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Effects of pesticides on mycorrhizal symbiosis in tea

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ABSTRACT: The experiment was conducted at BTRI farm to evaluate the effect of pesticides on mycorrhizal symbiosis in tea. Different pesticides were used along with mycorrhizal fungi. In the results, variable effects of pesticides on the symbiosis were observed. The systemic fungicide Calixin @ 1.12 L ha^{-1} (T8) significantly (P=0.01) suppressed the mycorrhizal activities in tea. 33% mycorrhizal colonization in roots and 54% spore populations in rhizosphere soil were reduced by application of Calixin. From the results, it can also be seen that Calixin significantly (P=0.01) decreased the total plant height (2.9%), fresh weight (4.6%), dry mass (16.4%) and number of leaves (4.8%) of tea saplings, compared to without Calixin application (T_2). The adverse effect on AM fungal activities was also recorded by using systemic herbicide Roundup (T_{14}). No harmful effects were observed in the treatment of fungicides Knowin and Macuprax; herbicides Gramoxone and Bimastar on the symbiosis in tea. The application of Knowin, Macuprax, Gramoxone and Bimastar along with AM fungi significantly (P=0.01) increased plant height, fresh and dry mass, number of leaves, percentage root colonization of mycorrhizal fungi and spore population in rhizosphere soil. The insecticides Fentox, Thiodan and Carbofuran had also no harmful effect on mycorrhizal symbiosis in tea.

KEYWORDS: *Mycorrhiza; Symbiosis; Pesticides; Rhizosphere soil; Colonization*

Introduction

Application of pesticides for controlling pests and diseases is a common practice in crop protection over the world. Knowledge of the possible environmental hazards posed by use of such biocides is much more available on cultivation of general crops compared to tea culture. Reports are available on the effects of pesticides on non-target soil organisms[1–3] and reports addressing the environmental effects of herbicides,[4,5] insecticides,[3] fungicides,[6] on soil organisms.[7] The systemic fungicides are more toxic to the AM fungi than most other compounds like contact fungicides.[8]

The fungicides are used to control a broad spectrum of plant diseases; hence they have the potential to cause critical changes in soil microbial populations including mycorrhizal fungi. Tu[7] and Zelles *et al.*[9] have described an initial flush of activity of non-target organisms, following fungicides applications, leading to increased overall microbial activity. Published reports on the effect of a specific pesticide on mycorrhizal fungi are sometimes contradictory. For example, propiconazole markedly reduced P uptake by a mycorrhizal root system as observed by Hetrick *et al.*,[10] while Kling and Jakobsen[11] found no negative effect of propiconazole on hyphal P uptake.

Arbuscular mycorrhizal (AM) fungi are affected by a number of pesticides, especially fungicides.[8,12] Consequently, the application of fungicides decreased positive effect of the AM fungi[13] but increased plant growth following root colonization by AM fungi is mainly due to an improvement in the phosphorus supply to the plant.[14] External hyphae of AM fungi take up and transport P to the host plant.[15] This P uptake into the host plant *via* external hyphae of AM fungi was shown to be hampered by some fungicides.[10,11,16,17]

All of these studies have been done with agricultural crops, but no work has received on adverse effect of pesticides on mycorrhizal symbiosis in tea ecosystems like P deficient acidic soils. Therefore, the objective of the present study was to evaluate the effects of some commonly used tea pesticides (fungicides, herbicides and insecticides) on arbuscular mycorrhizal fungi symbiosis.

Materials and Methods

Preparation of Tea Cuttings and Primary Bed

The tea cuttings were collected from BT5 mother bushes of BTRI farm. Each cutting consists of one full leaf with a stalk of ~2.5–3.0 cm in length. Hard red wood of the shoot is the indicative to the best cutting. The portion of the hard green wood of the shoot is first cut obliquely

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over the axillary bud, followed by a second cut parallel to the first cut by means of a sharp blade. The cuttings were kept moistened in a water bowl till planting out in the nursery bed, measuring 1.5 m × 10 m. Cuttings were planted at 6–7 cm spacing in triangular from. Leaves were not allowed to touch the soil surface. Low shades were provided on the bed with bamboo-split lathe-frame of 1.5 × 1.2 m with about 23-cm height. Cultural operations like weeding, forking and watering were done as and when necessary.

Preparation of Secondary Bed

The secondary bed measured 1.5 m × 10 m with slanting towards east at an inclination of 15 cm. The drain around the bed was 30 cm × 15 cm. A sand layer of 2 cm was spread on the bed to absorb excess water during watering. High shades were provided of 1.2 m on the beds with bamboo tarja. The soil (potting media) was collected from Experimental Farm of BTRI. The soil was light textured. The chemical analysis of the soil was done at Soil Science Laboratory, BTRI.

