COMPATIBILITY STUDY OF TRICHODERMA ISOLATES WITH CHEMICAL FUNGICIDES

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ABSTRACT

Chemical use in agricultural farming in recent years has led to many threats concerning the environment and human health. Trichoderma spp. has been used as a biocontrol agent and is gaining popularity in recent years. Integrated use of Trichoderma with compatible chemicals is one disease management strategy which would aid in immediate action on plant pathogens and provide control over pathogens in long term as well. In vitro compatibility test of five Trichoderma isolates with thirteen different chemical fungicides at two different concentrations was carried out in the laboratory of Plant Pathology Division. The results showed that four of the tested chemical pesticides viz; Bavistin (Carbendazim 50% WP), Cryzole (Hexaconazole 5% SC), Benlate (Benomyl 50% WP) and Saaf (Carbendazim 12% Mancozeb 63% WP) exhibited complete inhibition of Trichoderma, irrespective of the isolates tested. Seven of them were compatible with all Trichoderma isolates and two chemicals Krilaxyl (Metalaxyl 8% + Mancozeb 64% WP) and Aver up (Chlorothalonil 75% WP) showed some degree of inhibition of two Trichoderma isolates, while the rest of the isolates were fully compatible. In all the chemical treatments it was noted that growth of Trichoderma decreased as the concentration of pesticides increased. Integration of safer and compatible chemical pesticides and Trichodermacan provide an effective and long-term solution against plant diseases in agricultural farming.

Keywords: Biocontrol, chemical pesticides, compatibility test, *Trichoderma*

INTRODUCTION

Trichoderma sp. is well established as a biopesticide, biofertilizer in agriculture and is the most exploited bioagent used for the control of various plant pathogens (Kumar et al., 2014). It can be used as seed treatment, seed biopriming, seedling treatment, soil applications, and foliar applications (Benitez et al., 2004).However, there are also findings, in which *Trichoderma* give excellent control of plant pathogens in greenhouse and pot trials while they fail to perform at the same level in fields. This may be primarily due to the time taken to adapt to new ecological niches with variable microclimate

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and interaction with several other microorganisms. Scientists have often explained of the lag phase of *Trichoderma* to provide control of plant pathogens as they would need time to colonise the root system or the diseased foliar parts of plants to provide ultimate benefit of plant protection (Mutawila et al., 2015). Hence the beneficial effect of *Trichoderma* may be exhibited in long term. Chemicals on the other hand act relatively very quicker than the biopesticides. But, the detrimental effect of chemical pesticides on environment and health is a serious issue. Most chemicals have short term effect and need to be applied continuously. Pesticide use in Nepal is low (0.396 kg a.i. perha) as compared to other countries (Sharma, 2015). However, the data for the use of chemical pesticides in Nepal in agricultural commodities suggests an indiscriminate use of the pesticides, particularly in vegetable farming (Bhandari et al., 2018; Gyawali, 2018).This also induces resistance in pathogens and therefore an integrated approach of using both chemicals and *Trichoderma* may enhance the effectiveness of disease control and provide better management of plant diseases (Madhusudhan et al., 2010).There are also many findings of effective disease control with combined use of *Trichoderma* species and chemical pesticides in seed treatment or when applied in field conditions (Venkateswarlu et al., 2008; Mahesh et al., 2010; Animisha and Zacharia, 2011). Several chemicals however have negative effects on *Trichoderma* growth and colonization (Sushir et al., 2015; Tapwal et al., 2012) and hence firstly safer chemicals compatible with *Trichoderma* must be tested. This study was conducted to select the chemical fungicides, which are compatible with different *Trichoderma* isolates for their potential to be used in integrated disease management approach. Differences in response of various *Trichoderma* strains have also been observed by several authors and resistant isolates have been identified, which can tolerate field concentrations of stronger chemical pesticides (Silva et al., 2018). Different isolates of *Trichoderma* were used in this *in vitro* test to find out if there were variable responses among the isolates to the chemicals under study.

