Research Article

IN-VITRO COMPATIBILITY OF SOME NATIVE ISOLATES OF *Trichoderma* spp. WITH COMMONLY USED PESTICIDES

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ABSTRACT

Trichoderma is an effective bio-control agent against a wide range of disease-causing pathogens of various crops and potential to be integrated into the disease management program. The study was conducted to find out the compatible pesticide/s with *Trichoderma* for integrated pest management (IPM). This study includes *in-vitro* evaluation of four chemical fungicides (carbendazim, copper-oxychloride, captan and tebuconazole + sulphur), three chemical insecticides (malathion, imidachlorpid and chlorpyriphos + cypermethrin) and one botanical insecticide (azadirachtin) for compatibility with seven different native isolates and two known species of *Trichoderma* through poisoned food technique. Copper-oxychloride was found compatible with all the tested isolates. In contrast, carbendazim and tebuconazole + sulphur were found incompatible. The insecticides, malathion, imidachlorpid, chlorpyriphos + cypermethrin, azadirachtin and the fungicide, captan showed some degree of compatibility. It is concluded that the tested *Trichoderma* isolates and the given species could be used together in combination with the given compatible fungicide such as copper-oxychloride and the given insecticides. These results would be helpful to set out the possibility of combining *Trichoderma* spp. as a biocontrol agent with pesticides to be used through IPM approach.

Keywords: *Compatibility test, In-vitro, pesticides, Trichoderma*

INTRODUCTION

Trichoderma is a cosmopolitan free living antagonistic microorganism found in all types of soil and colonies in the root system (Harman *et al*., 2004; Mutawila *et al*., 2015). It acts as an effective well documented bio-control agent against a wide range of disease-causing pathogens of various crops persisting in soil and interacts symbiotically with crops and several other microorganisms (Srivastava *et al*., 2015; Manandhar *et al*., 2020). The biodiversity of soil antagonistic microorganisms is challenged by the application of pesticides including fungicides, insecticides, herbicides and other agrochemicals which

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leads to weak natural antagonistic activity of microorganisms (Tapwal *et al*., 2012). Therefore, there is a need for judicious combined use of bio-control agents with compatible chemical pesticides for effective and better management of the plant diseases (Madhusudhan *et al*., 2010). Chemical pesticides are generally harmful or have negative effects on growth and colonization of *Trichoderma*, however, some are reported to be compatible (Sushir *et al.,* 2015; Tapwal *et al*., 2012) and more knowledge on compatibility needs to be explored. Hence, this study was conducted to select safer and compatible pesticide/s among the commonly used chemical fungicides and insecticides including azadirachtin with different Trichoderma isolates to incorporate in IPM.

MATERIALS AND METHODS

Seven native isolates of *Trichoderma* spp. and two known species were assessed in-vitro by poisoned food technique with eight major pesticides commonly being used in vegetable cultivation. The experiment was organized in a completely randomized design (CRD) with three replications (one plate as one replication) where pesticides were used as the treatments.

Experimental site

The test was performed in the laboratory of National Plant Pathology Research Centre (NPPRC), Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur during June, 2019 - July, 2019.

Selection of treatments

Seven *Trichoderma* isolates used in this study were the fresh native isolates isolated from the soil of various places of Nepal and two known *Trichoderma* species (*T. viride* and *T. harzianum*) from the laboratory of NPPRC (Table 1). Eight pesticides as treatments assessed for compatibility with *Trichoderma* are given in Table 2.

Table 1. *Trichoderma* species and isolates used in the experiment

Isolates/Species	Location	Source/Fresh isolation from
Trichoderma-TPD	Pakhribas, Dhankuta	Vegetable field
Trichoderma-TTS	Tarahara, Sunsari	Rice field
Trichoderma-TRK	Raniban, Kathmandu	Sal forest area
Trichoderma-TRC	Rampur, Chitwan	Fallow grassland
Trichoderma-TLK	Lumle, Kaski	Potato field
Trichoderma-TRD	Rajhena, Dang	Maize field
Trichoderma-TRJ	Rajikot, Jumla	Apple orchard
T. viride	-	NPPRC
T. harzianum	-	NPPRC

Table 2. Details of the treatments (Pesticides)

