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Compatibility study of bio control agents with agrochemicals under *in vitro* conditions

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Abstract
The evaluation of fungicides/combination of fungicides, insecticides and herbicides with *Trichoderma* isolate-1 at different concentrations under *in vitro* using poisoned food technique. Among three fungicides tried, pyraclostrobin 13.3 + epoxiconazole 5 SE with 61.90 percent mycelial growth inhibition at 50 ppm concentrations respectively and proved significantly compatible over rest of the fungicide treatments. Whereas, the maximum mycelial growth inhibition was found in carbendazim 50 WP (94.51%) found incompatible with *Trichoderma* isolate-1. Among three promising insecticides tried, tetraniliprole 18.18 SC with 17.40, 25.94, 29.28 and 54.86 percent mycelial growth inhibition at 250, 500, 1000 and 1500 ppm concentrations respectively and proved significantly compatible over rest of the insecticide treatments. While, the maximum mycelial growth inhibition was found in quinalphos 25 EC (93.40%) found incompatible with *Trichoderma* isolate-1. Among three promising herbicides tried, imazamox 35 + imazethapyr 35 WG showed least mean mycelial growth inhibition (39.29%) at 50 ppm while, the maximum mycelial growth inhibition was found in quizalofop ethyl 10 EC (94.51%) found incompatible with *Trichoderma* isolate-1. The evaluation of agrochemicals with *P. fluorescens* isolate-6 at four different concentrations under *in vitro* condition using poisoned food technique. All nine agrochemicals completely inhibited the growth of *P. fluorescens* isolate-6 at all four different concentrations, showed an incompatible reaction with all the agrochemicals tested. The complete inhibition observed may be due either to the toxicity of the agrochemicals or to the sensitivity of *P. fluorescens* isolate-6.

Keywords: *Trichoderma*, *Pseudomonas*, agrochemicals, compatibility

1. Introduction
Notable success in disease control using antagonistic microorganisms has been achieved in the laboratory, greenhouse and field over the past several years. Based on this information, there is potential to develop biological control methods for plant diseases under field conditions. Commercial formulations of various biocontrol agents are already available in the market. However, inadequate information on the performance of these antagonists under varying environmental conditions remains a major constraint to the large-scale adoption of this technology and also lack of knowledge about compatibility of bioagents with agrochemicals. There is growing interest in combining fungal biocontrol with fungicides, and research on this form of integrated control is progressing more rapidly than on other combinations of control components (Lewis and Papavizas, 1991) [3]. A novel blending technique has been reported, in which biocontrol agents were used simultaneously with seed-dressing fungicides without exhibiting toxic effects on the antagonists. Integrated seed treatment with chemicals and compatible antagonists not only protects seeds and seedlings from soil-borne infections but also offers protection from seed-borne inoculum. Therefore, the use of compatible fungicides is essential for effective integrated disease management (Dubey and Patel, 2001) [2]. Considering this, the present study was undertaken to evaluate the compatibility of agrochemicals with *Trichoderma* isolate-1 and *Pseudomonas fluorescens* isolate-6, with the aim of formulating an integrated disease management approach.

2. Materials and Methods
The present investigation was carried out in the Main Oilseeds Research Station Laboratory, Junagadh Agricultural University, Junagadh during the year 2024-25. Compatibility study of biocontrol agents (both fungal and bacterial bioagents) with agrochemicals under *in vitro*

conditions using Factorial Completely Randomized Design (FCRD). Evaluation of fungal (*Trichoderma* isolate-1) and bacterial (*Pseudomonas fluorescens* isolate-6) compatibility test consists of nine agrochemical treatments with four different concentrations of each treatment.

