposition depends to a large extent on the number of acidic and basic sites present on the surface of the solid. According to this concept the following mechanism is proposed.

For acidic sites:-

$$A^+ + H_2O_2 \longrightarrow A^+HO_2^- + H^+$$

$$A^+HO_2^- + H_2O_2 \longrightarrow H_2O + O_2 + A^+OH$$

For basic sites:-

$$B^- + H_2O_2 \longrightarrow B^-H^+ + HO_2^-$$

$$HO_2^- + H_2O_2 \longrightarrow H_2O + O_2 + OH^-$$

Where A^+ is acidic site and B^- is basic site, present on the surface. It can be seen that the mechanisms are similar in both cases. HO_2^- formed being responsible for the decomposition ion of H_2O_2.

Electrical conductivity measurements may give us information about the type of charge carriers and the phases formed for the different calcination temperatures. Therefore, the electrical conductivity, σ, of calcined samples ZnO, silica gel and their mixtures was measured as a function of temperature in the range 25 to 600°C. The results obtained are represented by plotting ln σ against $\frac{1}{T}$, a typical plot is shown in Fig.(7). The plots of all calcined samples investigated show the same trend. They show minima and maxima in σ-values for pure silica gel and each one of the mixed oxides, while the plots of the pure ZnO show only a minimum in σ-values. The maxima in σ-values appearing at all samples investigated, except pure ZnO, at $\sim$ 370°C should be attributed to the presence of silica gel in the sample.

The observed decrease in σ-values with increasing the temperature, in the lower temperature range can be attributed to the elimination of adsorbed water molecules present in the samples investigated. The conductivity data of the pure oxides calcined at 1000°C was found to be good coincide with those reported in the literature[14,15,16].

Since the samples investigated are heterogeneous many non linear plots for the relation $\ln \sigma$ vs. $\frac{1}{T}$ are obtained. The activation energies are calculated using Arrhenius equation from the linear parts in these plots. The conductivity results are summarized and given in table (3). From which it can be seen a decrease in E_a-values with increasing SiO_2 contents in the samples calcined at 1000°C. This may be attributed to the formation of Zn_2SiO_4 in these samples at 1000°C.

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Table (1): Surface area (m^2/g) of ZnO-SiO_2.

Calc. Temp.	100% ZnO	85% ZnO	70% ZnO	50% ZnO	30% ZnO	15% ZnO	100% SiO_2
600	2.2	122	80.9	87	153	132	230

Table (2): Rate constants of H_2O_2 decomposition over ZnO - SiO_2 system at different calcination temperatures.

Calc. temp. °C	300°C						600°C						1000°C					
	45°C		55°C		65°C		45°C		55°C		65°C		45°C		55°C		65°C	
Composition	$k\times10^4$ min^{-1}	r	$k\times10^4$ min^{-1}	r	$k\times10^4$ min^{-1}	r	$k\times10^4$ min^{-1}	r	$k\times10^4$ min^{-1}	r	$k\times10^4$ min^{-1}	r	$k\times10^4$ min^{-1}	r	$k\times10^4$ min^{-1}	r	$k\times10^4$ min^{-1}	r
0% ZnO	1.954	0.999	2.521	0.999	3.299	0.995	1.753	0.999	2.682	0.997	3.367	0.997	1.446	0.991	1.741	0.991	2.187	0.9
15% ZnO	3.519	0.996	4.139	0.970	4.993	0.999	4.648	0.999	5.002	0.997	7.436	0.999	2.889	0.991	3.851	0.991	5.539	0.9
30% ZnO	6.281	0.994	7.429	0.999	8.019	0.996	7.491	0.989	8.541	0.995	10.458	0.995	4.591	0.946	5.216	0.996	6.389	0.9
50% ZnO	3.853	0.893	4.599	0.996	5.393	0.996	4.872	0.994	6.193	0.993	7.999	0.964	3.654	0.992	3.829	0.991	4.838	0.9
70% ZnO	5.281	0.989	7.276	0.999	8.922	0.996	5.921	0.991	8.329	0.996	8.529	0.998	5.147	0.996	6.415	0.996	8.769	0.9
85% ZnO	6.918	0.996	8.459	0.992	10.765	0.992	6.886	0.955	10.078	0.986	11.909	0.998	6.174	0.989	7.056	0.996	10.057	0.9
100% ZnO	3.749	0.995	4.182	0.989	5.003	0.985	3.496	0.998	3.569	0.988	4.583	0.989	3.416	0.994	3.735	0.997	4.598	0.9

r Regression coefficient.

Table (3): Electrical conductivity Data of ZnO - SiO_2-xH_2O system.

Calc. temp.	600°C					1000°C				
	T_b	before T_b		after T_b		T_b	before T_b		after T_b	
Comp.		E_{a_1} $kJ.mol^{-1}$	r	E_{a_2} $kJ.mol^{-1}$	r		E_{a_1} $kJ.mol^{-1}$	r	E_{a_2} $kJ.mol^{-1}$	r
100% ZnO	190	55.249	0.9759	104.990	0.9967	120	22.586	0.9702	67.783	0.9988
85% ZnO	140	26.884	0.8328	155.933	0.9835	140	9.897	0.9058	118.344	0.9203
70% ZnO	100	30.402	0.9805	101.144	0.9806	100	34.715	0.9292	117.072	0.9898
50% ZnO	180	34.926	0.9197	122.888	0.9679	200	48.000	0.8480	73.115	0.9905
30% ZnO	200	32.684	0.8626	42.887	0.9453	200	55.023	0.9561	66.039	0.9469
15% ZnO	140	25.357	0.9277	48.357	0.9910	200	32.255	0.8686	68.106	0.9883
0% ZnO	260	32.887	0.9280	76.636	0.9492	120	36.466	0.9877	43.150	0.9498

T_b break temp.

r regression coefficient.

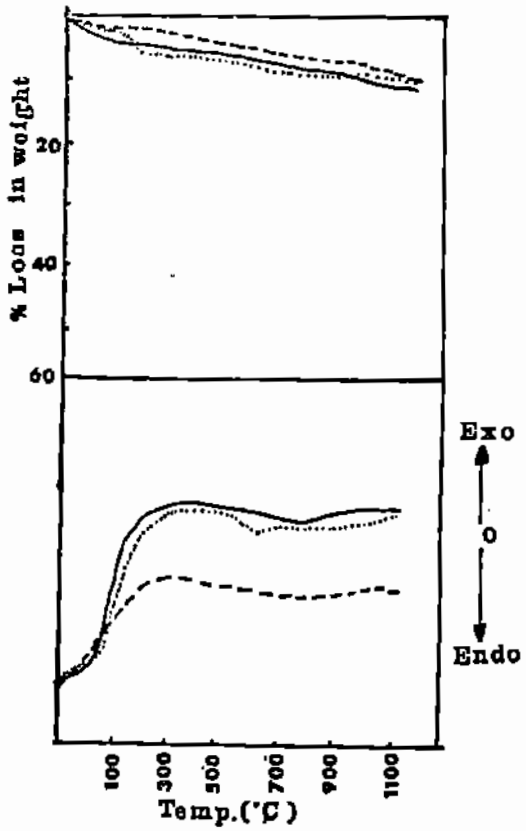

Fig.(1): DTA—TGA of $ZnO-SiO_2$ system calcined at 300°C
ZnO(— — —), $ZnO-SiO_2$(——), SiO_2(····)

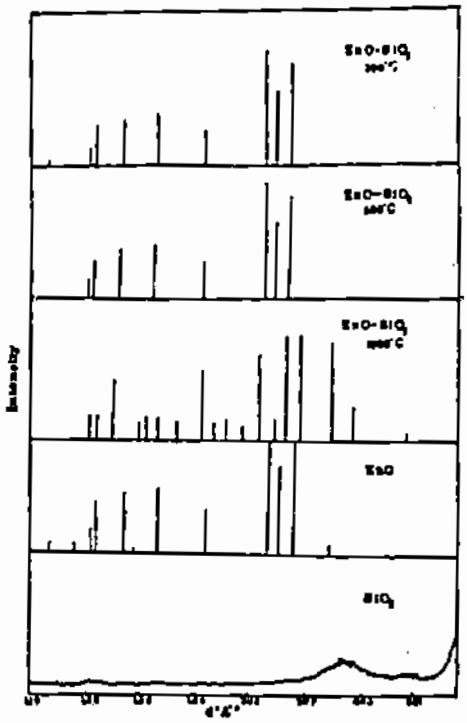

Fig.(2): X-ray diffraction patterns for ZnO-SiO system calcined at different temperatures.

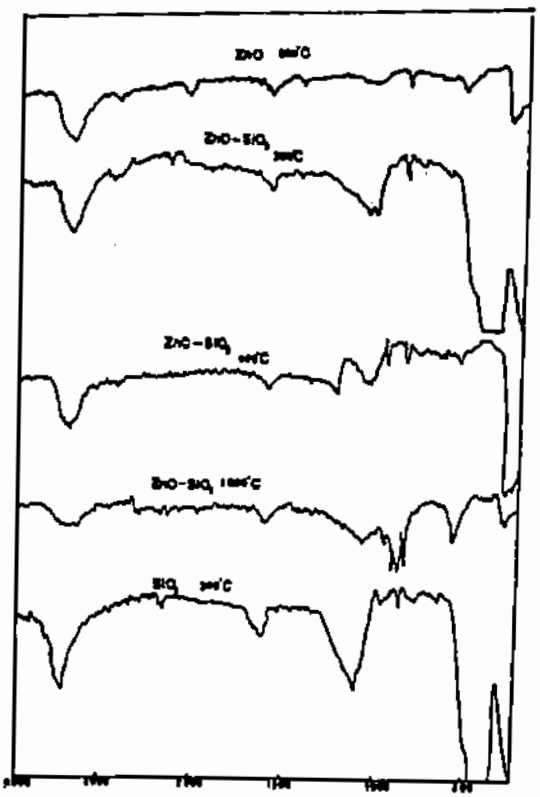

Fig.(3): I.R of $ZnO-SiO_2$ system calcined at different temperature.

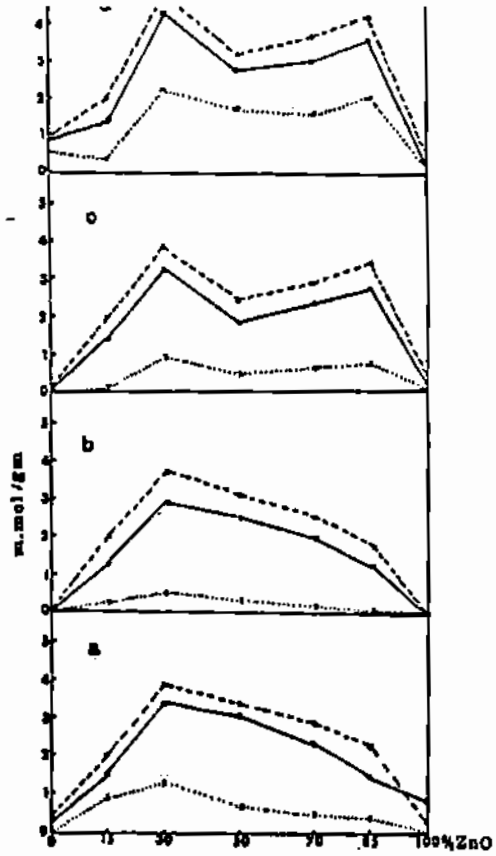

Fig.(4): Effect of composition on acidic properties of ZnO–SiO_2 for a – $pK_a = 1.5$ b – $pK_a = 3.3$ c – $pK_a = 4.0$ d – $pK_a = 6.8$ (• 300°C, ▪ 500°C, ▲ 1000°C)

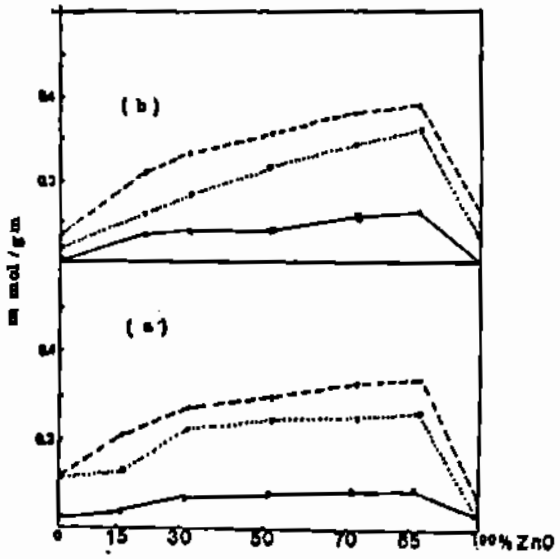

Fig.(5) The variation of basicity with the composition and calcination temperature. (a) $pK_a = 7.1$, (b) $pK_a = 9.3$ (• 1000°C, ▪ 500°C, ▲ 300°C.)

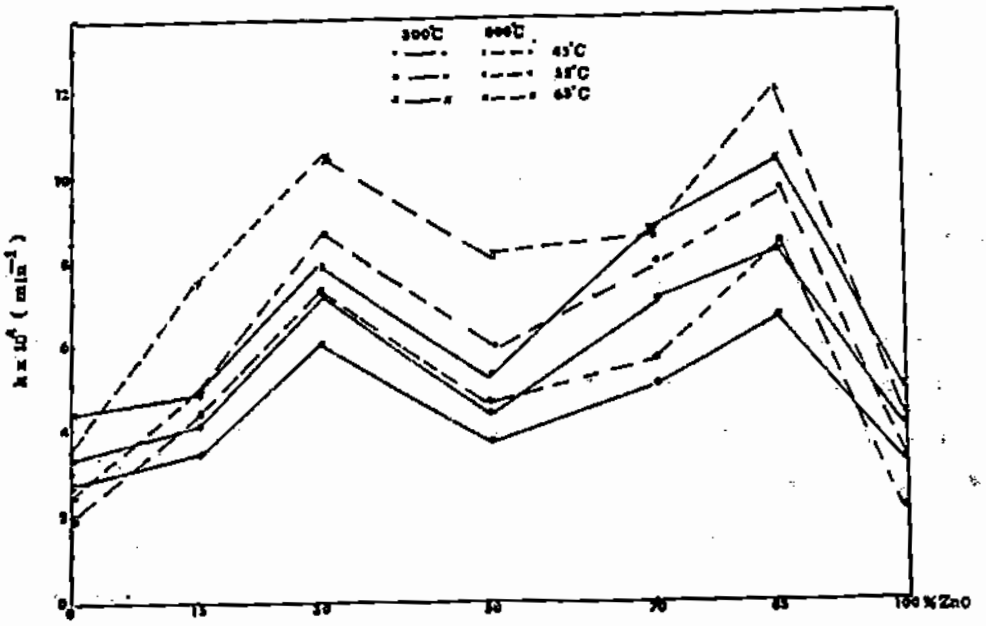

Fig.(6): Variation of rate constant of H_2O_2 decomposition on $ZnO-SiO_2$ system calcined at different temperature.

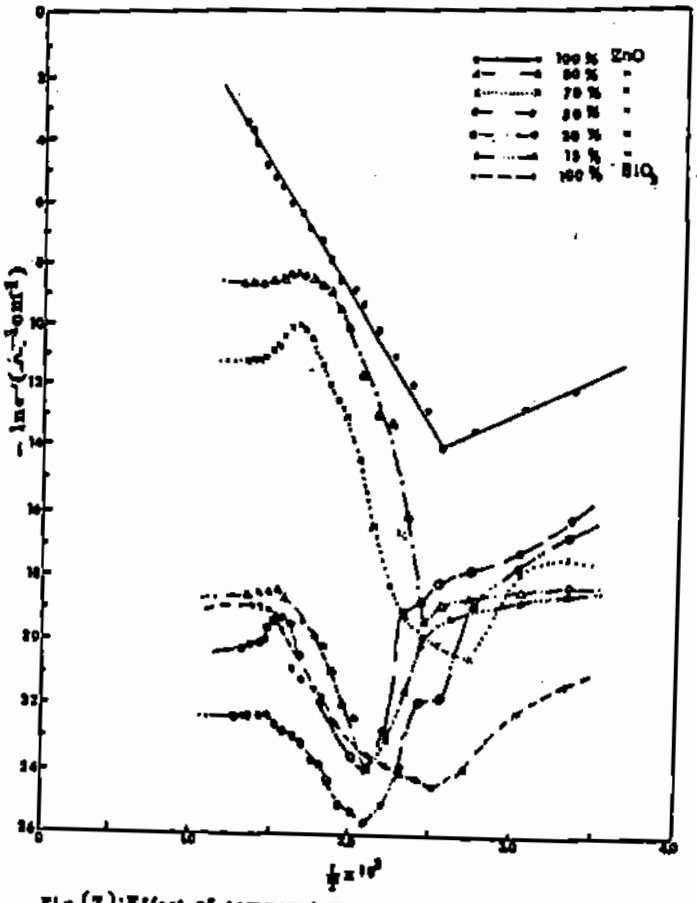

Fig.(7): Effect of temperature on the electrical conductivity of $ZnO-SiO_2$ x H_2O system calcined at 1000°C .

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Thermal and spectroscopic characterization of the products obtained from the reaction between Mn-Carbonate and Ammonium Dichromate at different temperatures

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Pure and mixed Mn-Cr oxides were prepared from the reaction between manganese carbonate and ammonium dichromate with molar ratios of 3:1, 1:1 and 1:3 with respect to Mn_2O_3:Cr_2O_3. Thermal decomposition for each of the mixture and the pure compound was studied using DTA and TG techniques. Pure and mixed salts were thermally treated at temperatures of 250°C, 500°, 750° and 1000°C and characterized by means of x-ray, diffraction analysis and IR absorption spectroscopy.

The results obtained revealed that the thermal treatment of mixtures at 250°C produced a well crystalline $MnCO_3$ and/or Cr-oxide phases depending on the composition of the mixture. At 500°C, poorly crystalline Mn_2O_3, Cr_2O_3 and amorphous manganese chromate intermediates were detected. Further increase in temperature of treatment was accompanied by the formation of $Mn_{3-x}Cr_xO_4$ compound in all mixtures. This compound decomposes at temperatures just lower than 700°C to form crystalline phase of Mn_3O_4 and Cr_2O_3.

Introduction

Many binary oxides are widely used in catalysis[1-4]. These binary systems were found to be more catalytically active than their separated oxide components[5,6]. In catalysis, it is well known that the activity of oxide catalysts depends on many factors, such as methods of preparation, calcination conditions and the interaction occurring between the different components of the catalyst, the latter is a very important factor and many investigations are cited in the literature concerning this subject[7-9].

In the present investigation, we studied the effect of temperature on the interaction between manganese and chromium salts in order to characterize the different products obtained at various temperatures of treatment. The techniques employed in this work were DTA, X-ray diffraction spectroscopy and IR absorption spectroscopy.

Experimental

The starting materials used in this investigation were pure ammonium dichromate and manganeses carbonate from BDH grade. Mixtures of molar ratios 3:1 (I), 1:1 (II) and 1:3 (III) with respect to Cr_2O_3:Mn_2O_3 were prepared by mixing and grinding the salts. Each one of the pure ammonium dichromate, manganese carbonate and their mixtures I, II and III was heated at temperatures of 250^o, 500^o, 750^o, or 1000^oC for 4 hours.

Thermal analysis for each of $(NH_4)_2Cr_2O_7$ and Mn-carbonate was carried out using DTA and TG unit of the NETZSCH Gertebau simultaneous thermal analysis system (STA 409, 6.223). The rate of heating was 10^oC min^{-1}.

The X-ray diffractograms of the samples were taken on a diffractometer Philips (Holland) with a scintillation counter and plus height analysis at 35Kv, 14 mA using Co-target radiation. The spectra were scanned at rate of $2^o min^{-1}$ in 2O.

IR spectra of the samples were recorded on a Beckman infrared spectrophotometric unit using the KBr disc technique.

Result and Discussion:

The thermal analysis diagrams for pure manganese carbonate, Fig. 1, show that the compound starts to decompose by losing the dehydration water at temperature range of 100-250°C. The weighing loss ≈ 29% occurring at temperature rang of 300-400°C refers to the decomposition of Manganese carbonate to MnO_2. This oxide dissociates to Mn_2O_3 (weight loss ≈ 9%) at a temperature range of about 450-500°C. The decomposition of $MnCO_3$ can be represented as follows:

$$MnCO_3.0.5H_2O \xrightarrow[\text{Loss} \approx 7\%]{< 300^oC} MnCO_3 + 0.5H_2O$$

$$MnCO_3 + 1/2\ O2 \xrightarrow[\text{Loss} \approx 22\%]{300\text{-}400^oC} MnO_2 + CO_2$$

$$2MnO_2 \xrightarrow[\text{Loss} \approx 9\%]{> 400^oC} Mn_2O_3 + 0.5O_2$$

The ammonium dichromate decomposes thermally in several steps, Fig. 2, with a total weighing loss of ≈ 65%.

The decomposition steps observed here agree with those reported by EZ-Eldin(10).

$$4(NH_4)_2Cr_2O_7 \xrightarrow{\approx 200^oC} 4Cr(OH)_3 + 2CrO_3 + 2CrOOH + 2NH_3 + 3N_2 + 6H_2O$$

$$2CrO_3 + 1/2CrOOH \xrightarrow{\approx 200^oC} 0.5CrO_2 + 0.25Cr_2(Cr_2O_7)_3 + 0.25H_2O + 0.25O_2$$

$$1.5CrOOH \xrightarrow{\approx 250^oC} 0.75Cr_2O_3 + 0.75\ H_2O$$

$$0.5CrO_2 \xrightarrow{> 350^oC} 0.25Cr_2O_3 + 0.125O_2$$

$$0.25Cr_2(Cr_2O_7)_3 \xrightarrow{> 350^oC} Cr_2O_3 + 1.125O_2$$

To identify the phases formed during the thermal treatment, the X-ray diffraction spectra for pure $MnCO_3$ heated at different temperatures 250^o, 500^o, 750^o and 1000^oC were recorded and summarized in Fig. (3,4 and 5).

For sample heated at 250^oC, crystalline phase of $MnCO_3$ (d-values 1.76, 2.17, 2.84 and 3.66 A^o)(11) was only detected. While the heated samples at temperatures of 500^o, 750^o and 1000^oC showed crystalline tetragonal phase for Mn_2O_3 (d-values 2.49, 2.76, 3.08 and 4.22 A^o)(12). The crystallinity of this phase increase with temperature. At temperature of 1000^oC crystalline phase of Mn_3O_4 (d-values 1.49, 2.10, 2.54 and 4.86 A^o)(13) could be detected beside the phase of Mn_2O_3.

The X-ray diffraction pattern of heated ammonium dichromate sample at 500°C, Fig. 3, support the above thermal decomposition results which showed the formation of Cr_2O_3 at 500°C(14).

The degree of crystallinity of Cr_2O_3 increases with increasing the heating temperature, Figs. 4 and 5.

The X-ray diffraction patterns for the mixture samples (I, II, III) heated at 250°C showed the absence of any crystalline phases. The disappearance of the patterns of crystalline phases of $MnCO_3$, which was observed in case of pure sample can be attributed to the interaction occurring between $MnCO_3$ and the composition products of ammonium dichromate.

For all mixtures heated at 500°C, the X-ray diffraction patterns showed crystalline phases of Mn_2O_3(12) and Cr_2O_3(14).

At temperature 750°C, crystalline phases of Cr_2O_3 and Mn_3O_4 could be detected. The formation of Mn_3O_4 at 750°C could be explained as a result of certain reaction to form $Mn_{3-x}Cr_xO_4$, which is then dissociate to Cr_2O_3 and Mn_3O_4, (at $500^\circ < T < 750^\circ$C).

$$\text{Mn-oxide + Cr-oxide} \xrightarrow{> 500^\circ\text{C}} (Mn_{3-x}Cr_xO_7) + Mn_3O_4 + XO_2 \xrightarrow{750^\circ\text{C}} Cr_2O_3$$

The mixed oxides of $Mn_{3-x}Cr_xO_4$ was also detected as a result of the reaction between manganese carbonate and chromium nitrate at about 500°C(15).

The x-ray diffraction patterns for the mixtures heated at 1000°C showed an increase in the intensity of patterns for crystalline phases of Mn_3O_4 and Cr_2O_3.

Figs. 6,7,8 and 9 illustrate the IR spectra for pure and mixed manganese and chromium salts heated at different temperatures. Fig. 6 shows the IR spectra of the pure salts and their mixtures heated at 250°C. The bands appeared at wave lengths of ≈1810, 1437, 1087, 870 and 728 cm^{-1} indicate the presence of carbonate group. The increase in the concentration of chromium in the mixtures led to decrease intensity of the manganese carbonate bands and at the same time an increase in the intensity of the corresponding bands of chromium oxide at 1100, 710, 650, 570, 555, 440 and 407 cm^{-1}. The IR-spectra for pure Mn-carbonate heated at 500°C showed the disappearance of carbonate bands of pure Mn-carbonate and the appearance of new bands at 1150, 980, 845, 690, 610, 485 and 410 cm^{-1} which are characterized for Mn_2O_3.

For mixture samples heated at 500°C, the IR spectrogram showed a broad band pointing to the presence of some sort of chromates, which formed as a result of solid state reactions between manganese carbonate and ammonium dichromate. The IR spectra of calcined mixtures at 750°C showed

bands corresponding to Cr_2O_3 and Mn_3O_4. Further increase in temperature, 1000^oC increases the intensity of Cr_2O_3 and Mn_3O_4 bands, which confirmed the results obtained from X-ray.

Figure Captions

Fig. 1: DTA and TG of manganese carbonate.

Fig. 2: DTA and TG of ammonium dichromate.

Fig. 3: X-ray diffraction patterns of ammonium dichromate, manganese carbonate and their mixtures calcined at 500°C.

1- Cr_2O_3 2- Mn_2O_3

Fig. 4: X-ray diffraction patterns of ammonium dichromate, manganese carbonate and their mixtures calcined at 750°C.

1- Cr_2O_3 2- Mn_2O_3 3- Mn_3O_4

Fig. 5: X-ray diffraction patterns of ammonium dichromate, manganese carbonate and their mixtures calcined at 1000°C.

1- Cr_2O_3 2-Mn_2O_3 3- Mn_3O_4

Fig. 6: IR-spectra of ammonium dichromate, manganese carbonate and their mixtures calcined at 250°C.

Fig. 7: IR-spectra of ammonium dichromate, manganese carbonate and their mixtures calcined at 500°C.

Fig. 8: IR-spectra of ammonium dichromate, manganese carbonate and their mixtures calcined at 750°C.

Fig. 9: IR-spectra of ammonium dichromate, manganese carbonate and their mixtures calcined at 1000°C.

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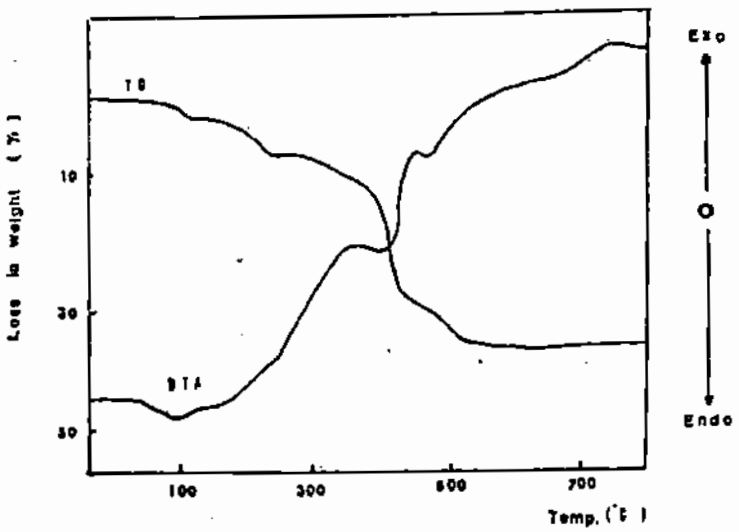
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DTA
Loss in weight (%)
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50
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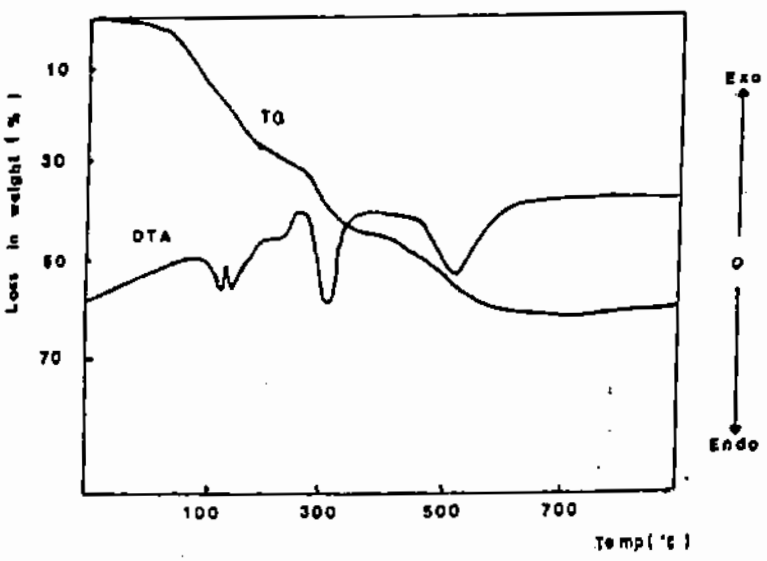
300
500
700
Temp. (°C)
Exo
O
Endo

Fig 1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DTA
Loss in weight (%)
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30
50
70
100
300
500
700
Temp (°C)
Exo
O
Endo

Fig 2

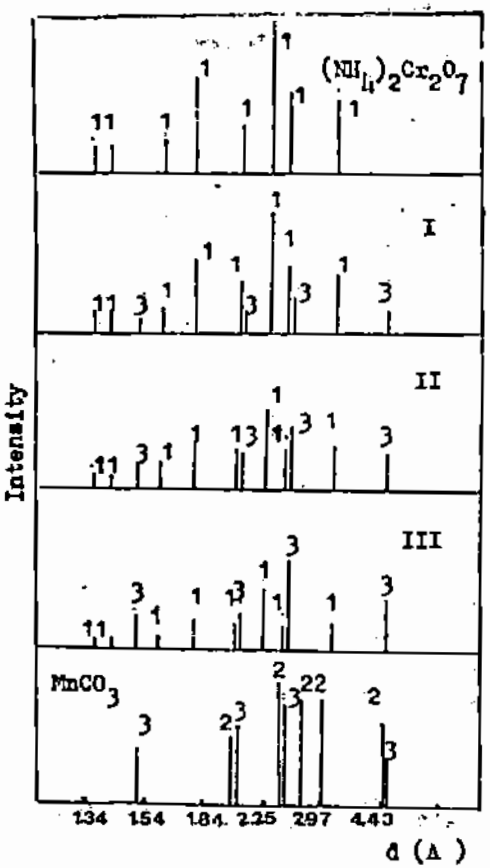

Fig 5

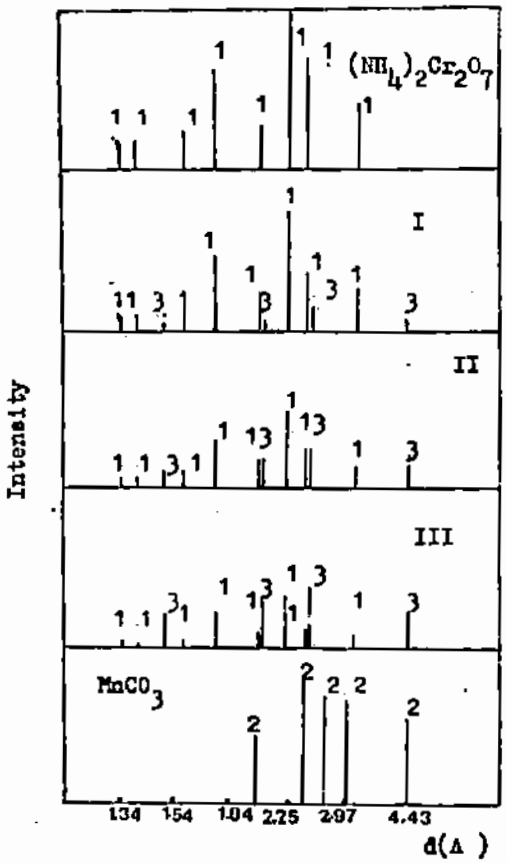

Fig 4

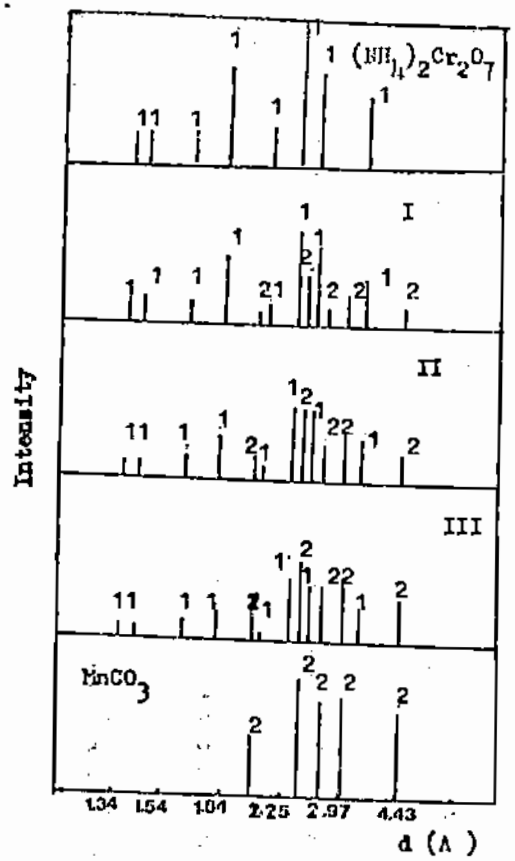

Fig 3

Absorbance
3000
2000
1500
500
Wave number(Cm⁻¹)
$(NH_4)_2Cr_2O_7$
I
II
III
$MnCO_3$
0

Absorbance
3000
2000
1500
500
Wave number(Cm⁻¹)
$(NH_4)_2Cr_2O_7$
I
II
III
$MnCO_3$

Fig 9

Absorbance
3000
2000
1500
500
Wave number(Cm⁻¹)
$(NH_4)_2Cr_2O_7$
I
II
III
$MnCO_3$

Absorbance
3000
2000
1500
500
Wave number(Cm⁻¹)
$(NH_4)_2Cr_2O_7$
I
II
III
$MnCO_3$

Fig 8

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Vol. 18 (1993).

The Inhibition Of Commercial Fatty Acid Sulphonate Towards The Corrosion Of Aluminium In Hydrochloric Acid

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ABSTRACT

The dissolution of aluminium in 2M hydrochloric acid in the presence of sodium salt of Soya bean oil sulphonate (SSS) as corrosion inhibitor has been studied using hydrogen evolution and thermometric methods. The two methods gave consistent results. The results obtained indicate that the inhibitive effect of the sulphonated mixture relates to chemisorption mechanism on the metal surface via the π electrons in the double bonds.

1- INTRODUCTION

The wide use of aluminium in industry makes its corrosion inhibition is of great importance. Some corrosion inhibitors were prepared through ethoxylation of commerically available unsaturated fatty acid mixtures tall oil, soya bean, cotton seed and linseed oil. These fatty acids have been tested as steel corrosion inhibitors in HCl and H_2SO_4 acid solutions[1]. They provide adequate inhibition of steel in HCl and H_2SO_4 at different concentration and at temperature up to 80°C and act as mixed inhibitors.

The present study aimed to investigate the effect of sulphonated soya bean oil as corrosion inhibitor for aluminium in 2M HCl

solution and to relate the inhibition mechanism to the additive structure.

2- EXPERIMENTAL

2.1- Preparation and properties of inhibitor

i- Separation and washed the fatty acid from soya bean oil[2]. The soya been oil so obtained is a mixture of the following fatty acids[3] given in Table I

Table I - Composition of the commercial fatty acid (Soya bean oil)

acid	%
Oleic	25
Linoleic	52
Linolenic	8
$C_{12} - C_{18}$	15
$C_{19} - C_{22}$	Trace

ii- Preparation of sulphonated fatty acid. 100 g of separated soya bean fatty acid (mixture) was stirred at 30°C while 56.7 g of concentrated $H_2S_2O_7$ (oleum) is slowly added over a period of 6-7 hrs. The product was extracted with 20 % NaCl solution and neutralized with 40 % NaOH solution[4]. The product have the general formula $R\text{-}COOSO_3Na$ where R is a mainly a mixture of $C_{17}H_{29}$, $C_{17}H_{31}$

and $C_{17}H_{33}$ also contains amounts of saturated fatty acids (C_{12}-C_{18}).

2.2- Hydrogen evolution measurement

Reactions in which gases are given off or taken up can be monitored by studying the change in the amount of gas over time. Since aluminium is readily soluble in aqueous acids with liberation of hydrogen, the hydrogen evolution method was used to measure the rate of dissolution of Al in HCl solutions[5-11]. The reaction vessel used in this method and the procedure for determining the dissolution of aluminium in the acid solution were the same as described elsewhere[12]. The efficiency of a given inhibitor can be evaluated as the percentage reduction in reaction rate K, % inhibitor efficiency (% I E)

$$\% \, I \, E = \frac{K_{uninhibited} - K_{inhibited}}{K_{uninhibited}} \times 100$$

2.3- Thermometric measurement

Recently, a simple and rapid method has been developed[13] based on the thermometric corrosion test of Mylius[14]. This method has been used successfully for comparing the inhibition efficiency of different organic additives in reducing the rate of dissolution of aluminium[15-17]. The procedure for the determination of metal dissolution rate by the thermometric method has been described previously[13]. The reaction number RN is defined as[18]

$$RN = (T_m - T_i) / t$$

where T_m and T_i are the maximum and initial temperature, respectively, and t is time (in minutes) required to reach the maximum temperature. The percent reduction in RN[11] is given as:

$$\% \ RR = \frac{RN_{uninhibited} - RN_{inhibited}}{RN_{uninhibited}} \times 100$$

The chemical composition of aluminium of sheet used (Riedel - de Haen, W. Germany) is given in Table 2.

Table 2. Chemical composition of aluminium

Element	Al	Fe	Cu	Si	Ti	Zn
Amount (%)	99	0.2	0.2	0.2	0.03	0.08

A stock solution of 2M HCl was used. Aluminium test pieces measuring 0.1 x 10 x 30 mm were used for the hydrogen evolution method, while 0.1 x 10 x 100 mm test pieces were used for the thermometric method. These were degreased and etched as previously described[19]. Each experiment was carried out with 100 ml of the acid solution in the hydrogen evolution method and with 15 ml of the acid solution in thermometric method.

The experimental data were analysed using a Kypro personal computer and curve fitting was carried out using a standard regression package.

3- RESULTS AND DISCUSSION

3.1- Hydrogen evolution measurements

Figure 1 shows the volume of hydrogen evolved as a function of time for dissolution of Al in 2M HCl at 30°C in the absence and presence of SSS over concentration range (0.1 - 150) x 10^2 mg/L. It was found that the hydrogen evolution increases linearly with time after a certain time interval which increases with an increase of SSS concentration. The initial time interval was attributed to an incubation period[20] representing the dissolution of the oxide film on the metal surface and the start of attack[21]. Accordingly, it can be stated that the dissolution of aluminium itself is linarly related to the reaction time, as seen from Fig. 2. This behaviour is characteristic of zero-order reaction[22], given by[8]:

$$V = K t$$

where V, is the volume of hydrogen evolved which is proportional to the fraction of the reactant converted into reaction product at time t, and K is specific reaction rate.

The experimental data, given in table 3, and Fig. 3 indicate that the corroson rate of aluminium decreases with increasing SSS concentration.

Figure 4 shows the plot of % I E as a function of log C_{SSS} which is invariably sigmoid in nature, indicating that SSS exhibits its inhibitive effect through its adsorption on the metal surface. The turn in the curve is rather sharp, suggesting the

Table 3- Effect of SSS concentration on the dissolution rate of Al in 2M HCl solution at 30°C

SSS concentration (mg/L)	0.0	37.3	186.5	373	1865	3730	1-920
Corrosion rate, K (cm^3/min)	10.82	7.79	6.80	5.90	2.55	1.67	..22
Incubation period (min)	13.45	15	18.83	21.75	28.75	43.67	82.88
% I.E	0.0	28	37.15	45.47	73.66	84.57	98.72

Table 4- Effect of temperature on corrosion of Al in 2M HCl + 3730 mg/L SSS

Temperature (°C)	30	40	50	60
Corrosion rate, K (cm^3 / min)	1.67	2.71	6.17	10.36
Incubation period (min)	43.67	14.42	6.5	3.63

formation of a monolayer of the inhibitor on the surface of the corroded metal.

