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Evaluation and compatibility of new fungicides with *Trichoderma harzianum* for managing the charcoal rot of soybean

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Abstract
Among many fungal diseases causing threat to soybean production, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. is a most threatening soil borne fungal pathogen. Fungal, bacterial and endophyte bioagents were evaluated against *M. phaseolina* by using dual culture technique. Among the bioagents tested, IOF isolate *Trichoderma harzianum* from UAS Dharwad recorded maximum mycelial inhibition (82.35 %) which was significantly superior over rest of the bioagents. Among contact, systemic and combi product fungicides tested Tebuconazole and, Trifloxystrobin 13.7 % + Penflufen 13.7% (Evergol) and Metalaxyl 4 % + Mancozeb 64 % (Ridomil gold) showed maximum mycelial inhibition. The *in vitro* evaluation of bioagents and fungicides compatibility tests were conducted. From studies confirmed that *T. harzianum* (IOF, Dharwad) was more effective compared to other bioagents. So *T. harzianum* (IOF, Dharwad) was used in compatibility studies with all the 14 fungicides. Results revealed that *T. harzianum* was more compatible with Trifloxystrobin 13.7% + Penflufen 13.7% (Evergol) (81.20%) followed by Metalaxyl 4 % + Mancozeb 64 % (Ridomil gold) (73.00 %) and mancozeb (71.80%). Least compatibility was observed in Carbendazim (12.46%).

Keywords: Soybean, *Macrophomina phaseolina*, Bioagents, *Trichoderma harzainum*, fungicides, combi-products, *in vitro* evaluation, compatibility

Introduction
Soybean is the miracle crop of the twenty first century, is a triple beneficiary crop, which contains about 20 per cent of oil and 38-42 per cent high quality protein possessing high level of essential amino acids except methionine and cystine (Kale, 1985) [6]. Now a day's great interest has been documented about the soybean cultivation and use, mainly on account of industrial, dietetic and agricultural importance. Over the past decade productivity trend of soybean indicates a plateau due to biotic and abiotic stress. The crop suffers from many diseases such as rust (*Phakopsora pachyrhizi*), charcoal rot (*Macrophomina phaseolina*), collar rot (*Sclerotium rolsfii*), purple seed stain (*Cercospora kikuchi*), fungal foliar spots. In India, the charcoal rot, which is used to be a minor disease of soybean until 2004, became a serious disease due to altered weather conditions particularly on due to longer dry spells during crop growth period. *Macrophomina phaseolina* is the soil inhabiting organism capable of infecting soybean at any crop growth stage also seed-borne in many crops including soybean. It produces microsclerotia in root and stem tissues of host plants, which enable it to survive in soil for 2- 15 years and act as primary source of inoculum (Gupta *et al*., 2004) [5]. Biological control through the use of antagonistic microorganisms is a potential non- chemical means of controlling plant diseases by reducing inoculum levels of the pathogens. Use of fungicides for the management of disease in the absence of resistant genotypes needs to be used judiciously based on their need, dosage and type of diseases. And especially for soil borne disease, if the combined application of bioagent and fungicide is adopted then we can reduce the dosages and hazardous to the environment. Hence the study was conducted to know the efficacy of bioagents, fungicides and compatibility of both for the management of charcoal rot of soybean.

Material and Methods
The following experiments were conducted at Department of Plant Pathology, College of Agriculture, Dharwad during the year 2019-2020.

***In vitro* evaluation of bioagents**

Different bioagents *viz*., *Pseudomonas fluorescens* and *Trichoderma harzianum* and endophytes were evaluated for their antagonistic properties against *M. phaseolina* by dual culture technique (Dennis and Webster, 1971) [4].

Fungal bioagents were evaluated by inoculating the pathogen on one side of Petri plate and the antagonist at exactly opposite side of the same plate by leaving 3-4 cm gap. For this actively growing culture of both fungal bioagent and pathogen were used.