MATERIALS AND METHODS

TRICHODERMA ISOLATES USED FOR THE STUDY

Five different isolates of *Trichoderma* were used for the study, which were Ts, Tv, T22, Th and T69. All isolates except T22 were the native isolates recovered from rhizosphere of vegetable crops in different places of Nepal. T22 was an isolate recovered from commercial Trichoderma product. Serial dilutions of the soils/commercial formulation were made in sterilized water and the suspension was spread on PDA plates for the isolation of *Trichoderma*. Plates were incubated at 25°C until the growth of *Trichoderma*. The colonies were observed under stereoscope and isolation of *Trichoderma*

was performed with the help of sterile needle onto PDA slants. The isolates T69, Th were identified as *T. harzianum*, Ts as *T. asperellum* and Tv as *T. viride* while T22 was not identified to species level.

CHEMICAL FUNGICIDES USED FOR THE STUDY

Thirteen commonly used chemical pesticides were tested for their compatibility with the *Trichoderma* isolates. These were AverUp (Chlorothalonil 75% WP), Aver Green (Mancozeb 64% + Cymoxanil 8% WP), Triazole (Tricyclazole 75% WP), Cryzole (Hexaconazole 5% SC), Sectin (Fenamidone 10%+ Mancozeb 50% WG), Antracol (Propineb 70% WP), Bavistin (Carbendazim 50% WP), COC(Copper oxychloride 50% WP), Nacrobat (Dimethomorph 69% WG), Uthane (Mancozeb 50% WP), Krilaxyl (Metalaxyl 8 % + Mancozeb 64% WP), Benlate (Benomyl 50% WP) and Saaf (Carbendazim 12% Mancozeb 63% WP). These were tested at two different concentrations of 50 ppm and 100 ppm of their active ingredients.

COMPATIBILITY TEST OF CHEMICAL FUNGICIDES AND TRICHODERMA ISOLATES

Chemical fungicides were mixed in sterilized PDA before pouring onto 8 cm plates. Five mm circular discs of 7 days old culture of *Trichoderma* were excised with sterile cork borer and placed at the centre of PDA plates under aseptic conditions. PDA plates without addition of chemical were treated as control. Each isolate of *Trichoderma* was tested in 3 replications of the test chemicals and control. The experiment was set in completely randomized design and plates were incubated at 26 ¤C. Colony diameter of *Trichoderma* in the treatments was measured every day for a week.

STATISTICAL ANALYSIS

M-Stat software was used for the statistical analysis of the data. The significance of differences between chemical treatments and the isolates used under study was tested at 1% level of significance.

RESULTS AND DISCUSSION

Four fungicides viz; Bavistin, Cryzole, Benlate and Saaf had complete inhibitory effect on all *Trichoderma*, irrespective of the isolates tested. Seven chemicals (Aver Green, Triazole, Sectin, Antracol, Blitox-50, Nacrobat, Uthane) were found to be compatible with all *Trichoderma* isolates (Figure 1a and 1b). These chemicals initially exhibited partial inhibition of the isolates as compared to control but eventually at the end of 7 days allowed for complete growth of the fungi. Sensitivity of the isolates TS and T22 was noted in the chemicals Aver up and Krilaxyl while the other *Trichoderma* isolates were compatible with these chemicals. Results (data of 3 and 7 days after *Trichoderma* inoculation) are presented in Table 1. Significant differences among the chemicals and between isolates in terms of colony growth could be seen from the study (Table 2 and Table 3).

