

# Compatability Studies with fungicides, insecticides and their combinations on *Trichoderma viridae* in invitro conditions

P. Vasundara, V. Rangaswamy, and M. Johnson.

**Abstract**— Invitro (lab) experiments were conducted to check the compatibility of two insecticides, three fungicides and their combinations on *Trichoderma Viridae*. It shows variable responses against the tested pesticides (fungicides and insecticides) and their combinations at recommended concentrations for field studies. The treatments of Mancozeb (3000 ppm), Imidacloprid (2000 ppm) and combination of Mancozeb (3000 ppm) + Imidacloprid (2000 ppm) showed high compatibility with *Trichoderma Viridae* by showing 7, 11 and 11 percent growth inhibition respectively. The treatments viz. Carbendazim (1000 ppm) + Chlorpyrifos (6000 ppm), Tebuconazole (1000 ppm) + Imidacloprid (2000 ppm) and Tebuconazole (1000 ppm) + Chlorpyrifos (6000 ppm) showed high incompatibility with 100 percent growth inhibition. While moderate compatibility were recorded in the treatments of Chlorpyrifos (6000 ppm) with 68 percent, Tebuconazole (1000 ppm) with 60 percent, Carbendazim (1000 ppm) + Imidacloprid (2000 ppm) with 57 percent, Mancozeb (3000 ppm) + Chlorpyrifos (6000 ppm) with 55 percent, carbendazim (1000 ppm) alone with 55 percent growth inhibition respectively.

**Keywords**— Fungicides, Insecticides, *Trichoderma viridae*, Compatibility, Invitro.

## 1 INTRODUCTION

Biological control of soil borne plant pathogens by species of *Trichoderma* is a vital area of plant pathological research all over the world in these days (Mukhopadhyay, 1987). Most of the soil borne diseases are not amenable for management through chemicals. Use of several antagonistic species of *Trichoderma* (*Trichoderma. viride*, *Trichoderma. harzianum* and *Trichoderma. virens*) against a range of economically important soil borne plant pathogens has been well documented (Cook and Baker, 1983; Chet, 1987; Raghuchander et al., 1997; Anitha and Tripathi, 2000; Mukerjee et al., 2001). *Trichoderma* species are known to utilize a wide variety of carbon and nitrogen sources for their growth and activity in soil. In the present day agriculture, the usage of pesticides has become an indispensable component. Seed treatment with combination of fungicides, insecticides and bioagents is the common method in groundnut Crop. Application of pesticides generally disturbs and alters the biological equilibrium in soil. The effect of pesticides on the growth and activity of the antagonistic fungi and bacteria has not been clearly studied yet.

The beneficial effects of the *Trichoderma viridae* is that it establishes symbiotic rather than parasitic relationships with the plant, by increasing plant growth and productivity, helping to

overcome stress stimulations, and improving nutrient absorption (Harman et al., 2004). Species of the *Trichoderma* genus are able to inhibit the growth of variety of potentially pathogenic fungi. A recent list of mechanisms are viz., mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogens enzymes (Lewis and Lumsden, 2001). Growth promotion due to *Trichoderma* spp. is also reported in several crop species (Manoranjitham et al., 1999).

In recent years, the search of biological control agents for the management of dreaded soil borne diseases has been advocated widely. Since, the biocontrol agents are applied either to seed or soil or both, there is every possibility of interaction and interference that would arise with the commonly used agrochemicals applied to seed, soil or both. The full expression of potential biocontrol is considered in terms of rhizosphere competence, suppression of pathogens, tolerance to pesticides, competitive saprophytic ability, adaptability to environment etc. Combined application of biocontrol agents with commonly used fungicides and insecticides may result either in synergism / antagonism between the two. However, in view of the complexities arising from the use of chemical pesticides, such as harmful effect on environment and non-target organisms including man, domestic animals, beneficial insects, wild life, the use of micro-organisms as biocontrol agents has provided a very promising alternative and less hazardous method for plant disease control. Antagonists may act against pathogens in one or more of the following mechanisms. Competition, antibiosis, parasitism and predation or induce resistance in plant of hydrolytic en-