For evaluation of bacterial bioagents, five mm mycelial disc of actively growing culture of *M. phaseolina* was placed on one side of the Petri plate and bacterial bioagent was streaked on other side of the plate. Each treatment was replicated three times. Then such plates were incubated 28 ± 1ºC till the control plate fully covered by the growth of the fungus. The radial growth of pathogen was measured and per cent inhibition over control was calculated by using the formula given by Vincent (1947) [11]. From the study, the best bioagent was further evaluated for the compatibility with the fungicides.

***In vitro* evaluation of fungicides**

The efficacy of non-systemic fungicides *i.e.*, Mancozeb, Copper oxychloride, Captan (@ 500, 1000 and 2000 ppm concentration) and systemic *i.e.*, Carbendazim, Hexaconazole, Propiconazole, Difenconazole, Thiophanate methyl, Tebuconazole (@ 250, 500 and 1000 ppm concentration) and combi product fungicides *i.e.*, Carbendazim 12 % + Mancozeb 63 %, Metalaxyl 4 % + Mancozeb 64 %, Trifloxystrobin 13.3% + Penflufen, Thiophanate methyl 45 % + Pyraclostrobin 5% (@500, 1000 and 1500 ppm concentration) were assayed under *in vitro* using poisoned food technique (Sharvelle, 1961) [10]. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control, that was calculated by using the formula given by Vincent (1947) [11].

Compatibility of effective biocontrol agent with fungicides

Compatibility tests were conducted under *in vitro* condition to find out safer fungicides against best biocontrol agent. The fungicide was added in the PDA medium after autoclaving. For this 20 ml of PDA medium was amended with fungicides and culture plates were seeded with the best biocontrol agent (5mm disk of 3 days old culture) in the centre of Petri plates. The plates without amendment were served as control. The plates were incubated at 28 °C in BOD. Based on the radial growth of bioagents in fungicides treated plates compatibility was decided. All the above mentioned fungicides were evaluated for compatibility study.

Results and Discussion

***In vitro* evaluation of bioagents**

The results from *in vitro* evaluation of bioagents against *M. phaseolina* revealed that, the fungal bioagents were better than bacterial bioagents in inhibiting the mycelium growth of the pathogen. Inhibition range was from 74.51 to 82.35 per cent for fungal bioagents while it was 59.22 to 73.33 per cent for bacterial bioagents. Among the fungal bioagents tested, *Trichoderma harzianum* (IOF, Dharwad) showed maximum inhibition (82.35%) of mycelial growth of *M. phaseolina*. Among bacterial bioagents tested *Pseudomonas fluorescence* (Multiplex Sparsha) showed maximum mycelial inhibition (73.33 %) (Table 1 and Plate 1). Naik *et al*. (2009) [8] reported that *T. viride* showed maximum mycelial inhibition of *M. phaseolina* without hyphal contact was due to secretion of some diffusible non-volatile compounds. Production of antibiotics or secretion of extracellular hydrolytic enzymes and cell wall degrading enzymes such as chitinases, glucanases that break down polysaccharides, chitins and β-glucanases, there by destroying cell wall integrity. Biological control through the use of antagonistic microorganisms is a potential non- chemical means of controlling plant diseases by reducing inoculum levels of the pathogens. These are the safe and cheaper means of disease management which reduce toxicity hazards and also helps in eco-friendly disease management approach as compared to chemicals (Kumar and Gupta, 1999) [7].

***In vitro* evaluation of fungicides**

Efficacy of contact, systemic and combi product fungicides were evaluated at three concentrations in the laboratory against *M. phaseolina* by following poisoned food technique. Among contact fungicides, Mancozeb and Captan showed 97.28 and 89.63 per cent inhibition of mycelial growth of *M. phaseolina* respectively whereas minimum inhibition was noticed in Copper oxychloride (88.15%). The mode of action of Mancozeb and Captan fungicide is to inhibit the germination, growth and multiplication of the fungus and they are directly toxic to the pathogen (Nene and Thapliyal 1973) [9] (Table 2 and Plate 2).