2.1 Evaluation of Compatibility of *Trichoderma* isolate-1 with Agrochemicals under *in vitro* Condition

Nine best agrochemicals with four different concentrations were evaluated against *Trichoderma* isolate-1 under laboratory condition following poisoned food technique of Bagchi and Das (1968) [1]. Experiment was laid out with nine treatments and each treatment repeated three times. Completely Randomized Design with Factorial concept was used for analyzing the experimental data. A control plate without poisoned medium was also maintained simultaneously to compare the growth of *Trichoderma* isolate-1 in treated plates. The observation on radial growth was taken when fungal biocontrol colony in control plate attain 90 mm and percent growth inhibition of fungus in each treatment was calculated by using formula given earlier in Equation (1). The details of nine best promising agrochemicals used are given in Table 1. The percent mycelial growth inhibition of the fungus in each of the treatment was calculated by using the following formula (Vincent, 1947) [6].

$$I = \frac{C\text{-}T}{C} \times 100 \quad \ldots. (1)$$

Where,
I = percent inhibition of mycelial growth
T = Mean radial growth of test pathogen in treated plate (mm)
C = Mean radial growth of test pathogen in control plate (mm)

Table 1: Compatibility evaluation of *Trichoderma* isolate-1 with agrochemicals

Tr. No.	Technical/active ingredient	Concentration (ppm)			
		1	2	3	4
T_1	Carbendazim 50 WP	50	100	250	500
T_2	Mancozeb 50 + carbendazim 25 WS	50	100	250	500
T_3	Pyraclostrobin 13.3 + epoxiconazole 5 SE	50	100	250	500
T_4	Quinalphos 25 EC	250	500	1000	1500
T_5	Pyriproxyfen 5 + diafenthiuron 25 SE	250	500	1000	1500
T_6	Tetraniliprole 18.18 SC	250	500	1000	1500
T_7	Quizalofop ethyl 10 EC	50	100	250	500
T_8	Imazamox 35 + imazethapyr 35 WG	50	100	250	500
T_9	Pendimethalin 30 EC	50	100	250	500
T_{10}	Control				

2.2 Evaluation of Compatibility of *P. fluorescens* isolate-6 with Promising Agrochemicals under *in vitro* Condition

Nine best agrochemicals with four different concentrations were evaluated against
P. fluorescens isolate-6 under laboratory condition following poisoned food technique of Bagchi and Das (1968) [1]. Experiment was laid out with nine treatments and each treatment repeated three times. 5 mm paper disc dipped in bacterial suspension placed in the centre of Petri plate. A control plate without poisoned medium was also maintained simultaneously to compare the growth of *P. fluorescens* isolate-6 in treated plates. Completely Randomized Design with Factorial concept was used for analysing the experimental data. The observation on radial growth was taken when bacterial colony in control plate attain 90 mm and percent growth inhibition of bacteria in each treatment was calculated by using formula given earlier in Equation (1). The details of nine best promising agrochemical used are given in Table 2.

Table 2: Compatibility evaluation of *P. fluorescens* isolate-6 with agrochemicals

Tr. No.	Technical/active ingredient	Concentration (ppm)			
		1	2	3	4
T_1	Carbendazim 50 WP	50	100	250	500
T_2	Mancozeb 50 + carbendazim 25 WS	50	100	250	500
T_3	Pyraclostrobin 13.3 + epoxiconazole 5 SE	50	100	250	500
T_4	Quinalphos 25 EC	250	500	1000	1500
T_5	Pyriproxyfen 5 + diafenthiuron 25 SE	250	500	1000	1500
T_6	Tetraniliprole 18.18 SC	250	500	1000	1500
T_7	Quizalofop ethyl 10 EC	50	100	250	500
T_8	Imazamox 35 + imazethapyr 35 WG	50	100	250	500
T_9	Pendimethalin 30 EC	50	100	250	500
T_{10}	Control				

3. Results and Discussion

3.1 Evaluation of Compatibility of *Trichoderma* isolate-1 with Agrochemicals under *in vitro* Condition

The least mycelial growth inhibited agrochemicals are compatible with fungal bioagent. The results obtained are communicated here under.

The data presented in Table-1, indicated that all agrochemicals were significantly reduced the mycelial growth of test fungal bioagent. Among the four concentrations, 50 and 250 ppm concentrations remained significantly compatible over rest of the concentrations with 66.67 percent mean mycelial growth inhibition.