Prepeartion of Inuculum

The indigenous mycorrhizal inoculum was obtained by collecting the propagules by wet sieving and decanting[18] from the soils of tea plantations. The crude inoculum (spores, hyphae and root bits) was multiplied and maintained in a small plot (1 m × 2 m) with sterilized soil. *Mimosa invisa* L. and *Calapogonium mucunoides* Desv. were grown conjointly for 16 weeks, after which the tops of the plants were removed. The roots were finely chopped and the dried root–soil mixture was mixed thoroughly to obtain a homogenous inuculum. Before inoculation, percentage root colonization and number of spores with the plants was assessed.

Preparation of Poly Bags

The soil was sieved with four mesh sieve and mixed thoroughly with decomposed cow dung in the ratio of 7:1 (Soil : Cow dung) and was sterilized properly. Poly bags (transparent) for the experiment was 23 cm in length and 9.6 cm in diameter (lay flat 15 cm and poly bag area was 7.235×10^{-3} m^2) and 0.04 mm thickness. The bag was filled uniformly with 1.6 kg soil per bag, watered regularly and kept under shade for 15 days for settling before putting the cuttings. Crude inoculum was placed 2 cm below the soil surface as a uniform thin layer containing about thousand infectious propagules per 100 g of the indigenous mycorrhizal fungi, comprising spores Gigaspora, Scutellospora and Glomus species, sporocarps, bits of hyphae and infected root segments per bag. Thereafter, pesticides (three fungicides *viz.* Knowin 50 WP, Macuprax 16 W/W and Calixin 75 EC; three herbicides like Gramoxone 20%, Bimastar 240/120 AS and Roundup 41% and three insecticides *viz.* Fentox 20 EC, Thiodan 35 EC and Carbofuran 3G) with their recommended doses (Knowin @ 0.75 kg, Macuprax @ 2.24 kg, Calixin @ 1.12 L, Gramoxone @ 2.24 L, Bimastar @ 3.50 L, Roundup @ 3.70 L, Fentox @ 1.25 L, Thiodan @ 1.5 L ha^{-1} and Carbofuran @ 0.275 kg m^{-3}) were applied. One tea sapling was transplanted per poly bag, which raised in primary bed. The experiment was conducted accompanied by 22 treatments with 3 replicates using randomized block design at BTRI farm. Plants were watered with normal pond water regularly and maintained for 1 year after transplantation. Collected data were analysed by means of a comparison test using DMRT.

Data were recorded every 6-month interval. The parameters of data included shoot length, fresh weight, dry weight and number of leaves of the plants. Percentage of mycorrhizal association in tea roots and population of mycorrhizal spores in rhizosphere soil were also recorded. The mycorrhizal colonization in roots was determined by staining the roots according to Phillips and Hayman[19] and mycorrhizal spores were determined according to the wet sieving and decanting technique.[18] Treatments preformed are described in Box 1.

Results and Discussion

The results of the present study are presented in Tables 1–3. Since the effect of pesticides on arbuscular mycorrhizal (AM) fungi obtained after 6 months was almost similar to that achieved after 1 year, the results obtained after 1 year are discussed. Result revealed that Calixin (tridemorph) @ 1.12 L ha^{-1} (T_8) significantly suppressed the mycorrhizal activities. This application resulted in a 33% reduction in mycorrhizal root colonization and 54% spore population in rhizosphere soil. By reducing AM fungal symbiosis, Calixin applications resulted in significant (P=0.01) decreases in total plant height, fresh weight, dry mass and number of leaves (2.9%, 4.6%, 16.4% and 4.8% reductions, respectively, compared to without Calixin T_2) as was found by Von Alten *et al.*[8] They observed that systemic fungicides affected AM symbiosis. Similar results were also found by Dodd and Jeffries,[13] who described the application of fungicides decreased positive effect of the AM fungi. Calixin, a systemic fungicide inhibits sterol biosynthesis in plant and AM fungi and thus results in stunting growth. On

Box 1: Treatments Performed

Treatments: 22

T_1 = Control (sterile soil)
T_2 = AM inoculum

Fungicides
T_3 = Knowin (Carbendazim)
T_4 = AM + Knowin
T_5 = Macuprax (B. mixture + Cupraneb)
T_6 = AM + Macuprax
T_7 = Calixin (Tridemorph)
T_8 = AM + Calixin

