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Title: Assessment of the Sensitivity of *Trichoderma harzianum* (Rifai.) to Seed and Soil Treating Chemicals

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ABSTRACT

An experiment was conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh to evaluate the sensitivity of *Trichoderma harzianum* to seeds and soil treating chemicals namely, Carbendazim (Bavistin), Mancozeb (Dithane M-45), 64% Mancozeb + 8% Metalaxyl (Ridomil 68 WP), Iprodione (Hayprodione 50 WP), Carboxin + Thiram (Provax 200 WP), Carbofuran (Furadan 5G), and Calcium hypochloride (Bleaching powder). All tested chemicals were applied at three concentrations: Bavistin, Dithane M 45, Ridomil, Hepridione 50 WP, Provex, and Furadan 5G at 1000, 2000, and 3000 ppm; and Bleaching powder at 25, 50, and 100 ppm. Results revealed that mycelial growth of *T. harzianum* was inhibited 100% at all concentrations of Bavistin, Hayprodione 50 WP and Provex. However, Mancozeb inhibited 100% mycelial growth at 3000 ppm concentration and 66.80% at 2000 ppm (recommended dose). At the recommended dose, Ridomil, Furadan 5G, and Bleaching powder inhibited mycelial growth of *T. harzianum* by 24%, 14.60%, and 22.60% respectively. Across different concentrations, Mancozeb, Ridomil, Carbofuran, and Bleaching powder showed inhibition rates of over 98%, 82%, 91%, and 83% respectively. These results suggest that *T. harzianum* is highly sensitive to Bavistin, Provex, Hepridione 50 WP, and Dithane M 45, while it shows relatively low sensitivity to Ridomil, Furadan 5G, and Bleaching powder at the recommended dose.

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INTRODUCTION

In the light of present-day constraints of plant disease control practices, biological control is increasingly gaining status as a possible practical agricultural method for soil-borne pathogen control. Biological control of soil-borne pathogens offers environmentally safe, durable and cost-effective alternative to chemicals (Woo et al., 2014; Brotman et al., 2008; Harman *et al.*, 2004). Soil-borne plant pathogens remain a major constraint in sustainable agriculture, contributing significantly to yield losses in a wide range of crops globally (Bosale and Borade, 2015). Soil borne plant pathogens can be controlled by the addition of antagonistic microorganism to soil is a potential mean of plant disease control (Shinde et al., 2023; Bhai and Thomas, 2010). Among the different bio-control agents so far identified, species of *Trichoderma* are the most effective in reducing disease incidence of various crops caused by different soil borne plant pathogens including *Sclerotium rolfsii* and *Rhizoctonia solani* (Henis et al., 1978 and Mukhopadhyay, 1994). *Trichoderma* is widely distributed all over the world and occurs nearly in all soils and natural habitats especially in those containing organic matter. It can be grown in both semi-solid and liquid fermentation. It can be applied either alone or in combination with low doses of fungicides to eventually establish the fungus in the soil (Mondal et al., 1995).

In recent years, considerable success has been achieved by introducing antagonists to soil. *Trichoderma* spp. was found to be effective against different *sclerotia* forming fungi including *S. rolfsii* (Elad et al., 1980: Ahmed et al., 2000). In addition, the combined use of bio-control agents and chemical pesticides has attracted much attention as a way to obtain synergistic or additive effects in the control of soil-borne pathogens (Nongmaithem, 2015; Benitez et al., 2004; Harman et al., 1996).

Trichoderma can also be used to treat seeds as a protection against seed-borne fungal diseases. But sometimes different types of fungicides are used in soil as soil drenching to control soil borne pathogen and seed treatment. When treated seeds are sown in soil chemicals are incorporated into soil and soil drenching fungicides also mixed in soil but we know soil is the harbors of *Trichoderma* spp. So, fungicides also effect on population of *Trichoderma* spp. in soil. Furthermore, *T. harzianum* has been widely reported to promote plant growth, improve nutrient uptake, and enhance soil health (Verma et al., 2007; Brotman et al., 2008). Therefore, this experiment was undertaken to investigate the potentiality of *Trichoderma harzianum* to grow over the effect of some seed and soil treating chemicals.