Compatibility study of fungicides with *Trichoderma* isolate-1

As the concentration of fungicides increases, the mycelial growth inhibition also increases which mean it is incompatible. Pyraclostrobin 13.3 + epoxiconazole 5 SE showed least mean mycelial growth inhibition (71.07%) showed compatible reaction, when compared to other fungicides which were found incompatible with test fungal bioagent.

The interaction effect between promising fungicides and concentrations shows that, pyraclostrobin 13.3 + epoxiconazole 5 SE found more compatible with 61.90 percent mycelial growth inhibition at 50 ppm concentration respectively and proved significantly compatible over rest of the fungicide treatments.

Carbendazim 50 WP and mancozeb 50 + carbendazim 25 WS at 500 ppm concentration reported that maximum inhibition of mycelial growth (94.51%) and (93.40%) of *Trichoderma* isolate-1 both were incompatible.

Compatibility study of insecticides with *Trichoderma* isolate-1

As the concentration of insecticides increases, the mycelial growth inhibition also increases which shows incompatible reaction. Tetraniliprole 18.18 SC showed least mean

mycelial growth inhibition (31.87%) which remained compatible with test fungal bioagent when compared to other insecticides were found incompatible with test fungal bioagent.

The interaction effect between promising insecticides and concentrations shows that, tetraniliprole 18.18 SC found more compatible with 17.40, 25.94, 29.28 and 54.86 percent least mycelial growth inhibition at 250, 500, 1000 and 1500 ppm concentrations respectively and proved significantly compatible over rest of the insecticide treatments. Quinalphos 25 EC and pyriproxyfen 5 + diafenthiuron 25 SE at 1500 ppm concentration reported that maximum inhibition of mycelial growth (93.40%) and (91.57%) both were incompatible with *Trichoderma* isolate-1.

Compatibility study of herbicides with *Trichoderma* isolate-1

As the concentration of herbicides increases, the mycelial growth inhibition also increases which shows incompatible reaction. Imazamox 35 + imazethapyr 35 WG showed least mean mycelial growth inhibition (65.05%) found significantly compatible with *Trichoderma* isolate-1.

Table 1: Compatibility evaluation of *Trichoderma* isolate-1 with agrochemicals under *in vitro* condition

Promising agrochemicals	Mycelial growth inhibition (%)				Mean (%)
Carbendazim 50 WP @ 50, 100, 250 & 500 ppm	71.62 (90.06)	72.71 (91.17)	73.87 (92.28)	76.45 (94.51)	73.66 (92.00)
Mancozeb 50 + carbendazim 25 WS @ 50, 100, 250 & 500 ppm	70.58 (88.94)	72.71 (91.17)	73.46 (91.89)	75.11 (93.40)	72.96 (91.35)
Pyraclostrobin 13.3 + epoxiconazole 5 SE @ 50, 100, 250 & 500 ppm	51.88 (61.90)	55.44 (67.83)	60.15 (75.23)	62.95 (79.32)	57.61 (71.07)
Quinalphos 25 EC @ 250, 500, 1000 & 1500 ppm	67.42 (85.25)	71.62 (90.06)	73.08 (91.53)	75.11 (93.40)	71.80 (90.06)
Pyriproxyfen 5 + diafenthiuron 25 SE @ 250, 500, 1000 & 1500 ppm	65.69 (83.05)	69.25 (87.45)	71.26 (89.67)	73.12 (91.57)	69.83 (87.94)
Tetraniliprole 18.18 SC @ 250, 500, 1000 & 1500 ppm	24.66 (17.40)	30.62 (25.94)	32.76 (29.28)	47.79 (54.86)	33.96 (31.87)
Quizalofop ethyl 10 EC @ 50, 100, 250 & 500 ppm	67.42 (85.25)	70.58 (88.94)	73.87 (92.28)	76.45 (94.51)	72.08 (90.25)
Imazamox 35 + imazethapyr 35 WG @ 50, 100, 250 & 500 ppm	38.81 (39.29)	55.44 (67.83)	58.71 (73.02)	63.47 (80.05)	54.11 (65.05)
Pendimethalin 30 EC @ 50, 100, 250 & 500 ppm	44.39 (48.93)	51.67 (61.53)	62.57 (78.78)	67.71 (85.61)	56.58 (68.71)
Mean	55.83 (66.67)	61.12 (74.66)	64.41 (79.33)	68.68 (85.61)	
	Promising agrochemicals (A)		**Conc. (C)**	**A × C**	
S. Em.±	0.37		**0.25**	**0.74**	
C.D. at 5%	1.04		**0.69**	**2.08**	
C.V.%	2.04				