Pesticides*	Trade name
Carbendazim 50% WP	G-Bestin
Copper-oxychloride 50% WP	P-Oxyride
Captan 50% WP	Criptan
Tebuconazole 10% + sulphur 65% WG	Sajha
Malathion 50% EC	Plant Malathion
Imidachlorpid 17.8% SL	Allmida
Chlorpyriphos 50% + cypermethrin 5% EC	Triveni
Azadirachtin 300 ppm	Nimbecidine
Control (Without any pesticide)	

***** All pesticides were tested at 10 ppm active ingredient.

Preparation of stock solutions of pesticides

The stock solutions of pesticides were prepared to make the precise quantity for each poisoned potato dextrose agar (PDA). First, required quantities of pesticides were calculated for a certain volume and poured in sterile distilled water in test tubes and serial dilution was performed to make the stock solution. Ten ppm of active ingredient was used for each pesticide's stock solution.

Preparation of poisoned PDA Petri plates

Borosil petri plates (9 cm diameter) containing poisoned PDA medium was used. For this, PDA and the stock solution to be added were prepared and calculated. Then after when the PDA cooled to 50-55 ^{0}C the calculated stock solution was added and mixed thoroughly. Then approximately 20 ml of this medium was poured in each plate. For control, only PDA with no pesticide was used.

Preparation of individual culture and inoculation of native isolates of *Trichoderma* spp. on PDA Petri plates

The seven native isolates of *Trichoderma* spp. used in the experiment were isolated from soil using *Trichoderma*-selective medium (TSM) with slight modification. TSM with composition of magnesium sulphate heptahydrate - 0.2 g, dipotassium hydrogen phosphate - 0.9 g, ammonium nitrate - 1.0 g, potassium chloride - 0.5 g, glucose - 3.0 g, agar - 20.0 g, distilled water - 1000 ml, rose bengal - 0.033 g and antibiotic (streptomycin - 0.2 g) was used as isolation medium. For fresh isolation, first the soil samples representing the top 15 cm of the land were collected from various locations representing different agro-ecological regions of Nepal and serially diluted up to seventh level taking 1 g in 10 ml of sterile distilled water at first and repeating by taking 1 ml to the next 9 ml sterile distilled water. 100 µl suspension as inoculum was inoculated at the center and spread over Petri plates

containing TSM. The plates were incubated for 7 to 10 days at 24±20C. Isolation and purification were carried out for the fresh *Trichoderma* isolates. The two known *Trichoderma* spp. used were provided by NPPRC, NARC, Khumaltar. During the experiment, cultures of *Trichoderma* spp. isolates were prepared on PDA Petri plates and five-day old cultures were used for the experiment. The five mm-sized mycelial discs were cut with cork borer and then inoculated inversely at the center of the poisoned PDA Petri plate.

Incubation and observation

The inoculated Petri plates were incubated at room temperature where minimum and maximum temperature were recorded as 210C and 290C respectively. The radial growth of the *Trichoderma* isolates was measured using Vernier caliper. The colony was measured every day. However, for the analysis purpose, the three-day data were used referencing the fully grown colony in the control Petri plates on that day. The percent growth inhibition was calculated by using the formula given by Edgington *et al.* (1971) and Hajieghrari *et al.* (2008).

Statistical analysis

The data recorded were tabulated in Microsoft Excel 2013 data worksheets. R-Studio version 1.2.1335 was used for the statistical analysis of the data with agricolae 1.3-1 and lsmeans 2.30-0 for analysis of variance (ANOVA). For mean separation, Duncan's multiple range test (DMRT) was performed at 5% level of significance. The Microsoft Excel 2013 was used for tabular and graphical representation.

RESULTS AND DISCUSSION

In-vitro assessment for the compatibility of *Trichoderma* isolates with pesticides revealed that there were significant differences in compatibility between tested *Trichoderma* isolates and pesticides over the check (Table 3).

