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The in vitro effect of certain fungicides, insecticides, and biopesticides on mycelial growth in the biocontrol fungus *Trichoderma harzianum*

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Abstract: The in vitro effect of certain fungicides, insecticides, and biopesticides on mycelial growth of *Trichoderma harzianum* was evaluated. Seven systemic fungicides, 2 contact fungicides, and 4 biopesticides were tested. A progressive increase in percent inhibition of radial growth in the fungus was observed as the concentrations of the fungicides increased. Among the systemic fungicides, hexaconazole was the most toxic, followed by propiconazole and triflumizole. Toxicity of the contact fungicides was lower than that of the systemic fungicides, among which copper oxychloride and copper hydroxide were highly compatible; no inhibition was observed at lower concentrations. In terms of the insecticides tested, quinalphos and dicofol exhibited toxicity (quinalphos more so), even at the low concentration of 10 ppm. Endosulfan, fenpropathrin, and propargite were less toxic, which indicated the compatibility of these insecticides with *T. harzianum*. A varying level of inhibition was observed in response to the biopesticides. Although neem-based biopesticides completely inhibited the growth of the biocontrol strain, ovis was compatible with the growth of *Trichoderma harzianum*. The present results will help delineate the possibility of combining *T. harzianum* biocontrol agents and agrochemicals for use in an integrated pest management approach.

Key words: Biological control, *Trichoderma harzianum*, fungicides, insecticides, biopesticides

Trichoderma harzianum biyokontrol mantarının misel gelişimi üzerine belli fungusitlerin, insektisitlerin ve biyopestisitlerin in vitro etkisi

Özet: Denemeler, in vitro ortamda, *Trichoderma harzianum*'un misel gelişimi üzerine belli fungusitlerin, insektisitlerin ve biyopestisitlerin etkisini değerlendirmek için yapılmıştır. Yedi sistemik fungusit, iki kontakt fungusit ve dört biyopestisit test edilmiştir. Fungusit konsantrasyonunda artışla birlikte, mantarın radyal gelişimi üzerine yüzde inhibisyonda kademeli bir artış gözlenmiştir. Sistemik fungusitler arasında, hexaconzole'ün en toksik madde olduğu bulunmuş, bunu propiconazole ve triflumizole izlemiştir. Kontakt fungusitlerin toksitesi karşılaştırmalı olarak bakır oksiklorit ve bakır hidroksit gibi son derece uyumlu olan ve düşük dozda inhibisyon göstermeyen sistemik fungusitlerden daha düşüktür. İnsektisit testinde, 10ppm'in daha düşük konsantrasyonunda bile quinolphos, toksisite göstermiş bunu dicofol izlemiştir. Bu insektisitlerle uyumluluk gösteren endosülfan, fenpropathrin ve propargite, daha az seviyede toksisite göstermiştir. Biyopestisitlere dayanan neem, biyokontrol suşların gelişimini tamamiyle inhibe etmesine rağmen, ovisin *Trichoderma harzianum*'ın gelişimine uyumlu olacağı bulunmuştur. Mevcut sonuçlar, zararlılarla entegre bir mücadelenin yönetim yaklaşımı için, *T. harzianum*'un biyokontrol ajanları ve agrokimyasallarının birleştirilme olanaklarını tanımaya yardım edecektir.

Anahtar sözcükler: Biyolojik kontrol, *Trichoderma harzianum*, fungusitler, insektisitler, biyopestisitler

Introduction

Biological control involves the use of beneficial microorganisms to attack and control plant pathogens, and the diseases they cause. It is an environmentally acceptable approach to disease management. Among the fungal biocontrol agents, *Trichoderma* spp. have acquired much importance (1-3). *Trichoderma* spp. are not only among the most commonly isolated soil fungi, but are also potential biocontrol agents, especially against pathogenic fungi (4) such as *Macrophomina phaseolina* (5), *Rhizoctonia solani*, and *Pythium* species (6). On tea plantations *Trichoderma* spp. are used as biocontrol agents against primary and secondary root diseases (7,8), collar canker caused by *Phomopsis theae* (9), and some stem diseases.

Although use of biocontrol agents could reduce chemical application to a limited extent, it is less reliable and less efficient (10). Integrated pest management (IPM) is an approach involving the use of biological, physical, and chemical measures to manage pest and pathogen populations in a cost-effective ecological way. Within these complex plant protection strategies, one may need to combine biocontrol agents with chemicals to achieve the target (11). The combined use of biocontrol agents and chemical pesticides has attracted much attention as a way to obtain synergistic or additive effects in the control of soil-borne pathogens (12). The effect of certain fungicides and herbicides on *Trichoderma* spp. was reported earlier with an emphasis on practical applications (11).

The present study aimed to evaluate the in vitro effects of certain fungicides, insecticides, and biopesticides commonly used on tea plantations and to determine their influence on *Trichoderma harzianum*.

