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Influence of Malathion and Mancozeb on Mycorrhizal Colonization and Growth of *Zea mays* and *Vicia faba*

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Abatract: Mycorrhizal symbiosis of *Zea mays* and *Vicia faba* plants noticeably increased their growth parameters. Leaf pigments and element contents of N, P, Ca, K, Mg and Na, were also increased as compared with non-mycorrhizal plants. Infection of mycorrhizal plants and their sporulation were markedly influenced by the applied level of pesticides. Lower concentrations of both Malathion and Mancozeb were responsible for higher mycorrhizal colonization and sporulation, while higher concentrations were conducive to mycorrhizal activities. Malathion proved to be more deleterious to mycorrhizal colonization and sporulation compared with Mancozeb. Plant growth responded differently depending on pesticides concentration and mycorrhizal inoculations. At lower pesticides concentrations, mycorrhizal plants had high levels of growth parameters compared with pesticide free plants, while non-mycorrhizal plants show low levels. Higher concentrations of pesticides reduced growth of tested plants. The reduction in growth reached to 80% for some parameters. Moreover, the reduction of plant pigments b not exceeded 16%. Malathion affect the growth parameters of *Zea mays* and *Vicia faba*, either, mycorrhizal or non-mycorrhizal, compared with Mancozeb. The latter was most deleterious to elements contents at tested pesticides concentrations.

Key words: Pesticides · mycorrhizal symbiosis · malathion · mancozeb · *Zea mays* · *Vicia faba*

INTRODUCTION

The arbuscular mycorrhizal fungi are important rhizospheric microorganisms. They are the mutualistic symbiosis (non-pathogenic association) between soil-borne fungi and the roots of higher plants [1]. They are also described to improve the absorption of several nutrients by the roots of the growing plant as P, N, K, Mg, Cu, Ca and Fe [2-4]. So, mycorrhiza can increase plant uptake of nutrients and consequently, they increase root and shoot biomass and improve plant growth [5].

The deliberate use of pesticides and/or their inadvertent introduction into the soil environment is of concern, because many pesticidal compounds are known to influence pest populations by hampering biochemical and physiological processes that are common to both pest and non-pest populations [6]. Pesticides could disrupt plant productivity by inhibiting the development of symbiotic association between vesicular arbuscular mycorrhizal (VAM) fungi and plants [7]. Mycorrhiza can survive pesticides employed in commercial nursery and enhanced plant productivity [8]. However, their colonization could be reduced in field plots through application of some fungicides as a soil drench [9]. Their activity may be greatly limited by soil fumigation [10]. The objective of this study is to investigate the effects of two different pesticides; Flonex-M400 (fungicide) and Malathion-57% (insecticide) on mycorrhizal colonization and growth of *Zea mays* (a monocotyledon) and *Vicia faba* (a dicotyledon) plants.

MATERIALS AND METHODS

Soil: Sandy loam soil (1:1) was air dried, passed through 2 mm sieve, mixed thoroughly for homogenicity and sterilized by autoclaving at 121°C for 20 min to kill soil microflora. The soil is non-saline with pH 7.9 and has 1.35% organic matters. The total soluble salts were 1.17%, with total nitrogen content of about 0.89 mg kg^{-1} and phosphorus content of 0.042 mg kg^{-1}.

Test plants: Seeds of *Vicia faba* and grains of *Zea mays* were kindly provided from Hada Al-Sham farm, Faculty of Metrology, King Abdulaziz University, Jeddah,

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Saudi Arabia. Seeds and grains sizes and weights were homogenous. They were surface sterilized (0.1% $HgCl_2$ + 0.2% HCL for 5 min), followed by repeated washes with sterile distilled water. Seeds and grains were planted in presterilized plastic pots (18 cm in diameter and 13 cm in depth) loaded with 2 kg air dried sterilized sandy loam soil which is mixed with 0.3 g rock phosphate /kg and received 60 ml weekly of Hoaglands solution, minus phosphorus [11 12].

Pot experiments: Five seeds or grains were planted in each pot at equal intervals. They were irrigated until the plants seedlings emerge (about 5 cm height) and thinned to two plants per pot.

