Effect of Benomyl and Dithane M-45 on mycoflora, mycorrhizae and growth enhancement of *Helianthus annuus* L.

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Abstract: With the ever- increasing number of different kinds of fungicides and their wide spread use in modern agriculture has aroused concern about their possible interaction with non-target organisms that are directly or indirectly involved in the maintenance of soil fertility. In the present investigation, the effect of two fungicide viz. Dithane M-45 and Benomy on arbuscular mycorrhizal relation and soil mycoflora of *Helianthus annuus* L. was investigated in pot experiments under polyhouse conditions. It was evident from the results that the two fungicides had different effects on soil mycoflora. The Benomyl fungicide had a marked inhibitory effect on infection and subsequent colonization of *H. annuus* roots by indigenous AM fungi. As the concentration of fungicides increased, the growth, nutrient uptake and yield of *H. annuus* decreased and minimum growth was observed at higher concentration of fungicides. Both fungicides had deleterious effect on mycorrhizal spore number and percentage root colonization also.
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1. Introduction

Fungi are the top most cause of crop loss worldwide, and have a significant economic impact on quality and yield, so managing fungal diseases is an integral component of crop production. From ages, fungicides are being used for coping with adverse effect of harmful microorganisms to save our valuable crops. Fungicides are extensively used in agriculture system to control soil borne, seed borne or air borne fungal pathogens (McGrath, 2004).

With the ever- increasing number of different kinds of fungicides and their wide spread use in modern agriculture has aroused concern about their possible interaction with non-target organisms that are directly or indirectly involved in the maintenance of soil fertility. Suspensions of two wide-spectrum fungicides *i.e.*, Benomyl 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), and the latter is a non-systemic fungicide known to remain on plant surface.

Helianthus annuus L. commonly known as Sunflower belongs to family Compositae is a major oil seed crop, ranked second than soybean in the world. Due to the presence of monounsaturated fats and vitamins A, D, E and K, it is considered as a useful source of dietary fat in preventing heart disease (Weiss, 2000). The seeds contain hulls with high fiber, wax contents and low protein content, considered as a big obstacle for obtaining a better yield of oil and high quality protein meal.

Keeping in view the above information regarding the effect of fungicides on microorganisms including AM fungi, the present investigation was aimed to see the effect of two soil fungicides namely Benomyl and Dithane M-45 on soil mycoflora of Sunflower including AM fungi.

2. Materials and methods

2.1 Study site: Experiments were designed out under polyhouse of Botany Department, Kurukshetra University, Kurukshetra, Haryana, India during 2011-2013.

2.2 Experimental design: Soil was given the treatment of two fungicides i.e. Benomyl and Dithane M-45. Two kg. of soil was filled in each pot (30×25) and the recommended dose of Benomyl fungicide i.e. 200g per 100kg seed while in case of Dithane M- 45 fungicide i.e. 2.5g per kg were added (Anand, S. 2008; Huda, K. 2008). Healthy seeds of Sunflower were sown in each pot. The experiment was conducted in a 3×6 factorial design employing three concentrations of fungicides [half the recommended dose (low conc.), recommended dose (medium conc.) and double the recommended dose (high conc.)]. To maintain the moisture for the germination, the seeds were regularly watered. Soil samples were taken out for both mycofloral study and mycorrhizal study after 120 days. Control soil samples were kept without any treatment. Five pots of each treatment and control were taken.

2.3 Mycoflora study:

Waksman's soil dilution plate methods (1927) were used for quantitative studies of soil mycoflora.

2.4 Isolation and quantification of AM spores: AM spores were isolated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and quantified by grid line intersect method (Adholeya and Gaur, 1994).

2.5 Mass multiplication of AM spores: Dominant AM spores *Glomus mosseae* (Nicol. and Gerd.) and *Acaulospora laevis* (Gerd. and Trappe) were mass multiplied by using wheat as host plant (Tanwar *et al.,* 2010).

2.6 Mass culture of *T. viride*: The inoculum of *Trichoderma viride* (MTCC Number-2074) obtained from Institute of Microbial Technology (IMTECH), Chandigarh and then further mass produced by using wheat bran and saw dust medium which was prepared by using wheat bran, saw dust and distilled water in the ratio of 3:1:4.

Selected AM fungi (*Glomus mosseae* and *Acaulospora laevis*) and bioinoculants i.e. *Trichoderma viride* were used as single, double and consortium of all bioinoculants along with different doses of fungicides to investigate their potential as growth promoters and yield on different crops in pot under polyhouse condition.

