ELSEVIER

Applied Soil Ecology 14 (2000) 191–200

Applied Soil Ecology

www.elsevier.com/locate/apsoil

The impact of pesticides on arbuscular mycorrhizal and nitrogen-fixing symbioses in legumes

Mohamed Hemida Abd-Alla[a,*], Shukry Ahmed Omar[a,1], Sokol Karanxha[b]

[a] *Botany Department, Faculty of Science, Assiut University, Assiut 71516, Egypt*

[b] *Chemistry Department, Interfaculty Department, Agricultural University of Tirana, Tirana, Albania*

Received 30 August 1999; received in revised form 1 March 2000; accepted 6 March 2000

Abstract

Effects of the pesticides Afugan, Brominal, Gramoxone, Selecron and Sumi Oil on growth, nodulation and root colonisation by arbuscular mycrrhizal (AM) fungi of the legumes cowpea (*Vigna sinensis* L.), common bean (*Phaseolus vulgaris* L.) and Lupin (*Lupinus albus* L.) were determined. The growth of all plants was inhibited by pesticide application, but this effect varied with the pesticide and plant species. Nodule formation was significantly inhibited in cowpea after 20 days of planting by all pesticides tested. Following the initial decrease, there was recovery from the inhibitory effects at 40 and 60 days after planting. Although the number of nodules on common bean and lupin did not differ from control at 20 days after planting, differences were evident during the later stages of plant growth. The pesticides significantly inhibited AM root colonisation and the number of spores in all legumes, but on the other hand, spore formation was stimulated in pesticide-treated cowpea 60 days after planting. The accumulation of N, P and K in pesticide-treated plants was lower than in control plants. Growth and nutrient status of the legumes varied with nodulation and AM colonisation. The results suggest that pesticides affect plant growth, *Rhizobium*/*Bradyrhizobium* and AM fungi at different stages of plant growth and effects varied with pesticide and plant species.

Keywords: *Bradyrhizobium*; Legumes; Nodulation; Pesticides; *Rhizobium*; Arbuscular mycorrhiza

1. Introduction

Symbiotic nitrogen fixation in agriculture can mainly be attributed to legumes, abundantly cultivated not only because of their potential to fix nitrogen but also because there exist numerous high yielding varieties of several species (e.g. *Glycine max*, *Phaseolus vulgaris*, *Vigna*, *Lupinus*) which form an excellent source of protein and oils for human and animal consumption. Traditionally, these legumes have been widely grown in the Nile River Valley basin and as the need for production of legumes has increased, it has become necessary to bring reclaimed desert areas into production. Although the fertility of these desert soils is adequate for limited production, the decrease in indigenous populations of micro-organisms has restricted yields (Hemida et al., 1997).

Many previous studies have shown that inoculation of legumes with both rhizobia and arbuscular mycorrhiza (AM) increases plant growth to a greater extent than can be attributed to either inoculum when added singly (e.g. Kawai and Yamamoto, 1986; Vejsadova et al., 1989). Barea and Azcon-Aguilar (1983) attributed this increase in growth to the enhanced foliar N content due to scavenging of the soil for mineral N by

* Corresponding author.

E-mail address: mhadbdalla@hotmail.com (M.H. Abd-Alla)

[1] Present address: Institute of Plant Nutrition, Justus-Liebig University, Südanlage 6, D-35390 Giessen, Germany.

0929-1393/00/$ – see front matter
PII: S0929-1393(00)00056-1

AM. An increase in P nutrition and a resulting increase in N_2 fixation has also been suggested to account for the additional benefits of the synergistic relationship (Brown and Bethlenfalvay, 1987).

Pesticides are essential for controlling plant pests, and accordingly, improve the productivity of major crops including legumes. Pesticides applied to leguminous crops constitute a potential hazard to growth, nodulation and nitrogen accumulation (Abd-Alla and Omar, 1993). Nemec (1985) reported that nematicides had little or no effect on AM fungi, but Sreenivasa and Bagyaraj (1989) reported that pesticides were toxic to AM fungi even at recommended levels. Menge (1982) reported that systemic fungicides have a more harmful effect on AM fungi than on non-systemic fungicides.

The effects of pesticide application on dual symbiosis in temperate and tropical soils has been studied extensively (e.g. Hetrick and Wilson, 1991; Sugavanam et al., 1994). However, the effect of pesticides on these organisms and plant growth in desert soil is yet to be examined adequately. The objective of the current study was therefore to examine the effects of pesticide application on AM–legume–rhizobia/bradyrhizobia associations in calcareous sandy soil.

2. Materials and methods

2.1. Plant growth

Surface-sterilized seeds (five seeds per pot) of common bean (*P. vulgaris* L. cv. Kentucky Wonder), cowpea (*Vigna sinensis* L. cv. Azmerly), and lupin (*Lupinus albus* L. cv. Marita) were sown in plastic pots (28 cm diameter, 38 cm depth) containing 5 kg of sterilized calcareous sandy clay soil. The sterilization was carried out by heating the soil in metal buckets at 200°C for 24 h. The physical and chemical characteristics of the soil are shown in Table 1. Available Ca, Mg, and Na were determined according to Lanyon and Heald (1982), and soil P according to Olsen and Sommers (1982).