Materials and methods

A strain of *T. harzianum* obtained from the Center of Advanced Studies (CAS) in Botany, Guindy Campus, University of Madras, was used in the present study. This *T. harzianum* strain was isolated from tea rhizosphere (UPASI TRF farm) and maintained on potato dextrose agar (PDA) slants. In all, 7 systemic fungicides (tebuconazole [(Folicur 250 EC], tridemorph [Calixin 80 EC], propiconazole [Tilt 25 EC], hexaconazole [Contaf 5 EC], triflumizole [Procure 30 WP], bitertanol [Baycor 25 WP, and azoxystrobin [Amistar 25 SC]) and 2 contact fungicides (copper oxychloride [Fytolan 50 WP] and copper hydroxide [Excel 46.1% DF]) were tested. In addition, 5 insecticides (propargite [Omite 57 EC], endosulfan [Endocel 35 EC], fenpropathrin [Meothrin 30 EC], dicofol [Ditol 18.5 EC], and quinalphos [Ekalux 20 EC]) were evaluated. Among the biopesticides, 3 neem-based formulations viz. nimbecidine (0.03%), ponneem (0.05%), neem kernel aqueous extract (NKAE), and a formulation containing a mixture of plant extracts (Ovis) was tested at 2.5%, 5%, and 7.5% concentrations. The in vitro bioefficacy of the test compounds was determined using the poisoned food technique (13).

Stock solutions (1000 ppm) of agrochemicals were prepared by dissolving the required quantities of each into sterile distilled water. Appropriate quantities of the respective solutions were added to molten PDA medium (50 mL) from stock solution so as to obtain the required concentrations and were mixed thoroughly by gentle shaking. About 15 mL of sterilized medium was poured into 90-mm sterilized petri plates. After solidification, the plates were inoculated with 5-mm discs of 4-day-old *T. harzianum* culture. Four replicates were used for each concentration of every compound tested. *T. harzianum* in PDA plates without any added compounds served as controls. The inoculated plates were incubated at 25 ± 1 °C and radial colony diameter data were recorded 5 days after inoculation.

Inhibition of radial growth was measured based on control plate colony diameter using Sundar et al.'s formula (14):

$$\text{Percent Inhibition} = ((X - Y)/X) \times 100$$

where X is growth of the control plate and Y is growth of the treated plate.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and significant means were segregated by critical difference (CD) at various levels of significance.

Results and discussion

The effects of the fungicides on the growth of *T. harzianum* are presented in Table 1. Among the systemic fungicides tested, hexaconazole was the most toxic to the growth of *T. harzianum*, followed by propiconazole and triflumizole at the lowest concentration (5 ppm) tested. At the 5-ppm concentration growth inhibition caused by hexaconazole-, propiconazole-, and triflumizole-amended medium was 87.7%, 56.4%, and 36.2%, respectively. No growth was observed at the 10-ppm level with hexaconazole, the 25-ppm level with propiconazole, or the 50-ppm level with triflumizole. The antifungal activity of hexaconazole and propiconazole (both of the triazole group of fungicides) have been reported to be the result of their ability to inhibit ergosterol biosynthesis in fungi (15). Mizuno (16) reported the antifungal activity of triflumizole, which acts as a selective inhibitor of demethylation during ergosterol biosynthesis. A progressive increase in percent inhibition of radial growth in *T. harzianum* was observed as the concentration of all the fungicides increased. Although most of the systemic fungicides were able to completely suppress the growth of *T. harzianum* at the highest concentration (300 ppm) used in the present study, azoxystrobin and bitertanol were more compatible with the biocontrol strain. At the highest concentration of azoxystrobin and bitertanol, inhibition of the fungus was 41.1% and 71.3%, respectively. In the case of the contact fungicides, both copper oxychloride and copper hydroxide showed compatibility with *T. harzianum*. No inhibition of mycelial growth was noted at the 5- and 10-ppm levels of both chemicals. A gradual increase was observed in percent inhibition as the concentration increased, but inhibition was lower than that of the systemic fungicides. At the highest concentration inhibition was 60% and 33.1%, respectively, for the 2 chemicals. This can be explained in terms of the variation in sensitivity of the test fungus to the fungicides (17). Earlier reports suggest that biocontrol agents that can tolerate a certain level of fungicides were mixed with agrochemicals, resulting in eradication of diseases (18).

In terms of the insecticides tested in the present study, quinalphos exhibited the highest toxicity, followed by dicofol at the lowest concentration; at the

Table 1. Effect of fungicides on the mycelial growth of *Trichoderma harzianum*.