MATERIALS AND METHODS

Collection and multiplication of isolate of *T. harzianum*

The experiment was carried out in the Plant Protection Laboratory of Khulna University's Agrotechnology Discipline to evaluate the effect of seed treating and soil treating chemicals at different concentrations on the growth of *Trichoderma harzianum* as antagonistic fungi. For this reason, an isolate of *Trichoderma harzianum* was collected from the preserved isolates of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. PDA was poured in sterilized petridishes, 20 ml in each. A 5 mm block of the pure culture of *Trichoderma harzianum* was placed upside down at the center of each plate. The block was cut with the help of a flame sterilized corkborer (5 mm diameter). The inoculated petridishes were kept in the growth chamber at 27±2ºC temperature for observation. All the works were done in aseptic condition.

Preservation of *T. harzianum*

Prepared PDA medium before sterilization is poured into sterilized test tubes, 10 ml in each. Then the test tubes are sterilized in an autoclave at 121°C temperature for 20 minutes. After autoclaving, slanting of test tube was done at 45° angles to increase the surface area of the medium in the test tube. Seven days old fungal hyphae with the help of a flame sterilized niddle are placed in the test tube. The inoculated test tubes were kept in the growth chamber at 27±2ºC temperature. The preservation procedure was conducted in aseptic condition.

Collection of chemicals

Chemicals (for seed and soil treatment) were purchased locally. To carry out the experiment, the fungicides and chemicals were obtained as per Table 1.

Calculation of different concentrations of chemicals

Concentrations of the above chemicals were selected based on the recommended dose on the crops and soils. One is lower than recommended, one is recommended and one other higher than the recommended dose. All the chemicals' concentrations were taken in 1000 ppm, 2000 ppm and 3000 ppm respectively but bleaching powder concentrations were taken 25, 50 and 100 ppm concentrations respectively.

Preparation of different concentrations of chemicals

Food poison technique (Dhingra and Sinclair 1985) was followed in this experiment. PDA medium amended with fungicides at different concentrations. Different contact and systemic fungicides were used (systemic+systemic and contact+systemic). PDA without fungicides was used as control (0 ppm). Chemicals were weighted with the help of electric balance for each concentration. Then it was mixed in 100 ml warm PDA in a conical flask and mixed well by shaker and then autoclaved.

Measurement of radial growth and calculation of percent inhibition

Data on radial colony diameter will record when the colony growth of the pathogen completely covered the control plates. Two measures of the diameter of the colonies on PDA with and without fungicide will take at right angle. Average of the two measurements will be considered as the radial colony diameter of a fungus. Inhibition of radial growth will compute based on colony diameter on control plate using standard formula (Daouk et al., 1995) -

$$\% \text{ Inhibition} = \frac{X-Y}{X} * 100$$

Where,

X = Average growth of *Trichoderma harzianum* in control petridishes

Y = Average growth of *Trichoderma harzianum* in each chemicals treated petridishes

Inoculation and incubation

About 20 ml of the sterilized medium was poured in each 90 mm sterilized petridishes after autoclaving. Mycelia discs were cut by using a cork borer (5 mm diameter) from the margin of 3 days old culture of isolates. One disc of the isolate was placed reversely at the center of the petridish after solidification of PDA so that the mycelium and medium were in contact. Each treatment was replicated five times in a Completely Randomized Design. The inoculated plates will incubate on desk at room temperature (27±2 0C).

Experimental design and data analysis

The experiment was conducted in the Plant Protection Laboratory of the Agrotechnology Discipline at Khulna University. Each treatment was replicated five times using a Completely Randomized Design (CRD). Data were statistically analyzed using the MSTAT-C program, and treatment means were compared using Duncan's Multiple Range Test (DMRT).

Table1. List of chemicals that were used in the experiment

Trade Name	Chemical Name	Company Name	Concentration (ppm)
Dithane M-45	Mancozeb	Bayer CropScience Limited	1000, 2000 and 3000
Bavistin	Carbendazim	BASF Bangladesh Limited	1000, 2000 and 3000
Ridomyl 68 Wg	8% Metalaxyl + 64% Mancozeb	Syngenta Bangladesh Limited	1000, 2000 and 3000
Furadan 5 G	Carbofuran	Padma Oil Company Limited	1000, 2000 and 3000
Hayprodione 50 WP	Iprodione	Haychem Bangladesh Limited	1000, 2000 and 3000
Provax 200 WP	Carboxin+Thiram	Hossain Enterprise C.C. Limited	1000, 2000 and 3000
Bleaching powder	Calcium hypochloride	Techno Power Bangladesh	25, 50 and 100

