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# Compatibility of different insecticides USD in turmeric cultivation with combination of bio control agents under *in vitro* conditions

**M Lakshmi Naga Nandini, SK Nayab Rasool, CH Ruth and K Gopal**

**Abstract**
The compatibility of bio-control agents *Trichoderma viridae* and *Pseudomonas fluorescens* was assessed with commonly used chemical insecticides in turmeric *viz.,* thiamethoxam 25% WG (Cruiser), chloropyrifos 20% EC (Dursban), dimethoate 30% EC (Rogor), malathion 50% EC (Malathion) and phosphamidon 40% SL (Demecron) each at three (0.05%, 0.1% and 0.2%) different concentrations. The compatibility tests revealed that the response of the *Trichoderma viridae* isolate to different insecticides differed significantly. Cent per cent inhibition was noticed at all three concentrations of phosphamidon (40%) (Demecron). The lowest inhibition on growth of antagonist was noticed with dimethoate (30% EC) (Rogar) at 0.05 per cent concentration (19.75%) followed by thiamethoxam (25%) (Cruiser) at 0.1% recorded lowest inhibition of 21.36 per cent which were statistically on par with each other on the growth of the bio-agent where as in others the reduction in growth was in range of 24.70 to 84.44 per cent over control. The response of isolate *Pseudomonas fluorescens* to different insecticides at three concentrations varied significantly. The data revealed that the antagonist was found compatible with thiamethoxam (25%) (Cruiser) and phosphamidon (40%) (Demecron) at all the three concentrations with zero per cent inhibition. The other insecticides like chlorpyriphos (20%) (Dursban), dimethoate (30%) (Rogar) and malathion (50%) at all three concentrations showed the inhibition on the growth of antagonist in the range of 7.77 and 24.44 per cent. Moreover, the pesticide tolerance ability broadened the use as these bio-pesticides in conjugation with pesticides can be applied under integrated disease management for the management of soil borne plant pathogens.

**Keywords:** insecticides, *Trichoderma viridae*, *Pseudomonas fluorescens*, Compatibility, *In vitro*

## Introduction
Plant pathogens are destructive and cause tremendous yield losses to all kinds of crops. Control of plant diseases by the use of antagonistic microorganisms can be an effective means (Cook and Baker, 1983) [5]. Interaction between bio-control agents and plant pathogens has been studied extensively and application of bio-control agents to protect some commercially important crops is promising (Vesseur *et al*., 1990) [21]. A large number of plant diseases have been successfully controlled through fungal and bacterial antagonists (Sahebani and Hadavi, 2008; Federico *et al*., 2007; Cook and Baker, 1983; Vidhyasekaran *et al*., 1997) [19, 8, 5, 22]. Supplementation with specific compounds may provide a competitive advantage for the establishment of the introduced bio-control agents and improve the bio-control. In several disease management strategies, the addition of pesticides at reduced rates in combination with bio-control agents has significantly enhanced disease control, compared to treatments with bio-control agent alone (Frances *et al*., 2002; Buck, 2004) [9, 3]. Integrated use of bio-control agent with reduced dose of pesticide was effective against many plant diseases compared with the individual components of disease management.
Biological control is an alternative to the use of chemical pesticides. Biological pesticides may act to suppress the population of the pathogenic organisms, stimulate plant growth which may allow plants to quickly outgrow any pathogen effects, or damage the pathogen by means of toxins produced (Cook, 2000; Gilreath, 2002) [6, 10]. *Trichoderma spp*. has received the most attention for control of soil borne pathogens. *Trichoderma viridae* is a fungal bio-control agent that attacks a range of phyto pathogenic fungi. It can be used either alone or in combination with other *Trichoderma* spp. in biological control of several plant diseases (Papavizas, 1985; Chet, 1987; Samuels, 1996) [14, 4, 20]. 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) [11].

In recent years, the search of biological control agents for the management of dreaded soil borne diseases has been advocated widely. Since, the bio-control 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 bio-control is considered in terms of rhizosphere competence, suppression of pathogens, tolerance to pesticides, competitive saprophytic ability, adaptability to environment etc. Combined application of bio-control 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 bio-control 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 enzymes excreted by antagonists are a well-known feature of mycoparasitism (Henis and Chet, 1975) [12]. 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) [2]. Though few studies about the sensitivity of bio-control agents with certain fungicides and insecticides are available, studies / reports with special reference to commercially available bio-control agents of *Trichoderma* and *Pseudomonas* are major. Compatibility of living organisms with modern inputs in plant protection like fungicides, insecticides is a pre-requisite for disease management and increasing plant growth.