The experiment had six treatments as follows:

1. Control (autoclaved soil without any bioinoculant)
2. *Glomus mosseae* (G)
3. *Acaulospora laevis* (A)
4. *G. mosseae* + *T. viride* (G+T)
5. *A. laevis* + *T. viride* (A+T)
6. *G. mosseae* + *A. laevis* + *T. viride* (G+A+ T)

2.7 Growth study: After 120 days roots were uprooted, washed, blotted dry for determination of plant height, root and shoot fresh biomasses, fresh root weight and mycorrhizal root colonization and then oven dried for root dry weight, P content, phosphatase, protein and oil estimation. Leaf area (cm^2) was assessed by using leaf area meter (Systronics 211, Ahmedabad, India).

2.8 Percent of root colonization: For assessment of root colonization, rapid clearing and staining method of Philips and Hayman (1970) was followed and the percent infection was calculated by the following equation (Giovannetti and Mosse, 1980):

Percentage AM colonization of roots was calculated using: (Number of root segments colonized / number of root segments studied) × 100.

2.9 Phosphorus estimation: The phosphorus content of roots and shoots of all experimentally plants was estimated by 'Phospho-vanadomolybdate yellow colour method' (Jackson, 1973).

2.10 Phosphatase estimation: Phosphatase activity was assayed by using p-nitrophenyl phosphate (PNPP) as substrate, hydrolyzed by the enzyme to p-nitrophenol. For this, ice cold sodium acetate buffer (0.05 M with pH 4.8) for acid phosphatase and sodium carbonate-bicarbonate buffer (0.05 M with pH 10) for alkaline phosphatase activity was used (Tabatabai and Bremner, 1969).

2.11 Protein estimation: Protein was estimated by the method of Bradford (1976) using coomassive brilliant blue G-250 dye.

2.12 Oil extraction: Oil was extracted by petroleum ether of boiling range between 40-60^{0}C using the Soxhlet's procedure (AOCS, 1997). Five replicates of each treatment were taken.

3. Results and discussion

It is desirable for fungicides to act upon the pathogen alone leaving the rest of soil mycoflora undisturbed or at least not unfavorably affected.

3.1 Effect of fungicides on soil mycoflora:

A total number of 31 parasitic and saprophytic fungal species belonging to 13 genera were isolated from experimental soil of sunflower. While studying influence of different concentration of fungicides on soil fungi of sunflower, it was observed that low concentrations of Benomyl and Dithane M-45 fungicides are less harmful to soil fungi in comparison to medium and high concentration (Table- 1 & 2).

Most of the fungal species were absent at higher concentration of fungicide application. Fungal population was always less in fungicidal treated soil than in control. *Aspergillus niger* were found to be resistant to Benomyl as it was present in all the concentrations of fungicide, while *A. parasiticus, A. versicolor* and *A. fumigatus* were found only at low and medium concentration of Benomyl fungicide. A total number of 14 fungal species were isolated from low concentration and 4 fungal species were isolated at higher concentration of Benomyl. The present results may however be compared with the results obtained by Ponchet and Tramier (1971) who reported that Benomyl might also change the balance of soil mycoflora. A similar reduction was observed in number of species by Bertoldi *et al*. (1978) and Frahm (1973). The negative influence that the microflora suffers from fungicides has been investigated by other workers too (Agnihotri, 1974; Foster, 1975). Results obtained by Chiocchio *et al*. (2002) while studying effect of Benomyl on spore germination and hyphal length of AM fungus (*G. mosseae*) revealed that lower dose of Benomyl is beneficial for spore germination and root infection. In the present investigation, similar observations were seen as lower concentrations are beneficial as compared to higher concentrations of fungicides.

In the present study, Dithane M-45 also caused adverse effect on soil mycoflora and AM fungi as well (Table- 2).

Reduction in fungal species appeared to be

directly related with fungicidal concentration. Less number of fungal species were observed in all three concentration of Dithane M-45 in soil in comparison to control. Dithane M-45 was also having deleterious effect on *Mucor racemosus* like Benomyl. *Mucor* sp. was not affected much at low concentration of Dithane M-45. *Aspergillus fumigatus*, *A. nidulans*, *A. amstelodami*, *P. aurantiogriseum*, *P. citrinum*, *P. funiculosum*, *P. corylophilum*, *Dreschlera halodes*, *Stachybotrys atra*, *Arthrobotrys superba*, *Trichoderma viride* and *Cladosporium* sp. were sensitive to fungicide application and even lowest concentration inhibited their growth. *Penicillium* species could not tolerate higher concentration of Dithane M-45. *Trichoderma* sp. was recorded at higher concentration of Dithane M-45. A total number of 19 fungal species were isolated from low concentration of fungicide treatment. From the results, it is clear that Benomyl is more deleterious to soil fungi in comparison to Dithane M-45. Kuthubutheen and Pugh (1979) also recorded reduction in fungal population in soil after application of Dithane M-45 and other fungicides. Although both the fungicides were found capable of causing well-marked adverse effect on soil mycoflora yet there are certain soil fungi which showed resistance to these fungicides. Similarly, Carbendazim-Mancozeb fungicidal mixture applied at a concentration of 2.34mg/kg soil had a greater inhibitory effect at the recommended concentration of 1.67mg/kg soil (Fawole *et al*., 2009). This observation gets ample support from Bhutta *et al*., (2001); Afzal *et al*. (2010) who evaluated that two systemic fungicides viz., Topsin and Bayleton were found to be significantly effective in the elimination of seed- borne fungi.