- P.Vasundara currently pursuing doctoral research in microbiology in Sri Krishnadevaraya University, Anantapur, Andhrapradesh, India, PH: +91-9908625822. E-mail: vasu\_micro@yahoo.com
- Prof. V.Rangaswamy: Head, Department of microbiology, Sri Krishnadevaraya University, Anantapur, Andhrapradesh, India.
- M.Johnson: Principle Scientist, Department of plant pathology, Agriculture research station, Rekulakunta, Anantapur, Andhrapradesh, India. E-mail: johnson.manda@rediffmail.com

zymes excreted by antagonists are a well-known feature of mycoparasitism (Henis and Chet, 1975). Though, fungicides have enormous killing capacity but indiscriminate use of fungicides is not only hazardous to living being but disrupt the natural ecological balance by killing the beneficial soil microbe (Ansari, 1995). Though few studies about the sensitivity of biocontrol agents with certain fungicides and insecticides are available, studies / reports with special reference to commercially available biocontrol agents of *Trichoderma* and *Pseudomonas* are meager. Compatibility of living organisms with modern inputs in plant protection like fungicides, insecticides is a pre-requisite for disease management and increasing plant growth. Hence, there is need to test the compatibility studies on seed treating fungicides, insecticides and their combinations on *Trichoderma viridae* a biological control agent in invitro conditions as one of the objective for this study.

## 2 Materials and Methods:

The commercial biocontrol agent *Trichoderma viridae* in the form of talc was collected from biocontrol Laboratory, Agriculture research station, Rekulakunta, Ananthapuramu. Compatibility tests were conducted under invitro condition to check the compatibility of fungicides, insecticides and their combinations on *Trichoderma viridae*. The general laboratory techniques followed for the present study were those described by Nene and Thapliyal (1993), Dhingra and Sinclair (1995) and Aneja (2001) for the preparation of media, sterilization and maintenance of fungal cultures with slight modification wherever necessary. TSM (*Trichoderma* Selective Medium) was used for isolation of *Trichoderma viridae*. To isolate *Trichoderma viridae* from the commercial formulations, 4 g of the commercial formulation of the isolate was added to 100 ml sterile distilled water and 0.5 ml of the preparation was aseptically transferred into *Trichoderma* selective medium (The medium was prepared by adding required quantities of the components in 1000 ml distilled water and was sterilized in an autoclave at 15 kg / cm<sup>2</sup> (121.6 °C) for 20 minutes. This medium was used for isolation of *Trichoderma* spp. from commercial formulations) containing plates. The inoculated plates were incubated at 28±2 °C for one week and the resultant *Trichoderma* colonies were isolated and reidentified. Cultures of *Trichoderma* spp. were maintained on PDA by periodic transfers for further studies.

Efficacy of three fungicides, two insecticides and their combinations at recommended concentrations (Table 1) were evaluated against the *Trichoderma viride* by poisoned food technique as described by Dhingra and Sinclair (1995).

Table.1: Details of fungicides and insecticides evaluated

for the compatibility of *Trichoderma viridae*.

S. No.	Trade name	Chemical name	Active ingredient	Concentration (ppm)
1	Indofil M-45	Mancozeb	75% WP*	3000
2	Bavistin	Carbendazim	50% WP	1000
3	Raxil	Tebuconazole	2% DS	1000
4	Confidor	Imidacloprid	17-18% SL	2000
5	Force	Chlorpyrifos	20% EC	6000

\* WP: Wettable powder; DS: powder for dry seed treatment; SL: soluble liquid and EC: emulsifiable concentrate.

The chemicals were tested at recommended doses as used in the field experiment for each treatment 120 ml of potato Dextrose Agar (PDA) medium was taken in 250 ml conical flask and autoclaved. To this medium required concentrations of the chemicals viz, fungicides (Mancozeb 3000 ppm, Carbendazim 1000 ppm and Tebuconazole 1000 ppm) insecticides (Imidacloprid 2000 ppm, chlorpyrifos 2000 ppm) and the combination of fungicides insecticides were added at lukewarm temperature and mixed thoroughly by shaking the flask the poisoned medium distributed equally into six petriplates which were treated as six replications and allowed to solidify. The experiment was conducted in a complete randomized design (CRBD) with twelve treatments presented in table 2.

