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MYCOFLORA OF SUNFLOWER RHIZOSPHERE IN RELATION TO SOIL FUMIGATION

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SUMMARY

The present investigation was aimed at analyzing the role of fumigants, *i.e.*, different concentrations of carbon disulphide (CS_2) and formalin, on soil mycoflora including mycorrhizal fungi in the sunflower rhizosphere. Fungi were greatly reduced immediately after fumigant application but with the passage of time these started to reappear. In qualitative analyses of mycoflora, *Aspergillus niger, Aspergillus terreus* and *Penicillium nigricans* reappeared only after 20 days at all CS_2 concentrations. The quantitative study showed the boosting up of the population of *Trichoderma viride* with increasing concentrations of formalin and CS_2 after 20 days. High concentrations of both fumigants initially decreased mycorrhizal spore number. However, the mycorrhizal spore number increased later on. Mycorrhizal root colonization reached maximum after 40 days in treated soils. *Glomus mosseae* was resistant to CS_2 application but *Glomus geosporum* and *Acaulospora laevis* were inhibited by high concentrations of the fumigant.

Key words: sunflower, fumigants, mycoflora, VAM fungi

INTRODUCTION

Soil contains a large number of diverse microbial populations. Some of these microorganisms are inherently resistant to adverse environmental conditions as soil organic matter protects them from biocidal concentration. There were some situations in agriculture and horticulture where soilborne plant pathogens may be controlled by strong biocidal treatments that affect most sections of the soil microbial population. Carbon disulphide (CS_2) was the first chemical used as soil fumigant. Fungi including mycorrhizae are more readily killed by fumigants than many bacteria. Many workers have found *Trichoderma* and *Penicillium* spp. to be dominant in fumigated soils (Tiwari and Mehrotra, 1973; Kumar, 1995). Saksena (1960) studied the resistance of various soil fungi to fumigants and their ability to recolo-

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nize the fumigated soils. In view the above information, two fumigants, *i.e.*, carbon disulphide and formalin, were tested to see their effects on microorganisms including VAM fungi. Another objective was to see the effects of different doses of these fumigants on the population of *Trichoderma viride* which is used both as a biocontrol agent and a biofertilizer. The effect of two fumigants on plant growth and plant phosphorus content of sunflower was also investigated.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from the botanical garden of Botany Department, Kurukshetra University, Kurukshetra, Haryana, India. Soil was sieved for further treatment.

Treatments

Soil was treated with two fumigants, *i.e.*, formalin and CS_2. Two kg of soil were taken in each pot (size 30 × 25 cm) and fumigants were added to the soil at 2, 4, 6 and 11 ml per 2 kg of soil. The pots were covered with polythene bags after fumigation so that no fume could escape from them. Polythene covers were removed after 48 h. Healthy seeds of sunflower were sown in each pot. To maintain the moisture needed for seed germination and growth of plants, pots were watered regularly. Soil samples were taken out for mycofloral and mycorrhizal studies after 10, 20, 40 and 70 days. Control pots were kept without any treatment. Five pots of each treatment and control were taken for analyses.

Mycoflora study

For quantitative and qualitative studies of soil mycoflora, Warcup soil plate method (1950) and Waksman soil dilution method (1927) were used. For mycorrhizal study, the sunflower plants from the treated and control pots were uprooted after regular time intervals of 10, 20, 40 and 70 days. Isolation of VAM spores was done by the wet sieving and decanting technique of Gerdemann and Nicolson (1963). Root colonization of VAM fungi was studied by the rapid clearing and staining technique of Philips and Hayman (1970). Growth of sunflower plants was measured after 70 days. For this study, two parameters were measured, *i.e.*, height of plants and phosphorous (P) content of plant shoots and flowers. Phosphorus content of plant shoots and flowers was estimated by the vanadomolybdate phosphoric yellow color method as reported by Jackson (1973). Data were analyzed using the least significant difference test (LSD) and the analysis of variance.

