Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 1271-1276
Received: 21-09-2018
Accepted: 24-10-2018

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Compatibility of biocontrol agents with fungicides used in turmeric cultivation under *in vitro* conditions

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Abstract
An experiment was conducted to study the compatibility of fungicides with bacterial and fungal biocontrol agents under *in vitro* conditions with seven fungicides commonly used in management of turmeric diseases by poisoned food technique for fungi, inhibition zone technique for bacteria. Each fungicide was tested at three concentrations *viz.*, 0.05%, 0.1% and 0.2%. The results of the study proved that, bordeaux mixture and carbendazim (12%) + mancozeb (64%) were incompatible and showed cent percent inhibition on the growth of the fungal antagonist. Cymoxanil (8%) + mancozeb (64%) at 0.1 per cent concentration recorded lowest inhibition of 13.58 per cent on the growth of the fungal bioagent. Mancozeb (4%) + metalaxyl-M (64%), carbendazim (12%) + mancozeb (64%), Cymoxanil (8%) + mancozeb (64%), mancozeb, lower concentration of fenamidone (10%) + mancozeb (50%) were compatible with bacterial antagonist. The other fungicides recorded the inhibition in the growth of the bacterial antagonist in the range of 9.5 to 21.35 per cent. Among copper fungicides, bordeaux mixture was more inhibitory than copper oxychloride (50%) with bacterial antagonist.

Keywords: *Trichoderma viride, Pseudomonas fluorescens,* fungicides, compatibility, *in vitro,* turmeric diseases, inhibition zone technique, poisoned food technique

Introduction
Different biological control agents (BCAs) can be used for the control of plant diseases. These include fungi, bacteria and actinomycetes. The most important BCAs belong to the genus *Trichoderma* species, *Bacillus* species, *Pseudomonas* species and streptomycetes. Biological control of plant pathogens is an eye catching alternative to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains (Harman *et.al.,* 2004). A recent list of mechanisms are *viz.,* mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilisation and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogens enzymes (Lewis *et al.,* 2001) [10]. Apart from bio control ability, the BCAs possess other traits such as rhizosphere competence, tolerance of fungicides, saprophytic competitive ability, ability to tolerate high and low temperatures, adaptability to different edaphic conditions, good searching ability, host specificity, high reproduction rate, short life cycle, adaptability, well adapted to different stages of life cycle of target host, able to maintain itself after reducing host population (Okigbo and Ikediugwu, 2000) [15] have showed that *Trichoderma viride* displaced the naturally occurring mycoflora on the surface of the turmeric. To develop an effective disease management programme, the compatibility of potential bio agents with fungicides is essential. Combinations of fungicides and compatible bio agents in an IDM strategy protects the seeds and seedlings from soil borne and seed borne inoculum (Dubey and Patil, 2001) [7]. Integration of compatible bio agents with fungicides may enhance the effectiveness of disease control and provide better management of soil borne diseases (Papavizas and Lewis, 1981) [17]. The combination of BCAs with fungicides would provide similar disease suppression as achieved with higher fungicide use (Monte, 2001) [12]. Combining antagonists with synthetic chemicals eliminates the chance of resistance development and reduces the fungicide application. It is therefore, proposed to identify the compatibility of the potential bio agents with commonly used fungicides for the eco-friendly management of the tea diseases. As fungicides should have inhibitory effect on the pathogen but should not have deleterious effect on the antagonists, an understanding of the effect of fungicides on the pathogen and the antagonists would provide information for the selection of fungicides and fungicide resistant antagonists, through compatibility studies *in vitro.* In addition, this strategy may display even better control of resistant strains of fungal pathogens and May help the commercial growers to

