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In-vitro compatibility of *Trichoderma harzianum* with systemic fungicides

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Abstract

Trichoderma spp. are also known as effective bio-control agents against soil-borne pathogens of several important crops. Integration of chemicals and bio-control agents has been the subject of research during recent years. The potential value of bio-pesticides for the control of important plant pathogens requires investigation due to the general requirement for very low pesticides residues in foodstuffs and concerns over the increasing development of resistance to the different classes of chemicals. The aim of the present study was to check the compatibility of different fungicides viz., Hexaconazole, Nativo (Tebuconazole 50%+ Trifloxystobin 25%), Propiconazole and Tebuconazole were tested with their different doses of 10, 15, 20 and 25 ppm *in vitro* for their compatibility with *T. harzianum* strain IRRI-1 using poisoned food technique. All the four concentrations of Nativo were highly compatible with low toxic effect against *T. harzianum* strain IRRI-1 *in vitro*. As there was 74.42, 79.07, 93.02 and 95.35 percent inhibition of radial growth of *T. harzianum* strain IRRI-1 due to Nativo at 10ppm, 15ppm, 20ppm and 25ppm concentrations respectively, even after a prolonged exposure i.e. up to 96 hours (4 days). 15 and 20 ppm concentration of Propiconazole was completely inhibitory up to 48 hours but after 72 hours, a little growth of *T. harzianum* strain IRRI-1 was visible. 25 ppm concentration of Propiconazole was completely inhibitory up to 96 hours was noticed that the systemic fungicide i.e. Nativo and Propiconazole were comparatively safer, than Hexaconazole and Tebuconazole which exhibited acute toxicity for growth of *T. harzianum* strain IRRI-1.

Keywords: *Trichoderma harzianum*, fungicides, radial growth, percent inhibition

1. 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) [4]. 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) [2]. 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) [8]. The combination of BCAs with fungicides would provide similar disease suppression as achieved with higher fungicide use (Monte 2001) [2]. 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 plant 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) [11]. 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) [7]. The combined use of BCAs and chemical pesticides has attracted much attention in order to obtain synergistic or additive effects in the control of soil borne diseases (Locke *et al.*, 1985) [5].

In view of this, investigation was conducted to test the possibility of combining *Trichoderma* 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* 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 *in vitro* compatibility of different fungicides with *T. harzianum* strain IRRI-1.

2. Methods and Materials

The experiment was conducted during 2017-18 at the Bio-control laboratory in the Department of Plant Pathology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.) India.

A previously characterized drought tolerant isolates of *T. harzianum* strain IRRI-1 was selected on the basis of their cultural characteristics and allotted a specific number from Bio-control laboratory. The isolates of *T. harzianum* strain IRRI-1 was maintained on PDA slants and was time to time sub-cultured for its revival. *T. harzianum* strain IRRI-1 cultured main tend on PDA slants were stored in refrigerator for future use.

2.1 Preparation of PDA Media

250g of potato was peeled and cut into small and fine sliced pieces. 200g of potato pieces were weighed and placed into a stainless steel pan. 1000 ml of water was added to potato pieces and boiled gently for such a period until they are easily mashed by a glass rod. The decoction thus obtained was filtered through muslin cloth and squeezed out all the liquid in a measuring cylinder and potato pieces were discarded. Now sufficient amount of water was added to make the volume 1000 ml. Now pre-weighed 20g agar-agar was added (20g) slowly to the boiling solution so as to dissolve it. At the same time dextrose (20g) was also added in boiling solution (melted with agar) and final volume made up to 1 litre while boiling the solution was stored with glass rod to mix the agar-agar and dextrose properly. It was poured @ about 200 ml in each of four conical flasks of 500ml capacity and 10 ml per culture tube to prepare the PDA slants. Flasks and culture tubes both were tightly plugged with non-absorbent cotton and wrapped with butter paper and rubber bands. The culture tubes and flasks were placed vertically (mouths up) in wire baskets and then autoclaved at 1.1 kg/cm² pressure for 20 min at 121.6°C and cooled before pouring into Petri plates.

