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EFFECT OF BAVISTIN AND DITHANE M-45 ON THE *Mycorrhizae* AND *Rhizosphere* MICROBES OF SUNFLOWER

Ashok Aggarwal, Dipti Sharma, Vipin Parkash, Seema Sharma and Anil Gupta

Botany Department, Kurukshetra University, Kurukshetra, Haryana, 136119, India

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SUMMARY

In the present investigation, two fungicides viz. Bavistin and Dithane M-45 were tested for the effect on soil mycoflora with special reference to mycorrhizal fungi of sunflower crop. It was evident from the results that the two fungicides had different effects on soil mycoflora. Reduction of various species of soil fungi was more extensive in first twenty days in comparison with the later half of the experimental period. The fungicidal effect of both fungicides decreased with increase of time and it resulted in reappearance of certain fungi after a certain period of time. Regarding the effect of fungicides on sunflower growth and phosphorus content, it was found that as the concentration of the fungicides increased, the growth decreased and minimum growth was observed in 1% concentration of Bavistin as well as of Dithane M-45. Both fungicides had deleterious effect on mycorrhizal spore number and percentage mycorrhizal root colonization.

Key words: sunflower, fungicides, VAM fungi and soil fungi

INTRODUCTION

Fungicides have been used for many years to control various plant pathogens. These fungicides are used for seed treatment, as spray and soil drenches or soil mixing. A part of a toxicant administered as spray or dust on plant shoots reaches the soil as runoff or drift. While controlling plant pathogens with the help of these chemicals, the effect of these fungicides on the non-target organisms is however seldom realized. Suspensions of two wide-spectrum fungicides *i.e.*, Bavistin and Dithane M-45 are commonly used to control fungi infecting sunflower crops. The former is a systemic which may persist both in plant tissues and soil (Frahm, 1973), the latter is a non-systemic fungicide known to remain on plant surface.

Although much is known about new fungicides before their release for commercial use, it is still true that cropping information are usually focused on the inci-

dence of pathogens (Bertoldi *et al.,* 1977). There appears to be a conflict of interest between our need to control pathogenic fungi by fungicides and our desire to encourage root symbiosis, which involves vesicular arbuscular mycorrhizal (VAM) fungi. Although the majority of fungicides adversely affect the symbiosis, some of them do not appear to damage mycorrhizal fungi. In view of the above, the present investigation was undertaken to study the effect of two fungicides viz. Bavistin and Dithane M-45 on soil mycoflora with special reference to mycorrhizal fungi of sunflower crop.

MATERIALS AND METHODS

A. Collection of soil samples

Soil samples were collected from the Botanical Garden of Botany Department of Kurukshetra University, Kurukshetra. Soil was sieved prior to further treatment.

B. Treatments

Soil was treated with the two fungicides *i.e.* Bavistin and Dithane M-45. Two kg of soil was taken in each pot (size 30 × 25 cm) and fungicides were added in such a manner as to get the grading concentrations of 2.5%, 0.5%, 0.75% and 1%. Healthy seeds of sunflower were planted in each pot. To maintain the moisture for germination, the seeds were regularly watered. Soil samples were taken out for mycofloral and mycorrhizal studies after 10, 20, 40 and 70 days. Control samples were kept without any treatment. Five pots were taken per each treatment and control.

C. Mycoflora study

Warcup's plate method (1950) and Waksman's soil dilution method (1927) were used for quantitative and qualitative studies of soil mycoflora.

D. Mycorrhizal study

For mycorrhizal study, the sunflower plants from treated and control pots were uprooted after regular time intervals *i.e.*, 10, 20, 40 and 70 days.

Isolation of VAM spores was done by the wet sieving and decanting technique of Gerdemann and Nicolson (1963). Colonization of VAM fungi was studied by the rapid clearing and staining technique of Phillips and Hayman (1970).

E. Growth study

Plant growth was measured after 70 days of the treatment. For this study, two parameters were measured *i.e.*, height of plant and phosphorous (P) contents in plant shoot and flower. Phosphorous contents in plant shoot and flower were estimated by the vanadomolybdate phosphoric yellow color method of Jackson (1973). Data were also analyzed statistically by the LSD analyses of variance.