Figure 5 shows the incubation period (τ) increases with increasing the additive concentration and obeys a logarithmic relationship.

The effect of temperature on the rate of dissolution of Al in 2M HCl was studied between 35°C and 60°C in the presence of 3.730 g / L SSS the results given in table (4) and shown in figure (6). The corrosion rate increase with increasing temperature and obeys the Arrhenius law[23] in the temperature range used (Fig. 7). The activation energy for the corrosion process was evaluated to be 5.49 Kcal. / mole; indicating the corresion process is controlled by the surface reaction, since the activation energy for the corrosion process is above 5 Kcals[10].

It is found that the incubation period decreases with increase in temperature according to a linear relationship which satisfies the equation

$$\log \tau = 6.95 - 3.6 \text{ Log } T$$

3.2- Thermometric measurements

The dissolution of Al in 2M HCl was accompanied by temperature change. The maximum temperature was 58.4°C, and is attained within 65 min, corresponding to 0.668°C / min. To this solution increasing amounts of sodium soya sulphonate were added. The recorded thermometric curves are shown in Fig. 9. One re-

cognizes at first an incubation period, after which the temperature rises gradually with time. The rate of temperature rise increases progressively with time. Sodium soya sulphonate interferes with the dissolution of aluminium and lowers the R.N. This is produced mainly through a lowering in T_m and a corresponding increase in τ. This suggests strong adsorption of the additive[13] as indicated from the thermometric curves. Table 5 represents the effect of the additive concentration on the parameters of the thermometric curves. Figure 10 shows the variation of percentage reduction in reaction number (% R.R.) as function of log C_{SSS} for Al in 2M HCl. The curve has a sigmoid nature, substantiating the idea that SSS reduces the corrosion rate by way of adsorption leading to the formation of a monolayer of the inhibitor on the aluminium surface. In a plot of the time delay ($\triangle \tau$) vs log C, the turn in the curve observed (Fig. 11) indicates the formation of a monolayer of the inhibitor on the surface of the metal[10].

Figure 12 shows the incubation period (τ) varies with the SSS concentration of the following relationship can be deduced

$$\log \tau = 2.6 + 0.24 \log C$$

The experimental results suggest that the adsorption mechanism proceeds through a monolayer formation of sodium soya sulphonate on the surface of the metal. As seen from the chemical composition of the additive, its main constituent is the sulphonate salt of linoleic acid which contains two double bonds. This substantiates

the idea that inhibitive effect of SSS can be related to chemisorption mechanism on the metal surface via the π electrons in the double bonds.

It should be noted that the two different techniques demonstrate the agreement and conformity of the experimental results as to the type of inhibition of the corrosion of aluminium. Nevertheless, they showed small differences in absolute values for the inhibition efficiency. However, this observed discrepancy could be attributed to the different experimental conditions under which each technique was carried out.

Table 5. Effect of SSS concentration on parameters of thermometric curves for Al in 2M HCl

[SSS] mg/L	T_i °C	T_m °C	t min	R.N. °C/min	% R.R	Δt min	τ min
0.0	15	58.4	65	0.67	0.0	0.0	44
186.5	15	56.2	74	0.56	16.42	9	64
373	15	55.8	87	0.47	29.85	22	76
3730	15	45.6	137	0.22	67.16	72	127
14920	15	44.2	242	0.12	82.09	177	190

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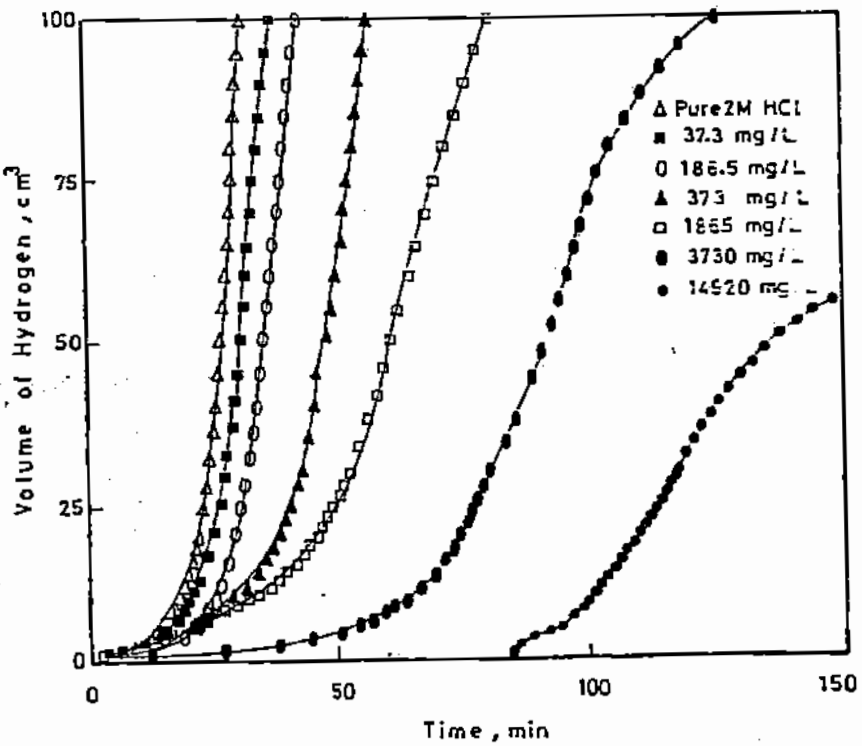
List of Figures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Time , min
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25
50
75
100
0
50
100
150
Δ Pure2M HCl
■ 37.3 mg/L
0 186.5 mg/L
▲ 373 mg/L
□ 1865 mg/L
● 3730 mg/L
● 14920 mg/L

Fig.(1)

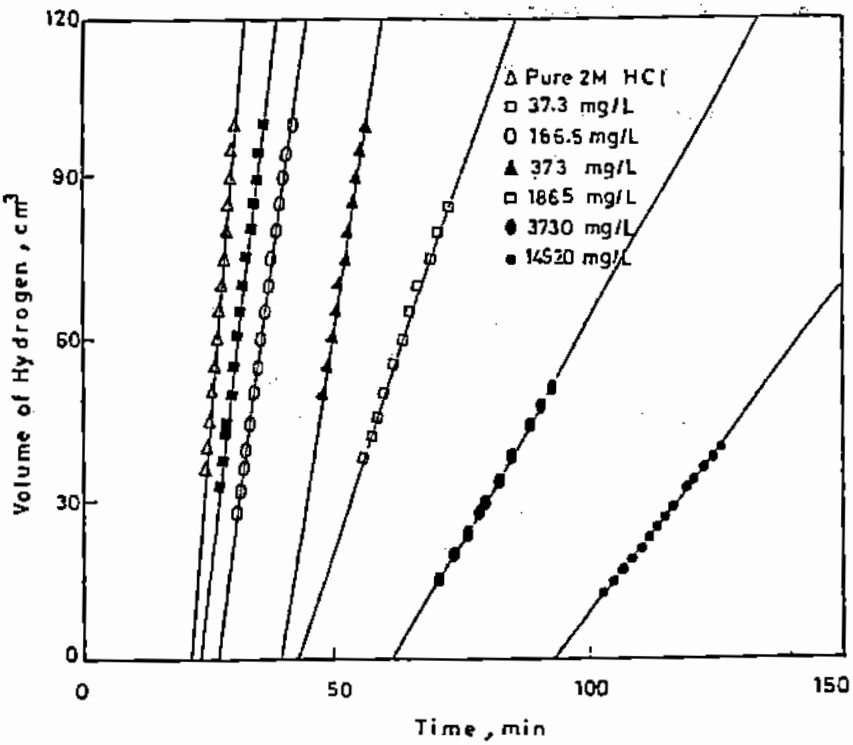
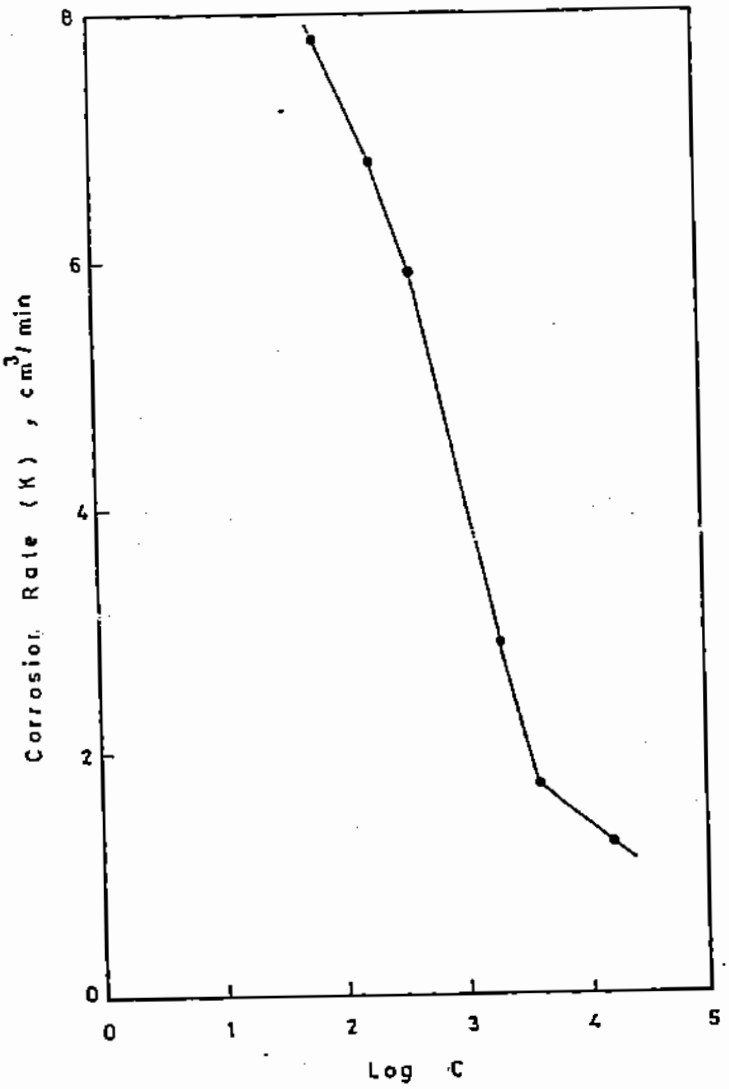

Δ Pure 2M HCl
□ 37.3 mg/L
O 166.5 mg/L
▲ 373 mg/L
□ 1865 mg/L
● 3730 mg/L
● 14520 mg/L
Volume of Hydrogen , cm³
120
90
60
30
0
0
50
100
150
Time , min

Fig.(2)



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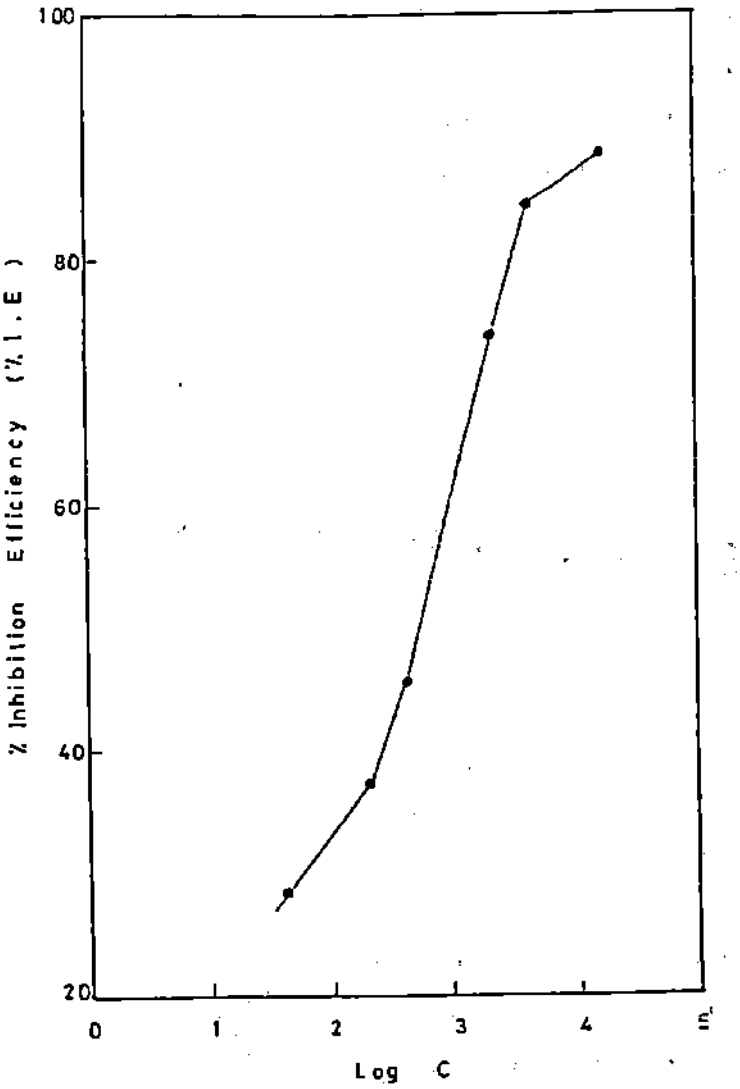

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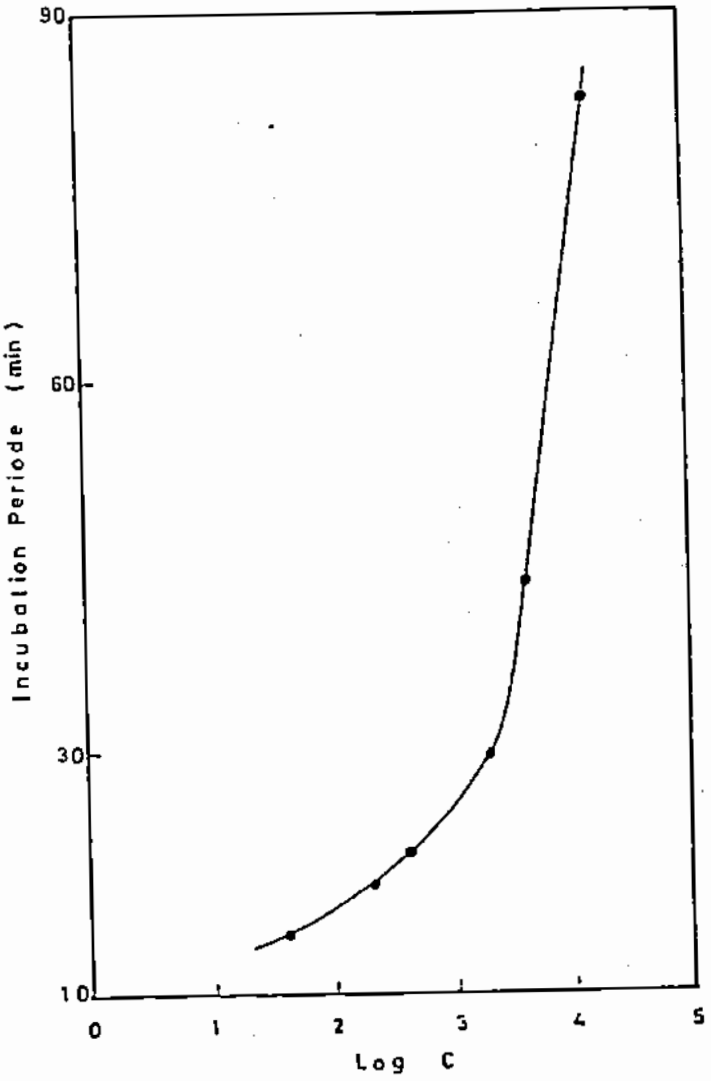

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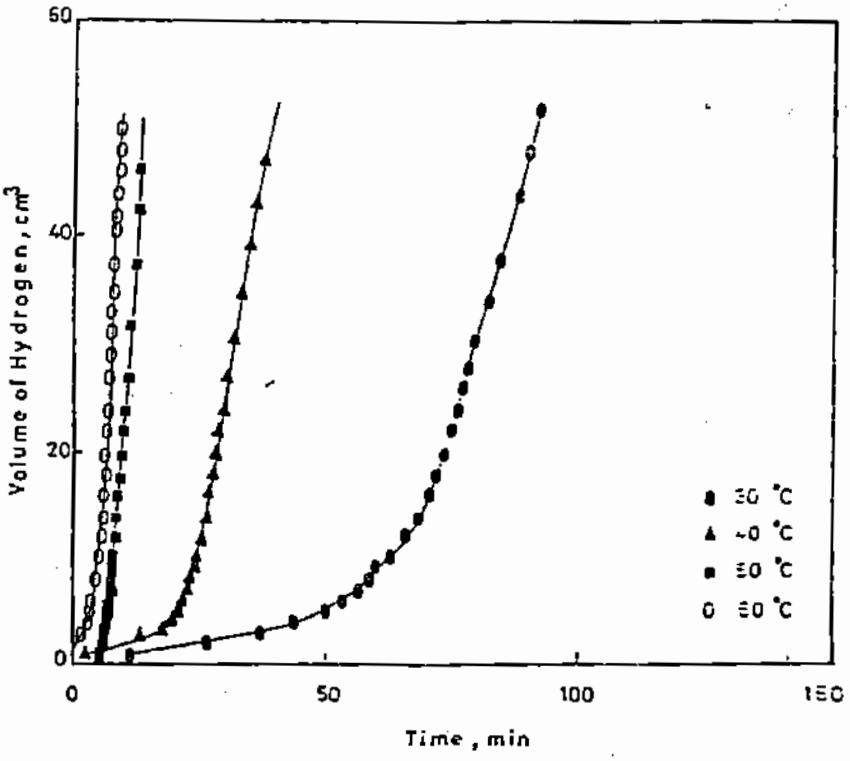

Fig.(6)

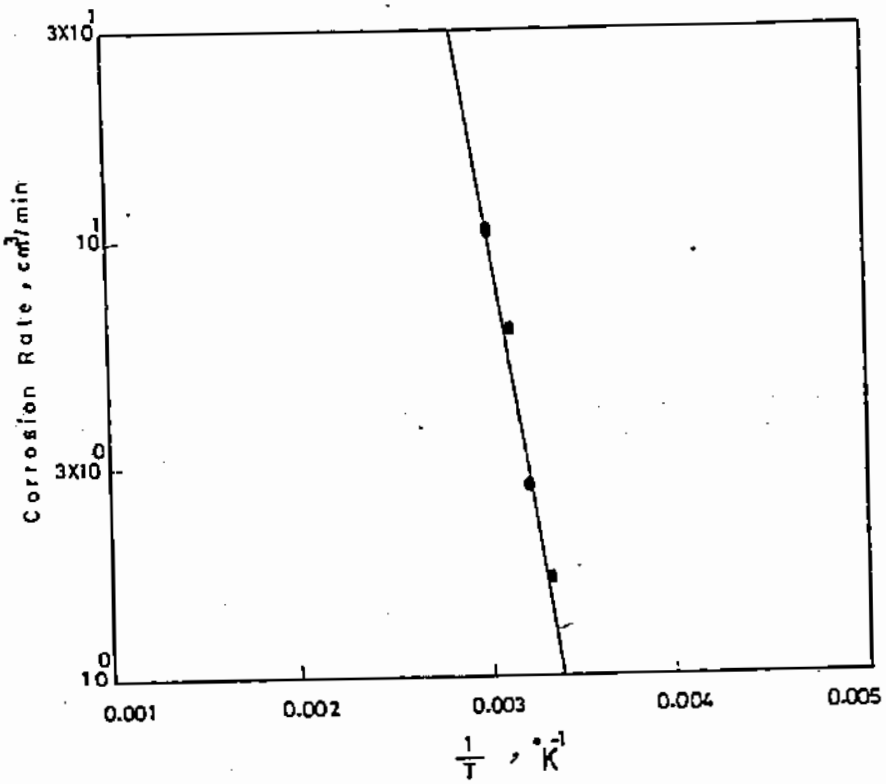
3X10^1
10^1
3X10^0
10^0
Corrosion Rate , cm³/min
0.001
0.002
0.003
0.004
0.005
1/T , °K⁻¹

Fig.(7)

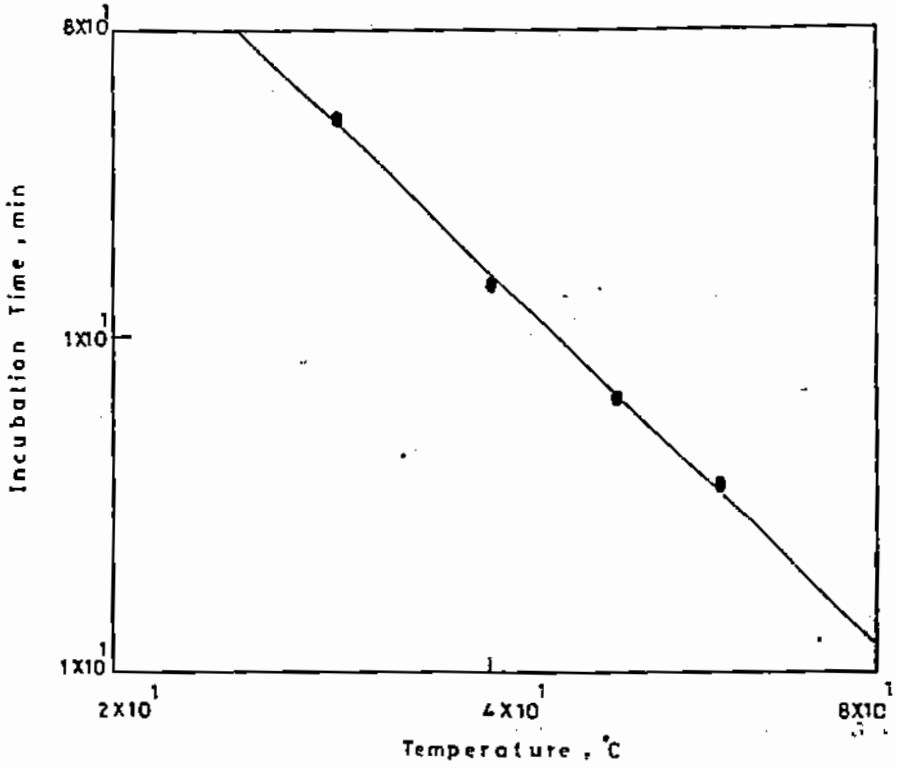
8X10
1X10
1X10
Incubation Time , min
2X10
4X10
8X10
Temperature , °C

Fig.(8)

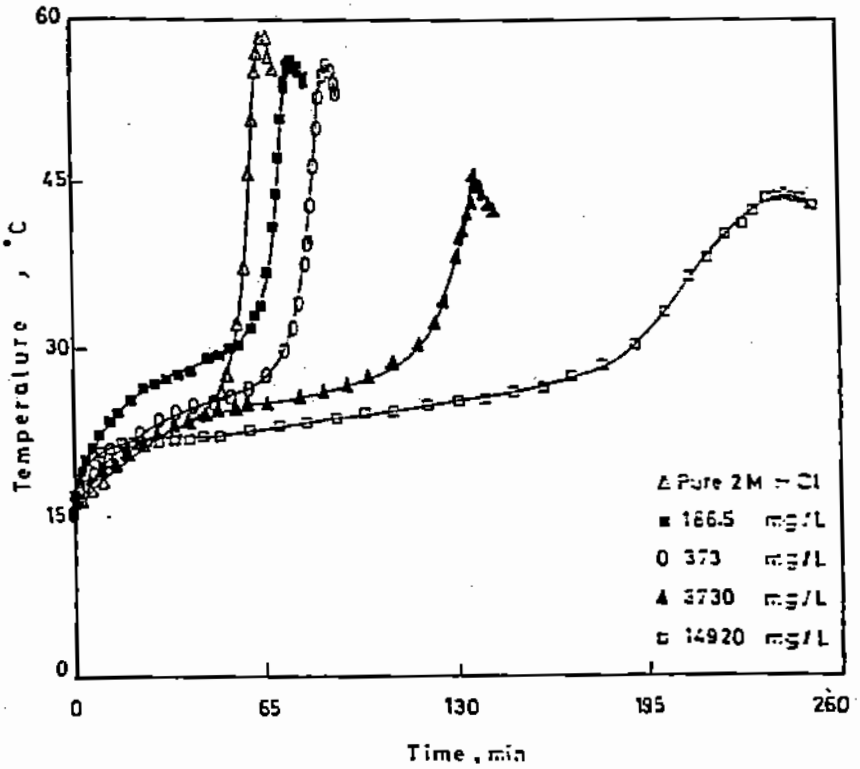
60
45
30
15
0
Temperature , °C
0
65
130
195
260
Time , min
Δ Pure 2M - Cl
■ 166.5 mg/L
0 373 mg/L
▲ 3730 mg/L
□ 14920 mg/L

Fig.(9)

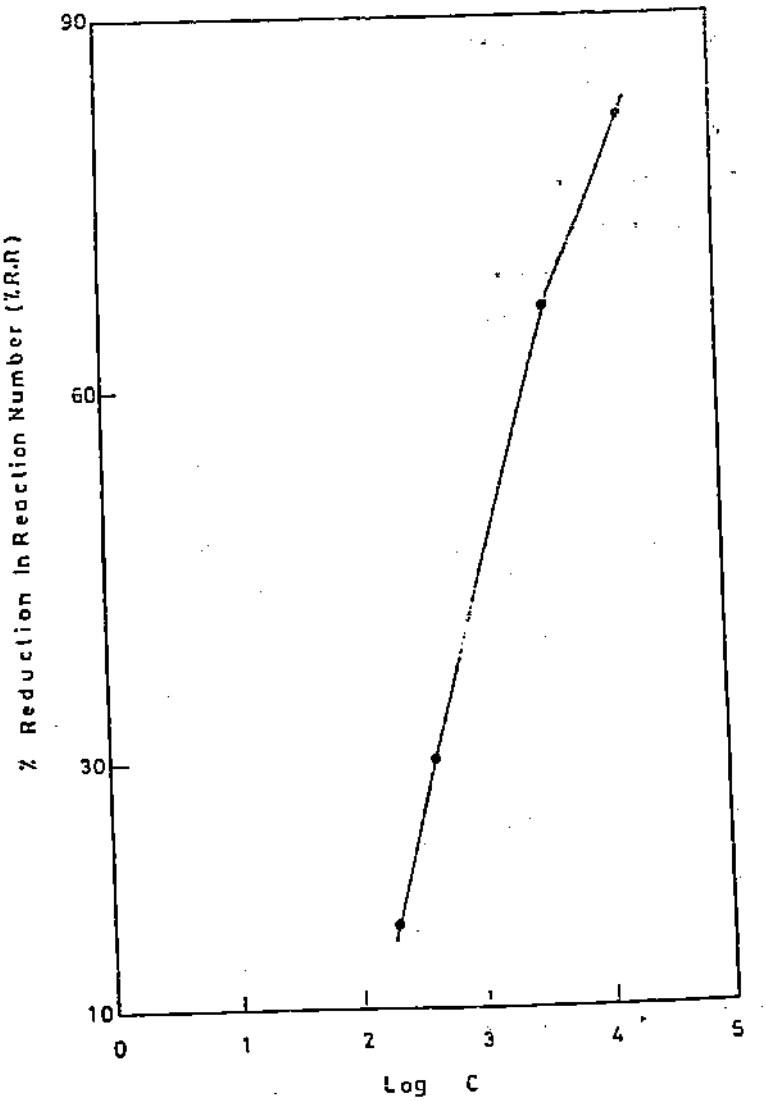

Fig.(10)

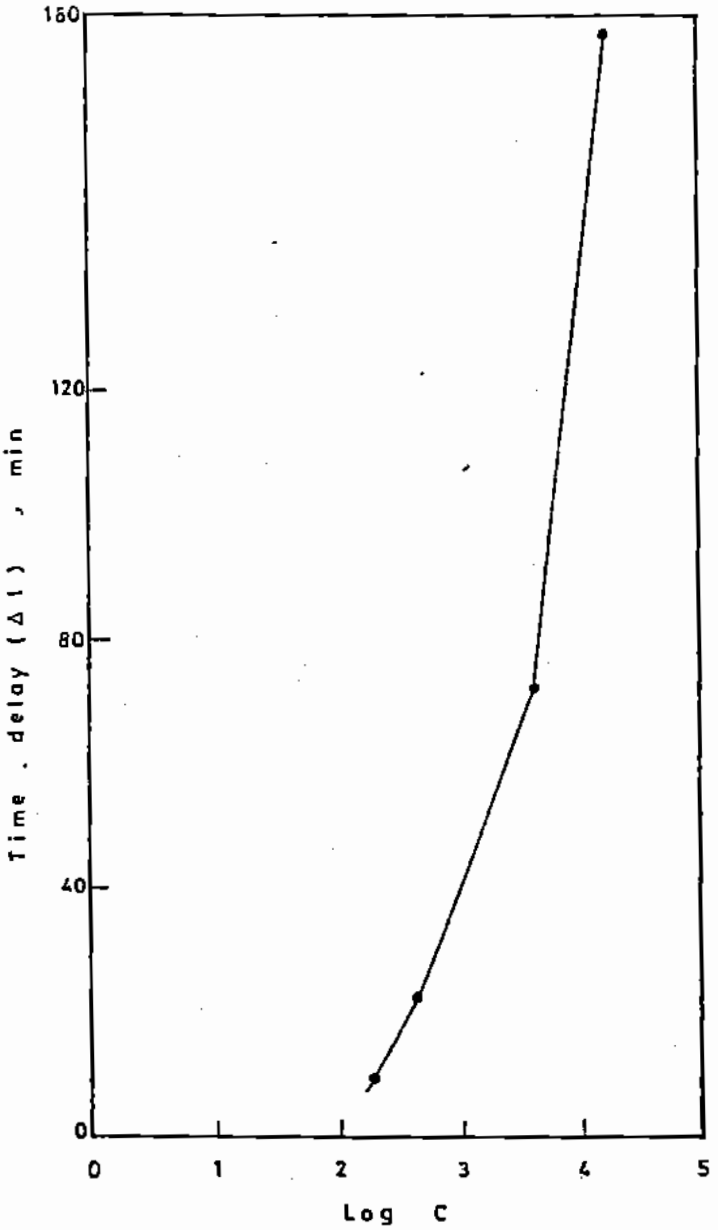

Fig. (11)

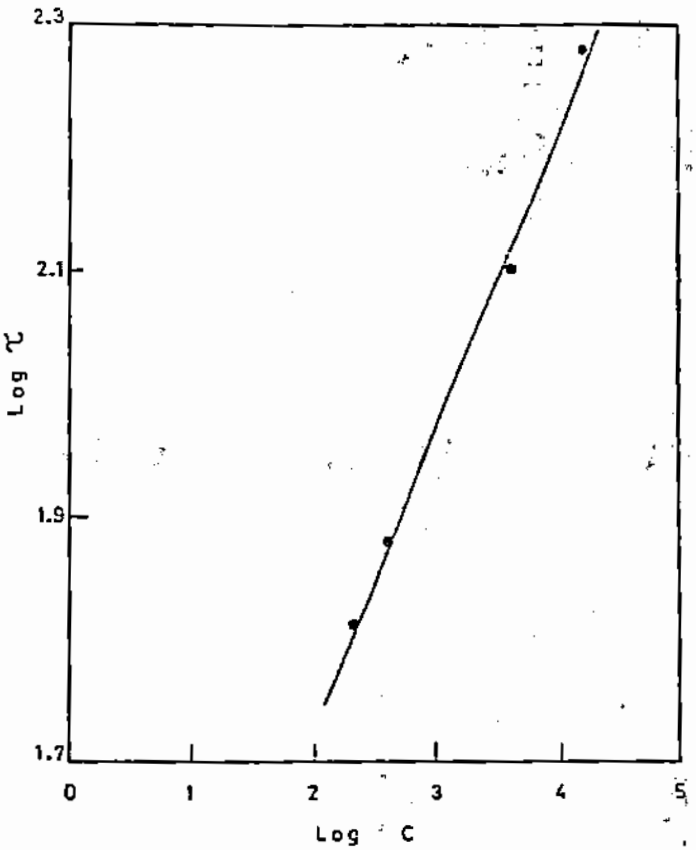

Fig.(12)

Vol. 18 (1993).

COMPARATIVE STUDIES ON GROWTH, NODULATION AND NITROGEN FIXATION OF TWO LEGUMINOUS PLANTS GROWING UNDER DIFFERENT LEVELS OF WATER SUPPLY AND IRRIGATION INTERVALS

By

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Abstract:

A greenhouse study was conducted at Heliopolis using loamy soil characterized by low water capacity retention to compare between growth, nodulation and nitrogen fixation of (Lupinus termis cv. Giza 4) and chickpea (Cicer arietinum cv. Giza 2) when subjected to different levels of water supply and irrigation intervals.

Four water amounts; 400, 300, 200 and 100 cm^3 and four water intervals, 2-day, 4-day, 6-day and 8-day intervals were applied for a period of five weeks in a factorial randomized block design with three replications.

Growth of the two plants were greatly affected by deficiency in water supply when they were grown either at low water amount (100 cm^3) or increased irrigation intervals.

Lupin gave best growth (dry matter) at water amount (300 cm^3) and 2-day irrigation interval, while in case of chickpea growth was excellent at 300 cm^3 and irrigated every 4-day interval. On the other hand, nodulation and nitrogen fixation were optimum for the two investigated plants irrigated every 2-day. Lupin shoot looked thick and short, with few nodes at low water amount (100 cm^3) and increased irrigation interval (4-day).

Furthermore, chickpea responded to water regime by formation of new lateral branches and long tap roots at water amount (100 cm^3) and irrigation interval of 4-day which manifested in rich dry matter accumulation at the same level of water amount.

Generally application of 100 cm^3 water at any irrigation interval resulted in very poor crop performance with respect to Lupin but not for chickpea.

Introduction:

Water is a very important factor affecting crop production in arid and semi arid regions (Labanauskas et al., 1981), it affects non legumes (Thakur and Ria, 1984; El-Zeiny and Kortam, 1985 and Batanouny et al., (1991), as well as several legumes; (Morandi et al., 1981 and Egli et al., 1983). Not only legumes growth but also legumes varieties, (Babalola, 1980 and Abou Bakr et al., 1993) and nodulation and nitrogen fixation (Finn and Brun, 1980; Dejong and Phillps, 1982; Mbagwa and Osuigwe, 1984 and Sprent and Sprent, 1990).

Some data indicate that water stress disrupts interactions between Rhizobium and host plant directly by altering nodule fine structure or enzyme activity (Sprent, 1976), other evidence suggested that it affects photosynthesis in plants (Finn and Brun, 1980) and root system in non legumes (Batanouny et al., 1991). It decreases nitrate reductase activity (Srivastava, 1980) and negatively affects various other aspects of N_2-metabolism (Hsiao, 1973).

Water stress decreases, plant heights and causes stem dwarfism (Taylor et al., 1982; Hutchnison et al., 1986; Kandil et al., 1988 and Abou Bakr et al., 1993).

Because Lupin and chickpea are very important crops in the developing countries and in semiarid regions where water is very important factor affecting plant growth, the present study was performed to through light upon growth behaviour, nodulation and nitrogen fixation of these two legumes which grow in many areas have deficient water supply to maximize their growth under sever water conditions, also to show the optimum amount of water used together with irrigation intervals so as to avoid wastage of water in those semiarid regions.

Materials and Methods:

Seeds of Lupin (Lupinus termis cv. Giza 4) and Chickpea (Cicer arietinum cv. Giza 2) were obtained from Agriculture Research Centre Ministry of Agriculture, Giza.

Seeds of each plant were inoculated separately with its specific Rhizobium; Rhizobium lupini and Rhizobium leguminosarum (local isolates) for Lupinus termis and Cicer arietinum respectively. Rhizobia were obtained from Microbial Culture Collection Centre (MIRCEN), the unit of Bio-fertilizer, Faculty of Agriculture, Ain Shams University, Shoubra, Egypt. The experiment was performed in the experimental garden of the Botany Department, Women's College, Ain Shams University. The soil was air dried, passed through a 2 mm sieve and adjusted at pH 7.2. Clay pots (30 cm diameter and 25 cm depth) were chosen for cultivation. In each pot, 4 Kg of clay loam soil were placed. Mechanical soil analysis were carried out following the method described by Jakson (1973), and given in Table (1). The soil have a 21% moisture content at field capacity, 1.04% organic matter, 1.40% total nitrogen and 0.6 ds/m electrical conductivity. Each pot was fertilized with 60 mg/Kg^{-1} N_2, 15 mg/Kg^{-1} p and 60 mg/Kg^{-1} K. Ten seeds of each plant Lupinus termis and Cicer arietinum were sown per pot. Two weeks after germination, plants were thinned to five plants per pot, then subjected to the treatments which consist of four water amounts (400, 300, 200 and 100 cm^3), and four irrigation intervals (2-day, 4-day, 6-day and 8-day), keeping three replication for each treatment.

Plants were uprooted weekly, washed and dried at 70°C to constant weight, growth parameters including root and shoot height and dry weight were made. Plants were ground and used for determination of nitrogen according to the method described by Jakson (1973). Nitrogen determination was determined by kjeldahl method. All the nodules per pot were carefully counted, dried and weighted. The obtained data were subjected to analysis of variance (Snedecor and Cochran, 1980). Because data were more obvious at 2-and 4-day intervals than 6-and 8-day intervals, comparative studies of the 2-tested plants were made at 4-day interval.

Results and Discussions:

Shoot length:

The obtained data (Fig. 1&2) show that the various growth criteria measured in the present study was affected by extending the period between the successive irrigation intervals as well as the amount of water used

The average shoot length of Lupinus termis after five weeks from the initiation of treatments was 9, 14.5, 14 and 10 cm in plants receiving water amount of 100, 200, 300 and 400 cm³ respectively, and irrigated every 4-day interval, while it was 19, 19.5, 18 and 17.5 cm in shoot height of Cicer arietinum irrigated at the same level of field capacity and irrigation interval. These results showed that Lupinus termis shoot, growth was short, thick and had few nodes but the shoot growth of Cicer arietinum was long and had normal shap at the same level of water amount and irrigation interval. Kramer (1969) pointed out that water deficit reduces enlargement and stem elongation. This observation was confirmed by Levitt (1980) who stated that decrease in cell enlargement must be the most sensitive response of the plant to water stress. Similar results were, however, obtained by other investigators (Morandi et al., 1983) (in case of Lupinus termis) and Abou Bakr et al., 1993). They also found that soil moisture deficit retarded plant growth, resulting in short stems and few nodes. Furthermore, our results on Lupinus termis were in agreement with the finding of the following workers (Hutchinson et al., 1986 and Kandil et al., 1988) and were in disagreement with our results on Cicer arietinum. The above mentioned investigators noticed that the increase in water stress by elongation of irrigation intervals resulted in stem dwarfism of many legumes.

Root length:

Data obtained (Fig. 1&2) on root length revealed that root system has been greatly affected by irrigation regime, thus root length of Cicer arietinum after five weeks from the initiation of treatment was 14, 12, 11.5, 10 cm in plant irrigated with 100, 200, 300 and 400 cm³ respectively, and every 4-day interval (long period interval) comparing with Lupinus termis root length which was 6, 6.5, 6.3 and 6.3 cm irrigated at the same level of water amount and days intervals. It is evident that Cicer arietinum root length was much longer than Lupinus termis root length under the same level of water supply. Batanouny et al., (1991) found that there is a close relationship between water supply and root system characters in Zea mays plant. They mentioned that decrease in water supply resulted in increase in the depth of penetration, increase in

lateral extension and increase in fresh and dry weight. Those authors attributed such increment to mechanisms by which plants increase the absorbing surface and exploiting large volume of soil surface.

Dry matter Content

It is clear from Fig. (3&4) that the dry matter of the whole plant exhibited considerable variation with depletion of water supply and irrigation intervals either with Lupinus termis or Cicer arietinum, but in general the first plant has increased amounts of dry matter accumulation than the second, one this is due not to the treatment itself but to the nature of plant growth and leaf area. On the other hand at the end of the experiment (5 weeks), Cicer arietinum showed the highest dry matter whereas Lupinus termis demonstrated the lowest dry weight at all irrigation treatments and at period intervals (4-day).

Cicer arietinum responded to water stress as mentioned previously by increase in lateral branches and increase in root length which intern enhance dry matter accumulation.

If we compare between the dry matter of the two investigated plants at the end of the experiment, it was found that Lupinus termis dry matter was best with the application of 300 cm^3 applied every 8-day, whereas best Cicer arietinum dry matter was obtained by application of 300 cm^3 applied every 4-day interval. These data revealed that Cicer arietinum could sustain water stress condition much better than Lupinus termis. This might be due to many factors such as increase in root depth and increase in lateral branch number which reflect increase in dry matter accumulation. Batanouny et al., (1991), found results similarly on Zea mays plant. They claimed that decrease in water supply, increase fresh and dry weights of root system. On the other hand, many authors attributed decreased yield in case of Lupinus termis associated with reduced amount of water to water stress condition which invariably resulted in stomatal closer. This has the overall effect of reducing the photosynthetic efficiency of the leaves with consequent reduction in yield.