Herbicides
T_9 = Gramoxone (Paraquat)
T_{10} = AM + Gramoxone
T_{11} = Bimastar (Glyphosate + 2,4-D)
T_{12} = AM + Bimastar
T_{13} = Roundup (Glyphosate)
T_{14} = AM + Roundup

Insecticides
T_{15} = Thiodan (Endosulfan)
T_{16} = AM + Thiodan
T_{17} = Carbofuran
T_{18} = AM + Carbofuran
T_{19} = Fentox (Fenvalerate)
T_{20} = AM + Fentox

Cocktail (fungicide+herbicide+insecticide)
T_{21} = Knowin + Roundup + Carbofuran
T_{22} = AM + Knowin + Roundup + Carbofuran

the other hand, *fungicides* – Knowin (carbendazim) and Macuprax (bordeaux mixture + cupraneb), *herbicides* – Gramoxone (paraquat) and Bimastar (combination of glyphosate and 2,4-D) and *insecticides* (Thiodan, Fentox and Carbofuran) did not affect on AM fungal symbiosis for the growth and development of tea plants. Nonetheless, in the presence of AM fungi these pesticides produced a significant (P=0.01) growth which was statistically similar to the treatment of only AM inoculated (T_2). About 3–6% plant growth was found to be increased by these pesticides with the presence of AM fungi, which supported to the results obtained by Smith and Read.[14] Similarly, the pesticides influenced significantly on mycorrhizal colonization and spore population (Table 3). The plant growth increased following root colonization by AM fungi is mainly due to an improvement in the phosphorus supply to the plant.

The Herbicide Roundup (T_{14}) was found to be more toxic compared to the other herbicides on AM symbiosis in tea (Fig. 1). Among the tested pesticides (fungicides, herbicides, insecticides), insecticides were least toxic to mycorrhizal symbiosis (Fig. 2). The cocktail application of pesticides (T_{22}) also decreased the AM activities such as 1.6%, 9.7%, 14.8% and 6.3% plant height, fresh weight, dry weight and number of leaves were decreased, respectively (Table 2).

The percent root colonization and spore population in the rhizosphere soils of the different treatments is pre-

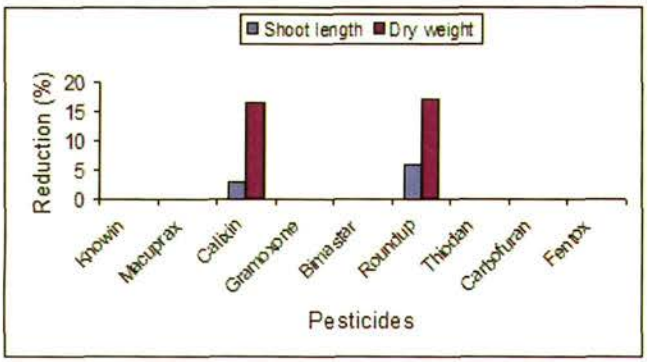

Figure 1: Percentage of Shoot length and dry weight reduction due to individual pesticides applications on AM symbiosis in tea nursery.

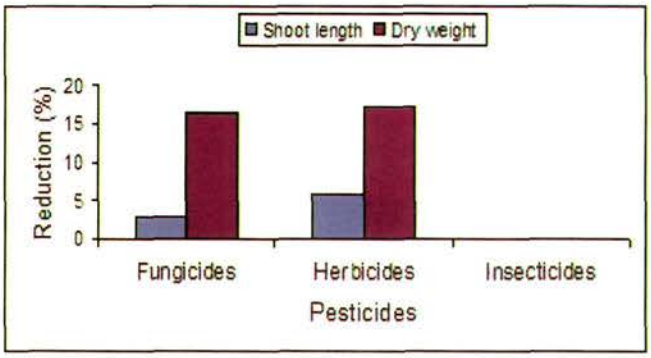

Figure 2: Comparison to the group of pesticides on AM symbiosis for percentage of shoot length and dry weight reduction in tea nursery.

