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In-vitro studies on the compatibility of *Trichoderma viride* with commonly used agrochemicals in the vegetable cropping system

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ABSTRACT: *In-vitro* studies were conducted to check the compatibility of microbial bioagent, *Trichoderma viride* with seven fungicides, eigtht insecticides, two herbicides, three fertilizers, and two antibiotics at different concentrations. The bioagent was compatible with insecticides *viz.,* imidacloprid 27.8% SL, diafenthiuron 50% WP, chlorantraniliprole 18.5% SC, flubendiamide 20% WG, azoxystrobin 23% SC, deltamethrin 2.8% EC, clothianidin 50% WDG, fenpropathrin 10% EC, and tiamethoxam 25% WG at all the four concentrations. However, fungicides hexaconazole 5% EC, propiconazole 25% EC, tebuconazole 25.9 % EC, and tebuconazole 50% + trifloxistrobin 25% WG and copper oxychloride 50% WP showed incompatibility with *T. viride.* Among the herbicides, glyphosate 41% SL was compatible, while paraquat dichloride 24% SL showed an incompatible reaction. Urea and muriate of potash (MOP) showed varied compatibility at different concentrations, while single super phosphate (SSP) showed a compatible reaction with the microbial bioagent. The results also indicate that antibiotic like streptomycin and gentamycin are compatible with *T. viride*. Knowledge of the compatibility of the microbes and the agrochemicals will be useful to apply biopesticide formulations in combination with inorganic pesticides, targeting different pests to reduce the cost and time.

Keywords: Agrochemicals, fungicides, insecticides, *Trichoderma viride*, compatibility

INTRODUCTION

Management of agricultural pests and pathogens using microbial biopesticides is a non-chemical and sustainable approach under climate-smart agriculture (Hanuman *et al.*, 2018) as the approach is cost-effective and eco-friendly. Sole application of microbial biopesticides is seemed to be useful in controlling agricultural pests and pathogens, however, when used under integrated pest management programmes (IPM) their compatibility with other chemical pesticides like fungicides, insecticides, herbicides, etc. needs to be tested for better bio-efficacy and results (Ons *et al*., 2020). Compatibility assessment of microbial bioagents against chemical pesticides is essential to confirm (i) if the pesticide particles are useful when mixed with the biocontrol agents or not (ii) for reduction of the incurred cost of a single spray (iii) to gather proper knowledge and understanding about detrimental effects of chemicals and pesticides on exposure time, durability, action mechanisms and mode of replication of microbial bioagents (Ons *et al*., 2020).

In several disease and pest management strategies, the addition of fungicides, insecticides, and herbicides at recommended rates in combination with biocontrol agents has tremendously escalated the management of disease and pests, as compared to treatments done with biocontrol agents or chemicals separately on different platforms individually (Cerda *et al*., 2004). Microbial bioagents *viz., Trichoderma viride, Trichoderma harzianum, Bacillus subtilis, Bacillus bassiana, Lecanicillium lecanii,* etc. are found as efficient biopesticides against a wide range of plant pests and pathogens (Reference). According to Shukla *et al.* (2019), there are issues related to microbial biopesticides such as effective spore load and viability (colony forming unit/ml), virulence, self-survival, etc., the application of recommended doses of plant protection formulations (PPFs) needs to be made along with efficacious biopesticides in IPM schedules. Thus, knowledge about compatibility among microbial biopesticides and recommended doses of agrochemicals needs to be explored for future sustainability in agriculture.

MATERIALS AND METHODS

In-vitro Compatibility of *Trichoderma viride* with agrochemicals

The poison food technique (Grover and Moore, 1961) was used for compatibility assessment. Potato Dextrose Agar (PDA) medium was used for carrying out the poison food technique. The media was then mixed with different insecticides (Table 1), fungicides, (Table 2) herbicides

Table 1. *In-vitro* compatibility of *Trichoderma viride* with some commonly used insecticides

Insecticide	Concentration (in ppm)	Mean (±) * (Diameter of mycelia in cm)	Per cent inhibition
Imidacloprid 17.8 SL	100	9.00	0.00
	500	8.95	0.58
	1000	7.81	13.25
	2000	7.63	15.22
Thiamethoxam 25% WG	100	8.44	6.22
	500	8.37	7.03
	1000	3.60	60.00
	2000	2.40	73.33
Deltamethrin 2.8% EC	100	8.73	3.03
	500	7.20	19.96
	1000	6.33	29.63
	2000	6.24	30.63
Diafenthiuron 50% WP	100	9.00	0.00
	500	8.93	0.77
	1000	8.66	3.74
	2000	7.58	15.77
Chlorantraniliprole 18.5%SC	100	9.00	0.00
	500	9.00	0.00
	1000	7.85	12.81
	2000	7.37	18.11
Fenpropathrin 10% EC	100	9.00	0.00
	500	9.00	0.00
	1000	5.74	36.25
	2000	1.75	80.55
Flubendiamide 20% WG	100	9.00	0.00
	500	9.00	0.00
	1000	8.96	0.41
	2000	8.90	1.11
Clothianidin 50% WDG	100	8.97	0.30
	500	7.57	15.88
	1000	7.31	18. 81
	2000	6.63	26.36
Control	Control	9.00	0.00
SE±(d)	**0.10**		
C.D. @ 5 %	**0.27**		