3.2 Effect of Fungicides on Mycorrhizae:

Present investigation indicated that low concentration of two fungicides were non toxic to AM fungi but to lesser extent stimulate their infection and spore numbers. Maximum AM spore number and highest mycorrhization was observed in sunflower plants treated with low concentration of Benomyl in comparison to medium and high concentration (Table-3). In the same way, Dithane M-45 (Table- 4) also showed inhibitory effect on spore number and root colonization with the increase in fungicide concentration. Lowest concentration of fungicides recorded the maximum spore number as well as highest root colonization. AM root colonization showed a decreasing trend with increasing concentration of Benomyl and Dithane M-45 and lowest was recorded in high concentration of fungicides. Less arbuscules, vesicles were observed in fungicides treatment than in control. It can be said that the recommended dose of the fungicide to sunflower plants considerably decreases the AM root colonization and spore numbers in comparison to the lowest concentration of both fungicides.

Benomyl has been found to affect negatively AM symbiosis by delaying or preventing the formation of AM symbiosis between fungi and roots and by decreasing plant p-uptake (Menge, 1982; Ocampo, 1993). Benomyl also suppresses mycelial growth by preventing nuclear division. In fact, this fungicide inhibits mitosis by blocking the formation of β-tubuline and microtubules when chromosomes are separated (Howard and Aist, 1980; Isaac, 1992). Our results showed that lower concentration of Benomyl and Dithane M-45 did not decrease AM spore number but inhibited the hyphal length, arbuscules and vesicles at higher concentration. This suggests that fungal hyphae, vesicles etc are more permeable to fungicides than the multistratified wall of the spores. Udaiyan *et al*. (1995) also have observed an inhibition of colonization and decrease AM spore count by the application of fungicides. Kumar and Abraham (2001) have studied the effect of Dithane M-45 and Bavistin on AM of *Gluta travancorica*, *Bentinckia condapanaa* and *Myristica malabarica* of Western Ghatts and reported the deleterious effect on non-target fungi. Fungicides are expected to affect mycorrhizal fungi most profoundly and its formation on hosts (Trappe *et al*., 1984). Udaiyan *et al*. (1995) have observed that Bavistin application exhibited measurable inhibition in root colonization. Reduction in number of mycorrhizal structure like vesicles and arbuscules might be associated with the inhibition of infection process. Several workers have reported the adverse effect of fungicides on soil fungi. Domsch (1965) reported that fungicides like Captan, Nabam, Vapam, Allyl alcohol had adverse effect on *Rhizoctonia soloni, Monilia prunicosa*, *Mucor hiemalis*, *Rhizopus nigricas*, *Fusarium solani* and *Aspergillus fumigatus*. But in the present investigation, none of the fungicide showed stimulatory effect at higher concentration. The observed reduction in AM spore number in present investigation by fungicide application is probably due to the reduction of AM infected root length or may be due to the exhaustion of extramatrical hyphae by toxic chemicals. According to Sreenivasa and Bagyaraj (1989) that Captan and Cabofuran fungicides were deleterious to AM at the recommended level but at half of the recommended dose it showed increased percent root colonization. In the present investigation, similar observations were observed that higher doses were inhibitory while half of the recommended doses showed positive results. Smith *et al*. (2000) also showed inhibitory effect of Benomyl on AM fungi. The results of the present investigation are in corroboration with earlier workers. Bharat (2011) reported that the effect of commonly used fungicides like, Mancozeb and Copper sulphate, Mancozeb

application resulted highest reduction in AM fungi colonization.

3.3 Effect of Fungicides along with bioinoculants on Sunflower plant growth:

In Sunflower, inoculations with AM fungi significantly increase plant growth as compared to uninoculated plants at lower concentration of Benomyl (Table-5 & 6).

However, maximum plant height was observed at lower concentration of fungicides in the plants treated with mix consortium of both AM fungi and *Trichoderma viride*. Root length was recorded maximal in *G. mosseae* + *A. laevis* + *T. viride* treated plants at lower concentration of Benomyl. Similar observation was made in case of shoot and root biomass. A decline in growth parameters was noticed with all bioinoculants with increase in concentration of fungicides. However, mycorrhizal colonization, arbuscule and vesicle formation significantly decreased with the increase in Benomyl application rate. The high rate of fungicides application i.e., medium concentration and high concentration, lead to antagonistic inhibition of mycorrhizal colonization whereas in lower dose, mycorrhiza was able to increase the root colonization significantly.