RESULTS AND DISCUSSION

Both fumigants had inhibitory effect on soil mycoflora. During the initial 10 days, the inhibitory effect was very pronounced but thereafter the fungitoxic effect decreased and resulted in recurrence of certain fungi. After 10 days, high concentrations of the fumigants (6 ml/2 kg) and (11 ml/2 kg) were completely inhibitory to fungi and no fungal species was recorded. *Aspergillus ruber, A. ochraceus*, *A. luchuensis*, *A. fumigatus* and *Penicillium funiculosum* were inhibited by carbon disulphide even at a very low concentration (2 ml/2 kg). *A. niger* and *Penicillium nigricans* were inhibited by the higher concentrations after 10 days (6 and 11 ml/2 kg). However, after 10 days, these fungal species reappeared in nearly all treatments. *A. terreus* and *Mucor racemosus* were recorded more frequently after 20 days. *Trichoderma viride* and *Curvularia lunata* reappeared in the treated soil after 40 days.

Formalin also had deleterious effect on soil mycoflora. *Aspergillus terreus* and *Fusarium oxysporum* were resistant to formalin application and these species were present in all the concentrations of the fumigant on all sampling days. Formalin was not completely fungitoxic to all the fungal species at the high concentrations. Inhibitory effect of formalin also decreased with passage of time. *Aspergillus ruber*, *A. ochraceus*, *A. fumigatus*, *A. candidus, A. luchuensis* and *Mucor racemosus* were inhibited by formalin. Of the above species, *Aspergillus ochraceus* and *A. fumigatus* reappeared after 70 days in the lowest concentration (2 ml/2 kg). *Aspergillus flavus* and *A. niger* were found in the 2 ml/2 kg concentration after 40 days. *Trichoderma viride* was the dominant fungus in the formalin-treated soil in all concentrations.

In the quantitative study, carbon disulphide completely inhibited soil fungi at high concentrations, *i.e.*, 6 ml to 11 ml, on the 10^{th} day but larger numbers of fungal species were recorded after 20 days in various concentrations of carbon disulphide. *Aspergillus niger*, *A. terreus* and *P. nigricans* were present in the low concentrations after 10 days and after 20 days were present in all concentrations. *Cladosporium cladosporioides*, *Alternaria alternata*, *Curvularia lunata* and *Mucor racemosus* were present in the low concentrations of carbon disulphide. *Trichoderma viride* reappeared after 20 days in the low concentrations and in all concentrations after 70 days.

Aspergillus ochraceus, A. fumigatus, Penicillum chrysogenum and *Mucor racemosus* were inhibited by formalin in all concentrations. *Aspergillus flavus, A. candidus, Fusarium solani, Penicillium funiculosum* and *Trichoderma viride* were present in the high concentrations of formalin after 40 days. *Aspergillus terreus* and *Fusarium oxysporum* were relatively resistant to formalin application. *Trichoderma viride* reappeared after 20 days.

Height of the untreated (control) plants was greater than that of the plants growing in the fumigant-treated soils. The P content increased in the CS_2 treatment with

4 ml/2 kg, decreased with 6 ml/2 kg and again increased with 11 ml/2 kg. Regarding the P content in flowers, it showed a decreasing trend up to the fumigant concentration of 6 ml/2 kg and then increased after treatment with 11 ml/2 kg (Table 1).

Phosphorus content in plants decreased with increasing formalin concentration but it showed a slight increase at 11 ml/ 2kg of formalin. In the case of P content in flowers, it decreased with increasing formalin concentration but showed an increase at the highest concentration, *i.e.*, 11 ml/2 kg soil (Table 1).

Table 1: Effect of carbon disulphide and formalin on the growth and P content of sunflower

Conc. (ml/2 kg)	Height of plants		P content in plant (ppm)		P content in flower (ppm)	
	CS_2	Formalin	CS_2	Formalin	CS_2	Formalin
2	$83^{a}\pm0.47$	$76^{a}\pm0.4$	$2055.6^{a}\pm0.4$	$2580.4^{a}\pm2.3$	$6612.6^{a}\pm0.4$	$4789.8^{a}\pm1.8$
4	$96^{b}\pm0.94$	$95^{b}\pm2.3$	$2950.4^{b}\pm0.4$	$2394.8^{b}\pm0.4$	$1513.4^{b}\pm1.4$	$4658.8^{b}\pm1.4$
6	$96^{b}\pm1.41$	$96^{b}\pm0.9$	$1732.2^{c}\pm0.9$	$2021.8^{c}\pm0.9$	$1542^{c}\pm0.9$	$4112.4^{c}\pm2.8$
11	$100^{bc}\pm0.27$	$98^{b}\pm1.3$	$2429^{d}\pm1.8$	$2778.4^{d}\pm1.4$	$4583.2^{d}\pm1.4$	$5043.8^{d}\pm0.9$
Control	$105^{c}\pm1.41$	$105^{c}\pm1.4$	$1362^{e}\pm2.35$	$1362^{e}\pm2.3$	$6684.4^{e}\pm1.8$	$6684.4^{e}\pm1.8$
LSD (P=0.05)	8.25	5.38	6.85	6.01	12.70	2.46