RESULTS AND DISCUSSION

Effect on mycoflora

It is evident from qualitative analyses that Bavistin had a deleterious effect on soil fungi at all concentrations, especially higher ones. However, the fungi-toxic effect of Bavistin decreased with increase of time and resulted in the reappearance of certain fungi after a particular time. Reduction in various species of soil fungi was more extensive in the first twenty days than in the later half of the experimental period for both fungicides. *Mucor racemosus* was susceptible to Bavistin up to 20 days after treatment but after 40^{th} day it was present in nearly all concentrations. *Aspergillus niger* and *Aspergillus terreus* were found to be resistant to Bavistin and were present in all concentrations. *Aspergillus flavus, Aspergillus ruber, Aspergillus ochraceus* and *Aspergillus fumigatus* were seldom recorded at 0.5% concentration.

Bavistin at 0.5 and 0.75% concentrations was toxic to most of the *Penicillia. Penicillium* ssp. were recorded at 0.25% at all the sampling days. *Alternaria alternata*, *Curvularia lunata, Fusarium equiseti* and *Fusarium solani* were more resistant to most of the concentrations of Bavistin in comparison with the other deuteromycetous fungi. *Trichoderma viride* was found to be more abundant in the later half of the experiment *i.e.*, after 20 days.

Dithane M-45 also adversely affected the soil fungi. Reduction in fungal species appeared to be directly related with fungicidal concentrations but with the increase of time interval the number of fungi increased again. Decreased numbers of fungal species were observed in all concentrations of Dithane M-45 in the soil in comparison with the control, up to the 70^{th} day. Dithane M-45 had a deleterious effect on *Mucor racemosus* just like Bavistin. *Mucor* sp. was not affected much at the lower concentrations *i.e.*, up to 0.5% but the higher concentrations of Dithane M-45 restricted *Mucor* sp. *A. niger* and *A. terreus* tolerated most of the concentrations of Dithane M-45 after 40^{th} day. *A. flavus, A. ruber* and *A. fumigatus* were sensitive to fungicide application and even the lowest concentration *i.e.*, 0.25%, inhibited their growth. *Penicillium* species could not tolerate high concentration of Dithane M-45. *P. chrysogenum* and *P. javanicum* showed disability to grow at 0.75 and 1% concentrations.

A. alternata and *C. lunata* were resistant to Dithane M-45 but *T. viride* was most frequent after 20 days *i.e.* in the later half of experiment. *Fusarium equiseti*, *F. solani Helminthosporium* sp., and *Cladosporium oxysporum* were more frequent in the later half.

Quantitative analysis also showed inhibitory effects of Bavistin on soil mycoflora. The fungi were expressed in 1×10^3 fungi/g of soil. The total number of species as well as the number of fungi/g of soil decreased with increase in Bavistin concentration in the first 20 days and after 20 days the fungal population increased. But even after 70 days the fungal population was always smaller in the treatment than in the control.

Mucoraceous fungi were sensitive to Bavistin during first twenty days, but their sensitivity decreased afterwards. *M. racemosus* was inhibited at 0.25% on the 10^{th}

Table 1: Effect of Bavistin on soil fungi of sunflower crop at different time intervals examined by soil dilution plate method (fungi expressed in 1×10^3 fungi/g of soil)