Reduction in dry matter accumulation as a result of water deficiency was pronounced by many workers on soybean (Scott, 1984 and Kandil, 1988) et al. and cowpea (Mbagwa and Osuigwe, 1984) as well as on Medicago sativa and its variety (Abou Bakr et al., (1993) in addition to Zea mays (Thakur and Ria, 1984; El-Zeiny and Kattam, 1985).

Nodulation and nitrogen fixation:

It is clear from Tables(2&3) that water depletion affects formation of nodules on Lupinus termis and Cicer arietinum roots. Optimum nodulation was obtained by the application of 400 cm^3 water and irrigated every day for the two tested plants, but the number of nodule or their weights were varied according to the nature of the plant itself. Water stress affects root growth and the magnitude was differed, being increased in Cicer arietinum and decreased in Lupinus termis.

Lupinus termis root looked short and thick, while Cicer arietinum root was long and penetrated deeper at 100 cm^3 and 4 irrigation intervals. All nodules were aggregated on the main root in case of Lupinus termis but in Cicer arietinum were on both main root and lateral roots, moreover, their number were more or less identical under the same treatment of water amount and irrigation intervals. Our results were disagreement, however, with the results obtained by Mbagwa and Osuigwe (1984) on cowpea nodulation and water deficiency. They reported that good nodulation was obtained by the application of 100% field capacity and irrigated every day while Habbish and Mahdi (1976) observed poor nodulation when watering bean and cowpea at 15% soil moisture, these results are in accordance with our results. We found bad nodule formation at 100 cm^3 at all irrigation intervals either in Lupinus termis or Cicer arietinum. Moreover, Karama (1976) reported that watering at field capacity or excess increased cowpea nodulation, although excess water than field capacity interferes with aeration (Abou Bakr et al., (1993).

Nitrogen fixed by two investigated plant were greatly affected by the treatments as well as by the nature of the plants themselves. Thus optimum nitrogen content was obtained by the application of 400 cm^3 water and irrigated every day for the two

tested plants. This holds true with the number of nodules. Nitrogen decreased by the decrease of water amount used (100 cm^3) and also by increase of irrigation interval (4-day).

Regrading Lupinus termis nitrogen content at the fifth week it was 4.82% at 400 cm^3 and 4-day interval, while Cicer arietinum nitrogen content at the same level of water amount and irrigation interval was 3.32%. On the other hand nitrogen content of Lupinus termis at the same week but at 100 cm^3 and 8-day of irrigation interval was 2.56%. Furthermore in case of Cicer arietirum, nitrogen content at the same levels of field capacity and irrigation interval was 1.75%.

From the above mentioned data, it was found that, low water amount (100 cm^3) and long irrigation interval (8-day) drastically affected nitrogen content of either Lupinus termis or Cicer arietinum.

Our results were in agreement with many other authors (water stress decreased symbiotic nitrogen fixation and growth of nodulated legumes) Dejong and Phillips, 1982; Finn and Brun, 1980 and Sprent and Sprent, 1990). Some others found that it disrupts fine structure of nodule membrane permeability and enzyme activity (Sprent, 1976).

Water stress is also known to decrease nitrate reductase activity (Srivastava, 1980) and negatively affects other aspects of N_2-metabolism (Hsiao, 1973).

Conclusion:

Cicer arietinum could sustain draught much better than Lupinus termis when grown in semiarid regions with depletion in water supply and elongation of irrigation interval. It is also inferred that Cicer arietinum could be a popular choice for inter cropping because its drought susceptibility is intermediate, the closer of its stomates under stress allows it to conserve moisture for the main crop and its deeper rooting habit is also an advantage under stress conditions in semiarid regions.

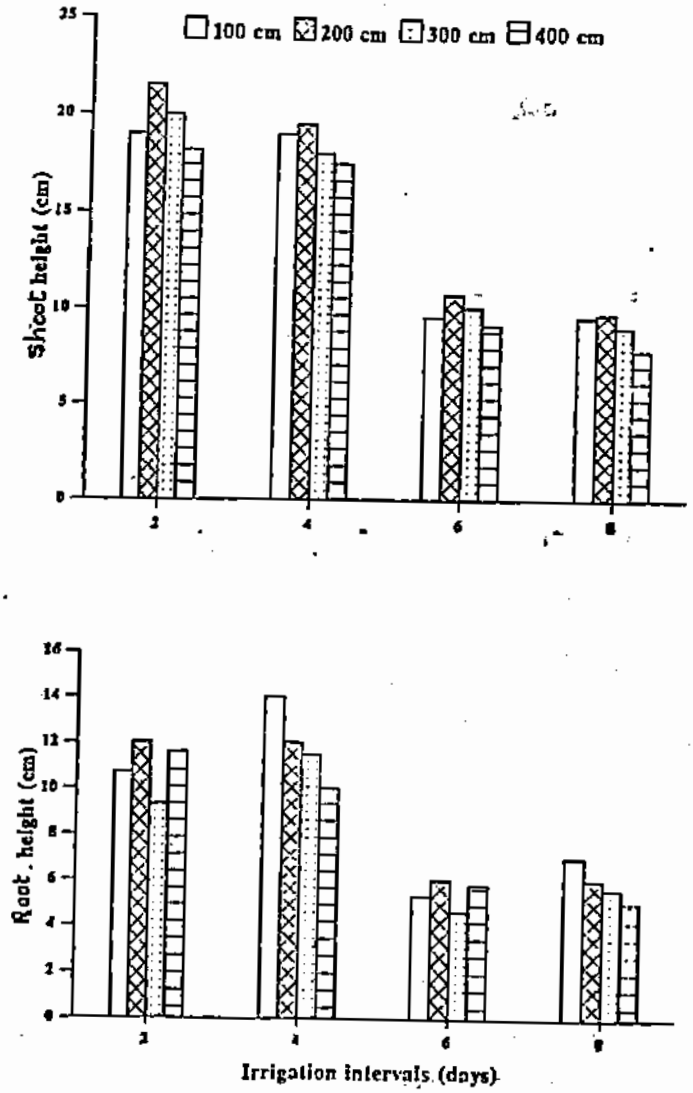

Fig. (1): Effect of irrigation intervals (days) and water amount (cm^3) on plant height (cm) of Cicer arietinum at 5th week

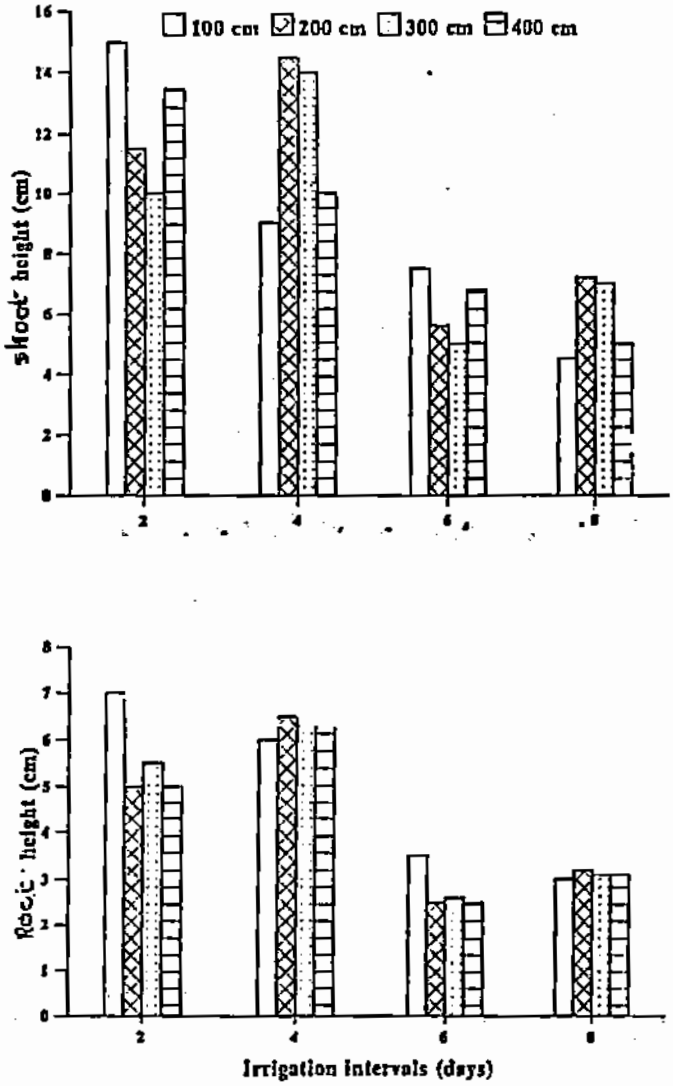

Fig. (2): Effect of irrigation intervals (days) and water amount (cm)^3 on plant height (cm) of Lupinus termis at 5th week

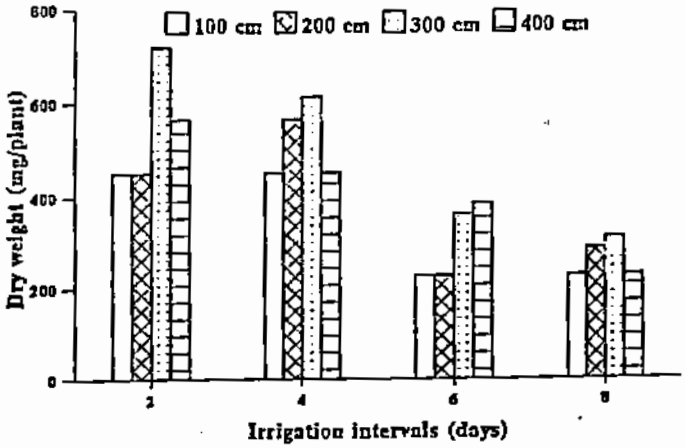

Fig. (3): Effect of irrigation intervals (days) and water amount (cm)^3 on dry weight (mg/plant) of Lupinus termis at 5th week

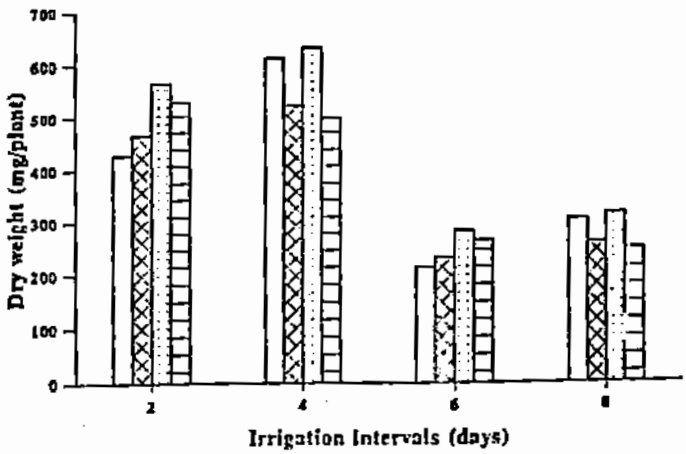

Fig. (4): Effect of irrigation intervals (days) and water amount (cm)^3 on dry weight (mg/plant) of Cicer arietinum at 5th week

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Table (1)

Mechanical analysis of the soil used in the experiment

Total sand	37.2%
Silt	30.6%
Clay	32.2%
Soil texture	clay. loam

Table (2) : Effect of irrigation intervals and water amounts on nodulation and nitrogen content of Lupinus ternis (at 5 th. weak).

Irrigation intervals (days)	Applied water amount (cm^3) 400			300			200			100		
	N%	Nodule no/pot	Nodule wt. mg./pot	N%	Nodule no/pot	Nodule wt. mg./pot	N%	Nodule no/pot	Nodule wt. mg./pot	N%	Nodule no/pot	Nodule wt. mg./pot
2	4.82	70	0.12	4.54	30	0.03	4.20	18	0.02	3.17	10	0.001
4	3.90	35	0.08	4.67	24	0.02	3.20	15	0.02	3.67	6	0.001
6	4.50	30	0.02	4.12	19	0.01	3.11	8	0.01	2.8'	4	0.001
8	4.50	16	0.01	3.84	13	0.01	2.92	5	0.01	2.5[illegible]	2	0.001
Means	4.43	38	0.03	4.29	22	0.02	3.36	12	0.015	3.05	5	0.001

* L.S.D for nitrogen content at 0.05 = 0.6274

* L.S.D. for nodule no./pot at 0.05 = 14.3588

* L.S.D for nodule wt. (mg./pot) at 0.05 = 0.0293

Table (3) : Effect of irrigation intervals and water amounts on nodulation and nitrogen content of *Cicer arietinum* (at 5 th. week).

Irrigation intervals (days)	Applied water amount (cm^3) 400			300			200			100		
	NI	Nodule no/pot	Nodule wt. mg./pot	NI	Nodule no/pot	Nodule wt. mg./pot	NI	Nodule no/pot	Nodule wt. mg./pot	NI	Nodule no/pot	Nodule wt mg./pot
2	3.32	50	0.10	2.37	28	0.05	3.17	20	0.010	2.43	8	0.002
4	3.36	38	0.03	2.31	26	0.02	3.00	17	0.005	2.80	5	0.002
6	3.20	25	0.03	2.17	17	0.01	3.30	14	0.005	2.10	4	0.001
8	3.07	20	0.02	2.00	12	0.01	2.19	8	0.002	1.75	2	0.001
Means	3.22	34	0.04	2.12	21	0.02	2.90	15	0.005	2.00	5	0.002

* L S D for nitrogen content at 0.05 = 0.31932

* L S D for nodule (no./pot) at 0.05 = 11.9529

L.S.D. for nodule wt. (mg./pot) at 0.05 = 0.0349

دراسة مقارنة على النمو و التعقيد و تثبيت النتروجين لنباتين بقولين ناميين تحت مستويات مختلفة من الأمداد المائى و فترات الرى

د . فاطمة عبد الوهاب حليش — د . منى اسحق فهمى
كلية البنات — جامعة عين شمس — مصر الجديدة — القاهرة
مصر

ملخص

تم أجراء دراسة مقارنة على النمو و التعقيد و تثبيت النتروجين لكل من نباتى الترمس (جيزة ٤) و الحمص (جيزة ٢) الناميين فى تربة طمية تمتاز بقوة أحتفاظ مائية قليلة و ذلك فى صوبا زراعية بمصر الجديدة تحت تأثير مستويات مختلفة من الأمداد المائى و فترات الرى و قد استعملت اربع كميات من الماء ٤٠٠ — ٣٠٠ — ٢٠٠ — و ١٠٠ سم٣ و كذلك اربع فترات للرى ٤ يوم ، ٦ يوم ، ٨ يوم لمدة خمس أسابيع .

و قد أوضحت النتائج الآتى :—

تأثر نمو النباتان مع الامداد المائى القليل سواء عند الرى بكمية ماء قليلة (١٠٠ سم٣) أو الرى على فترات طويلة (٤ — يوم) و قد كان نمو نبات الترمس أحسن (مادة جافة) عند استعمال كمية ماء تكافئ ٣٠٠ سم٣ و بعد فترة رى (٢ — يوم) بينما كان هناك نموا ممتازا لنبات الحمص عند استعمال كمية ماء تكافئ ٣٠٠ سم٣ أيضا و لكن بعد فترة رى (٤ — يوم) من ناحية أخرى كان التعقيد و تثبيت النتروجين أمثل لكلا النباتان المختبرا بن باستعمال كمية ماء تكافئ ٤٠٠ سم٣ مع رى النباتات كل ٢ يوم . و قد ظهرت سيقان الترمس سميكة و قصيرة و ذات عقد قليلة عند كمية الماء القليلة (١٠٠ سم٣) — و فترة الرى الطويلة (٤ — يوم) و علاوة على ذلك فقد استجاب الحمص للرجيم المائى بتكوين أفرع هوائية جديدة و جذور طويلة عند فترة الرى الطويلة (٤ — يوم) التى ظهرت جليا فى زيادة تراكم المادة الجافة عند نفس مستوى كمية الماء .

و على العموم فأن استعمال كمية ماء قليلة (١٠٠ سم٣) عند أى فترة للرى انما تكشف عن محصول ذات كفاءة فقيرة بالنسبة للترمس و ليس العكس .

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STUDIES ON INTERACTIONS OF THE PETROLEUM DERIVATIVES (PROPANIL - SO_2) WITH HERBICIDE AND INSECTICIDE ON BARNYARDGRASS ADN RICE

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SUMMARY

Pot experiments were carried out to estimate the efficiency of the synthesized petroleum aromatic derivative (propanil - SO_2) as herbicide either separately or in combination with commercial herbicide thiobencarb and insecticides (Carbaryl, Carbofuran and Malathion) on barnyardgrass (Echinochloa crus - galli (L.) P. Beauv) and /or rice (Oryza sativa cv Giza 176).

The determination of the effectivenes of propanil - SO_2 in controlling barnyardgrass showed no significant results. After application of propanil - SO_2 with thiobencarb the injury symptoms appeared on the treated seedlings of either barnyardgrass or rice were nearly similar to those sprayed with thiobencarb alone.

The interaction effects between propanil - SO_2 and the examined insecticides showed slight reduction in rice dry weight and grain yield ranged from 1 - 3% after the application of the insecticides 1 to 4 days before and after propanil - SO_2 treatment respectively The abtained data was not significant.

The vegetative growth and yield of rice plants were less injured when the time interval between insecticides and propanil treatment increased.

Rice plants were not affected by the application of the used insecticides plus proparil - SO_2 .

INTRODUCTION

Chemical weed control is a miracle of our technological age and it has been at the fore front in technological achievement. It involves knowledge in the fields of chemistry and biology (Ashton and Crafts, 1981).

New and better herbicides are being continuously synthesized and developed. Yields of cereals, soybeans, cotton, sugar beet, and in any other crops have increased in some cases 100% after application of synthetic organic herbicides (Ashton & Crafts, 1981).The herbicides are classified into two major groups inorganic and organic.

Propanil is a member of amide herbicides. It is especially valuable in rice culture because of its high selectivity. Rice plants are 40 times more tolerant of propanil than barnyardgrass (Matsunaka, 1965). The resistance of rice to propanil has been attributed to the ability of these species to degrade propanil more rapidly then most weed species (Adachi, et. al. 1966).

Chemical weed control in direct - seeded rice demands astrict herbicides application timing because rice and weeds are of the same grawth stage (De Datta and Bernasor, 1973). The ideal herbicides for weed control in rice

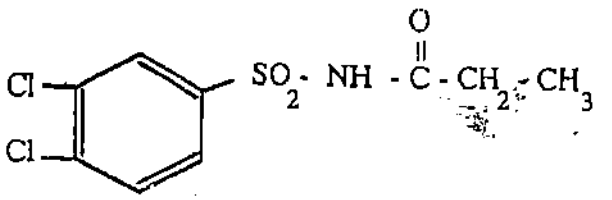
combines high selectivity and efficacy with safe application methods (Hassan et al , 1990).

Interaction between herbicides and insecticides were reported by (Smith and Tugwell, 1975 and Mukhopadhyay and sen, 1981). It was hound that the organophosphate and carbamate insecticides interact with the herbicide propanil to increase injury to rice (Bowling and Hudgins, 1966).

The aim of the present study was to test the efficiency of the synthesized propanil SO_2 as herbicides either separately or in combination with commercial herbicide and insecticide on the growth of barnyardgrass and rice.

MATERIALS AND METHODS

Preparation of N-propanil 3, 4 - dichlorobenzene sulphonamide (propanil SO_2)



This compound was prepared in three steps:

A. Mixing 1,2 dichlorobenzene with Cl SO_3 H in a solvent of CH cl_3 (Stewart, 1922).

B The produced dichlorobenzene sulphonyl chloride was treated with NH_4 OH according to (Somasekhara, 1968) to prepare 3,4- dichlorobenzene sulphonamide. C. 3,4 - dichlorobenzene sulphonamide (0.005 mol) was mixed with propionyl chloride (0.007 mal) and glacial acetic acid (5 ml) on boiling bath. The solid product was filtered, dissolved in Na HCO_3 .

acidified with glacial acetic acid and crystallized by ethanal to produce N-propionyl 3,4 - dichlorobenzene sulphonamide (El-Dib, 1978).

Commercial herbicides and insecticides used

Pesticides	Common	Chemical name	Trade name	Formulation
Herbicides	Propanil	3,4 - dichloropropionanilide	Stam F -34	EC
	Thiobencarb	S [(4 - chlorophenyl) methyl] diethyl caarbamothioate.	Saturn 50%	EC
Insecticides	Carbaryl	1-naphthyl methyl carbamate	Siven 85%	WP
	Carbofuran	2,3 dihydro - 2 - 2 - dimethyl - 7 - benzofuranyl methyl carbamate.	Furadan 5%	G
	Malathion	o,o- dimethyl phosph orodithioate ester of diethyl mercaptosuccinoate.	Malathion 80%	EC

Ec = Emulsifiable

WP = Wittable Powder

G = Granules

pot experiment:

The experiments were carried out at Rice Research and Training Centre, Sakka, Kafr El-Sheikh under greenhouse conditions (average temperature 28 + 2 C during the day time and 18 C at night). seeds were obtained from the same Research Centre.

The effect of the synthesized herbicides propanil - SO_2 was tested either separately or in combination with desired commercial herbicides or insecticides on barnyardgrass (Echinochloa crus - galli (L.) P. Beauv) and rice (Oryza sativa)

c. Giza 176. Plants were grown in plastic pots 20 cm depth and 20 cm diameter. In each pot 4 Kg of clay loam soil was placed. Some chemical analysis of the used soil was as follows:

pH	Ec ds/m	Co_3 Meq/L	HCo_3 Meq/L	OM %	NH_4 ppm	No_3 ppm	P ppm	K ppm	Zn ppm
7.8	1.1	1.0	1.9	2.1	2.9	19.3	11	625	1.9

Fifteen seeds each of barnyardgrass and rice were planted per pot, after emergence the seedlings were thinned to 10 plants per pot. Pots were irrigated by saturating the soil several times during the early season. It was flooded first when the plants ranged from 15 to 25 cm tall. Water was maintained on the plants during the season except for draining one day before and reflooding one day after each treatment. Rice plants received 40 Kg nitrogen /fed as urea 46% at 25 days after seeding.

The synthesized propanil - SO_2 and all commercial herbicides and insecticides were used as Kg/fed in a total water emulsion of 200L/fed., with a CO_2 - pressurized back - pack sprayer at 2.11 Kg/cm^2 fitted with flat - fan spray nozzles # 1004. The boam was positioned 25 cm above each pot.

The Herbicides were applied at half -, one- two- and three- leaf stages of barnyardgrass and rice at different rates.

Growth stages of rice plants when treated with insecticides in relation to

propanil application were listed in the fallowing Table:

Application time of insecticides		Rice growth stage	
10	DBP	1	Leaf stage
7	DBP	2	Leaf stage
4	DBP	2.5	Leaf stage
2	DBP	3	Leaf stage
1	DBP	3.5	Leaf stage
1	DAP	3.5	Leaf stage
2	DAP	4	Leaf stage
4	DAP	4.5	Leaf stage
7	DAP	5	Leaf stage
10	DAP	5.5	Leaf stage

DBP = days before propanil application.
DAP = days after propanil application.

The insecticides carbaryl, carbofuran and malathion were applied at the rates of 1.2 Kg ai/fed wettable powder, 0.8 Kg ai/fed granules and 1.2 Kg ai/fed emulsifiable concentrate respectively in relation to the propanil or propanil - SO_2 treatment at the rate of 1.05 Kg ai/fed emulsifiable concentrate.

Fresh and dry weights of shoots of barnyardgrass and rice, plant height and leaf area of rice plant were recorded at 30 days after seeding. leaf area was determined using LI-3100 Area Meter. Total chlorophyll content of rice leaves were estimated at 5 days after seeding according to th procedure (Arnon, 1959).

After maturity the harvested grains were adjusted to 14% moisture (Smith, 1974). Rice grain yield in grams per pot were obtained. The total nitrogen

content of rice grains was determined (Jackson, 1967). The starch,content of rice grains was estimated using the method of (Yoschida et al.,1972).

The results were statistically analyzed and significance of treatment differences was estimated through L.S.D. (Snedecor and Cochron, 1969).

Effect of propanil, propanil - SO_2, thiobencarb and their combinations on barnyardgrass and rice:

Effective weed control programms have been developed for the most severe weeds of rice (barnyardgrass) were based primarily one the use of propanil and other herbicides (Smith et al., 1977 and Eastin, 1981).

In the present investigation we tried to compare the effects of either propanil or propanil - So with thiobencarb when applied at different stages of growth for controlling branyardgrass.

Both barnyardgrass and rice plants developed dark greed leaves and stunting within 1 - 3 days after thiobencarb application,while the plants showed scorching and stunting following the application of propanil plus thiobencarb. The symptoms were obvious at higher concentrations and when the treatments were done at the early stages of growth one - and two - leaf stage. Rice plants recovered from the injury within 7 - 15 days after treatments.

Table (1) represented that either propanil or thiobencarb caused significant reduction in fresh and dry weights of barnyardgrass at one - leaf stage. Weed injury was ranged from 16 - 87% and 28 - 64% following the application of propanil and thiobencarb respectively. The barnyardgrass - control

was decreased at late stages of growth (two - and three - leaf stage). However,propanil tank-mixed with thiobencarb provided excellent control of barnyardgrass (72 - 100%) at higher concentrations of propanil plus thiobencarb 0.35 + 1.0, 0.70 + 0.5, 0.70 + 0.75, 0,70 + 1.0, 1.05 + 0.5, 1.05 + 0.75 and 1.05 + 1.0 Kg ai/fed when applied at one - and two - leaf stage of growth.

It was clear from the experimental results that postemergence tank - mixture of propanil + thiobencarb caused an effective control of barnyardgrass more than single treatments. The phytotoxic effects were apparent when the herbicides were applied at lower stages of growth and at higher concentrations.

The obtained results were in aggrement with (Smith and Khodayari, 1985 and Khodayari et al., 1989) who reported that propanil tank - mixed with thiobencarb postemergence provided an effective control of barnyardgrass than sequential application of them.

It was reported by different investigators (Smith 1975, Smith et al., 1977 and Hassan and Mahrous, 1989) and confirmed by the present work, that postemergence tank - mixture of propanil with thiobencarb was most effective for controlling barnyardgrass at one - and two - leaf stage of growth, while larger barnyardgrass plants were frequently not controlled.

Tables (2,3) showed that the effect of propanil, thiobencarb and their combinations on plant height, fresh weight, dry weight,leaf area and total chlorophyll content of rice. At one - leaf stage of rice there was severe reduction on the growth parameters after the application of the herbicides. The reduction was clearly observed as the rate of herbicides increased. At one - leaf

stage of growth plant height, fresh weight, dry weight and leaf area were reduced by 61% , 55%, 54% and 72% respectively following the application of thiobencarb at 1.0 Kg. ai/fed. This injury decreased at two - and three - leaf stage of growth. Postemergence tank - mixtures of propanil plus thiobencarb enhanced the injury of rice plants greatly at lower stages of growth and at higher concentrations of the herbicides.

Propanil at 1.05 Kg ai/fed, propanil + thiobencarb at 1.05 + 0.5, 1.05 + 0.75 and 1.05 + 1.0 Kg ai/fed caused 20% reduction in the total chlorophyll content of rice leaves. This reduction was not singnificant as shown in Table (3).

Table (4) represented that rice grains harvested from plant treated with the herbicides were severely affected at the early stages of growth. When the plants ware sprayed with either propanil or thiobencarb at one - leaf stage the observed reduction in yield ranged from 29 - 54%, 29 - 56% respectively. This harmfull effect increased by using tank - mixture of both herbicides at the same stage of growth. however, the reduction in rice grain yield was not statistically significant when the plants were treated with the herbicides either alone or in combination at the late stage of growth (three - leaf stage).

The total nitrogen content, crude protein and starch content of rice grains were not significantly affected by the herbicidal treatments at all rates and times of application as illustrated in Table (4).

Results from this investigation showed that the application of propanil in combination with thiobencarb increased rice injury as compared with single

treatments. This injury was clearly observed at one - and two - leaf stage of growth and at higher rates of the combined herbicides.

Previous investigators (Smith et al., 1977, Richard et al., 1981 and Smith, 1981) found that mixtures of propanil with thiobencarb applied early postemergence injured rice moderately, but the rice recovered and the grain yield was developed which supports the data reported herein.

Some herbicides injured rice more than propanil but injury for all treatments was inconsistent (Smith and Khodayari, 1985).

Trials were made to examine the phytotoxicity of propanil - SO_2 when used in combination with thiobencarb as shown in Table (5). After application of propanil - SO_2 with thiobencarb, the injury symptoms appeared on the treated seedlings of either barnyardgrass or rice were nearly similar to those sprayed with thiobencarb alone. Postemergence application of propanil - SO_2 at 0.35, 0.70 and 1.05 Kg ai/fed, in combination with thiobencarb at 0.50, 0.75 and 1.0 kg ai/fed resulted in significant reduction in fresh and dry weights of barnyardgrass at lower stages of growth and at higher concentrations. The percent injury ranged from 74 to 77% and 41 to 44% at one - and two - leaf stage of growth respectively at higher rates of propanil - SO_2 + thiobencarb 0.70 + 1.0 and 1.05+1.0 kg ai/fed as shown in Table (5).

Propanil - SO_2 in combination with thiobencarb was also tested on the vegetative growth and the crop yield of rice at different rates and times of application as indicated in Tables (6,7 & 8). The phytotoxic effects increased at lower stages of growth (one - and two - leaf stage) and at higher rates of

propanil - SO_2 + thiobencarb 0.7 + 1.0, 1.05 + 0.50,1.05 +0.75 and 1.05 + 1.0 kg a.i/fed

Rice grain yield and total nitrogen content, crude protein & starch content of rice grains as influenced by propanil - SO_2 and thiobencarb were illustrated in Table (8). There was singnificant reduction in rice grain yield ranged from 65 - 68% at one - leaf stage when propanil - SO_2 + thiobencarb applied at rates of 0.70 + 1.0 and 1.05 + 1.0 kg a.i/fed respectively. However, the percent injury of the total nitrogen content, crude protein and starch content of rice grains was not significant.

Herbicides - insecticides interactions on rice:

Losses in the yield of rice crop due to weeds and insects are quite severe. Biological - control is at present perhaps impractical for large scale use to combat these pestes. Large number of investigations have been made on the use of herbicides and insecticides separately, but very few investigations have been made on the combined use of herbicides and insecticides in rice crop. Very often weeds and insects infest the rice crop at the same time (Gifford, 1973 and Smith and Seaman, 1973). Hence herbicides and insecticides are needed at about the same time and one application of these combined pesticides will help to reduce the cost of operation.Therefore, it was of great necessity to study in detail the interaction of these herbicides and insecticides combinations.

With these ideas trials were made in the present investigation to drive more knowledge about the interaction of propanil or propanil - SO_2 with some insecticides on rice when they were applied at various times and rates before or after herbicidal treatments.

Propanil or propanil - SO_2 and insecticides interactions on rice:

Observations indicated that leaf chlorosis and necrosis of rice plants were less as the time interval between insecticides and propanil treatment increased. The rice recovered from the interacting effects within a 5 - week period after propanil treatment. Older rice plants recovered more quickly than younger ones.

Propanil is widely used as a selective herbicide for controlling barnyardgrass in rice and usually causes no significant injury to rice plants. However certain insecticides interact adversely with propanil result in phytotoxic injury to rice plants.

The above observations were in agreement with those reported by (Smith and Tugwell, 1975 and Khodayari et al., 1986). The application of propanil plus carbofuran caused yellowing and burning of rice leaves but rice recovered about five weeks after application (Mukhopadhyay and Sen, 1981).

Synergistic phytotoxicity was apparent on rice plants when propanil was applied one day after carbaryl treatment where most rice plants were killed. Slight injury was noticed on plants treated with propanil two weeks after carbaryl treatment (Yih et al., 1968 b).

Table (9) showed that all insecticides (carbaryl, carbofuran and malathion) interacted with propanil at all times of application. Their interactions injured rice vegetatively. The degree of injury is influenced both by the type and the application time of the used insecticides in relation to the propanil application. The reduction in rice dry weight expressed as percent proved an

excellent indication of phytotoxicity. The synergistic effects in rice resulting in dry weight loss from 39% to 69% when the isecticides were applied 4 days to 1 day before or after propanil treatment respectively. Injury was lowered from 28% to 0% with time intervals of 7 to 10 days before or after propanil application.

After recovery of the vegetatively injured rice plants, grain yield was developed. Rice grain yield was reduced as the same trend observed in rice dry weight as shown in Table (9).

The phytotoxic symptoms exhibited by rice plants following the interaction of insecticides and propanil may be attributed to the inhibiting effect of the insecticides on the activity of rice arylacylamidase enzyme which detoxifies propanil causing loss of selectivity in rice.

Results of previous researchs obtained by different investigators (Bowling and Hudgins, 1966, Bowling and Flinchum, 1968 and Yih et al., 1968 a) indicated that the degree of rice plants injury was directly correlated with the degree of propanil hydrolyzing-enzyme inhibition caused by certain insecticides. The basis for the interaction between propanil and certain insecticides appeared to be an inhibition of rice arylacylamidase enzyme which metabolizes propanil to non - toxic compounds 3,4 - dichloroaniline and propionic acid (Frear and Still, 1968, Matsunaka, 1968, El-Refai and Mowafy, 1973 and Matsunaka, 1981).

A mixture of propanil at 3 lb/acre and carbaryl at 0.5 lb/acre were applied to rice 4 inches tall caused 55% injury, compared with 5 and 0% injury from either propanil or carbaryl respectively (Smith, 1968).

Table (10) represented the interaction effects between propanil SO_2 and the examined insecticides. Slight reduction was observed in rice dry weight and grain yield ranged from 1% to 3% after the application of the insecticides 4 days to 1 day before or after propanil SO_2 treatment respectively. The obtained data was not significant.

CONCLUSION:

The results from the present study revealed that the response injury of barnyardgrass and rice to tested herbicides depends on the leaf - stage of growth at which they were applied. It is preferable to apply the herbicides at one - or two - leaf stage of barnyardgrass to achieve maximum control. However, it is safe to use herbicides after three - leaf stage of rice to avoid any phytotoxic effect.

The synthesized herbicides need knowledge in the fields of chemistry and biology and at least observational experience in the responses of common weeds and crops to them. Also weed and crop ecology and appreciation of the factors determining selectivity of the new herbicides are very important.

Generally herbicides must be applied at lowest rates by which maximum control of weeds is achieved.

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Table 1: Effect of propanil , thiobencarb and their combinations on the fresh and dry weights of barnyardgrass (Echinochloa crus-galli) at 30 days after seeding .

Herbicides	Rate of application Kg ai / fed	Fresh weight (g / pot)						Dry weight (g / pot)					
		Leaf stage & % reduction						Leaf stage & % reduction					
		1 lf	%	2 lf	%	3 lf	%	1 lf	%	2 lf	%	3lf	%
Control (untreated)	-	21	0	21	0	21	0	3.67	0	3.51	0	3.69	0
Propanil	0.35	17.6	16	19.4	8	19.7	6	3.1	16	3.24	8	3.47	6
	0.70	4.6	78	7.8	63	12.5	41	0.8	78	1.3	63	2.13	42
	1.05	2.8	87	3.1	85	7.5	64	0.4	89	0.56	84	1.37	63
Thiobencarb	0.5	15.2	28	20.4	3	21.	0	2.69	27	3.40	3	3.69	0
	0.75	11.5	45	19.7	6	20.2	4	2.0	46	3.32	5	3.55	4
	1.0	7.5	64	16.8	20	18	14	1.3	65	2.8	20	3.15	15
Propanil + thiobencarb	0.35 + 0.5	14.5	31	19.8	6	20.8	1	2.53	31	3.38	4	3.60	2
	0.35 + 0.75	11.2	47	19.1	9	18.3	13	1.95	47	3.20	9	3.22	13
	0.35 + 1.0	5.9	72	14.8	30	15	29	1.03	72	2.45	30	2.63	29
Propanil + thiobencarb	0.70 + 0.5	3.5	83	7.0	67	11.7	44	0.60	84	1.17	67	2.05	44
	0.70 + 0.75	2.8	87	6.6	69	11.5	45	0.53	86	1.10	69	2.02	45
	0.70 + 1.0	2.3	89	5.9	72	11.3	46	0.40	89	1.03	71	2.0	46
Propanil + thiobencarb	1.05 + 0.5	2.0	91	6.4	70	8.9	58	0.34	91	1.08	69	1.59	57
	1.05 + 0.75	1.9	91	6.2	71	7.6	64	0.31	92	1.05	70	1.28	65
	1.05 + 1.0	0.0	100	0.0	100	6.6	69	0.0	100	0.0	100	1.2	68
$L.S.D_{0.05}$		1.9		1.3		1.7		0.32		0.14		0.23	

Table : 2 Effect of propanil , thiobencarb and their combinations on plant height, fresh weight and dry weight of rice (Oryza sativa) cv Giza 176 at 30 days after seeding.

Herbicides	Rate of application Kg ai / fed	Plant height (cm / plant)						Fresh weight (g / pot)						Dry weight (g / pot)					
		Leaf stage & % reduction						Leaf stage & % reduction						Leaf stage & % reduction					
		1 lf	%	2 lf	%	3 lf	%	1lf	%	2 lf	%	3lf	%	1lf	%	2lf	%	3lf	%
Control (untreated)	-	28	0	28	0	28	0	13	0	13	0	13	0	2.35	0	2.29	0	2.30	0
Propanil	0.35	24	14	28	0	28	0	11.6	11	12.9	1	13	0	2.1	11	2.31	0	2.33	0
	0.70	21	25	26.7	5	28	0	9.2	29	12.2	6	12.9	1	1.7	28	2.17	5	2.31	0
	1.05	13	54	23	18	26.9	4	5.2	60	10.9	16	12.5	4	0.95	60	1.9	17	2.22	4
Thiobencarb	0.5	18	36	28.1	0	28	0	9.5	27	13.5	0	13.7	0	1.7	28	2.34	0	2.36	0
	0.75	13	54	27.8	1	27.9	0	7.8	40	13	0	13.1	0	1.4	40	2.32	0	2.31	0
	1.0	11	61	27.3	3	27.8	1	5.9	55	12	8	12.9	1	1.08	54	2.11	9	2.28	1
Propanil + thiobencarb	0.35 + 0.5	17	39	27.9	0	28	0	7.0	46	12.9	1	13	0	1.3	45	2.29	0	2.31	0
	0.35 + 0.75	12	57	27.5	2	27.7	1	6.1	53	12.5	4	13	0	1.1	53	2.23	3	2.30	0
	0.35 + 1.0	10	64	26.9	4	27.4	2	4.9	62	12.1	7	12.9	1	0.9	62	2.10	8	2.28	1
Propanil + thiobencarb	0.70 + 0.5	15	46	26.5	5	27.6	1	5.9	55	12.6	3	13.2	0	1.08	54	2.23	3	2.32	0
	0.70 + 0.75	11	61	26.4	6	27.3	3	4.9	62	12.4	5	12.9	0	0.95	60	2.20	4	2.30	0
	0.70 + 1.0	9	67	25.2	10	26.5	5	3.8	71	10.4	20	11.4	12	0.68	71	1.8	21	2.0	13
Propanil + thiobencarb	1.05 + 0.5	10	64	23.1	18	27	4	2.0	85	9.21	29	11.4	12	0.33	86	1.6	30	2.0	13
	1.05 + 0.75	8	71	22.9	18	26.4	5	1.8	86	9.0	31	11.1	15	0.30	87	1.57	31	1.95	15
	1.05 + 1.0	7	75	22.2	21	26.2	6	1.38	89	8.5	35	10.9	16	0.28	88	1.48	35	1.90	17

Table 3 : Effect of propanil , thiobencarb and their combinations on leaf area and total chlorophyll content of rice (Oryza sativa) cv Giza 176.

Herbicides	Rate of application Kg ai / fed	Leaf area (cm^2 / pot) at 30 days after seeding						Total * chlorophyll mg/g fresh wt.	
		Leaf stage & % reduction							
		1 lf	%	2 lf	%	3 lf	%	2lf	% red
Control (untreated)	-	96	0	96	0	96	0	5	0
Propanil	0.35	83	14	96	0	97	0	6	0
	0.70	61	37	89	7	96	0	5	0
	1.05	34	65	69	28	95	1	4	20
Thiobencarb	0.5	68	29	92	4	98	0	6	0
	0.75	44	54	90	6	97	0	6	0
	1.0	27	72	89	7	95	1	5	0
Propanil + thiobencarb	0.35 + 0.5	63	34	90	6	96	0	6	0
	0.35 + 0.75	41	57	89	7	95	1	5	0
	0.35 + 1.0	21	78	87	9	94	2	5	0
Propanil + thiobencarb	0.70 + 0.5	57	41	89	7	95	1	5	0
	0.70 + 0.75	39	59	89	7	94	2	5	0
	0.70 + 1.0	20	79	82	15	93	3	4	20
Propanil + thiobencarb	1.05 + 0.5	23	76	66	31	93	3	4	20
	1.05 + 0.75	20	79	64	33	91	5	4	20
	1.05 + 1.0	10	90	63	34	90	6	4	20
L.S.D$_{0.05}$		11		8		N.S		N.S	

* At 45 days after seeding

Table 4 : Effect of propanil , thiobencarb and their combinations on grain yield and total nitrogen , crude protein & starch content of rice-grains(Oryza sativa) cv Giza 176.