Although use of bio-control agents could reduce chemical application to a limited extent, it is less reliable and less efficient (Monte, 2001) [15]. Integrated pest management is an approach involving the use of biological, physical and chemical measures to manage pest and pathogen populations in a cost- effective ecological way. Within these plant protection strategies, one may need to combine bio-control agents with chemicals to achieve the target (Kredics *et al.*, 2003) [13]. The combined use of bio-control agents and chemical pesticides has attracted much attention as a way to obtain synergistic or additive effects in the control of soil borne pathogens (Locke *et al.*, 1985) [14]. The objective of the present study is to test the growth of bio-control agents *Trichoderma viridae* and *Pseudomonas fluorescens* with commonly used insecticides at different concentrations under *in vitro* conditions for the control of plant pathogens.

## Materials and Methods

The commercial bio-control agent *Trichoderma viridae* in the form of talc was isolated from the turmeric rhizome samples collected from Kurnool, Kadapa, Guntur, Visakhapatnam, West Godavari of Andhra Pradesh Zone, India. Compatibility tests were conducted under *invitro* condition to check the compatibility of insecticides on *Trichoderma viridae.* The general laboratory techniques followed for the present study were those described by Nene and Thapliyal (1993) [16], Dhingra and Sinclair (1995) [7] and Aneja (2001) [1] 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 / $cm^2$ (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±2ºC 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. In case of *Pseudomonas fluorescens,* the healthy rhizome samples were collected from turmeric growing regions used for isolation of *Pseudomonas fluorescens* on selective King'S Agar media.

Efficacy of five insecticides at recommended concentrations (Table 1) were evaluated against the *Trichoderma viride* by poisoned food technique as described by Dhingra and Sinclair (1995) [7], in case of bacteria by inhibition zone technique (Vincent, 1947) [23].

**Table 1:** Details of insecticides evaluated for the compatibility of *Trichoderma viridae* and *Pseudomonas fluorescens.*

| Sl. No. | Chemical name | Active ingredient | Trade name | Concentrations (per cent) |
|---|---|---|---|---|
| 1. | Dimethoate | 30 % EC | Rogor | 0.05, 0.1, 0.2 |
| 2. | Chloropyrifos | 20 % EC | Dursban | 0.05, 0.1, 0.2 |
| 3. | Thiamethoxam | 25% WG | Cruiser | 0.05, 0.1, 0.2 |
| 4. | Malathion | 50% EC | Malathion | 0.05, 0.1, 0.2 |
| 5. | Phosphamidon | 40% SL | Demecron | 0.05, 0.1, 0.2 |

WG: Wettable granules; 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,* insecticides (thiamethoxam 25% WG (Cruiser), chloropyrifos 20% EC (Dursban), dimethoate 30% EC (Rogor), malathion 50% EC (Malathion) and phosphamidon 40% SL (Demecron) with 0.05%, 0.1% and 0.2% concentrations were added at luke warm temperature and mixed thoroughly by shaking the flask the poisoned medium distributed equally into three petriplates which were treated as three replications and allowed to solidify. The experiment was conducted in a complete randomized design (CRD) with five treatments presented in table 2 & 3.

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 three petriplates, which were treated as three 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 bio-control agent over the control was calculated by using the following formula.

I=(C-T)/C× 100

Where I= percent inhibition

C= colony diameter at bio-control agent in control

T=colony diameter at bio-control agent in treatment.

In case of bacteria, the different concentrations of the pesticides were prepared in nutrient agar. Desired concentration is poured in Petriplates and left over night to observe contamination if any. There after 0.1 ml of overnight culture of *P. fluorescens* was spread over the solidified plates with spreader. These plates were incubated at 30±2$^0$C and *P. fluorescens* colonies were identified and counted after 24h. The observations on growth of *P. fluorescens* on media containing different concentrations of various chemicals were recorded and Percent Inhibition over Control (PIOC) of insecticides for *P. fluorescens* was calculated by using above formula.

I=(C-T)/C× 100

Where I= percent inhibition

C= colony diameter at biocontrol agent in control

T=colony diameter at biocontrol agent in treatment.

**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) [17].

**Results and Discussion**

It is essential to test the compatibility of bio-control agents with the commonly used pesticides for their successful integration under IDM strategy of crop protection. Therefore, studies were undertaken on these aspects. The compatibility tests revealed that the sensitivity of five insecticides *viz.*, thiamethoxam 25% WG (Cruiser), chloropyrifos 20% EC (Dursban), dimethoate 30% EC (Rogor), malathion 50% EC (Malathion), phosphamidon 40% SL (Demecron) each at three different concentrations was tested under *in vitro* conditions (Plate 1). The response of the *Trichoderma viridae* isolate to different insecticides differed significantly. Cent per cent inhibition was noticed at three concentrations of phosphamidon (40%) (Demecron). The lowest inhibition on growth of antagonist was noticed with dimethoate (30% EC) (Rogar) at 0.05 per cent concentration (19.75%) followed by thiamethoxam (25%) (Cruiser) at 0.1% recorded lowest inhibition of 21.36 per cent which were statistically on par with each other on the growth of the bioagent where as in others the reduction in growth was in range of 24.70 to 84.44 per cent over control (Table 2 & Plate 1).