Table 1. Colony diameter of *Trichoderma* isolates (cm) in different chemical fungicide treatment (50 ppm and 100ppm of active ingredients) at 3 and 7 days of inoculation

Chemical fungicides		Colony diameter (cm) of *Trichoderma* isolates									
		Ts		Th		T22		T69		Tv	
		3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Aver Up (Chlorothalonil 75% WP)	50 ppm	1.43	2.03	1.97	8.00	1.67	2.37	1.90	7.23	1.67	5.20
	100 ppm	1.20	1.87	1.73	8.00	1.43	2.20	1.67	6.57	1.63	4.23
Aver green (Mancozeb 64% + Cymoxanil 8% WP)	50 ppm	8.00	8.00	8.00	8.00	8.00	8.00	7.16	8.00	8.00	8.00
	100 ppm	6.37	8.00	7.37	8.00	6.30	7.70	4.70	8.00	7.57	8.00
Triazole(Tricyclazole 75% WP)	50 ppm	7.73	8.00	6.90	8.00	4.50	8.00	3.53	7.63	7.97	8.00
	100 ppm	4.80	8.00	2.77	5.23	8.00	8.00	3.10	6.30	5.30	8.00
Cryzole(Hexaconazole 5% SC)	50 ppm	0.50	0.50	0.50	1.27	0.50	0.50	0.50	0.50	0.50	0.50
	100 ppm	0.50	0.50	0.50	1.33	0.50	0.50	0.50	0.50	0.50	0.50
Sectin(Fenamidone 10%+ Mancozeb 50% WG)	50 ppm	6.10	8.00	7.60	8.00	6.70	8.00	5.20	8.00	6.90	8.00
	100 ppm	5.73	8.00	7.13	8.00	5.50	8.00	4.27	8.00	6.70	8.00
AntracolPropineb 70 % WP)	50 ppm	7.07	8.00	8.00	8.00	8.00	7.23	6.47	8.00	8.00	8.00
	100 ppm	6.97	8.00	8.00	8.00	8.00	8.00	5.70	8.00	7.93	8.00
Bavistin (Carbendazim 50% WP)	50 ppm	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
	100 ppm	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
COC(copper oxychloride 50% WP)	50 ppm	6.90	8.00	6.07	8.00	7.07	8.00	5.43	8.00	6.50	8.00
	100 ppm	4.50	8.00	5.80	8.00	4.80	8.00	5.13	8.00	6.20	8.00

Nacrobat(Dimethomorph 69% WG)	50 ppm	7.30	8.00	7.07	8.00	7.20	8.00	7.23	8.00	6.85	8.00
	100 ppm	7.03	8.00	6.83	8.00	6.77	8.00	6.83	8.00	6.60	8.00
Uthane(Mancozeb 50% WP)	50 ppm	5.77	8.00	5.70	8.00	5.67	8.00	6.90	8.00	5.35	8.00
	100 ppm	5.30	8.00	5.37	8.00	5.27	8.00	6.53	8.00	5.15	7.70
Krilaxyl(Metalaxyl 8% + Mancozeb 64% WP)	50 ppm	2.90	6.60	5.20	7.80	2.80	6.37	6.30	8.00	6.20	7.90
	100 ppm	2.47	4.83	4.87	7.60	2.50	5.60	5.97	8.00	5.60	8.00
Benlate (Benomyl 50% WP)	50 ppm	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
	100 ppm	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Saaf(Carbendazim 12% Mancozeb 63% WP)	50 ppm	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
	100 ppm	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Control (PDA without chemical)		8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00

Table 2. Effect of different chemicals (50 ppm and 100 ppm concentration) on colony diameter of *Trichoderma* sp. at 3 days and 7 days after inoculation