Means in each column that have different letters differ significantly at the 0.05 level of probability

On the 10^{th} day, the mycorrhizal spore number decreased as the concentration of CS_2 increased. On the 20^{th} day, the mycorrhizal spore number showed increases at lower concentrations but it showed a decrease at the highest concentration of 11 ml/2 kg soil. After 40 days onward, the mycorrhizal spore count and root colonization showed a rise with increase in the concentration of the fumigants (Table 2). Table 2 showed that within the treatments, the mycorrhizal spore number increased initially with formalin concentrations increasing up to 6 ml/2 kg soil and then it decreased at the concentration of 11 ml/2 kg.

Table 2: Effect of carbon disulphide and formalin on mycorrhizal spore number/100 g of sunflower at different time intervals

Conc. (ml/2 kg)	10^{th} day		20^{th} day		40^{th} day		70^{th} day	
	CS_2	Formalin	CS_2	Formalin	CS_2	Formalin	CS_2	Formalin
Control	$840^{a}\pm4.7$	$840^{a}\pm4.7$	$936^{a}\pm2.8$	$936^{a}\pm2.8$	$648^{a}\pm3.7$	$648^{a}\pm3.7$	$628^{a}\pm3.7$	$628^{a}\pm3.7$
2	$800^{b}\pm9.4$	$890^{b}\pm4.7$	$950^{ab}\pm4.7$	$1028^{b}\pm3.7$	$400^{a}\pm9.4$	$600^{b}\pm9.4$	$432^{b}\pm0.9$	$488^{b}\pm4.2$
4	$775^{c}\pm2.3$	$920^{c}\pm11.4$	$965^{b}\pm7.4$	$1112^{c}\pm4.7$	$448^{b}\pm3.7$	$612^{b}\pm5.6$	$556^{c}\pm2.8$	$516^{c}\pm7.5$
6	$750^{d}\pm2.8$	$920^{c}\pm14.1$	$990^{c}\pm18.8$	$1270^{d}\pm4.7$	$450^{b}\pm20.4$	$368^{c}\pm3.7$	$580^{d}\pm4.7$	$348^{d}\pm3.7$
11	$700^{e}\pm9.4$	$600^{d}\pm20.4$	$800^{d}\pm14.1$	$920^{a}\pm9.4$	$504^{c}\pm1.8$	$292^{d}\pm0.9$	$628^{e}\pm3.7$	$360^{d}\pm4.7$
LSD (P=0.05)	22.12	27.16	22.31	22.62	14.07	20.69	11.60	24.18

Means in each column that have different letters differ significantly at the 0.05 level of probability

Glomus mosseae was resistant to CS_2 application. *Glomus versiforme*, *Gigaspora margarita*, *Gigaspora gigantea* were inhibited by CS_2 application. Effect of the fumigants was inhibitory during the initial 20 days after treatment and then this deleterious effect decreased. *Glomus reticulatum*, *Glomus aggregatum*, *Glomus fasciculatum*, *Acaulospora foveata* and *Acaulospora* sp. were recorded

after 20 days at the high concentrations. In the case of formalin treatment, the VAM species decreased in comparison with the untreated control. Fungal species recorded after the 20th day were more numerous than before that. *Glomus geosporum*, *Glomus gilmorei* and *Glomus diaphanum* were inhibited by formalin. *Glomus intraradices*, *Glomus reticulatum* and *Glomus fasciculatum* were resistant to formalin application. *Glomus mosseae*, *Glomus versiforme*, *Acaulospora laevis* and *Glomus* sp. I were recorded more frequently, even at high concentrations, after 20 days but *Acaulospora foveata*, *Glomus* sp. II and *Glomus caledonicum* were recorded seldom.

Mycorrhizal root colonization decreased with increase in CS_2 concentration of up to the 40th day but after the 70th day it leveled off. The number of vesicles present in the various concentrations of CS_2 was low, with minimum numbers being present at the highest concentration of CS_2 (Table 3).