Herbicides	Rate of application Kg ai / fed	Grain yield (g / pot)						Total nitrogen		Crude Protein		Starch content	
		Leaf stage & % reduction						mg / g grain					
		1 lf	%	2 lf	%	3 lf	%	2 lf	% red	2 lf	% red	2lf	%red
Control (untreated)	-	41	0	40	0	41	0	21	0	125	0	43	0
Propanil	0.35	29	29	39	3	41	0	20	5	125	0	42	2
	0.70	28	32	38	5	39	5	18	14	113	10	40	7
	1.05	19	54	37	8	38	7	18	14	113	10	40	7
Thiobencarb	0.5	29	29	40	0	42	0	19	10	119	5	42	2
	0.75	25	39	39	3	41	0	18	14	113	10	40	7
	1.0	18	56	39	3	39	5	17	19	106	15	40	7
Propanil + thiobencarb	0.35 + 0.5	18	56	38	5	42	0	19	10	119	5	41	5
	0.35 + 0.75	9	78	37	8	41	0	18	14	113	10	40	7
	0.35 + 1.0	7	83	36	10	39	5	17	19	106	15	40	7
Propanil + thiobencarb	0.70 + 0.5	8	81	37	8	41	0	18	14	113	10	40	7
	0.70 + 0.75	7	83	36	10	40	2	18	14	113	10	40	7
	0.70 + 1.0	6	85	34	15	39	5	17	19	106	15	39	9
Propanil + thiobencarb	1.05 + 0.5	4	90	32	20	40	2	18	14	113	10	40	7
	1.05 + 0.75	3	93	31	23	39	5	17	19	106	15	39	9
	1.05 + 1.0	2	95	30	25	39	5	17	19	106	15	39	9
L.S.D$_{0.05}$		3		6		N.S		N.S		N.S		N.S	

Table 5 : Effect of propanil - SO_2 , thiobencarb and their combinations on the fresh and dry weights of barnyardgrass (Echinochloa crus-galli) at 30 days after seeding .

Herbicides	Rate of application Kg ai / fed	Fresh weight (g / pot)						Dry weight (g / pot)					
		Leaf stage & % reduction						Leaf stage & % reduction					
		1 lf	%	2 lf	%	3 lf	%	1 lf	%	2 lf	%	3lf	%
Control (untreated)	-	23	0	23	0	23	0	3.89	0	3.97	0	3.91	0
Propanil SO_2	0.35	23.1	0	23.2	0	23.6	0	3.90	0	3.99	0	3.93	0
	0.70	22.8	1	22.7	1	22.9	0	3.84	1	3.93	1	3.90	0
	1.05	21.5	7	21.9	5	22.5	2	3.65	6	3.79	5	3.85	2
Thiobencarb	0.5	15.9	31	21.5	7	22.9	0	2.7	31	3.65	8	3.90	0
	0.75	13.3	42	21	9	22.1	4	2.3	41	3.60	9	3.81	3
	1.0	7.1	69	18.5	20	21.5	7	1.19	69	3.15	21	3.69	6
Propanil-SO_2 +thiobencarb	0.35 + 0.5	15	35	21.2	8	22.5	2	2.55	34	3.60	9	3.83	2
	0.35 + 0.75	12.7	45	20.5	11	22.1	4	2.17	44	3.54	11	3.71	5
	0.35 + 1.0	6.9	70	17.6	23	21	9	1.15	70	3.07	23	3.57	9
Propanil-SO_2 +thiobencarb	0.70 + 0.5	14	39	21	9	22.1	4	2.4	38	3.63	9	3.74	4
	0.70 + 0.75	12.2	47	20.4	11	21.5	7	2.05	47	3.52	11	3.64	7
	0.70 + 1.0	5.9	74	13.5	41	20.5	11	1.0	74	2.34	41	3.40	13
Propanil-SO_2 +thiobencarb	1.05 + 0.5	13.3	42	20.5	11	22.1	4	2.3	41	3.54	11	3.71	5
	1.05 + 0.75	11.5	50	18.7	19	21.5	7	1.95	50	3.20	19	3.59	8
	1.05 + 1.0	5.3	77	12.9	44	20.3	12	0.9	77	2.22	44	3.33	15
$L.S.D_{0.05}$		2.91		3.15		N.S		0.31		0.47		N.S	

Table : 6 Effect of propanil-SO_2 , thiobencarb and their combinations on plant height, fresh weight and dry weight of rice (Oryza sativa) cv Giza 176 at 30 days after seeding.

Herbicides	Rate of application Kg ai / fed	Plant height (cm / plant)						Fresh weight (g / pot)						Dry weight (g / pot)					
		Leaf stage & % reduction						Leaf stage & % reduction						Leaf stage & % reduction					
		1 lf	%	2 lf	%	3 lf	%	1 lf	%	2 lf	%	3 lf	%	1lf	%	2lf	%	3lf	%
Control (untreated)	-	29	0	29	0	29	0	17	0	17	0	17	0	2.87	0	2.89	0	2.89	0
Propanil-SO_2	0.35	29	0	29.1	0	28.9	0	17.1	0	17.1	0	17.3	0	2.90	0	2.88	0	2.89	0
	0.70	28.4	2	28.6	1	28.8	1	16.5	3	16.6	2	16.8	1	2.81	3	2.83	2	2.86	1
	1.05	28	3	28.4	2	28.5	2	16.1	5	16.3	4	16.5	3	2.74	5	2.79	4	2.81	3
Thiobencarb	0.5	16.8	42	29	0	29.1	0	12.3	28	16.4	4	17	0	2.06	28	2.77	4	2.89	0
	0.75	13.4	54	28.1	3	28.8	1	9.9	42	16.0	6	16.6	2	1.7	41	2.73	6	2.83	2
	1.0	10.7	63	28.1	3	28.4	2	7.0	59	15.5	9	16.3	4	1.18	59	2.63	9	2.79	4
Propanil-SO_2 +thiobencarb	0.35 + 0.5	16.2	44	28.9	0	29	0	12.0	29	16.3	4	16.8	1	2.03	29	2.78	4	2.86	1
	0.35 + 0.75	13.0	55	28.2	3	28.8	1	9.7	43	16.0	6	16.6	2	1.65	43	2.73	6	2.83	2
	0.35 + 1.0	10.1	65	28	3	28.3	2	6.8	60	15.3	10	16.1	5	1.15	60	2.63	9	2.74	5
Propanil-SO_2 +thiobencarb	0.70 + 0.5	15.9	45	28.8	1	29.3	0	11.5	32	16.0	6	16.5	3	1.98	31	2.73	6	2.81	3
	0.70 + 0.75	12.2	58	28.2	3	28.5	2	9.5	44	15.9	7	16.1	5	1.65	43	2.69	7	2.74	5
	0.70 + 1.0	9.5	67	27.9	4	28.4	2	6.5	62	15.3	10	15.8	7	1.10	62	2.59	10	2.70	7
Propanil-SO_2 +thiobencarb	1.05 + 0.5	15.0	48	28.4	2	29	0	11.0	35	15.8	7	16.3	4	1.89	34	2.70	7	2.78	4
	1.05 + 0.75	11.8	59	28	3	28.5	2	8.7	49	15.6	8	16.1	5	1.48	48	2.66	8	2.75	5
	1.05 + 1.0	9.2	68	27.8	4	28	3	6.0	65	15.2	11	15.9	7	1.01	65	2.59	10	2.69	7
L.S.$D_{0.05}$		3.4		N.S		N.S		2.71		2.3		N.S		0.42		0.37		N.S	

Table : 7 Effect of propanil- SO_2 , thiobencarb and their combinations on leaf area and total chlorophyll content of rice (Oryza sativa) cv Giza 176.

Herbicides	Rate of application Kg. ai / fed	Leaf area (cm^2 / pot) at 30 days after seeding						Total * chlorophyll mg/g fresh wt.	
		Leaf stage & % reduction							
		1 lf	%	2 lf	%	3 lf	%	2lf	% red
Control (untreated)	-	103	0	103	0	103	0	5	0
Propanil-SO_2	0.35	104	0	103	0	103	0	6	0
	0.70	102	1	102	1	102	1	6	0
	1.05	100	3	101	2	101	2	5	0
Thiobencarb	0.5	61	41	98	5	103	0	6	0
	0.75	35	66	97	6	102	1	5	0
	1.0	27	74	95	8	101	2	5	0
Propanil-SO_2 +thiobencarb	0.35 + 0.5	57	45	97	6	102	1	6	0
	0.35 + 0.75	32	69	95	8	102	1	5	0
	0.35 + 1.0	26	75	94	9	101	2	5	0
Propanil-SO_2 +thiobencarb	0.70 + 0.5	56	46	96	7	102	1	6	0
	0.70 + 0.75	30	71	95	8	101	2	5	0
	0.70 + 1.0	25	76	93	10	100	3	4	20
Propanil-SO_2 +thiobencarb	1.05 + 0.5	55	47	96	7	101	2	5	0
	1.05 + 0.75	27	74	94	9	101	2	5	0
	1.05 + 1.0	24	77	93	10	100	3	4	20
$L.S.D_{0.05}$		9		N.S		N.S		N.S	

* At 45 days after seeding

Table 8 : Effect of propanil - SO_2, thiobencarb and their combinations on grain yield and total nitrogen, crude protein & starch content of rice grains(Oryza sativa) cv Giza 176.

Herbicides	Rate of application Kg ai / fed	Grain yield (g / pot)						Total nitrogen		Crude Protein		Starch content	
		Leaf stage & % reduction						mg / g grain					
		1 lf	%	2 lf	%	3 lf	%	2 lf	% red	2 lf	% red	2lf	%red
Control (untreated)	-	37	0	36	0	36	0	21	0	125	0	43	0
Propanil-SO_2	0.35	37	0	38	0	38	0	20	5	125	0	43	0
	0.70	36	3	35	3	37	0	20	5	125	0	43	0
	1.05	35	5	35	3	35	3	19	10	119	5	42	2
Thiobencarb	0.5	26	30	37	0	38	0	19	10	119	5	41	5
	0.75	22	41	35	3	36	0	18	14	113	10	40	7
	1.0	15	60	34	6	35	3	17	19	106	15	40	7
Propanil-SO_2 +thiobencarb	0.35 + 0.5	25	32	36	0	37	0	19	10	119	5	41	5
	0.35 + 0.75	21	43	35	3	35	3	18	14	113	10	40	7
	0.35 + 1.0	14	62	34	6	35	3	17	19	106	15	40	7
Propanil-SO_2 +thiobencarb	0.70 + 0.5	23	38	35	3	36	0	18	14	113	10	41	5
	0.70 + 0.75	19	49	35	3	35	3	18	14	113	10	40	7
	0.70 + 1.0	13	65	34	6	35	3	17	19	106	15	39	9
Propanil-SO_2 +thiobencarb	1.05 + 0.5	21	43	35	3	35	3	18	14	113	10	40	7
	1.05 + 0.75	18	51	34	6	35	3	18	14	113	10	40	7
	1.05 + 1.0	12	68	34	6	34	6	17	19	106	15	39	9
L.S.$D_{0.05}$		5		N.S		N.S		N.S		N.S		N.S	

Table: 9 Rice dry weight (30 days after seeding) and grain yield as influenced by propanil and insecticides interactions .

Time of * insecticide application	Carbaryl				Carbofuran				Malathion			
	Dry wt. (g/pot)	% red.	Grain yield (g/pot)	% red.	Dry wt. (g/pot)	% red.	Grain yield (g/pot)	% red.	Dry wt. (g/pot)	% red.	Grain yield (g/pot)	% red.
Control (untreated)	4.25	0	58	0	4.25	0	58	0	4.25	0	58	0
10 DBP	3.9	8	54	7	4.05	5	55	5	4.16	2	57	2
7 DBP	3.08	28	51	12	3.29	23	52	10	3.59	16	55	5
4 DBP	2.0	53	32	45	2.2	48	34	41	2.6	39	40	31
2 DBP	1.5	65	29	50	1.6	62	31	47	1.78	58	37	36
1 DBP	1.3	69	22	62	1.5	65	25	57	1.7	60	31	47
1 DAP	1.65	61	33	43	2.14	50	38	35	2.14	50	41	29
2 DAP	1.74	59	35	40	2.11	50	40	31	2.42	43	44	24
4 DAP	2.28	46	39	33	2.6	39	45	22	2.48	42	49	16
7 DAP	3.8	11	52	10	3.9	8	51	12	3.83	10	55	5
10 DAP	3.9	8	55	5	4.1	4	54	7	4.25	0	58	0
L.S.D $_{0.05}$	4		3		4		3		4		3	

* DBP = days before propanil application
DAP = days after propanil application

Table: 10 Rice dry weight (30 days after seeding) and grain yield as influenced by propanil-SO_2 and insecticides interactions .

Time of * insecticide application	Carbaryl				Carbofuran				Malathion			
	Dry wt. (g / pot)	% red.	Grain yield (g / pot)	% red.	Dry wt. (g / pot)	% red.	Grain yield (g / pot)	% red.	Dry wt. (g / pot)	% red.	Grain yield (g / pot)	% red.
Control (untreated)	3.19	0	50	0	3.17	0	50	0	3.17	0	50	0
10 DBP	3.2	0	52	0	3.18	0	52	0	3.18	0	51	0
7 DBP	3.16	1	51	0	3.16	0	51	0	3.13	2	50	0
4 DBP	3.15	1	50	0	3.15	1	50	0	3.11	2	49	2
2 DBP	3.12	2	49	2	3.11	2	49	2	3.09	3	49	2
1 DBP	3.08	3	48	4	3.07	3	49	2	3.07	3	48	4
1 DAP	3.1	3	48	4	3.08	3	48	4	3.08	3	49	2
2 DAP	3.14	2	49	2	3.12	2	49	2	3.12	2	49	2
4 DAP	3.16	1	49	2	3.15	1	50	0	3.1	2	50	0
7 DAP	3.19	0	50	0	3.15	1	51	0	3.14	1	50	0
10 DAP	3.20	0	51	0	3.17	0	52	0	3.17	0	51	0
L.S.D 0.05	N.S		N.S		N.S		N.S		N.S		N.S	

* DBP = days before propanil - SO_2 application

DAP = days after propanil - SO_2 application

دراسات على تداخل المشتق البترولى
(بربانيل ـ كب أ$_{٢}$) مع مبيدات الحشائش
ومبيدات الحشرات وتأثيره على الدنيبه والارز

مهرشان طه المقدم ـ زينب يوسف محمد ابو بكر ـ فايدة احمد شراره

الملخص العربى

اجريت تجارب فى الاصص لتقدير تأثير المشتق النطرى البترولى المخلـــــــق (بربانيل ـ كب أ$_{٢}$) كمبيد حشائش على الدنيبة او الارز • وقد تم استخدامه امـــا منفردا او مختلط مع مبيد الحشائش التجارى (ثيوبنكارب) او مع المبيدات الحشرية التجارية (كاربارىل او كاربوفيوران او مالاثايون) على الدنيبه او الارز •

وقد لوحظ ان نباتات الدنيبة لم تظهر نتائج معنوية عند معاملتها بالبربانيـــل كب أ$_{٢}$) ولكن عند معامله بادرات الدنيبة والارز بمخلوط من بروبانيل ـ كب أ$_{٢}$ مـــع ثيوبنكارب ظهرت اعراض الاصابة على النباتات مشابهه تقريبا لتلك الناتجه بالمعاملـــه بالثيوبنكارب •

ويخلط البربانيل ـ كب أ$_{٢}$ مع المبيدات الحشرية المختبره اظهرت النتائـــــج انخفاض طفيف فى الوزن الجاف ومحصول الارز تراوح مابين ١ ـ ٣ ٪ بعد المعاملـــــه بالمبيدات الحشرية سواء كانت المعامله قبل او بعد البربانيل بمده ١ ـ ٤ ايام علـــى التوالى وكانت النتائج المتحصل عليها غير معنوية •

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RESPONSE OF BARNYARDGRASS (ECHINOCHLOA CRUS - GALLI) AND RICE (ORIZA SATIVA) TO SOME PETROLEUM AROMATIC DERIVATIVES AS HERBICIDES TREATMENTS

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SUMMARY

The present investigation was performed to synthesize and test some petroleum aromatic derivatives (propanil - SO_2 , sodium salt of propanil - SO_2 and O - , P - , & m - xylene sulphonamides) as herbicides on barnyardgrass (Echinochloa crus - galli (L.) P. Beauv.) and rice (Oryza sativa) cv Giza 176.

The application of the synthesized herbicides propanil - SO_2 and its sodium salt have no effect on fresh and dry weights of barnyardgrass at one - two - and three - leaf stage when applied at the rates of 0.70, 1.05, 1.40 and 1.75 Kg ai/fed. Rice plants showed the same trend toward propanil - SO_2 and its sodium salt as barnyardgrass.

By appling either O-, m- or p- xylene sulphonamides at the rates of 250, 500 and 1000 ppm to barnyard grass at half -, one - and three - leaf stage, the m- isomer was the only herbicide affected the growth of the grass when applied at 500 and 1000 ppm.

Rice plants showed no response toward the O-, P- and m- xylene sulphonamides at all rates and times of application.

INTRODUCTION

Rice (Oryza sativa) is economically an important crop from production, consumption and export point of view in Egypt. It is grown in 420,000 hectare annually. Its production is best suitable to the soil climatic conditions of the Nile-Delta, where 97% of the Egyptian rice is produced in six governorates.

Weeds are one of the major causes of grain yield losses in rice culture in Egypt. The major weed problem is barnyardgrass (Echinochloa crus-galli (L.) P. Beauv.) Hassan and Mahrous, 1989. The ecological requirements of barnyardgrass and rice are very similar : this leads to high competition and as a result to potentially great yield losses.

Herbicides are classified into two major groups, inorganic and organic. Their application involves four categories : band, broadcast, spot treatments, and directed spraying (Ware, 1983).

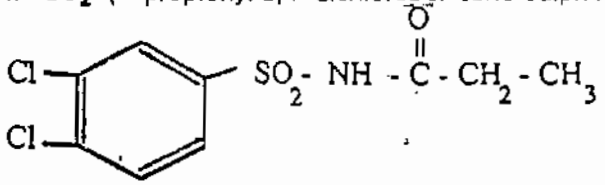
It was reported that the commercial herbicide stam F-34 (propanil) has a significant effect in controlling a broad spectrum of weeds especially that in rice fields (Matsunaka, 1965 and yih et al., 1968 a).

In the present investigation it was plained to prepare and test some petroleum aromatic derivatives (Propanil - SO_2, sodium salt of propanil - SO_2 and O-, p- and m- xylene sulphonamides) as herbicides on barnyardgrass (Echinochloa crus - galli) and rice (Oryza sativa).

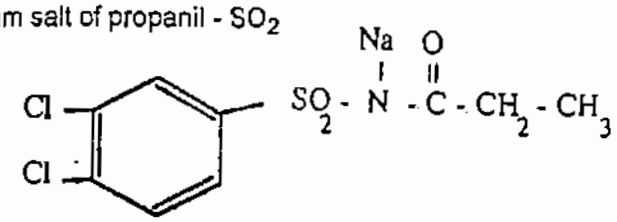
MATERIALS AND METHODS

ne synthe sized herbicides:

1 - Propanil - SO_2 (N-propionyl 3,4- dichlorobenzene sulphonamide).



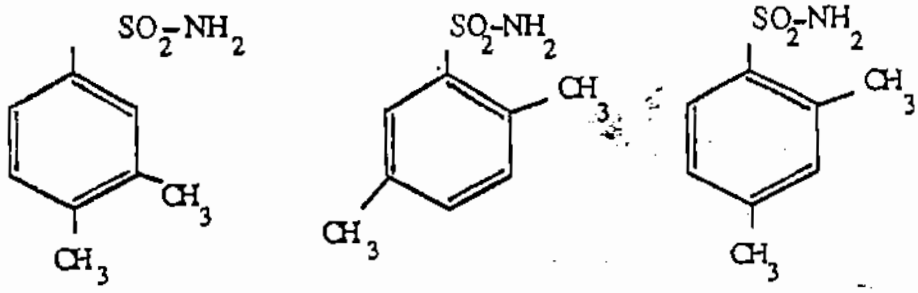
2 - Sodium salt of propanil - SO_2



3 - O- xylene sulphonamide (3,4- sulphonamide)

4 - P- xylene sulphonamide (2,5- sulphonamide).

5 - m- xylene sulphonamide (2,4- sulphonamide).



3,4 - Xylene sulphonamide | 2,5 - Xylene sulphonamide | 2,4 - Xylene sulphonamide

o - Xylene sulphonamide | p - Xylene sulphonamide | m - Xylene sulphonamide

Propanil - SO_2 and its sodium salt were prepared using the methods adopted by somasekhara, 1968 and El-Dib, 1978. The compounds of o- , p- and m- xylene sulphonamides were prepared according to the methods described by (Hurt and Allison, 1939).

Green house experiments:

The herbicidal efficiency of the synthesized herbicides as well as the commercial herbicide propanil (3,4 - dichloropropionanilide) (Stam F- 34) were tested on barnyardgrass (Echinochloa crus-galli (L.) P. Beaur) and Rice (Oryza sativa) cv Giza 176 under greenhouse conditions at Rice Research and Training Centre, Sakka, Kafr El-Sheikh. Seeds were obtained from the same Research Centre. Plants were grown in cylindrical plastic pots 20 cm depth and 20 cm diameter. In each pot 4 Kg of clay loam soil was placed. Some chemical analysis of the used soil was as follows:

PH	Ec ds/m	Co_3 Meq/L	HCo_3 Meq/L	OM %	NH_4 ppm	No_3 ppm	P ppm	K ppm	Zn ppm
7.8	1.1	1.0	1.9	2.1	2.9	19.3	11	625	1.9

Fifteen seeds of each of barnyardgrass and rice were planted per pot at a depth of 1-1.5 cm. After emergence the seedlings were thinned to 10 plants per pot. The plants were grown in a greenhouse (average temperature 28 ± 2°C during the day time and 18°C at night).

Pots were irrigated by saturating the soil several times during the early season to facilitate germination, emergence and growth of young barnyardgrass and rice seedlings. It was flooded first when the plants ranged from 15 to 25 cm tall. Water was maintained on the plants during the season except for draining one day before and reflooding one day after each treatment. Water was drained

from all pots in the fall after rice matured. Rice plants received 40 Kg. nitrogen / fed as urea 46% at 25 days after seeding.

The synthesized herbicides o-, p- and m- xylene sulphonamides were applied us as ppm, while propanil SO_2, sodium salt of propanil SO_2 commercial herbicides was used as Kg ai / fed in a total water emulsion of 200 L / fed., with a Co_2 - pressurized back - pack sprayer at 2.11 Kg / cm^2 fitted with flat - fan spray nozzles # 1004. The boarn was positioned 25 cm above each pot.

The herbicides were applied at half -, one -, two - and three - leaf stages of barnyardgrass and rice at different rates.

All treatments were applied postemergence as sprays to unflooded pots through CO_2 back - pack sprayer.

The eaperimental design was randomized complete block with 10 replications. The pots were rated visually for plants injury withen 7 days after herbicide application.

The results were statistically analyzed and significance of treatment differences was estimated through L.S.D. (Snadecor and Cochron, 1969).

Estimations:

Fresh and dry weights of shoots of barnyardgrass and rice were recorded at 30 days after seeding. Rice plant height and leaf area were recorded at 30 days after seeding however, total chlorophyll content of, rice leaves were

estimated at 45 days old according to the procedure of (Arnon, 1959). Leaf area was determined using LI - 3100 Area Meter.

At maturity the harvested grains were adjusted to 14% moisture (Smith, 1974). Rice grain yield in grams per pot were abtained. Total nitrogen content of rice grains was determined (Jackson, 1967). The starck content of rice grains was estimated using the method of (Yoschida et. al., 1972).

RESULTS AND DISCUSSION

The present study was aimed to drive more knowledge about the utilization of the synthesized petroleum aromatic derivatives (propanil - SO_2, sodium salt of propanil - SO_2, and o-, p - & m - xylene sulphonamides) as herbicides for managing barnyardgrass which causes severe losses in rice crop yield.

Effect of propanil and thesynthesized herbicides propanil - SO_2 and sodium salt of propanil - SO_2 on barnyardgrass (Echinochloa crus - galli (L.) P. Beauv.) and rice (Oryza sativa L.).

A. Barnyardgrass:

Table (1) illustrated the effect of propanil and the synthesized herbicides propanil - SO_2 and sodium salt of propanil - SO_2 on the fresh and dry weights of barnyardgrass.

Barnyardgrass at one-, two - and three - leaf stage treated with propanil at rates of 0.70, 1.05, 1.40 and 1.75 Kg ai/fed was injured moderately to severely.

One to three days after propanil application, both barnyardgrass and rice showed scorching, necrosis and chlorosis.

Leaf chlorosis response of the treated plants might be attributed to the photosynthetic- inhibitory effect of propanil. These observations are in aggrement with previous investigations (Nakamura et al., 1968, Ashton and Crafts, 1981 and Matsunaka, 1981) who reported that propanil alters several biochemical changes including photosynthesis.

The injury symptoms of barnyardgrass increased with time till death at 4 - 7 days after propanil treatment. However, injured riceplants recovered at 7 - 15 days after treatment. It is clear from Table (1) that concentrations higher than 0.70 Kg ai/fed caused complete death of barnyardgrass.

It has been established that the synthesized chemical compounds contain one aryl group linked by $-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-NH$ radical have high significant effect on suppressing great varieties of bacteria and fungi (Swiss patent, 1949, Raffa, 1966 and Marei et al., 1981), Therefore, trials were made to test these derivatives as herbicides in rice crop.

The application of the synthesized herbicides propanil - SO_2 and its sodium salt have no effect on the fresh and dry weights of barnyardgrass at one-, two- and three- leaf stage when applied at the rates of 0.70, 1.05, 1.40 and 1.75 Kg ai/fed as shown in Table (1). The percent reduction ranged from 0 to 5% which was not singnificant.

Previous researchs proved the effectivness of propanil in controlling barnyardgrass (Matsunaka, 1965 and Yih et al., 1968 a). Sierra and Vega (1967) reported that propanil caused a reduction in dry weight of barnyardgrass which became more severe with increasing concentrations.

Propanil applied at 3 to 5 l b/acre controlled barnyardgrass one to three inches tall in the one - to four - leaf stage of growth (Smith et al., 1977).

Propanil was found to be a strong inhibitor of RNA and protein synthesis (Moreland et al., 1969). Transpiration has been reported to be reduced by propanil (Ivanova, 1970). In addition abnormal and degenerating chloroplasts as well as deteriorating cellular membranes were found following the use of propanil (Ashton and Crafts, 1981).

B. Rice:

Tables (2&3) showed the effect of time and rate of propanil application on the vegetative growth of rice. The percent reduction in plant height ranged from (1 to 79%), fresh weight (1 to 81%), dry weight (1 to 80%) and leaf area (1 to 89%). Generally the reduction decreased as the rate of the herbicide increased from 0.70 to 1.75 Kg ai/fed; it decreased as the treatment was delayed from one- to three- leaf stage. Rice plants did not show any response toward the synthesized herbicides (propanil - SO_2 and its sodium salt) at different rates and times of application. The total chlorophyll content of rice leaves was reduced by 20% when propanil applied at rates of 1.05, 1.4 and 1.75 Kg ai/fed. However, propanil- SO_2 and its sodium salt reduced the total chlorophyll content by 20% only at 1.75 Kg ai/fed as shown in Table (3).

Rice grain yield generally reflects the level of the vegetative gowth as affected by propanil, propanil - SO_2 and sodium salt of propanil - SO_2 . Time of application and rate of propanil affected grain yield when applied at one-leaf stage, where the percent reduction ranged from 53 to 85% only at higher rates 1.05, 1.40 and 1.75 Kg ai/fed. The reduction decreased as the time of application was delayed at two - and three - leaf stage of growth. However, rice grain yield was not affected due to the application of propanil - SO_2 and its sodium salt at all rates and times of application as shown in Table (4).

It is clear from the above results that rice plants are more tolerant of propanil than barnyardgrass at the same stage of growth. The injury symptoms exhibited by rice plants after propanil treatment were inconsistent. Even when the injury seems severe rice plants usually recover within two weeks and new leaves that emerge after treatment show no injury symptoms. This may be due to the inhibitory effect of rice enzyme arylacylamidase which was observed to detoxify propanil).

These results were in accordance with previous research reports (Frear and Still, 1968, Akatsuka et al., 1973 and Kodama and Akatsuka, 1975). They found that, in the leaves of rice an arylacylamidase enzyme detoxifies propanil to 3,4 - dichloroaniline and propionic acid, while barnyardgrass leaves contained small amount of the same enzyme.

Previous investigators (Adachi et al. 1966, Still and Kuzirian, 1967 and Yih et al., 1968 b) stated that rice was at least 20 times more effective than barnyardgrass in inactivating propanil which selectively kills barnyardgrass, while rice was slightly injured.

Temporary chlorosis and tip burn on rice leaves may occur soon after treatments with propanil at rates of 3 to 5 lb/acre, but permanant injury does not occur even at rates as high as 12 lb/acre (Smith et al., 1977).

Effect of the synthesized herbicides o-, p- and m- xylene sulphonamides on barnyardgrass and rice.

A. Barnyardgrass:

Table (5), and Photo. (1 & 2) showed that the synthesized herbicides 0- and p- xylene suplhonamides at rates of 250, 500 & 1000 ppm applied to barnyardgrass at half-, one- and two- leaf stage of growth failed to control barnyardgrass satisfactory where no changes were observed on either the morphological characters or the fresh and dry weights of barnyardgrass, while the m-isomer was the only herbicide affected the growth of barnyardgrass. The plants were stunted within 1 to 3 days following the application of the m-xylene sulphonamide compared with the untreated control. These symptoms increased with time until death. The m-xylene sulphonamide caused greater phytotoxicity on the fresh and dry weights of barnyardgrass. Generally barnardgrass - control increased as the rate of the m-xylene sulphonamide increased from 250 to 1000 ppm at lower stage of growth. The m-xylene sulphonamide controlled barnyardgrass effectively by 29 and 87% decrease from the untreated control in the half - leaf stage of growth at the rates of 500 and 1000 ppm respectively. The effect decreased as the treatment was delayed from half- to two- leaf stage of growth.

It is clear from the above results that m-xylene sulphonamide controlled barnyardgrass more than o- and p-xylene sulphonamides. This may be attributed to the greater solubility of m-xylene sulphonamide in methyl and ethyl alcohols than o- and p-xylene sulphonamides.

B.Rice:

The data in Tables (6 & 7), illustrated that rice plants showed no response toward the synthesized herbicides o- , p- and m-xylene sulphonamides at all rates and times of application. The m-xylene sulphonamide caused slight stunting of rice plants within 1 - 3 days after treatments rather than o- and p-isomers. The injured rice plants recovered within 7 - 15 days and the new leaves that emerged after treatments were uninjured.

The effect of the synthesized herbicides o- , p- and m- xylene sulphonamides on rice grain yield and total nitrogen content, crude protein & starch content of rice grains was illustrated in Table (8). It was clear that rice grain yield showed no response toward the tested herbicides at all rates used. The percent reduction in rice grain yield at half-, one- and two- leaf stage of growth ranged from 0 - 8%, while the percent reduction in total nitrogen content and starch content ranged from 0 - 10% and 0 - 7% respectively. However, all these differences were statistically non-significant.

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Table : 1 Effect of propanil and the synthesized herbicides propanil - SO_2 and sodium salt of propanil - SO_2, on the fresh and dry weights of barnyardgrass(Echinochloa crus-galli) at 30 days after seeding.

Herbicides	Rate of application Kg ai /fed	Fresh weight (g / pot)						Dry weight (g / pot)					
		Leaf stage & % reduction						Leaf stage & % reduction					
		1 lf	%	2 lf	%	3 lf	%	1 lf	%	2 lf	%	3 lf	%
Control (untreated)	-	23.9	0	23.9	0	23.9	0	3.95	0	3.95	0	3.95	0
Propanil	0.70	6.01	75	8.7	64	13	46	0.98	75	1.45	63	2.17	45
	1.05	2.4	90	3.5	85	8.7	64	0.4	90	0.6	85	1.42	64
	1.40	0	100	0	100	2.6	89	0	100	0	100	0.45	89
	1.75	0	100	0	100	0	100	0	100	0	100	0	100
Propanil- SO_2	0.70	23.9	0	24	0	24	0	3.95	0	3.94	0	4.0	0
	1.05	23.7	1	23.8	1	23.9	0	3.90	1	3.91	1	3.91	1
	1.40	23.2	3	23.3	3	23.5	2	3.85	3	3.83	3	3.89	2
	1.75	22.7	5	22.8	5	23.1	3	3.76	5	3.79	4	3.87	2
Sodium salt of propanil - SO_2	0.70	23.9	0	23.9	0	24	0	3.94	0	4.0	0	4.0	0
	1.05	23.6	1	23.5	2	23.8	1	3.89	2	3.90	1	3.90	1
	1.40	22.9	4	23	4	23.5	2	3.82	3	3.84	3	3.88	2
	1.75	22.7	5	22.8	5	23.3	3	3.77	5	3.78	4	3.86	2
$L.S.D_{0.05}$		3.2		4.7		5.9		0.45		0.68		0.83	

Table : 2 Effect of propanil and the synthesized herbicides propanil - SO_2 and sodium salt of propanil - SO_2 on plant height, fresh weight and dry weight of rice (Oryza sativa) cv Giza 176 at 30 days after seeding.

Herbicides	Rate of application Kg ai /fed	Plant height (cm/plant)						Fresh weight (g/pot)						Dry weight (g/pot)					
		Leaf stage & % reduction						Leaf stage & % reduction						Leaf stage & % reduction					
		1 lf	%	2 lf	%	3 lf	%	1 lf	%	2 lf	%	3 lf	%	1 lf	%	2 lf	%	3 lf	%
Control (untreated)	-	25	0	25	0	25	0	17	0	17	0	17	0	2.87	0	2.87	0	2.87	0
Propanil	0.70	19	24	22.9	8	24.6	1	12.8	24	16.1	5	16.9	1	2.14	25	2.71	6	2.83	1
	1.05	11.3	55	22	12	23.9	4	8.5	50	15.3	10	16.3	4	1.43	50	2.67	7	2.74	5
	1.40	6.8	73	19.3	23	21	16	6.1	64	12.8	24	14.8	13	1.03	64	2.24	23	2.51	13
	1.75	5.3	79	17	32	19	24	3.2	81	12.2	28	13.5	21	0.57	80	2.07	28	2.3	20
Propanil- SO_2	0.70	25	0	24.9	0	25	0	16.8	1	16.9	1	17	0	2.89	0	2.90	0	2.88	0
	1.05	24.1	3	24.5	2	24.7	1	16.6	2	16.8	1	16.9	1	2.86	1	2.84	1	2.85	1
	1.40	23.5	6	23.3	6	24.1	3	16.1	5	16.3	4	16.7	2	2.75	5	2.79	4	2.81	2
	1.75	22.6	9	22.8	8	23.9	4	15.8	7	15.9	6	16.3	4	2.69	7	2.73	6	2.78	4
Sodium salt of propanil - SO_2	0.70	25	0	25	0	24.9	0	16.9	1	16.9	1	17	0	2.90	0	2.88	0	2.90	0
	1.05	24.5	2	24.3	2	24.3	2	16.5	3	16.6	2	16.8	1	2.83	2	2.85	1	2.84	1
	1.40	23.1	7	23.6	5	24	4	16	6	16.1	5	16.6	2	2.73	6	2.75	5	2.79	4
	1.75	22.7	9	22.9	8	23.8	4	15.7	7	15.8	7	16.2	5	2.66	8	2.69	7	2.77	4
$L.S.D_{0.05}$		3.8		3.6		N.S		2.57		2.41		N.S		0.39		0.47		N.S	

Table 3-: Effect of propanil and the synthesized herbicides propanil - SO_2 and sodium salt of propanil - SO_2 on leaf area and total chlorophyll content of rice (Oryza sativa) cv Giza 176.

Herbicides	Rate of application Kg ai / fed	Leaf area (cm^2 / pot) at 30 days after seeding						Total * chlorophyll mg/g fresh wt.	
		Leaf stage & % reduction							
		1 lf	%	2 lf	%	3 lf	%	2lf	% red
Control (untreated)	-	85	0	85	0	85	0	5	0
Propanil	0.70	57	33	81	5	84	1	5	0
	1.05	32	62	68	20	83	2	4	20
	1.40	16	81	57	33	81	5	4	20
	1.75	9	89	44	48	76	11	4	20
Propanil- SO_2	0.70	84	1	86	0	86	0	6	0
	1.05	84	1	84	1	85	0	5	0
	1.40	82	4	82	4	84	1	5	0
	1.75	80	6	81	5	83	2	4	20
Sodium salt of propanil- SO_2	0.70	84	1	85	0	85	0	6	0
	1.05	83	2	84	1	84	1	5	0
	1.40	81	5	82	4	84	1	5	0
	1.75	80	6	80	6	83	2	4	20
$L.S.D_{0.05}$		9		7		N.S		N.S	

* At 45 days after seeding

Table : 4 Effect of propanil and the synthesized herbicides propanil- SO_2 and sodium salt of propanil -SO_2 on grain yield and total nitrogen, crude protein & starch content of rice grains(Oryza sativa) cv Giza 176.

Herbicides	Rate of application Kg ai /fed	Grain yield (g / pot)						Total nitrogen		Crude protein		Starch content	
		Leaf stage & % reduction						mg / g grain					
		1 lf	%	2 lf	%	3 lf	%	2 lf	% red	2 lf	% red	2 lf	% red
Control (untreated)	-	34	0	34	0	34	0	21	0	125	0	43	0
Propanil	0.70	22	35	33	3	33	3	19	10	119	5	40	7
	1.05	16	53	30	12	32	6	18	14	113	10	40	7
	1.40	12	65	27	21	31	9	17	19	106	15	39	9
	1.75	5	85	24	29	30	12	17	19	106	15	39	9
Propanil- SO_2	0.70	34	0	35	0	35	0	21	0	126	0	43	0
	1.05	33	3	33	3	34	0	21	0	125	0	42	2
	1.40	32	6	33	3	33	3	19	10	119	5	42	2
	1.75	31	9	32	6	33	3	19	10	119	5	41	5
Sodium salt of propanil - SO_2	0.70	34	0	34	0	34	0	21	0	125	0	43	0
	1.05	33	3	33	3	34	0	20	5	125	0	42	2
	1.40	32	6	32	6	33	3	19	10	119	5	41	5
	1.75	31	9	32	6	33	3	19	10	119	5	41	5
$L.S.D_{0.05}$		5		4		N.S		N.S		N.S		N.S	

Table : 5 Effect of the synthesized herbicides o -, p -, and m- xylene sulphonamides on the fresh and dry weights of barnyardgrass (Echinochloa crus-galli) at 30 days after seeding .

Herbicides	Rate of application (ppm)	Fresh weight (g / pot)						Dry weight (g / pot)					
		Leaf stage & % reduction						Leaf stage & % reduction					
		1/2 lf	%	1 lf	%	2 lf	%	1/2 lf	%	1 lf	%	2 lf	%
Control (untreated)	-	17.1	0	17.1	0	17.1	0	2.83	0	2.83	0	2.83	0
o-Xylene sulphonamide	250	16.9	1	17	1	17.1	0	2.8	1	2.81	1	2.85	0
	500	16.5	4	16.8	2	17	1	2.76	3	2.78	2	2.8	1
	1000	16	6	16	6	16.6	3	2.65	6	2.66	6	2.74	3
p-Xylene sulphonamide	250	16.9	1	17	1	17.1	0	2.8	1	2.81	1	2.9	0
	500	16.3	5	16.8	2	16.8	2	2.68	5	2.77	2	2.78	2
	1000	15.9	7	15.9	7	16.1	6	2.63	7	2.65	6	2.68	5
m-Xylene sulphonamide	250	16.5	4	16.8	2	16.9	1	2.75	3	2.77	2	2.85	0
	500	12.1	29	16.1	6	16.3	5	2.0	29	2.69	5	2.7	5
	1000	2.3	87	15.8	8	16	6	0.38	87	2.6	8	2.66	6
$L.S.D_{0.05}$		5		N.S		N.S		0.5		N.S		N.S	

Table: 6 Effect of the synthesized herbicides o -, p -, and m- xylene sulphonamides on plant height, fresh weight and dry weight of rice (Oryza sativa) cv Giza 176 at 30 days after seeding.