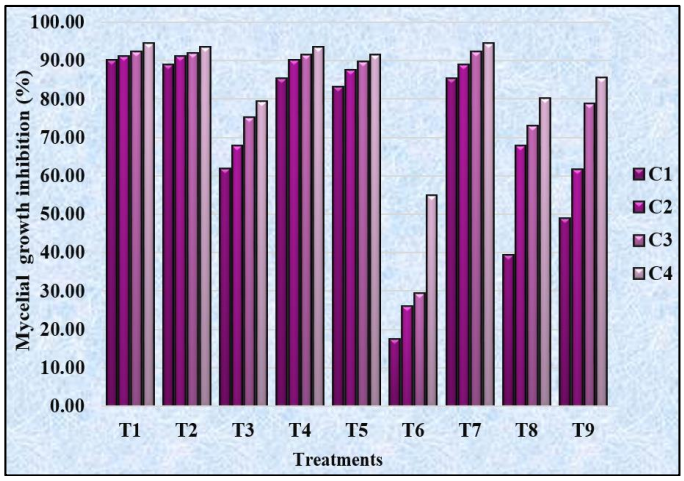
Note: Data outside the parentheses are arcsine transformed, whereas inside are retransformed values.

The next compatible treatment was Pendimethalin 30 EC showed 68.71 percent mean mycelial growth inhibition of test bioagent over rest of insecticides which were found incompatible.

The interaction effect between promising insecticides and concentrations shows that, imazamox 35 + imazethapyr 35 WG found more compatible with 39.29 percent least mycelial growth inhibition at 50 ppm concentration respectively and proved significantly compatible over rest of the herbicide treatments. Pendimethalin 30 EC found next moderately compatible treatment at 50 ppm concentration and gave 48.93 percent mycelial growth inhibition. Quizalofop ethyl 10 EC at 1500 ppm concentration showed maximum mycelial growth inhibition (94.51%) of *Trichoderma* isolate-1 was found incompatible reaction.

The results obtained in present study of promising agrochemicals with *Trichoderma* isolate were corroborate with more or less similar type of results as obtained by Mohiddin and Khan (2013) [4], Saxena *et al*. (2014) [5] and Vyas (2020) [7] while working on *Trichoderma*. isolates.

Fig 1: Evaluation of compatibility of *Trichoderma* isolate-1 with promising agrochemicals under *in vitro* condition



Treatments-(Concentrations- C_1, C_2, C_3 & C_4)

T_1: Carbendazim 50 WP (50, 100, 250 & 500 ppm)

T_2: Mancozeb 50 + carbendazim 25 WS (50, 100, 250 & 500 ppm)

T_3: Pyraclostrobin 13.3 + epoxiconazole 5 SE (50, 100, 250 & 500 ppm)

T_4: Quinalphos 25 EC (250, 500, 1000 & 1500 ppm)

T_5: Pyriproxyfen 5 + diafenthiuron 25 SE (250, 500, 1000 & 1500 ppm)

T_6: Tetraniliprole 18.18 SC (250, 500, 1000 & 1500 ppm)

T_7: Quizalofop ethyl 10 EC (50, 100, 250 & 500 ppm)

T_8: Imazamox 35 + imazethapyr 35 WG (50, 100, 250 & 500 ppm)

T_9: Pendimethalin 30 EC (50, 100, 250 & 500 ppm)