Phosphorus uptake in shoots and roots was also significantly higher as compared to uninoculated treatments, highest being at low concentration of fungicides. Again, treatment of *G. mosseae* + *A. laevis* + *T. viride* proved to be the best after 120 days of inoculation. With reference to phosphatase activity, alkaline phosphatase was found to be more active than acid phosphatase. All the plants inoculated with bioinoculants harbored higher enzyme activity than non-inoculated plants. However, higher activity was recorded in mix consortium of *G. mosseae*, *A. laevis* and *T. viride* at all concentrations of fungicides with maximal at low concentration followed by medium and high concentrations. Likewise, head diameter, protein content and oil content were also observed best at low concentration of fungicides in mix consortium of *G. mosseae* + *A. laevis* + *T. viride*. A decline in yield was noticed along with bioinoculants at increase in fungicide concentration. These results indicate that in presence of AM fungi, the sunflower plant survived better in the lower fungicide concentration and enhance the plant growth.

Table-7 & 8 showed the effect of Dithane M-45 on growth and yield of Sunflower. Plants treated with recommended concentration and high concentration showed a decrease in plant growth, where as the lower concentration of Dithane M-45 along with bioinoculants showed increase in plant height. Root length was observed maximum at lower concentration of Dithane M-45 in the plants treated with mix consortium of both AM fungi and *Trichoderma viride*. Shoot and root biomass also recorded maximal in *G. mosseae* + *A. laevis* + *T. viride* treated plants at lower concentration of fungicide. However, mycorrhizal colonization, arbuscule and vesicle formation significantly decreased with the increase in Dithane M-45 application rate. The higher rate of fungicides application i.e., medium concentration and high concentration, lead to antagonistic inhibition of mycorrhizal colonization whereas in lower dose, mycorrhiza was able to increase the root colonization significantly. Likewise, AM spore population as well as root colonization was observed best at lower concentration of fungicides.

Similar observation was made in shoot and root P. A decline was noticed in growth of Sunflower with increase in concentration of fungicides. However, higher phosphatase activity was recorded in mix consortium of *G. mosseae*, *A. laevis* and *T. viride* at all concentration of fungicides with maximal at low concentration followed by medium and high concentrations after inoculation. Likewise, head diameter, protein content and oil content were observed best at low concentration of fungicides in mix consortium of *G. mosseae* + *A. laevis* + *T. viride*. A decline was noticed in yield of bioinoculants with increase in fungicides concentration. These results indicate that in presence of AM fungi, the sunflower plant survived better in the lower fungicide concentration and enhance the plant growth. If we compare both fungicides, it can be said that Benomyl is more deleterious to plant growth as compared to Dithane M-45.

In the present investigation, high concentration of fungicides i.e. Benomyl and Dithane M-45 reduced growth and yield of plant. The Benomyl fungicide inhibits mycorrhizal growth and development because of the activity of methyl 1, 2-benzimidazole carbomate which is a product of benomyl hydrolysis. Moreover, application of Benomyl also damages external hyphae of mycorhhiza and prevents root colonization, reducing water and nutrient uptake, photosynthesis and plant growth. Recently, Abbasian *et al.* (2012) have also reported that application of Benomyl interferes with plant-mycorrhiza symbiosis and inhibits root system development, absorption and growth. Similarly Abbasian *et al*. (2012), Saleh and Saleh (2006) also reported that lower concentration of Mancozeb was responsible for higher mycorrhizal colonization and sporulation, while higher concentration was inhibitory to mycorrhizal activities. In accordance to these findings, it was reported by many workers that mycorrhizal inoculation increased growth yield of many plants (Marin *et al*., 2002; Khaliel *et al*., 1994; Rao, 2002).

Table- 1: Effect of Benomyl fungicide on soil mycoflora

Sr. No.	Species diversity	Fungicide concentration			
		Control	Low	Medium	High
1.	*Aspergillus niger*	+	+	+	+
2.	*A. flavus*	+	+	-	-
3.	*A. parasiticus*	+	+	+	-
4.	*A. ochraceus*	+	+	-	-
5.	*A. candidus*	+	-	-	-
6.	*A. fumigatus*	+	+	+	-
7.	*A. nidulans*	+	-	-	-
8.	*A. versicolor*	+	+	+	-
9.	*A. amstelodami*	+	-	-	-
10.	*A. repens*	+	-	-	-
11.	*A. terreus*	+	+	+	-
12.	*Penicillium atramentosum*	+	+	-	-
13.	*P.aurantiogriseum*	+	-	-	-
14.	*P. citrinum*	+	+	-	-
15.	*P. commune*	+	-	-	-
16.	*P. chrysogenum*	+	+	-	-
17.	*P. funiculosum*	+	-	-	-
18.	*P. corylophilum*	+	-	-	-
19.	*Rhizopus nigricans*	+	+	-	-
20	*Mucor racemosus*	+	-	-	-
21.	*Mucor hiemalis*	+	-	-	-
22.	*Fusarium oxysporum*	+	+	-	+
23.	*Drechslera halodes*	+	-	-	-
24.	*Rhizoctonia solani*	+	+	+	-
25.	*Stachybotrys atra*	+	-	-	-
26.	*Arthrobotrys superba*	+	-	-	-
27.	*Cladosporium* sp.	+	+	+	+
28.	*Alternaria* sp.	+	+	-	+
29.	*Curvularia lunata*	+	-	+	-
30.	*Trichoderma viride*	+	-	-	-
31.	*Verticillium*	+	-	+	-