reduce the amount of fungicide use, thus lowering the amount of chemical residue in the marketed products. Combined applications of BCAs followed by small quantities of fungicides may help the antagonists and the relative cost of the formulations (Thoudam and Dutta, 2014) [24]. *Trichoderma* species are known to suppress infection of root by soil borne pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* species and *Pythium* species on various crops (Ehteshamul-Haque, *et al.*, 1990; Benítez *et al.*, 2004; Adekunle *et al.*, 2001) [8, 4, 1]. Species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (Dubey *et al.*, 2007; Yedidia *et al.*, 1999) [6, 26]. *Trichoderma harzianum* has shown effective control of root infecting fungi and root-knot nematodes (Spiegel and Chet, 1998; Sun and Liu, 2006) [22, 23]. *Trichoderma harzianum* isolated from rhizome rot suppressive soils reduced the disease and increased plant growth and yield (Ram *et al.*, 1999) [18]. It has been reported that many *Trichoderma* species has an innate and/or induced resistance to many fungicides but the level of resistance varies with the fungicide (Omar, 2006). The combined use of BCAs and chemical pesticides has attracted much attention in order to obtain synergistic or additive effects in the control of soilborne diseases (Locke *et al.*, 1985) [11]. *Pseudomonas fluorescens* is a Plant Growth Promoting Rhizobacteria (PGPR) as well as a broad spectrum biocontrol agent for soil borne as well as foliar pathogens including nematodes (Omar, 2006). This is an ideal candidate in organic agriculture and plays significant role in integrated disease management of turmeric. In view of this, investigation was conducted to test the possibility of combining *Trichoderma* and *Pseudomonas* species with fungicides under laboratory condition. The long term goal is to develop an effective IDM package for managing soil borne plant disease as well as to prevent the resistance development in pathogens to fungicides. Integrating chemical resistant *Trichoderma* and *Pseudomonas* species has an importance in the framework of integrated disease management. Disease prevention can be increased by using such tolerant species that keeps pathogens under sufficient pressure so that they cannot thrive. Keeping the above in view, the present work was designed to observe the compatibility of different fungicides with the BCA that is., *Trichoderma viride* (AUT1) and *Pseudomonas fluorescens* (AUP1) *in vitro*.

Materials and Methods

Collection of *Trichoderma viride, Pseudomonas fluorescens* and fungicides

The species of *Trichoderma viride* and *Pseudomonas fluorescens* were used to study its compatibility with fungicides under *in vitro* conditions and they were designated as AUT1 and AUP1 which were isolated from the turmeric rhizome samples collected from Kurnool, Kadapa, Guntur, Visakhapatnam, West Godavari of Andhra Pradesh Zone, India. They are further checked for purity and are used for experimentation. The seven fungicides used were copper oxychloride 50% (Blitox), mancozeb 4% + metalaxyl 64% (Ridomil Gold MZ), carbendazim 12% + mancozeb 64% (Saaf), bordeaux mixture, mancozeb 75% (Indofil M-45), fenamidone 10% + mancozeb 50% (Sectin) and Cymoxanil 8% + mancozeb 64% (Curzate M-8) each at three different concentrations *viz.*, 0.05%, 0.1% and 0.2% were evaluated for this study. These fungicides were obtained from College of Horticulture, Dr. Y.S.R. Horticultural University. The details of fungicides used are given in Table1.

The poisoned food technique

The purpose of this experiment was to evaluate the efficacy of tested fungicides at different concentrations against *Trichoderma viride* which were available currently on market to control fungal pathogens. The quantity of fungicides needed to get the desired concentration was added to 100 ml sterilized, molten PDA medium in 250 ml conical flask, mixed well and poured in sterilized Petri dishes at the rate of 15-20 ml per plate. To avoid contamination, all ten fungicides were exposed to UV light for a period of 30 min before adding it into the medium. After solidification of the medium, mycelial discs of 8 mm diameter from actively growing fungal antagonist were cut and placed at the centre of the each Petri dish. Control consisted of PDA medium alone inoculated with the antagonist. Three replications were maintained for each concentration. The inoculated plates were incubated at room temperature and observations on the mycelial growth of the fungal antagonist were taken when control plates showed full growth. The relative growth reduction for each fungicide was calculated by the equation below.

$$L= C-T/C \times 100$$

Where L is percentage of inhibition in growth of *Trichoderma viride*; C is radial growth of the *Trichoderma viride* in control; T is radial growth (mm) of the *Trichoderma viride* in the presence of the fungicides (Rita and Tricita, 2004) [19].

The Inhibition Zone Technique

The purpose of this experiment was to evaluate the efficacy of tested fungicides at different concentrations against *Pseudomonas fluorescens* which were available currently on market to control fungal pathogens. Sterile filter paper discs of 8 mm diameter were soaked in different concentrations of each fungicide. The discs were placed at the center of Petri dishes containing the NA medium seeded with 48 h. old culture of the *Pseudomonas fluorescens*. Control consisted of filter paper disc soaked in sterile distilled water. Three replications were maintained. The inoculated plates were incubated at room temperature and the observations on inhibition zone were recorded after 48 h. The per cent reduction in radial growth over control was calculated by using the following formula.

$$L= C-T/C \times 100$$

Where, L=Percentage reduction in growth of *Pseudomonas fluorescens* C=Radial growth (mm) of *Pseudomonas fluorescens* in control T=Radial growth (mm) of the *Pseudomonas fluorescence* in treatment (Nene and Thapliyal, 1993) [14].