T_{10}: Control

Fig 2: Evaluation of compatibility of *Trichoderma* isolate-1 with promising agrochemicals under *in vitro* condition

3.2 Evaluation of Compatibility of *P. fluorescens* isolate-6 with Agrochemicals under *in vitro* Condition

Table 2: Evaluation of compatibility of *P. fluorescens* isolate-6 with agrochemicals

Promising agrochemicals	Mycelial growth inhibition (%)				Mean (%)
Carbendazim 50 WP @ 50, 100, 250 & 500 ppm	66.81 (84.50)	68.63 (86.72)	71.26 (89.67)	72.71 (91.17)	69.85 (88.02)
Mancozeb 50 + carbendazim 25 WS @ 50, 100, 250 & 500 ppm	79.62 (96.75)	90.05 (100.00)	90.05 (100.00)	90.05 (100.00)	87.44 (99.19)
Pyraclostrobin 13.3 + epoxiconazole 5 SE @ 50, 100, 250 & 500 ppm	64.28 (81.16)	65.66 (83.01)	69.58 (87.83)	71.62 (90.06)	67.78 (85.52)
Quinalphos 25 EC @ 250, 500, 1000 & 1500 ppm	65.10 (82.28)	65.95 (83.39)	67.71 (85.61)	70.58 (88.94)	67.33 (85.05)
Pyriproxyfen 5 + diafenthiuron 25 SE @ 250, 500, 1000 & 1500 ppm	61.15 (76.72)	64.28 (81.16)	67.71 (85.61)	69.58 (87.83)	65.68 (82.83)
Tetraniliprole 18.18 SC @ 250, 500, 1000 & 1500 ppm	58.95 (73.40)	61.41 (77.10)	63.47 (80.05)	66.81 (84.50)	62.66 (78.76)
Quizalofop ethyl 10 EC @ 50, 100, 250 & 500 ppm	60.40 (75.61)	63.47 (80.05)	65.95 (83.39)	72.71 (91.17)	65.63 (82.55)
Imazamox 35 + imazethapyr 35 WG @ 50, 100, 250 & 500 ppm	65.95 (83.39)	67.71 (85.61)	69.58 (87.83)	71.96 (90.41)	68.80 (86.81)
Pendimethalin 30 EC @ 50, 100, 250 & 500 ppm	66.52 (84.12)	67.40 (85.23)	69.67 (87.93)	71.96 (90.41)	68.89 (86.92)
Mean	65.42 (81.99)	68.28 (84.70)	70.55 (87.55)	73.11 (90.50)	
	Agrochemicals(A)		Conc. (C)	A × C	
S. Em.±	0.28		0.19	0.57	
C.D. at 5%	0.80		0.53	1.60	
C.V.%	1.41				

Note: Data outside the parentheses are arcsine transformed, whereas inside are retransformed values.