Each pot was inoculated with 5 ml of appropriate rhizobia or bradyrhizobia suspension containing 10^7 cells ml^{-1}, prepared from 4-day old cultures grown in nutrient broth and added below the soil surface. *Rhizobium leguminosarium* bv. *phaseoli* TAL 182, *Bradyrhizobium* (cowpea) CB 1015, and *Bradyrhizobium* (lupin) WPBS 3201 D were used for inoculating common bean, cowpea, and lupin, respectively. Spores of *Glomus fasciculatum* (Hall and Fish, 1979; Trappe, 1982) were isolated from the soil using a wet sieving technique (Gerdemann and Nicolson, 1963) and multiplied on onion roots (Omar, 1995, 1998). AM inoculum (50 gm of onion rhizosphere soil) was added 2–3 cm below the soil surface. Ten days after emergence, seedlings were thinned to three plants per pot. Plants were grown under greenhouse conditions. Temperatures during the experimental period averaged 28/12 (day/night). Dilute (0.25 ionic strength, Somasegaran and Hoben, 1985) nitrogen-free nutrient solution was added once a week and the soil water content was adjusted to 60% water-holding capacity of the soil with distilled water every 2 days.

Table 1
Physical and chemical characteristics of the soil used

Property	Value
Sand (%)	55
Silt (%)	12
Clay (%)	23
$CaCO_3$ (%)	19
Electrical conductivity ($dS\,m^{-1}$)	1.8
pH (1.2 soil:water)	7.7
Organic matter (%)	0.36
Total N (%)	0.02
Exchangeable cation ($mg\,kg^{-1}$)	
Ca^{2+}	106
Mg^{2+}	28
Na^{+}	75

2.2. Pesticide application

Five pesticides were used in this investigation. The fungicide Afugan is commonly known as Pyrazophos with 30% active ingredient (a.i.). Chemically, it is 0,0-diethyl 0-(6-ethoxycarbonyl-5-methylpyrazolo-[1,5-a]pyrimidin 2-yl)phosphorothioate. Two herbicides were also used: Brominal (Bromoxynil or Labuctrill-25), a benzoic acid herbicide (3,5-dibromo-4-hydroxybenzonitrile) with 24% a.i. formulation and Gramoxone (Paraquat), a bipyridyl herbicide (1,1-dimethyl-4,4-bipyridylium dichloride) in 20% a.i. formulation. Afugan, Brominal and Gramoxone were manufactured by Hoechst AG, Frankfurt, Germany and Plant Protection Limited, Fernhurst, Haslemore, Surrey, England, respectively. The

insecticide Selecron (Profenfos), an organophosphorus pesticide (0-(4-bromo-2-chlorophenyl)0-ethyl *S*-*n*-propyl-phosphorothioate), in 72% a.i. formulation produced by Ciba Geigy Limited, Basel, Switzerland and Sumi Oil (formulated product of Suimithion, 0,0-dimethyl 0-(3-methyl-4-nitrophenyl)phosphorothiate) in 8% a.i. formulation produced by Kafar El-Zayat Pesticides and Chemicals Co., Egypt. Afugan, Bromnial, Gramoxone, Selecron and Sumi Oil were applied at the rate of 3.0, 0.6, 0.75, 0.9 and 0.13 mg a.i. (kg soil)$^{-1}$, respectively. These concentrations are equivalent to the recommended field dose. Conversion of field application rates to milligrams of pesticide per kilogram of soil was calculated assuming an even distribution of the pesticide in the plough layer. Pesticides were diluted with water and added to the top layer of the soil with irrigation water 2 days after planting and pots not receiving pesticides were used as control. Pots were arranged in a randomized block design with four replicates and left to grow under the conditions described above (Section 2.1) until harvesting.

2.3. Measurements

Plants were harvested every 20 days up to 60 days after sowing, with almost the entire root system intact. From each pot for each treatment, one plant was assessed after each harvest. Plant replicates for each treatment were of near uniform size and canopy shape. The shoots were severed at the soil level, dried at 70°C for 48 h and weighed. Roots were carefully separated from the soil together with rhizosphere soil particles. Root and soil from each individual pot were carefully wet-sieved to collect the spores (Gerdemann and Nicolson, 1963). The spores were kept refrigerated and each sample was adjusted to a suitable volume. Aliquots measuring 1 ml were spread on grid filter paper and counted using a dissecting microscope. After spore sieving, the roots were washed in distilled water and the nodules on each root were counted. After nodule counting, each root mass was divided into two equal parts. One-half of the root mass was dried at 70°C and weighed. This root mass when doubled represented the total dry mass of the original sample. The other part of the roots was cut into short lengths (3–5 mm) to estimate the proportion of root length colonized with AM fungi after clearing the roots in 2.5% KOH at 90°C and staining with trypan blue, 0.05% in lactophenol (Phillips and Hayman, 1970).

Dry matter from 60-day old plants was ground and digested in a triple acid mixture, and plant tissue P was determined according to Olsen and Sommers (1982). N was estimated by the Kjeldahl method (Black et al., 1965) and K was estimated by a flame photometric method (David, 1962).