Four fungicides were tested with their different doses of 10, 15, 20 and 25ppm, *in vitro* for their compatibility with *T. harzianum* strain IRRI-1 using poisoned food technique. Required amount of fungicides were added in each 250 ml

capacity flask, containing 100, ml sterilized PDA media to obtain 10ppm, 15ppm, 20ppm, and 25ppm concentration of each test fungitoxicant. It was mixed thoroughly by shaking the flask prior to pouring in sterilized Petri Plates. The medium was allowed to solidify over night and then 5 mm discs from seven days old actively growing culture of *T. harzianum* strain IRRI-1 was placed in center of each Petri Plate. PDA medium mixed with distilled water only served as check. Three replications were maintained for each treatment and inoculated plates were incubated at 28 ± 2°C in BOD incubator. Observations were recorded on radial growth of *T. harzianum* strain IRRI-1 at an interval of each 24 hrs, up to 5 days. Percent inhibition was calculated by using the formula given below and analyzed under complete randomized design (CRD) (Gomez and Gomez, 1984) [3].

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

Where

C = Growth of fungus in control

T = Growth of fungus in treatments

3. Results and Discussion

3.1 Results

In total four fungicides *viz.* Hexaconazole, Nativo (Tebuconazole 50%+ Trifloxystobin 25%), Propiconazole and Tebuconazole at four concentration *viz.* 0ppm, 15ppm, 20ppm, and 25ppm were tested for their compatibility with *T. harzianum* strain IRRI-1 *in vitro* and the data recorded have been presented in Table 1 and Table 2. After thorough review of data table, it is evident that all the four concentrations of Nativo were highly compatible with low toxic effect against *T. harzianum* strain IRRI-1 *in vitro*. As there was 74.42, 79.07, 93.02 and 95.35 percent inhibition of radial growth of *T. harzianum* strain IRRI-1 due to Nativo (Tebuconazole 50%+ Trifloxystobin 25%) at 10ppm, 15ppm, 20ppm and 25ppm concentrations respectively, even after a prolong exposure *i.e.* up to 96 hours (4 days).

Propiconazole was quite toxic to *T. harzianum* strain IRRI-1 but next in the order of compatibility after Nativo (Tebuconazole 50%+ Trifloxystobin 25%). In the initial period of incubation *i.e.* 48 hours, there was almost complete inhibition at 15, 20, and 25 ppm but as days of incubation increased it seems that toxic effect of chemical get decreased and fungal growth was visualized at 10 ppm concentration of Propiconazole, at a period of 48 hours. Similarly 15 and 20 ppm concentration of Propiconazole was completely inhibitory up to 48 hours but after 72 hours, a little growth of *T. harzianum* was visible. 25 ppm concentration of Propiconazole was completely inhibitory up to 96 hours.

In the initial period of incubation *i.e.* at 24 and 48 hours all the concentrations of Hexaconazole were 100 per cent inhibitory of radial growth. However at 72 hours also 15, 20 and 25 ppm concentration of Hexaconazole was completely inhibitory but 10 ppm concentration resulted in 91.18 per cent inhibition. With prolonging incubation period of 96 hours it seems that level of toxicity get reduced and resulted in 88.37, 90.70, 93.02 and 95.35 per cent inhibition due to 10, 15, 20 and 25 ppm concentration of Hexaconazole.

In the initial period of incubation *i.e.* at 24 hours all the concentrations of Tebuconazole were 100 per cent inhibitory to radial growth. However at 48 and 72 hours also 15, 20 and 25 ppm concentrations were completely inhibitory but 10 ppm concentration resulted in 92.30 and 91.18 percent inhibition. With prolonging incubation period of 96 hours, it seem that

level of toxicity get little reduced and resulted in 81.40, 90.70, and 95.35 per cent inhibition due to 10, 15 and 20 ppm concentrations but 25 ppm concentration was completely inhibitory to radial growth at 96 hrs also.

Overall, it was noticed that the systemic fungicide *i.e.* Nativo (Tebuconazole50%+ Trifloxystrobin25%) and Propiconazole

were comparatively safer, than Hexaconazole and Tebuconazole which exhibited acute toxicity for growth of *T. harzianum* strain IRRI-1. Compatibility of these chemicals couldn't be assessed beyond 96 hrs because the Petri Plate main tend without any fungi toxicant exhibited 100 percent growth within 96 hrs only.