Among combi product fungicides tested maximum mean mycelial inhibition noticed in Trifloxystrobin 13.7% + Penflufen 13.7% (Evergol) (95.93%), Metalaxyl 4 % + Mancozeb 64 % (Ridomil Gold) (93.46%), Carbendazim 12% + Mancozeb 63% (Saaf) (86.42%) and Carboxin 37.5 % + Thiram 37.5% (Vitavax power) (89.13%). Least inhibition of 84.08 per cent was observed in Thiophanate methyl 45 % + Pyraclostrobin 5% (Xelora) (Table 2 and Plate 2).

Efficacy of systemic fungicides tested against *M. phaseolina* revealed that, Tebuconazole recorded 91.23 per cent inhibition of mycelial growth of *M. phaseolina* followed by Thiophenate methyl (95.55 %) and Propiconazole (88.40 %). Least inhibition of mycelial growth was observed in Carbendazim (72.22%). Tebuconazole affects the cell walls of fungi by suppressing spore germination, and hinders the growth of pathogen (Table 3 and Plate 3).

Penflufen is a novel pyrazole fungicide with SDHI (Succinate Dehydrogenase Inhibitor). It targets the succinate dehydrogenase, one of the enzymes in respiratory chain within the mitochondria of the fungus. Trifloxystrobin interferes in process of building the structure of fungal cell wall. Finally it inhibits the reproduction and further growth of fungus. Hence the combination of these two were more effective compare to other fungicides. The results are in agreement with Nene and Thapliyal (1973) [9] who reported effectiveness of triazoles because they are known to inhibit the biosynthesis pathway in fungi.

Compatibility of *Trichoderma harzianum* (IOF, Dharwad) with fungicides

From studies confirmed that *T. harzianum* (IOF, Dharwad) was more effective compared to other bioagents. So it was used in compatibility studies with all the 14 fungicides. Results revealed that *T. harzianum* is more compatible with Trifloxystrobin 13.7% + Penflufen 13.7% (Evergol) (81.20%)

followed by Metalaxyl 4 % + Mancozeb 64 % (Ridomil gold) (73.00 %) and mancozeb (71.80%). Least compatibility was observed in Carbendazim (12.46%) (Table 4a & b and Plate 4a & b).

Similar work was also carried out by Chakrabarty *et al.* (2013) [3] who reported the compatibility between *Trichoderma viride* and fungicides like Calixin, Ridomil MZ-72, Blitox and Bavistin. Ashish *et al*. (2019) [2] conducted the compatibility study and results revealed that 100 per cent compatibility was between *T. harzianum* and Mancozeb at 50 ppm concentration and observed the least compatibilty with carbendazim even at 50 ppm concentration. The studies conducted by Ajay and Prasad (2018) [1] revealed that, all the four concentrations of Mancozeb were highly compatible with almost negligible toxic effect against *Trichoderma harzianum in vitro*.

Conclusion and Acknowledgement

Among the tested bioagents against *M. phaseolina*, maximum inhibition was recorded in *Trichoderma harzianum* (IOF, Dharwad). Mancozeb and Captan were effective with maximum inhibition among contact fungicides. Among systemic fungicide, Tebuconazole and Thiophenate methyl were found effective which recorded maximum mycelial inhibition. Among the combi product tested, Trifloxystrobin 13.7 % + Penflufen 13.7% (Evergol) and Metalaxyl 4 % + Mancozeb 64 % (Ridomil gold) showed maximum mycelial inhibition. *T. harzianum* was more compatible with Trifloxystrobin 13.7% + Penflufen 13.7% (Evergol) followed by Metalaxyl 4 % + Mancozeb 64 % (Ridomil gold). Further, these *in vitro* results have been utilized for the confirmation under field condition for the year 2020-21 and 2021-22.