2.4. Statistical analysis

Data were subjected to one-way analysis of variances with four replicates using a computer programme (PC-stat). Treatment means were compared using the least significant difference (LSD) test when significant *F* values occurred.

3. Results

3.1. Effects on plant growth

Most of the pesticides tested significantly inhibited shoot dry matter accumulation in cowpea after 20 and 60 days but not after 40 days (Table 2). Common bean plants had a significantly lower shoot dry mass 20 days after planting when grown in soil treated with Afugan, Brominal and Sumi Oil, but after 40 and 60 days, the decrease was evident in plants treated with Afugan and Brominal only. No initial effect of pesticides was observed on the shoot dry mass in lupin, but 60 days after planting, the shoot dry matter was reduced in all cases (Table 2).

Negative effects of pesticides on root growth of cowpea were not observed at 20 days after planting, but inhibition was observed after 40 and 60 days. Most of the pesticides used significantly decreased the root dry mass of common bean plants, especially after 40- and 60-day periods. The pesticides had no significant inhibitory effect on the root dry mass in lupin except Afugan and Brominal at 20 days after planting (Table 2).

3.2. Effects on nodule formation

All pesticides significantly reduced the number of nodules on cowpea after 20 days, but after the longer periods (40 and 60 days), this effect was confined to Selecron and Summi Oil (Fig. 1). No significant reduc-

Table 2
Effects of pesticide application on dry matter yields of some legumes grown in sandy soil inoculated with rhizobia/bradyrhizobia and arbuscular mycorrhiza[a]

Pesticide	Cowpea			Common bean			Lupin		
	20 DAP	40 DAP	60 DAP	20 DAP	40 DAP	60 DAP	20 DAP	40 DAP	60 DAP
Shoot dry mass ((g plant)$^{-1}$)									
Control	0.28	0.40	0.66	0.33	1.59	2.39	0.48	1.59	3.19
Afugan	0.21	0.41	0.50	0.27	0.78	1.50	0.39	1.29	2.12
Brominal	0.22	0.44	0.61	0.26	0.79	1.37	0.28	1.33	2.15
Gramoxone	0.22	0.46	0.53	0.31	1.33	2.05	0.45	1.39	2.18
Selecron	0.21	0.48	0.50	0.34	1.46	2.27	0.38	1.38	2.25
Sumi Oil	0.27	0.42	0.52	0.22	1.32	2.21	0.36	0.88	2.36
LSD ($P \leq 0.05$)	0.03	0.09	0.13	0.06	0.29	0.37	0.23	0.35	0.28
Root dry mass ((g plant)$^{-1}$)									
Control	0.06	0.42	0.59	0.20	0.49	0.58	0.18	0.42	0.59
Afugan	0.05	0.10	0.40	0.25	0.32	0.41	0.10	0.33	0.45
Brominal	0.06	0.12	0.35	0.13	0.34	0.43	0.08	0.39	0.48
Gramoxone	0.10	0.11	0.33	0.25	0.33	0.44	0.18	0.41	0.49
Selecron	0.18	0.21	0.42	0.14	0.35	0.52	0.20	0.46	0.50
Sumi Oil	0.07	0.33	0.39	0.17	0.29	0.40	0.22	0.35	0.44
LSD ($P \leq 0.05$)	0.05	0.12	0.09	0.06	0.14	0.12	0.06	0.11	0.18

[a] Each value represents the mean of four replicates; DAP: days after planting.

tion in nodule number on common bean was recorded after 20 days, but nodule number was significantly decreased with Brominal, Gramoxone and Sumi Oil after 40 days; and with Afugan and Brominal, only after 60 days. A deleterious effect of pesticides on nodulation of lupin was observed only for Afugan, after 60 days.

3.3. Effects on AM root colonisation and spore number

In the case of cowpea plants, the proportion of root length colonized by AM fungi was significantly decreased with all pesticides used 20 days after planting, but the effect of Afugan and Brominal had disappeared after 60 and 40 days, respectively (Table 3). Patterns of common bean root colonisation with AM fungi in the presence of these pesticides were similar to those for cowpea. On the other hand, root colonisation of lupin with AM fungi was significantly reduced with all pesticides and after all growth periods.

The number of AM spores sieved from the rhizosphere of cowpea was significantly decreased with all pesticides after 20 days, but the effect of Gramoxone had disappeared after 40 days (Table 3). On the other hand, pesticide application increased spore number after 60 days. Except for Afugan and Sumi Oil after 20 days, the pesticides significantly reduced the number of AM spores collected from the rhizosphere of common bean after all experimental periods. AM spore formation in the rhizosphere of lupin was inhibited with all pesticides and after all experimental periods.

3.4. Effects on plant nutrient status

Except in the case of Brominal, N and P contents of cowpea were significantly decreased compared to control plants 60 days after planting in soil treated with pesticides (Fig. 2). The K content of cowpea was decreased with Afugan and Brominal only. All pesticides used significantly decreased the N and P contents of common bean. Gramoxone had no significant effect on the K content of common bean, but all remaining pesticides significantly reduced the concentration of this element. Elemental (N, P and K) contents of lupin were significantly decreased with all pesticides.