+ = Present, - = absent

Table- 2: Effect of Dithane M-45 fungicide on soil mycoflora

Sr. No.	Species Diversity	Fungicide Concentration			
		Control	Low	Medium	High
1.	*Aspergillus niger*	+	+	+	+
2.	*A. flavus*	+	+	+	-
3.	*A. parasiticus*	+	+	+	-
4.	*A. ochraceus*	+	+	-	-
5.	*A. candidus*	+	+	-	-
6.	*A. fumigatus*	+	-	-	-
7.	*A. nidulans*	+	-	-	-
8.	*A. versicolor*	+	+	+	-

9.	*A. amstelodami*	+	-	-	-
10.	*A. repens*	+	+	-	-
11.	*A. terreus*	+	+	+	+
12.	*Penicillium atramentosum*	+	+	+	+
13.	*P.aurantiogriseum*	+	-	-	-
14.	*P. citrinum*	+	-	-	-
15.	*P. commune*	+	+	-	-
16.	*P. chrysogenum*	+	+	-	-
17.	*P. funiculosum*	+	-	-	-
18.	*P. corylophilum*	+	-	-	-
19.	*Rhizopus nigricans*	+	+	-	-
20	*Mucor racemosus*	+	+	-	-
21.	*Mucor hiemalis*	+	+	-	-
22.	*Fusarium oxysporum*	+	+	+	-
23.	*Dreschlera halodes*	+	-	-	-
24.	*Rhizoctonia solani*	+	+	+	-
25.	*Stachybotrys atra*	+	-	-	-
26.	*Arthrobotrys superba*	+	-	-	-
27.	*Cladosporium* sp.	+	-	-	+
28.	*Alternaria* sp.	+	+	-	+
29.	*Curvularia lunata*	+	+	+	-
30.	*Trichoderma viride*	+	-	+	+
31.	*Verticillium*	+	+	+	-

+ = Present, - = absent

Table-3: Effect of different concentration of Benomyl on AM fungi associated with sunflower

Treatments	AM spore number/10 g^{-1} soil	AM root colonization (%)	Type of Infection		
			M	V	A
Control	143	78.42	+	+	+
Low conc	142	79.31	+	+	-
Med conc	128	58.14	+	-	-
High conc	79	14.98	-	-	-

M- Mycelium, V-Vesicles, A-Arbuscules
+= Present, -= absent

Table- 4: Effect of Dithane M-45 on AM fungi associated with Sunflower

Treatments	AM spore number/10 g^{-1} soil	AM root colonization (%)	Type of Infection		
			M	V	A
Control	143	78.42	+	+	+
Low conc	147	81.42	+	+	+
Med conc	134	63.11	+	-	-
High conc	92	34.12	-	-	-

M- Mycelium, V-Vesicles, A-Arbuscules; + = Present, - = absent

Table- 5: Effect of different concentrations of Benomyl on some growth parameters and on mycorrhization of Sunflower after 120 days of inoculation