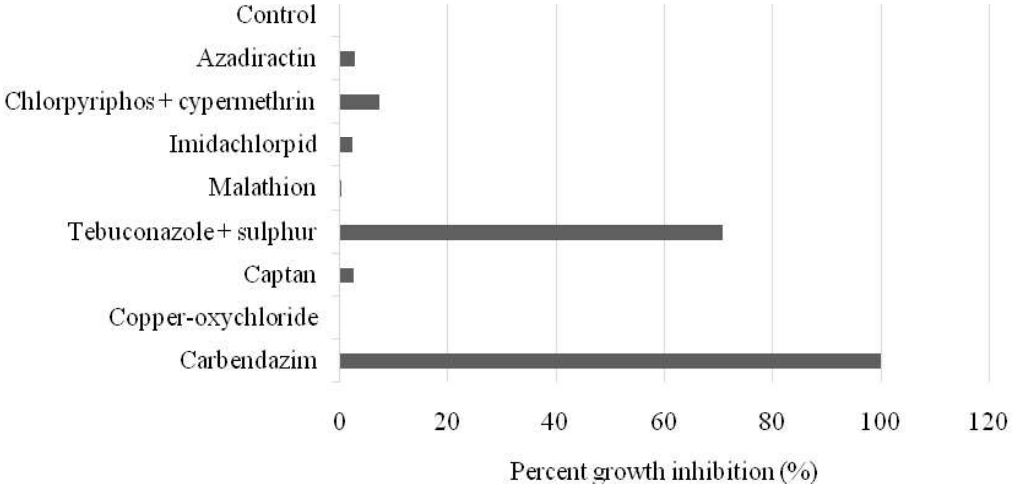
Table 3. *In-vitro* effect of different pesticides on mean radial growth of *Trichoderma* isolates through poisoned food technique at 72 hours after inoculation

Pesticides	Radial growth of *Trichoderma* isolates (cm)								
	TPD	**TTS**	**TRK**	**TRC**	**TLK**	**TRD**	**TRJ**	**TV**	**TH**
Carbendazim	0.5^{c} (0.7)	0.5^{c} (0.7)	0.5^{d} (0.7)	0.5^{c} (0.7)	0.5^{c} (0.7)	0.5^{d} (0.7)	0.5^{c} (0.7)	0.5^{d} (0.7)	0.5^{c} (0.7)
Copper-oxychloride	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)
Captan	9.0^{a} (3.0)	9.0^{a} (3.0)	8.8^{a} (3.0)	8.3^{a} (2.9)	9.0^{a} (3.0)	9.0^{a} (3.0)	8.4^{a} (2.9)	9.0^{a} (3.0)	8.4^{a} (2.9)
Tebuconazole + sulphur	3.7^{b} (1.9)	2.8^{b} (1.7)	1.6^{c} (1.2)	2.9^{b} (1.7)	3.4^{b} (1.8)	3.0^{c} (1.7)	2.7^{b} (1.6)	2.0^{c} (1.4)	1.5^{b} (1.2)
Malathion	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	8.6^{a} (2.9)
Imidachlorpid	9.0^{a} (3.0)	9.0^{a} (3.0)	8.1^{b} (2.8)	9.0^{a} (3.0)	7.9^{a} (2.8)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)
Chlorpyriphos + cypermethrin	9.0^{a} (3.0)	8.2^{a} (2.9)	8.8^{a} (3.0)	9.0^{a} (3.0)	7.7^{a} (2.7)	7.6^{b} (2.8)	9.0^{a} (3.0)	7.9^{b} (2.8)	7.7^{a} (2.7)
Azadirachtin	9.0^{a} (3.0)	8.5^{a} (2.9)	8.9^{a} (3.0)	8.6^{a} (2.9)	9.0^{a} (3.0)	9.0^{a} (3.0)	8.9^{a} (3.0)	8.3^{ab} (2.9)	8.4^{a} (2.9)
Control	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)	9.0^{a} (3.0)
F-test	***	***	***	***	***	***	***	***	***
S.Em±	0.023	0.055	0.028	0.043	0.109	0.042	0.043	0.061	0.105
LSD (≤0.05)	0.07	0.16	0.08	0.13	0.32	0.12	0.13	0.18	0.31
CV (%)	1.53	3.68	1.90	2.89	7.34	2.80	2.87	4.16	7.31

R_g = Radial colony growth, Values of disease severity in parentheses were square root transformed and Means in a column followed by same letter do not differ significantly at 5% percent probability by DMRT, *** = Highly significant, S.Em = Standard error of mean, LSD (≤0.05) = Least significance difference, showing 5% level of significance, CV (%) = Covariance percentage, TPD = *Trichoderma* – Pakhribas, Dhankuta, TTS = *Trichoderma* – Tarahara, Sunsari, TRK = *Trichoderma* – Raniban, Kathmandu, TRC = *Trichoderma* – Rampur, Chitwan, TLK = *Trichoderma* – Lumle, Kaski, TRD = *Trichoderma* – Rajhena, Dang, TRJ = *Trichoderma* – Rajokot, Jumla, TV = *T. viride*, TH = *T. harzianum*

Among the tested chemical fungicides, carbendazim 50% WP was found completely inhibiting the radial colony growth of all *Trichoderma* isolates. While, copper-oxychloride 50% WP was not found to inhibit the growth at all. Captan 50% WP also did not inhibit the growth of most of the tested *Trichoderma* isolates. Similarly, tebuconazole 10% + sulphur 65% WG was also found to inhibit colony growth of the tested *Trichoderma* isolates ranging from 58.55% to 82.89%.

