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EFFECT OF CHEMICAL AND BIOLOGICAL INSECTICIDES ON GROWTH, PIGMENTATION, PHOTOSYNTHETIC ACTIVITY AND YIELD PRODUCTIVITY OF *VICIA FABA* PLANTS

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A pot experiment was conducted to study the effect of foliar application of chemical insecticide (malathion) and biological insecticide (yeast emulsion) on growth vigor, pigments content, photosynthetic activity, growth regulators content of bean plants and the quality of yielded seeds as well as its chemical composition.

In the majority of cases, the two insecticides appeared to cause pronounced effect on growth parameters during growth of bean plants. Malathion application induced slight increase or marked decrease in pigments content and photosynthetic activity of 50 and 85 days old plant, respectively. On the other hand, yeast emulsion appeared to cause non-significant effect on the above-mentioned parameters, all over the growth period of bean plants.

Foliar application of malathion caused significant decrease in growth promotery substances (i.e. IAA, GA_3 and cytokinin) with simultaneous increase in IAA-oxidase as well as abscisic acid content of bean plant. The application of yeast emulsion appeared without significant effect on growth regulators content of bean plants. Moreover, yield and yield attributes as well as the biochemical aspects of yielded seeds showed variable changes in response to malathion and yeast emulsion application.

Key words: growth, insecticides, *Vicia faba*, yield

INTRODUCTION

All chemical insecticides are now being viewed with suspicion by peable interested in protecting our world from broad scale pollution (Martin and Charles 1976). Chemical insecticides are widely used in Egypt and other countries in the modern agriculture in order to minimize the loss in economic crops due to insect invading (Barakat 1997).

Many investigators have noticed the deleterious effect of insecticides, thus the inhibitory effect of organophosphate insecticides (the group to which malathion belong) on plants have been discussed by many authors

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(Berisford and Ayres 1976, Draughon and Ayres 1981, Gruzdyev *et al.* 1983, Kallqvist *et al.* 1994, Sudhakar *et al.* 1998).

Rozek (1984) working on *Phaseolus vulgaris* plant observed that, treating this plant with malathion and dimethoate accelerated the degradation of chlorophyll *a* and *b* and carotenoids. Furthermore Preusser *et al.* (1984) working on *Zea mays* and *Vicia faba* found that higher concentrations of endosulfan insecticide caused distinct inhibition of carbohydrates and protein contents.

Ewais (1988) stated that the organophosphorus insecticides (malathion, dimethoate and tamaron) inhibited not only chlorophylls, total soluble carbohydrates and protein biosynthesis in treated sunflower plants, but also caused inhibition of the biological activities of auxins and gibberellins. Recently Saafan (1995) found that, treatment of Squash plants with insecticide nuvacron significantly decreased chlorophyll contents, total soluble carbohydrates of leaves and protein content and also caused marked reduction in the activities of auxins and gibberellins accompanied by an increase in the activities of growth inhibitors. The degree of reduction was found to depend on the age of the plant.

In the light of the above-mentioned reviews, it was thought of particular interest to study the effect of chemical insecticide malathion as compared with the biological insecticide yeast emulsion as a foliar application on growth parameters, pigments, photosynthetic activity and growth regulators content of *Vicia faba* plant as well as their effect on yield components and chemical composition of the yielded seeds.

MATERIALS AND METHODS

Pure strain of *Vicia faba* var. Giza 402 was obtained from the Agricultural Research Centre, Ministry of Agriculture, Giza. The insecticides applied in this investigation are: commercial malathion as a chemical insecticide which used at the recommended dose (according to Ramulu 1983). It belongs to organophosphate group with the formula S–1,2 di(ethoxy carbonyl) ethyl 00-dimethyl phosphorodithioate. The second insecticide is the yeast emulsion as a biological insecticide which composed of 1.5 g yeast powder, 1 g wheat meal and 1 g molas in 100 ml water (according to announcement of Ministry of Agriculture of Egypt).