Table 1: Effects of pesticides on AM symbiosis in the growth and development of tea plants under nursery conditions after 6-month (average of 3 replications)

Treatments	Shoot length (cm/plant)	Fresh weight (g/plant)	Dry weight (g/plant)	No. of leaves/ plant
T_1	35.80 b	9.42 ef	2.60 ef	25.25 b
T_2	37.85 a	10.07 c	3.30 c	27.67 a
Fungicides				
T_3	36.10 b	9.47 e	2.61 ef	25.33 b
T_4	38.00 a	10.15 bc	3.45 b	27.70 a
T_5	36.00 b	9.41 ef	2.62 ef	25.10 b
T_6	37.88 a	10.17 bc	3.27 c	27.50 a
T_7	35.25 bc	9.43 ef	2.54 efgh	25.27 b
T_8	35.75 b	9.45 ef	2.50 fgh	25.67 b
Herbicides				
T_9	36.00 b	9.43 ef	2.58 efg	25.57 b
T_{10}	38.10 a	10.25 b	3.37 bc	27.33 a
T_{11}	35.25 bc	9.43 ef	2.56 efgh	25.23 b
T_{12}	38.25 a	10.23 b	3.15 d	27.43 a
T_{13}	34.00 c	9.40 ef	2.46 gh	25.30 b
T_{14}	35.00 bc	9.50 e	2.45 gh	25.40 b
Insecticides				
T_{15}	35.65 b	9.47 e	2.50 fgh	25.33 b
T_{16}	37.45 a	10.24 b	3.31 c	27.50 a
T_{17}	35.15 bc	9.47 e	2.62 ef	25.30 b
T_{18}	37.65 a	10.67 a	3.80 a	27.60 a
T_{19}	35.45 b	9.45 e	2.65 e	25.60 b
T_{20}	37.55 a	10.13 bc	3.72 a	27.67 a
Cocktail of pesticides				
T_{21}	35.35 b	9.43 ef	2.53 efgh	25.40 b
T_{22}	36.45 b	9.66 d	2.59 efg	25.45 b
$P = 0.1$	(0.438)	(0.054)	(0.044)	(0.408)

Note: Means followed by same letters are not significantly different from each other at 1% level. Values in parentheses are *SE* values.

Table 2: Effects of pesticides on AM symbiosis in the growth and development of tea plants under nursery conditions after 1 year (average of 3 replications)

Treatments	Shoot length (cm/plant)	Fresh weight (g/plant)	Dry weight (g/plant)	No. of leaves/ plant
T_1	54.67 fg	14.66 f	3.70 bcde	35.33 b
T_2	56.63 cd	15.68 d	4.40 a	37.80 a
Fungicides				
T_3	55.00 f	14.73 f	3.72 bcd	35.40 b
T_4	58.33 ab	15.09 e	4.55 a	37.67 a
T_5	54.67 fg	14.66 f	3.74 bc	35.67 b
T_6	57.00 c	16.78 a	4.48 a	37.65 a
T_7	53.55 gh	14.67 f	3.62 cde	35.45 b
T_8	55.00 f	14.96 ef	3.68 bcde	36.00 b
Herbicides				
T_9	55.33 ef	14.66 f	3.60 def	35.40 b
T_{10}	58.67 a	15.44 d	4.48 a	37.73 a
T_{11}	53.33 gh	14.65 f	3.58 ef	35.20 b
T_{12}	58.67 a	15.54 d	4.45 a	37.67 a
T_{13}	52.67 h	14.67 f	3.70 bcde	35.30 b
T_{14}	53.33 gh	14.89 ef	3.65 bcde	35.40 b
Insecticides				
T_{15}	53.00 h	14.88 ef	3.75 b	35.33 b
T_{16}	56.33 cde	16.41 b	4.53 a	37.70 a
T_{17}	52.67 h	14.66 e	3.65 bcde	35.30 b
T_{18}	56.53 cd	16.07 c	4.52 a	37.60 a
T_{19}	53.50 gh	14.70 f	3.70 bcde	35.37 b
T_{20}	57.33 bc	16.29 bc	4.50 a	37.65 a
Cocktail of pesticides				
T_{21}	52.67 h	14.72 f	3.50 f	35.47 b
T_{22}	55.67 def	14.16 g	3.75 b	35.40 b
$P = 0.1$	(0.408)	(0.115)	(0.040)	(0.548)

Note: Means followed by same letters are not significantly different from each other at 1% level. Values in parentheses are *SE* values.

sented in Table 3. The root colonization and spore populations were found to be higher than that of un-inoculated treatments. The range colonization and spore populations were observed from 40% to 50% and from 75% to 110%, respectively, at end the experiment. AM status was not found in all un-inoculated treatments. The standard deviation (±) of colonization and spore populations were observed ±5 and from 5 to 10, respectively. The AM structural colonization like arbuscules and vesicles was observed to be poor, though 10% arbuscles were present, but vesicles were absent (Table 3). The lower colonization showed lower intensity of arbuscules as was supported by Saif,[20] where the author found that the plants with low mycorrhizal colonization and the abuscules were either absent or low in number. Arbuscules are the most effective and functional unit of the AM fungal symbiosis and higher abundance of arbuscules is the evidence of effective AM association.[21] The viable AM spore and colonization of the root was enhanced sig-