Table 1: Fungicides evaluated for compatibility to *Trichoderma viride* and *Pseudomonas fluorescens.*

S. No	Chemical name	Trade name	Concentrations (per cent)
1	Copper oxychloride 50% WDP	Blitox	0.05, 0.1, 0.2
2	Mancozeb 4% + Metalaxyl-M 64% w/w	Ridomil Gold MZ 68 WG	0.05, 0.1, 0.2
3	Carbendazim 12% + Mancozeb 64% WP	Saaf	0.05, 0.1, 0.2
4	$CuSO_4$ + Lime + Water	bordeaux mixture	0.05, 0.1, 0.2
5	Mancozeb 75% WP	Indofil-M45 WP	0.05, 0.1, 0.2
6	Fenamidone 10% + Mancozeb 50% WG	Sectin	0.05, 0.1, 0.2
7	Cymoxanil 8% + Mancozeb 64% WP	Curzate M-8	0.05, 0.1, 0.2

Methods of data analysis

The statistical analysis of mycelia growth diameters of bio control agents and per cent of inhibition were tested. The data obtained in these experiments were statistically analyzed by using completely randomized design (CRD). Mean comparisons of different parameters were conducted using the procedures of SPSS statistical analysis software version 16. Mean separation was determined according to Duncan's multiple range test ($P<0.05$).

Results and Discussion

In vitro compatibility tests were done with seven fungicides on *Trichoderma viride* and *Pseudomonas fluorescens.* Among the treatments the mean radial growth of *Trichoderma viride* varied from 0.0 to 90 mm (Fig-1). It is evident from the data presented in table 2, combination of Cymoxanil (8%) + mancozeb (64%) (Curzate M-8) at lower concentration (Fig-1) showed more compatibility with *Trichoderma viride* and luxuriant growth of antagonist was found in all the petriplates containing poisoned medium and the observed mean radial growth of *Trichoderma viride* was 77.77 mm with 13.58 percent growth inhibition. Mancozeb (75%) (Indofil-M45) alone and combination of mancozeb (4%) + metalaxyl (64%) (Ridomil Gold MZ) at lower concentration (Fig-1) are also showed compatibility by recording radial growth of 72.22 mm and growth inhibition percentage is 19.75 percent in both treatments. All these three treatments, combination of mancozeb (4%) + metalaxyl (64%), Cymoxanil (8%) + mancozeb (64%) and mancozeb (75%) treatments are on par with bio control agent *Trichoderma viride* (Fig-1) and were significantly superior over all other treatments. Bordeaux mixture and carbendazim (12%) + mancozeb (64%) (Saaf) were (Fig-1), showed high incompatible with *Trichoderma viride* and the observed mean radial growth was of 0.0 mm and 100 percent growth inhibition was recorded. Copper oxychloride (50%) (Blitox) alone and combination of fenamidone 10% + mancozeb 50% (Sectin) (Fig-1) showed moderate compatibility with *Trichoderma viride.* The mean radial growth recorded in these treatments were 40.22 mm with 54.44 percent growth inhibition and 39.51 cm with 53.31 percent growth inhibition respectively.

Ramarethinam *et al.* (2001) [20] reported that the fungicides like carbendazim (50% WP), hexaconazole (5% EC) completely inhibited the growth of *Trichoderma viride* centration *in vitro.* Desai *et al.* (2002) [5] 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) [13] Sharma and Mishra (1995); Abha Agarwal and Tripathi (1999) [2], who also found good growth of *Trichoderma* isolates at low and medium concentrations of various fungicides. These results were similar to the reports of Bagwan (2010) [3] who reported that mancozeb was found comparatively safer against *Trichoderma harzianum* and *Trichoderma viride*.

In case of bacterial antagonist, it is evident from the data presented in table 3 and Fig. 2, the copper fungicides, copper oxy chloride and bordeaux mixture was incompatible with bacterial bioagent at all concentration tested (Table 2). The perusal of literature revealed that the strains of *P. fluorscens* recorded compatibility with all the concentration of fungicides like Cymoxanil 8% + Mancozeb 64%, Mancozeb 75%, Carbendazim 12% + Mancozeb 64% and Mancozeb 4% + Metalaxyl 64%. The Dithiocarbamate fungicide Fenamidone 10% + Mancozeb 50% WG, was compatible at 0.05 and incompatible at 0.1 and 0.2%. The compatibility of Mancozeb 4% + Metalaxyl 64% with *P. fluorescens* was not reported earlier. The tolerance of *P. fluoresens* up to 0.2% of mancozeb was reported earlier (Vidhyasekaran and Muthumilan, 1995) [25].