A homogeneous lot of *Vicia faba* seeds were surface sterilized with 0.001 M $HgCl_2$ solution for three minutes and then washed thoroughly with distilled water. The sterilized seeds were sown in earthen ware pots (30 cm in diameter) filled with 3 kg soil (sand: clay 2/1 v/v). The pots were kept in greenhouse and the plants were subjected to natural day/night conditions (minimum/maximum temperature and relative humidity were 29.2/33.2 and 35/45%, respectively, at mid-day during the experimental period) and irrigated with tap water. After two weeks, the pots were divided into three sets, the plants of the 2nd and 3rd sets sprayed with malathion and yeast emulsion, respectively, while the plants of the 1st set sprayed with normal tap water to serve as control.

Growth measurement, pigments and photosynthetic activity as well as growth regulators contents of *Vicia faba* plants started after 50 days from sowing and finished after 85 days from sowing. In addition, the yield components and chemical composition of the yielded seeds were detected.

A number of uniform plants (ten) from each group was taken for growth and yield determinates, while triplicate samples were used for pigments and photosynthetic activity. Other samples were collected and deep frozen in methanol, for at least 24 hours, before the determination of growth regulators. Data were obtained and the mean values were computed for the previous parameters.

Estimation of growth regulating substances. The extraction and separation of plant growth substances followed the procedures described by Shindy and Smith (1975). For bioassay of auxins, the straight growth test of *Hordeum* coleoptile sections as described by Foda and Radwan (1962) was used. For measurement of gibberellin substances, the lettuce hypocotyl bioassay adopted by Frankland and Wareing (1960) was followed. The technique used to assay the activity of cytokinins was that described by Esashi and Leopold (1969); cotyledons of *Xanthium brasilicum* seeds were used as the test specimen. ABA was bioassayed by the straight-growth test of *Triticum* coleoptile segments as recommended by Wright (1969). To assay the IAA-oxidase, the plant material was extracted following the method of Kar and Mishra (1976). The activity of this enzyme was assayed following the method of Gorden and Weber (1951) as described by Darby-Shire (1971).

Estimation of photosynthetic pigments. Pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) were measured by the spectrophotometric method as recommended by Metzner *et al.* (1965).

Estimation of photosynthetic activity (^{14}C-light fixation). As described by Shaddad (1979) and modified by Aldesuquy (2000) a definite fresh mass of 2nd leaf discs was introduced into the fixation apparatus (Fig. 1). An aque-

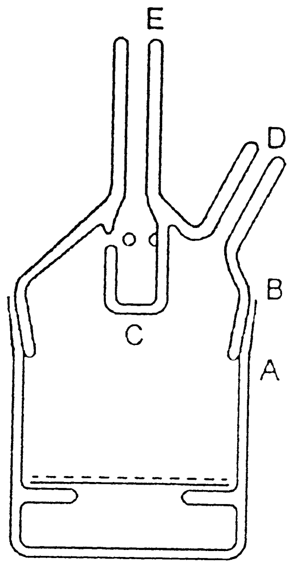

Fig. 1. $^{14}CO_2$-fixation apparatus. A: main container, B: lid, C: inner container (1 cm^3 capacity), D and E: side arms

ous solution of ^{14}C-sodium carbonate of known activity (3.7 MBQ cm^{-3}) was pipetted into the apparatus followed by H_2SO_4 (10%). The evolved $^{14}CO_2$ passed over and radioactivity of the green leaf discs was measured using Packard Scintillation Counter model 526.

Estimation of carbohydrates. The method of extraction was essentially those described by Younis *et al.* (1969). The polysaccharides were determined in the dry residue after alcohol extraction according to Naguib (1963).

Estimation of protein content. The method adopted for estimation of protein was essentially those described by Bradford (1976).

The results were first subjected to the analysis of variance (Anova). When Anova showed a significant ($p < 0.05$) effect, the least significant differences were used to compare treatments (Snedecor and Cochran 1976).