Fungicide Concentration	Parameters→ Treatments	Plant height (cm)	Shoot biomass (g)		Root biomass (g)		Root Length (Cm)	Leaf area (Sq.Cm.)	AM Root colonization (%)	AM Spore number/10 g soil
			Fresh	Dry	Fresh	Dry				
Low Concentration	Control	70.64±4.069	12.74±2.100	2.93±0.746	0.79±0.105	0.41±0.092	8.60±0.718	42.96±1.498	8.88±1.675	5.2±1.483
	G. mosseae	79.70±3.701	20.31±1.583	4.45±0.956	1.49±0.367	0.69±0.213	15.48±1.050	72.09±0.853	43.09±4.186	53.0±4.980
	A. laevis	73.58±2.299	15.23±1.562	3.16±0.355	1.45±0.126	0.50±0.072	10.48±1.108	65.51±1.805	32.51±0.782	52.4±2.332
	G+T	93.52±3.746	22.70±1.083	5.78±0.976	1.64±0.053	0.64±0.100	16.28±0.593	77.53±1.211	43.32±1.492	56.8±2.713
	A+T	75.92±2.951	25.57±1.575	6.69±0.844	1.56±0.176	0.58±0.100	12.50±0.803	67.39±1.640	39.47±0.665	33.0±2.280
	G + A.+ T	98.81±2.808	30.70±3.018	7.85±1.514	1.72±0.199	0.72±0.038	19.83±2.258	82.39±0.775	56.87±7.235	86.4±2.332
Medium Concentration	Control	59.94±2.530	8.18±1.961	1.74±0.716	0.58±0.099	0.31±0.064	8.10±1.102	36.61±0.633	5.66±1.099	2.60±1.35
	G. mosseae	72.52±2.983	15.73±1.121	3.58±0.525	1.41±0.297	041±0.065	9.16±0.483	60.56±1.111	36.38±1.417	31.4±2.332
	A. laevis	70.66±2.176	12.94±1.242	2.91±0.675	1.30±0.151	0.35±0.053	12.42±0.598	54.37±0.973	27.65±1.249	26.2±1.720
	G+T	74.40±2.053	19.52±1.105	4.15±0.800	1.57±0.353	0.46±0.062	14.72±1.889	67.67±0.602	39.25±0.692	40.8±3.311
	A+T	71.48±1.861	13.36±1.343	3.14±0.759	1.36±0.254	0.38±0.045	11.45±1.218	70.33±0.782	22.64±1.830	341.4±2.800
	G + A + T	83.96±2.681	26.42±1.248	5.68±1.690	1.59±0.045	0.59±0.020	16.51±1.715	72.44±0.833	48.13±1.632	60.2±2.926
High Concentration	Control	34.92±0.740	7.70±0.787	0.79±0.065	0.66±0.170	0.15±0.043	4.59±0.925	28.17±0.688	2.14±0.517	00±00
	G. mosseae	47.72±1.033	9.15±1.474	1.40±0.387	1.03±0.338	0.19±0.061	10.55±1.653	27.39±0.489	12.40±2.612	22.8±2.786
	A. laevis	40.28±2.111	7.04±0.778	0.93±0.210	0.98±0.213	0.17±0.053	8.47±1.926	25.22±2.667	19.20±3.419	12.4±1.020
	G+T	51.70±3.141	12.59±1.170	2.38±0.755	1.23±0.082	0.27±0.057	9.34±1.014	31.31±1.365	20.50±3.490	24.4±5.535
	A+T	45.16±1.627	14.33±1.395	2.15±0.497	1.07±0.162	0.17±0.043	6.88±1.001	25.23±0.796	14.06±2.420	19.0±1.673
	G + A + T	65.20±1.503	16.12±1.336	2.94±0.788	1.39±0.309	0.35±0.042	5.64±1.674	36.10±0.547	32.80±0.964	50±7.239

G: *Glomus mosseae*; A: *Acaulospora laevis*, T- *Trichoderma viride*
± Standard deviation

Table- 6: Effect of different concentrations of Benomyl on phosphatase activity, phosphorus content and yield parameters of Sunflower after 120 day of inoculation

Fungicide Concentration	Parameters→ Treatments ↓	Phosphatase (IUg^{-1}Fresh Weight)		Phosphorus content (%)		Yield		
		Acidic	Alkaline	Shoot	Root	Head Diameter	Protein content(%)	Oil content(%)
Lower concentration	Control	0.09±0.044	0.19±0.041	0.92±0.047	1.01±0.055	7.08±0.635	12.02±0.595	25.34±0.434
	G. mosseae	0.34±0.057	0.95±0.060	1.42±0.265	1.72±0.110	10.42±0.370	17.85±0.338	32.26±0.802
	A. laevis	0.29±0.033	0.92±0.035	1.40±0.103	1.53±0.096	9.28±0.229	14.51±0.599	29.50±0.781
	G+T	0.38±0.046	0.99±0.029	1.49±0.110	1.79±0.094	10.62±0.311	19.57±0.636	34.42±1.126
	A+T	0.49±0.075	0.96±0.024	1.53±0.164	1.64±0.093	11.52±0.290	18.60±0.477	31.27±0.305
	G + A.+ T	0.52±0.080	1.05±0.105	1.59±0.106	1.98±0.164	11.97±0.422	21.94±0.320	35.63±0.540
Medium concentration	Control	0.05±0.033	0.16±0.061	0.67±0.132	0.85±0.083	5.26±0.584	9.27±0.529	18.16±0.413
	G. mosseae	0.24±0.120	0.71±0.129	1.19±0.113	1.53±0.125	7.52±0.086	13.23±0.343	24.33±0.950
	A. laevis	0.16±0.036	0.60±0.048	1.27±0.170	1.44±0.070	6.03±0.281	10.27±0.713	21.36±0.751
	G+T	0.24±0.024	0.79±0.079	1.23±0.070	1.58±0.087	9.21±0.380	15.14±0.571	26.24±1.088
	A+T	0.19±0.030	0.64±0.073	1.32±0.095	1.47±0.116	7.29±0.405	12.34±0.459	23.56±1.124
	G + A + T	0.32±0.065	0.88±0.123	1.39±0.179	1.69±0.158	10.25±0.358	18.06±0.845	29.06±0.730
High concentration	Control	0.03±0.027	0.08±0.051	0.34±0.063	0.54±0.100	3.11±0.185	5.24±0.651	10.51±1.266
	G. mosseae	0.14±0.019	0.58±0.118	0.58±0.068	0.77±0.006	6.25±0.197	9.72±0.325	13.55±0.782
	A. laevis	0.06±0.030	0.42±0.049	0.43±0.020	0.59±0.063	5.03±0.598	7.66±0.313	11.94±0.918
	G+T	0.14±0.040	0.59±0.072	0.66±0.036	0.86±0.137	7.07±0.097	10.15±0.410	17.70±0.812
	A+T	0.07±0.029	0.46±0.127	0.47±0.159	0.75±0.144	6.62±0.401	8.34±0.312	15.26±0.971
	G + A + T	0.20±0.075	0.69±0.045	0.76±0.020	1.03±0.139	7.75±0.685	11.05±0.238	19.70±0.675