Table 1: *In vitro* efficacy of bioagents against *Macrophomina phaseolina* causing charcoal rot of soybean

Sl. No	Bioagents	Per cent inhibition of mycelial growth
1	*Trichoderma harzianum* (IOF, Dharwad)	82.35 (65.13)*
2	*Trichoderma viride* (Multiplex Nisarga)	75.69 (60.43)
3	*Trichoderma harzianum* (UAS Raichur isolate)	74.51 (59.65)
4	*Pseudomonas fluorescens* (Multiplex Sparsha)	73.33 (58.89)
5	*Pseudomonas fluorescens* (UAS Raichur isolate)	64.31 (53.30)
6	*Pseudomonas fluorescens* (IOF, Dharwad)	59.22 (50.29)
7	Endophyte (*Neofusicoccum parvum*)	77.65 (61.76)
SEm±		0.75
C.D.@1%		3.14

*indicates angular transformed values

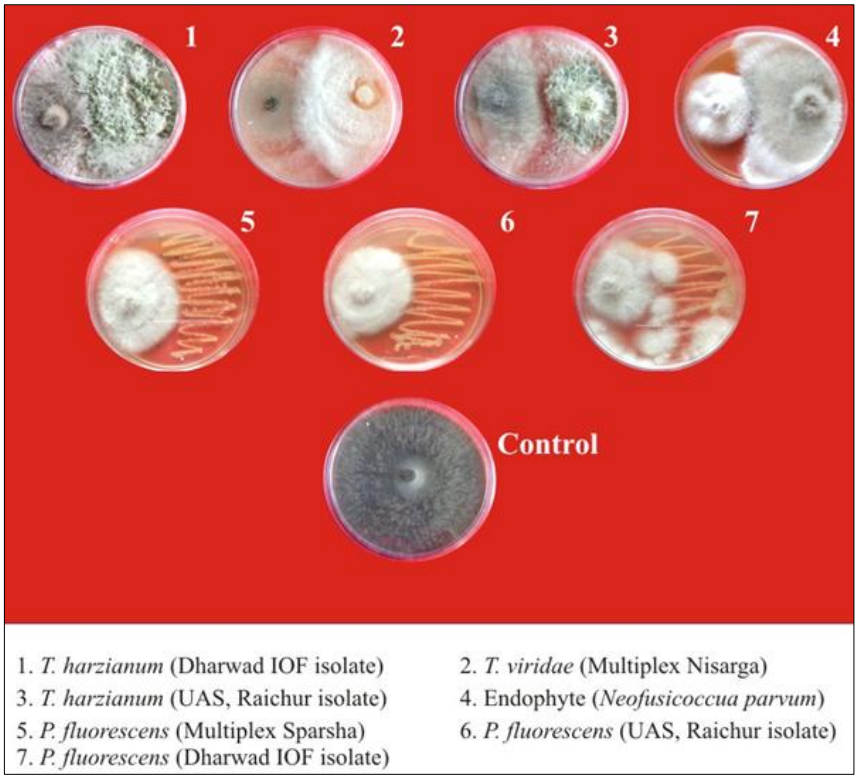

Plate 1: *In vitro* efficacy of bioagents against *Macrophomina phaseolina*

Table 2: *In vitro* efficacy of contact and combi fungicides against *Macrophomina phaseolina* causing charcoal rot of soybean

Contact fungicide	Trade name	Per cent inhibition of mycelial growth			
		Concentrations (ppm)			Mean
		500	1000	2000	
Mancozeb 75% WP	Dithane M-45	95.56 (77.80)*	97.04 (80.06)	99.26 (85.03)	97.28 (80.48)
Captan 50% WP	Captaf	87.78 (69.51)	90.00 (71.54)	91.11 (72.62)	89.63 (71.19)
Copper oxy chloride 50% WP	Blitox	85.93 (67.94)	88.52 (70.17)	90.00 (71.54)	88.15 (69.83)
Combi fungicide	**Trade name**				
(Trifloxystrobin 13.7% + Penflufen 13.7%) 50% SL	EverGol	94.07 (75.88)	95.93 (78.32)	97.78 (81.39)	95.93 (78.32)
(Metalaxyl 4 % + mancozeb 64 %) 68% WP	Ridomil Gold	91.11 (72.62)	92.59 (74.18)	96.67 (79.45)	93.46 (75.15)
(Carboxin 37.5 % + Thiram 37.5%) 75% WP	Vitavax power	83.33 (65.88)	90.00 (71.54)	94.07 (75.88)	89.13 (71.10)
(Carbendazim 12%+ Mancozeb 63%) 75% WP	Saaf	82.22 (65.04)	87.04 (68.87)	90.00 (71.54)	86.42 (68.48)
(Thiophanate methyl 45 % + Pyraclostrobin 5%) 50% SL	Xelora	78.52 (62.36)	85.19 (67.34)	88.52 (70.10)	84.08 (66.62)
		Fungicide(F)	**Concentration(C)**	**F×C**	
SEm±		1.98	1.21	0.91	
C. D @1%		0.52	0.32	3.44	