Herbicides	Rate of application (ppm)	Plant height (cm / Plant)						Fresh weight (g / pot)						Dry weight (g / pot)					
		Leaf stage & % reduction						Leaf stage & % reduction						Leaf stage & % reduction					
		1/2 lf	%	1 lf	%	2 lf	%	1/2 lf	%	1 lf	%	2 lf	%	1/2 lf	%	1 lf	%	2 lf	%
Control (untreated)	-	24.9	0	24.9	0	24.9	0	11	0	11	0	11	0	1.83	0	1.83	0	1.83	0
o-Xylene sulphonamide	250	25	0	24.9	0	25	0	11	0	11.1	0	11.1	0	1.84	0	1.83	0	1.84	0
	500	24.5	2	24.7	1	24.8	0	10.9	1	10.9	1	10.9	1	1.81	1	1.82	1	1.82	1
	1000	23.9	4	24	4	24.5	2	10.7	3	10.8	2	10.9	1	1.78	3	1.80	2	1.81	1
p-Xylene sulphonamide	250	24.9	0	25.1	0	25.1	0	11.1	0	11.1	0	11	0	1.83	0	1.83	0	1.83	0
	500	24.3	2	24.5	2	24.7	1	10.8	2	10.9	1	10.9	1	1.82	1	1.81	1	1.81	1
	1000	23.8	4	24	4	24.4	2	10.6	4	10.8	2	10.8	2	1.77	3	1.79	2	1.81	1
m-Xylene sulphonamide	250	24.8	0	24.9	0	25	0	10.9	1	11	0	11	0	1.83	0	1.83	0	1.83	0
	500	24	4	24.1	3	24.7	1	10.7	3	10.8	2	10.9	1	1.77	3	1.79	2	1.82	1
	1000	23.6	5	23.8	4	24.3	2	10.5	5	10.7	3	10.8	2	1.75	4	1.77	3	1.81	1
$L.S.D_{0.05}$		N.S		N.S		N.S		N.S		N.S		N.S		N.S		N.S		N.S	

Table 7 : Effect of the synthesized herbicides o -, p -, and m-xylene sulphonamides on leaf area and total chlorophyll content of rice (Oryza sativa) cv Giza 176.

Herbicides	Rate of application (ppm)	Leaf area (cm^2 / pot) at 30 days after seeding						Total * chlorophyll mg/g fresh wt.	
		Leaf stage & % reduction							
		1/2 lf	%	1 lf	%	2 lf	%	2lf	% red
Control (untreated)	-	82	0	82	0	82	0	5	0
o-Xylene sulphonamide	250	82	0	83	0	82	0	6	0
	500	80	2	81	1	81	1	5	0
	1000	79	4	79	4	80	2	4	20
p-Xylene sulphonamide	250	83	0	82	0	83	0	6	0
	500	81	1	80	2	82	0	5	0
	1000	78	5	79	4	80	2	4	20
m-Xylene sulphonamide	250	81	1	82	0	82	0	5	0
	500	79	4	79	4	81	1	5	0
	1000	77	6	78	5	80	2	4	20
$L.S.D_{0.05}$		N.S		N.S		N.S		N.S	

* At 45 days after seeding

Table 8 : Effect of the synthesized herbicides o-, p-, and m-xylene sulphonamides on grain yield and total nitrogen, crude protein & starch content of rice grains(Oryza sativa) cv Giza 176.

Herbicides	Rate of application (ppm)	Grain yield (g / pot)						Total nitrogen		Crude protein		Starch content	
		Leaf stage & % reduction						mg / g grain					
		1/2 lf	%	1 lf	%	2 lf	%	2 lf	% red	2 lf	% red	2 lf	% red
Control (untreated)	-	40	0	40	0	40	0	21	0	125	0	42	0
o-Xylene sulphonamide	250	39	3	41	0	41	0	20	5	125	0	42	0
	500	38	5	39	3	40	0	19	10	119	5	40	5
	1000	37	8	38	5	39	3	19	10	119	5	40	5
p-Xylene sulphonamide	250	39	3	40	0	41	0	20	5	125	0	42	0
	500	38	5	38	5	40	0	19	10	119	5	40	5
	1000	37	8	38	5	39	3	18	14	113	10	39	7
m-Xylene sulphonamide	250	38	5	39	3	40	0	19	10	119	5	41	2
	500	37	8	38	5	39	3	18	14	113	10	39	7
	1000	37	8	37	8	38	5	18	14	113	10	39	7
$L.S.D_{0.05}$		N.S		N.S		N.S		N.S		N.S		N.S	

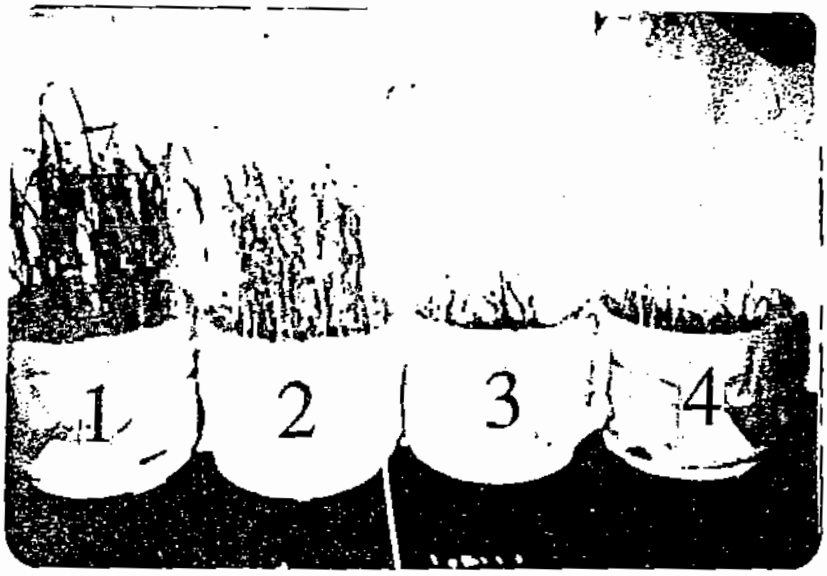

Photo (1) Effect of the synthesized herbicide m - xylene sulphonamide applied at 250, 500, 1000 ppm on the growth of barnyardgrass (Echinochloa crus-galli) at half- leaf stage.

Photo (2) Effect of the synthesized herbicide m - xylene sulphonamide applied at 250, 500, 1000 ppm on the growth of barnyardgrass (Echinochloa crus-galli) at one - leaf stage.

1. Control
2. At 250 ppm
3. At 500 ppm
4. At 1000 ppm

استجابة الدنيبه (ايكانوكلوا كرس جالى) والارز (أوريزا ساتيفا) لبعض المشتقات البترولية العطرية كمبيدات حشائش

زينب يوسف محمد بكر - مهرشان طه المقدم - فايدة احمد شراره

الملخص العربى

تم فى هذا البحث تخليق بعض المشتقات العطرية البترولية (بربانيل - كب أ٢ والملح الصوديومى له وأرثو.وبارا وميتا زيلين سلفونامید . واختبارها كمبيدات للحشائش على حشيشه الدنيبه والارز .

لوحظ عدم تأثر كل من نباتات الدنيبه والارز فى مراحل النمو المختلفة (١ ، ٢ ، ٣ ورقة) عند معاملتها بتركيزات متعدده (٧ر ، ١ر٠٥ ، ١ر٤ ، ١ر٧٥ كجم مادة فعاله للفدان) من المبيدات المحضره معمليا (بربانيل - كب أ٢ والملح الصوديومى له) .

وباستخدام المبيدات المخلقه (ارثو وميتا وبارا زيلين سلفونامید برشها على حشيشة الدنيبه بتركيزات ٢٥٠ ، ٥٠٠ ، ١٠٠٠ جزء فى المليون اثناء مراحل نمو مختلفة (½ ، ١ ، ٢ ورقه) وجد ان المركب ميتا - زيلين سلفونامید اكثرهم فعاليه عند تركيز ٥٠٠ ، ١٠٠٠ جزء فى المليون حيث تأثر به نمو حشيشه الدنيبه .

كما لوحظ ايضا عدم استجابة نباتات الارز عند رشها بأرثو وبارا وميتا - زيلين سلفونامید عند معاملتها بكل التركيزات فى جميع مراحل النمو المختلفة .

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SPECTROPHOTOMETRIC STUDY ON PINACYANOL CHLORIDE AT DIFFERENT TEMPERATURES.

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Spectral changes of a cyanine dye, pinacyanol chloride, were observed. The aggregation ability of the dye in water, where only the monomer-dimer equilibrium exists, was investigated.The equilibrium constant, K_a, and thermodynamic parameters of dimerization were determined. The influence of the dye concentration and temperature variation on the dye aggregation was discussed. The results show that the dimer formation is favoured by negative enthalpy and by increase in entropy.

INTRODUCTION:

Very little work on cyanine dyes has been carried out with regard to their aggregation in aqueous and non aqueous solutions.Cyanines are currently investigated as sensitizers of solar-energy conversion systems through photochemical processes, with special attention being paid to their J-aggregates[1,2].

Cyanine dyes exist in solution in the monomeric form in equilibrium with the dimeric aggregates and/or the poly-

aggregates [2-5]. The concentration of the dyes in solution strongly affects these equilibria:

$$KD \rightleftharpoons m\ [D.D] \rightleftharpoons n\ [DD....D]$$

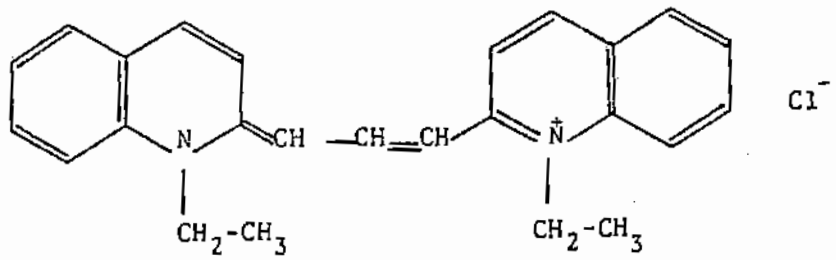
The monomeric form persists in diluted solution, while the aggregated forms predominate in concentrated solutions. Aggregation of pinacyanol chloride in aqueous SiO_2-colloids showed that the aggregation process is a result of high local dye concentration in the vicinity of the colloid owing to the binding of the positively charged dyes to the negatively charged colloidal interface[1].

Absorption spectra of the cyanine dyes are strongly affected by the aggregation[6]. A variety of environmental factors such as hydrophobic dispersions and electrostatic factors affect the dye-dye interactions[3]. It is well known that solutions of cyanine dyes in polar organic solvents at room temperature follow Beer's law over an extended concentration range[5]. The effect of concentration on the aggregation of the Pyronine G dye has been studied using spectrophotometry[7]. It was shown that the dye spectra can be interpreted as being those of the dimerized dye.

In the present investigation we report a study on the effect of concentration, solvents and temperature variations on the aggregation parameters of pinacyanol chloride.

EXPERIMENTAL:

Quinaldine Blue, C.I. pinacyanol chloride, was obtained from Sigma Chemical Company, England. It was used as received. Its structure is:



Analar acetone was used. The spectrophotometric measurements were carried out on a Perkin-Elmer Spectrophotometer model 555. Quartz cells of 0.5 and 1.0 cm standard path length were used.

The dye solutions were prepared in distilled water and in acetone. The dye concentration range extended from 0.04-1.2 X 10^{-4} M/dm^3 in H_2O and 0.1-3 X 10^{-5} M/dm^3 in acetone. The same measurements were carried out at different temperatures: 18, 25, 35 and 45°C. The aggregation parameters were calculated using the maximus slope method[9].

RESULTS AND DISCUSSIONS:

The spectra of pinacyanol chloride have been measured at various concentrations ranging from 0.04 to 1.2 X 10^{-4}

M/dm³ in distilled water and from 0.1 to 3 X 10^{-5} M/dm³ in acetone and at 18,25, 35 and 45°C. The spectra are shown in Fig.(1). It is quite clear that there are two λ_{max} for the dye in water at 600 and 547 nm and a shoulder at 520 nm. Pronounced effects of water on the absorption spectra of the dye can be noticed from the figures. The aggregation of the dye with increasing concentration is shown by the reduction in intensity of the molecular maximum at 600 nm and the appearance of a new maximum at 522 nm instead of a shoulder. These spectral changes may be attributed to the decrease of both the delocalization of the π-electron system and the charge transfer character of the two quinolinium moieties.

There is also a big shift and increase in intensity of the other band which appears in acetone at 560 nm and in water at 547 nm.

The dye solutions in acetone follow Beer's law at the concentration range 0.04 X 10^{-4} to 1.2 X 10^{-4} M/dm³. The shape of the spectrum in acetone is typical for the molecular spectrum of cyanine dyes[5]. In aqueous medium, however, on increasing the dye concentrations, a peak corresponding to an isobestic point appears at shorter wavelength. According to the well known properties of the isobestic point, two coloured species are in equilibrium, the monomer form and the dimer form of the dye[6].

In Fig.(1), the position of the peak at 600 nm is generally similar in water and in acetone either on increasing the dye concentration or the temperature. This indicates the predominance of the monomeric species of the dyes under these conditions. From the above results, the following monomer/dimer equilibrium can be assumed $2M \rightleftharpoons D$.

Hence, the monomer and the dimer concentrations (C_M and C_D respectively) change according to the law of mass action as follows:

$$K_n = C_D/C^2_M,$$

where K_n is the equilibrium constant for dimer formation. Plots of log C_D against log C_m at different temperatures and various concentrations are shown in Fig. (2). It is clear from the results that, for pinacyanol chloride, the slopes at 18 and 25°C have a value of 1.8. This value decreases on increasing the temperature to 45°C. These results are comparable with those obtained by other authors[1,2].

In the present study, the aggregation constant, K_n and the aggregation number, n, were determined using the maximum slope method according to the equation:

$$\log C\ (\epsilon_1 - \epsilon) = n \log C\ (\epsilon - \bar{\epsilon}_{\infty}) + \log nK_n\ (\epsilon_1 - \bar{\epsilon}_{\infty})^{1-n}$$

Where ϵ = experimentally measured molar absorptivity for dye solution.

ϵ_1 = monomer molar absorptivity.

ϵ_n = polymer molar absorptivity.

$\bar{\epsilon}_{no} = \epsilon_{n/n}$

This maximum slope method is described in detail elsewhere[8]. K_n, and n were calculated from the intercept and slope of the plots of log C $(\epsilon_1 - \epsilon)$ against log C $(\epsilon - \bar{\epsilon}_{no})$ Fig. (3). The results are shown in Table (1).

The standard free-energy change for the dimer formation, ΔG, and the change of entropy, ΔS, have been calculated using the standard equations:

$$\Delta G = - RT \ln K_n$$

and $$\Delta S = \frac{\Delta H - \Delta G}{T}$$

The values of ΔH were calculated by using the slope obtained from linear regression analysis.

The results in Table (1) showed that the dye dimerizes in aqueous solutions at 18°C. The increase of temperature causes the aggregation constant, K_n, to decrease significantly. This may be due to disaggregation on increasing the temperature which agrees with the results

obtained by other authors[9]. This can also be explained on the basis of the disruption of hydrogen bonds since the aggregation of the pinacyanol chloride is believed to be due to hydrogen bonding[1].

The calculated K, (9.8 X 10^4) for the pinacyanol chloride dye is found to be consistent with those for cyanine dyes[1,6,10]. For cyanine and thiocarbocyanine dyes the value of K were vary from 10^3 to 10^5.

The importance of London-Van der Waal's forces and the contribution of dispersion forces associated with delocalized electrons to the self association of dyes[10,11] is well recognized. The influence of hydrophobic forces may be included. Water molecules at the hydrocarbon portion of the dissolved dyes have been considered to form structured region of low entropy, the so-called icebergs. The hydrocarbon-hydrocarbon interactions would displace this water and causes the system to gain entropy, leading to dye association[2].

It is clear from this study that the dye is almost molecularly dispersed at all temperatures in the non-aqueous medium using acetone. However, in aqueous media it has been

suggested that the aggregation of dyes may involve sandwiched water molecules as intermediates[12]. So acetone may prevent aggregation by making such intermediates water molecules unavailable. The effect of acetone on icebergs around the dye ions should, on the other hand, affect both monomers and the aggregated species.

The thermodynamic parameters of aggregation of the dyes in aqueous and non aqueous solutions at different temperatures are given in Table (2). The results show that dimer formation is accompanied by positive entropy change. The most likely candidate for the source of this positive entropy change is the Frank and Evance type of icebergs or structured regions of water around non polar solute[13]. Addition of solvents to the dimeric systems causes disaggregation and makes the entropy more negative. Thus, there is less negative free energy change of formation corresponding to disaggregation.

The low values for ΔH indicate weakly bonded species[10]. The ΔH value may arise from the combination of several types of interactions, particularly dispersive interaction of London type and hydrogen bonding. Hence,the results obtained are comparable with those reported for the dye pyronine G[7] (ΔG = -18.9 KJ/mol, ΔH = -21 KJ/mol) and with the results obtained using other techniques[9].

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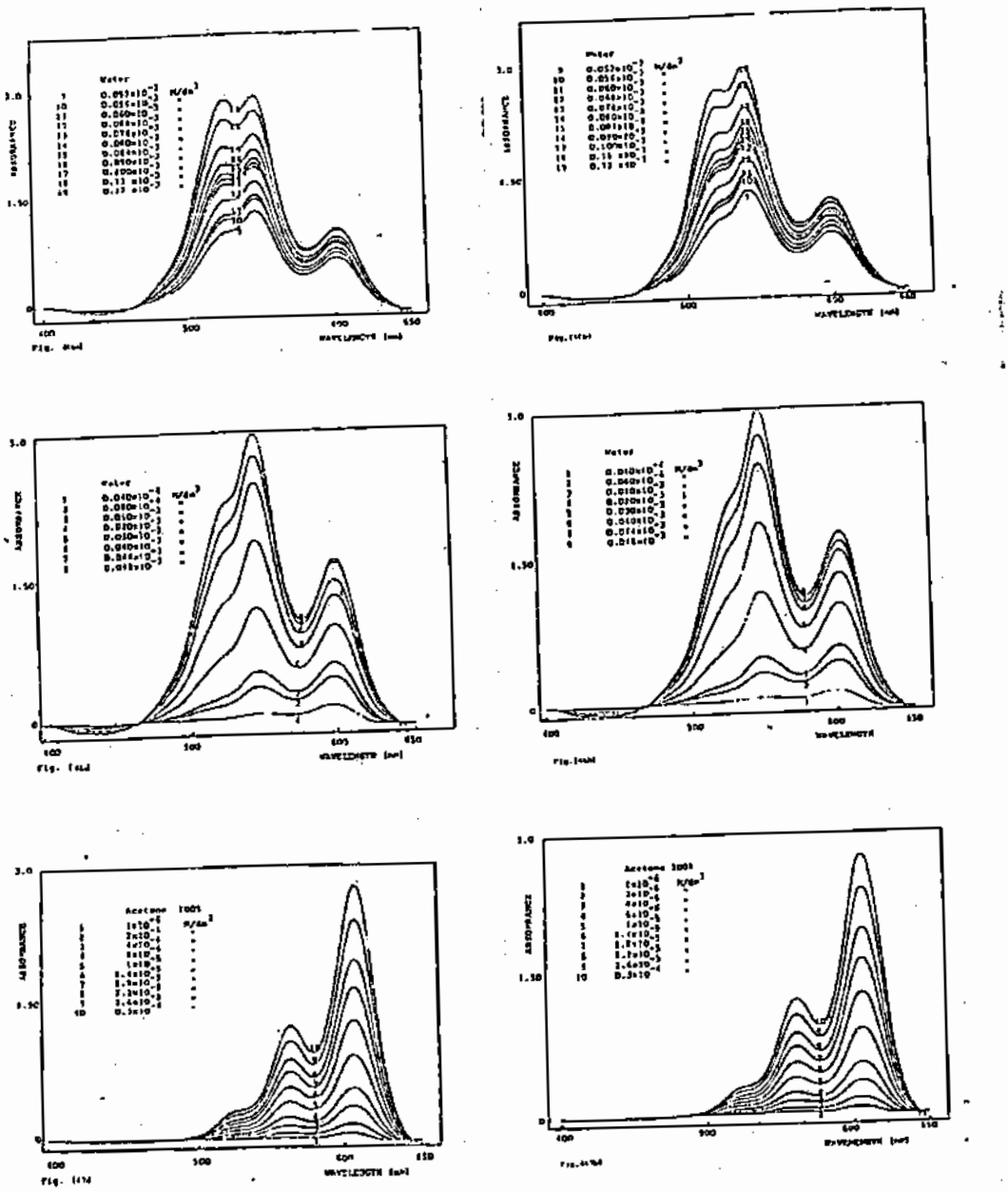
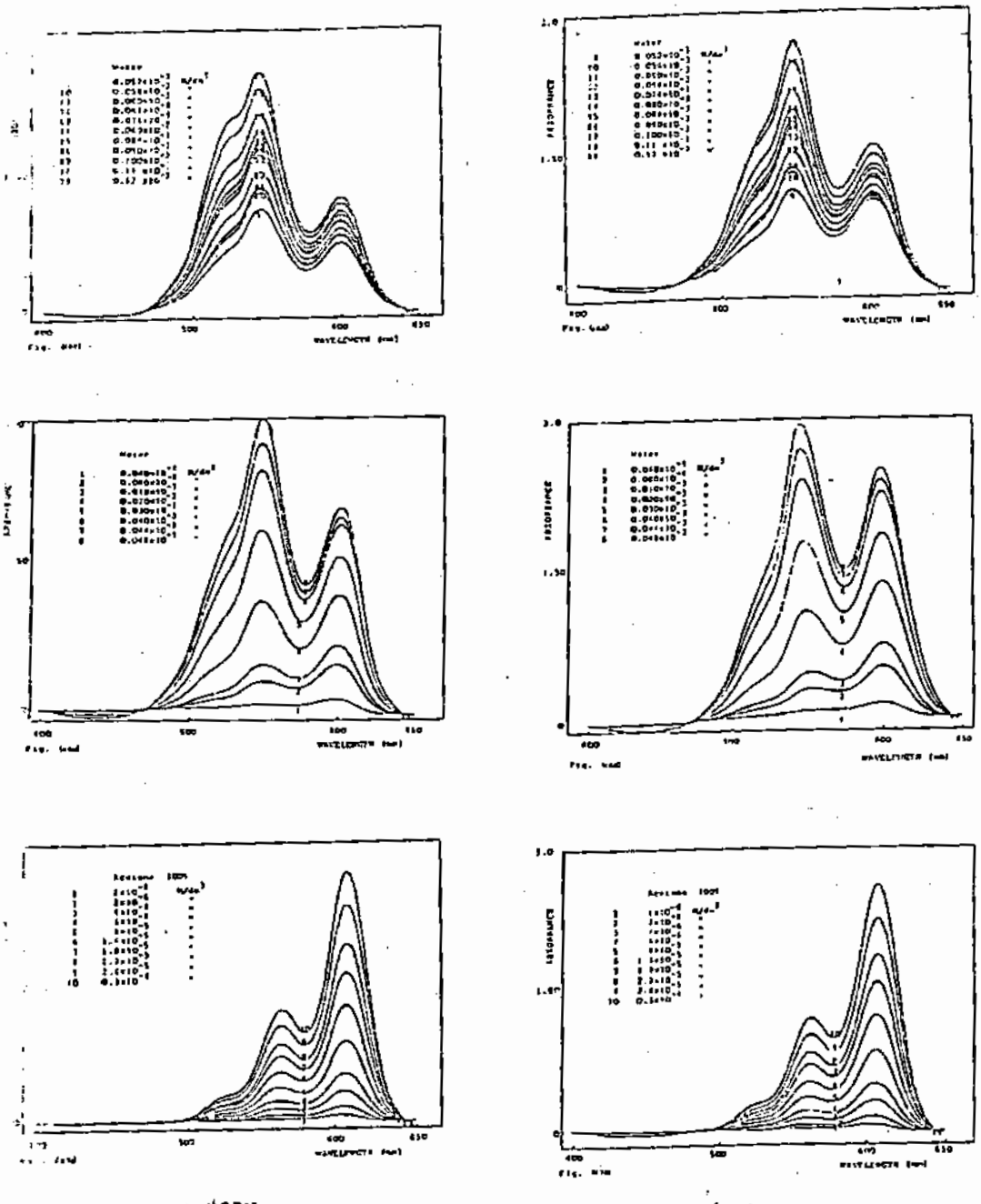


at 18°C at 25°C

Fig. (I) Absorption spectra of pinacyerol chloride at different temperature



at 35°C

at 45 °C

Fig. (I) Continued. Absorption Spectra of pinacyanol Chloride at different temperatures

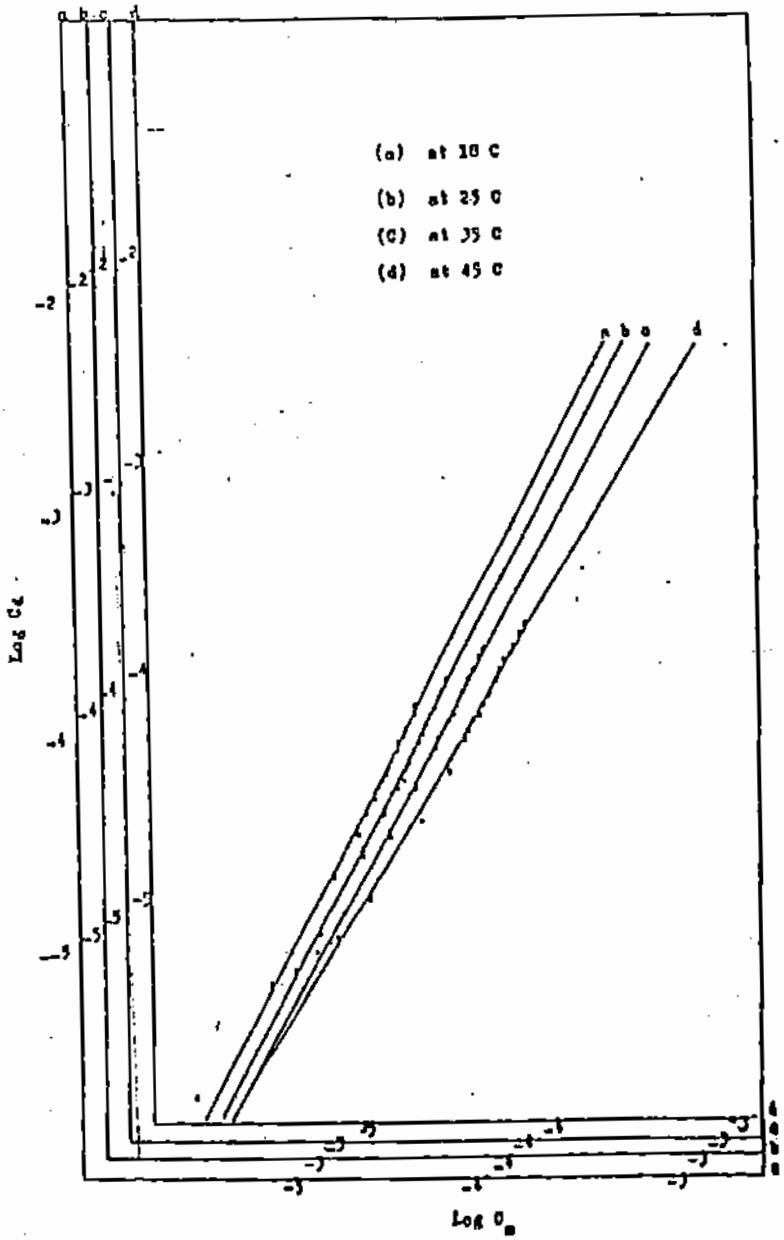
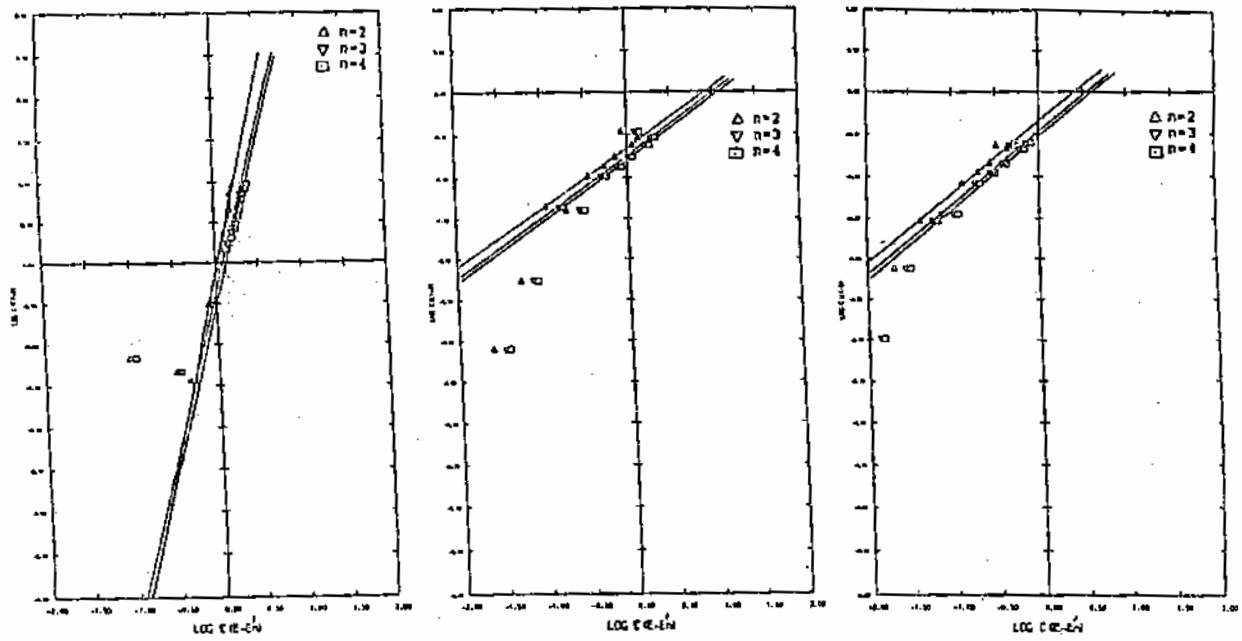


Fig.(2): Log θ_d Vs. Log C_m for Pinacyanol chloride in distilled water at different temperatures.



dist. water at 600 nm acetone 100% at 605 nm acetone 100% at 562 nm

Fig. (3): Relation between log C ($\epsilon_1 - \epsilon$) and log C ($\epsilon - \dot{\epsilon}_\infty$) in dist. water and acetone for pinacyanol chloride at 25 °C.

Table (1) The aggregation Constants and Aggregation Numbers for Pinacyanol Chloride From The Maximum Slope Method.

18°C.			25°C.		
Solv.Conc.(1V/V)	Agg.Cons.(K_n)	Agg.Number(n)	Solv.Conc.(1V/V)	Agg.Cons.(K_n)	Agg.Number(n)
Water	7.59×10^{-3}	2.4	Water	5.98×10^{-4}	2.2
Acetone 100(λ =605 nm)	0.0062×10^{2}	1.1	Acetone 100(λ =605 nm)	0.0052×10^{2}	1.0
Acetone 100(λ =562 nm)	0.0053×10^{2}	0.9	Acetone 100(λ =562 nm)	0.005×10^{2}	0.7
35°C			45°C		
Solv.Conc.(1V/V)	Agg.Cons.(K_c)	Agg.Number(n)	Solv.Conc.(1V/V)	Agg.Cons.(K_n)	Agg.Number(n)
Water	4.64×10^{-3}	2.0	Water	3.98×10^{-3}	2.0
Acetone 100(λ =605 nm)	0.0056×10^{2}	0.9	Acetone 100(λ =605 nm)	0.0040×10^{2}	0.9
Acetone 100(λ =562 nm)	0.0040×10^{2}	0.7	Acetone 100(λ =562 nm)	0.0030×10^{2}	0.7

Table (2) Thermodynamic Parameters for pinacyanol chloride.

Solvent (% V/V)	T= 291 °C		T= 298°C		T=303°C		T=318°C		
	ΔG KJ/mole	Δ S J/deg/mole	ΔG KJ/mole	ΔS J/deg/mole	Δ G KJ/mole	Δ S J/deg/ mole	Δ G KJ/mole	Δ S J/deg/ mole	ΔB KJ/mole
H_2O	-27.19	+30.94	-27.21	+30.28	-27.52	+30.29	-28.00	+30.85	-18.19
Acetone At 605nm.	-1.16	-37.44	-1.62	-36.58	-1.78	-35.39	-2.42	-34.40	12.64
At=562	-1.54	31.89	-1.72	31.14	-2.35	-30.13	-3.18	-29.18	-11.49

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PROLONGATION OF STORAGE PERIOD OF PEAR FRUITS THROUGH INACTIVATION OF CERTAIN ENZYMES BY USING ETHYLENE-INHIBITING SOLUTIONS

By

M. KORD AND T. HATHOUT

ABSTRACT

Ethylene-inhibiting substances such as n-propylagallate, silver nitrate and benzothiadiazole at different concentrations were sprayed on pear tree at 10, 15 days after full bloom. The fruits were harvested at maturity. Silver nitrate and benzothiadiazole at 100 mg L^{-1} concentration were found to be most effective in slowing down the polygalacturonase, cellulase and malic dehydrogenase enzyme activities of the fruit after harvest and during storage at 0±1 °C temperature and 90-95 percent relative humidity. These prolonged the shelf life of the fruits.

INTRODUCTION

Pear is one of the popular fruits. Though it has fairly good shelf-life but still it is desirable to further enhance the shelf-life, so that the fruits may be available for longer period for consumption and also can fetch better price. Several post-harvest treatments like use of calcium compounds, ethylene-inhibiting solutions, storage in perforated polyethylene bags and use of growth regulators are reported to extend the storage life of fruits (Ahlawat et al., 1984, Banik et al., 1987, 88; Singh, 1988, Kumar and Chauhan, 1990 and Chattopadhyay et al., 1992).

The fruits at maturity undergo an increase in respiration and ethylene production accompanied by marked changes in composition and texture such as enzyme synthesis, flesh softening, conversion of starch to sugars and synthesis of volatiles etc. The softening of fruit tissue during ripening is an important process affecting the edible quality of fruit as well as the length of time for which they can be stored (Knee and Bartley, 1981). Textural changes are result of the changes in the structure and composition of the cell wall. The enzymes involved in softenning of fruits are polygalacturonase (PGS) and cellulases (Knee, 1973; Bartley, 1978; Huber 1983). The respiratory enzyme, malic dehydrogenase is an important mitochondrial enzyme and its activity is reported to increase during ripening of apple (Hulme et al., 1964). Softening of the fruits also occurs during storage as a result of which the keeping quality of the fruit is reduced. In the present investigation, an attempt has been made to study the effect of preharvest application of ethylene inhibitors of the polygalaturonase, cellulase and malic dehydrogenase activities of delicious pear during cold storage with a view to reduce the softening and improve the keeping quality of the fruits, and to prolong their storage period.

MATERIALS AND METHODS

Pear trees (*Pyrus communis* L.) of uniform size and vigour were selected in Dana Farm, El-Nobbarrya, 151 Kilo Cairo-Alex. way. In each tree, 3 well spaced uniform branches with average to good crop load were selected for preharvest sprays. These branches constituted three replications for each treatment and the experiment was laid out in randomized block design. Two sprays of ethylene inhibitors viz. n-pro-pylgallate (100, 200

and 300 mg L^{-1}), $AgNO_3$ (25, 50 and 100 mg L^{-1}) and benzothiadiazole (25, 50 and 100 mg L^{-1}) were given to the pear fruits and surrounding foliage on the selected limbs at 10 and 15 days after full bloom. (These concentrations were used on apple fruits by Mahajand and Chopra, 1992). The control plants were sprayed with water.

Fruits were harvested at maturity and packed in standard size corrugated fibre board cartoon and stored in cold chamber maintained at 0± 1°C and 90-95 percent relative humidity. Ten fruits per replication from each treatment were drawn at 0, 90, 150, 180 and 210 days of storage and used to study the polygalacturonase (PG), cellulase and malic dehydrogenase enzyme activities.

PG and cellulase enzyme activities were determined according to the methods described by Abeles and Takeda (1990), and Mahadevan and Sridhar (1982). PG was extracted from pear fruits by homogenising 10 g of tissue with 50 ml of 0.15 M aqueous solution of sodium chloride. Then passed through four layers of cheese cloth and centrifuged at 10,000 g for 20 minutes at 4°C. The supernatant was used as source of enzyme. The enzyme reaction mixture consisted of 2 ml of enzyme extract, 4 ml of sodium polypectate in sodium acetate-acetic acid buffer (pH 5.2) and 1 ml of acetate buffer (pH 5.2). The contents were mixed thoroughly and incubated at 37°C for 16 hours. In a similar way the assay of cellulase was performed using carboxymethyl cellulose (CMC) as substrate. Rest of the procedudral steps are the same as described for PG activity.

Malic dehydrogenase activity was determined following the reduction of oxaloacetic acid with coupled oxidation of NADH to NAD^+ (Mallick and Singh, 1980). The enzyme activity was expressed as change in OD per minutes.

RESULTS AND DISCUSSION

A glimpse to the data on PG activity (Table 1) revealed that it was quite low at the time of sampling, increased with the storage upto 150 days and then declined towards the end of the sampling. The PG activity was the lowest in $AgNO_3$ (100 mg L^{-1}) and benzothiadiazole (100 mg L^{-1}). Control fruits on the other hand recorded the maximum activity upto 150 days and thereafter a sharp decline was observed.

The polygalacturonase due to their ubiquitous distribution and their temporal association with ripening are most often implicated in the wall metabolism responsible for softening (Huber, 1983). Softening of the fruit during ripening is normally accompained by an increase in the concentration of soluble pectic polysaccarides (Bartley and Knee, 1982; Huber, 1983). Hobsen et al. (1984) observed a decrease in PG activity of silver thiosulphate treated tomato fruits, which is also confirmed in the present study. They further suggested that silver may be involved in inhibition of PG activity by binding to the sites for ethylene action.

The cellulase activity at harvest was low in $AgNO_3$ (100 mg L^{-1}) treated fruits. The activity increased very slowly upto 150 days and then

declined gradually till the last sampling date. The control fruits on the other hand showed rapid decline in this regard.

Cellulases are responsible for the hydrolysis of cellulose to glucose. Knee (1973) has reported the loss of wall cellulose in some fruits during ripening. Abeles and Takeda (1990) have more recently reported that the slower steady loss of flesh firmness of apples may be caused by the continued action of cellulase already present in the fruit. However, Bartley (1976) suggested that pear has no cellulase activity. The lack of detailed information regarding the great numbers and specificity of fruit cellulases renders any attempt to assign a specific role to these enzymes in ripening, purely speculative (Huber, 1983).

The malic dehydrogenase activity was low in those fruits which were sprayed with $AgNO_3$ and benzothiadiazole (50 and 100 mg L^{-1}). These treatments slowed down the enzyme activity. The slowing down of malic dehydrogenase activity in some fruits may be attributed to the retardation of aerobic respiration and intermediatory metabolism in which the TCA cylce substrates are involved (Price and Thimann, 1954).

From the data it is obvious that silver nitrate and benzothiadiazole at 100 mg L^{-1} concentration were found to be most effective in slowing down the polygalacturonase, cellulase and malic dehydrogenase enzyme activities during storage and so these are the most effective in prolongation the storage period.

Table (1): Effect of preharvest application of ethylene inhibitors on polygalacturonase and cellulase enzyme activities of pear stored at 0±1°C and 90-95% relative humidity.