The antagonist *Trichoderma viride* was cut into 5 mm discs from the periphery of actively growing colony with sterilized cork bore and transferred to the centre of each plate containing poisoned medium (different chemicals) control was maintained by placing *Trichoderma viride* discs in plates containing untreated (not poisoned) medium. For this treatment 120 ml of potato dextrose agar (PDA) medium was taken in 250 ml conical flask and autoclaved. The non poisoned medium (serves as control) was distributed equally into six petriplates, which were treated as six replications and allowed to solidify. All the inoculated petriplates were incubated at 28±2 °C in BOD incubator. The colony diameter of *Trichoderma viride* in the treatments was measured and compared with check (control) and reduction in growth was taken as a measure of toxicity. Percent inhibition of the growth of biocontrol agent over the control was calculated by using the following formula.

$$I = (C - T) / C \times 100$$

Where I= percent inhibition

C= colony diameter at biocontrol agent in control

T=colony diameter at biocontrol agent in treatment

## 3 Stastical analysis:

The data obtained in these experiments were statistically analyzed by using completely randomized design (CRD). The

data pertaining to percentages were angularly transformed. (Table 2) Results were analyzed by following appropriate statistical methods as per the procedure suggested by Panes and Sukhatme (1978).

#### 4 Results and Discussion:

In vitro compatibility tests were done with three fungicides, two insecticides and their combinations on *Trichoderma viridae*. Among the treatments the mean radial growth of *Trichoderma Viridae* varied from 0.0 to 9.0 cm (Fig-1). It is evident from the data presented in table 2, mancozeb (Fig-2) showed more compatibility with *Trichoderma Viridae* and luxuriant growth of antagonist was found in all the petriplates containing poisoned medium and the observed mean radial growth of *Trichoderma viridae* was 8.4 cm with 7 percent growth inhibition, combination of mancozeb and imidachloprid, imidachloprid alone (Fig-3) are also showed compatibility by recording radial growth of 8.0 cm and 8.0 cm, growth inhibition percentage in both treatments is 11 percent. All these three treatments mancozeb, imidachloprid and combination of mancozeb + imidachloprid treatments are on par with control agent *Trichoderma Viridae* (Fig-4) and were significantly superior over all other treatments. Combination of the treatments carbendazim and chlorpyrifos (Fig-5), Tebuconazole and imidachloprid (Fig-6), Tebuconazole and chlorpyrifos (Fig-7), shows high incompatible with *Trichoderma Viridae* and the observed mean radial growth was of 0.0 cm and 100 percent growth inhibition was recorded. Carbendazim, Tebuconazole (Fig-8), Chlorpyrifos, alone and combination of mancozeb and chlorpyrifos (Fig-9), carbendazim and imidachloprid (Fig-10), showed moderate compatibility with *Trichoderma viridae*. The mean radial growth recorded in these treatments were 4.1 cm with 55 percent growth inhibition, 3.6 cm with 60 percent growth inhibition, 2.9 cm with 68 percent growth inhibition, 4.0 cm with 55 percent growth inhibition, and 3.9 cm with 57 percent growth inhibition respectively.

Ramarethinam et al. (2001) reported that the fungicides like carbendazim (50% WP), hexaconazole (5% EC) completely inhibited the growth of *Trichoderma viridae* centration in vitro. Desai et al. (2002) also reported that mancozeb at 500 ppm recorded a lower inhibition of hyphae (5.70%) and sporulation (11.02%) of *Trichoderma harzianum*. The results are also in agreement with the works of Mukhopadyay et al. (1986) Sharma and Mishra (1995); Abha Agarwal and Tripathi (1999), who also found good growth of *Trichoderma* isolates at low and medium concentrations of various fungicides.