Fig. 1. Percent growth inhibition on poisoned PDA by different pesticides at 10 ppm a.i. under consideration for the tested *Trichoderma* isolates. (Each datum is the mean for the tested *Trichoderma* isolates.)



Furthermore, among the tested chemical insecticides, malathion 50% EC was found not inhibiting most of the tested *Trichoderma* isolates but 4.97 percent growth inhibition was observed for *T. harzianum*. In the same way, imidachlorpid 17.8% SL also did not inhibit the growth of most of the tested *Trichoderma* isolates but *Trichoderma* isolates from Raniban and Lumle had percent growth inhibition of 9.89 and 11.83, respectively. Chlorpyriphos 50% + cypermethrin 5% EC was found to inhibit the radial colony growth of most of the tested *Trichoderma* isolates with least growth inhibition for *Trichoderma* isolate of Raniban (2.01%) and highest for *Trichoderma* isolate of Rajhena (15.08%). Azadirachtin exhibited less percent growth inhibition of the *Trichoderma* isolates ranging from 0.64 to 8.17.

Copper-oxychloride was found to be compatible with all the tested isolates while carbendazim was incompatible. Compatibility between copper-oxychloride and *Trichoderma* has been reported by several research workers (Sarkar *et al.*, 2008; Bagwan, 2010; Tapwal *et al.*, 2012). Similarly, incompatibility of *Trichoderma* with carbendazim has been mentioned by many workers (Madhavi *et al.*, 2011; Tapwal *et al*., 2012; Dhanya *et al*., 2016; Kumar *et al*., 2018; Shashikumar *et al*., 2019). Both the compatibility with copper-oxychloride and the incompatibility with carbendazim have also been documented in Nepal by Plant Pathology Division of NARC (Annual Report, 2019). In the present study, tebuconazole + sulphur showed some degree of incompatibility. Other researchers found *Trichoderma* highly sensitive to tebuconazole (McLean *et al*., 2001; Bagwan, 2010; Madhavi *et a*l., 2011; Thoudam and Dutta, 2014) and completely compatible with sulphur (Sharma *et al*., 2016). Based on the present results, we put captan in a compatible group.

Bagwan (2010) and Madhavi *et al*. (2011) reported *Trichoderma* slightly sensitive to captan. The differences in results between the present and the previous research by others could be due to varied isolates of *Trichoderma* used in different experiments and also the concentrations used.

Interestingly, all the tested chemical insecticides and azadirachtin were found compatible with the isolates of *Trichoderma* in varying levels. This might be due to inherent resistance of *Trichoderma* to the insecticides and their capacities to degrade the chemicals (Bhai and Thomas, 2010; Nandini *et al.*, 2018). Malathion's nature of incompatibility between *Trichoderma* and insecticides have been noticed (Ahanger *et al*., 2014; Karumari & Singh, 2015; Nandini *et al*., 2018). However, some reports showed only partial compatibility of *Trichoderma* with imidachlorpid and chlorpyrifos + cypermethrin (Madhavi *et al*., 2011; Dhanya *et al*., 2016; Siddhartha *et al*., 2017) and also with neem oil (Patel & Biswas, 2016; Mallaiah & Rao, 2016). In the present study, we found almost complete compatible reactions between the insecticides, including neem oil (azadirachtin) and the isolates of *Trichoderma*. The limitation of the present study is that we did test the pesticides at only 10 ppm concentration.

From the present study, it can be concluded that the tested *Trichoderma* may be used in integration with the copper-oxychloride and captan, however, should never be used with carbendazim and tebuconazole + sulphur. Almost all of the tested insecticides in the given concentration are safe to use in combination with *Trichoderma*. Compatibility studies between *Trichoderma* and the pesticides along with others should be carried out at different higher concentrations to determine the most effective and safer application under field conditions for additive effects in an IPM approach.

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