



Evaluation of *trichoderma viride* and *trichoderma harzianum* consortia compatibility with chemical fungicides for integrated management of soil borne diseases

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Abstract

A concurrent and promising effect has been achieved by using microbial inoculants on bio control in recent agricultural practices, an environment friendly as future concern to food safety. The present study aims to evaluate the mycelia growth of *Trichoderma viride* and *Trichoderma harzianum* consortium on Potato dextrose agar enriched with different chemical fungicides used to control wilt diseases in many vegetables. It is evident from the data presented that 100 % inhibitory effect was showed in Carbendazim 50 % WP. Highest radial mycelia growth was observed 43.45 mm, 29.89 mm after 72 hr incubation in presence of EDTA-Copper 12% WP and Zineb 75 % WP@ 1 g/L and 0.5 g/L with 3.2 and 33.4 percent inhibition, respectively. The present investigation clearly demonstrates that, the high incompatibility was observed in the treatment of systemic fungicides at all concentrations. Therefore, soil borne plant pathogens can be well managed by combined application of biopesticides with systemic or contact fungicides like EDTA-Cu, Zineb at lower doses. *Trichoderma* consortia compatible with Copper EDTA and Zineb as GAP in IPM.

Keywords: Biocontrol, consortia, mycelia, inhibitory, IPM, GAP

1. Introduction

Biological control of plant pathogens is considered as a potential control strategy in recent years, because chemical control results in accumulation of harmful chemical residues, which may lead to serious ecological problems. Now days, effective management of plant diseases & microbial contamination in agricultural field is generally controlled by the use of chemical pesticides. However, the high & indiscriminate use of these chemical fungicides has caused health problems in animals & humans, due to residual toxicity. Because of their undesirable effects such as high and acute toxicity, large numbers of chemical fungicides have been banned in western countries. Many pathogenic microorganisms have developed resistance against chemical fungicides. Due to this the management of diseases on agricultural plants is difficult. Now a day's eco-friendly pest management in sustainable agriculture has been well recognized. Fungi belonging to the genus *Trichoderma*, and bacteria such as *Pseudomonas* and *Bacillus* are the most promising bio control agent against a range of plant pathogens under a variety of environmental conditions. Among the microbial bio-control agents *Trichoderma viride* and *Trichoderma harzianum* are commonly used for the management of most of the diseases in vegetable and horticultural Crops. *Trichoderma* genus is a group of antagonistic cellulolytic fungi, capable of controlling a number of diseases. They are considered as biocontrol agent of foliar diseases and soil borne diseases (Papavizas, 1985) [1].

As an antagonist, *Trichoderma* spp. produce antibiotics and antifungal toxic metabolites and also inhibit pathogens by secreting enzymes like gluconase, cellulase, chitinase, protease etc, which disintegrate the cell wall of pathogen and act as a competitor for mineral nutrients. Generally application of bio-control agents simply leads to

inconsistent performance because a single bio-control agent is not likely to be active against all kinds of soil environments and agricultural eco-systems. In such conditions the use of combination of strains which have different modes of action will be more useful to improve suppression. A combination of *Trichoderma viride* and *Trichoderma harzianum* were having more effect on the control. Supplementation of fungicides at reduced rates in combination with biocontrol agents has significantly enhance disease control, as compared to treatments' with biocontrol alone. The objectives of the present study is to evaluate the growth of *Trichoderma* consortia with commonly used fungicides at different concentrations under in vitro conditions which are commonly used for the control of soil borne pathogen.

2. Materials and Methods

Three fungicides viz. Carbendazim 50 % WP, EDTA copper 6 % WSP & Zineb 75 % WP were procured from local pesticide shop. Biocontrol agent *Trichoderma viride* and *Trichoderma harzianum* procured from Pratik Industries, Kupwad MIDC Sangli (MH). In-vitro studies were carried out under aseptic conditions in laboratory. Sub culturing and inoculation work were carried out in laminar air flow before start working LAF platform was sterilized by glowing ultraviolet light for half an hr prior. All glassware's such as petriplates, beakers, conical flasks, test tubes were sterilized in hot air oven at 180⁰ C and culture medium were sterilized in autoclave at 121⁰ C, 15 lbs pressure for 15 minutes.

Preparation of Consortium

Preisolated, pure and Sub cultured *Trichoderma viride* and *Trichoderma harzianum* in PD broth as mother culture were used for the preparation of consortium. Equal amount of *Trichoderma viride* and *Trichoderma harzianum* were

mixed aseptically and stored under room temperature. This mixed culture was used for further studies.

The Poisoned Food Technique

Compatibility of *Trichoderma viride* and *Trichoderma harzianum* with three fungicides viz. Carbendazim 50 % WP, EDTA copper 6 % WSP & Zineb 75 % WP @ 0.5g/L, 1g/L, 1.5g/L, 2g/L were tested by using Poison Food Technique. Required concentration of each fungicide was added to an autoclaved Potato Dextrose Agar (PDA- Merck) to obtain the desired concentration of all fungicides. The same medium PDA without fungicide served as Control.