The pots are classified into two sets; each has 70 pots. The first set had no mycorrhizal inoculations, while the second was inoculated with 300 fungal spores per pot. Half of the pots in each set were planted with *Zea mays* and the other half was planted with *Vicia faba*. Three weeks after sowing, thirty pots of each sets were sprayed with the fungicides, Flonex-MZ400 (Mancozeb, zinc ion, manganese ethylene bisdithiocarbamate), at different concentrations (0.75, 1.4 recommended and 2.8 ml l^{-1}) and the other thirty of each set were sprayed by the insecticide, Malathion -57% (0.0-dimethyl (1,2-dicarbeethoxyethyl) dithiophosphate), at different levels (0.75, 1.12 recommended and 2.2 ml l^{-1}). The rest were not treated with pesticides and served as controls.

Zea mays and *Vicia faba* seedlings were allowed to grow under greenhouse conditions (28°C and illumination period of 13 h/day) for eight weeks. At the end of the tested growth period, plants were carefully uprooted, washed thoroughly with tap water and rinsed twice with distilled water. After washing, the roots and foliar system were separated, weighed, dried at 70°C, ground and stored for further analyses.

Mycorrhizal spores: Spores of VAM fungi were collected from agricultural soils at Asfan region, Saudi Arabia. They extracted from their cultivation and propagation pots, planted with *Zea mays* (to have high population of viable spores), using wet sieving and decanting method [13]. The spore suspension was diluted with water, so that each ml has 30 spores. For soil inoculation, the surface soil crushed and 10 ml of the spore suspension were distributed on the soil surface, after which the crushed soil was return back into its pots.

Growth parameters of the test plants: The foliar (shoot) system height (cm) and root system length (cm) were measured. Dry weights of root and shoot systems were also determined.

Plant pigments: Chlorophyll a, b and carotenoids were estimated spectro-photometrically [14], after their acetone extraction from fresh leaves.

Determination of nitrogen: It was measured colrurimetrically using Nesslers reagent [15, 16].

Determination of P, Ca, K, Na and Mg: Plant materials were digested in nitric-perchloric acid mixture (5:3) and analyzed colourimetrically with malachite green reagent to determine P concentration and by atomic absorption spectrophotometry [17], Perkin-Elmer 500, for Ca, K, Na and Mg.

Mycorrhizal root infection: Roots were carefully washed from adhering soil using tap water. A sample of approximately 0.5 g fresh roots from each pot was removed and cut into 1 cm length to estimate the degree of VAM infection using grid-line intersect method [18], for examination of cleared and stained root samples, Phillips and Hayman [19] method was used.

Data were statistically analyzed using one way analysis of variance ANOVA on the basis of which L.S.D values ($p = 0.05$ for $N = 5$) for any two compared means were calculated.

RESULTS AND DISCUSSION

Response of test plants to mycorrhizal infection: Inoculation of *Zea mays* and *Vicia faba* plants, with VAM spores noticeably increased their growth parameters (Table 1). *Zea mays* shoot and root systems showed about 28, 40% length increase. Biomass outcome increase of about 80, 188%, respectively, as plant grown in soil infected with VAM spores. Plant pigments of chlorophyll a, b and carotenoids increased by 36, 14 and 26%, respectively, under mycorrhizal compared with non-inoculated plants. Assayed plant elements showed range of increase between 10% for K and 142% for P. It is noteworthy to conclude that phosphorus content, root dry weight and to a lesser extent shoot dry weight and nitrogen content were the most growth parameters responded effectively to mycorrhizal inoculation. The rest parameters showed good range of increases (10-54%).

As for the dicotyledonous plant *Vicia faba*, mycorrhizal inoculation resulted in a detectable increasing in plant parameters ranged between 10% up to

Table 1: Effect of mycorrhizal inoculation on some growth parameters of *Zea mays* and *Vicia faba* plants after 8 weeks

	Zea mays		*Vicia faba*	
Growth parameters	-VAM	+VAM (increase %)	-VAM	+VAM (increase %)
Shoot length (cm) /plant	53.30	28.0	48.30	33.1
Root length (cm)	14.70	40.0	14.50	27.6
Root dry weight (g)	0.11	118.0	0.25	146.6
Shoot dry weight (g)	0.82	80.0	0.68	87.0
Leaf pigments (mg g^{-1}):				
Chlorophyll a	4.20	36.0	6.56	12.0
Chlorophyll b	3.40	14.0	4.20	20.0
Carotenoids	2.50	26.0	3.20	19.0
Element content (mg g^{-1}):				
N	9.20	88.0	14.10	67.0
P	0.92	142.0	0.81	161.0
Ca	7.80	53.0	6.40	53.0
K	0.98	10.0	1.01	10.0
Mg	5.10	54.0	3.10	90.0
Na	1.30	30.0	1.20	33.0