Name of fungus		10th day					20th day					40th day					70th day				
		Percent concentration																			
		C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1
1	*Aspergillus niger*	0.4	-	-	-	-	0.5	0.5	0.1	-	0.2	0.5	-	0.1	0.1	0.1	0.2	-	-	0.3	0.3
2	*A. candidus*	0.3	1.1	2.1	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	*A. flavus*	0.4	-	-	-	-	0.4	1.1	0.1	-	0.6	0.5	0.6	1	0.2	0.6	0.9	-	-	0.3	0.2
4	*A. flavipes*	0.2	-	-	-	-	-	-	-	-	-	-	-	-	0.4	0.1	-	-	-	0.3	-
5	*A. nidulans*	0.2	-	-	-	0.1	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-
6	*A. fumigatus*	2	-	-	0.3	0.1	0.1	-	0.3	-	-	0.1	-	0.5	0.5	0.3	0.1	0.1	0.1	0.2	0.2
7.	*A. ruber*	0.4	-	0.3	0.3	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.	*A. luchuensis*	-	-	-	-	-	-	-	-	-	-	0.6	0.4	0.2	0.1	-	0.5	-	-	-	-
9.	*A. ochraceus*	-		-	-	-	0.3	0.3	0.5	1.0	-	1.4	0.3	0.6	0.3	0.3	0.3	-	-	0.2	-
10	*A. tamarii*	-	-	-	-	0.1	-	0.3	0.6	-	-	-	-	-	-	-	-	-	-	-	-
11.	*A. terreus*	-	-	-	-	-	0.1	-	-	-	0.4	1.4	0.4	0.4	-	0.3	0.4	0.3	0.2	-	-
12.	*Penicillium rubrum*	0.3	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13.	*P. citrinum*	0.8	-	-	-	-	-	0.8	-	-	0.5	-	-	0.2	-	0.2	-	-	-	0.3	0.1
14.	*P. capsulatum*	-	-	-	0.2	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15.	*P. funiculosum*	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-
16.	*Fusarium solani*	-	0.7	0.3	-	-	-	-	-	-	-	.4	0.2	0.4	-	0.4	0.4	-	-	-	-
17.	*F. oxysporum*	4.5	-	-	-	-	-	1.5	-	-	-	-	0.2	0.2	-	-	-	-	-	0.3	-
18.	*F. compactum*	-	1.0	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-
19.	*F. equiseti*	-	-	-	-	-	0.3	0.2	0.1	-	-	0.2	0.1	-	-	-	0.2	0.1	-	-	-
20.	*F. javanicum*	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	*Mucor racemosus*	1.7	0.1	-	-	.	1	1	1.4	-		0.6	0.1	0.3	0.3	0.1	0.6	0.1	0.2	0.1	0.2
22.	*Trichoderma viride*	0.2	0.1	0.1	0.2	0.2	0.2		0.2	0.3		0.1	0.2			0.2					
23.	*Trichurus sp.*	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	0.9	-	-	-	0.1	-
24.	*Trichothecium sp.*	-	0.1	-	-	0.5	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25.	*Trichosporium sp.*	-	-	0.3	-	2.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26.	*Choanephora sp.*	0.4	-	-	0.3	0.1	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-
27.	*Gliocladium sp.*	-	-	-	-	-	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28.	*Curvularia lunata*	0.2	1.5	-	0.8	-	-	0.5	-	-	1.7	0.4	0.3	0.5	0.5	-	0.4	-	0.2	0.3	0.1
29.	*Humicola sp.*	-	-	-	0.7	0.3	-	0.1	-	-	-	-	0.4	-	0.7	-	-	-	-	-	-
30.	*Alternaria alternata*	1.8	-	-	-	1.4	1	-	0.6	0.8	-	1.1	-	1	0.9	0.8	1.2	1	0.9	0.9	0.9
31.	*Helminthosporium* sp.	-	-	-	-	3.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32.	*Cladosporium cladosporioides*	-	-	-	-	0.2	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
33.	*Verticillium* sp.	-	-	-	-	-	0.1	0.1	-	-	-	0.2	0.1	-	-	-	0.1	-	-	-	-
34.	*Acremonium* sp.	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35.	*Monilia* sp.	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-
36.	*Phoma fineti*	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-
37.	*Cunninghamella blakeslea*	0.1	-	-	-	-	0.1	-	-	-	0.2	0.2	0.1	-	0.1	0.1	0.2	0.1	-	0.1	0.1

Table 2: Effect of Dithane M-45 on soil fungi of sunflower crop at different time intervals examined by soil dilution plate method (fungi expressed in 1×10^3 fungi/g of soil)