4. Discussion

Our results show that all five pesticides, when used at field application rates, reduced growth and related microbial activity in cowpea, common bean and lupin,

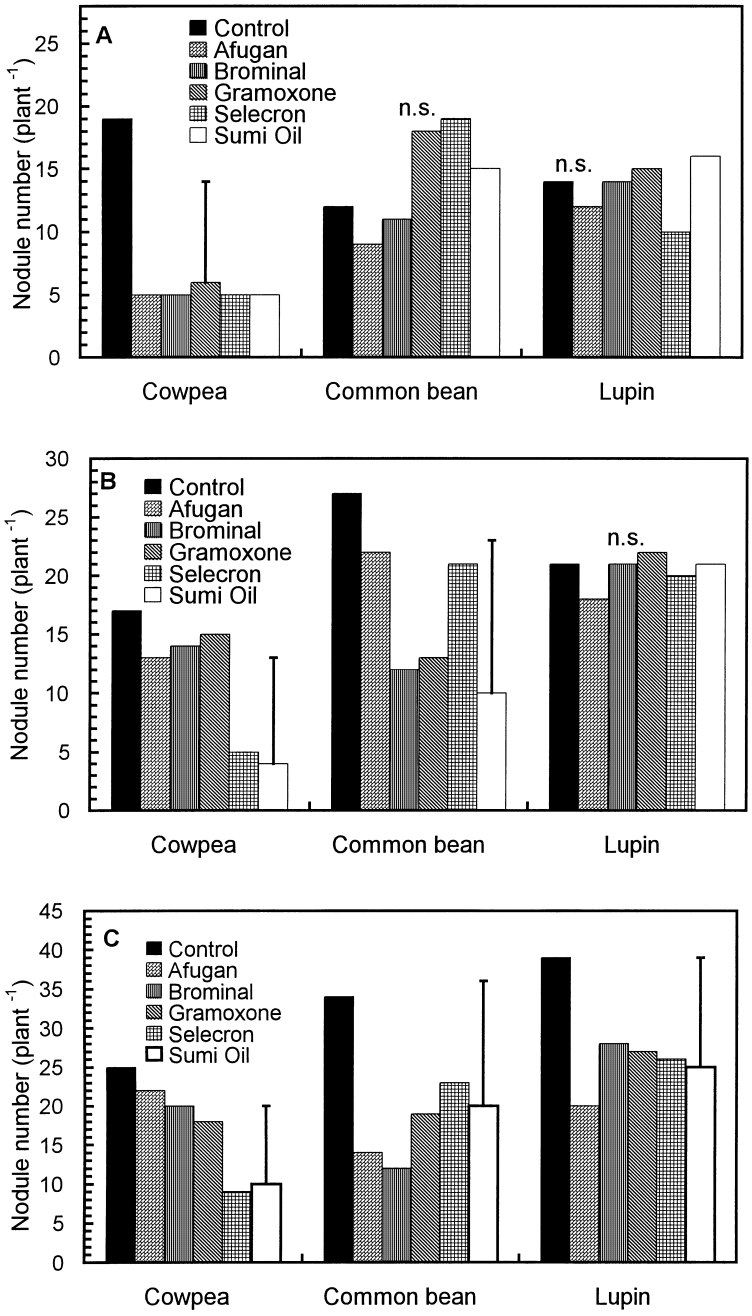

Fig. 1. Effect of pesticides on the nodule number on cowpea, common bean and lupin grown in sandy soil and inoculated with rhizobia/bradyrhizobia–arbuscular mychorrhiza and harvested at (A) 20 days, (B) 40 days, and (C) 60 days after planting. Values represent the means of four replicates for each species and vertical bars are LSDs at the 0.05 level; n.s. denotes non-significant differences among treatments at $P \leq 0.05$.

Table 3
Effects of pesticide application on arbuscular mycorrhizal root colonisation and arbuscular mycorrhizal fungal spore number in the rhizosphere soil of some legumes inoculated with rhizobia/bradyrhizobia and arbuscular mycorrhiza[a]

Pesticide	Cowpea			Common bean			Lupin		
	20 DAP	40 DAP	60 DAP	20 DAP	40 DAP	60 DAP	20 DAP	40 DAP	60 DAP
Root colonisation (%)									
Control	95	96	98	65	86	95	85	93	98
Afugan	65	85	93	43	59	92	35	46	52
Brominal	80	94	95	46	60	90	32	41	55
Gramoxone	75	79	84	48	68	91	18	28	85
Selecron	63	80	85	44	64	85	16	25	86
Sumi Oil	60	67	83	42	60	84	15	21	82
LSD (P=5%)	9.2	8.8	7.8	13	11.8	7.9	13.9	12.5	11.3
Spore number ((g soil)$^{-1}$)									
Control	18	22	28	12	25	37	22	30	37
Afugan	3	8	33	9	12	22	5	10	12
Brominal	4	9	34	3	15	25	3	12	15
Gramoxone	6	15	39	4	13	21	6	14	17
Selecron	5	7	40	5	14	27	5	11	15
Sumi Oil	6	8	43	8	16	28	4	8	11
LSD (P=5%)	3.4	7.3	10.8	4.2	7.9	8.6	6.4	9.3	10.3