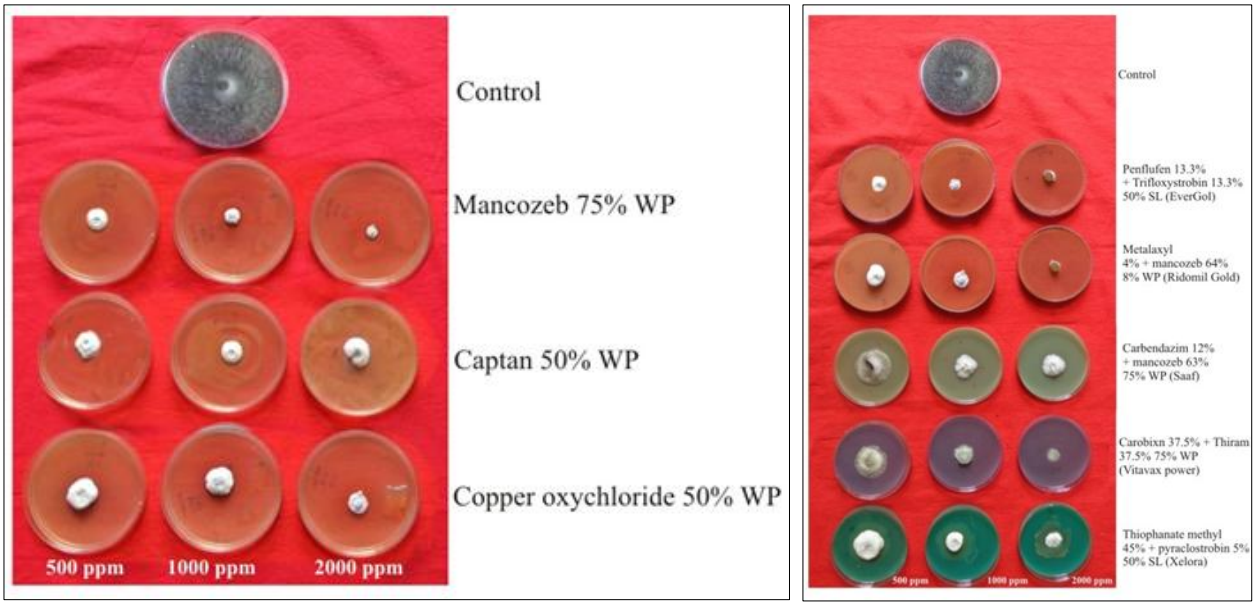

Plate 2: *In vitro* efficacy of contact and combi products fungicides against *Macrophomina phaseolina*

Table 3: *In vitro* efficacy of systemic fungicides against *Macrophomina phaseolina* causing charcoal rot of soybean

Fungicides	Trade name	Per cent inhibition of mycelial growth			
		Concentrations (ppm)			Mean
		250	500	1000	
Tebuconazole 25% WP	Raxil	90.00 (71.54)*	91.48 (73.00)	92.22 (73.78)	91.23 (72.75)
Thiophanate methyl 70% WP	Roko	83.33 (65.88)	89.26 (70.84)	92.59 (74.18)	88.40 (70.05)
Difenoconazole 25% EC	Score	81.11 (64.21)	85.56 (67.64)	89.63 (71.19)	85.43 (67.54)
Hexaconazole 5% EC	Contaf	78.52 (62.36)	83.33 (65.88)	91.11 (72.62)	84.32 (66.65)
Propiconazole 25% EC	Tilt	64.07 (53.15)*	69.26 (56.30)	88.52 (70.17)	73.95 (59.29)
Carbendazim 50% WP	Bavistin	63.33 (52.71)	72.96 (58.65)	80.37 (63.68)	72.22 (58.17)
		Fungicide (F)	**Concentration (C)**	**F×C**	
SEm±		0.68	0.48	1.18	
C.D.@1%		2.62	1.85	4.54	