	Name of fungus	10th day					20th day					40th day					70th day				
		Percent concentration																			
		C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1
1	*Aspergillus niger*	0.4	-	0.2	0.3	0.4	0.5	-	0.1	0.2	0.4	0.5	-	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1
2	*A. candidus*	0.3	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	*A. flavus*	0.4	-	0.4	0.1	-	0.4	0.2	0.3	0.1	-	0.5	1.1	0.4	0.1	-	0.9	0.6	0.5	0.2	0.1
4	*A. flavipes*	0.2	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	*A. nidulans*	0.2	-	-	-	0.2	-	-	-	0.4	-	-	0.2	-	-	-	-	-	-	-	-
6	*A. fumigatus*	2	0.6	-	-	-	0.1	0.2	-	0.6	0.3	0.1	0.1	0.4	0.1	-	0.1	0.1	-	0.1	0.1
7	*A. ruber*	0.4	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	*A. luchuensis*		0.2	-	0.1	0.2	-	-	-	-	-	0.6	-	-	-	-	0.5	0.3	0.2	-	-
9	*A.ochraceus*	-	0.1	-	-	-	0.3	0.3	-	-	0.7	1.4	-	-	1.0	1.1	0.3	-	-	-	-
10	*A. terreus*	-	-	0.1	-	-	0.1	0.1	-	-	-	1.4	-	-	-	-	0.4	0.3	0.2	-	0.1
11	*Penicillium rubrum*	0.3	-	0.1	-	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	*P. citrinum*	0.8	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	*P. capsulatum*	-	-	-	-	-	-	-	0.1	0.3	0.4	-	0.2	-	-	-	-	-	-	-	-
14	*P. funiculosum*	-	-	0.3	-	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	*P. chrysogenum*	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.1	-	-	-	-	0.3	-
16	*P. notatum*	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	*Fusarium solani*	-	0.2	-	-	-	-	-	-	-	-	0.4	-	-	-	-	0.4	-	-	-	-
18	*F. oxysporum*	4.5	-	-	-	-	-	0.2	-	-	-	-	-	-	0.3	-	-	-	-	-	-
19	*F. equiseti*	-	-	0.1	0.1	-	0.3	0.2	0.1	-	-	-	0.1	-	-	-	0.1	0.2	0.3	0.1	0.1
20	*Mucor racemosus*	1.7	-	-	-	-	1	-	0.2	0.1	0.1	0.6	-	-	-	-	-	-	-	-	-
21	*M. pusillus*	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	*Trichoderma viride*	0.2	0.4	-	-	0.4	0.2	-	-	-	0.1	0.3	0.2	0.1	0.1	-	0.4	0.3	0.2	0.1	0.1
23	*Trichothecium sp.*	-	-	-	-	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	*Trichosporium sp.*	-	0.2	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	*Gliocladium sp.*	-	0.7	-	-	-	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	*Curvularia lunata*	0.2	-	-	-	-	0.4	-	-	-	0.1	0.4	0.3	0.2	2.4	-	0.4	-	0.3	0.2	0.2
27	*Alternaria alternata*	1.8	-	-	-	0.1	1	0.6	0.5	0.5	-	1	0.6	0.5	0.5	0.1	0.9	0.1	0.2	0.1	0.1
28	*Helminthosporium* sp.	-	-	-	-	0.1	-	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-
29	Cladosporium cladosporioides	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	*Acremoniun vitis*	-	0.1	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	*Verticillium* sp.	-	-	-	-	-	0.1	-	-	-	-	0.1	-	-	-	-	0.2	0.1	0.2	-	0.1
32	*Gleotrichum* sp.	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	*Torula* sp.	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	*Pythium* sp.	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	*Choanephora* sp.	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-

day and at 0.5% on the 20th day, whereas *Cunninghamella blakeslea* was inhibited even at the lowest concentration *i.e.*, 0.25%. Both fungi were present nearly in all concentrations after 20 days, but their number of cells/g of soil was always less than that of the control. *A. niger, A. flavus, A. fumigatus, A. ocharceus, A. terreus, C. lunata* and *A . alternata* were frequently recorded in this experiment but generally most of them were found susceptible to Bavistin in higher concentrations up to the end of experiment *i.e.* on the 70th day. *A. niger, A. flavus, A. fumigatus, C. lunata* and *A. alternata* were recorded to reappear in increased concentrations after 20 days of treatment. Numbers of viable cells/g soil of *A. niger* and *A. fumigatus* were more in higher concentrations *i.e.*, 0.75 and 1% than in the control. *P. capsulatum, P. funiculosum, F. compactum, Trichurus* sp., *Trichosporium* sp., *Humicola* sp., *Helminthosporium* sp., *Monilia* sp. and *Phoma fineti* were recorded only in certain concentrations of Bavistin. *P. rubrum*, *F. oxysporum, F. javanicum, Trichothecium* sp., *Gliocladium* sp. and *Cladosporium cladosporioides* were seldom recorded in the treated and control variants of the experiment. *Verticillium* sp. could tolerate only 0.25% of Bavistin and their number of viable cells/g of soil were always less than that of the control (Table 1).