[a] Each value represents the mean of four replicates; DAP: days after planting.

the degree of inhibition varying, to some extent, with pesticides and plants. Similar inhibitory effects of herbicides have previously been reported. MacRae and Alexander (1965) found that the growth of alfalfa in two types of autoclaved soil was strongly inhibited by the addition of the herbicide 4-(2,4-DB) to the soil. Bertholet and Clark (1985) reported that Trifluralin and Metribuzin reduced the dry mass of faba bean plants, while the shoot and root dry mass of faba bean were also decreased when grown in soil treated with Brominal and Gramoxone, even at field application doses (Abd-Alla and Omar, 1993). The effects of these chemicals on plant growth could be direct or indirect. Most reports attribute the inhibitory effect of pesticides on plant growth to the suppression of growth-promoting micro-organisms in the rhizosphere (De Bertoldi et al., 1978; Siti et al., 1982; Koch et al., 1997).

Our results indicate that root nodule formation was inhibited in all three legumes, the effect being most evident in the case of cowpea. Reduction in the number of nodules on soybean was recorded following Trifluralin application at rates of 0.74 and 1.1 kg^{-1} (Kust and Struckmeyer, 1971). Nodule dry mass of soybean was also decreased in five soil types treated with pesticides (Dunigan et al., 1972). Nodulation of broad bean was inhibited by the herbicides Trifluralin and Metribuzin (Bertholet and Clark, 1985). Similar results were also obtained by Eberbach and Douglas (1991) using the herbicides Paraquat and Glyphosate. Abd-Alla and Omar (1993) recorded a significant reduction in nodule formation by faba bean plants grown in herbicide-treated pot soil. Curley and Burton (1975) attributed the inhibition in nodulation, concomitant with pesticide application, to the inability of rhizobia/bradyrhizobia to multiply in the presence of pesticides. Pesticides may inhibit nodulation through their effect on cellulolytic and pectolytic enzyme production by rhizobia, as occurs in other micro-organisms (Mahmoud and Omar, 1995). Production of these enzymes by rhizobia is essential for root hair penetration (Hansen, 1994). Nodulation inhibition with pesticides may also be due to alteration of root hair morphology or to alteration in the quality and quantity of root exudation (Ratnayake et al., 1978) including flavenoid compounds and lectin that play an important role in the attraction and attachment of rhizobia to root hairs (Hansen, 1994).

Deleterious effects of pesticides on root colonisation with AM fungi and mycorrhizal spore forma-