Treatments	Polygalacturonase					Cellulase				
	Days in storage					Days in storage				
	0	90	150	180	210	0	90	150	180	210
Control	16.5	24.8	31.2	21.8	17.4	11.2	12.2	13.2	9.4	8.0
n-Propylagallate										
100 mg L^{-1}	15.1	22.1	28.4	22.0	17.3	11.4	12.3	13.1	9.3	8.3
200 mg L^{-1}	14.8	21.6	25.6	22.7	17.6	10.5	11.2	11.8	9.5	8.4
300 mg L^{-1}	13.7	21.8	27.8	22.6	17.2	10.5	11.0	11.6	9.7	8.6
LSD 5%	1.004	1.239	1.132	0.213	0.067	0.137	0.081	0.066	0.068	0.038
1%	1.566	1.958	1.732	0.334	0.103	1.199	0.129	0.102	0.108	0.060
Control	16.4	24.9	31.5	21.4	17.9	11.4	12.1	13.5	9.5	8.1
$AgNO_3$										
25 mg L^{-1}	13.8	20.9	26.0	22.7	18.6	9.7	10.2	10.5	9.3	8.5
50 mg L^{-1}	11.9	18.3	24.8	22.9	18.5	9.0	9.5	10.2	9.6	8.3
100 mg L^{-1}	11.5	17.8	24.7	23.8	18.5	8.4	9.0	10.3	9.8	8.9
LSD 5%	0.817	0.284	0.731	0.275	0.374	0.284	0.417	0.352	0.831	0.113
1%	1.283	0.449	1.155	0.428	0.587	0.442	0.654	0.553	1.280	0.179
Control	16.5	24.9	31.2	21.7	17.8	11.3	12.3	13.3	9.3	8.0
Benzothiadiazole										
25 mg L^{-1}	13.6	19.8	25.6	23.2	17.7	9.6	10.2	11.4	9.2	8.8
50 mg L^{-1}	12.0	18.7	24.6	23.4	18.2	9.0	9.9	10.2	9.6	8.9
100 mg L^{-1}	11.5	18.1	24.8	23.7	18.4	8.9	9.3	10.1	9.9	9.0
L.S.D. 5%	1.063	0.980	1.097	0.993	0.984	1.132	0.976	0.966	1.365	0.783
%	1.648	1.548	1.711	1.559	1.525	1.766	1.533	1.507	2.1[illegible]	1.206

Table (2): Effect of preharvest application of ethylene inhibitors on malic dehydrogenase activity of pear stored at 0±1°C and 90-95% relative humidity.

Days of storage	Malic dehydrogenase				
Treatments	0	90	150	180	210
Control	0.079	0.135	0.190	0.065	0.020
n-Propylagallate					
100 mg L^{-1}	0.072	0.132	0.186	0.083	0.031
200 mg L^{-1}	0.068	0.130	0.184	0.086	0.028
300 mg L^{-1}	0.065	0.127	0.178	0.089	0.029
LSD 5%	0.012	0.021	0.009	0.004	0.019
1%	0.019	0.033	0.014	0.006	0.030
Control	0.079	0.135	0.190	0.065	0.020
$AgNO_3$					
25 mg L^{-1}	0.065	0.121	0.167	0.074	0.025
50 mg L^{-1}	0.062	0.109	0.158	0.081	0.029
100 mg L^{-1}	0.058	0.095	0.131	0.085	0.033
LSD 5%	0.010	0.018	0.011	0.006	0.014
1%	0.016	0.028	0.017	0.010	0.022
Control	0.079	0.135	0.190	0.065	0.020
Benzothiadiazole					
25 mg L^{-1}	0.061	0.107	0.155	0.080	0.028
50 mg L^{-1}	0.059	0.103	0.127	0.085	0.030
100 mg L^{-1}	0.055	0.075	0.119	0.087	0.036
L.S.D. 5%	0.002	0.019	0.018	0.003	0.016
1%	0.003	0.029	0.028	0.005	0.025

إطالة مدة تخزين ثمار الكمثرى عن طريق تثبيط نشاط بعض الانزيمات باستعمال محاليل منبطة للإثيلين

ميمونة كرد و تهاني حتحوت

تم رش أشجار الكمثرى ببعض مثبطات الايثلين هي: Silver, n-propylagallate, nitrate and benzothiadizole وذلك بتركيزات مختلفة بعد تمام النضج، وقد وجد أن Benzothiadiazole, silver nitrate عند تركيز ١٠٠ مجم/ لتر، كان لهما اكبر تأثير على تثبيط نشاط انزيمات بوليجالاكتيوروناز والسليولاز والديهايدروجيناز أثناء التخزين عند درجة حرارة واحد مئوية ورطوبة نسبية مقدارها ٩٠-٩٥٪. كما أدت هذه المعاملات الى اطالة فترة تخزين هذه الثمار.

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SYNTHESIS AND EVALUATION OF SOME PEPTIDE CHAINS USING THE LIQUID PHASE METHOD AS BIOLOGICALLY ACTIVE SUBSTANCES

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ABSTRACT

Some peptide chains different in lengthes and sequences were synthesized using polyethylene glycol as polymeric support and t-butyl oxy carbonyl group as N-terminal group. p-Bromoethyl benzoyl chloride was used as anchoring group. 18 crown ether-6 was employed to catalyse the esterification of the first amino acid to the anchoring group.The yield of the esterification was improved to 95.5% in case of Butyl oxy carbonyl (BOC) Glycine and 79.2% in case of BOC Tyrosine. The strategy of the liquid phase method was employed.

The antimicrobial activity of the pure synthesized peptides were tested after cleavage of the polymeric support agoinst two Gram + va bacteria, two Gram-ve bacteria, two filamentous fungi and yeast.

INTRODUCTION

The liquid phase method of peptide synthesis using soluble polyethylene glycol was introduced by Bayer et al.[1,2].

Since then, several biological active peptides have been synthesized using this method. It offers many advantages over classical and solid phase methods of peptide synthesis. The use of an insoluble support as COOH-terminal protecting group, as realized in the solid-phase synthesis by Merrifield[3], enables easy separation of excess reagents from the polymer-

bound peptide but suffers from inherent deficiencies induced by the polymeric, heterogeneous matrix. The use of soluble polymeric supports was devised to eliminate some of the short-comings of solid-phase synthesis while retaining the enormous advantages of a polymer-mediated synthesis. From the great number of functionalized linear polymers available, polyethylene glycol (PEG) proved to be most compatible with respect to the physical and chemical properties necessary for "liquid-phase" strategy[4,5]. The liquid-phase method, as developed by Bayer et al[1]is therefore primarily based upon the use of PEG, which can be regarded as solubilizing polymeric protecting group, as the COOH-terminal "liquid support". Viewed from the chemical methodology, liquid-phase synthesis is identical to classical solution methods, however, the presence of a solubilizing macromolecular protecting group affords (i) higher solubility of peptides during stepwise synthesis and (ii) facilitated separation of low- molecular- weight compounds from the polymeric peptide ester.

The present work represents the synthesis of five peptides chains using liquid phase method, aiming to study their antimicrobial activity towards two Gram + ve bacteria, two Gram - ve bacteria, two filamentous fungi and yeast.

RESULTS AND DISCUSSION

The following peptide chains were synthesized using the liquid phase method:

1. H-Glu-Ala-Leu-Val- Lys-Gly-OH.
2. H-Leu-Tyr-Leu-Val-Cys-Gly-OH.
3. H-Lys-Val-Tyr-OH.
4. H-Gly-Ala-Leu-Tyr-Leu-Cys-Gly-OH.
5. H-Gly-Ala-Leu-Tyr-Cys-Gly-OH.

These peptides are co-valently bound to monofunctional polyethylene glycol monomethyl ether (PEGM) of molecular weight 5000. The esterification of the polymer with first amino acid was catalysed by 18 crown-6[6,7]

in DMF at 60 °C, which was found to be the best condition for quantitative esterification. The incorporation of the first amino acid with p-bromomethyl benzoyl polyethylene glycol monomethyl ether in this reaction was resulted in 95.5% yield in case of BOC Gly and 79.2% yield in case of BOC Tyr. Without using of this catalyst the yield of esterification of these two amino acids were 79% nad 68% respectively. This avoid any undesirable side reactions.

All the synthesized peptides were chromatographically separated and purified. Analytical controls were carried out after each step of the synthesis, purity were indicated by amino acid analysis and thin-layer chromatography. All the amino acids were BOC-protected at o -amino group.

The BOC-group was removed by treatment of MPEGA-peptide for 30 min with trifluoroacetic acid-dichloromethane (1 : 1) using (10 ml) of the deprotecting agent per (1.0 g) MPEGA- peptide. The volume of th solution was then reduced by flash evaporation to an oil and the polyethylene glycol-peptide was precipitated by the addition of anhydrous ether under vigorous stirring. The mixture was stirred over 15-30 min at 30°C, the precipitate was filtered, washed with ether, and dried under vacuum. The coupling reactions were carried out by symmetrical anhydride method[8] applying excess anhydride component. To this end, the BOC-protected amino acid derivative was dissolved in a minimum amount of dichloromethane and the solution was cooled to 0 °C, equivalent of dicyclohexylcarbodiimide (DOC) in a 2 M stock solution of dichloromethane was added and the mixture was allowed to stand 30 min at 0 °C. The precipitated dicyclohexylurea was removed by filtering the anhydride solution directly into a flask containing the deprotected aminocomponent in dichloromethane.

The extent of coupling was mentioned first by qualitative UV test on thin-layer plates. Quantitative ninhydrin tests[9] were carried out after isolation of protected poly ethylene glycol- peptide by precipitation. The purity was tested after each step by thin-layer chromatography.

The amino acid analysis of the synthesized peptide:

1. H-Glu-Ala-Leu-Val-Lys-Gly-OH

	Glu	Ala	Val	Lys	Gly	Leu
calculated:	1	1	1	1	1	1
found:	0.89	0.97	1	1.02	1	1

2. H-Leu-Tyr-Leu-Val-Cys-Gly-OH

	Leu	Tyr	Val	Cys	Gly
Calculated:	2	1	1	1	1
found:	2	0.78	1	0.91	1

3. H-Lys-Val-Tyr-OH

	Lys	Val	Tyr
Calculated:	1	1	1
found:	1	1	0.8

4. H-Gly-Ala-Leu-Tyr-Leu-Cys-Gly-OH

	Gly	Ala	Leu	Cys	Tyr
calculated:	2	1	2	1	1
found:	1.9	1	1.98	1	0.77

5. H-Gly-Ala-Leu-Tyr-Cys-Gly-OH

	Gly	Ala	Tyr	Cys	Leu
calculated:	2	1	1	1	1
found:	1.91	1	0.78	0.91	1

Biological Activity:

The antimicrobial properties of five pure synthesized peptides were tested after cleavage of the polymeric support against two Gram +ve bacteria (Bacillus subtilis and Staphylococcus aureus), two Gram-ve bacteria (Escherichia coli and Pseudomonas aeruginosa), yeast (Candide albicans) and two filamentous fungi (Aspergillus niger and Penicillium chrysogenum). Results in the present investigation indicated that no inhibition zones caused on the growth of all tested microorganisms by the water soluble pure peptides at all applied concentrations (500, 1000, 5000 and 10000 ppm). By increasing the incubation period the tested microorganisms were grown on the water soluble synthesized peptides. It can be concluded from these results that the five tested synthesized peptides have no activity against the tested bacteria and fungi, and the tested microorganisms may use these peptides as nutrient substances.

EXPERIMENTAL

1. Amino- acid Analysis: (By using Beckmann Unichrom Analyser)

0.1 (uM) of the peptide was hydrolysed by 3-4 ml 6 N HCl in evacuated sealed tube for 24hr at 110 °C. Then the solvent was removed in vacuum to give a residue analyzed according to Moore method[10].

2. Thin-Layer Chromatography (TLC)[11,12] :

Thin-layer chromatography is useful for estimating the purity both of starting materials for liquid-phase peptide synthesis. TLC plates may be spread using Brinkman Silica Gel G or Silica Gel H, both give essentially the same Rf's.

Thin-layer plates used were Merk Silica Gel 60 with and without fluoresence indicator. The plates were developed by Ninhydrin solution (300 mg Ninhydrin dissolved in 100 ml n- butanol and 3 ml Acetic acid[13,14]

Solvent system used is 1- butanol: acetic acid: water (30: 10: 10)

3. Synthesis:

3.1 Synthesis of BOC-amino-acid[15] :

A suspension of (0.05 mole) of the amino acid and (0.055 mole) of BOC azide[16] in (10 ml) of water and (10 ml) of dioxane, was placed in a vessel of a pH-stat(autotitrator). The reservoir of the pH-stat was filled with (4N) NaOH. The pH control of the instrument was advanced to the point where continued uptake of base indicates that the reaction is proceeding at a reasonable rate. A few amino acids will react at pH 8.5, and most at pH 9.8, a few require pH 10.2 for a reasonable rate of reaction. The reaction is usually completed in a few hours, although the reaction with certain amino acids is quite sluggish and may requires more than 24 hr. The end of the reaction is indicated by cessation of base uptake. Extraction of the solution 3 times with ether to remove unreacted azide, then chilling the aqueous phase in ice, the reaction mixture was then acidified to pH 2 with HCl in a pH-stat, then extracted 3 times with ethyl acetate. The ethyl acetate extract was washed 3 times with small portions of water (saturated NaCl solution for water-soluble derivatives), dry over $MgSO_4$, and evapourate under reduced pressure.

3.2 Purification of MPEG:

MPEG was dissolved in a least amount of distilled methylene chloride and reprecipitated by dropwise addition of dry ether under cooling and vigorous stirring. The precipitate was filtered, washed with dry ether without suction. The process was repeated several times till pure crystalline substance was obtained and dry under vacuo for 2 hr.

TLC; Rf= 0, solvent system 1- butanol (30), acetic acid (10), water (10).

3.3. Synthesis of p-bromomethyl benzoyl chloride[17] :

3.3.1. Synthesis of p-bromomethyl benzoic acid:

Benzoyl peroxide (0.2 g) and N-bromosuccinimide (17.8 g; 100 m.mol) (recrystallized from hot water) were added to a suspension of p-toluic acid (13.6g; 100 m.mol) (recrystallized from $CHCl_3$- MeOH) in dry benzene (100 ml). The mixture was heated under reflux for 24 hr. Removal of the solvent in vacuo gave a white residue which was suspended in (100 ml) of boiling water, collected by filtration and washed with boiling water (4 x 100 ml). The crude product was dried and recrystallized from hot MeOH to give pure acid (17.5 g, 81.4%): mp. 225-227°C (Lit. 224 - 226°C)[18] Anal calcd. for $C_8H_7BrO_2$: C, 44.68, H, 3.28; Br, 37.15 found : C, 44.50; H, 3.18; Br, 37.02.

3.3.2. Synthesis of the acid chloride:

(10.75 g; 0.05 M) of p-bromomethyl benzoic acid was refluxed with (0.075m) thionyl chloride for 6 hr, the excess of the thionyl chloride was distilled over a water bath. The product was crystallized from Pet. ether (60 - 80%) to give the pure acid chloride (10.05 g. -90%) mp 57°C (Lit. 56°C)[19].

4. Esterification of p-bromomethyl benzoyl chloride with MPEG:

(50 g; 10mM) MPEG and (7g, 30 mM) p-bromomethyl benzoyl chloride were dissolved in (500 ml) dry toluene and refluxed in a three necked flask in nitrogen atmosphere for 24 hr. The excess of toluene was distilled under vacuo till the total volume becomes (60 ml) after cooling, the product was crystallizd by dropwise addition of dry ether during vigorous stirring, then filtered, redissolved in methylene chloride and reprecipitated by dry ether. The last step was repeated twice till pure product was obtained.

TLC; Rf = O, solvent system l-butanol (30), acetic acid (10), water (10).

5. Synthesis of BOC amino acid potassium salt[20] :

(1.0 g) of a BOC amino acid was dissolved in a mixture of ethanol (6 ml), H_2O (4 ml) and 1.0 equivalent of a (1.0 N) KOH solution, the solvent

was removed by azeotropic distillation in the presence of toluene and dried in vacuo over P_2O_5, the resultant hygroscopic white salt was used without further treatment.

5. Coupling reaction of potassium salts of BOC amino acids with p-bromomethyl benzoyl polyethylene glycol monomethyl ether (MPEGA) catalyzed by 18- crown-6:

6.1. Coupling reaction of p-bromomethyl benzoyl polyethylene glycol monomethyl ether with different BOC amino acid potassium salts:

A mixture of (1 mM) BOC amino acid potassium salt, (2.61 g; 0.5 mM) MPEGA and (0.279; 1 mM) 18-crown-6 were dissolved in DMF and refluxed for 48 hr at 60°C. The reaction mixture then, dried under vacuo and the residue was dissolved in CH_2Cl_2 and reprecipitated by dry ether with cooling and vigorous stirring. Then, the product was filtered, dried and redissolved several times in CH_2Cl_2 and reprecipitated by dry ether till chromatographically pure product was obtained. The yield of incorporations are about 100%.

For cleavage of BOC group, 12 g of MPEGA- bound peptide dissolved in (120 ml) TFA CH_2Cl_2 (1:1) and the solution was stirred for 30 min at RT. Then, the reaction mixture was distilled under vacuum, the produced oil was dissolved in CH_2Cl_2 and the product was precipitated by dropwise addition of dry ether under cooling and vigorous stirring. The pure product was filtered and dried under vacua, yield 91.7%.

TLC: Rf = O, solvent l-butanol (30), acetic acid (10), water (10).

5.2. Cupling reaction of Gly with the second amino acid:

(2 mM) MPEGA- Gly was dissolved in 20 ml dichloromethane, in another flask, (10 mM) of the second amino acid was dissolved in (10 ml) dichloromethane and cooled to 0°C, 0.48 equivalent (5 mM) of dicyclohexylcarbodiimide dissolved in dichloromethane was added and the mixture was allowed to stand for 30 min at 0°C while stirring. The precipitate of dicyclohexyl urea was removed by filtrating the anhydride solu-

tion directly into the flask containing the deprotected amino component in dichloromethane with slow stirring. The above solution was neutralized by N-methyl morpholine till pH=7, then the solution was concentrated to about (10 ml) and stirred over night at RT. The product was precipitated by dropwise addition of dry ether under vigorous stirring while cooling, then the product was recrystallized twice till pure crystals were obtained. The coupling was controlled by quantitative ninhydrine test.

TLC; Rf = 0, solvent system l-butanol (30) acetic acid (10), water (10).

other amino acids were coupled by the above described method.

7. Investigation of antimicrobial activity:

The antimicrobial activity for each of the five water soluble synthesized peptides at different applied concentrations (500, 1000, 5000 and 10000 ppm) were investigated against 2 Gram +ve bacteria (Bacillus subtilis and Staphylococcus aureus), 2 Gram-ve bacteria (Escherichia coli and Pseudomonas aeruginosa), two filamentous fungi (Aspergillus niger and Penicillium chrysogenum) and yeast (Candide albicans).

The bacterial tested organisms were cultivated on nutrient agar medium, where as yeast and moulds were cultivated on malt extract agar and Czapek's Dox agar media, respectively according to Harrigan and McCane[21].

The antimicrobial activity of the studied peptides were assayed by measuring the inhibition zones of microbial growth caused by known volume of the water soluble peptide. The method was essentially as foolows:

Petri-dishes containing base layer of (2%) agar in water and an upper layer of the specific culture medium seeded with the tested microorganism. Hold glass microfibre filters discs (0.6 cm) with saturated known volume (0.1 ml) of each different concentration of water soluble peptide put on the surface of the medium. Then, the plates were incubated at 37°C for 2 days for bacteria, and at 28°C for 4 days for yeast and fungi, and the diameter of inhibition zones were measured.

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تحضير وتقييم بعض البيتيدات بطريقة الوسط السائل كمواد ذات نشاط بيولوجى

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اهتم هذا البحث بتحضير خمسة سلاسل بيتيدية مختلفة فى الطول ونوع الأحماض الأمينية فى السلسلة بطريقة الوسط السائل بهدف دراسة تأثيرها على أنواع مختلفة من البكتريا والفطريات والخميرة وقد استخدم البولى إيثيلين جليكول كمدعم للطرف الكربونى للسلسلة وكذلك مجموعة البيوتيل الثلثى أوكسى كوبونيل كحامية لمجموعة الأمين كما ربط البوليمر بالحمض الأمينى الأول من خلال مجموعة اروماتية هى بارا- برومو - ميثيل - كلوريد البنزويل. واستخدم الكربن ايثير كعامل حفز لتفاعل الاستره بين الحمض الأمينى الأول ومجموعة الربط الأروماتيه بهدف تحقيق أعلى نسبة لارتباطه بالبوليمر لحل مشكلة السلاسل القير مرغوب فيها والتى تتكون مع تحضير السلسلة الرئيسية ويصعب فصلها وقد تحقق ذلك برفع نسبة ارتباط الجليسين من ٧٩.٢٪ إلى ٩٥.٥٪ وحمض التيروزين من ٦٨٪ إلى ٧٩٪ وقد تمت دراسة تأثير هذه البيتيدات بعد نقائها كروماتوجرافيا على بعض الكائنات الدقيقة.

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Thermal and spectroscopic characterization of the products obtained from the reaction between Mn-Carbonate and Ammonium Dichromate at different temperatures

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Pure and mixed Mn-Cr oxides were prepared from the reaction between manganese carbonate and ammonium dichromate with molar ratios of 3:1, 1:1 and 1:3 with respect to Mn_2O_3:Cr_2O_3. Thermal decomposition for each of the mixture and the pure compound was studied using DTA and TG techniques. Pure and mixed salts were thermally treated at temperatures of 250°C, 500°, 750° and 1000°C and characterized by means of x-ray, diffraction analysis and IR absorption spectroscopy.

The results obtained revealed that the thermal treatment of mixtures at 250°C produced a well crystalline $MnCO_3$ and/or Cr-oxide phases depending on the composition of the mixture. At 500°C, poorly crystalline Mn_2O_3, Cr_2O_3 and amorphous manganese chromate intermediates were detected. Further increase in temperature of treatment was accompanied by the formation of $Mn_{3-x}Cr_xO_4$ compound in all mixtures. This compound decomposes at temperatures just lower than 700°C to form crystalline phase of Mn_3O_4 and Cr_2O_3.

Introduction

Many binary oxides are widely used in catalysis[1-4]. These binary systems were found to be more catalytically active than their separated oxide components[5,6]. In catalysis, it is well known that the activity of oxide catalysts depends on many factors, such as methods of preparation, calcination conditions and the interaction occurring between the different components of the catalyst, the latter is a very important factor and many investigations are cited in the literature concerning this subject[7-9].

In the present investigation, we studied the effect of temperature on the interaction between manganese and chromium salts in order to characterize the different products obtained at various temperatures of treatment. The techniques employed in this work were DTA, X-ray diffraction spectroscopy and IR absorption spectroscopy.

Experimental

The starting materials used in this investigation were pure ammonium dichromate and manganeses carbonate from BDH grade. Mixtures of molar ratios 3:1 (I), 1:1 (II) and 1:3 (III) with respect to Cr_2O_3:Mn_2O_3 were prepared by mixing and grinding the salts. Each one of the pure ammonium dichromate, manganese carbonate and their mixtures I, II and III was heated at temperatures of 250^o, 500^o, 750^o, or 1000^oC for 4 hours.

Thermal analysis for each of $(NH_4)_2Cr_2O_7$ and Mn-carbonate was carried out using DTA and TG unit of the NETZSCH Gertebau simultaneous thermal analysis system (STA 409, 6.223). The rate of heating was 10^oC min^{-1}.

The X-ray diffractograms of the samples were taken on a diffractometer Philips (Holland) with a scintillation counter and plus height analysis at 35Kv, 14 mA using Co-target radiation. The spectra were scanned at rate of 2^omin^{-1} in 2O.

IR spectra of the samples were recorded on a Beckman infrared spectrophotometric unit using the KBr disc technique.

Result and Discussion:

The thermal analysis diagrams for pure manganese carbonate, Fig. 1, show that the compound starts to decompose by losing the dehydration water at temperature range of 100-250°C. The weighing loss ≈ 29% occurring at temperature rang of 300-400°C refers to the decomposition of Manganese carbonate to MnO_2. This oxide dissociates to Mn_2O_3 (weight loss ≈ 9%) at a temperature range of about 450-500°C. The decomposition of $MnCO_3$ can be represented as follows:

$$MnCO_3.0.5H_2O \xrightarrow[\text{Loss} \approx 7\%]{< 300^oC} MnCO_3 + 0.5H_2O$$

$$MnCO_3 + 1/2\ O2 \xrightarrow[\text{Loss} \approx 22\%]{300\text{-}400^oC} MnO_2 + CO_2$$

$$2MnO_2 \xrightarrow[\text{Loss} \approx 9\%]{> 400^oC} Mn_2O_3 + 0.5O_2$$

The ammonium dichromate decomposes thermally in several steps, Fig. 2, with a total weighing loss of ≈ 65%.

The decomposition steps observed here agree with those reported by EZ-Eldin(10).

$$4(NH_4)_2Cr_2O_7 \xrightarrow{\approx 200^{o}C} 4Cr(OH)_3 + 2CrO_3 + 2CrOOH + 2NH_3 + 3N_2 + 6H_2O$$

$$2CrO_3 + 1/2CrOOH \xrightarrow{\approx 200^{o}C} 0.5CrO_2 + 0.25Cr_2(Cr_2O_7)_3 + 0.25H_2O + 0.25O_2$$

$$1.5CrOOH \xrightarrow{\approx 250^{o}C} 0.75Cr_2O_3 + 0.75\ H_2O$$

$$0.5CrO_2 \xrightarrow{> 350^{o}C} 0.25Cr_2O_3 + 0.125O_2$$

$$0.25Cr_2(Cr_2O_7)_3 \xrightarrow{> 350^{o}C} Cr_2O_3 + 1.125O_2$$

To identify the phases formed during the thermal treatment, the X-ray diffraction spectra for pure $MnCO_3$ heated at different temperatures 250°, 500°, 750° and 1000°C were recorded and summarized in Fig. (3,4 and 5).

For sample heated at 250°C, crystalline phase of $MnCO_3$ (d-values 1.76, 2.17, 2.84 and 3.66 A°)(11) was only detected. While the heated samples at temperatures of 500°, 750° and 1000°C showed crystalline tetragonal phase for Mn_2O_3 (d-values 2.49, 2.76, 3.08 and 4.22 A°)(12). The crystallinity of this phase increase with temperature. At temperature of 1000°C, crystalline phase of Mn_3O_4 (d-values 1.49, 2.10, 2.54 and 4.86 A°)(13) could be detected beside the phase of Mn_2O_3.

The X-ray diffraction pattern of heated ammonium dichromate sample at 500°C, Fig. 3, support the above thermal decomposition results which showed the formation of Cr_2O_3 at 500°C(14).

The degree of crystallinity of Cr_2O_3 increases with increasing the heating temperature, Figs. 4 and 5.

The X-ray diffraction patterns for the mixture samples (I, II, III) heated at 250°C showed the absence of any crystalline phases. The disappearance of the patterns of crystalline phases of $MnCO_3$, which was observed in case of pure sample can be attributed to the interaction occurring between $MnCO_3$ and the composition products of ammonium dichromate.

For all mixtures heated at 500°C, the X-ray diffraction patterns showed crystalline phases of Mn_2O_3(12) and Cr_2O_3(14).

At temperature 750°C, crystalline phases of Cr_2O_3 and Mn_3O_4 could be detected. The formation of Mn_3O_4 at 750°C could be explained as a result of certain reaction to form $Mn_{3-x}Cr_xO_4$, which is then dissociate to Cr_2O_3 and Mn_3O_4, (at $500^0<T<750^0$C).

$$\text{Mn-oxide} + \text{Cr-oxide} \xrightarrow{>500^0\text{C}} (Mn_{3-x}Cr_xO_7) \xrightarrow{750^0\text{C}} Cr_2O_3 + Mn_3O_4 + XO_2$$

The mixed oxides of $Mn_{3-x}Cr_xO_4$ was also detected as a result of the reaction between manganese carbonate and chromium nitrate at about 500^oC(15).

The x-ray diffraction patterns for the mixtures heated at 1000^oC showed an increase in the intensity of patterns for crystalline phases of Mn_3O_4 and Cr_2O_3.

Figs. 6,7,8 and 9 illustrate the IR spectra for pure and mixed manganese and chromium salts heated at different temperatures. Fig. 6 shows the IR spectra of the pure salts and their mixtures heated at 250^oC. The bands appeared at wave lengths of ≈1810, 1437, 1087, 870 and 728 cm^{-1} indicate the presence of carbonate group. The increase in the concentration of chromium in the mixtures led to decrease intensity of the manganese carbonate bands and at the same time an increase in the intensity of the corresponding bands of chromium oxide at 1100, 710, 650, 570, 555, 440 and 407 cm^{-1}. The IR-spectra for pure Mn-carbonate heated at 500^oC showed the disappearance of carbonate bands of pure Mn-carbonate and the appearance of new bands at 1150, 980, 845, 690, 610, 485 and 410 cm^{-1} which are characterized for Mn_2O_3.

For mixture samples heated at 500^oC, the IR spectrogram showed a broad band pointing to the presence of some sort of chromates, which formed as a result of solid state reactions between manganese carbonate and ammonium dichromate. The IR spectra of calcined mixtures at 750^oC showed

bands corresponding to Cr_2O_3 and Mn_3O_4. Further increase in temperature, 1000^oC increases the intensity of Cr_2O_3 and Mn_3O_4 bands, which confirmed the results obtained from X-ray.

Figure Captions

Fig. 1: DTA and TG of manganese carbonate.

Fig. 2: DTA and TG of ammonium dichromate.

Fig. 3: X-ray diffraction patterns of ammonium dichromate, manganese carbonate and their mixtures calcined at 500°C.

1- Cr_2O_3 2- Mn_2O_3

Fig. 4: X-ray diffraction patterns of ammonium dichromate, manganese carbonate and their mixtures calcined at 750°C.

1- Cr_2O_3 2- Mn_2O_3 3- Mn_3O_4

Fig. 5: X-ray diffraction patterns of ammonium dichromate, manganese carbonate and their mixtures calcined at 1000°C.

1- Cr_2O_3 2-Mn_2O_3 3- Mn_3O_4

Fig. 6: IR-spectra of ammonium dichromate, manganese carbonate and their mixtures calcined at 250°C.

Fig. 7: IR-spectra of ammonium dichromate, manganese carbonate and their mixtures calcined at 500°C.

Fig. 8: IR-spectra of ammonium dichromate, manganese carbonate and their mixtures calcined at 750°C.

Fig. 9: IR-spectra of ammonium dichromate, manganese carbonate and their mixtures calcined at 1000°C.

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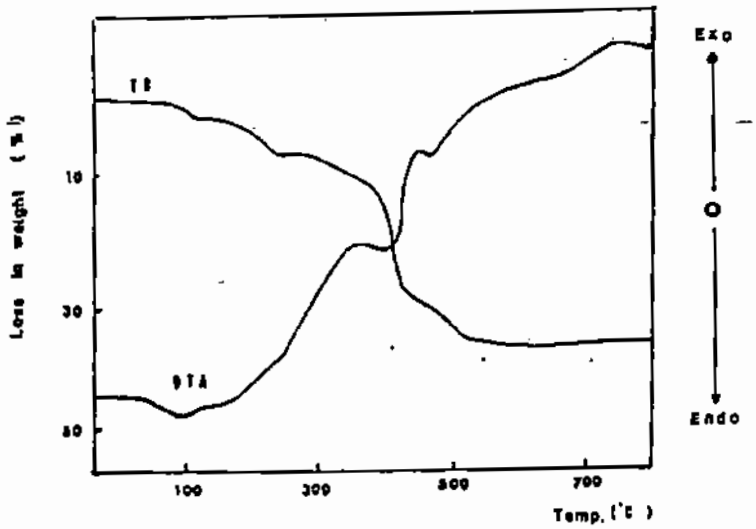
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DTA
Loss in weight (%)
10
30
50
100
300
500
700
Temp. (°C)
Exo
Endo

Fig 1

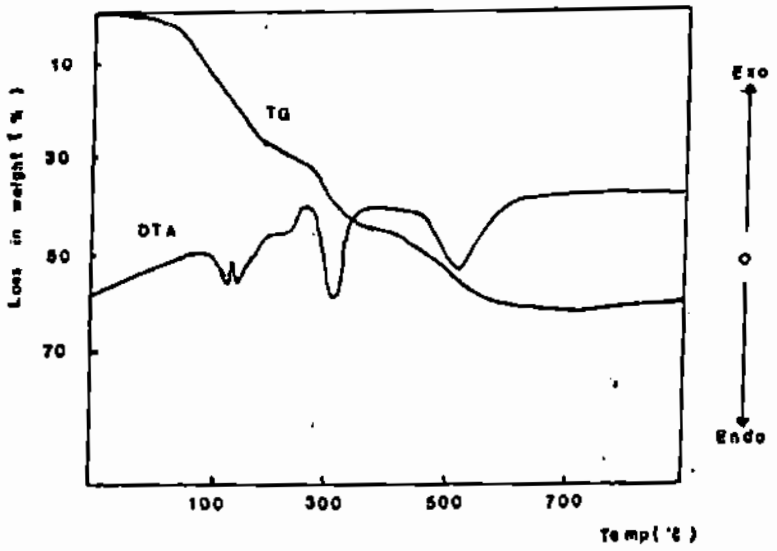
TG
DTA
Loss in weight (%)
10
30
50
70
100
300
500
700
Temp(°C)
Exo
Endo

Fig 2

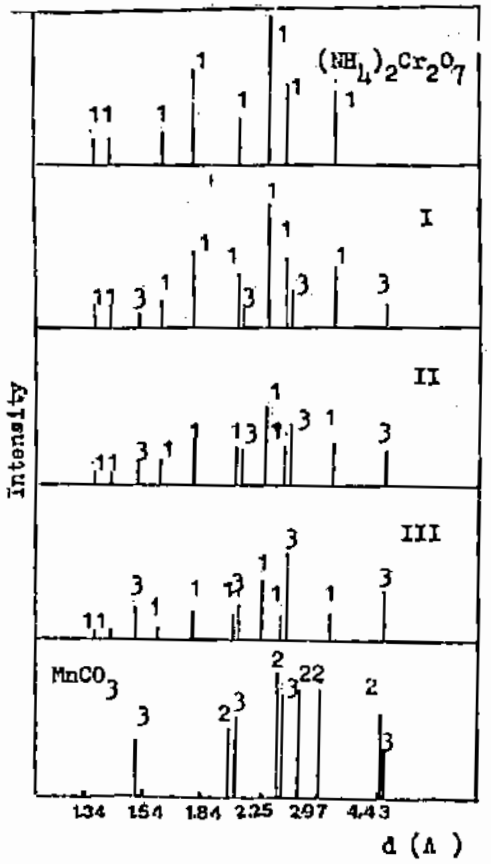

Fig 5

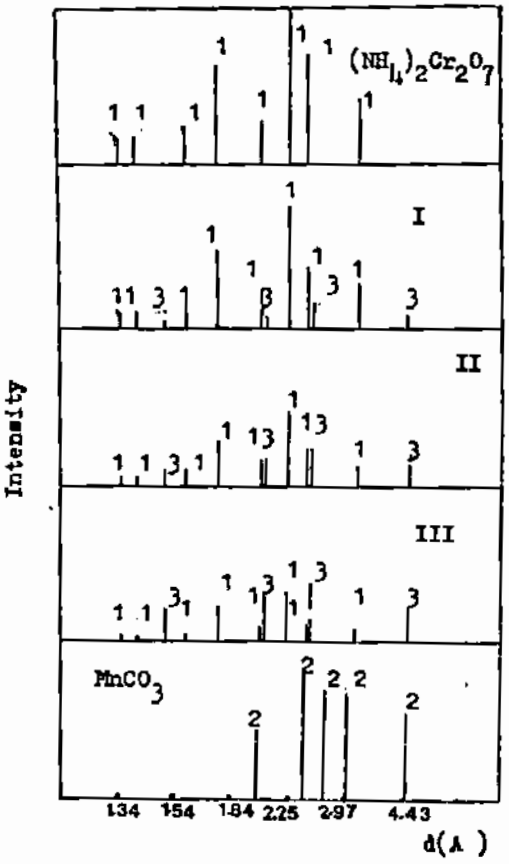

Fig 4

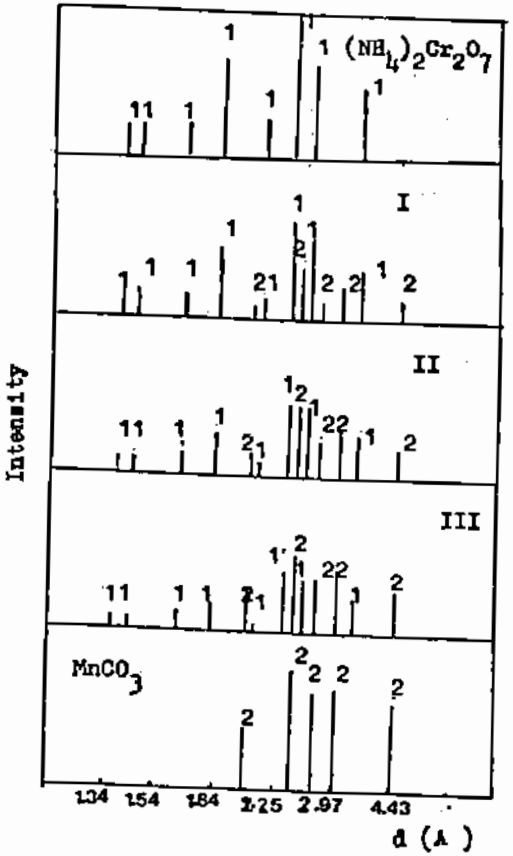

Fig 3

Fig 5

Fig 6

Fig 8

Fig 9

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CHEMICAL CONTROL OF SOME TOMATO AND PEPPER DISEASES

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Abstract

The test of eleven systemic and non-systemic fungicides was carried out in laboratory and pots . three fungicides namely Benlate , Quinolate and Rizolex-T. were used in concentrations 5,10,20,50,100,250 and 500 ppm to control growth of Fusarium oxysporum f.sp. lycopersici and Fusarium solani and eight fungicides namely polyram, Topsin M, Trimiltox - forte, Nemispore , Tridex , Sandofan , Ridomil MZ and Dithane M45 were used in same concentrations to control growth of Alternaria solani.

It was found that the lethal effect of the different fungicides used increased with increase in concentration till at 500 ppm., no growth was observed and also at 250 ppm when quinolate and benlate were used .

Seeds of seven CVS. tomato were used ; Castle rock, Strain B, Redstare, Super Marmmend, Super Queen, Ice and Val icc.

It was found that Strain B CV.of tomato was resistant to wilt symptoms while Ice, Redstar and Super Queen showed high susceptibility to wilt infection .

Rizolex-T. followed by Benlate showed high efficiency for many CVS. used when the effect of different fungicides on the length of root system was studied, while Quinolate gave maximum efficiency when the effect of different fungicides on length of shoot was studied.

Two CVS. of tomato Ice and Super Queen and two CVS. of pepper California wonder and Yolo wonder L were used in planting in infested soil with 3% rate of Fusarium solani, other groups of seeds were treated with the tested fungicides at

recommended dose of each . It was found that the percentage of pre-emergence damping off was high and the maximum number of survival plants was obtained when seeds of tomato and pepper were treated with Rizolex T. and Benlate, respectively.

Two CVS. of tomato ; Castle rock and Strain B and two CVS. of pepper California wonder and Yolo wonder L. was sprayed with Alternaria solani. It was found that maximum efficiency was observed when Trimiltox forte and Sandofan were used and the efficiency in number reduction was found to be 91.26 and 91.00 respectively for Castle rock CV. but in case of Strain B CV. Sandofan followed by Tridex and Ridomil showed maximum activity reach to 95.67, 95.06 and 91.31 respectively . In case of pepper CV. Ridomil followed by Trimiltox showed maximum efficiency in reduction of spots number.

INTRODUCTION

Fungicides have been used by numerous investigators for controlling tomato diseases by several means of applications '.

Kalra and Sohi (1984) observed that the systemic fungicides inhibited growth of F.oxysporum. The systemic fungicides Benomyl, Bavistin (carbendazim) and NF 44 (thiophanate-methyl) but not catixin, completely inhibited growth of F.oxysporum .

Difolatan , Dithane M-45 and Thiram reduced it considerably but Diathane Z-78 (Zineb) and Blitox (copper oxychloride) proved almost ineffective .

Kamlesh et al. (1986) concluded that Blitox 50(copper oxychloride) was the most effective for controlling Alternaria solani followed by Difolatan (coptafol) and Dithane M-45 (mancozeb) and these may be recommended, especially during the rainy season.

In field trials carried out by moeso (1991), captafol (Difolatan). chlorotholonil (Daconil) and Fentin acetate (Brestan) gave the best performance in 1981-82; while chlorothalonil (Daconil), fentin acetate + maneb(as Brema), copper oxychloride + maneb + zineb (as caprosan, copper salts + mancozeb (as Trimeltox forte) the most effective in 1982-83 .

The aim of the present study was to investigate the effect of some fungicides on growth of Fusarium oxysporum, F.solani and Alternaria solani. Efficacy of these

fungicides on domping - off and early blight an tomato and pepper plants was also studied .