G: *Glomus mosseae*; A: *Acaulospora laevis,* T- *Trichoderma viride*
± Standard deviation

Table- 7: Effect of different concentrations of Dithane M-45 on some growth parameters and on mycorrhization of Sunflower after 120 days of inoculation

Fungicide Concentration	Parameters→ Treatments ↓	Plant height (Cm)	Shoot biomass (g)		Root biomass (g)		Root Length (Cm)	Leaf area (Sq.Cm.)	AM Root colonization (%)	AM Spore number/10 g soil
			Fresh	Dry	Fresh	Dry				
Low Concentration	Control	78.07±2.100	14.28±1.683	4.76±0.309	1.96±0.076	0.67±0.072	11.10±0.925	47.64±1.923	10.28±1.554	00±00
	G. mosseae	86.55±2.717	20.42±0.710	6.46±0.586	3.22±0.342	0.97±0.086	21.38±0.769	81.12±1.274	51.12±1.174	71.0±4.796
	A. laevis	82.42±4.871	18.32±0.708	5.05±0.535	3.12±0.259	0.88±0.064	18.40±1.565	74.23±1.675	50.52±2.062	68.2±7.497
	G+T	93.98±3.086	25.42±1.112	8.24±0.666	3.43±0.476	0.99±0.079	20.12±1.781	90.38±0.192	58.16±2.584	80.8±1.304
	A+T	97.43±2.600	28.22±1.037	9.08±0.572	3.54±0.716	0.99±0.090	23.48±0.750	94.47±1.395	69.56±1.805	84.2±7.596
	G + A.+ T	106.86±2.873	29.41±0.516	9.23±0.591	3.73±0.434	1.04±0.091	25.66±0.929	99.11±0.532	75.07±3.034	90.2±5.450
Medium Concentration	Control	63.98±2.069	9.72±0.649	2.54±0.329	1.04±0.061	0.42±0.157	8.12±0.650	40.40±0.805	28.23±0.751	00±00
	G. mosseae	73.76±2.916	17.27±1.160	4.06±0.041	2.10±0.530	0.65±0.081	15.14±0.921	64.26±0.665	43.27±1.151	59.6±4.336
	A. laevis	70.46±4.964	12.32±0.721	3.19±0.086	2.00±0.706	0.52±0.076	13.38±0.719	60.40±0.967	39.22±1.564	53.4±6.348
	G+T	75.66±1.557	19.82±0.584	5.06±0.777	2.83±0.149	0.66±0.051	17.48±0.409	71.53±0.305	50.47±1.978	69.2±8.258
	A+T	79.90±2.414	23.66±0.802	6.61±0.217	2.70±0.119	0.65±0.055	16.40±0.731	79.28±0.791	52.21±1.626	75.4±4.775
	G + A + T	89.28±3.717	25.81±0.143	7.83±0.767	3.00±0.120	0.89±0.054	20.28±0.798	85.14±0.622	62.95±0.869	78.2±8.843
High Concentration	Control	37.80±1.704	7.54±0.684	0.86±0.032	0.73±0.092	0.20±0.047	6.50±0.283	31.12±0.555	00±00	00±00
	G. mosseae	51.04±3.191	9.47±0.909	1.58±0.192	1.03±0.097	0.22±0.059	8.12±0.687	34.17±0.252	17.46±0.925	29.0±7.616
	A. laevis	52.86±3.866	7.14±0.496	0.97±0.199	1.27±0.055	0.30±0.033	8.62±0.585	36.20±0.480	19.14±0.677	22.6±.3.209
	G+T	56.60±2.280	12.26±0.669	2.51±0.074	1.38±0.040	0.29±0.052	11.10±0.561	45.22±0.638	23.14±1.450	33.2±8.438
	A+T	61.52±2.521	14.44±0.901	2.82±0.059	1.41±0.043	0.32±0.048	11.98±0.563	40.23±0.847	29.16±1.619	38.4±3.286
	G + A + T	65.58±1.928	16.19±0.588	3.42±0.155	1.59±0.035	0.37±0.037	12.32±0.705	48.42±0.943	32.71±1.220	**45.4**±6.189