Table 1: Effect of different concentrations of fungicides on radial growth of *Trichoderma harzianum* strain IRRI-1 at 24, 48, 72 and 96 h of their inoculation

S. No.	Fungicide/ Treatments	Concentrations	Radial growth (mm) 24 h	Radial growth (mm) 48 h	Radial growth (mm) 72 h	Radial growth (mm) 96h
1.	Hexaconazole	10ppm	0.00	0.00	6.00	10.00
2.		15ppm	0.00	0.00	0.00	8.00
3.		20ppm	0.00	0.00	0.00	6.00
4.		25ppm	0.00	0.00	0.00	4.00
5.	Nativo (Tebuconazole and Trifloxystrobin)	10ppm	0.00	8.00	12.00	22.00
6.		15ppm	0.00	6.00	10.00	18.00
7.		20ppm	0.00	0.00	0.00	6.00
8.		25ppm	0.00	0.00	0.00	4.00
9.	Propiconazole	10ppm	0.00	4.00	10.00	16.00
10.		15ppm	0.00	0.00	8.00	12.00
11.		20ppm	0.00	0.00	4.00	10.00
12.		25ppm	0.00	0.00	0.00	0.00
13.	Tebuconazole	10ppm	0.00	4.00	6.00	16.00
14.		15ppm	0.00	0.00	0.00	8.00
15.		20ppm	0.00	0.00	0.00	4.00
16.		25ppm	0.00	0.00	0.00	0.00
17.	Control	-	30.00	52.00	68.00	86.00
SE(m)			0.252	0.444	0.597	0.804
C.D.			0.728	1.282	1.724	2.32