The results of the compatibility study of fungal antagonist with insecticides revealed that in general all insecticides showed varying levels of compatibility. Bhai and Thomas (2010) conducted similar studies with Quinalphos (Fig. 1). The differential response of *Trichoderma viride* to various insecticides in the present study might be due to their inherent resistance to the insecticides and their ability to degrade these chemicals.

The *in vitro* sensitivity of five insecticides *viz.*, thiamethoxam 25% WG (Cruiser), chloropyrifos 20% EC (Dursban), dimethoate 30% EC (Rogor), malathion 50% EC (Malathion), phosphamidon 40% SL (Demecron) each at three concentrations were tested against *Pseudomonas fluorescens*. The response of isolate *Pseudomonas fluorescens* to different insecticides at three concentrations varied significantly. From data given in (Table 3 and Fig. 2) it revealed that the antagonist was found compatible with thiamethoxam (25%) (Cruiser) and phosphamidon (40%) (Demecron) at all the three concentrations with zero per cent inhibition. The other insecticides like chlorpyriphos (20%) (Dursban), dimethoate (30%) (Rogar) and malathion (50%) at all three concentrations showed the inhibition on the growth of antagonist in the range of 7.77 and 24.44 per cent. On comparing the effect of different insecticides tested it was found that, except highest concentration of dimethoate (30%) and malathion (50%) (0.2 per cent) all other insecticides recorded less than 20 per cent inhibition on the growth of all bacterial antagonists. Hence they may be considered as incompatible with their antagonist.

A similar type of study was conducted by Elkins and Lindow (1999). They found the bacterial antagonists were compatible with thiamethoxam and phosphamidon at various concentrations. Therefore, these insecticides could be recommended for the insect control without much adverse effect against the bacterial antagonists. Mathew (2003) reported *P. fluorescens* was incompatible with recommended doses of dimethoate, chlorpyriphos and malathion.

The result of the present screening would help in the selection of biological control agents, which can be used, with reduced dose of selected pesticides for the control of plant pathogens and pests. Therefore care should be taken while selecting components in the integrated disease management programme. So it is evident that there lies in the potential of two biocontol agents to be used along with plant protection chemicals as a control of integrated control packages. However, the performance of these selected bioagents in different turmeric growing areas is to be ascertained before recommending to the farming community as an eco-friendly management practice against the disease. Pesticides those are inhibitory against a narrow spectrum of plant pathogen but not against biocontrol agent offer a chance for integration of chemical and biocontrol agents.