RESULTS AND DISCUSSION

Growth parameters (*i.e.* shoot and root length, number of branches, number of leaves, leaf area, number of bacterial nodules, fresh and dry weight of shoot and root) were decreased ($p < 0.05$) in response to mala-

Table 1

Effect of foliar application of malathion or yeast emulsion on growth parameters of Vicia faba plants

a. Shoot

Growth parameters	Shoot length (cm)		No. of branch/plant		No. of leaves/plant		Leaf area (cm^2)		Fresh wt. of shoot (g/plant)		Dry wt. of shoot (g/plant)		Water content of shoot (g/plant)		Water content of shoot (%)	
Day of sowing	50	85	50	85	50	85	50	85	50	85	50	85	50	85	50	85
Control	25.2	42.8	0.44	1.73	8.0	19.5	24.8	38.6	13.8	37.3	1.26	3.95	12.54	33.35	90.87	89.41
Malathion	21.6*	41.0	0.33*	1.40*	6.8*	17.3*	21.0*	35.1*	11.7*	33.0*	1.17*	3.36*	10.53*	29.64*	90.00*	89.81
Yeast emulsion	24.9	41.9	0.37	1.63	7.6	18.9	24.2	38.2	13.1	35.2	1.24	3.78	11.86	31.42	90.53	89.26
L.S.D. 5% level	1.29	2.98	0.08	0.11	0.59	1.75	0.78	2.02	0.94	2.56	0.09	0.23	1.01	2.71	0.72	0.8

b. Root

Growth parameters	Root length (cm)		No. of bacterial nodules		Fresh wt of root (g/plant)		Dry wt of root (g/plant)		Water content of root (g/plant)		Water content of root (%)	
Day of sowing	50	85	50	85	50	85	50	85	50	85	50	85
Control	15.8	22.6	68.0	79.5	5.4	8.5	0.32	0.80	5.08	7.7	94.07	90.59
Malathion	14.9*	21.8	49.1*	53.4*	3.9*	6.9*	0.27*	0.60*	3.63*	6.3*	93.08	91.30
Yeast emulsion	15.5	22.3	62.5	75.6	5.3	8.10	0.30	0.66	5.00	7.44	94.34	91.85
L.S.D. at 5% level	0.86	1.99	6.30	6.46	0.18	0.45	0.03	0.15	0.93	0.61	1.32	1.44

* = significant increase or decrease

thion treatment, throughout the growth and development of bean plants (Table 1). On the other hand, the above-mentioned growth criteria appeared to be non-significantly affected by yeast emulsion treatment (Table 1).

The reduction in growth parameters seems to be a common effect of most insecticides (Westlake and Gunther 1966, Munnecke 1978, Batra and Stavely 1993, Savitri *et al.* 1998). In this connection Steven *et al.* (1999) stated that malathion exposure frequently caused significant decrease in algal biomass accrual and they found that the inhibition of algal biomass accrual by malathion was more severe under conditions of enhanced nutrient supply.

In general, malathion increased ($p < 0.05$) the total chlorophylls and carotenoids of bean leaves after 50 days from sowing (Table 2). On the other hand, these pigments decreased ($p < 0.05$) in response to malathion particularly after 85 days from sowing. Yeast emulsion appeared without significant effect on pigment content of bean leaves during the two stages of bean growth (Table 2).

It is apparent from Table 3 that the photosynthetic activity in the bean plant (soluble, insoluble and total photosynthates) increased ($p < 0.05$) and decreased ($p < 0.05$) after 50 and 85 days from sowing, respectively, with malathion application. Generally yeast emulsion treatment seemed without significant effect on photosynthetic activity. On the other hand, the ratio of soluble to insoluble photosynthate was non-significantly affected by malathion or yeast emulsion, during the two stages of plant growth.

The increase in pigment content and photosynthetic activity of bean leaves after 50 days from sowing as a result of malathion treatment may be attributed to that malathion (Tables 2 and 3) may provide a source of phosphorus to the plant (Steven *et al.* 1999). Also Ebrahim (1999) stated that gaucho application to cotton plant induced a progressive and significant increase in chlorophyll *a* and *b* content as compared with that of control plants. Furthermore, he attributed this increment to the influence of the insecticide gaucho on the developmental processes leading to synthesis of photosynthetic apparatus and chlorophyll, and/or to the effect on activities of chloroplast enzymes.