These results were similar to the findings of Tiwari et al. (2004) studied non target effect of insecticide on mycelial growth of *Trichoderma harzianum* and reported that chlorpyrifos and carbosulfan was highly inhibitory to the growth of

*Trichoderma harzianum* while imidachloprid was found to be highly compatible at 500 and 1000 ppm concentrations. Study on the compatibility of diafenthiuron with antagonistic microorganisms of plant pathogens viz., *Trichoderma viride* and *Pseudomonas fluorescens* revealed that diafenthiuron had some inhibitory effect on the mycelial growth of *Trichoderma viride*. Diafenthiuron did not affect the growth of *Pseudomonas fluorescens* (Stanley et al., 2010). These results were similar to the reports of Bagwan (2010) who reported that mancozeb was found comparatively safer against *Trichoderma harzianum* and *Trichoderma viride*. These results are similar to the reports of Madhavi et al., (2011) who reported that *Trichoderma viride* showed a high compatibility with the insecticide imidachloprid (7.6 cm mycelial growth) followed by mancozeb (6.3 cm) and tebuconazole (3.7 cm).

Anitha et al. (2001) screened carboxin and metalaxyl against fungal and bacterial antagonists in the laboratory and found that carboxin and metalaxyl did not inhibit the growth of *Trichoderma viride*, while little inhibition of *Gliocladium virens* was noticed at 0.1 per cent concentration of carboxin.

Kumar (1998) reported that there was significant reduction in the radial growth and sporulation of *Trichoderma viride* when tested at 7000 ppm of imidachloprid. Martinez and Toledo et al. (1992) also reported cent per cent inhibition of both hyphae and sporulation of native isolate of *Trichoderma viride* with chlorpyrifos in addition to methyl pyrinofos. Similar results were also obtained by Bhat and Sabalpara, (2001); Prasanna et al., (2002); Desai et al. (2002).

Girija and Umamaheswaran (2003) reported the compatibility of *Trichoderma virens* with carbendazim invitro at three concentrations (100, 500 and 1000 ppm) concentrations and observed that the antagonist *Trichoderma virens* was compatible with carbendazim at 100 ppm concentration.

Bagwan (2010) studied that compatability tests were conducted under invitro condition to find out safer fungicides, pesticides, different cakes and botanicals against *Trichoderma*. For this different fungicide, pesticides, cakes and botanicals were tested against *Trichoderma harzianum* (Th 09) and *Trichoderma viride* (Tv 11). Results indicate that among the fungicides tested, thiram (0.2%), copper oxychloride (0.2%) and mancozeb (0.2%) were found comparatively safer against *Trichoderma harzianum* and *Trichoderma viride* as compared to other fungicides. *Trichoderma* was most sensitive to captan, tebuconazole, vitavax, propiconazole and chlorothalonil. But *Trichoderma* was tolerant to all the pesticides and weedicides tested. None of the pesticide and weedicide inhibited the growth of *Trichoderma*. Among the botanicals tested, 10% fresh leaf extract of karanj leaves (*Pongamea pinnata*) and cumin leaves inhibited 32.19% 27.15% growth of *Trichoderma*, respectively as compared to control. Another interesting thing observed that,

neem oil (5%), neem leaves extract (10%), wild sorghum leaves extract (10%), neem cake, castor cake and mustard cake extract (10%) enhanced the growth of *Trichoderma*. This finding indicates that seed treatment or furrow applications of *Trichoderma* would be compatible with thiram, copper oxychloride, mancozeb, pesticides, weedicides, neem oil, neem leaves extract, wild sorghum leaves extract, neem cake, castor cake and mustard cake extracts for the integrated management of soil borne diseases of groundnut.