Table 3: Effect of carbon disulphide and formalin on percentage mycorrhizal root colonization of sunflower at different time intervals

Conc. (ml/2 kg)	10th day		20th day		40th day		70th day	
	CS_2	Formalin	CS_2	Formalin	CS_2	Formalin	CS_2	Formalin
Control	84.61^{a}±0.47	84.16^{a}±0.47	86.36^{a}±0.4	86.36^{a}±0.4	100^{a}±0	100^{a}±0	100^{a}±0	100^{a}±0
2	83.33^{a}±0.95	100^{b}±0	85^{a}±2.3	100^{b}±0	100^{a}±0	100^{a}±0	100^{a}±0	100^{a}±0
4	37.50^{b}±1.65	91.66^{c}±0.4	50^{b}±0.9	94^{c}±0.4	75^{b}±2.3	100^{a}±0	100^{a}±0	100^{a}±0
6	37.50^{b}±0.47	91.66^{c}±0.9	50^{b}±2.3	94^{c}±0.5	75^{b}±0.4	100^{a}±0	100^{a}±0	100^{a}±0
11	36.30^{b}±0.94	73.68^{d}±1.8	45^{b}±0.9	78^{d}±0.4	69^{b}±0.9	88^{b}±0.9	100^{a}±0	100^{a}±0
LSD (P=0.05)	1.93	1.93	5.38	5.38	11.1	9.8	2.71	5.90

Means in each column that have different letters differ significantly at the 0.05 level of probability.

In the case of formalin (Table 3), the percentage of mycorrhizal root colonization increased initially at 2 ml concentration and then it showed a decreasing trend. After 40 days, the mycorrhizal root colonization reached 100% in all formalin concentrations but the highest one (11 ml).

In the present investigation, the fumigants, *i.e.*, carbon disulphide and formalin, had an inhibitory effect on soil microorganisms up to the 10th day after treatment. After 10 days both the fumigants were less inhibitory in comparison to control but *Trichoderma viride* population increased with formalin treated soil after 10 days. As mentioned earlier the *Trichoderma* population increased after 10 days with formalin treatment, Wainwright (1977) suggested that dominance of this fungus might be due to their ability to utilize NH_4^+-N, which is amply available in fumigated soil, or to resistance of mycostasis caused by NH_3. Martin *et al.* (1963) reported that there is marked reduction of fungal population after fumigation with carbon disulphide, chloropicrin and ethylene dibromide.

In the case of formalin treatment, lower concentrations were stimulatory but higher concentrations were inhibitory to VAM fungi. According to Bird *et al.* (1974), O'Bannon and Nemec (1978), most of the fumigants except ethylene dibromide were toxic to VAM fungi. Hayman (1970) indicated that formalin applied in previous year decreased the number of *Endogone* sp. in subsequent year. Fumigation of soil with

broad-spectrum biocides such as methyl bromide, formalin and CS_2 can greatly reduce vesicular-arbuscular mycorrhizal formation (An *et al.,* 1993; Trappe *et al.,* 1984). Hayman (1970) reported of mycorrhizal fungi surviving high concentrations of fumigation, which indicated that that the tested fungal populations were resistant to fumigation.

In the present investigation, CS_2 showed different effect on VAM species at different concentrations. *Glomus mosseae* appeared to be resistant to all concentrations up to the 70^{th} day. Similarly, the effect of formalin on VAM species showed that *Glomus intraradices*, *Glomus reticulatum* and *Glomus fasciculatum* were resistant to most of the concentrations of both fumigants. Spokes and McDonald (1978) showed that fumigants did not affect mycorrhizal root colonization by *Glomus fasciculatum* but these fumigants reduced the colonization by *Glomus mosseae* to 2/3 of the control. Soil fumigation may kill beneficial microorganisms such as bacteria and actinomycetes that can improve spore number and root colonization of VAM fungi.