Table 2: Effect of different concentrations of the fungicide, mancozeb and the insecticide malathion on mycorrhizal infection and spore numbers in *Zea mays* and *Vicia faba* roots and rhizospheric soil, after 8 weeks of planting

	Zea mays		*Vicia faba*	
Pesticide concentration (ml l^{-1})	VAM infection (%)	No. of spores/100 g dry soil	VAM infection (%)	No. of spores/100 g dry soil
Control	61.1	29.0	53.4	22.0
Fungicide: Flonex-M400:				
0.75	71.3	34.0	57.2	27.0
1.40	66.3	33.0	47.5	17.0
2.80	43.1	21.0	33.1	13.0
Insecticide: Malathion-57%:				
0.75	67.2	31.0	56.1	26.0
1.12	48.6	21.0	45.2	18.0
2.24	27.1	13.0	20.9	9.0
L.S.D	13.02	11.28	09.06	8.80

160% comparing to non-inoculated plants. To some extend, the figure was almost parallel to that recorded in *Zea mays* plants for phosphorus, root and shoot dry weights and nitrogen. However, Mg uptake increased significantly to about 90% in VAM inoculated *Vicia faba*, while its pigments content generally showed lower increases at VAM inoculation as compared to *Zea mays* pigmentation, except for chlorophyll b. In accordance to these findings, it was reported by many workers that mycorrhizal inoculation increased growth yields of many plants [8, 20-23]. Mycorrhizal inoculations to many plants increase their essential metal uptake of P, N, Mg, K, Na, Ca, etc., [24-26]. It was indicated that VAM colonization increased photosynthetic activity [27, 28]. It was found that VAM symbiosis in legume plants has been attributed to high P uptake necessary for nodulation and N_2-fixation and also improve N absorption [4, 29, 30].

Effect of pesticides on mycorrhiza: The influence of different concentrations of the fungicide (Mancozeb) and the insecticide (Malathion) on mycorrhizal root infection and spores numbers (Table 2), indicated that low applied concentrations of the two pesticides were non toxic to VAM, but to lesser extent stimulate their infection and spore numbers. However, spraying *Zea mays* plants with the recommended concentration of the fungicide (1.4 ml l^{-1}), still promoting fungal infection and spore numbers. Generally, it is safe to conclude that, at the recommended doses of the fungicide to *Vicia faba* or the insecticide to both plants, detectable decreases in

Table 3: Effect of different concentrations of malathion on some growth parameters of *Zea mays* and *Vicia faba* plants either mycorrhizal or non-mycorrhizal after 8 weeks of planting

		Zea mays		*Vicia faba*	
Growth parameters	Insecticide level (ml l^{-1})	+VAM	-VAM	+VAM	-VAM
Shoot length (cm) /plant	0.75	70.10	49.40	67.20	43.30
	1.12	59.80	43.20	53.60	38.20
	2.24	32.10	32.20	31.40	21.30
Root length (cm) /plant	0.75	22.30	12.60	19.1	12.9
	1.12	16.20	9.80	15.3	12.3
	2.24	10.20	8.90	9.2	6.4
Shoot dry weight (g)	0.75	1.59	0.58	1.38	0.57
	1.12	1.24	0.40	0.85	0.41
	2.24	0.79	0.24	0.38	0.21
Root dry weight (g)	0.75	0.36	0.09	0.57	0.19
	1.12	0.24	0.06	0.31	0.17
	2.24	0.13	0.04	0.25	0.09
Leaf pigments (mg g^{-1}):					
Chlorophyll a	0.75	6.80	3.50	7.50	6.30
	1.12	6.20	3.40	7.30	6.10
	2.24	5.70	3.30	7.00	5.90
Chlorophyll b	0.75	4.20	2.80	5.00	3.90
	1.12	3.80	2.70	4.90	3.60
	2.24	3.60	2.60	4.50	3.30
Carotenoids	0.75	3.30	2.20	3.90	3.00
	1.12	3.00	2.10	3.70	2.70
	2.24	3.00	2.00	3.50	2.60
Elements content (mg g^{-1}):					
N	0.75	17.70	5.90	24.30	8.40
	1.12	11.30	2.80	19.40	6.00
	2.24	8.90	1.00	11.00	3.00
P	0.75	2.40	0.60	2.20	0.40
	1.12	2.00	0.30	1.90	0.10
	2.24	1.20	0.10	0.70	0.08
Ca	0.75	12.40	5.50	10.50	4.00
	1.12	9.60	3.80	7.80	3.00
	2.24	6.10	1.90	5.10	1.70
K	0.75	1.10	0.60	1.20	0.80
	1.12	0.90	0.40	0.90	0.50
	2.24	0.70	0.20	0.50	0.30
Mg	0.75	8.10	4.00	6.00	2.00
	1.12	4.50	3.00	4.00	1.10
	2.24	2.90	1.80	2.50	0.70
Na	0.75	1.70	1.00	1.60	0.80
	1.12	1.30	0.90	1.20	0.60
	2.24	1.00	0.70	1.00	0.30