Material and Methods

Isolation of the causal pathogens

the causal pathogens of tomato and pepper wilt and root rot diseases were isolated from untreated seeds and diseased stems and roots of tomato and pepper plants . The untreated tomato seed CV. money maker and untreated pepper seeds CV. California wonder were obtained from Agricultural Research Center-Ministry of Agriculture , Giza , Arab Republic of Egypt . The casual pathogen of early blight was isolated from infected tomato leaves from Ismaillia Governorate.

Tomato and pepper seeds and plants showing the characteristic wilt , root rot and early blight symptoms (Dimond et al 1952, Walker , 1957) were cut into small pieces, surface sterlized , then transferred to the surface of PDA medium in petri dishes and incubated at 28 for 2-4 days .

The developed fungi were carefuly transferred to PDA slants . pure cultures were obtained for each isolate using the single spore and hyphal tip (Riker and Riker , 1936) .

Chemical Control

laboratory studies - effect on linear growth

Effect of some fungicides on fungal growth of Fusarium - oxysporm , Fusarium-solani and Alternaria-solani were studied.

Eleven fungicides differing in their active ingredients as show in table (1) were evaluated in vitro using 5,10,20,50,100,250 and 500 ppm. of active ingredient for the poisoned technique using PDA medium (tohamy et al.1991) .

Four petri dishes , of each fungicide , were inocubated with the three fungi Fusarium Oxysporum , Fusarium solani and Alternaria solni , petridishes were incubated at 28,28 and 25C respectively, growth linear diameter was measured daily until the control plates were covered with fungus growth . for counting the spores,10 ml. sterilized water were added to each petri dish and spores were gently transferred using a camel brush .

b) pots experiments

1) Effect of fungicides on early blight .

Two tomato CVS (Castle rok and Strain B) and two pepper CVS .(California wonder and Yolo wonder) were sown in pots 30 cm. in diameter containing (1/1) - sand-clay soil .

Five replicates in case of tomato and three replicates in case of pepper were used for every treatment . Seedlings , 21 days old , were inoculated using spore suspension (100,000 spores / ml.) of Alternaria solani .

Plants in each pot were covered with muslin covers for 24 hours fungicides at recommended dose (table 1) were sprayed after 15,30 and 45 days .

2) Effect of fungicides on damping off and root rot .

Two tomato CVS (Ice and Super Queen) and two pepper CVS (California wonder and Yolo wonder) were planted in soil infested with Fusarium solani . Pots ,30 cm. in diameter , containing (1/1) sand - clay soil , were infested with the fungus at rate of 3 % of soil weight , left for 5 days , then seeds treated with each of the 3 fungicides (table 1) , were planted with 30 seeds / pot and 5 replicates , in case of tomato CVS. and 20 seeds / pot and 3 replicates in case of pepper CVS.

percentage of pre and post emergence damping off for both tomato and pepper plants . and precentage of survival plants were calculated .

3) varietal reactions :-

seven tomato CVS. were tested for thier reaction to Fusarium wilt - seeds were planted in soil infested with the fungus F. oxysporum F.sp Iycopersici . Notes on wilt were recorded at the end of the experiment after 3 monthes .

RESULTS

Fungicides were tested in the laboratory and on potted plants.

a) Laboratory experiments

1) Effect of fungicides on growth of Fusarium SP:-

Results summarized in Fig.(1) show the effect of different concentration of the three fungicides ; Benlate, Quinolate and Rizolex-T. on the growth of Fusarium oxysporum f.sp. lycopersici, fungal growth was measured as diameter in cm. daily from the first day of incubation and until control plates were covered with growth .

It was found that Benlate and Quinolate were the most effective on fungal growth followed by Rizolex.T. Diameter of colonies gradually decreased when the concentration of the fungicide was increased from 5 to 100 ppm. , while no growth was observed at 250 and 500 ppm. in case of Benlate and Quinolate respectively.

Data in fig.(2) , illustrate the effect of the different concentrations of the three fungicides on growth of Fusarium solani . It was clear that Quinolate gave maximum effect when compared with Benlate and Rizalex.T. growth gradually decreased with the increase in fungicide concentration , while no growth was observed at 250 and 500 ppm. of Quinolate , while Rizolex.T. resulted in the least effect when compared with Quinolate and Benlate .

2) Effect of fungicides on growth of A.solani.

Data obtained (fig. 3) show the effect of concentration of 8 fungicides on growth of Alternaria solnai at 25 C on solid PDA medium . Topsin at 500 ppm was the only fungicide which completely inhibited growth of the fungus .

On the other hand , Ridomil MZ58 followed by Tridex at all concentrations resulted in maximum reduction in growth of A.solani . Also , Topsin followed by Trimiltox forte were effective and followed Ridomil M258 and Tridex in their effect at all concentrations . All fungicides used showed decrease in diameter of growth when concentration was increased . However Polyram , Sandofan , Nemispore and Ditnan M45 were the least effective

3) Effect of fungicides on sporulation of Fusarium Sp:-

Data illustrated (fig.4) show the effect of different fungicides concentration on number of spores / ml. of Fusrium oxysporum f. sp. lycopersici after 10 days on solid PDA medium. Number of spores /ml. decreased with the increase of fungicides concentration , Quinolate followed by Benlate were the most effective as no spores were produced at 250 and 500 ppm.

``Also , results in fig (5) , indicate that the three fungicides were effective at the different concentration on the number of total spores / ml. of Fusarium solani . however Quinolate was the most effective (no spores produced at 250 ppm) followed by Benlate (no spores produced at 500 ppm) , while Rizolex T. was the least effective (spores produced up to 500 ppm) .

Effect of fungicides on sporulation of A.solani :-

Results summarized in fig (6) show the effect of fungicides concentration on number of spores / ml. of Alternaria solani after 10 days growth on PDA medium . Topsin M was the most effective as no spores were produced at 500 ppm . It was followed by Redomil then Trimoltox which the least number of spores at 500 ppm followed by Tridex then Polyram . Reduction in number of spores may start sharp as in case of Tridex and Trimitox forte (6000 spores) at 5 ppm or gradualy as Dithane M45 (8000 at 5ppm) which may be considered the least effective on spore production . In general all fungicides in thier different concentraion affected spore production with different degree .

Pots experiments

Effect of fungicides on early blight caused by Alternaria solani :

Data obtained (fig. 7) illustrate the effect of spraying with 8 fungicides on the number of spots on leaves of tomato CV. Castle rok inoculated with A. solani after the 3rd fungicides application . Trimiltox and Sandofan resulted in maximum efficiency as the percent age reduction of number of spots was 94.97 % followed by Ridomil MZ58 and Dithane M45 as efficiency or percentage reduction reach 91,26 and 91,00 % for the two fungicides , respectivley (fig.7) . Differences between treatments were highly significant . Also fig. (8) summarize results on the effect of spraying with the same 8 fungicides on the number spots on leaves of tomato CV. strain B . Sandofan followed by Tridex and Ridomil MZ58 showed maximum efficiency in which percentage of reduction in number of spots to the sprayed plant were equal and Dithane M45 showed high efficiency as percentage reduction reaches 88.27 % .

In case of pepper CV. California wonder and Yellow wonder L , Ridonil followed by Trimiltox forte showed maximum percentage of reduction in number of spots compared with the other fungicides (fig. 9).

Effect on seedling damping off :

fig.(10 & 11) show the effect of diffrent fungicides on seedling damping off of tomato CV. Ice and Super Qeen grow in soil infested with Fusarium solani from the data , it can be concluded that percentage of pre-emergence damping off was more than that of post -emergance for the two tomato CVS. Also , the least percentage of damping off and the highest percentage survival plants was observed when seeds of the tomato CVS.were treated with Rizolex T. between Benlate and Quinolate and between Quinolate and Rizolex T. for the two tomato CVS.

The same effect was also noted when seeds of the two pepper CVS. , California wonder (fig. 12) and Yolo wonder L (fig. 13) were treated with the three fungicides . Percentage of pre-emergence damping off was more that of post-emergence Benlate proved to be the best fungicides for seed treatment ; percentage of pre-emergance damping off was the least and equal to 46.5 and 28.00 % for the two pepper CVS. , respctivley . Also, percentage of survival plants reashed the maximum when Benlate was used .

Effect of fungicides on tomato wilt caused by Fusrium oxysporum f. sp. lycopersici

The effect of different fungicides on percentage of infection with tomato wilt disease is summarized in table (2) .

It can be observed that seed of Castle rok , strain B , Super-Queen val Ice and Ice CVS. with Rizolex T. at the rate of 39/kg resulted in the least percentage of wilt , while Benlate treated seed (at rate of use 2g/kg) showed the least percentage with supermammend and red star .

- Further more diffrence between fungicides for length of root and shoot table (2) were significant-Rizolex T. followed by Benlate resulted in highest length of root , while Rizolex T. resulted in highest length of shoot. defference between fungicides and control were also significant .

DISCUSSION

From data obtained , it was observed that diameter of growth was gradually decreased when the concentration of fungicides used increased from 5 to 100 ppm, when the effect of different concentration of different fungicides on the growth of the three isolated fungi studied in laboratory . It was also observed that no growth was observed at 250 and 500 ppm. when Benlate and Quinolate were used to controlling growth of Fusarium oxysporum f. sp. lycopersici and Fusarium solani . In this case Quinolate followed by Benalte gave the best control effect , Bechet & Lordach (1983) and kalra & Sohi (1984 9) . in case of Alternaria solani , Ridomil followed by Tridex gave the maximum reduction of growth . No growth was observed at 500 ppm. concentration of Topsin , Fadi et al (1985) .

From the same experiment the number of spores were determined and it was found that Quinolate followed by Benlate highly reduced the total number of spores /ml. after 7 days of growth of both f. solani on solid PDA medium . in case of growth of Alternaria solani on solid PDA medium containing 8 different fungicides at different conc. the total number of spores /ml. was highly reduced after 10 days growth with Ridomil followed by Tridex , Karla & Sohi (1984 a) and Fadi et al. (1985) .

pots experiment were carried out using the same group of systemic and non - systemic fungicides to control different fugal diseases appeared due to artificial infestation and from the data obtained it can be observed that Trimiltox forte and Sandofan showen maximum efficiency in controlling early blight caused by A solani . Also Ridomil and Dithane gave high control effect in reduction the number of spots appeared after the third application on tomato plants CV. Castle . The percentage of reduction reached 94.97 when Trimiltox used highly significant differences can be observed from result obtained .

Sandofan , Tridex and Ridomil showed high efficiency in reduction in number of spots appeared on strain B CV. of tomato plaw (95.67, 95.06 and 91.39) respectivly , Kamlesh et al (1986) and Moesa et al (1991) .

Redomil folowed by Trimiltox showed maximum effect in controlling the disease on leaves , Rajagopal and Vidhyasekaram (1985) .

Fusarium solani which cause root rot of tomato and pepper plants and leaded to pre and post emergence damping off of seedlings , was also controlled with the same fungicides and it was found that least precentage of damping off and highest percentage of survival plants was obtained when Ice and Super - Queen CV. of tomato plants treated with Rizolex T. also high difference was obtianed when California wonder and Yolo wondor L. CV. of pepper seeds were treated with Benlate , Bechet and Iordach (1983) .

When the same group of fungicides was used to control vascular wilt disease of tomato plants caused by F. oxysporum , Ice , Super Queen and Redstar CV. showed to be highly susceptible to wilt disease , while strain B , CV. showed a resistance to wilt symptoms . Rezolex.T. and Benlate gave high effect on length of shoot system , Beihn (1973) and Nikolaeva (1978) .

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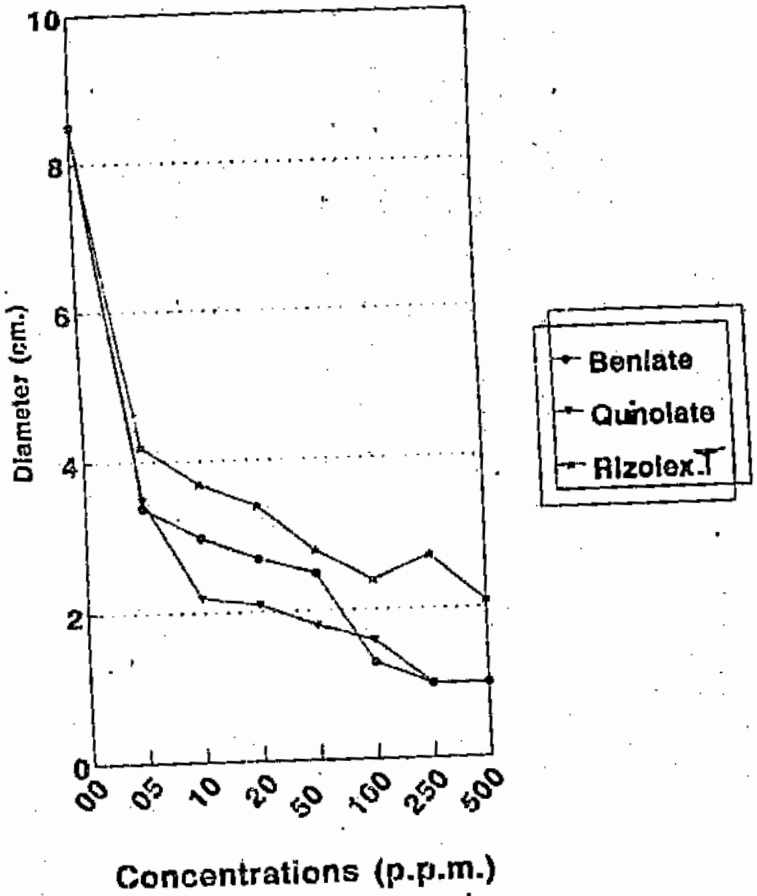


Fig. (1): Effect of different fungicides concentrations on growth of F. oxysporum f. sp. lycopersici at 28 °C..

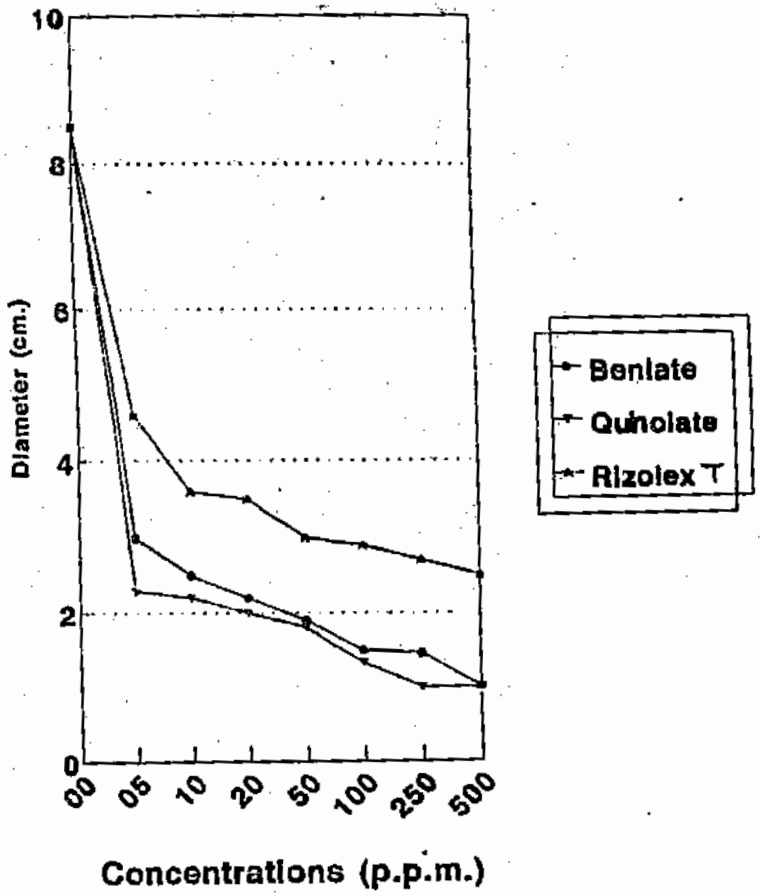


Fig. (2): effect of different fungicides concentrations on growth of Fusarium solani at 28 °C

(268)

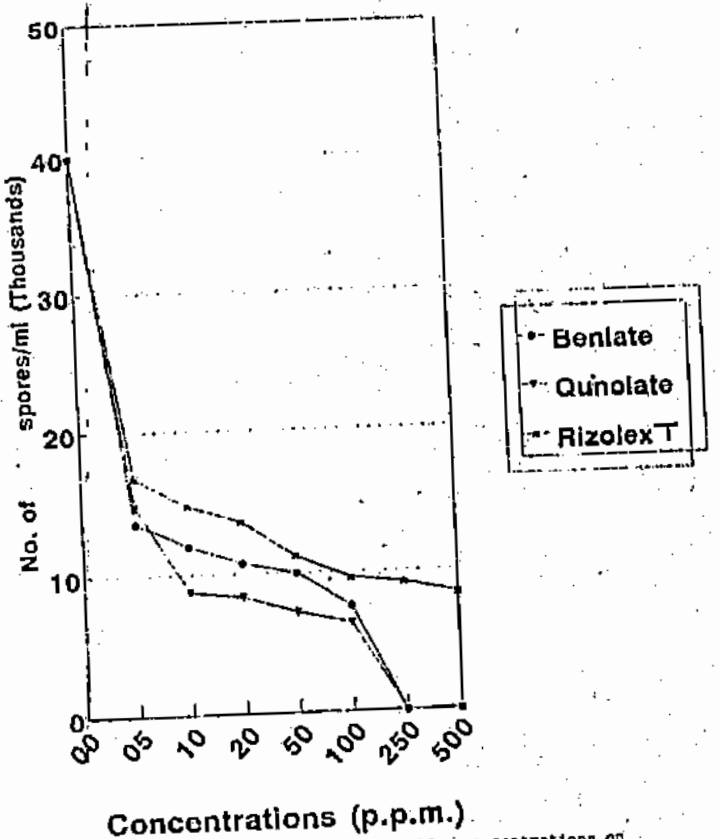


Fig. (4): Effect of different fungicides concentrations on number of spores / ml. of F. oxysporum f. sp. lycopersici after 10 days growth on PDA medium.

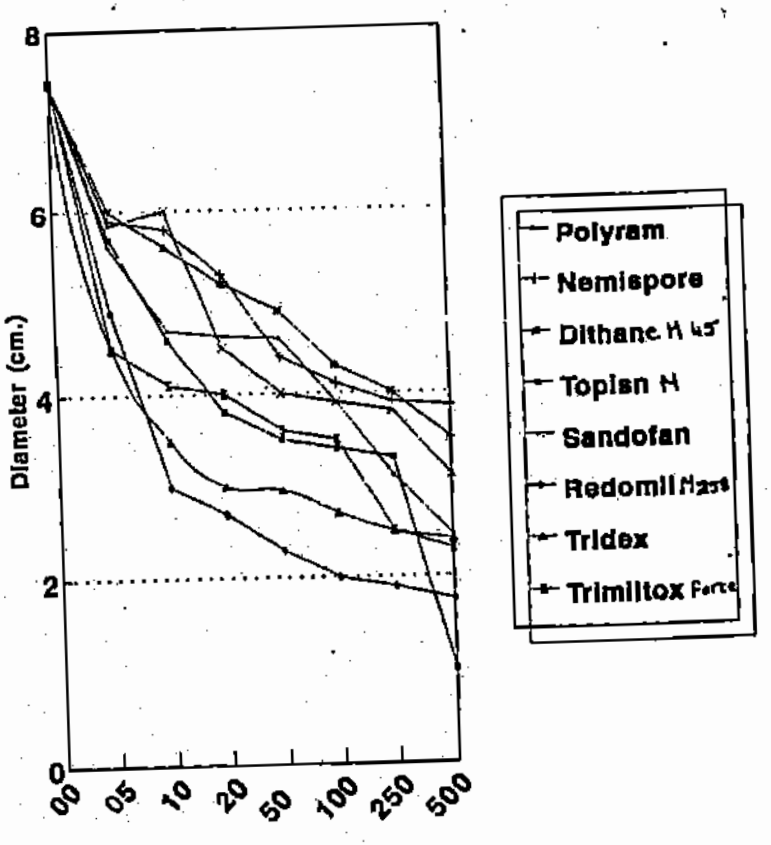


Fig. (3): Effect of different fungicides concentrations on growth of Alternaria solani at 25 °C

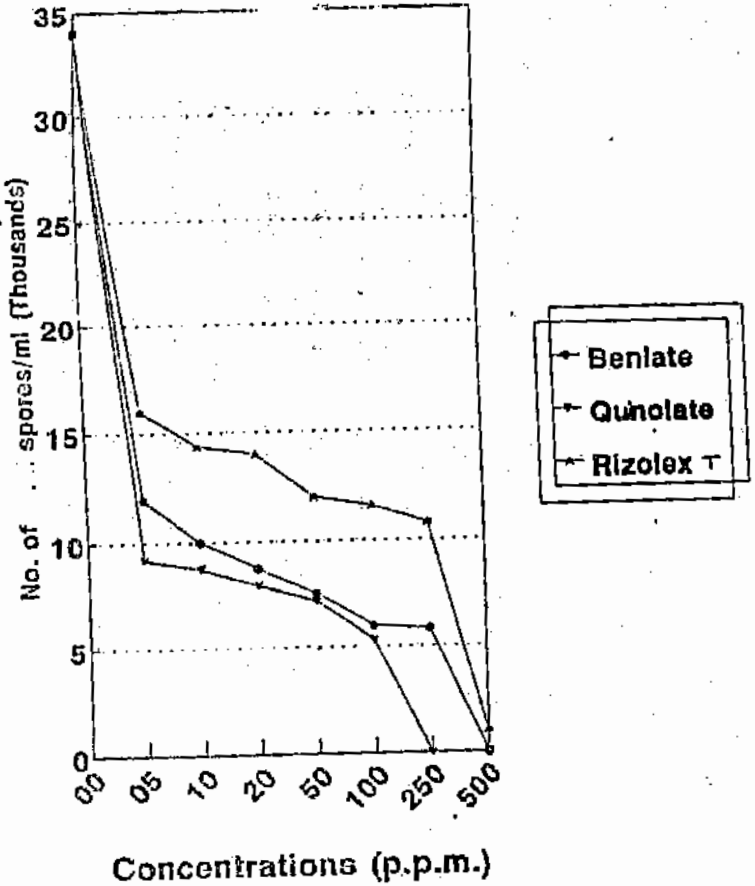


Fig. ( ): Effect of different fungicides concentrations on number of spores/ml. of Fusarium solani after 7 days growth on PDA medium.

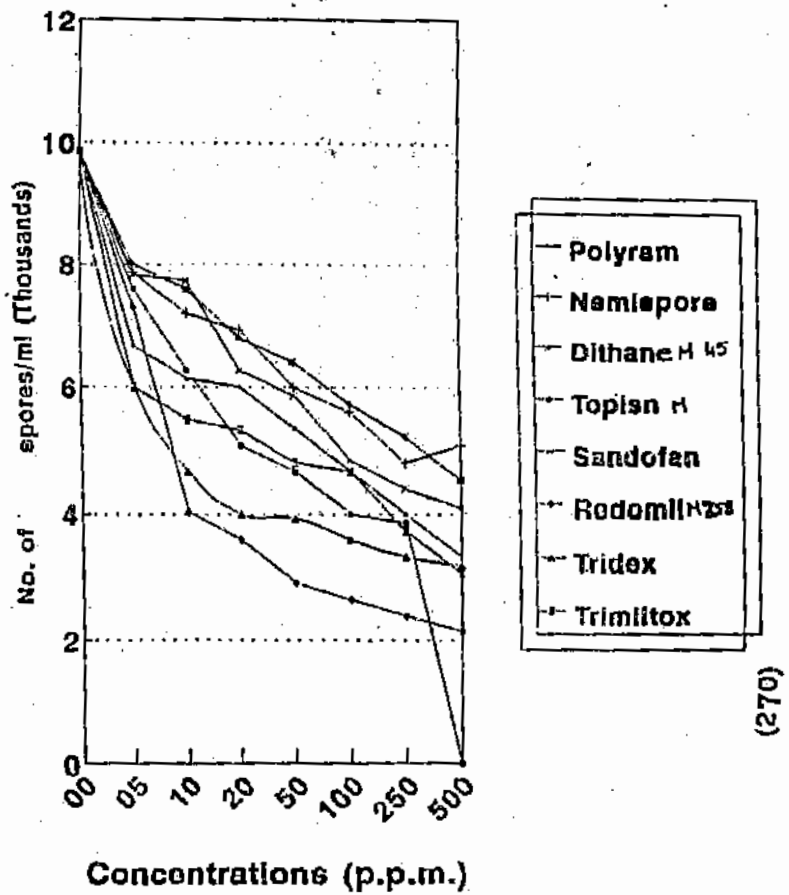


Fig. ( ): Effect of different fungicides concentrations on number of spores/ml of Alternaria solani after 10 days growth on PDA medium.

Fig. 17 : The effect of spraying with different fungicides on the number of spots on tomato leaves cv. Castle rock.

Fig. (18) : The effect of spraying with different fungicides on the number of spots on tomato leaves cv. Strain B.

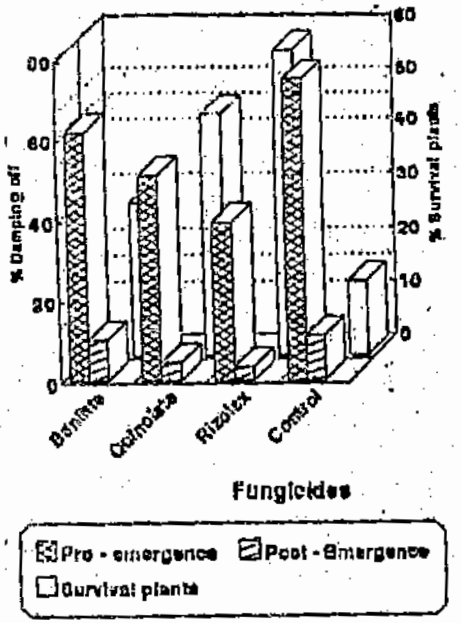


Fig. (10): Effect of different fungicides on percentage damping off (pre and post emergence) and survival tomato plants cv. Ica.

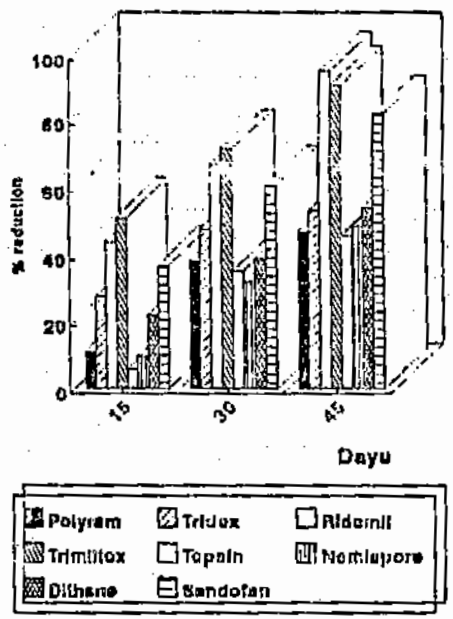
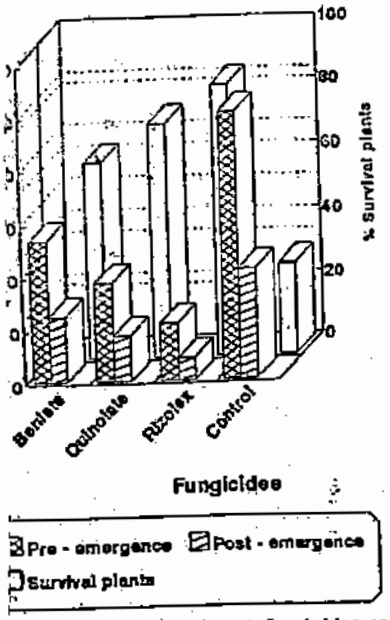


Fig. (9): The effect of spraying with different fungicides on the number of spots on pepper leaves cv. California wonder.



(11): Effect of different fungicides on percentage damping off (pre and post emergence) and survival tomato plants cv. Super-queen.

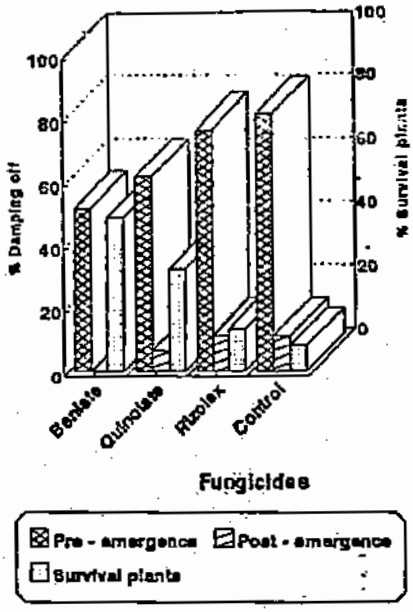


Fig. (12): Effect of different fungicides on percent damping off (pre and post emergence) survival pepper plants cv. California wor

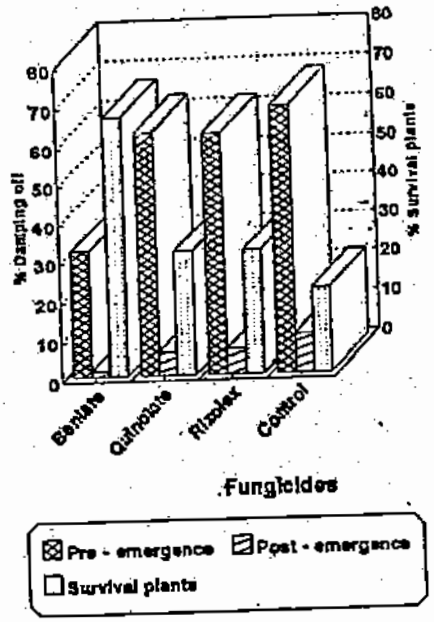


Fig. (13): Effect of different fungicides on percentage damping off (pre and post emergence) and survival pepper plants cv. Yolo wonder L.

Table (1): List of systemic and non-systemic fungicides .

Trade name	Chemical constituent	Manufcturer
1- Sandofan	Oxadixyl 8% + Mancozeb 56% 2- methoxy -N- (2- oxy- 1,3 - oxazolidin - 3 - yl) acet - 2,6 - xylidide.	Sandoz AG.
2- Dithan M45 (Mancozeb)	1,2- ethanediybis = (Carbamo-dithioato) [2-] manganese	Rohm and Hass
3- Quinolate (Oxine-Copper)	Bis (quinolin - 8 - olato) copper	Bayer
4- Benlate (Benomyl)	Methyl 1-(butylcarbamoyl) ben-zimidazol -2- ylcarbamate	E.I. dupont
5- Trimiltox forte	20% mancozeb + 21% cupper salts + 6 stimulant additive	Sandoz AG.
6- Rizolex -T 50% WP	20% tolcofos-methyl + 30% thiram o-2,6-dicloro -4- methylphenyl o,o- dimethyl phosphorothiate + Bis (dimethyl thiocarbamoyl) disulphide.	Sumitomo chemical Co.
7- Polyram (Metiram)	Zinc ammoniate ethylenebis (di-thiocarbamate) -pdy (ethylenebis (thiuram disulphide)	BASF, AG.
8- Topsin M 70 (Thiopphanate)	Diethyl 4,4 - (o-phenylene) bis (3- thioallophanate)	Nippon Soda Co.
9- Ridomil MZ 58	Metalaxyl + Mancozeb Methyl D,L -N- (2,6 dimethyl-phenyl)-N- (2- methoxyacetyl) - alaninate+ Dithiocarbamate	Ciba - Geigy AG.
10- Nemispore	80% (16% Manganese + 2% Zinc + 62% Ethylene bisdithiocarbamate)	Lotus
11- Tridex	44% FL + ethanediy bis= (Carba-modithioate) (2-) manganese	Ron Blank

Table (2): Effect of different fungicides on percentage of infection with tomato wilt disease.

Fungicides	Rate of use /kg. seed	Percentage of wilt						
		Castle rock	Strain B	Super-marmmed	Super-Queen	Valice	Redstar	Ice
Benlate	2 g.	12.1	11.0	11.8	19.1	15.7	11.6	28.8
Quinolate	3 g.	14.6	9.5	18.8	24.3	18.1	23.9	33.4
Rizolex T	3 g.	9.1	5.4	14.2	14.9	12.6	16.6	22.1
Control		22.7	14.7	27.0	35.2	23.4	30.5	44.4
L.S.D =		0.138	0.130	0.261	0.131	0.133	0.264	0.262

Table (2): Effect of different fungicides on length of root system of tomato plants grown in soil infested with F. oxysporum .

Fungicides	Rate of use /kg. seed	Length of root (cm.)						
		Castle rock	Strain B	Super-marmmed	Super-Queen	Valice	Redstar	Ice
Benlate	2 g.	11.8	9.2	8.4	7.9	9.0	9.9	11.0
Quinolate	3 g.	12.7	6.3	11.2	7.0	9.0	8.8	10.0
Rizolex T	3 g.	16.0	8.2	8.4	8.3	12.1	10.2	9.3
Control		11.5	5.2	7.4	6.0	6.9	8.5	7.8
L.S.D =		0.98	0.74	0.53	0.39	0.32	0.45	0.44

Table (2): Effect of different fungicides on length of shoot system of tomato plant grown in soil infested with F. oxysporum .

Fungicides	Rate of use /kg. seed	Length of shoot (cm.)						
		Castle rock	Strain B	Super-marmmed	Super-Queen	Valice	Redstar	Ice
Benlate	2 g.	17.0	13.2	10.2	14.2	15.2	13.2	18.0
Quinolate	3 g.	12.8	9.4	14.2	13.9	17.0	13.0	17.3
Rizolex T	3 g.	15.7	9.3	11.2	15.2	20.0	14.0	12.2
Control		11.9	8.3	10.0	13.0	13.5	12.3	10.4
L.S.D =		0.226	0.238	0.226	0.302	0.272	0.130	0.228

التحكم الكيميائى لبعض امراض الطماطم والفلفل

منى اسحاق فهد - زينب حسين خير الله - امانى احمد يسرى
قسم النبات - كلية البنات - جامعة عين شمس
القاهره - مصر

تد تم اختبار احدى عشر مبيد فطرى جهازى وغير جهازى فى المعمل والاصيص . ثلاثه منهم (بنلات - كينولات - وريزولتكس) استعملت بتركيزات ٥ ، ١٠ ، ٢٠ ، ٥٠ ، ١٠٠ ، ٢٥٠ ، ٥٠٠ جزئ فى المليون للتحكم فى نمو فيوزيريم لوكس سبورم (ليكوبرسى) وفيوزيريم سولانى والثمانية (بوليرام - توبسين - تريمولتكس فورت - نميسبور - تريدكس - ساندوفان - ريدوميل - والديثان) استخدمو كذلك بنفس التركيزات للتحكم فى نمو الالترناريا سولانى .

وقد وجد أن التأثير القاتل لهذه المبيدات المختلفة تزيد بزيادة التركيز حتى ٥٠٠ جزئ فى المليون - لم يلاحظ اى نمو عند ٢٥٠ جزئ فى المليون عند استخدام الكينولات والبنلات .

استخدم بذور سبعه سلالات من الطماطم (كاستل روك - السلاله (ب) - ريدستار - سوبر مارمند - سوبر كوين - أيس وفال أيس) . وجد أن (السلاله ب) مقاومة لاغراض التبول بينما أيس - ريدستار والسوبر كوين لهم قابلية للاصابة بالذبول .

زيزولكس ثم البنلات لهم كفاءة عالية لانواع كثيرة من السلالات عندما درس تأثير المبيدات على طول الجهاز الجذرى ، بينما اعطى الكينولات كفاءة عالية عند درس تأثير المبيدات على طول الساق .

سلالتان من الطماطم (أيس وسوبر كوين) وسلالتان من الفلف كاليفورنيا وندر وبولووندر زرعت فى تربه معاملة بـ ٣٪ فيوزيريم سولانى ومجموعة اخرى من البذور عوملت بالمبيدات المختبرة بالجرعة المحررة لكل منها .

وجد أن النسبة المئوية للذبول عالية عندما عوملت بذور الطماطم والفلفل بالريزولتكس ثم البنلات .

سلالتان من الطماطم (كاستل روك وسلالة ب) وسلالتان من الفلفل (كاليفورنيا وندر ويلو وندر) رشت بالألترناريا سولانى .

ووجد أعلى كفاءة عند استعمال تريمولتكس فورت وساندوفان حيث أن النقص كان ٩١,٢٦ ، ٩١ بالترتيب لكاستل روك بينما فى حالى (السلالة ب) ساندوفان ثم تريدكس وريدومول اعطى لحسن كفاءة وصلت الى ٩٥,٦٧ - ٩٥,٠٦ ، ٩١,٣١ .

وأما فى حالى الفلفل - ريدوميل ثم تريمولتكس اعطى اعلى كفاءة فى اختزال عدد البقع .

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Effect of the herbicide "Treflan" on the mitosis of *Vicia faba*

By

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INTRODUCTION

The toluidine herbicide "Treflan" [α,α,α, trifluoro 2,6-dinitro-N,N-dipropyl -P-toluidine (trifluralin)] (Fig. 1), controls most grass and broad leaf weeds, Ballada et al. (1971), Poole and Conover (1971), Harvey et al. (1972) and Baskakov et al. 1982 . It used as a herbicide for weed control in soyabean (Kudinov 1980).

Continuous use of pesticides may cause its accumulation in the soil. The aim of the present study is to evaluate the genotixic effect of pure Treflan on *Vicia faba* plant.

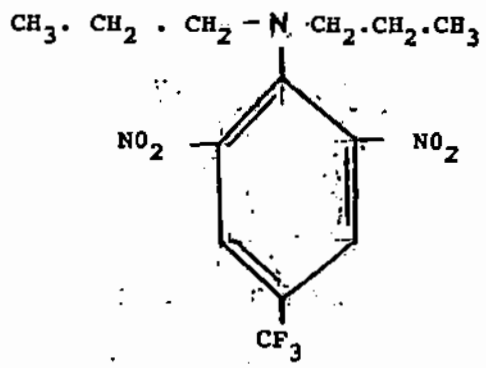

Fig. (3): Treflan

MATERIALS AND METHDOS

Sterilized seeds of Vicia faba (var. Giza 2) and pure Treflan 100 % were used in this study. The herbicide was dissolved in distilled water.

Two types of treatments were conducted.

I. Irrigation :

Seeds were soaked for 24 hrs. in tap water then cultivated in 10 pots, (5 pots for treatment and 5 pots for control, each pot contains 5 plants, i.e. 25 seedling for each treatment and control). The plants were irrigated twice for one week once with the saturated "Treflan" solution (390 ppm) and the other with water. The other plants in 5 pots were irrigated twice with water and used as control. The roots were washed thoroughly with water cut and fixed, after one week only.

II. Seed-soaking :

Seeds were soaked in tap water for 24 hrs, then soaked in four different concentrations (390, 195, 97.50 and 48.75 ppm) of the herbicide solution for another 24 hrs, control seeds were soaked in distilled water. Seeds were germination in rolles of filter paper which were placed in containers with tap water at the bottom. Main roots were cut when they were about 2-3 cm in length and fixed in 1:3 acetic acid: ethanol for 24 hours and the roots were stored in 70 % ethyl alcohol in a refrigerator.

Five replicates (five roots) were performed for each treatment and the control (one root from each pot of irrigated seeds and one root from each roll of seed-soaked).

Examination of the roots was done in permanent root-tip squash preparations stained by the Feulgen technique.

Abnormalities were counted in the different mitotic stages (abnormal mitosis) and interphase cells. The data were analyzed according to the t-test.

RESULTS AND DISCUSSION

Both treatments with the herbicide did not affect the mitotic index in Vicia faba root tip-meristems (Table I). However, a statistically significant percentage of abnormal cells was observed in root mitosis after both treatments with the herbicide (Table II). It may be mentioned that other pesticides did not affect cell division but induced abnormal mitosis e.g. the insecticide "Dursban" and "Methamidophos" (Amer and Farah, 1983, 1985).

Most of the abnormal cells were observed in the metaphase and anatelophase stages (Table II). Hussein et al. (1984) found also that the highest frequency of abnormal cells in Allium cepa root-tips treated with "Trifluralin" were observed in the metapha and telophase stages.

Treflan-treatments induced cells with chromosome abnormaliti which increased in number as the concentration of the herbicide increased (Table II).

Disturbed pro-,meta-and anaphases where the chromosomes sprea allover the cell comprised the main type of the induced abnormalities after the two treatments (Table III, Fig. 2). The arrangeme

of chromatin threads was abnormal in the case of disturbed prophases (Compare Kabarity, 1966). Disturbed meta- and anaphases may be due to the disturbance of the spindle apparatus, and so the chromosomes spread irregularly all over the cell(Amer and Ali, 1969), and(Selim et al. 1981). Disturbed metaphases with contracted chromosome were observed (e.g. Fig. 3). Such phenomenon was observed after treatment with the herbicide isopropyl phenyl carbamate (Story and Mann, 1967), Ortho- and Paranitrophenols (Amer and Ali, 1969).