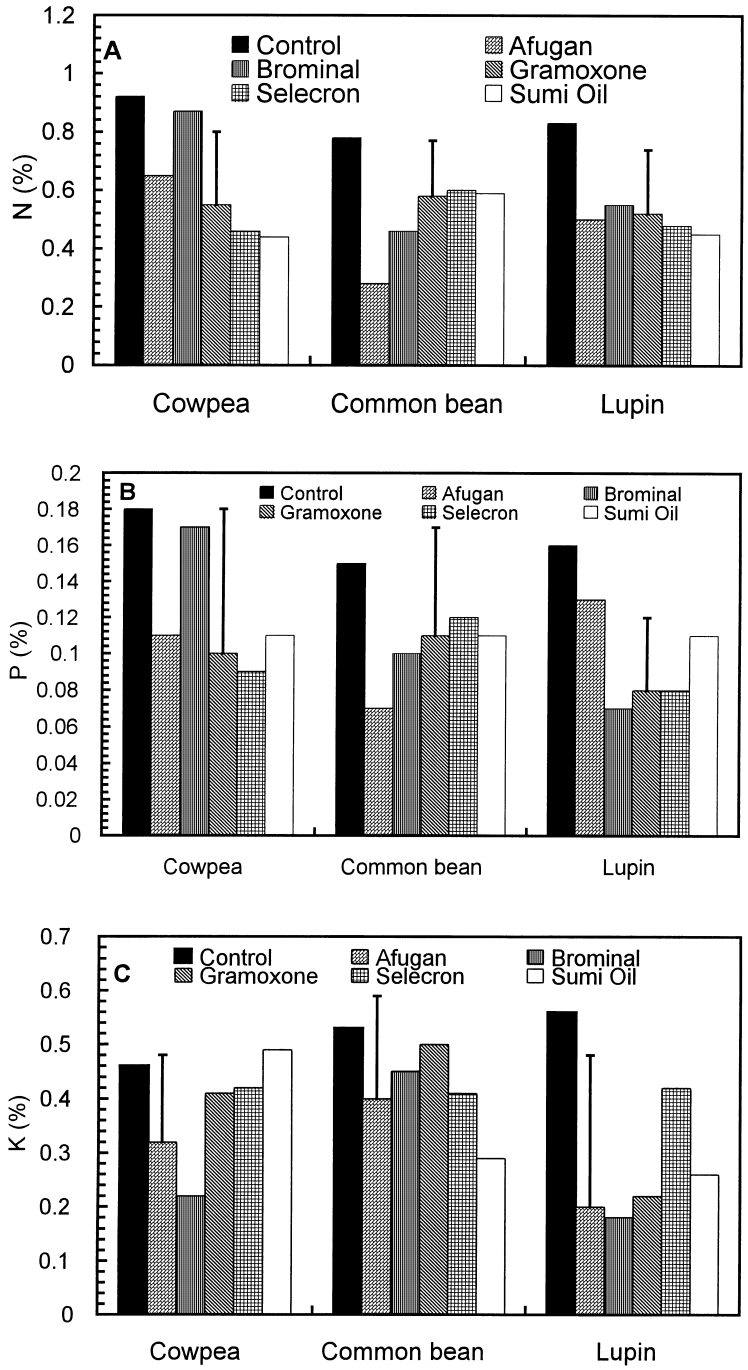

Fig. 2. Effect of pesticides on tissue (A) N, (B) P, and (C) K of cowpea, common bean and lupin grown in sandy soil and inoculated with rhizobia/bradyrhizobia–arbuscular mychorrhiza and harvested at 60 days after planting. Values represent the means of four replicates for each species and vertical bars are LSDs at $P \leq 0.05$.

tion have also been reported earlier. Sreenivasa and Bagyaraj (1989) found a reduction in root colonisation with AM fungi and spore number by Copper-oxychloride and Carbofuran. Most reports attribute the negative effects of pesticides on root colonisation with AM fungi to their effects on mycorrhizal spore germination to initiate infection (Nemec, 1980; Smith and Gianinazzi-Pearson, 1988; Dodd and Jeffries, 1989). Also, pesticides may affect root exudation from the macrosymbiont (Ratnayake et al., 1978; Schwab et al., 1982) or the production by AM hyphae of cell wall-degrading enzymes that are essential for root infection with AM fungi, in a manner similar to the case of root hair infection by rhizobia. In our study (Table 3), spore counts were increased in some treatments 60 days after cowpea planting, but this increment was not accompanied by a corresponding increase in root colonisation level with AM fungi. This could be due to the inability of spores to germinate in the presence of pesticides. Decrease in the number of AM spores sieved from the rhizosphere soil after most experimental periods is the result of inhibition in hyphal growth as measured in terms of root colonisation level. In addition to the importance of spore density in the rhizosphere soil to initiate infection during the growing season, spores also represent the main source of infection for the next crop (Brundrett et al., 1999; McGonigle and Miller, 1999).

The decrease in the nitrogen content of plant tissue reported here is not surprising and is mainly due to the side effects of pesticides on rhizobia/bradyrhizobia or AM fungi. The main source of plant nitrogen was N_2 fixed in nodules as the N content of the soil was very low (Table 1). The decrease in nodule number on pesticide-treated plants may explain the decrease in tissue N. Similarly, Abd-Alla and Omar (1993) reported a reduction in nitrogen content, concomitant with a decrease in nodule number, in faba bean plants grown in herbicide-treated soil. The decrease in AM root colonisation by pesticides may also have contributed to the decrease in plant nitrogen content in our experiment as it has been reported that mycorrhizal fungi participate in the acquisition of soil nitrogen by colonized roots (Singh, 1996). Also, mycorrhizal fungi may affect plant nitrogen content through their effect on the sugar supply for nitrogen fixation by rhizobia and bradyrhizobia in nodules (Hayman, 1982; Koch et al., 1997). In agreement with these suggestions, Vejsadova et al. (1989) have shown that mycorrhizal plants fix considerably more N_2 than non-colonized ones.

The decrease in P and K contents of the plants tested (Fig. 2) with pesticide application is mainly due to the harmful effects of these chemicals on root colonisation with AM fungi. The role of mycorrhizal fungi in P uptake by plants is well documented and considered as the chief mechanism involved in plant–mycorrhiza symbiosis (Aziz and Habte, 1987; Omar, 1995, 1998). Also, some authors demonstrated conclusively that plant colonisation with AM fungi enhanced K uptake from the soil and improved the K status of colonized plants (Sylvia et al., 1993; Subramanian and Charest, 1997). Once again, the negative effects of pesticides on P and K uptake by plants, owing to the effects of these pesticides on root colonisation with AM fungi, may be indirectly involved in decreasing nitrogen fixation, and consequently, total plant nitrogen through the decrease in adequate supply of these elements to roots and nodules (Barea and Azcon-Aguilar, 1983; Mathew and Johri, 1989). In accordance with these suggestions, Mosse et al. (1976) reported that many tropical legumes nodulated in phosphate-deficient soils only when they were mycorrhizal. Also, Abd El-Maksoud et al. (1988) and Ishac et al. (1994) have shown that the colonisation of legumes with AM fungi is an important perquisite for adequate yield of plant grown in calcareous soil. Enhancement of growth by AM fungi could possibly be due to phytohormonal production by these micro-organisms (Smith and Gianinazzi-Pearson, 1988) that may be changed by the action of pesticides.