root infection (17% for *Vicia faba*, 28, 19% for *Zea mays* and *Vicia faba*, respectively) and spore numbers (37% for *Vicia faba*, 32 and 31% for *Zea mays* and *Vicia faba*, respectively) were estimated, as compared to the lowest concentration of both pesticides. However, doubling the recommended concentration of the fungicide and insecticide resulting in marvel VAM root infection to both plants, ranging between 40-63% and spore numbers (38-73% less) as compared to the lower pesticides concentration. The results clearly indicated that the lower concentration of both pesticides and the recommended concentration of fungicide stimulated mycorrhizal colonization and sporulation in both plants compared with control. On the other hand, the insecticide

Table 4: Effect of different concentrations of mancozeb on some growth parameters of *Zea mays* and *Vicia faba* plants either mycorrhizal or non-mycorrhizal after 8 weeks of planting

		Zea mays		*Vicia faba*	
Growth parameters	Fungicide level (ml l^{-1})	+VAM	-VAM	+VAM	-VAM
Shoot length (cm) /plant	0.75	73.20	51.10	71.20	44.20
	1.12	70.60	47.00	69.30	40.30
	2.24	57.80	43.70	53.40	33.10
Root length (cm) /plant	0.75	23.50	13.60	22.20	13.20
	1.12	22.10	13.20	19.20	11.70
	2.24	18.10	10.90	16.20	9.90
Shoot dry weight (g)	0.75	1.76	0.68	1.43	0.54
	1.12	1.59	0.59	1.38	0.41
	2.24	0.98	0.48	0.80	0.29
Root dry weight (g)	0.75	0.52	0.09	0.60	0.240
	1.12	0.49	0.08	0.58	0.200
	2.24	0.25	0.06	0.33	0.120
Leaf pigments (mg g^{-1}):					
Chlorophyll a	0.75	6.90	3.90	7.70	6.40
	1.12	6.10	3.60	7.50	6.30
	2.24	5.80	3.50	7.10	6.00
Chlorophyll b	0.75	4.40	3.00	5.30	4.00
	1.12	4.30	2.90	5.10	3.90
	2.24	3.80	2.70	4.80	3.50
Carotenoids	0.75	4.00	2.30	4.10	3.10
	1.12	3.90	2.20	3.90	2.90
	2.24	3.40	2.10	3.60	2.70
Elements content (mg g^{-1}):					
N	0.75	20.60	7.10	29.20	10.30
	1.12	18.90	4.00	26.40	7.50
	2.24	13.60	3.10	17.20	4.20
P	0.75	2.70	0.70	2.50	0.50
	1.12	2.40	0.50	2.30	0.30
	2.24	1.90	0.20	1.70	0.10
Ca	0.75	13.20	6.10	12.10	4.30
	1.12	13.00	4.20	11.00	3.10
	2.24	8.90	3.00	6.30	1.90
K	0.75	1.20	0.80	1.20	0.90
	1.12	1.10	0.60	1.10	0.70
	2.24	0.90	0.40	1.00	0.40
Mg	0.75	9.60	4.00	7.80	2.20
	1.12	8.80	3.20	6.70	1.30
	2.24	6.10	2.10	3.50	0.90
Na	0.75	1.80	1.20	1.70	1.00
	1.12	1.80	1.10	1.60	0.70
	2.24	1.50	1.00	1.30	0.60

(malathion) was more deleterious to both mycorrhizal infection and sporulation in both plants compared with fungicide at some tested concentrations. In accordance to these findings it was indicated that pesticides might reduce VAM infection and sporulation depending on the type and rate of pesticides [31-33]. The application of the recommended concentration of some pesticides decreased root colonization by VAM and their sporulation [34] and the mycorrhiza can survive to pesticide concentrations employed in commercial nursery [8]. It had been also

reported that some pesticides have not affect VAM colonization and sporulation or to enhance them, while high doses decreased root colonization and sporulation [35-37].