Dithane M-45 was also found to be effective to most of the soil fungi at higher concentrations *i.e.* 0.75 and 1%. *A. niger, A. flavus, A. fumigatus, A. terreus, F. equiseti* and *Verticillium* sp. were present in nearly all concentrations on the 70th day after the treatment but the numbers of viable cells/g soil of these fungi in the treatments were always less than the values in the control. *T. viride, C. lunata* and *A. alternata* were highly sensitive to Dithane M-45 even at lowest concentrations but their sensitivity decreased with time and they were present in all concentrations on the 40th and 70th day. All the species of *Penicillia* were restricted by Dithane M-45 application. *Pythium* sp., *Torula* sp., *Acremonium vitis* and *Trichosporium* sp. were recorded in lower concentrations of Dithane M-45 (Table 2).

Both fungicides at different concentrations reduced significantly the growth and P content of sunflower. Maximum growth was observed in the control and flowering was initiated sooner in the control than in the various fungicide treatments. As the concentrations of both fungicides increased, the growth decreased. Minimum growth was observed in 1% concentrations of Bavistin and Dithane M-45 (Table 3).

Table 3: Effect of fungicides on growth and P content of sunflower after 70 days

Concentration (%)	Height of plant (cm)		P content of plant (ppm)		P content of flower (ppm)	
	Bavistin	DithaneM-45	Bavistin	DithaneM-45	Bavistin	DithaneM-45
0.25	65	66.6	1267.8	1271.3	6024	6231
0.50	50[a]	54	1250.8	1208	6009.2	6122.6
0.75	45[a]	44[a]	1152	1171.6	5908	5962.3
1	30	41.3[a]	1100	1150.6	5500	5502
Control	90	92.3	1350	1351.3	6664.4	6664
LSD (0.05)	5.81	7.05	4.18	2.50	4.91	5.98

Mean of three replicates

In each column, the means followed by same letters do not differ significantly at P=0.05 level

Regarding the P content (Table 3), it showed decreasing trends with increases in the concentration of both fungicides. P content was high in flowers as compared with plant shoots. Phosphorus content in untreated plant of the control was higher than the contents in either treatment.

Effect of fungicides on *Mycorrhizae*

Tables 4 showed the effect of the two fungicides on mycorrhizal spore number. It is evident from the Table 4 that Bavistin had a significant inhibitory effect on mycorrhizal spore number. On the 10^{th} day, the mycorrhizal spore number decreased at all concentration of the fungicide. The same trend was seen on the 20^{th} day but the number of spores at 1% was less than in the control. After 40 and 70 days there were regular decreases in mycorrhizal spore number. The lowest numbers were recorded at 1% concentration as compared with the other treatments and time intervals.

Dithane M-45 too exhibited a significant inhibitory effect on spore number (Table 4). On the 10^{th} day, the spore number initially decreased and then again increased. The lowest spore number was recorded at 1% concentration. After 20 days there was a regular decrease in spore number with increase in the concentration of Dithane M-45. The lowest value was recorded at 1% *i.e.*, at the highest concentration.

Table 4: Effect of fungicides on mycorrhizal spore count/100 g of sunflower soil at different time intervals

Concentration (%)	10^{th} day		20^{th} day		40^{th} day		70^{th} day	
	Bavistin	Dithane M-45	Bavistin	Dithane M-45	Bavistin	Dithane M-45	Bavistin	Dithane M-45
Control	704.6	711	772.3	771.6	731.6	729	707.3	707.3
0.25	493.6	619.3	444.6	762	634	705	665.6	692
0.50	483	654	434.3a	719	602	651.3	651	668
0.75	474.6	494	432a	673.3	550.3	638.3	602	508.3
1	462	452.3	421.3	594.3	501	602	484.6	493.6
LSD (0.05)	5.30	7.71	4.64	3.36	3.22	7.31	6.96	10.8

Mean of three replicates

In each column, the means followed by same letters do not differ significantly at P=0.05 level