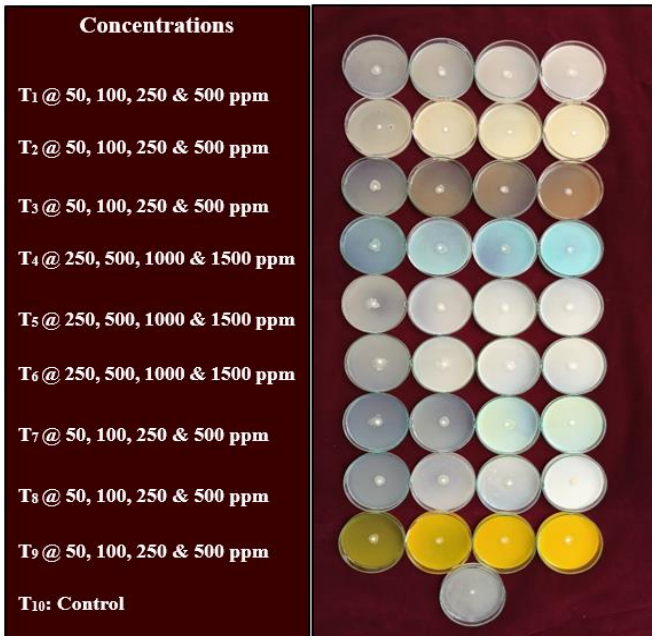
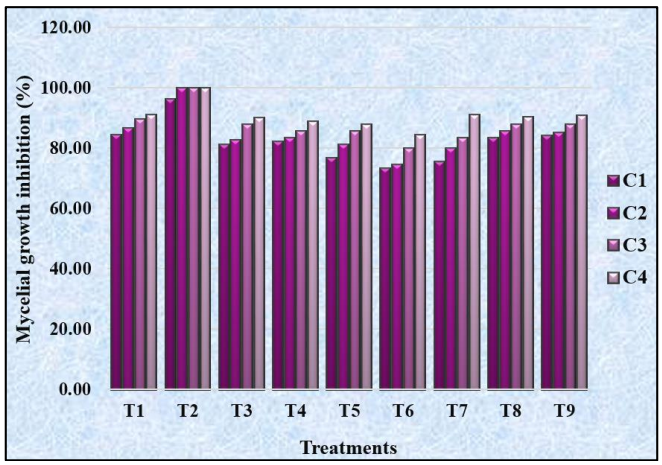


Fig 3: Evaluation of compatibility of *P. fluorescens* isolate-6 with promising agrochemicals under *in vitro* condition

Nine best selected agrochemicals (three fungicides, three insecticides, three herbicides) with different concentrations were evaluated against *P. fluorescens* isolate-6 under *in vitro* condition following poisoned food technique replicated thrice. A control plate without poisoned medium was also maintained simultaneously to compare the growth of *P. fluorescens* isolate-6 in treated plates. percent growth inhibitions in each of the treatment were calculated after seven days of inoculation. The least percent growth inhibited agrochemicals are compatible with bacterial bioagent. The results obtained are communicated here under.

The data presented in Table 2 indicated that all nine agrochemicals completely inhibited the growth of *P. fluorescens* isolate-6 at all four different concentrations, showed an incompatible reaction with all the agrochemicals tested. The complete inhibition observed may be due either to the toxicity of the agrochemicals or to the sensitivity of *P. fluorescens* isolate-6.



Treatments-(Concentrations- C_1, C_2, C_3 & C_4)
T_1: Carbendazim 50 WP (50, 100, 250 & 500 ppm)
T_2: Mancozeb 50 + carbendazim 25 WS (50, 100, 250 & 500 ppm)
T_3: Pyraclostrobin 13.3 + epoxiconazole 5 SE (50, 100, 250 & 500 ppm)
T_4: Quinalphos 25 EC (250, 500, 1000 & 1500 ppm)
T_5: Pyriproxyfen 5 + diafenthiuron 25 SE (250, 500, 1000 & 1500 ppm)
T_6: Tetraniliprole 18.18 SC (250, 500, 1000 & 1500 ppm)
T_7: Quizalofop ethyl 10 EC (50, 100, 250 & 500 ppm)
T_8: Imazamox 35 + imazethapyr 35 WG (50, 100, 250 & 500 ppm)
T_9: Pendimethalin 30 EC (50, 100, 250 & 500 ppm)
T_{10}: Control

Fig 4: Evaluation of compatibility of *P. fluorescens* isolate-6 with promising agrochemicals under *in vitro* condition

4. Conclusions

On the basis of this investigation, we can conclude that fungal bio control agent under the study can be applied to crop along with fungicides or insecticides or herbicides. Among the treatments, the fungicide pyraclostrobin 13.3 + epoxiconazole 5 SE at concentrations of 50 ppm; the insecticide tetraniliprole 18.18 SC at 250, 500, 1000, and 1500 ppm; and the herbicide imazamox 35 + imazethapyr 35 WG at 50 ppm, were found to be more compatible with *Trichoderma* isolate-1 compared to other treatments. While bacterial bio agent was found incompatible with agrochemicals.

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7. Conflict of interest

No conflict of interests exists

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