Table 3: Effect of pesticides on AM structural colonization (%) and spore population of young tea plants inoculated with AM fungi under nursery condition

Treatments	After 6 Months					After 1 Year				
	Mycorrhizal Status		AM Structure (%)			Mycorrhizal Status		AM Structure (%)		
	Root Infection (%)	No. of Spores/ 100 g soil	M	A	V	Root Infection (%)	No. of Spores/ 100 g soil	M	A	V
T_1	00	00	00	00	00	00	00	00	00	00
T_2	45 ± 5	110 ± 5	45	10	00	50 ± 5	115 ± 5	50	10	00
T_3	00	00	00	00	00	00	00	00	00	00
T_4	40 ± 5	95 ± 5	40	10	00	45 ± 5	110 ± 5	45	10	00
T_5	00	00	00	00	00	00	00	00	00	00
T_6	40 ± 5	80 ± 5	40	10	00	45 ± 5	105 ± 5	45	10	00
T_7	00	00	00	00	00	00	00	00	00	00
T_8	30 ± 5	50 ± 10	30	00	00	40 ± 5	75 ± 5	40	10	00
T_9	00	00	00	00	00	00	00	00	00	00
T_{10}	40 ± 5	95 ± 5	40	10	00	50 ± 5	110 ± 5	50	10	00
T_{11}	00	00	00	00	00	00	00	00	00	00
T_{12}	40 ± 5	105 ± 5	40	10	00	45 ± 5	115 ± 5	45	10	00
T_{13}	00	00	00	00	00	00	00	00	00	00
T_{14}	35 ± 5	75 ± 5	35	00	00	40 ± 5	110 ± 5	40	10	00
T_{15}	00	00	00	00	00	00	00	00	00	00
T_{16}	35 ± 5	70 ± 5	35	00	00	45 ± 5	90 ± 5	45	00	00
T_{17}	00	00	00	00	00	00	00	00	00	00
T_{18}	40 ± 5	75 ± 5	40	10	00	45 ± 5	90 ± 5	45	10	00
T_{19}	00	00	00	00	00	00	00	00	00	00
T_{20}	45 ± 5	105 ± 5	45	10	00	50 ± 5	110 ± 5	50	10	00
T_{21}	00	00	00	00	00	00	00	00	00	00
T_{22}	40 ± 5	80 ± 5	40	10	00	50 ± 5	95 ± 5	50	10	00
$P = 0.1$	00	00	00	00	00	00	00	00	00	00

Note: Mean ± SD of 3 samples. M= Mycelia, A= Arbuscule, V= Vesicle.

nificantly by inoculation compared to the un-inoculated control. This finding has supported by Olsen and Habte[22] who reported that the AM status was significantly higher when the plants were inoculated with AM fungi.

The application of insecticides and fungicides to the field crops to control insects and diseases is a common practice. Soil accumulation of these chemicals could have detrimental effects on plant growth by limiting the function of the AM fungi symbiosis.[23] High doses of Knowin (carbendazim) and Bimastar (glyphosate + 2,4-D) had the most negative effect on AM fungal activities in the present study. These results are similar to the findings of Dodd and Jeffries,[13] Larsen *et al.*,[16] and Merryweather and Fitter.[17] They described that Carbendazim, a benzimidazole fungicide, is a breakdown product of benomyl and at high doses of both chemicals were found to be severely inhibited the formation and function of AM fungi.

Similarly, 2.4-D is also a plant growth regulator, but at high concentration it may be used as plant killer. Being a systemic fungicide, Calixin has been used for controlling various plant diseases at the rate of 1.12 L ha^{-1} in tea plantations. Higher concentrations of the fungicide prevented the germination of AM fungal spores and inhibited the microbial activities into soil fungi.[13] For these reasons, any systemic chemicals should be used carefully and judiciously.

This study concludes that among the tested pesti-

cides, systemic fungicide-Calixin (Tridemorph) and herbicide-Roudup (glyphosate) are found to be more toxic than others to AM symbiosis in tea. Hence, its use should be restricted for saving AM activities in tea. At present, AM fungi are indispensable as bio-fertilizer and bio-controlling agents for organic and sustainable farming system. So that according to the toxicity, systemic fungicides should be used judiciously to save the beneficial organisms like AM fungi in tea.

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