*indicates angular transformed values

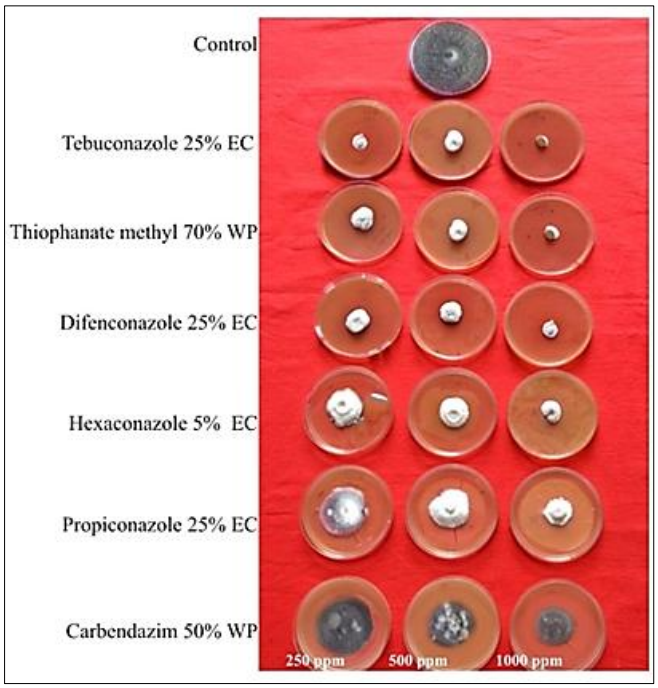

Plate 3: *In vitro* efficacy of systemic fungicides against *Macrophomina phaseolina*

Table 4a: Compatibility of effective biocontrol agent *Trichoderma harzianum* (IOF, Dharwad) with contact and combi fungicides

Sl. No.	Contact fungicide	Trade name	Mycelial growth in mm			
			Concentrations in ppm			
			500	1000	2000	Mean
1	Mancozeb 75% WP	Dithane M-45	77.40	70.60	67.40	71.80
2	Captan 50% WP	Captaf	68.60	67.40	62.60	66.20
3	Copper oxy chloride 50% WP	Blitox	68.60	66.60	46.00	60.40
	Combi fungicides	**Trade name**				
1	(Trifloxystrobin 13.7% + Penflufen 13.7%) 50% SL	EverGol	84.00	81.40	78.60	81.20
2	(Metalaxyl 4 % + mancozeb 64 %) 68% WP	Ridomil Gold	75.40	73.40	70.60	73.00
3	(Carboxin 37.5 % + Thiram 37.5%) 75% WP	Vitavax power	61.40	42.00	36.00	46.40
4	(Carbendazim 12% + Mancozeb 63%) 75% WP	Saaf	29.40	23.40	20.00	24.20
5	(Thiophanate methyl 45 % + Pyraclostrobin 5%) 50% SL	Xelora	28.60	21.40	20.60	23.40
6	Control		90.00	90.00	90.00	90.00
			Fungicide (F)	**Concentration(C)**	**F×C**	
	SEm±		0.24	0.17	0.48	
	C.D.@1%		0.88	0.62	1.76	

Plate 4a: *In vitro* compatibility of effective biocontrol agent *Trichoderma harzianum* (IOF, Dharwad) with contact and combi fungicides

Table 4b: Compatibility of effective biocontrol agent *Trichoderma harzianum* (IOF, Dharwad) with systemic fungicides

Sl. No.	Systemic fungicide	Trade name	Mycelial growth in mm			
			Concentration in ppm			Mean
			250	500	1000	
1	Tebuconazole 25% WP	Raxil	72.00	66.00	67.40	68.46
2	Hexaconazole 5% WP	Contaf	46.00	40.60	36.00	40.86
3	Difenoconazole 25% EC	Score	45.4	36.60	29.40	37.12
4	Thiophanate methyl 70% WP	Roko	24.00	21.40	20.6	22.00
5	Propiconazole 25% EC	Tilt	25.40	19.40	16.00	20.26
6	Carbendazim 50% WP	Bavistin	16.60	11.40	9.40	12.46
7	Control		90.00	90.00	90.00	90.00
			Fungicide (F)	**Concentration(C)**	**F×C**	
	SEm±		0.66	0.54	1.33	
	C.D.@1%		2.48	2.03	4.96	