In conclusion, the results of this experiment reveal that pesticide application, even at field application rates, caused reduction in plant growth. The effect could be directly on the plant itself or indirectly on root microflora (rhizobia and mycorrhiza). The results indicated that the effect on micro-organisms may be the main reason for growth inhibition of plants. These results emphasize the need for evaluating the side effects of pesticides on soil micro-organisms, that play a vital role in mineral nutrition of plants, before recommending them for use especially in newly reclaimed calcareous soil with low populations of indigenous micro-organisms.

References

Abd-Alla, M.H., Omar, S.A., 1993. Herbicides effects on nodulation, growth and nitrogen yield of faba bean induced by indigenous *Rhizobium leguminosarium*. Zentralbl. Mikrobiol. 148, 593–597.

Abd El-Maksoud, H.K., Boutros, B.N., Lotfy, A.A., 1988. Growth response of sour-orange, *Citrus aurantium* L., to mycorrhizal inoculation and superphosphate fertilization in sandy and calcareous soils. Egypt J. Soil Sci. 28, 385–391.

Aziz, T., Habte, M., 1987. Determining vesicular mycorrhizal effectiveness by monitoring P status of leaf disks. Can. J. Microbiol. 33, 1097–1101.

Barea, J.M., Azcon-Aguilar, C., 1983. Mycorrhizas and their significance in nodulating nitrogen fixing plants. Adv. Agron. 36, 1–54.

Bertholet, J., Clark, W., 1985. Effect of Trifluralin and Metribuzin on faba bean growth, development and symbiotic nitrogen fixation. Can. J. Plant Sci. 65, 9–20.

Black, C.A., Evens, D.D., Emsminger, L.E., 1965. Methods of Soil Analysis. Agronomy, Series No. 9. American Society of Agronomy, Madison, WI, pp. 1171–1174.

Brown, M.S., Bethlenfalvay, G.J., 1987. *Glycine–Glomus–Rhizobium* symbiosis. VI. Photosynthesis in nodulated mychorrhizal, or N- and P-fertilized soybean plants. Plant Physiol. 85, 120–123.

Brundrett, M.C., Abott, L.K., Jasper, D.A., 1999. Glomalean mycorrhizal fungi from tropical Australia. 1. Comparison of the effectiveness and specificity of different isolation procedures. Mycorrhiza 8, 305–314.

Curley, R.L., Burton, J.C., 1975. Compatibility of *Rhizobium japonicum* with chemical seed protectants. Agron. J. 67, 807–808.

David, D.J., 1962. Emission and absorption spectrochemical methods. In: Peech, K., Tracey, M.V. (Eds.), Modern Methods of Plant Analysis, Vol. 5. Springer, Berlin, pp. 1–25.

De Bertoldi, M., Rambelli, A., Griselli, M., 1978. Effects of Benomyl and Captan on rhizosphere fungi and the growth of *Allium cepa*. Soil Biol. Biochem. 10, 265–268.

Dodd, J.C., Jeffries, P., 1989. Effect of fungicides on three vesicular–arbuscular mycorrhiza fungi associated with winter wheat (*Triticum aestivum* L.). Biol. Fertil. Soils 7, 120–128.

Dunigan, E.P., Erey, J.P., Allen, L.D., McMahon Jr., A., 1972. Herbicidal effects on nodulation of *Glycine max* (L.) Merrill. Agron. J. 64, 806–808.

Eberbach, P.L., Douglas, L.A., 1991. Effect of herbicide residues in a sandy loam on the growth, nodulation and nitrogenase activity (C_2H_2/C_2H_4) of *Trifolium subterraneum*. Plant and Soil 131, 67–76.

Gerdemann, J.W., Nicolson, T.H., 1963. Spores of mycorrhizal *endogone* species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46, 235–244.

Hall, I.R., Fish, B.J., 1979. A key to the Endogonaceae. Trans. Br. Mycol. Soc. 73, 261–270.

Hansen, A.P., 1994. Symbiotic N_2 Fixation of Crop Legumes: Achievement and Perspectives, Hohenheim Tropical Agricultural Series. Margraf Verlag, pp. 31–36.

Hayman, D.S., 1982. The physiology of vesicular–arbuscular endomycorrhizal symbiosis. Can. J. Bot. 61, 944–963.

Hemida, S.A., Omar, S.A., Abdel-Mallek, A.Y., 1997. Microbial populations and enzyme activity in soil treated with heavy metals. Water Air Soil Pollut. 95, 13–22.

Hetrick, B.A.D., Wilson, G.W.T., 1991. Effect of mycorrhizal fungus species and Metalaxyl application on microbial suppression of mycorrhizal symbiosis. Mycologia 83, 97–102.

Ishac, Y.Z., Angle, J.S., El-Borollo, M.E., El-Demer, M.E., Mostafa, M.I., Fares, C.N., 1994. Growth of *Vicia faba* as affected by inoculation with vesicular–arbuscular mycorrhizae and *Rhizobium leguminosarium* bv. *viceae* in two soils. Biol. Fertil. Soils 17, 27–31.