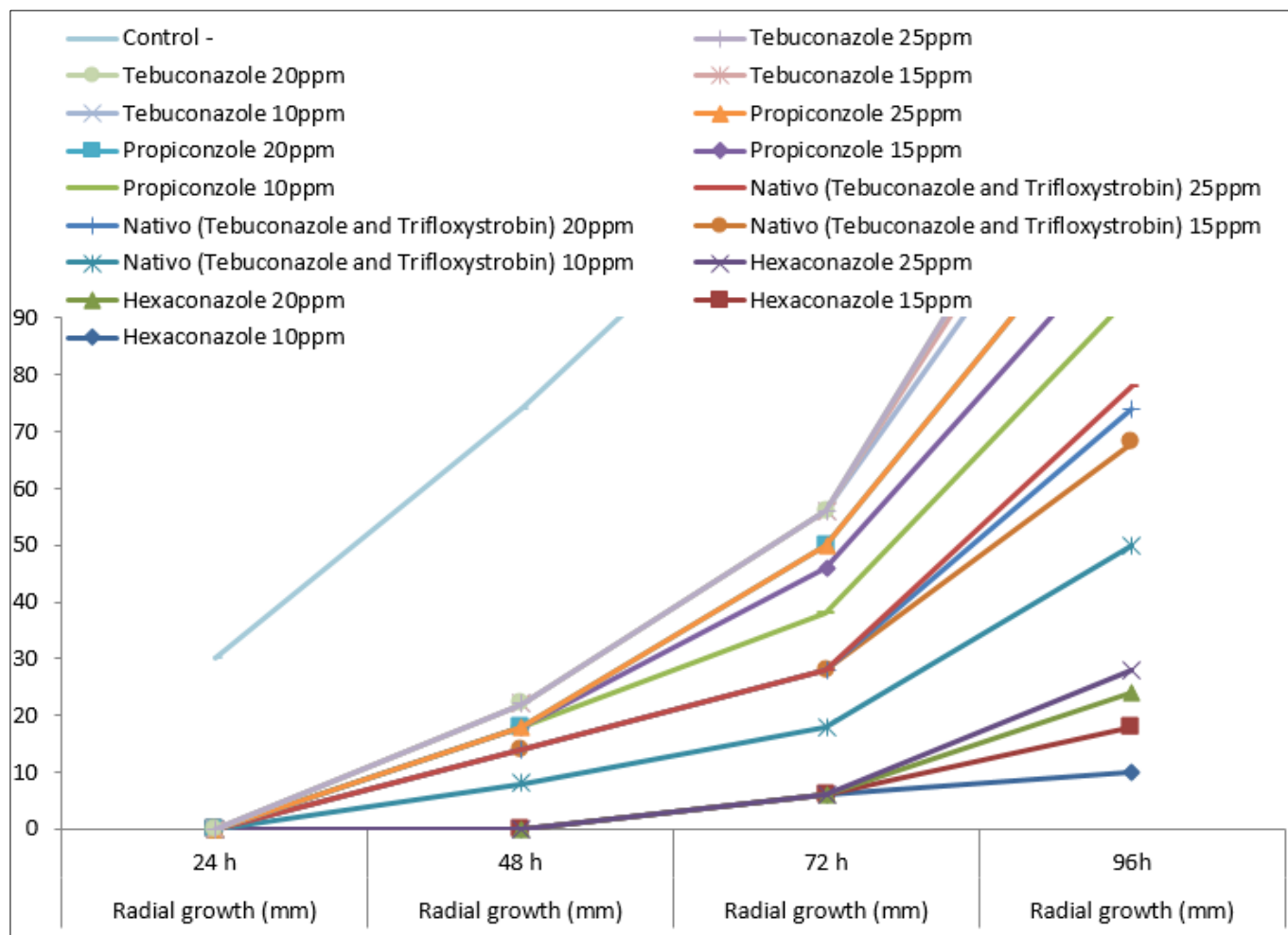
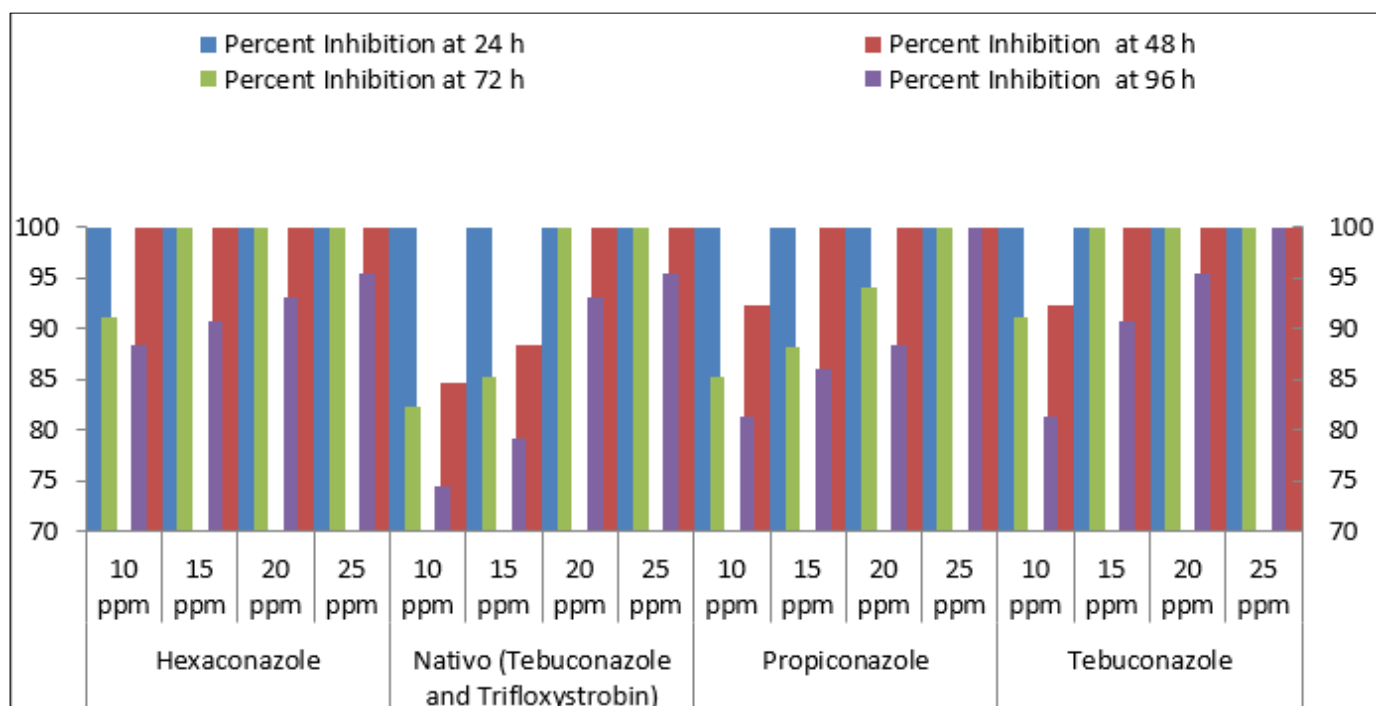