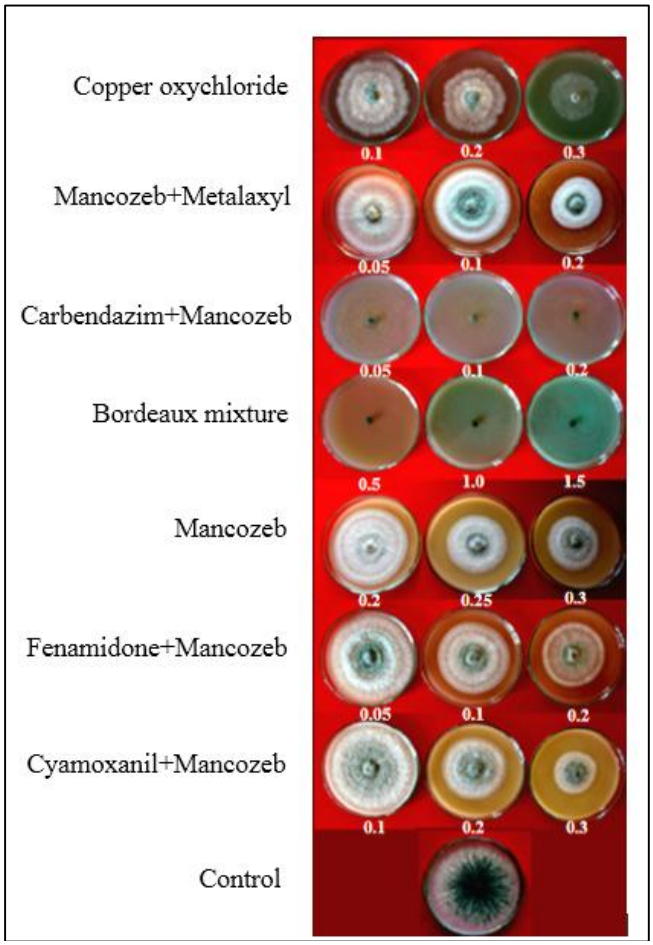
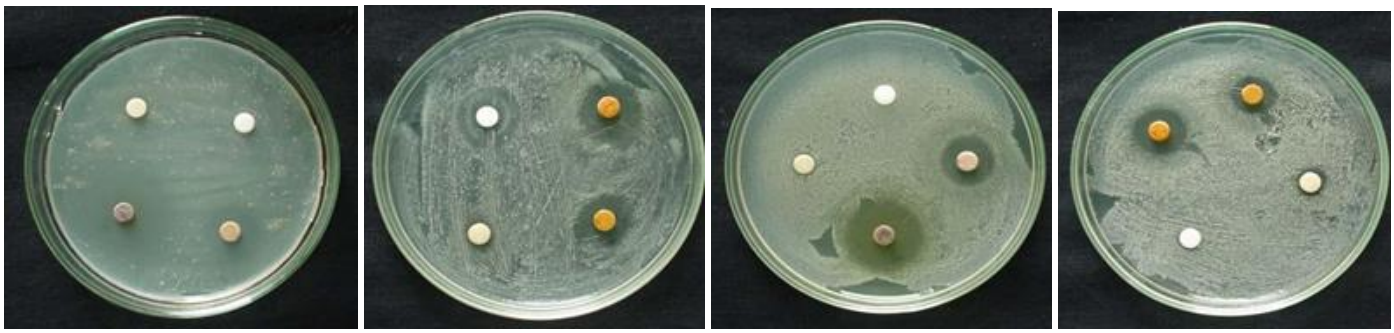


Fig 1: Compatibility of *Trichoderma viride* with fungicides in *in vitro* conditions



Mancozeb+ Metalaxyl | Copper oxychloride | Fenamidone 10% + Mancozeb 50% | Bordeaux mixture

Fig 2: Compatibility of *Pseudomonas fluorescens* with fungicides in *in vitro* conditions