Treatment	Concentration (ppm)						
	5	10	25	50	100	200	300
Tebuconazole	29.0(35.5) [a]	24.5(45.5)	16.8(62.8)	15.5(65.5)	10.2(77.3)	0.0(100)	0.0(100)
Tridemorph	31.0(31.1)	27.3(39.3)	21.2(52.8)	17.7(60.6)	12.7(71.7)	9.0(80.0)	0.0(100)
Bitertanol	43.0(4.4)	39.0(13.3)	36.2(19.5)	19.7(56.2)	18.9(58)	17.6(60.8)	12.9(71.3)
Hexaconazole	5.5(87.7)	0.0(100)	0.0(100)	0.0(100)	0.0(100)	0.0(100)	0.0(100)
Propiconazole	19.6(56.4)	8.1(82)	0.0(100)	0.0(100)	0.0(100)	0.0(100)	0.0(100)
Azoxystrobin	40.0(11.1)	35.5(21.1)	33.7(25.1)	31.0(31.1)	30.4(32.4)	29.0(35.5)	26.5(41.1)
Triflumizole	28.7(36.2)	19.7(56.2)	11.2(75.1)	0.0(100)	0.0(100)	0.0(100)	0.0(100)
Copper oxychloride	45.0(0.0)	45.0(0.0)	43.3(3.77)	41.2(8.4)	35.0(22.2)	22.5(50)	18.0(60)
Copper hydroxide	45.0(0.0)	45.0(0.0)	44.0(2.2)	43.0(4.4)	39.2(12.8)	34.5(23.3)	30.1(33.1)
Control [b]	45.0	45.0	45.0	45.0	45.0	45.0	45.0
CD at 5% [c]	0.84	0.54	0.51	0.44	0.47	0.55	0.45

[a] Radial growth on PDA medium after 5 days' incubation on the treated plates. Values in parentheses indicate percent inhibition of the mycelial growth compared to the control plates.

[b] Radial growth on PDA medium after 5 days' incubation on control plates

[c] Critical difference

10-ppm level the percent inhibition of mycelial growth was 17.5% and 11.3%, respectively. No inhibition was observed in response to the other pesticides at this concentration. Although a gradual increase in inhibition was observed as the concentration of pesticides increased, none of the chemicals completely suppressed the growth of the fungus, even at the highest concentration, except dicofol (Table 2). At the 300-ppm level dicofol completely suppressed fungal growth, followed by quinalphos (75.5%). Other pesticides, viz. endosulfan, fenpropathrin, and propargite, exhibited a lesser degree of toxicity towards *T. harzianum*, which indicates their compatibility with the test fungus. The compatibility of endosulfan with beneficial fungi was reported earlier (19).

The biopesticides tested in the present study exhibited varying levels of inhibition on the growth of *T. harzianum*. The results indicate that neem-based biopesticides (nimbecidine and ponneem) were incompatible with *T. harzianum*. Both biopesticides completely inhibited growth at the 5% concentration (Table 3). On the other hand, lower sensitivity to the formulation containing other plant extracts (Ovis) was observed. At the 5% concentration the percent inhibition was only 13.1%. Neem kernel aqueous extract (NKAE) exhibited 80.8% inhibition at the 5%

Table 2. Effect of pesticides on the mycelial growth of a biocontrol strain of *Trichoderma harzianum*.

Treatment	Concentration (ppm)					
	10	25	50	100	200	300
Dicofol	39.9(11.3)[a]	33.2(25.5)	24.1(46.4)	20.3(54.8)	0.0(100)	0.0(100)
Endosulfan	45.0(0.0)	43.6(3.1)	42.3(6.0)	39.5(12.2)	34.3(23.7)	30.0(33.3)
Fenpropathrin	45.0(0.0)	45.0(0.0)	44.6(0.8)	44.0(2.2)	43.1(4.2)	39.0(13.3)
Propargite	45.0(0.0)	43.8(2.6)	42.7(5.11)	39.6(12.0)	34.7(22.8)	32.0(28.8)
Quinolphos	37.1(17.5)	29.7(34.0)	18.8(58.2)	16.1(64.2)	13.0(71.1)	11.0(75.5)
Control [b]	45.0	45.0	45.0	45.0	45.0	45.0
CD at 5% [C]	0.68	0.62	0.63	0.70	0.65	0.61

[a] Radial growth on PDA medium after 5 days' incubation on the treated plates. Values in parentheses indicate percent inhibition of the mycelial growth compared to the control plates.
[b] Radial growth on PDA medium after 5 days' incubation on control plates
[c] Critical difference

Table 3. Influence of different biopesticides on the mycelial growth of *Trichoderma harzianum*.

Treatment	Radial growth (mm) at*		
	2.5%	5%	7.5%
Nimbicidine	22.8(49.3) [a]	0.0 (100)	0.0 (100)
Ponneem	25.9(42.4)	0.0 (100)	0.0 (100)
NKAE	23.6(47.5)	26.5(80.8)	0.0 (100)
Ovis	43.6(3.1)	39.1(13.1)	33.2(26.2)
Control [b]	45.0	45.0	45.0
CD at 5% [c]	0.66	0.69	0.67

[a] Radial growth on PDA medium after 5 days' incubation on the treated plates. Values in parentheses indicate percent inhibition of the mycelial growth compared to the control plates.
[b] Radial growth on PDA medium after 5 days' incubation on control plates
[c] Critical difference

level, which may have been due to the azadirachtin present in the kernels (20). High antimicrobial activity with extracts of different parts of neem has been reported (21).

In conclusion, the present study reported the in vitro influence of some common agrochemicals and biopesticides used on tea plantations on the growth of *T. harzianum*. Data on combining agrochemicals and biocontrol agents for developing an integrated approach to pest and disease management on tea plantations are limited. The current findings will provide base-level data in this context. Further research is needed to evaluate the practical application of these chemicals and biopesticides with *T. harzianum* biocontrol agents in the field.

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