Fig 1: Effect of different concentrations of fungicides on radial growth of *Trichoderma harzianum* strain IRRI-1 at 24, 48, 72 and 96 h of their inoculation

Table 2: Effect of different concentrations of fungicides on percent inhibition of *Trichoderma harzianum* strain IRRI-1 at 24, 48, 72 and 96 h of their inoculation

S. No.	Fungicide/ Treatments	Concentrations	Percent Inhibition at 24 h	Percent Inhibition at 48 h	Percent Inhibition at 72 h	Percent Inhibition at 96 h
1.	Hexaconazole	10ppm	100.00	100.00	91.18	88.37
2.		15ppm	100.00	100.00	100.00	90.70
3.		20ppm	100.00	100.00	100.00	93.02
4.		25ppm	100.00	100.00	100.00	95.35
5.	Nativo (Tebuconazole and Trifloxystrobin)	10ppm	100.00	84.61	82.35	74.42
6.		15ppm	100.00	88.46	85.29	79.07
7.		20ppm	100.00	100.00	100.00	93.02
8.		25ppm	100.00	100.00	100.00	95.35
9.	Propiconazole	10ppm	100.00	92.30	85.29	81.40
10.		15ppm	100.00	100.00	88.24	86.05
11.		20ppm	100.00	100.00	94.12	88.37
12.		25ppm	100.00	100.00	100.00	100.00
13.	Tebuconazole	10ppm	100.00	92.30	91.18	81.40
14.		15ppm	100.00	100.00	100.00	90.70
15.		20ppm	100.00	100.00	100.00	95.35
16.		25ppm	100.00	100.00	100.00	100.00
17.	Control	-	0.00	0.00	0.00	0.00

**Fig 2:** Effect of different concentrations of fungicides on percent inhibition of *Trichoderma harzianum* strain IRRI-1 at 24, 48, 72 and 96 h of their inoculation

4. Discussion

It was found that the two systemic fungicides *i.e.* Hexaconazole and Tebuconazole during the course of investigation were found to be comparatively more toxic and incompatible at any concentration tested against *T. harzianum* strain IRRI-1 than the other two systemic fungicides *i.e.* Nativo (Tebuconazole50%+ Trifloxystrobin25%) and Propiconazole. However, Tebuconazole was comparatively more toxic than Hexaconazole, to *T. harzianum* strain IRRI-1 at all concentration tested.

Overall, it was noticed that the two fungicides *i.e.* Nativo and Propiconazole were safer, as compared to Hexaconazole and Tebuconazole which acute toxicity for growth of *T. harzianum* strain IRRI-1.

The literatures suggest that bio control agents that can tolerate a certain level of fungicides were mixed with agrochemicals,

resulting in eradication of diseases (De Cal *et al.*, 1994). The results are also in agreement with the works of Mukhopadyay *et al.*, (1987)^[6] Sharma and Mishra, (1995)^[10] who also found good growth of *Trichoderma* isolates at low and medium concentrations of various fungicides. Among fungicides, Captan, Thiram, Chlorothalonil and Copper hydroxide were found compatible with the test antagonist up to 100µg a.i. /ml, while Mancozeb up to 250 µg a.i. /ml, as these did not adversely affect the growth of test antagonist. However, Benomyl, Thiophanate methyl, Bayleton and Ipridione were found incompatible with the test antagonist even at 25 µg a.i. /ml (Saxena *et al.*, 2014)^[9]. Present findings indicated that revealed that seed treatment or soil application of *Trichoderma* could be exploited along with compatible fungicides at their lower concentrations under bio-intensive integrated disease management practices.