Shukla (2011) reported the compatibility of *Trichoderma viride* with Bavistin (0.1 %) and carbosulfun (0.05%) recorded cent percent inhibiting in the growth and sporulation of the fungus. Similar observation were made by Bheemaraya et al (2012) of the five fungicides tested at 0.1 and 0.2 percent concentration, metalaxyl-M + mancozeb and mancozeb were compatible with growth of *Trichoderma* spp. while carbendazim, captan and propiconazole completely inhibited radial mycelial growth hence, were not compatible. Among insecticides evaluated at 0.1 and 0.2 percent concentration, imidacloprid showed little compatibility with isolates. But, chlorpyrifos, carbofuran and indoxacarb were highly incompatible. Hilmdia (imidachloprid) was found to be most compatible with V1 strain of *Trichoderma harzianum* in both the liquid media as it shows nil percentage reduction of mycelium. It was concluded that Decis (Deltamethrin), Hilcron (Monocrotophos), Hilmdia (imidachloprid) and Rogar (Dime-thoate) are compatible insecticides with *Trichoderma harzianum*. While some insecticides viz., Ekalux (Quinolpos), Marshal (Carbosulfan), and Rocket (Profenophos+ Cyper-metrin) inhibits the growth of *Trichoderma* spp. (Vinit Pratap Singh et al., 2012). Nadeesha et al., (2013) reported that, out of four systemic and two non-systemic fungicides tested under in vitro for compatibility with potential bioagent, mancozeb was found highly compatible with *Trichoderma* spp. (TAG-2). The differential response of *Trichoderma viride* to various fungicides, insecticides and their combination in the present study might be due to their inherent resistance to the fungicides, insecticides and their ability to degrade these chemicals.

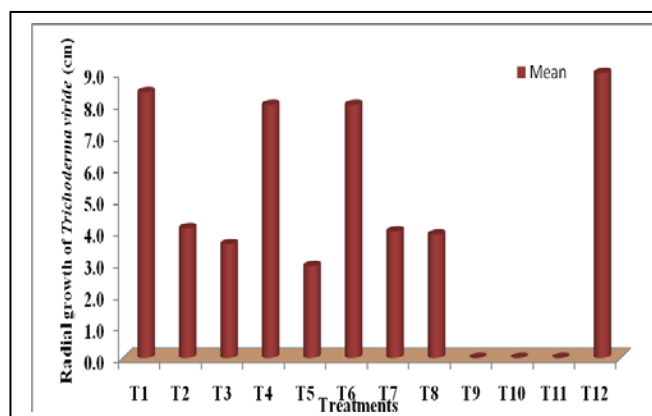


Fig-1: In vitro evaluation of fungicides and insecticides and their combinations on *Trichoderma viride*.

Treat ment	Fungicides / Insecticides	Con- centra- tion (PPM)	Radial growth of Tricho- derma viride (cm)				% Inhibi- tion over control
			R1	R2	R3	Mean	
T1	Mancozeb	3000	8.5	8.6	8.2	8.4* (16.9) **	7
T2	Carbendazim	1000	4.2	4.0	4.2	4.1 (11.7)	55
T3	Tebuconazole	1000	4.4	3.3	3.2	3.6 (10.9)	60
T4	Imidachloprid	2000	8.0	7.8	8.2	8.0 (16.4)	11
T5	Chlorpyrifos	6000	3.0	2.7	2.9	2.9 (9.7)	68
T6	T1 + T4	3000+ 2000	5.0	4.8	5.5	8.0 (16.4)	11
T7	T1+ T5	3000+ 6000	4.0	3.7	4.2	4.0 (11.5)	55
T8	T2+ T4	1000+ 2000	4.0	3.8	3.8	3.9 (11.3)	57
T9	T2+ T5	1000+ 6000	0.0	0.0	0.0	0.0 (0.0)	100
T10	T3+T4	3000+ 2000+ 6000	0.0	0.0	0.0	0.0 (0.0)	100
T11	T3+T5	1000+ 2000+ 6000	0.0	0.0	0.0	0.0 (0.0)	100
T12	Trichoderma viride	-	9.0	9.0	9.0	9.0 (17.5)	-
CD (1%)				0.61			
CV (%)				4.49			

Table 2: In vitro evaluation of fungicides and insecticides and their combinations on *Trichoderma viride*.



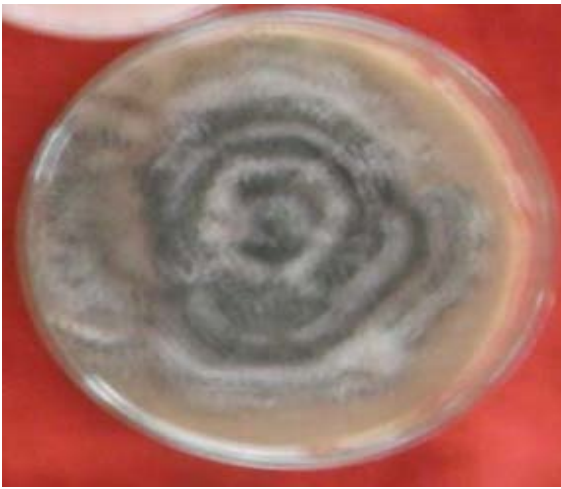


Fig-2: In vitro evaluation of mancozeb fungicide (T1) on *Trichoderma viride*.