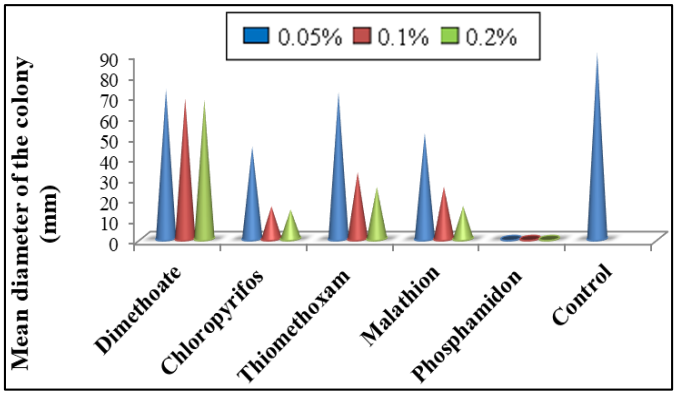


**Fig 1:** Compatibility of *Trichoderma viridae* with insecticides

**Table 2:** Compatibility of *Trichoderma viridae* with insecticides

| Sl. No | Insecticides | Concentration (per cent) | *Trichoderma viridae* | |
|---|---|---|---|---|
| | | | Mean diameter of the colony (mm)* | PIOC* |
| 1 | Dimethoate 30% EC | 0.05 | 72.22 | 19.75 (26.38) ** |
| | | 0.1 | 67.77 | 24.70 (29.79) |
| | | 0.2 | 67.00 | 25.55 (30.35) |
| 2 | Chloropyrifos 20% EC | 0.05 | 44.44 | 50.62 (45.34) |
| | | 0.1 | 15.55 | 82.72 (65.41) |
| | | 0.2 | 14.00 | 84.44 (66.74) |
| 3 | Thiamethoxam 25% WG | 0.1 | 70.77 | 21.36 (27.52) |
| | | 0.2 | 32.00 | 64.44 (53.37) |
| | | 0.3 | 25.00 | 72.22 (58.17) |
| 4 | Malathion 50% EC | 0.05 | 50.9 | 43.44 (41.21) |
| | | 0.1 | 25.00 | 72.22 (58.17) |
| | | 0.2 | 15.55 | 82.72 (65.41) |
| 5 | Phosphamidon 40% SL | 0.05 | 0 | 100 (89.97) |
| | | 0.1 | 0 | 100 (89.97) |
| | | 0.2 | 0 | 100 (89.97) |
| 6 | Control | - | 90 | - |
| | S.Em ± | | 1.019 | 0.732 |
| | C D (P = 0.05) | | 2.949 | 2.117 |

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

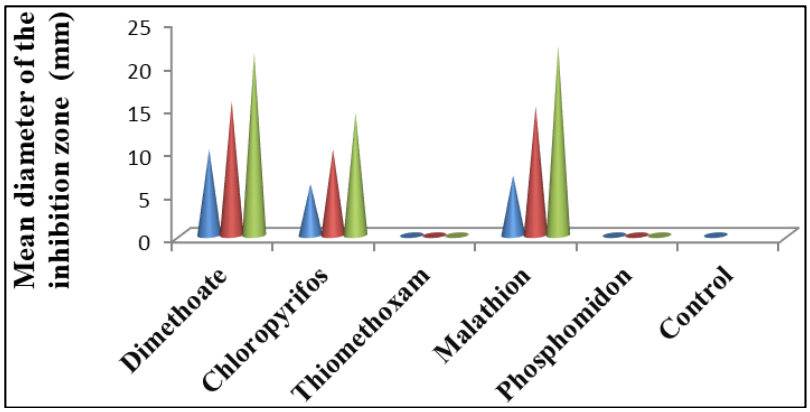


**Fig 2:** Compatibilty of *Pseudomonas fluorescens* with insecticides

**Table 3:** Compatibility of selected *Pseudomonas fluorescens* with insecticides

| Sl. No | Insecticides | Concentration (per cent) | *Pseudomonas fluorescens* | |
|---|---|---|---|---|
| | | | Mean diameter of Inhibition zone (mm)* | PIOC* |
| 1 | Dimethoate 30% EC | 0.05 | 10 | 11.11 (3.40)** |
| | | 0.1 | 15.55 | 17.27 (4.21) |
| | | 0.2 | 21.22 | 23.57 (4.90) |
| 2 | Chloropyrifos 20% EC | 0.05 | 6.00 | 6.66 (2.67) |
| | | 0.1 | 10.00 | 11.11 (3.40) |
| | | 0.2 | 14.22 | 15.8 (4.03) |
| 3 | Thiamethoxam 25% WG | 0.1 | 0 | 0 (0.71) |
| | | 0.2 | 0 | 0 (0.71) |
| | | 0.3 | 0 | 0 (0.71) |
| 4 | Malathion 50% EC | 0.05 | 7.00 | 7.77 (2.87) |
| | | 0.1 | 15.00 | 16.66 (4.14) |
| | | 0.2 | 22.00 | 24.44 (4.99) |
| 5 | Phosphamidon 40% SL | 0.05 | 0 | 0 (0.71) |
| | | 0.1 | 0 | 0 (0.71) |
| | | 0.2 | 0 | 0 (0.71) |
| 6 | Control | - | 0 | 0 (0.71) |
| | S.Em ± | | 0.388 | 0.044 |
| | C D (P = 0.05) | | 1.123 | 0.128 |

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

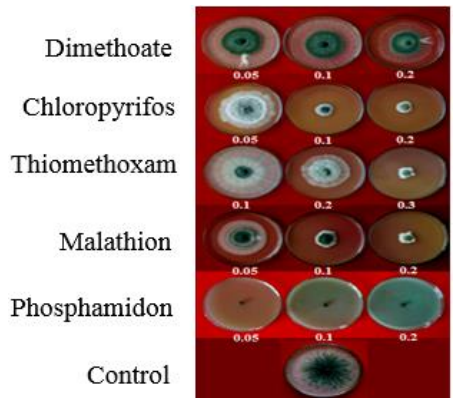


**Plate 1:** Compatibility of *Trichoderma viridae* with insecticides in *in vitro* conditions

## Conclusion

From the above experiments it was concluded that if the formulations made by the recommended dose of insecticides with the bio-control agent *Trichoderma viridae* and *Pseudomonas fluorescens* and used for the management of various plant pests show promising effect than the chemicals alone. It is cost effective and environment friendly also.

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