Biodiversity and natural occurrence of VAM spores were higher in control soil than in treated soil. *Glomus mosseae* and *Glomus geosporum* were resistant to Bavistin (Table 5). *Glomus versiforme*, *G. intraradices*, *G. macrocarpum*, *Acaulospora laevis* and *Sclerocystis coremoides* were present in the low concentrations and were absent in the high concentrations. *Gigaspora gigantea* and *Acaulospora* sp. were completely inhibited by Bavistin application even at the lowest concentration *i.e.*, 0.25%. *Entrophospora* sp. was present at high concentrations of the fungicide. In the case of Dithane M-45 (Table 6), *Glomus geosporum*, *Glomus macrocarpum*, *Glomus intraradices*, *Glomus aggregatum* and *Acaulospora fove-*

Table 5: Effect of Bavistin on VAM fungi of sunflower crop at different time intervals

	Name of VAM fungus	10th day					20th day					40th day					70th day				
		Percent concentration																			
		C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1
1.	*Glomus mosseae*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	*G.geosporum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	*G.versiforme*	+	+	-	+	-	+	+	-	-		+	+	-	+	-	+	-	-	+	-
4.	*G.macrocarpum*	+	+	+	-	+	+	+	+	-	+	+	+	-	+	-	+	+	+	-	+
5.	*G.intraradices*	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+
6.	*G.fasciculatum*	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-
7.	*G.aggregatum*	+	-	+	+	-	+	-	-	+	-	-	-	-	+	-	-	-	-	+	-
8.	*Glomus sp.*	+	+	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	-
9.	*Glomus sp. (black)*	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.	*G.reticulatum*	+	-	-	+	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-
11.	*Gigaspora margarita*	+	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-
12.	*G. gigantea*	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-
13.	*Acaulospora laevis*	+	+	-	+	-	-	+	-	+	-	+	+	-	+	-	+	-	-	+	-
14.	*Acaulospora* sp.	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15.	*Sclerocystis coremioides*	+	-	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-
16.	*Scutellospora* sp.	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+
17.	*Entrophosphora* sp.	+	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+

Table 6: Effect of Dithane M-45 on VAM fungi of sunflower crop at different time intervals

	Name of VAM fungus	10th day					20th day					40th day					70th day				
		Percent concentration																			
		C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1	C	0.25	0.5	0.75	1
1.	*Glomus geosporum*	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
2.	*G.versiforme*	+	+	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-
3.	*G. macrocarpum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	*G. intraradices*	+	-	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+
5.	*G. mosseae*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.	*G. leptoticum*	+	+	+	-	-	+	+	+	-	-	+	-	+	-	-	+	+	-	-	-
7.	*G. fasciculatum*	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	-	-	+	+
8.	*Glomus sp.*	+	-	+	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-
9.	*G. aggregatum*	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
10.	*G. reticulatum*	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-
11.	*Gigaspora margarita*	+	-	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	-	-	-
12.	*Acaulospora foveata*	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
13.	*A. mellea*	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
14.	*Sclerocystis sinuosa*		+	+	-	+	-	+	-	-	+	-	+	+	-	+		+	+	-	+
15.	*Sclerocystis coremioides*	+	-	+	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	-	+
16.	*Glomus* sp. (black)	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
17.	*Scutellospora* sp.	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-

ata were resistant to fungicide application. *Acaulospora mellea, Glomus reticulatum*, and *Glomus leptoticum* were inhibited at high concentrations. *Sclerocystis coremioides* and *Sclerocystis sinuosa* were registered at 0.5 and 1% concentrations of Dithane M-45. Unidentified (black) and *Endogone microcarpus* were seldom reported.

Table 7 showed the effect of Bavistin and Dithane M-45 on the percentage of mycorrhizal root colonization. Both Bavistin and Dithane M-45 showed inhibitory effect on percentage mycorrhizal root colonization. It may be mentioned that arbuscules, vesicles and intramatrical hyphae were seen in both control and fungicide treated plants but the degree of mycorrhizal root colonization varied. Mycorrhizal root colonization showed a decreasing trend with increasing concentrations of Bavistin, with minimum mycorrhizal root colonization occurring at 1% concentration (Table 7). Less arbuscules were observed in the Bavistin treatment than in the control.