Chemicals	Colony diameter of *Trichoderma* sp. (cm)			
	3 days 50 ppm	3 days 100 ppm	7 days 50 ppm	7 days 100 ppm
Aver Up (Chlorothalonil 75% WP)	1.71G	4.65C	1.54G	4.24D
Aver green (Mancozeb 64% + Cymoxanil 8% WP)	7.84AB	8.00A	6.40C	8.00A
Triazole(Tricyclazole 75% WP)	6.66D	7.95 A	4.04F	7.08B
Cryzole(Hexaconazole 5% SC)	0.50H	0.62D	0.50H	0.64E
Sectin(Fenamidone 10%+ Mancozeb 50% WG)	6.45D	8.00A	5.81D	8.00A
AntracolPropineb 70% WP)	7.51BC	8.00A	7.13B	8.00A
Bavistin (Carbendazim 50% WP)	0.50H	0.50E	0.50H	0.50F
COC(copper oxychloride 50% WP)	6.40D	8.00A	5.24E	8.00A
Nacrobat(Dimethomorph 69% WG)	7.51BC	8.00A	6.81B	8.00A
Uthane(Mancozeb 50% WP)	5.90E	8.00A	5.52DE	7.95A

Krilaxyl(Metalaxyl 8% + Mancozeb 64% WP)	4.54F	7.34B	4.12F	6.71C
Benlate (Benomyl 50% WP)	0.50H	0.50E	0.50H	0.50F
Saaf(Carbendazim 12% Mancozeb 63% WP)	0.50H	0.50E	0.50H	0.50F
Control (PDA without chemical)	7.95A	8.00A	8.00A	8.00A
Grand mean	3.82	4.71	3.40	4.60
Lsd	0.00	0.00	0.00	0.00
CV	3.14	2.08	2.93	2.40
P Value	***	***	***	***

Data are mean of three replicates. Figures followed by different letters along the column are significantly different ($p<0.001$)

Table 3. Variation in colony diameter of *Trichoderma* isolates in response to chemicals (50 ppm and 100 ppm concentration) at 3 days and 7 days after inoculation

Trichoderma isolates	Colony diameter of different *Trichoderma* isolates (cm)			
	3 days 50 ppm	3 days 100 ppm	7 days 50 ppm	7 days 100 ppm
Ts (*T. asperellum*)	3.72BC	4.41C	3.24B	4.28C
Th (*T. harzianum*)	3.96A	5.06A	3.57A	4.88A
T22 (Unidentified species)	3.84AB	4.45C	3.24B	4.37C
T69 (*T. harzianum*)	3.61C	4.88B	3.24B	4.75AB
Tk (*T. koningii*)	4.00A	4.75B	3.72A	4.67B
Grand mean	3.82	4.71	3.40	4.60
Lsd	0.00	0.00	0.00	0.00
CV	3.14	2.08	2.93	2.40
P value	***	***	***	***

Data are mean of three replicates. Figures followed by different letters along the column are significantly different ($p<0.001$)

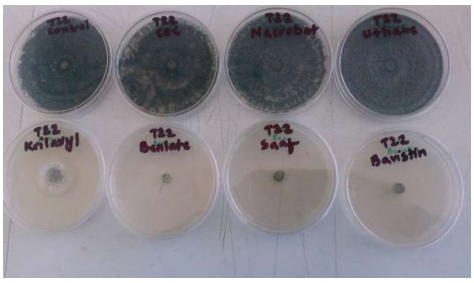

Figure 1a. Growth of *Trichoderma* isolate T22 in different chemical treated plates

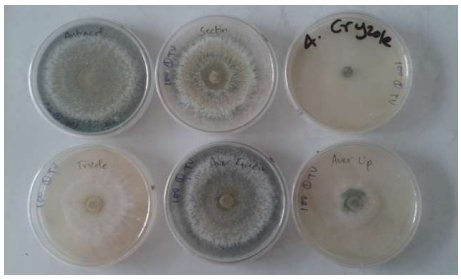

Figure 1b. Growth of *Trichoderma* isolate Tv in different chemical treated plates