The data in Tables 2 and 3 showed that malathion treatments decreased the pigments and photosynthetic activity of bean leaves after 85 days from sowing. These results are in accordance with those obtained by Rozek (1984) who reported that the use of the insecticides malathion and dimethoate had significantly reduced the chlorophyll contents of *Phaseolus vulgaris* plants. Furthermore, it has been suggested that these chemicals

Table 2

Effect of foliar application of malathion or yeast emulsion on photosynthetic pigments (mg/g dry wt) of *Vicia faba* plants

Parameters	Chlorophyll a		Chlorophyll b		Total chlorophylls		Carotenoids		Total pigments	
Day of sowing	50	85	50	85	50	85	50	85	50	85
Control	2.60	2.10	1.31	1.30	3.91	3.40	0.92	0.81	4.83	4.21
Malathion	2.72*	1.85*	1.39*	1.17*	4.11*	3.02*	1.06*	0.80	5.17*	3.82*
Yeast emulsion	2.70	2.10	1.38	1.36	4.08	3.46	1.00	0.83	5.08	4.29
L.S.D. at 5% level	0.12	0.14	0.08	0.075	0.18	0.13	0.091	0.071	0.26	0.13

Table 3

Effect of foliar application of malathion or yeast emulsion on photosynthetic activity of *Vicia faba* plants (Cpm × 10^{-3}/g fresh wt)

Parameters	Soluble		Insoluble		Total photosynthates		Soluble/insoluble	
Day of sowing	50	85	50	85	50	85	50	85
Control	42.98	56.43	11.05	16.81	54.03	73.24	3.89	3.35
Malathion	46.18*	46.98*	12.33*	13.92*	58.51*	60.90*	3.75	3.37
Yeast emulsion	43.11	56.46	11.82	16.92	54.93	74.38	3.65	3.34
L.S.D. at 5% level	2.45	3.26	1.05	2.34	3.32	5.66	0.46	0.33

* = significant increase or decrease

might have accelerated the degradation of chlorophyll *a* and *b* and carotenoids of the tested plants (Saafan 1992). The decline in chlorophyll content as a result of insecticide treatment has also been reported by Saafan (1995) working with nuvacron.

Growth stimulators (*i.e.* IAA, GA3 and kinetin) of bean plants appeared to decrease ($p < 0.05$) or non-significantly affected in response to application of malathion or yeast emulsion, respectively, all over the growth period (Table 4). As compared to control plants malathion increased ($p < 0.05$) the level of IAA oxidase or ABA content of bean plants during growth and development. On the other hand, yeast emulsion application resulted in a non-significant effect on IAA oxidase level and ABA content (Table 4).

These results are in accordance with those of Ewais (1988), who observed disturbances in the auxin levels of *Cicer arietinum* seeds as a result of endosulfan treatment. The observed decrease in auxins equivalent to IAA as a result of insecticide application may result from the increase in activity of IAA oxidase. These results were in conformity with those obtained by Volynets and Pal'chenko (1977) who showed that IAA oxidase was activated by phenolic compounds isolated from lupine plants treated with 2,4-D, metaxon and dalapon. Also Cole (1982) stated that the pesticides enhanced the oxidative destruction of IAA.

The decline in gibberellins equivalent to GA_3 accompanied with insecticide treatment may probably be due to the insecticide inhibition on its biosynthesis from its precursors. In this connection, Wilkinson (1982) observed that incorporation of ^{14}C-mevalonate into GA-precursor was inhibited as a result of treating the etiolated sorghum coleoptile with the pesticide. On the other hand, Chakraborti *et al.* (1983) stated that the simultaneous use of IAA or GA_3 with different concentrations of malathion tended to overcome the inhibitions caused by the insecticides as regarded the seedling growth, and the rate of application of root of *Vigna sinensis*. Recently Saafan and Mostafa (1995) found that treatment of maize plants with methomyl led to a significant decrease in the percentage and activity of growth promoting substances, while the growth inhibitors showed the opposite trend; thus significantly increased in number and activity with the increase in insecticide treatment and the age of plants. The same results were obtained by Saafan (1995) when treated squash plant with nuvacron.