RESULTS AND DISCUSSION

Effects of seed and soil treating chemicals on mycelia growth inhibition of *T. harzianum* at different concentrations

The different seed and soil treating chemicals in different concentrations inhibited the mycelia growth of *T. harzianum* significantly (p<0.01). Inhibition percentage increased with the increase of concentrations in all chemicals. No mycelia growth of *T. harzianum* was found at any concentration of Carbendazim (Bavistin), Carboxin + Thiram (Provex), Iprodione (Hayprodione 50 WP) i.e., percentage inhibition was 100% at all concentrations. The lowest percent inhibition was observed in Metalaxyl + Mancozeb (3.60) at 1000 ppm concentrations. *Trichoderma* spp. is highly sensitive to Cabendazim, Caboxin + Thiram, Ipridione and comparatively less sensitive to Metalaxil+ Mancozeb, Carbofuran, Medium sensitive to Mancozeb in concentration of 1000 ppm and 2000 ppm but were highly inhibited (100%) in 3000 ppm. Mancozeb and Thiram were found highly sensitive to *Trichoderma harzianum* by Mclean et al., (2001). Present study also found Mancozeb was sensitive. In case of Carbofuran and Bleaching powder the differences of inhibition (%) between two concentrations of the same chemicals were less. (Table 2, Fig. 1).

Table 2. Effect of different seed treating and soil treating chemicals at different concentrations on mycelia growth inhibition of *T. harzianum*

Sl. No.	Chemicals	Concentration (ppm)	Radial growth inhibition (%)
	Control		-
1.	Mancozeb (Dithane M-45)	1000 2000 3000	44.40 d 66.80 c 100.0 a
2.	Carbendazim (Bavistin)	1000 2000 3000	100.0 a 100.0 a 100.0 a
3.	Carboxin + Thiram (Provax 200 WP)	1000 2000 3000	100.0 a 100.0 a 100.0 a
4.	Iprodione (Hayprodione 50 WP)	1000 2000 3000	100.0 a 100.0 a 100.0 a
5.	Metalaxyl + Mancozeb (Ridomil)	1000 2000 3000	3.60 i 24.0 ef 82.60 b
6.	Carbofuran (Furadan 5G)	1000 2000 3000	9.40 h 14.60 g 16.40 g
7.	Bleaching Powder	25 50 100	14.20 g 22.60 f 26.80 e
CV (%)			2.69
LSD Value			3.059

Means followed by different letters are significantly different at 1% level.

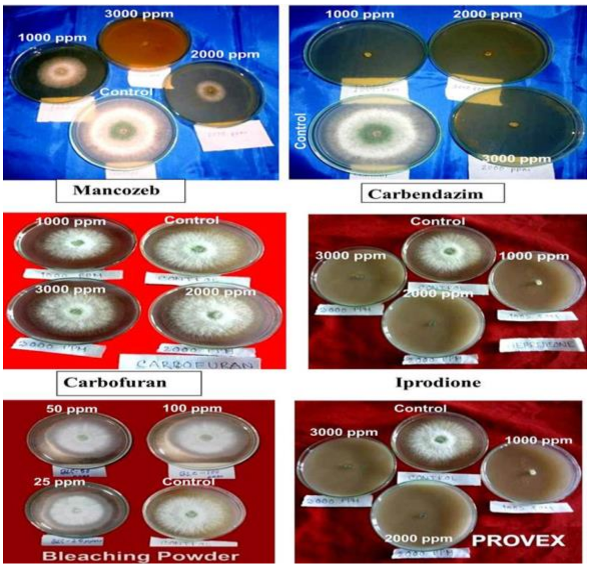

Fig. 1. Effect of seed and soil treating chemicals on mycelia growth inhibition percentages of *Trichoderma harzianum* at different concentrations

Effect of seed and soil chemicals on mycelial growth inhibition of *Trichoderma harzianum*

According to the regression equation, after applying seed and soil treating chemicals (Mancozeb, Ridomil, Carbofuran, and Bleaching Powder) in various concentrations, it inhibits the mycelial growth. Results showed that, inhibition percentage was 98% in mancozeb (Fig. 2), 82% in ridomyl (Fig. 2), 91% in carbofuran (Fig. 3) and 83% in bleaching powder (Fig. 3) treated experiments, which may explain the reason the variation increases with concentration.