Screening of *Trichoderma* consortia with different Fungicides

The culture medium was poured into sterilized 90 mm Petri plates in 3 replicates and on solidification, each Petri plate was inoculated with 5mm filter paper disc dipped in prepared consortia with active mycelia and spores of *Trichoderma viride* and *Trichoderma harzianum*. The inoculated Petri plates were incubated for 7 days at 27±2°C temperature. After incubation radial growth was measured with digital Vernier Caliper and percent growth inhibition

was calculated by applying the formula (Vincent, 1947) [2]

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

Where, C= Growth of fungus in Control in mm
T= Growth of fungus in treatment in mm

3. Results and Discussions

The results were depicted in table 1 and figure 1 indicated that growth of *Trichoderma viride* and *Trichoderma harzianum* consortium was compatible with Zineb 75 % WP @ 0.5 g/L by showing lowest percent inhibition. Where as maximum inhibition with Carbendazim 50 % WP @ 0.5 g/L at 72 hrs. The compatibility test of *Trichoderma* consortium revealed that all four treatments 0.5,1,1.5 & 2 g/L Carbendazim were most sensitive and resulted in total inhibition but EDTA-Cu and Zineb shown more compatibility with fungicide dose 0.5 & 1 g/L with better radial growth 42.20, 43.45 and 29.89, 23.98 mm respectively with 6.0, 3.2 & 33.4, 44.6 % inhibition.

Table 1: Compatibility of *Trichoderma* consortium with different concentrations of different fungicides

Treatments	Fungicide Dose g/l	Radial growth of mycelia (mm)						Percent inhibition					
		EDTA-Cu 6% WSP		Zineb 75 % WP		Carbendazim 50 % WP		EDTA-Cu 12% WSP		Zineb 75 % WP		Carbendazim 50 % WP	
		48h	72h	48h	72h	48h	72h	48h	72h	48h	72h	48h	72h
T1	0.5	33.99	42.20	13.2	29.89	0.0	0.0	5.2	6.0	16.6	33.4	100	100
T2	1.0	33.40	43.45	8.5	23.98	0.0	0.0	6.8	3.2	33.1	44.6	100	100
T3	1.5	32.96	42.04	4.6	20.74	0.0	0.0	8.1	6.4	42.1	53.8	100	100
T4	2.0	32.60	41.50	0.0	10.15	0.0	0.0	9.0	7.6	71.6	77.4	100	100
Control	Nil	35.86	44.92	35.86	44.92	35.86	44.92	0	0	0	0	0	0
Mean (T)		33.23	42.29	6.57	21.19	0.0	0.0	7.2	5.8	40.8	52.3	100	100
SEM±		0.32	0.41	0.06	0.20	0	0	0.07	0.05	0.39	0.51	0.97	0.97
CD@5%		1.66	2.11	0.32	1.05	0	0	0.36	0.29	2.04	2.61	5.00	5.00



Fig 1: Radial mycelia growth and its measurement with Vernier Caliper

These finding are correlates with Upadhyay *et al.*, (2004) [3] found that Bavistin (Carbendazim @ 25 g/l/L) resulted complete inhibition of growth and sporulation of *Trichoderma viride* In-vitro. Thoudam and Dutta (2014) [4] found the radial mycelia growth of *Trichoderma* was 100 % inhibited in Carbendazim. Similar results are reported by

Bagwan NB (2010) [5] that Thiram, copper oxychloride and Mancozeb at 0.2 % are compatible with *Trichoderma harzianum* and *Trichoderma viride*. 100 ppm, 200 ppm and 300 ppm concentration of Dithane, Carbendazim and Ridomil were highly compatible, but Blue copper and Captaf were highly insensitive to *Trichoderma spp.* (Tapwal

A, Kumar S, Gautam N, Pandey S 2012)^[6]. But Muthamilan and Jeyarajan (1996)^[7] reported that *T. harzianum*, *Rhizobium* and carbendazim reduced the groundnut root rot caused by *S. rolfsii*. Ramarethinan *et al.*, (2001)^[8] reported that the fungicides like Carbendazim (50% WP) completely inhibited the growth of *Trichoderma* In –vitro. Our demonstration clearly showed that at higher concentrations of EDTA-Cu 12 % and Zineb 75 WP directly proportional to the growth inhibition of *Trichoderma* consortium (*Tv +Th*) as 42.04 mm, 41.50 mm and 20.74 mm, 10.15 mm with 6.4, 7.6 & 53.8, 77.4 % growth inhibition at 1.5, 2 g/L fungicide application. These fungicides are in agreement with Mukhopadhyay *et al.*, (1987)^[9] Sharma and Mishra, (1995)^[10] they reported that considerable growth of *Trichoderma* isolates at low and medium concentration of various fungicides. Contact fungicide Zineb 75 WP at selected concentration of 500,100 & 1500 ppm were found to be safer to *Trichoderma harzianum* isolates than systemic fungicides like Carbendazim 50%WP (Sonavane P and Venkataravanappa, 2017)^[11].

4. Conclusion

Biocontrol involves the use of biological organisms to control the insect pest or causal organism responsible for the diseases. The microbial inoculants as bio control agents are effective, economical, easy to apply and environment friendly alternative in present and future concern to food safety. Present study aims to evaluate the mycelia growth of *Trichoderma viride* and *Trichoderma harzianum* consortium on Potato dextrose agar enriched with different chemical fungicides. It is evident from the data presented that 100 % inhibitory effect was showed in Carbendazim 50 % WP. Highest radial mycelia growth was observed 43.45 mm, 29.89 mm after 72 hr incubation in presence of EDTA-Copper 12% WP and Zineb 75 % WP@ 1 g/L and 0.5 g/L with 3.2 and 33.4 percent inhibition, respectively. The present investigation clearly demonstrates that, the high incompatibility was observed in the treatment of systemic fungicides at all concentrations. Therefore, soil borne plant pathogens can be well managed by combined application of biopesticides with systemic or contact fungicides like EDTA-Cu, Zineb at lower doses. *Trichoderma* consortia compatible with Copper EDTA and Zineb as GAP in IPM. So, soil borne plant pathogens can be well managed by combined application of biopesticides with systemic or contact fungicides like Copper, Zineb at lower doses. Hence pesticide residue in soil and in or on fruits, vegetables will not affect the effectiveness of biocontrols and it may be a better tool for Integrated Diseases Management practices.

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