G: *Glomus mosseae*; A: *Acaulospora laevis*, T- *Trichoderma viride*
± Standard deviation

Table 8: Effect of different concentrations of Dithane M-45 on Phosphatase activity, phosphorus content and yield parameters of Sunflower after 120 days of inoculation.

Fungicide Concentration	Parameters→ Treatments ↓	Phosphatase (IUg^{-1}Fresh Weight)		Phosphorus content (%)		Yield		
		Acidic	Alkaline	Shoot	Root	Head Diameter	Protein content(%)	Oil content(%)
Lower concentration	Control	0.09±0.030	0.23±0.022	1.01±0.016	1.11±0.014	9.12±0.596	15.38±0.718	32.95±1.197
	G. mosseae	0.38±0.036	1.09±0.025	1.80±0.009	2.01±0.015	13.15±0.611	19.61±0.960	41.16±1.351
	A. laevis	0.37±0.028	1.04±0.012	1.66±0.011	1.69±0.019	12.68±0.399	18.52±0.536	39.49±0.213
	G+T	0.50±0.015	1.08±0.027	1.93±0.007	2.42±0.084	13.71±0.403	20.75±0.261	42.70±0.360
	A+T	0.54±0.009	1.14±0.013	1.99±0.007	2.46±0.023	14.18±0.626	22.49±0.340	43.57±0.430
	G + A.+ T	0.65±0.018	1.16±0.005	2.14±0.114	3.81±0.016	15.09±0.447	22.22±0.168	44.14±0.654
Medium concentration	Control	0.06±0.011	0.18±0.004	0.93±0.017	0.99±0.012	7.10±0.644	12.22±0.085	26.10±0.620
	G. mosseae	0.36±0.055	0.96±0.013	1.47±0.018	1.72±0.084	10.25±0.264	17.62±0.231	31.08±0.687
	A. laevis	0.26±0.009	0.92±0.005	1.44±0.015	1.50±0.122	9.16±0.562	17.26±0.355	28.46±0.814
	G+T	0.36±0.008	0.99±0.027	1.51±0.016	1.73±0.023	10.68±0.627	18.69±0.334	35.22±1.341
	A+T	0.46±0.008	0.96±0.008	1.56±0.008	1.62±0.019	11.14±0.282	19.59±0.583	34.24±0.680
	G + A + T	0.49±0.009	1.03±0.015	1.60±0.009	1.84±0.016	11.83±0.629	20.06±0.340	38.24±0.643
High concentration	Control	0.04±0.009	0.16±0.005	0.54±0.008	0.66±0.017	4.18±0.586	8.59±0.928	17.48±0.818
	G. mosseae	0.12±0.008	0.56±0.007	1.19±0.013	1.12±0.012	7.55±0.808	11.51±0.849	24.46±0.643

	A. laevis	0.16±0.005	0.43±0.011	1.22±0.008	1.23±0.013	6.23±0.338	9.36±0.811	21.34±0.896
	G+T	0.18±0.008	0.66±0.007	1.22±0.018	1.30±0.008	7.60±0.387	12.22±0.774	25.22±1.190
	A+T	0.21±0.009	0.77±0.011	1.32±0.012	1.51±0.007	8.91±0.350	10.34±0.772	26.40±1.385
	G + A + T	0.25±0.008	0.81±0.011	1.36±0.005	1.54±0.013	9.15±0.713	12.27±0.829	28.28±0.370

G: *Glomus mosseae*; A: *Acaulospora laevis,* T- *Trichoderma viride*
± Standard deviation

Conclusion:

Both the fungicides are capable of inhibiting certain soil mycoflora at certain conditions. The ultimate sink of the fungicides when applied to agricultural soil which is the store house of microbes, in significant amount disturb the ecological balance. This may affect the overall microbial population, or some of them may be selectively inhibited. The fungicides alter the physiology of rhizosphere, which is turn alters the rhizosphere mycoflora. It should be desirable for fungicides to act upon the pathogen alone leaving rest of the microflora undisturbed, but in present investigation, none of the fungicide appears to possess such an ideal nature. It is suggested that a judicious use of fungicides may have less effect on soil mycoflora.

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