Regarding the effect of both fumigants on sunflower growth and P content, it was clear that the height of plants decreased in comparison with the control. However, the P content in plants increased but the content in flowers decreased in comparison with the control. There had been consistent reports of plant stunting following fumigation (Lambert *et al.,* 1979; Vyas and Singh, 1992). After prolonged research, the problem of stunting following fumigation was explained by Filler and Tayler (1968), who demonstrated that mycorrhizal inoculation increased the growth of tree seedlings in the soil fumigated with methyl bromide. Afek *et al.* (1990) reported that fresh weight and yield were one to two times greater in non-fumigated than in fumigated soil in the cases of cotton, onion and pepper. Fumigants with relatively high vapor pressure potential are able to move over greater distances in the soils than the compounds with low volatility (Laiho and Mialo, 1965). Finally, soil fumigants may not persist for a long period but delay in reintroduction of mycorrhizal fungi may produce disastrous stunting of host plants over several years. It may be concluded from this investigation that microorganisms including mycorrhizae are vital components of most agronomic systems. The effects of fumigants on non-target microorganisms including mycorrhizal fungi must be assessed carefully and fumigants should be intelligently used in agricultural systems.

CONCLUSIONS

High concentration of the two investigated fumigants initially decreased the mycorrhizal spore number but later on the number was increased in all concentrations of the fumigants. Mycorrhizal root colonization reached 100% on the 70^{th} day both in the control and in the fumigated soil. It was found that the fumigants inhibited the growth of all test fungi. Growth of sunflower plants increased concurrently with the increase in the concentration of fumigants. Phosphorous content of the treated plants showed a decrease followed by an increase at the higher fumigant concentrations. Thus, the high dosages of both fumigants caused adverse effects on soil microflora, mycorrhizal fungi and plant growth. Therefore, the use of both

fumigants at high dosages as well as their continuous application should be conducted in a judicious manner.

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MICROFLORA DE LA RIZÓSFERA DE GIRASOL EN RELACIÓN A LA FUMIGACIÓN DEL SUELO

RESUMEN

El objetivo de la presente investigación fue analizar el rol de los fumigantes disulfuro de carbono (CS_2) y formalin a diferentes concentraciones sobre la microflora del suelo incluyendo las micorrizas de la rizósfera del girasol. La población de hongos se redujo fuertemente inmediatamente después de la aplicación del fumigante pero con el paso del tiempo ésta comenzó a reaparecer. En análisis cuantitativos de microflora *Aspergillus niger*, *Aspergillus terreus* y *Penicillium nigricans* hicieron su aparición sólo después de veinte días de la aplicación de CS_2 a todas las concentraciones. Estudios cuantitativos mostraron el incremento de la población de *Trichoderma viride* con el aumento de la concentración de Formalin y CS_2 después de los veinte días. Inicialmente ambos fumigantes disminuyeron el número de esporas de micorrizas a las concentraciones más altas. Sin embargo, el número de esporas de micorrizas se incrementó más adelante. La colonización de raíces con micorrizas fue máxima después de cuarenta días en suelos tratados. *Glomus mosseae* fue resistente a la aplicación de CS_2 pero *Glomus geosporum* y *Acaulospora laevis* se inhibieron a altas concentraciones.

LA MYCOFLORE DE LA RHIZOSPHÈRE DU TOURNESOL EN RAPPORT AVEC LA FUMIGATION DU SOL

RÉSUMÉ

Cette recherche avait pour but d'analyser le rôle des fumigants - disulfure de carbone (CS_2) et formaline - à différentes concentrations sur la mycoflore du sol, y compris les champignons mycorrhiziens, de la rhizosphère du tournesol. La présence des champignons a été considérablement réduite immédiatement après l'application du fumigant mais avec le temps, ils ont commencé à réapparaître. Dans les analyses qualitatives de la mycoflore, *Aspergillus niger*, *Aspergillus terreus*, *Penicillium nigricans* sont réapparus seulement 20 jours après l'application du CS2, quelque soit la concentration. L'étude quantitative a montré une augmentation de la population de *Trichoderma viride* avec l'augmentation des concentrations de Formaline et de CS2, 20 jours après le traitement. Les deux fumigants ont fait décroître initialement le nombre de spores mycorrhiziennes aux concentrations les plus fortes. Toutefois, le nombre de spores mycorrhiziennes a été en augmentation plus tard. La colonisation mycorrhizienne des racines est devenue maximale dans les sols traités 40 jours après le traitement. *Glomus mosseae* s'est montré resistant à l'application de CS_2 application mais *Glomus geosporum*, *Acaulospora laevis* ont été inhibés aux concentrations les plus fortes.