Carbendazim, Benomyl, Hexaconazole even when tested at very low concentrations (<100 ppm) have been found to be inhibitory to *Trichoderma* growth by several authors (Sirohi et al., 2009; Bagwan, 2010; Madhusudhan et

al., 2010; Ranganathswamy et al., 2012; Kumar and Mane, 2017). Sushir et al. (2015) also found Benomyl as most toxic against *T. harzianum*. The fungicides seen as completely inhibitory in our study had the same chemicals as active ingredients. Variability of different *Trichoderma* strains in their response to chemicals has previously been explained by several authors (Chaparro et al., 2011; Silva et al., 2018). Hence it is of great significance to test the compatibility of bioagents and chemicals to be used in integration. There are also *Trichoderma* tolerant strains that can survive field concentrations of chemical fungicides and insecticides (Chaparro et al., 2011; Tang et al., 2009). Reports of mutant *Trichoderma* strains are also available, which are even resistant to strong chemicals, like benzimidazole (Mukherje et al.,1999; Mutawila et al., 2015).

The compatible chemicals seen in this study have also been reported as safer chemicals by previous authors (Bagwan, 2010; Gaur and Sharma, 2010; Madhusudhan et al., 2010). But Ranganathswamy et al. (2012)found Tricyclazole and Chlorothalonil as inhibitory to *T. harzianum* and *T. virens*contrary to our result. Sirohi et al. (2009) also found that chemicals copper oxycholride and metalaxyl at lower concentrations upto 100 ppm were compatible with *Trichoderma* but the same chemicals at higher concentrations exhibited inhibition of the bioagent. The chemicals at concentrations of 50ppm and 100 ppm were only evaluated in our experiment and hence it may be needed to test the chemicals at higher concentrations as well. Colony diameter of *Trichoderma* sp. was also seen to increase after 7 days of inoculation as compared to 3 days for most of the chemicals and the same pattern of increasing compatibility with time was reported by Khirallah et al. (2016).

The combined effect of *Trichoderma* along with chemicals has also been tested *in vivo* by several authors. Venkateswarlu et al. (2008) found that seed treatment with T. *virens* @ 4g and mancozeb @ 2g/kg seed was able to reduce the wilt incidence in tomato (caused by *F. oxysporum*f. sp. *lycopersici*) to greater extent than using the treatments alone. Similarly, Pandey and Upadhyay (1999) in their study found that seed treatment with *T. viride* and thiram gave maximum disease control of 81% in pigeonpea while combined use of *T. harzianum* and thiram provided 68% wilt control in pigeonpea. Sharma et al. (2003) reported the combined use of *Trichoderma* spp. and thiram as the most effective treatment in reducing linseed wilt caused by *F. oxysporum*f. sp. *lini*.

Trichoderma provides long term protection against pathogens and chemicals provide short term control. However, *Trichoderma* may take time to establish in newer ecological niches and colonise the roots to provide protection. Chemicals on the other hand act in shorter time (Mutawila et al., 2015) but

the disadvantages of excessive use of chemical pesticides is not new to anyone's knowledge. Hence combining *Trichoderma* and safer chemicals can be an effective tool for the management of diseases, where chemicals are needed in lesser doses and the threat of resistance development in pathogens can be minimized (Bhatnagar and Kumari, 2013). The compatibility of *Trichoderma* with the afore mentioned chemicals opens up probability for the integrated use of *Trichoderma* and chemicals for plant disease control so as to reduce chemical pesticide over exploitation in commercial farming.

CONCLUSION

The present finding shows that some chemical fungicides inhibit growth of *Trichoderma* spp. and some are compatible with *Trichoderma*. The chemicals compatible with *Trichoderma* spp. can be selected to be used in combination for the integrated disease management of agricultural crops. Variation among the *Trichoderma* isolates with regard to their response to chemicals is also possible and hence it is better to test the compatibility of individual *Trichoderma* spp. to be used in integration rather than generalize the effect of chemicals on *Trichoderma* growth. The combined use of bioagent and chemical can provide immediate control over pathogens and for a longer period of time. Field trials with combination of the *Trichoderma* spp. and compatible chemicals found in this study needs to be carried out to find out the effect on disease control.

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