Table 7: Effect of fungicides on percentage mycorrhizal root colonization on sunflower crop at different time intervals

Concentration (%)	10^{th} day		20^{th} day		40^{th} day		70^{th} day	
	Bavistin	Dithane M-45	Bavistin	Dithane M-45	Bavistin	Dithane M-45	Bavistin	Dithane M-45
Control	72[a]	71.6	82	81	98	98.6	97	99
0.25	70.3[a]	66.6	72.6	71.6	74.6[a]	73.3	74.6[a]	75.3
0.50	69.6[a]	58	65.3[a]	61	72.3[a]	64[a]	73.6[a]	71
0.75	63.3[b]	51	65[a]	54	65[b]	61[a]	68[b]	69
1	62[b]	34	61.6[a]	41.6	63[b]	52	64[b]	63
LSD (0.05)	3.36	2.27	4.07	2.25	4.36	3.74	4.86	2.20

Mean of three replicates

In each column, the means followed by same letters do not differ significantly at P=0.05 level

In the case of Dithane M-45, the percentage of mycorrhizal root colonization was inversely proportional to fungicide concentration as lowest mycorrhizal root colonization was observed at 1% concentration of the fungicide (Table 7).

It would be most desirable for a fungicide to act upon the pathogen/s alone, leaving the rest of soil mycoflora undisturbed or at least not unfavorably affected. In the present investigation none of the fungicide appeared to possess such an ideal nature.

Bavistin, a systemic fungicide, was found to be deleterious to soil fungi including VAM fungi. The presented results are similar to those obtained by Edgington and Barron (1967) and Edgington *et al.* (1971). In the case of another systemic fungicide, DCMOD (F_{461}), it was highly toxic to basidiomycetous fungi. The effect of soil treatment with Benomyl on the rhizosphere fungi of onion was investigated by Bertoldi *et al.* (1978) and Benomyl was found to reduce the population of fungi. In the present investigation, the influence of Bavistin on rhizospheric fungi seemed to be more persistent during the initial twenty days although the number of

rhizospheric fungi was reduced even after seventy days as compared with the control.

A similar reduction in the number of fungal species was observed by Bertoldi *et al.* (1978) and Frahm (1973). Bollen *et al.* (1983) observed that colonization ratio of roots was unaffected but the species composition was still markedly influence by Benomyl. The negative influence that the mycoflora suffers from fungicides has been investigated by a large number of workers (Domsch, 1964, 1970; Agnihotri, 1974; Foster, 1975).

In the present study, Dithane M-45 caused adverse effects on soil mycoflora and VAM fungi as well. Soil fungi however appeared to be more severely affected by both fungicides at the high concentrations. Kuthubutheen and Pugh (1979) also recorded reduction in fungal population in soil after application of Dithane M-45 and other fungicides. A number of other workers (Balasubramanium *et al.,* 1973; Agnihotri, 1973) reported an inhibitory effect of Dithane M-45 on soil fungi. Although both fungicides in the present investigation were found capable of causing well-marked adverse effects on soil mycoflora, there were certain soil fungi that showed resistance to these fungicides. Mucorales were found to more resistant to both fungicides than the other species of fungi. Among the deuteromycetous forms, *Alternaria alternata, Curvularia lunata, Fusarium equiseti* and *Trichoderma viride* were relatively less affected by both fungicides and their reappearance usually took place after 40 days.

In the present investigation, both fungicides reduced the growth and P content of plants. These results are in agreement with the observations of Boatman *et al.* (1978), Hale and Sanders (1982) and Manjunath and Bagyaraj (1984) who also reported the inhibition of growth as well as P content after fungicide application.

In the present investigation, both fungicides showed inhibitory effects on VAM fungi. Both fungicides reduced VAM root colonization and spore number as compared with the control. The inhibitory effect on mycorrhizal colonization and spore number was higher at the high concentrations after 20 days. Udaiyan *et al*. (1995) also have observed an inhibition of mycorrhizal colonization and spore count by the application of fungicides. Recently, Vijaykumar and Abraham (2001) have studied the effect of Dithane M-45 and Bavistin on VAM fungi of *Gluta travancorica, Bentinckia condapanaa* and *Myristica malabarica* of the Western Ghats and reported the deleterious effect on non-target fungi.

Fungicides are expected to affect profoundly mycorrhizal fungi and their formation on host plants. However, most of them selectively affect some fungi or can elicit their differential response (Edington *et al.,* 1971).

It was reported that the effect of Bavistin persists for a long period while the toxicity of Dithane M-45 becomes neutralized in a short period of time (Sugavanam *et al.,* 1994). Udaiyan *et al.* (1995) observed that Bavistin application exhibited measurable inhibition in root colonization. Reduction in number of mycorrhizal

structures like vesicles and arbuscules might be associated with the inhibition of infection process.