Fig-5: In vitro evaluation of combination of Carbendazim + Chloropyriphos (T9) on *Trichoderma viride*.



Fig-3: In vitro evaluation of imidacloprid insecticide (T4) on *Trichoderma viride*.

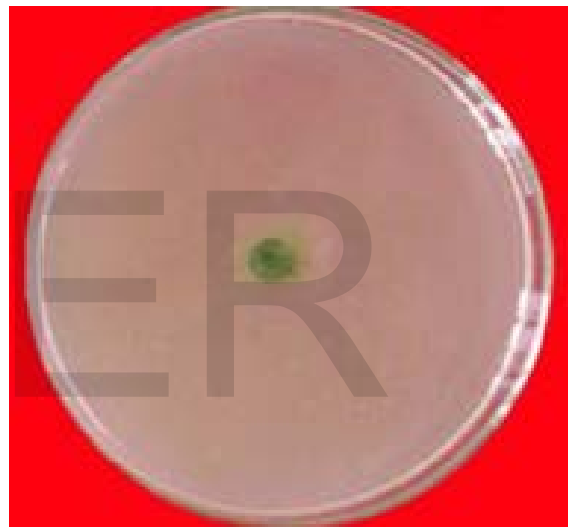


Fig-6: In vitro evaluation of combination of Tebuconazole + imidacloprid (T10) on *Trichoderma viride*.



Fig-4: In vitro evaluation of *Trichoderma viride* (T12).

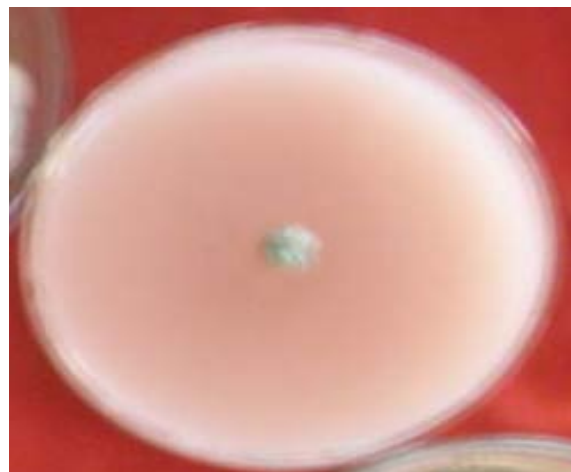


Fig-7: In vitro evaluation of combination of Tebuconazole + chloropyriphos (T11) on *Trichoderma viride*.



Fig-8: In vitro evaluation of Tebuconazole fungicide (T3) on *Trichoderma viride*.



Fig-9: In vitro evaluation of combination of Mancozeb + chlorpyrifos (T7) on *Trichoderma viride*.



Fig-10: In vitro evaluation of Carbendazim + Imidacloprid insecticide (T8) on *Trichoderma viride*.

## 5 CONCLUSION

Present findings indicated that seed treatment of *Trichoderma viridae* would be high compatible with fungicide mancozeb at 3000 ppm concentration, followed by combination of mancozeb with imidacloprid 3000 + 2000 ppm, imidacloprid at 2000 ppm concentration respectively. High incompatibility was observed in the treatments of Carbendazim (1000 ppm) + Chlorpyrifos (6000 ppm), Tebuconazole (1000 ppm) + Imidacloprid (2000 ppm) and Tebuconazole (1000 ppm) + Chlorpyrifos (6000 ppm). Moderate compatibility were recorded in the treatments of Chlorpyrifos (6000 ppm), Tebuconazole (1000 ppm), Carbendazim (1000 ppm) + Imidacloprid (2000 ppm), Mancozeb (3000 ppm) + Chlorpyrifos (6000 ppm), carbendazim (1000 ppm) alone.

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