The number of pods per bean plant, the number of seeds per pod, the total number of seeds per plant and the fresh and dry weights of seeds per pod were decreased significantly and non-significantly in response to foliar application with malathion and yeast emulsion, respectively (Table 5).

Table 4

Effect of foliar application of malathion or yeast emulsion on growth regulators content (µg/g fresh wt) and on IAA oxidase (activity/g. fresh wt/hr) of *Vicia faba* plants

Parameters	IAA		GA3		Cytokinin		ABA		IAA oxidase	
Day of sowing	50	85	50	85	50	85	50	85	50	85
Control	5.53	8.36	2.01	3.41	6.01	6.73	3.65	4.81	489.28	543.80
Malathion	4.97*	7.60*	1.82*	3.00*	5.32*	6.11*	4.35*	5.22*	584.28*	668.59*
Yeast emulsion	5.32	8.31	1.95	3.26	5.81	6.56	3.78	4.80	511.13	538.14
L.S.D. at 5% level	0.31	0.43	0.10	0.19	0.22	0.41	0.32	0.32	31.708	44.974

Table 5

Effect of foliar application of malathion or yeast emulsion on yield components and physiological aspects of developing seeds of *Vicia faba* plants

Parameters	No. of pods/plant	No. of seeds/pod	Total number of seeds/plant	Fresh wt of seeds (g/pod)	Dry wt of seeds (g/pod)	Total carbohydrates (mg/100 g dry wt)	Total protein (mg/100 g dry wt)
Control	13.8	3.4	48.5	2.7	0.28	16100	31640
Malathion	12.8*	3.0*	46.0*	2.4*	0.23*	15600	30580
Yeast emulsion	13.5	3.3	47.5	2.6	0.25	15900	30913
L.S.D. at 5% level	0.98	0.31	2.15	0.19	0.04	953	1217

* = significant increase or decrease

On the other hand, as compared to control plant, protein and total carbohydrates content of the yielded seeds of the plants treated with malathion or yeast emulsion, appeared to be non-significantly decreased (Table 5).

The reduction in yield components and the chemical composition of the yielded seeds in response to application of insecticides are in accordance with those obtained by Deka *et al.* (1998) who studied the relative field efficiency of five different insecticides (among them malathion) on mung bean crop during three successive seasons and found that the tested insecticides are effective in decreasing the grain yield of mung bean. In this respect Mathur *et al.* (1982) observed that the contents of reducing sugars in cotyledons of *Vigna mungo* seeds soaked in the insecticide dimethoate were increased, whereas contents of sugars in the axes were decreased with the increasing in the insecticide concentrations as being compared with those of control seeds.

Saafan (1995) recorded that the decreased level of protein in the tested plants (squash) by insecticide nuvacron is similar to that earlier, reported by Rozek (1984) who stated that the application of dimethoate or malathion to *Phaseolus vulgaris* plants altered the protein level of the yielded seeds. On the other hand, Almand (1995) and Burris *et al.* (1995) found that gaucho treatments enhanced all yield components of cotton plant significantly.

The observed reduction in yield component and protein as well as carbohydrates of the yielded seeds as a result of insecticide treatment is consistent with the decrease in the leaf area, number of bacterial nodules, pigments and consequently the photosynthetic activity (see Tables 1–3). In this connection Ebrahim (1999) stated that the increase of chlorophyll content with gaucho treatment could be referred to increasing nitrogen content which is the main component of the chlorophyll molecule.

Malathion is one of the safest organophosphorus compounds (Ramulu 1983), while the obtained results in this investigation, emphasized significant differences between treated and untreated plants, and hence it may be concluded that pest control is not only dependent upon the quality of the used pesticide, but also on their doses. Furthermore, it is clear from these data that malathion as a chemical insecticide is more dangerous to *Vicia faba* plants as compared with the used biological insecticide (yeast emulsion). This observation also recorded by Barakat (1997) who studied the genotoxicity of the chemical pesticide goal 2E as compared with the new biological pesticide biofly, by their effect on cell division and their capacity to induce chromosomal abnormalities in *Allium cepa* and *Vicia faba* plants.

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