There are many reports on the inhibitory effect of fungicides on VAM colonization (Parvathi *et al.,* 1985; Sugavanam *et al.*, 1994; Udiayan *et al.,* 1995; Wilson and Hartnet, 1998; Gupta and Borse, 1997; Pandey *et al.,* 1996; Sukarno *et al.,* 1993; Miller and Jackson, 1998). However, there are also reports on the stimulatory effects of fungicides on VAM colonization (Jabaji- Hare and Kendrick, 1985). In the present investigation, none of the fungicides showed a stimulatory effect at the high concentrations after 40 days. On the contrary, both fungicides showed stimulatory effects on mycorrhizal root colonization after 20 days of treatment, but less than the control. Since the root exudate is an important factor governing mycorrhizal colonization (Graham *et al*., 1980), any alteration in the quality or quantity of root exudation by fungicide application may increase or decrease mycorrhizal colonization. The reduction in spore number by fungicide application observed in the present investigation was probably due to the reduction of infected root length or due to the exhaustion of extramatrical hyphae by toxic chemicals. Dodd and Jaffries (1989) reported differential response of three *Glomus* ssp. to fungicides. This differential response appeared to be related to the tolerance of VAM fungi to toxic chemicals.

These results showed that fungicides had adverse effects on useful microorganisms as well as on plants. Therefore, overuse of fungicides both quantitatively and qualitatively may prove to be counterproductive.

CONCLUSIONS

It can be concluded that both fungicides at high dosage rates caused remarkable adverse effects on the soil mycoflora including mycorrhizal fungi inhabiting the sunflower crop. Therefore, use of fungicides in high doses as well as their continuous application to the soil should be resorted to carefully and judiciously. In order to regulate the usage of fungicides, periodic microbial monitoring should be done.

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INFLUENCIA DE BAVISTIN Y DITHANE M-45 EN MICROORGANISMOS *Mycorrhizae* y *Rhizosphere* EN GIRASOL

RESUMEN

En esta investigación fue estudiada la influencia de dos fungicidas, Bavistin y Dithane M 45, en micoflora del suelo con el hincapié especial en los hongos micorrizos de las plantaciones de girasol. Los resultados demostraron que los fungicidas investigados tenían diferentes influencias en la micoflora de suelo. La disminución del número de especies de los hongos de suelo era mayor durante los primeros 25 días que en la segunda parte del período del experimento. La influencia fungicida de ambos fungicidas iba disminuyendo en proporción con el tiempo, lo que condujo a reaparición de algunos hongos, tras cierto tiempo. En cuanto a la influencia de fungicidas en el crecimiento de girasol y el contenido de fósforo en girasol, fue determinado que el incremento de concentración de ambos fungicidas disminuía el crecimiento de hongos. El menor crecimiento fue determinado con la concentración de 1% tanto de Bavistin como de Dithane M-45. Ambos fungicidas tenían influencia negativa en el número de esporas micorrizas y en el porcentaje de colonización de micorriza en la raíz de girasol.

EFFET DU BAVISTIN ET DU DITHANE M-45 SUR LES MICROORGANISMES *Mycorrhizae* et *Rhizosphere* DU TOURNESOL

RÉSUMÉ

Cette recherche portait sur l'effet de deux fongicides, le Bavistin et le Dithane M45 sur le mycoflora avec une attention spéciale accordée au champignon mycorhize du tournesol. Les résultats ont démontré que les deux fongicides avaient un effet différent sur le mycoflora de sol. La réduction de différentes espèces de champignons de sol était plus grande dans les premiers vingt jours que dans la deuxième moitié de l'expérience. L'effet fongicide des deux fongicides avait diminué avec le temps et on a pu constater la réapparition de certains champignons après un certain temps. Pour ce qui est de l'effet des deux fongicides sur la croissance du tournesol et le contenu de phosphore, il a été constaté qu'une augmentation de la concentration des deux fongicides diminuait la croissance du champignon. La croissance la plus réduite a été établie avec une concentration de 1% autant de Bavistin que de Dithane M-45. Les deux fongicides avaient eu un effet négatif sur le nombre de spores de mycorhize et sur le pourcentage de colonisation de mycorhizes sur la racine du tournesol.