Kawai, Y., Yamamoto, Y., 1986. Increase in nodule formation and nitrogen fixation of soybean nodules by VA mycorrhiza. Plant Cell Physiol. 27, 399–405.

Koch, M., Tanami, Z., Bodani, H., Winnger, S., 1997. Field application of vesicular–arbuscular mycorrhizal fungi improved garlic yield in disinfected soil. Mycorrhiza 7, 47–50.

Kust, C.A., Struckmeyer, B.E., 1971. Effects of Trifluralin on the growth, nodulation and anatomy of soybean. Weed Sci. 19, 147–152.

Lanyon, L.E., Heald, W.R., 1982. Magnesium, calcium, strontium and barium. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil analysis. Part 2. Chemical and Microbiological Properties. Am. Soc. Agron., Madison, WI, pp. 247–262.

MacRae, I.C., Alexander, M., 1965. Microbial degradation of selected herbicides in soil. J. Agric. Food Chem. 13, 72–76.

Mahmoud, A.-L.E., Omar, S.A., 1995. Growth, cell wall-degrading enzymes and aflatoxin production by lemon-rotting fungi in relation to insecticide application. Microbiol. Res. 150, 195–200.

Mathew, J., Johri, B.N., 1989. Effect of indigenous and introduced VAM fungi on growth of mungbean. Mycol. Res. 92, 491–493.

McGonigle, T.P., Miller, M.H., 1999. Winter survival of extraradical hyphae and spores of arbuscular mycorrhizal fungi in the field. Appl. Soil Ecol. 12, 41–50.

Menge, J.A., 1982. Effect of soil fumigants and fungicides on vesicular–arbuscular fungi. Phytopathology 72, 1125–1132.

Mosse, B., Powell, C.L., Hayman, D.S., 1976. Plant growth responses to vesicular–arbuscular mycorrhiza. IX. Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol. 76, 331–342.

Nemec, S., 1980. Effect of 11 fungicides on endomycorrhizal development in sour orange. Can. J. Bot. 58, 522–526.

Nemec, S., 1985. Influence of selected pesticides on *Glomus* species and their infection in *citrus*. Plant and Soil 85, 543–552.

Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil analysis. Part 2. Chemical and Microbiological Properties. Am. Soc. Agron., Madison, WI, pp. 403–430.

Omar, S.A., 1995. Growth effects of the vesicular–arbuscular mycorrhiza fungus *Glomus constrictum* on maize plants in pot trials. Folia Microbiol. 40, 503–507.

Omar, S.A., 1998. The role of rock-phosphate-solubilizing fungi and vesicular–arbuscular-mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J. Microbiol. Biotechnol. 14, 211–218.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and VA mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 159–161.

Ratnayake, M., Leonard, R.T., Menge, J.A., 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. New Phytol. 81, 543–552.

Schwab, S.M., Johnson, E.L.V., Menge, J.A., 1982. Influence of Simazine on formation of vesicular–arbuscular mycorrhiza in *Chenopodium quinona* wild. Plant and Soil 64, 283–287.

Singh, C.S., 1996. Arbuscular mycorrhiza (AM) in association with *Rhizobium* sp. improves nodulation, N_2 fixation, and N utilization of pigeon pea (*Cajanus cajan*), as assessed with ^{15}N technique, in pots. Microbiol. Res. 15, 87–92.

Siti, E., Cohen, E., Katan, J., Mordechi, M., 1982. Control of *Ditylenchus dipsaci* in garlic by bulb and soil treatments. Phytoparasitica 10, 93–100.

Smith, S.E., Gianinazzi-Pearson, V., 1988. Physiological interactions between symbionts in vesicular–arbuscular mycorrhizal plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39, 221–244.

Somasegaran, P., Hoben, H.J., 1985. Methods in legume–*Rhizobium* technology. NIFTAL project and MIRCEN, University of Hawaii, Paia.

Sreenivasa, M.N., Bagyaraj, D.J., 1989. Use of pesticides for mass production of vesicular–arbuscular mychorrhizal inoculum. Plant and Soil 119, 127–132.

Subramanian, K.S., Charest, C., 1997. Nutritional, growth, and reproductive responses of maize (*Zea mays* L.) to arbuscular mycorrhizal inoculation during and after drought stress at tasseling. Mycorrhiza 7, 25–32.

Sugavanam, V., Udaiyan, K., Manian, S., 1994. Effect of fungicides on vesicular–arbuscular mycorrhizal infection and nodulation in ground nut (*Arachis hypogea* L.). Agric. Ecosyst. Environ. 46, 285–293.

Sylvia, D.M., Hammond, L.C., Bennet, J.M., Has, J.H., Linda, S.B., 1993. Field response of maize to VAM fungus and water management. Agron. J. 85, 193–198.

Trappe, J.M., 1982. Synoptic keys to the genera and species of zygomycetous mycorrhizal fungi. Phytopathologia 72, 1102–1108.

Vejsadova, H., Hrselova, H., Prikryl, Z., Vancura, V., 1989. Effect of different phosphorus and nitrogen levels on development of VA mycorrhiza, rhizobia activity and plant growth. Agric. Ecosyst. Environ. 29, 429–434.