(Table 3), fertilizers (Table 4), and antibiotics (Table 5) at 2000, 1000, 500, and 100 ppm concentrations, and the prepared media were poured into sterile Petri dishes. After the media solidified which was placed inside the laminar, a 5 mm diameter disc of *T. viride* was cut using a cork borer from the growing mycelium colony and it was placed in the center of the solidified Petri dishes. Petri dishes with no agrochemicals in the medium served as checks. For each treatment, 3 replications were maintained, and these Petri dishes were incubated at 28 ±1° C inside a BOD incubator. Observations were recorded periodically for radial growth and sporulation. The inhibition percentage was also recorded for checking compatibility. The record was done by measuring the radial growth, of the colony in each treatment and the inhibition percentage of growth was calculated using the formula given by Vincent (1927).

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

where **"I"** stands for percent growth inhibition; **"C"** stands for radial growth in the control(cm) plate; **"T"** stands for radial growth in the treated plates (cm)

RESULTS AND DISCUSSIONS

In-vitro compatibility of *Trichoderma viride* with different insecticides

Fenpropathrin 10% EC, flubendiamide 20% WG, and chlorantraniliprole18.5% SC had the lowest percent inhibition (%) of the eight insecticides tested, with 0% inhibition at 100 and 500 ppm, followed by imidacloprid 17.8% SL and diafenthiuron 50% WP, both with 0% inhibition at 100 ppm. fenpropathrin 10% EC showed the highest inhibition percentage of 80.55% at 2000 ppm and the results are tabulated in Table 1. Since flubendiamide 20% WG was shown to be extremely compatible with *T. viride* at all the tested concentrations, these findings demonstrated that it is the safest of all the compounds to be used with *T. viride*.

In-vitro compatibility of *Trichoderma viride* with different fungicides

Azoxystrobin 23% SC showed the least per cent inhibition (%) at all four different concentrations, thus, recorded its efficacy in disease reduction. The triazole fungicides were shown to be the most incompatible ones. The highest levels of inhibition were determined to be hexaconazole 5% EC, propiconazole 25% EC, tebuconazole 25.9% EC, and tebuconazole 50%

Table 2. *In-vitro* compatibility of *Trichoderma viride* with some commonly used fungicides

Fungicide	Concentration (ppm)	Mean (±) * (Diameter of mycelia in cm)	Per cent inhibition
Hexaconazole 5% EC	100	0.00	100.00
	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
Propiconazole 25% EC	100	0.00	100.00
	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
Tebuconazole 25.9% EC	100	0.00	100.00
	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
Tebuconazole 50% + Trifloxistrobin 25% WG	100	0.00	100.00
	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
Azoxystrobin 23% SC	100	9.00	0.00
	500	9.00	0.00
	1000	8.38	6.89
	2000	8.25	8.30
Chlorothalonil 75% WP	100	4.18	53.59
	500	3.49	61.19
	1000	0.00	100.00
	2000	0.00	100.00
Copper Oxychloride 50%WP	100	4.32	52.00
	500	4.11	54.33
	1000	3.77	58.08
	2000	3.34	62.89
Control	Control	9.00	0.00
SE±(d)	**0.06**		
C.D. @ 5%	**0.13**		

+ trifloxystrobin 25%, all of which showed 100% inhibition of the fungus's mycelia development at all dosages. Additionally, mycelia growth was inhibited by chlorothalonil 75% WP and COC 50% WP, with more than 50% inhibition (Table 2 and Fig. 1).

In-vitro compatibility of *Trichoderma viride* with different herbicides

Two herbicides were tested namely, glyphosate 41% SL and paraquat dichloride 24% SL, out of which glyphosate 41% SL comparatively was found to be compatible showing an inhibition rate of less than 50 %, and paraquat dichloride 24% SL showed the highest inhibition at 2000 ppm of 62.81% thus proving it to be highly incompatible, as shown in Table 3.

In-vitro compatibility of *Trichoderma viride* with different fertilizers

Three fertilizers (Urea SSP and MOP) were tested and amongst them, SSP showed excellent results when combined with the fungal bioagent, *T. viride*. It was found to be highly compatible at all the employed concentrations showing per cent inhibition (%) up to zero. Urea and MOP showed varied compatibility at different concentrations. Mycelial growth of *T. viride* was found to be inhibited at 100% application doses of urea and MOP at 2000 ppm and 85.37% (urea) and 85.92 % (MOP) at 1000 ppm, respectively. urea and MOP showed compatibility at 100 and 500 ppm concentrations (Table 4).

In-vitro compatibility of *Trichoderma viride* with different antibiotics

Table 3. *In-vitro* compatibility of *Trichoderma viride* with herbicides.