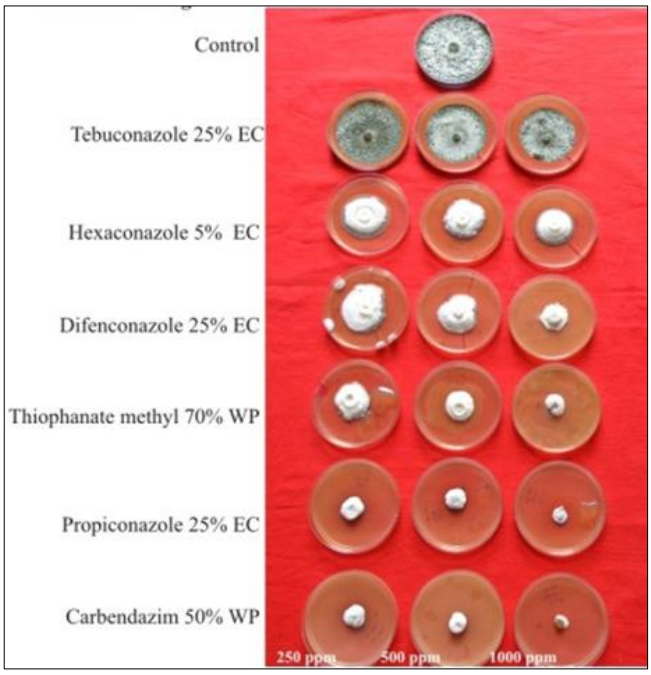

Plate 4b: *In vitro* compatibility of effective biocontrol agent *Trichoderma harzianum* (IOF, Dharwad) with systemic fungicides

References

1. Ajay T, Prasad D. Compatibility of *Trichoderma harzianum* with systemic and two non systemic fungicides under *in vitro*. *Asian Journal of Crop Science.* 2018;10(4):38-42.
2. Ashish K, Rani DB, Yogesh KC. Compatibility of *Trichoderma viride* with Fungicides for Plant Disease Management. *International Journal of Pure & Applied Bioscience*. 2019;7(3):44-51.
3. Chakrabarty R, Acharya GC, Sarma TC. Effect of fungicides, *Trichoderma* and plant extracts on mycelial growth of *Thielaviopsis paradoxa*, under *in vitro* condition. *The Bioscan*. 2013;8(1):55-58.
4. Dennis C, Webster J. Antagonistic properties of species group of *Trichoderma* III hyphal interaction. *Transaction of the British Mycological Society.* 1971;57:363-369.
5. Gupta GK, Singh N, Chauhan G, Vyas K, Joshi P. Soybean diseases and their management. *Soybean Production and Improvement in India*. Indore, India, NRC for Soybean, 2004, pp. 145-168.
6. Kale FS. Soybean, its value in dietetics, cultivation and uses, International Books and Periodicals Supply Services, India, 1985, p. 479.
7. Kumar A, Gupta JP. Variation in enzyme activity of tebuconazole tolerant biotypes of *Trichoderma viride. Indian Phytopathology*. 1999;52(3):263-266.
8. Naik MK, Madhukar HM, Devika Rani GS. Evaluation of biocontrol efficacy of *Trichoderma* isolates and methods of its applications against wilt of chilli caused by *Fusarium solani. Journal of Biological Control*. 2009;23:31-36.
9. Nene YL, Thapliyal PN. Fungicides in Plant Disease Control. Oxford and IBH publishing house, New Delhi, India, 1973, p. 163.
10. Sharvelle EG. The Nature and Use of Modern Fungicides. Burges Publications Co., Minnesota, USA, 1961, p. 308.
11. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. *Nature.* 1947;22:159-850.