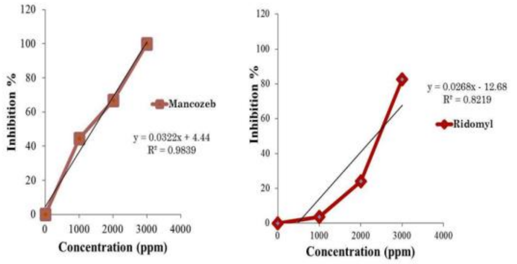

Fig. 2. Functional relationship among concentration and mycelial growth inhibition percentage by Mancozeb and Ridomyl

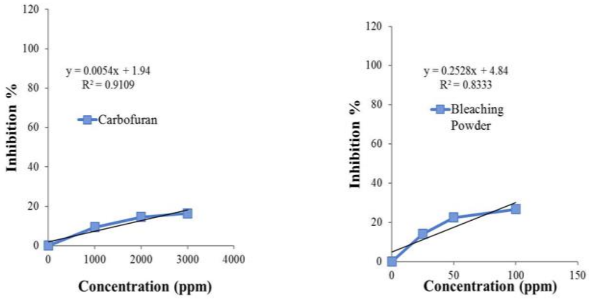

Fig. 3. Functional relationship among concentration and mycelial growth inhibition percentage by Carbofuran and Bleaching Powder

DISCUSSION

The present study aimed to assess the sensitivity of *Trichoderma harzianum* to various seed and soil treating chemicals widely used in crop protection. Results demonstrated that the inhibitory effects varied significantly depending on the type and concentration of the chemical used. Notably, Carbendazim, Carboxin with Thiram, and Iprodione completely inhibited the mycelial growth of *T. harzianum* at all tested concentrations (1000, 2000, and 3000 ppm), indicating high toxicity. These findings are consistent with the reports by Mclean *et al*. (2001), who found that Carbendazim and Thiram significantly reduced the viability and sporulation of *Trichoderma* spp.

Mancozeb showed a moderate inhibition at 1000 ppm and complete inhibition at 3000 ppm. This aligns with the findings of Elad *et al.* (1980), who observed reduced growth and biocontrol efficacy of *T. harzianum* when exposed to Mancozeb residues. The study by Escudero-Leyva *et al.* (2022) also emphasized that synthetic fungicides such as Mancozeb may adversely impact the antagonistic potential of *Trichoderma* spp. through disruption of cellular metabolism.

Metalaxyl with Mancozeb (Ridomyl) exhibited comparatively less toxicity at 1000 ppm but showed significant inhibition at higher concentrations. These results support the findings of Ahmed *et al*. (2000), who noted that the combined formulation of systemic and contact fungicides could suppress beneficial soil microbes, depending on application dose and exposure duration.

Carbofuran and Bleaching Powder displayed the lowest inhibitory effects on *T. harzianum*. Though toxicity increased with concentration, inhibition levels remained significantly lower than those caused by Carbendazim or Iprodione. This relative tolerance suggests the potential compatibility of *T. harzianum* with low doses of Carbofuran and Bleaching Powder under integrated disease management frameworks. Bhai and Thomas (2010) highlighted that compatibility assessments are critical for maintaining the biocontrol efficiency of *Trichoderma*, particularly when combined with agrochemicals in seed or soil treatments.

The analysis further confirmed the dose-dependent response of *T. harzianum* to the applied chemicals, as reported in earlier compatibility studies (Nongmaithem, 2015). The current findings underscore the necessity of careful selection and dose optimization of chemical agents to safeguard the beneficial roles of *Trichoderma harzianum* in sustainable agriculture.

CONCLUSION

Trichoderma harzianum is an effective soil biocontrol agent, but its successful integration into disease management programs depends on its compatibility with commonly used agrochemicals. This study demonstrated that several seed and soil-applied chemicals significantly inhibited the mycelial growth of *T. harzianum*. Complete inhibition was observed with Carbendazim (Bavistin), Carboxin + Thiram (Provex), and Iprodione (Hepridione 50 WP) at all tested concentrations. Mancozeb and Metalaxyl + Mancozeb also showed strong inhibitory effects at higher doses. In contrast, Carbofuran (Furadan 5G) and Bleaching Powder caused comparatively lower inhibition. These results highlight the need for compatibility assessment when combining chemical treatments with biocontrol agents to ensure sustainable and effective management of soil-borne plant pathogens.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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