Effect of pesticides on growth parameters of mycorrhizal and non-mycorrhizal plants: The influence of different concentrations (0.75, 1.12 recommended and 2.24 ml l^{-1}) of malathion on some growth parameters of *Zea mays* and *Vicia faba* plants, either mycorrhizal or non-mycorrhizal (Table 3) revealed that applying the lower concentration (0.75 ml l^{-1}) resulted in detectable decreases in the tested growth parameters in non-mycorrhizal plants compared with mycorrhizal infected plants. These results indicate that in presence of mycorrhiza, the test plants might survived the lower pesticide concentration and enhance plant growth, the results in live with that reported by Marin *et al*. [8]. Higher insecticide concentrations (1.12 and 2.24 ml l^{-1}) resulted in a drastic decrease in the growth parameters, where at the higher concentration (2.24 ml l^{-1}), the reduction in growth parameters was approximately 50% compared to low insecticide concentration. However, plant pigmentation of chlorophyll a, b and carotenoids reduced by about 16% at the same concentration. Results revealed that plant pigments were less susceptible to the action of the insecticide, either in mycorrhizal infected or non-infected plants. Moreover, an indirect relationship was observed between insecticide concentrations and elements content. P and N levels in non-mycorrhizal plants were about one third or less that detected in mycorrhizal infected plants. The results generally indicated that mycorrhizal infected plants had higher growth parameters compared with non-mycorrhizal plants (Table 2).

As for the fungicide mancozeb, the same trend of reduction in growth parameters was reported mainly at 0.75 ml l^{-1} concentration (Table 4). At all mancozeb concentration, mycorrhizal colonization stimulated plants growth parameters compared with non-mycorrhizal plants. These findings indicated that lower pesticides concentrations encourage mycorrhizal root infection and sporulation in both plants and these in harmony with the results in Table 2. Also, it is safe to conclude that the applied insecticide was more deleterious to growth parameters, mycorrhizal colonization and sporulation compared with fungicide, under same tested concentrations. Reduction of root and shoot lengths did not exceed 29%, at the highest concentration of fungicide (2.4 ml l^{-1}). Also reduction of dry weights of both systems ranged between 26-52% was reported at the same concentration of fungicide for both plants either mycorrhizal colonized or none. The pigmentations of the two plants were also less susceptible to the fungicide treatment (only 2-16% reduction). This may indicate that under pesticide stress, the activity of photosynthetic apparatus, including pigmentation, increased to form metabolites that counter the undesirable effect of the pesticides.

The elements content (N, P, Ca and K) of plants were drastically affected as the fungicide concentrations increased especially in absence of mycorrhizal infection whereas the reduction ranges between 50-80% compared with only 50% reduction in the case of mycorrhizal colonization. Phosphorus followed with N were the most reduced elements in absence of VAM (71-80% for P and 56-59% for N) and the two elements were less susceptible to fungicide action under mycorrhizal plants colonization. These findings revealed the importance of mycorrhizal symbiosis in elements uptake, especially P and N.

The results generally indicated that mancozeb was more deleterious, to tested plant growth parameters and leaf pigmentation. Mancozeb was more drastic to metal uptake, either in non-mycorrhizal or mycorrhizal colonized plants. In accordance to these findings, it was reported that pesticides have negative effect on plant growth and activity of mycorrhizal symbiosis, as well as elements content of N, P, K [7, 20, 38]. It was also indicated that pesticides have positive influence in plant growth at low levels, but disturb the symbiotic relationship between VAM and the host plant [6, 34].

Many workers revealed that lower doses of pesticides have positive influence in plant pigments of chlorophyll a, b and carotenoids, while higher concentrations have adverse effect [39, 40].

It is well known that mycorrhizal colonization enhancing P uptake, which increases nutrient use efficiency [25]. Increased pesticide levels resulted in sharp decrease in P uptake [41].

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