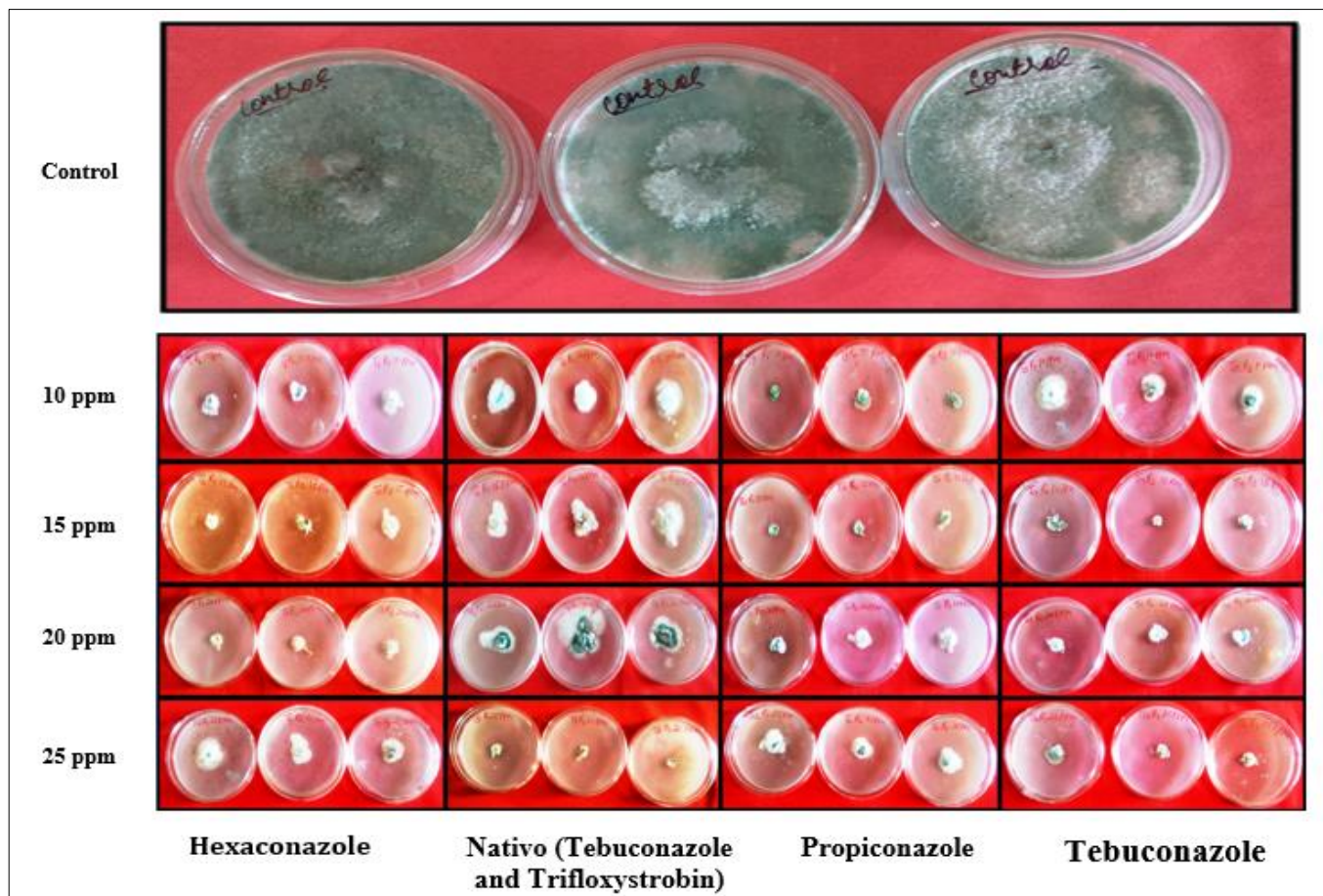


Fig 3: Percent inhibition of *Trichoderma harzianum* over control in presence of different concentrations of systemic fungicides

5. Conclusion

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 (100 and 200 ppm for both fungicides) with BCAs are recommended, in which judicious use of fungicides and their integration with BCAs is favored. 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.

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