Table 2: Compatibility of *Trichoderma viride* with fungicides

S. No	Fungicides	Concentration (Per cent)	*Trichoderma viride*	
			Mean diameter of colony (mm)*	PIOC*
1	Copper oxychloride 50% WDP	0.05	54.44	39.51 (38.93)**
		0.1	48.00	46.66 (43.07)
		0.2	40.22	53.31 (46.88)
2	Mancozeb 4% + Metalaxyl 64% W/W	0.05	72.22	19.75 (26.38)
		0.1	67.77	24.70 (29.79)
		0.2	50.9	43.44 (41.21)
3	Carbendazim 12% + Mancozeb 64% WP	0.05	0	100 (89.97)
		0.1	0	100 (89.97)
		0.2	0	100 (89.97)
4	Bordeaux mixture	0.05	0	100 (89.97)
		0.1	0	100 (89.97)
		0.2	0	100 (89.97)
5	Mancozeb 75% WP	0.05	72.22	19.75 (26.38)
		0.1	40.00	55.55 948.17)
		0.2	15.00	83.33 (65.88)
6	Fenamidone 10% + Mancozeb 50% WG	0.05	60.00	33.33 (35.25)
		0.1	50.9	43.44 (41.21)
		0.2	34.44	61.73 (51.76)
7	Cymoxanil 8% + Mancozeb 64% WP	0.05	77.77	13.58 (21.62)
		0.1	44.44	50.62 (45.34)
		0.2	37.00	58.88 (50.10)
8	Control	–	90	–
	S.Em ±		0.905	0.839
	C D (P = 0.05)		2.588	2.399

* Mean of three replications
******Figures in parenthesis are angular transformed values
PIOC = Per cent Inhibition over Control

Table 3: Compatibility of *Pseudomonas fluorescens* with fungicides

S. No	Fungicides	Concentration (Per cent)	*Pseudomonas fluorescens*	
			Mean diameter of Inhibition zone (mm)*	PIOC*
1	Copper oxychloride 50% WDP	0.05	10.00	11.11 (3.40)**
		0.1	12.55	13.94 (3.80)
		0.2	13.22	14.68 (3.89)
2	Mancozeb 4% + Metalaxyl 64% W/W	0.05	0	0 (0.71)
		0.1	0	0 (0.71)
		0.2	0	0 (0.71)
3	Carbendazim 12% + Mancozeb 64% WP	0.05	0	0 (0.71)
		0.1	0	0 (0.71)
		0.2	0	0 (0.71)
4	Bordeaux mixture	0.5	8.55	9.5 (3.16)
		1	12.55	13.74 (3.77)
		0.2	19.22	21.35 (4.67)
5	Mancozeb 75% WP	0.05	0	0 (0.71)
		0.1	0	0 (0.71)
		0.2	0	0 (0.71)
6	Fenamidone 10% + Mancozeb 50% WG	0.05	0	0 (0.71)
		0.1	9	10 (3.24)

		0.2	12.55	13.94 (3.8)
7	Cymoxanil 8% + Mancozeb 64% WP	0.05	0	0 (0.71)
		0.1	0	0 (0.71)
		0.2	0	0 (0.71)
8	Control	-	0	0 (0.71)
	S.Em ±		0.236	0.037
	C D (P = 0.05)		0.673	0.105

* Mean of three replications
******Figures in parenthesis are square root √+0.5 transformed values
PIOC = Per cent Inhibition over control

Conclusion

Present findings indicated that treatment of fungal bioagent would be high incompatible with bordeaux mixture and carbendazim (12%) + mancozeb (64%) and showed cent percent inhibition on the growth of the fungal antagonist. Cymoxanil (8%) + mancozeb (64%) at 0.1 per cent concentration recorded lowest inhibition of 13.58 per cent on the growth of the fungal bioagent. Mancozeb (4%) + metalaxyl-M (64%), carbendazim (12%) + mancozeb (64%), Cymoxanil (8%) + mancozeb (64%), mancozeb, lower concentration of fenamidone (10%) + mancozeb (50%) were compatible with bacterial antagonist. The other fungicides recorded the inhibition in the growth of the bacterial antagonist in the range of 9.5 to 21.35 per cent. As BCAs cannot handle the disease entirely when bulky size infection is already recognized in the field, farmers prefer fungicides for managing the crop diseases. But fungicides are harmful to the environment and also injurious for the soil, efficiency and human and animal health. Due to the disadvantages of fungicides, IDM programs (0.05%, 1% and 2% for fungicides) with BCAs are recommended, in which judicious use of fungicides and their integration with BCAs is favoured. As fungicides may have harmful effect on antagonists, an indebted of the effect of fungicides on antagonists would provide information on the selection of selective fungicides and fungicides resistant antagonists for compatibility studies as has been suggested in the present paper.

Acknowledgment

I would like to acknowledge College of Horticulture, Dr. Y.S.R. Horticultural University especially, Department of Plant Pathology for all round assistance and allowing me to carry out the research in the laboratory. All materials used in this work have been dually acknowledged.

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