Prophase-metaphase stage where, the chromosomes retained their arrangement as in prophase stage were also observed, and classified under disturbed type (Amer and Ali 1986). Lagging and sticky chromosomes were observed in a considerable percentage (Table III, Fig. 4).

It seems that the effect of "Treflan" on the cells differs in the different plants. Grigorenko et al. (1986) mentioned that when maize grains were soaked in a solution of "Treflan" the main aberrations were chromosome fragments and bridges. In the present study fragments and bridges were observed in a low percentage in the two treatments (Table III, Fig. 5). Micronuclei were observed in a low percentage in seed-soaked treatment only. In the present study polyploid cells were not observed as Kabarity and Nahas, (1979) found in Allium root tip treatments with "Treflan". Sawamura and Jackson (1968) found also that Trifluralin was more toxic to Vicia faba than to Tradescantia cells.

SUMMARY

The effect of pure, Treflan has been studied on root-mitosis of Vicia faba plant (var. Giza 2). Two types of treatments were conducted after the seeds were soaked in tap water for 24 hours. I. Seeds were cultivated in pots then irrigated twice/week once with Treflan solution (390 ppm) and the other with water. Control seeds were irrigated twice/week with water. II. Seeds were soaked for 24 hours in four different concentration of Treflan solution and water as control.

The two treatments induced a significant increase in the percentage of abnormal mitoses, but did not affect the mitotic index.

The types of the induced abnormalities were: disturbed pro-meta-, and ana-telophases, lagging and sticky chromosomes. Fragments, bridges and micronuclei were observed in a low percentage.

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Table (I): Mitotic index in Vicia faba root-tip meristems after treatment with "Treflan" solutions.

Type of treatment	Count. cells No.	Mitosis No.	Mitotic index Mean	±	S.E.
I. Irrigation :					
1. Control	5658	563	99.20	±	9.42
2. Treflan 390 ppm	5796	693	119.51	±	4.43
II. Seed-soak treatment :					
1. Control	5782	581	100.49	±	11.02
2. Treflan 390 ppm	5899	706	119.03	±	9.73
" 195 ppm	6035	774	128.13	±	14.50
" 97.50 ppm	6269	768	121.78	±	12.98
" 48.75 ppm	5834	459	78.57	±	8.35

Table (II): Percentage of abnormal cells in the different mitotic stages in the root-tip meristems of Vicia faba with Treflan solutions.

Type of treatment	% of abn. div.cells	% of abn. proph.cells	% of abn. meta-cells	% of abn. ana-telo-cells	% of abn. inter.cells
	mean ± S.E.	mean ± S.E.	mean ± S.E.	mean ± S.E.	mean ± S.E.
I. Irrigation :					
1. Control	0.78 ± 0.34	---	2.35±2.35	1.76 ± 1.18	--
2. Treflan 390 ppm	5.57 ± 0.76*	1.58 ± 0.65	17.26±3.23*	9.63 ± 3.35	--
II. Seed soak treatment :					
Control	2.12 ± 0.42	1.53 ± 0.43	2.18 ± 0.76	3.64 ± 1.80	0.18 ± 0.06
Treflan 390 ppm	8.09 ± 1.16*	3.78 ± 0.84	13.23 ± 3.50*	13.17 ± 2.25*	0.50 ± 0.11
" 195 ppm	7.33 ± 0.80*	2.72 ± 1.35	11.68 ± 1.53**	11.77 ± 1.86**	0.26 ± 0.08
" 97.50 ppm	5.29 ± 1.10*	1.80 ± 0.66	7.39 ± 1.02**	10.16 ± 2.36	0.32 ± 0.08
" 48.75 ppm	3.08 ± 0.60	2.11 ± 0.56	3.07 ± 1.38	4.71 ± 1.26	0.30 ± 0.20

* Significant at 0.05 level (t-test).

** " at 0.01 " (t-test).

Table (III): Percentage of different types of the abnormalities* occurring in the mitosis of Vicia faba roots after treatment with Treflan solutions.

Type of treatment	No. of all mitosis	No. of abn. mitosis	% of the different types of abnormalities relative to No. of abn. mitosis					
			Dist.	Lag.	Frag.	Stick.	Brid.	Micro.
I. Irrigation :								
Treflan 390 ppm	693	38	63.16	10.53	2.63	15.79	13.16	--
II. Seed-soak treatment :								
Treflan 390 ppm	1509	117	61.54	17.09	1.71	21.37	1.71	0.85
" 195 ppm	1433	106	58.49	27.36	--	8.49	2.83	2.83
" 97.5 ppm	3080	170	51.18	25.29	1.77	18.24	2.35	1.77
" 48.75 ppm	2480	79	39.24	27.85	3.80	25.32	2.53	6.33

* In the analysis of the different types of abnormalities, a cell containing more than one type of abnormalities was recorded under those types in the same time (e.g. a cell with stickiness and fragments).

Fig. (2)

Fig. (3)

Fig. (4)

Figs. (2-4): Disturbed metaphase (2), slight sticky metaphase (3), and telophase with bridge (4), after irrigated (2,4) and seed soaked treatment (3).

تاثير مبيد الاعشاب التريفلان على الانقسام الميتوزى لنبات الفول البلدى

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الملخص

درس تاثير مبيد الاعشاب التريفلان على الانقسام الميتوزى لجذور نبات الفول البلدى بان اجريت معاملتين على البذور بعد نقعها فى الماء لمدة ٢٤ ساعة .

المعاملة الاولى : وضعت البذور فى اصص ثم رويت مرتين لمدة اسبوع مرة بالمبيد واخرى بالماء اما بذور المقارنة فقد رويت المرتين بالماء ثم اخذت البذور وفصلت وتم فحص الجذور بعد ذلك .

المعاملة الثانية : نقعت البذور فى عدة تركيزات من المبيد المستخدم والماء المقطر لمدة ٢٤ ساعة اخرى ثم تركت لتنبت ثم فحصت الجذور بعد ذلك .

وجد فى كلتا المعاملتين تاثير معنوى على نسبة التذوذ الكروموسومى للخلايا المنقسمة . كانت اعلى نسبة لانواع التذوذ الكروموسومى فى الجذور المعاملة بالرى والنقع هى تبعثر الكروموسومات فى الدورين الاستوائى والانفصالى وبنسبة قليلة فى الدور التمهيدى يلى ذلك تخلف الكروموسومات واللزوجة ـ اما بالنسبة للقناطر والتقطع والانوبة الصغيرة فقد وجدت بنسبة اقل .

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Isolation and Identification of Mycotoxins Produced by Fusarium spp. and Alternaria solani

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Abstract:

Fusarium oxysporum f. sp. lycopersici, Fusarium solani and Alternaria solani were isolated from tomato and pepper seeds and diseased plants. Fusarium spp. and Alternaria solani were grown on artificial media produced toxic substances which increased in concentration with the age of culture. These toxic substances inhibited growth of radicles or (hypocotyl, cotyledonary leaf and plumules) when seeds were germinated in the filtrate of the fungus. Inhibitory effect of extract appeared to be due primarily to phytotoxicity.

Studies were extended to test the biological activity of fungal toxins. Toxins produced by Fusarium spp. and Alternaria solani, at different temperatures, pHs, carbon and nitrogen sources, inhibited growth of Bacillus megaterium and Bacillus mycoides which differed in its toxicity to bacterial growth.

Fusarium spp. secreted zearalenone and trichothecenes in liquid media. Maximum amounts of zearalenone and trichothecenes were produced by Fusarium oxysporum and Fusarium solani at 25-28°C and pH 5.

Growing Fusarium oxysporum on different carbon sources, amounts of zearalenone were in the order of xylose > sucrose > glucose > maltose > lactose > mannose. While, trichothecene amounts were in the order of glucose > maltose > sucrose > xylose > lactose > mannose. In case of F. solani the efficiency for toxin production differ from F. oxysporum using the same carbon sources.

Ammonium phosphate gave high levels of zearalenone and trichothecene (24.73 and 92.35 μg/ml). In case of F. solani, zearalenone was high in presence of casein (319 μg/ml). On the other hand, ammonium molybdate gave a high concentrations of trichothecene.

Introduction:

The genus Fusarium comprised large complex group of fungi with ascomycete teleomorphs and contains numerous species that produce noxious secondary metabolites and/or cause serious plant diseases (Nelson el al., 1983 and Marasas el al., 1984). Members of the genus have wide geographic and host ranges. Several species of Fusarium are associated with stalk rots (El-Meleigi el al., 1983 and Gilbertson el al., 1985), leaf spots (Schieber and Muller, 1968) and Fusarium wilt.

Several investigators have reported the natural occurrence of Fusarium mycotoxins, including Deoxynivalenol (DON), Nivalenol (NIV), Zearalenone (ZEA, Zearalenol, T-2 and Trichothecenes, secondary metabolites of several Fusarium species, as one of the causative agents of toxification of plants (Ueno el al., 1986; Lu el al., 1988; Luo, 1988 and Plattner el al., 1989). In addition, some kinds of Fusarium mycotoxins have been suspected to be involved in human chronic mycotoxicoses, such as esophageal cancer and Kashin-Beck disease (Hsia el al., 1983; Sokoloff, 1985; Jone el al., 1987 and Yang, 1989). Ueno el al., (1975) and Bacon el al., (1977) and Krivobok el al., (1987). They developed rapid and sensitive methods for identifying toxin production by toxicogenic strains of Fusarium cultivated in liquid medium.

Nemec el al., (1991) concluded that Fusarium solani (Mart) Sacc. produced at least 11 structural related "naphthazarin" toxins, but did not produce detectable levels of fusaric acid or trichothecenes. In culture naphthazarin toxins may be synthesized in diseased roots.

A metabolic product of Alternaria solani, has been isolated, and identified as alternaric acid and zinniol, which possessed marked phytotoxic. Alternaric acid produced symptoms characteristic of early blight on tomatoes with leaf lesions,chlorosis and necrosis (Brian el al., 1951 and 1952; Pound and Stahmann, 1951 and Maiero el al., 1991).

Siler and Gilchrist, (1983) isolated phytotoxic fractions from extracts of necrotic leaves of tomato plants infected with A. alternata f. sp. lycopersici (AAL); these fractions were indistinguishable with those isolated from the pathogen Alternaria.

Cotty and Misaghi (1984) tested thirty one isolates of 10 pathogenic Alternaria spp., for production of Zinniol, a non selective phytotoxin. Whereas, Clouse el al.,

(1985), developed simplified rapid procedure for the purification of 2 phytotoxic metabolites (TA, TB) from cell free culture filtrates of the tomato pathogen Alternaria alternata.

The study presented here has directed to investigate toxin production by the three fungi, carbon and nitrogen sources on mycotoxins produced by the investigated fungi.

Material and Methods:

1- Isolation of fungi:

Fusarium oxysporum, Fusarium solani and Alternaria solani were isolated and identified (Kheiralla el al., 1994).

2- Effect of toxins produced by F. oxysporum, F. solani and A. solani on germination of tomato and pepper seeds:

The three isolated fungi were grown on medium containing 200 g glucose, 0.5 g KCl, 0.5 g $MgSO_4$, 1.0 g KH_2PO_4 and 1.0 g yeast extract, and 1000 ml distilled water.

The liquid medium (100 ml) was dispensed in 250 ml Erlenmyer flasks, inoculated with 1 ml fresh prepared spore suspension (10^5-10^6) and incubated for one and two weeks at 28°C for the two Fusarium spp. and at 25°C for Alternaria solani. Fungal filtrates were used instead of water, to germinate tomato seeds (Kastle rock) and pepper seeds (California Wonder). Seeds were placed on surface of sterile filter papers wetted with fungi filtrates in sterile petri-dish. For control treatment, filter paper was wetted with sterile water. Percentages of germination, length of radicle and (hypocotyl, cotyledonary leaf and plumules) were recorded.

3- Standards toxins:

Standards of Zearalenone, Trichothecenes, Alternol (AOH), Altemuene (ALT) and Alternol mobomethyl ether (AME)were obtained from Sigma Chemical Co., P.O. BOX 14508, St., Louis, Mo 63178, USA and were used as reference standards.

4- Extraction and toxin analysis:

One ml of spore suspension from cultures of the two Fusarium spp. and Alternaria solani (10^6 /ml) were added to 100 ml of liquid medium in 250 ml Erlenmeyer flasks. The flasks were incubated at different temperatures or fungi grown on medium with different pH, carbon or nitrogen sources. Mycotoxins extraction and quantification were carried out using the method of A.O.A.C. (1984).

The culture filtrate or whole culture was extracted twice in separatory funnel with an equal volume 50 ml of ethyl acetate and the second extraction with 50 ml chloroform. Sodium chloride was added during shaking. The combined extracts were filtered over an hydrous sodium sulphate. The extracts were combined and evaporated to dryness on a rotary evaporator or under nitrogen to a small volume (1-2 ml). Purification of mycotoxins were carried out using SEP-PAC silica cartridge. Water Associates, Inc., Milford, MA, 01757. The final extract was then evaporated and stored at 3-5°C, to be used for microbial assay and chromatographic analysis.

All residues were analyzed by dissolving in 0.2 ml acetone or ethyl acetate or chloroform-acetone (9:1 v/v), spotting on thin-layer chromatography (TLC) sheet 13179 silica gel without a fluorescent indicator, activated at 110°C for 30 min, and development in unlined, unequilibrated tanks containing 100 ml solvent. Zearalenone and trichothecenes were determined on plate according to the method of Gimeno (1979). Initially, the plate was developed with hexane-ethyl ether-acetic acid (70:30:2 v/v/v) subsequently the plate was developed with hexane-ethylacetate (1:3 v/v). Plate was detected under UV light (365 and 254 nm) which appeared as a greenish blue fluorescent spot under shortwave, and confirmed by spraying with a fresh solution of 50% sulphuric acid in methanol and then heated for 10-20 min at 120°C. Zearalenone turns yellow and then brown, trichothecenes gave a blue colour.

Alerniol (AOH), Alter monomethyl ether (AME) and Altemune (ALT) were determined on plate using developing solvent which include chloroform-acetone (88-12 v/v) and toluene-ethyl acetate-formic acid (5:4:1 v/v/v). The compounds were visible as blue spots under long, and short wave UV light, AME and AOH being brighter under the latter. AME and AOH spots remained fluorescent blue after spraying

with 50% ethanolic sulphuric acid, but changed to greenish fluorescent-after-spraying and heating for 5 min at 100°C.

The intensity of the mycotoxins were measured with DESAGA CD_{60} fluirodensitometer at an excitation wave length of 365 um and emission wave length of 443 nm. The amount of mycotoxins extraction was the mean of three replicate samples on one TLC plate, and each spot was scanned twice.

5- Toxicity test and Biological assay:

Biological assay for toxicity was carried out using Bacillus megaterium strain 1057 and Bacillus mycoides EMCC 1084 (Ain Shams University, Microbiological Resource Centre, Cairo, Egypt). The organism was grown in 10 ml tryptone yeast glucose broth (TYG) Stott and Bullerman, 1975) for 24 h at 37° and then transferred to saline (0.85% w/v NaCl), diluted to a cell density of approx 10^6 ml. Aliquots 0.1 ml were inoculated on (TYGA) plates, B. megaterium for Fusaria toxins, while B. mycoides for Alternaria toxins. The plates were dried at 30°C for 60 min. To sterile filter-paper discs (5 mm diameter) 25 µl toxin extract was applied, (25 µl chloroform in control) After drying, the discs were placed on the inoculated plates which were incubated at 10-15°C for 1 h and then at 37°. After 24 h the width of the zone of inhibition round the edge of the disc was measured in duplicate experiments with 2 replicates for each extract concentration.

Statistical analysis was done using the complete randomized design in factorial arrangement (F-test). The least significant difference (L.S.D.) was used for comparing treatment means (Snedecor and Cochran, 1980).

Results and Discussion:

Fusarium spp. and Alternaria solani, when grown under artificial conditions, produced toxic diffusible substances which increased in concentration with the age of culture. These toxic substances inhibited growth of the radicels or (hypocotyl, cotyledonary leaf and plumules) when seeds were placed in the filtrate of the fungus.

Data in Tables (1 &2) show the effect of toxins produced by F. oxysporum and A. solani on germination of tomato seeds. It is clear that percentage of germination was

lower when tomato seeds were treated with filtrates of these fungi as compared with control; (water). Also the length of seedlings radicels and (hypocotyl, cotyledonary leaf and plumules) were less than control after the different periods of germination. Filtrates produced by these fungi were more toxic when fungi were incubated for two weeks compared with one week period. Percentage of germination decreased from 60 to 50 and from 70 to 60 incase of F. oxysporum and A. solani respectively.

The toxic effects of toxins produced by F. oxysporum and A. solani at different incubation periods when tested on the growth of pepper (hypocotyl, cotyledonary leaf and plumules) and radicle length decreased with increasing the incubation period till two weeks which were sensitive to phytotoxins Table (3 & 4). Also, percentage of germination decreased from 70 to 60 and from 90 to 80 when pepper seeds were treated with the two weeks old filtrates of F. solani and A. solani compared with the filtrates from one week old cultures.

Growth of hypocotyl, cotyledonary leaf and plumules was retarded and did not appear till after 6 days from the start of germination, i.e. reached 0.3 mm on the 6th day compared with the length hypocotyl, cotyledonary leaf and plumules incase of control (15.6 mm) Table (3), using filtrate of one week old culture of F. solani and A. solani. Where as no hypocotyl, cotyledonary leaf and plumules appeared till th 8th day i.e. length was 2-3 mm and 3-3 mm incase of F. solani and A. solani, using the filtrate of two week old culture, length of pepper radicle was affected by the fungal filtrate. The reduction in radicle length of the treated seeds reached approximately half that of the control.

These data show that all fungi isolates has the potential to produce phytotoxins which caused deformation of hypocotyls, chlorosis of cotyledons and stunting of seedlings. These toxins could be absorbed by the root after formation in the rhizosphere, or could be produced within the root cortex by invading pathogenic fungi, causing wilt or vessel plugging.

Results are in agreement with those obtained by Nemec el al., (1977) and Nemec, (1978) who reported that toxin produced by F. solani, was readily spread through the cortex and stele of rough lemon fibrous roots, causing growth reductions and chlorosis. Only inorganic salts and glucose, which would be available in the root

cortex are required for the elaboration of these toxins. F. solani are known to vary widely in toxin production (Kern and Naef-Roth 1965).

Appearance and spread of toxin-generating strains may be random, or may be favoured by changes in nutritional status or stress of the plants. Toxins produced by F. solani are known to disrupt plant metabolism by inhibiting anaerobic and oxidative decarboxylation reactions, (Kern et al., 1970).

Nedelnik, (1993) reported that 6 purified toxic substances, belonging to the trichothecenes, zearalenone and fusaric acid groups, at concentrations of 100, 10 and 1 g/ml, were phytotoxic on seeds of 12 varieties of Medicago sativa (Lucerne) and Trifolium pretense. Fusaric acid and acetoscirpenol at 100 g/ml had the most inhibitory effect on germination, followed by deoxynivalenol and H 1-2 toxins. Trichothecenes were significantly more phytotoxic than zearalenones. These toxins also caused deformation of hypocotyls, chlorosis of cotyledons and stunting of seedlings.

Results in Fig. (1-4) indicate the biological effect of different fungal extracts produced at different temperatures, pH, carbon and nitrogen sources on the growth of Bacillus megaterium 1057 and Bacillus mycoides EMCC 1084 as a possible biological confirmation for detecting toxins.

Toxins produced by F. oxysporum, F. solani showed the highest inhibition zones, at 28°C and pH 5, while toxins produced in media of pH 3 and 9 had lower effect on both bacteria. Also, sucrose and glucose were the best carbon sources, and led to a higher inhibition zones in case of F. oxysporum and F. solani respectively. Bacillus megaterium showed more inhibition by toxins produced by the two Fusarium spp. when using casein and ammonium chloride as nitrogen sources.

In case of A. solani, the highest inhibition zone for B. mycoides was detected at 25°C and at pH 7, while toxins were not detected when the investigated fungi were incubated at 40°C. Also, glucose was the best carbon source for growth and production of toxins by A. solani and led to higher inhibition zones Fig. (1-4). On the other hand, toxins produced by A. solani showed inhibitory effects on B. mycoides and was more pronounced in case of ammonium molybdate and ammonium chloride.

Statistical analysis of the previous data showed significant differences among the different factors under study. The biological effects of zearalenone and the F_2 series

were widely discussed by Mirocha et al., (1971). Zearalenone was non-mutagenic to Salmonella typhimurium in the Ames test. Zearalenone showed a narrow range of antibacterial activity limited to some Gram-positive aerobic spore-forming bacteria. Alternaria toxins (AME, AOH and ALT) were toxic to Bacillus mycoides and Hela cells, and weakly toxic to mice when administrated as a single dose (Betina, 1984).

Fusarium spp. secreted zearalenone and trichothecenes into the medium when grown in liquid cultures at different incubation temperatures, pH, carbon and nitrogen sources. The results in Table (5) indicate that the optimal temperature for both zearalenone and trichothecenes production by F. oxysporum was at 25°C (15,27 and 169.02 μg/ml), while the total toxin levels was 184.29 μg/ml. In case of F. solani, maximum amount of trichothecenes. (32.19 μg/ml) was detected at temperature 28°C while the greatest levels of zearalenone 10.43 μg/ml was observed at 25°C. At 25°C, maximum production of total toxins was 39.34 μg/ml. Data in Table (5) show that trichothecenes were the most frequently detected mycotoxins while zearalenone was present in low levels.

The effect of pH on toxin production by both F. oxysporum and F. solani is summarized shown in Table (6). The greatest amounts of both trichothecenes and zearalenone were achieved at pH 5. No toxins were detected for both fungi at pH 3.

Results in Table (7)show the effect of different carbon sources on the production of zearalenone and trichothecenes. The amounts of zearalenone produced by F. oxysporum were in the order of xylose> sucrose> glucose> maltose> lactose> mannose, while for the other toxin, trichothecene, the amounts were in the order of; glucose> maltose> sucrose> xylose> lactose> mannose. Generally, the best carbon source for total toxins production (72.64 μg/ml) was glucose as compared with the other carbon sources.

In case of F. solani, the efficiency of toxin production differed for F. oxysporum, using the same carbon sources. The amounts of zearalenone were in the order of xylose> glucose> mannose> maltose> lactose> sucrose. While for the other toxin, trichothecene, the amounts were in order of maltose> xylose> sucrose> mannose> glucose> lactose. The lowest amounts toxins, produced by F. solani, were detected using lactose as a carbon source.

Table (8) shows the effect of concentrations of the two toxins produced by F. oxysporum in presence of different nitrogen sources. Ammonium phosphate gave high levels of zearalenone and trichothecenes; 24.73 and 92.35 μg/ml respectively. While casein resulted in high levels of trichothecenes were lower than ammonium phosphate. In case of F. solani, there was a high concentration of zearalenone in presence of casein, 319.03 μg/ml. On the other hand, using ammonium molybdate, a high concentration of trichothecenes was detected, 183.68 μg/ml.

Much attention should be devoted to the natural occurrence of trichothecenes together with zearalenone and the combined effects of these toxin on human health. Thrane (1986), detected, zearalenone, T-2, HT-2, diacetoxyscripenol, neosolaniol, deoxnivalenol, fusaranon, produced by 114 Fusarium isolates. Also Krivobok el al., (1987) detected citrinin and zearalenone, 4 toxigenic from Fusarium spp. grown in liquid medium.

Data obtained in Table (9 & 10) show the effect of temperature and pH on quantities of Alternoil monomethyl ether (AME), Alternoil, (AOH) and Altenune (ALT) toxins produced by Alternaria solani. The higher quantities of AME, AOH and ALT were produced at 28°C. Also, large amounts of total toxins were produced at 28°C, white, the lowest amounts of toxins were detected at 35°C. Meanwhile, at pH 7 higher amounts of total toxins were produced, 184.38 μg/ml Table (8).

The production of the various mycotoxins by A. solani in the different carbon sources are given in (Table 11). It was observed that the high amounts of AME were detected in the presence of the order xylose> sucrose> mannose> lactose> maltose> glucose. while ALT was detected in the order glucose> xylose> sucrose> mannose> maltose> lactose. A high concentration of AOH was recorded in the presence of glucose followed by xylose.

The results in Table (12) indicate that the greatest amounts of (AME) were observed in ammonium molybdate and ammonium sulphate 0.8 μg/ml, while the maximum (ALT) was observed in the presence of casein, (AOH) production followed a similar pattern. Generally, casein and ammonium molybdate were the most favourable nitrogen sources for mycotoxins production by A. solani. Where as, ammonium

chloride, ammonium phosphate and ammonium sulphate, showed lower efficiency for toxin production.

Toxin production by a given fungius has been shown to depend on different environmental factors. Inadequate storage conditions, such as high moisture and warm temperature (25-30°C), can create conditions favourable for the growth of a fungus and production of mycotoxins (CAST, 1989).

The trichothecenes mycotoxins are potent inhibitors of protein and DNA synthesis in eukaryotic cells, and the bone marrow, thymus and intestinal epithelia are the target organs Ueno, (1987). Only limited mutagenic activity of the trichothecenes has been demonstrated in several short-term tests, such as the Ames test.

Zearalenone (ZEA) [6-(10-hydroxy-6-oxo-trans-1 undecenyl)-Bresorcylic acid μ-lactone] is an oestrogenic secondary metabolite produced by various species of Fusarium, which causes hyperestrogenism in swine as well as in other mammals (Schuh and Glawisching, 1980).

Alternaria is one of the most commonly occurring postharvest fungi in the decay of plants including many fruits and vegetables. In human foods, studies indicated that some of the mycotoxins produced by Alternaria, such as alternariol methyl ether (AME) and tenuzonic acid (TA), could be high in apple and tomato products, (Jelinek el al., 1989). Mycotoxins produced by Alternaria include dibemz [a] pyrone-type toxins, such as alternariol (AOH), AME, altenunene, tetramic acid types of metabolites, such as TA and perylene derivatives, such as altertoxins I, II and III. Crude extracts of A. alternata have been shown to be positive in the Ames test, Scott and Stoltz, (1980).

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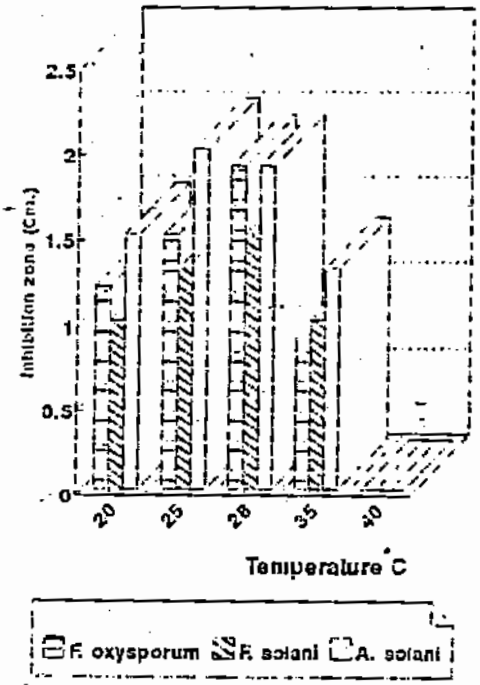

Fig. (1): Effect of toxin produced by three fungi at different temperatures on growth of Bacillus megaterium and Bacillus mycoides.

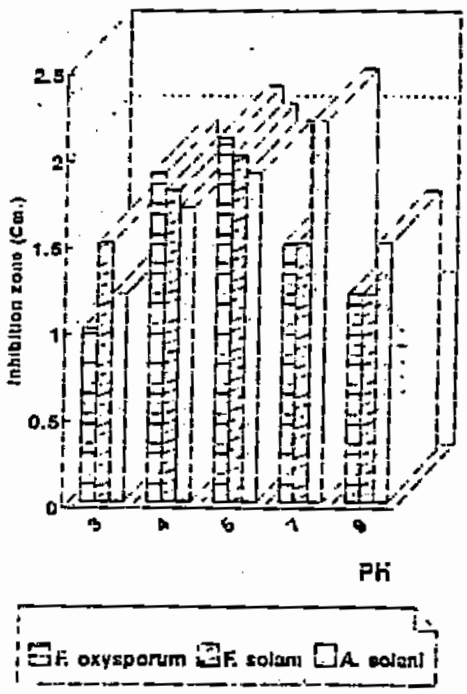

Fig. (2): Effect of toxin produced by three fungi at different pH's on growth of Bacillus megaterium and Bacillus mycoides.

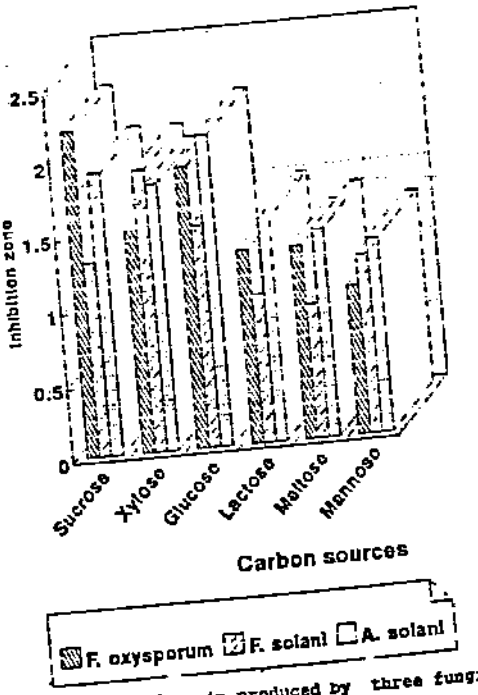

Fig. (3): Effect of toxin produced by three fungi using different carbon sources on growth of Bacillus megaterium and Bacillus mycoides.

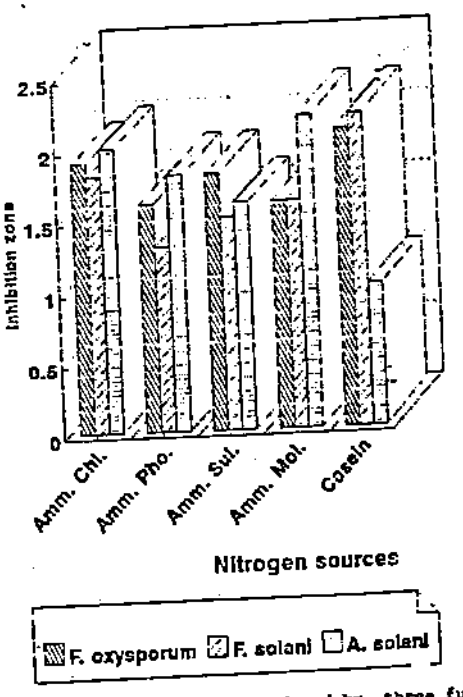

Fig. (4): Effect of toxin produced by three fungi using different nitrogen sources on growth of Bacillus megaterium and Bacillus mycoides.

Table (1): Effect of toxin produced by Fusarium oxysporum f.sp. lycopersici and Alternaria solani from one week old culture. on growth of tomato seedlings .

Treatment	Length of radicle (cm.)				Length of plumule (cm.)				Germination %
	Days after germination				Days after germination				
	2	4	6	8	2	4	6	8	
1	2.0	4.6	10.0	19.3	0.0	0.0	5.6	9.3	60.00
2	1.6	11.3	17.3	28.0	0.0	4.3	16.0	27.6	70.00
Control	6.0	18.6	24.3	32.6	5.3	12.3	29.6	40.0	100.00

*1- Seeds watered with F. oxysporum filtrate.

*2- Seeds watered with A. solani filtrate.

* Control seeds watered with sterile water

Table (2): Effect of toxin produced by Fusarium oxysporum f. sp. lycopersici and Alternaria solani from two weeks old culture. on growth of tomato seedlings.

Treatment	Length of radicle (cm.)				Length of plumule (cm.)				Germination %
	Days after germination				Days after germination				
	2	4	6	8	2	4	6	8	
1	2.6	3.6	5.0	11.0	0.0	0.0	5.0	10.6	50.00
2	2.6	10.0	13.3	19.0	6.0	7.6	9.0	15.0	90.00
Control	6.0	18.0	24.3	32.6	5.3	12.3	29.6	40.0	100.00

*1- Seeds watered with F. oxysporum filtrate.

*2- Seeds watered with A. solani filtrate.

* Control seeds watered with sterile water

Table (3): Effect of toxin produced by Fusarium solani and Alternaria solani from one week old cultures. on growth of pepper seedlings.

Treatment	Length of radicle (mm.)				Length of plumule (mm.)				Germination
	Days after germination				Days after germination				%
	2	4	6	8	2	4	6	8	
1	1.3	5.0	9.6	16.0	0.0	0.0	0.3	3.3	70.00
2	3.3	7.0	11.6	17.3	0.0	1.3	5.0	8.0	90.00
Control	4.3	12.3	17.0	22.3	0.6	3.3	15.6	21.0	90.00

*1- Seeds watered with F. solani filtrate.

*2- Seeds watered with A. solani filtrate.

* Control seeds watered with sterile water

Table (4): Effect of filtrates produced by Fusarium solani and Alternaria solani from two weeks old culture, on growth of pepper seedlings.

Treatment	Length of radicle (mm.)				Length of plumule (mm.)				Germination
	Days after germination				Days after germination				%
	2	4	6	8	2	4	6	8	
1	2.6	3.6	8.3	14.0	0.0	0.0	0.0	2.3	60.00
2	2.3	5.0	9.0	16.0	0.0	0.6	0.0	3.3	80.00
Control	4.3	12.3	17.0	22.3	0.6	3.3	15.6	21.0	90.00

*1- Seeds watered with F. solani filtrate.

*2- Seeds watered with A. solani filtrate.

* Control seeds watered with sterile water

able (5): Effect of temperature on concentration of toxins produced by two Fusarium species .

Fungus	Temperature C	Toxin production ug./ml.		Total toxin ug./ml.
		Zearalenone	Trichothecenes	
F. oxysporum	20	1.012	58.940	59.952
	25	15.265	169.020	184.285
	28	12.749	61.675	74.424
	35	1.803	45.631	47.434
F. solani	20	8.685	10.719	19.404
	25	10.429	12.347	22.776
	28	7.152	32.190	39.340
	35	1.333	6.098	7.431

Table (6): Effect of pH on concentration toxins produced by two Fusarium species.

Fungus	pH	Toxin production ug./ml.		Total toxin ug./ml.
		Zearalenone	Trichothecenes	
F. oxysporum	3	0.000	0.000	0.000
	5	18.230	88.030	106.260
	7	1.211	36.043	37.254
F. solani	3	0.000	0.000	0.000
	5	15.145	13.871	29.016
	7	8.780	9.147	17.927

7?): Effect of different carbon sources on concentration of toxins produced by two Fusarium species .

Fungus	Carbon sources	Toxin production ug./ml.		Total toxins ug./ml.
		Zearalenone	Trichothecenes	
xysporum	Sucrose	34.957	19.795	54.752
	Glucose	18.917	53.727	72.644
	Lactose	5.950	7.451	13.401
	Maltose	16.812	33.830	50.642
	Mannose	3.352	4.392	7.744
	Xylose	31.080	16.900	47.980
solani	Sucrose	1.379	62.272	63.651
	Glucose	8.416	43.702	52.118
	Lactose	1.860	22.283	24.143
	Maltose	2.301	88.682	90.983
	Mannose	2.440	53.78	56.220
	Xylose	8.500	71.180	79.680

Table (8): Effect of different nitrogen sources on concentration of toxin produced by two Fusarium species.

Fungus	Nitrogen sources	Toxin production ug./ml.		Total toxin ug./ml.
		Zearalenone	Trichothecenes	
F. oxysporum	Amm. chloride	19.750	2.172	21.922
	Amm. molybdate	10.789	7.024	17.813
	Casein	6.210	49.452	55.662
	Amm. phosphate	24.731	92.349	117.080
	Amm. Sulphate	5.013	4.564	9.577
F. solani	Amm. chloride	9.410	4.638	14.048
	Amm. molybdate	15.650	183.677	199.317
	Casein	319.028	53.549	372.580
	Amm. phosphate	12.200	35.261	47.461
	Amm. Sulphate	14.085	2.082	16.167

e(9): Effect of temperature on concentration of toxins produced by Alternaria solani.

Temperature C	Toxins (ug/ml.)			Total toxins ug./ml.
	AME	ALT	AOH	
20	79.200	0.880	36.552	116.632
25	57.600	2.550	126.734	186.885
28	144.800	2.497	163.480	310.777
35	48.000	0.900	48.000	96.900

* AME= Alternoil monomethyl ether
* ALT= Altenune
* AOH= Alternoil

(10): Effect of pH on concentration of toxins produced by Alternaria solani

pH	Toxins (ug/ml.)			Total toxins ug./ml.
	AME	ALT	AOH	
5	83.200	1.100	15.790	100.090
7	168.000	0.383	16.000	184.383
9	11.200	2.680	18.410	32.290

* AME= Alternoil monomethyl ether
* ALT= Altenune
* AOH= Alternoil

Table (11): Effect of different carbon sources on concentration of toxins produced by Alternaria solani.

Carbon sources	Toxin production ug./ml.			Total toxins ug./ml.
	AME	ALT	AOH	
Sucrose	261.096	10.440	37.556	309.092
Glucose	0.128	57.136	46.250	103.514
Lactose	160.450	0.136	18.945	179.531
Maltose	82.590	1.016	19.548	103.154
Mannose	164.240	2.208	5.355	171.803
Xylose	305.984	18.440	42.710	367.134

* AME = Alternoil monomethyl ether
* ALT = Altenune
* AOH = Alternoil

Table (12): Effect of different nitrogen sources on concentration of toxins produced by Alternaria solani.

Nitrogen sources	Toxin production ug./ml.			Total toxins ug./ml.
	AME	ALT	AOH	
Amm. chloride	0.080	4.880	7.832	12.792
Amm. molybdate	0.800	3.640	22.426	26.866
Casein	0.264	14.430	54.493	69.187
Amm. phosphate	0.560	0.056	15.531	16.147
Amm. Sulphate	0.800	1.580	11.380	13.760

* AME = Alternoil monomethyl ether
* ALT = Altenune
* AOH = Alternoil

عزل وتعريف السميات الفطرية المنتجة بواسطة أجناس الفيوزيريم والألترناريا

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تم عزل فطريات الفيوزيريم أوكس سبورم (ليكوبرس)، فيوزيريم سولانى والألترناريا سولانى من بذور الطماطم والفلفل والنباتات المريضة. نميت فطريات الفيوزيريم والألترناريا على بيئات صناعيه منتجة للمواد السامة التى يزيد تركيزها بزيادة عمر المزرعة وهذه المواد السامة ثبطت نمو الجذير أو (السويقة الجنينية السفلى - الأوراق الفلقية والرويشات) عندما نبتت البذور فى رشيح هذه الفطريات. ويظهر التأثير المبدئى المثبط للمستخلص إلى السمية الضوئيه.

وقد إمتدت الدراسة لإختبار النشاط البيولوجى لسميات الفطريات. السموم المنتجه بأجناس الفيوزيريم والألترناريا عند (درجات الحرارة، الأرقام الأيدروجينية، مصادر الكربون والنيتروجين) المختلفه قد ثبطت نمو الباسيلس ميجاتريم والباسيلس ميكويدس التى تختلف فى سميتها لنمو البكتيريا.

أفرزت أجناس الفيوزيريم الزراليون والتريكوثسين فى البيئة السائلة. وكان أعلى معدل لها عند درجات حرارة ٢٥-٢٨°م ورقم أيدروجينى (٥) عند إنماء الفيوزيريم اوكس سبورم على مصادر كربونية مختلفه، كان كمية الزراليون على هذا النحو ... زيلوز > سكروز > جلوكوز > مالتوز > لاكتوز > مانوز. بينما كانت كميات التريكوثسين على هذا النحو .. جلوكوز > مالتوز > سكروز > زيلوز > لاكتوز > مانوز.

إختلفت قدرة الفيوزيريم سولانى على إنتاجية التوكسين عن الفيوزيريم اوكس سبورم عند إستعمال نفس المصادر الكربونيه السابقة. قد أعطى فوسفات الأمونيوم أعلى معدل لإنتاج الزراليون والتريكوثسين (٢٤,٧٣ ، ٩٢,٣٥ ميكرو جرام/ملى). فى حالة الفيوزيريم سولانى كان إنتاج الزراليون أعلى فى وجود الكازين (٣١٩ ميكرو جرام/ملى)، بينما أعطى الأمونيوم موليبدات أعلى تركيزات من التريكوثسين.