Agrochemicals	Concentrations(in ppm)	Mean (±) * (Diameterof mycelia in cm)	Per cent inhibition
Glyphosate 41% SL	100	6.85	23.89
	500	6.01	33.19
	1000	5.63	37.44
	2000	5.60	37.78
Paraquat Dichloride 24%SL	100	8.48	5.78
	500	7.29	19.00
	1000	6.99	22.33
	2000	3.35	62.81
Control	Control	9.00	0.00
SE±(d)	**0.17**		
C.D. @ 5 %	**0.37**		

Data in table 5 and figure 2 depict the compatibility of all the antibiotics (streptomycin and gentamycin) with *T. viride* at all tested concentrations. For streptomycin, the inhibition percentage was recorded as (0%, 1.63%, 3.70%, and 5.63%) at 100, 500, 1000, and 2000 ppm in combination with *T. viride*, and for gentamycin, it gave (0%, 1.44%,6.00% and 9.11%) at 100, 500, 1000 and 2000 ppm. The result of the present investigation depicts that antibiotics can be used along with the fungal biopesticides for better bio-efficacy and results.

The present investigation was found to be relatable to the findings of Mareeswaran *et al.* (2016) who did a detailed study on the impact of fungicides, pesticides, and herbicides (propargite, glyphosate, and ammonium salt of glyphosate, propiconazole, hexaconazole, Combination of carbendazim and mancozeb) on the mycelia characteristics of the fungal bio-agents and found that among all the agrochemicals tested Ammonium salt of glyphosate was found to be incompatible with *T. viride*. The compatible nature of the growth media along with the pesticides suggests that these bio-agents may thus be used for bioremediation of pesticides contaminated soil study. The incompatible nature may be due to the toxic trait of the chemicals at a higher concentration, limiting the growth of the mycelium of the fungal bio-agents. Similar results were found by Sharma *et al.* (2016) who did an experiment on the compatibility of biocontrol agents with agrochemicals including azadirachtin 5EC, bifenthrin 8SC, clothianidin 50 WDG, deltamethrin 2.8EC, fenpropathrin 90 EC, thiamethoxam 25WG, COC 50WP, propiconazole 25EC, hexaconazole 5EC, boric acid, zinc, urea, SSP and MOP at 2500 ppm, 1250ppm, 625 ppm, 1000 ppm,2000 ppm, 750 ppm, 375 ppm, 100 ppm, 50 ppm, 25 ppm, 125 ppm, 62.5 ppm, and 31.2 ppm as well and found that the fungal bio-agents

Table 4. *In-vitro* compatibility of *Trichoderma viride* with fertilizers

Fertilizer	Concentration (ppm)	Mean (±) * (Diameterof mycelia in cm)	Per cent inhibition
Urea	100	8.93	0.78
	500	8.99	0.03
	1000	1.32	85.37
	2000	0.00	100.00
MOP	100	9.00	0.00
	500	8.95	0.59
	1000	1.27	85.92
	2000	0.00	100.00
SSP	100	9.00	0.00
	500	9.00	0.00
	1000	9.00	0.00
	2000	9.00	0.00
Control	control	9.00	0.00
SE±(d)	**0.11**		
C.D. @ 5 %	**0.22**		

were found to be highly compatible in nature except for zinc, hexaconazole, propiconazole, boric acid. The compatible reactions of the antagonistic microorganism with the various fungicides may be due to their ability to degrade chemicals and inherent resistance to most of the fungicides (Papavizas, 1985). The compatible reactions may also be due to the high tolerance potential of the native *Trichoderma* spp.

In the present study, all the triazoles showed inhibition against the *T. viride*. The results were in accordance with Gayatri *et al.* (2016) who studied the compatibility nature of the fungal and bacterial bio-agents with the fungicides and fertilizers (copper oxychloride, chlorothalonil, mancozeb, SSP, MOP, and urea) at various concentrations and found that among all the fungicides only mancozeb was incompatible at various concentrations and others were compatible. Also, the findings of Shashikumar *et al.* (2016); Dutta *et al.* (2016); Vyas *et al.* (2020) respectively were found to be similar, where they tested the fungal bio-agents including *Trichoderma viride* against carbendazim, copper oxychloride, mancozeb, mancozeb + carbendazim, methyl -o– demeton, cartap hydrochloride, chloropyrifos, quizalofop, pendimethalin, fenoxyprop – p – ethyl, propiconazole, hexaconazole, trifloxystrobin + tebuconazole, tebuconazole and concluded that mancozeb, carbendazim, and combination of mancozeb and carbendazim were found to be incompatible in nature.

Table 5. *In-vitro* compatibility of *Trichoderma viride* with antibiotics

Chemical	Concentrations (ppm)	Mean (±) * (Diameter of mycelia in cm)	Per cent inhibition
Streptomycin	100	9.00	0.00
	500	8.85	1.63
	1000	8.67	3.70
	2000	8.49	5.63
Gentamycin	100	9.00	0.00
	500	8.87	1.44
	1000	8.46	6.00
	2000	8.18	9.11
Control	control	9.00	0.00
SE±(d)	**0.22**		
C.D. @ 5 %	**0.46**		

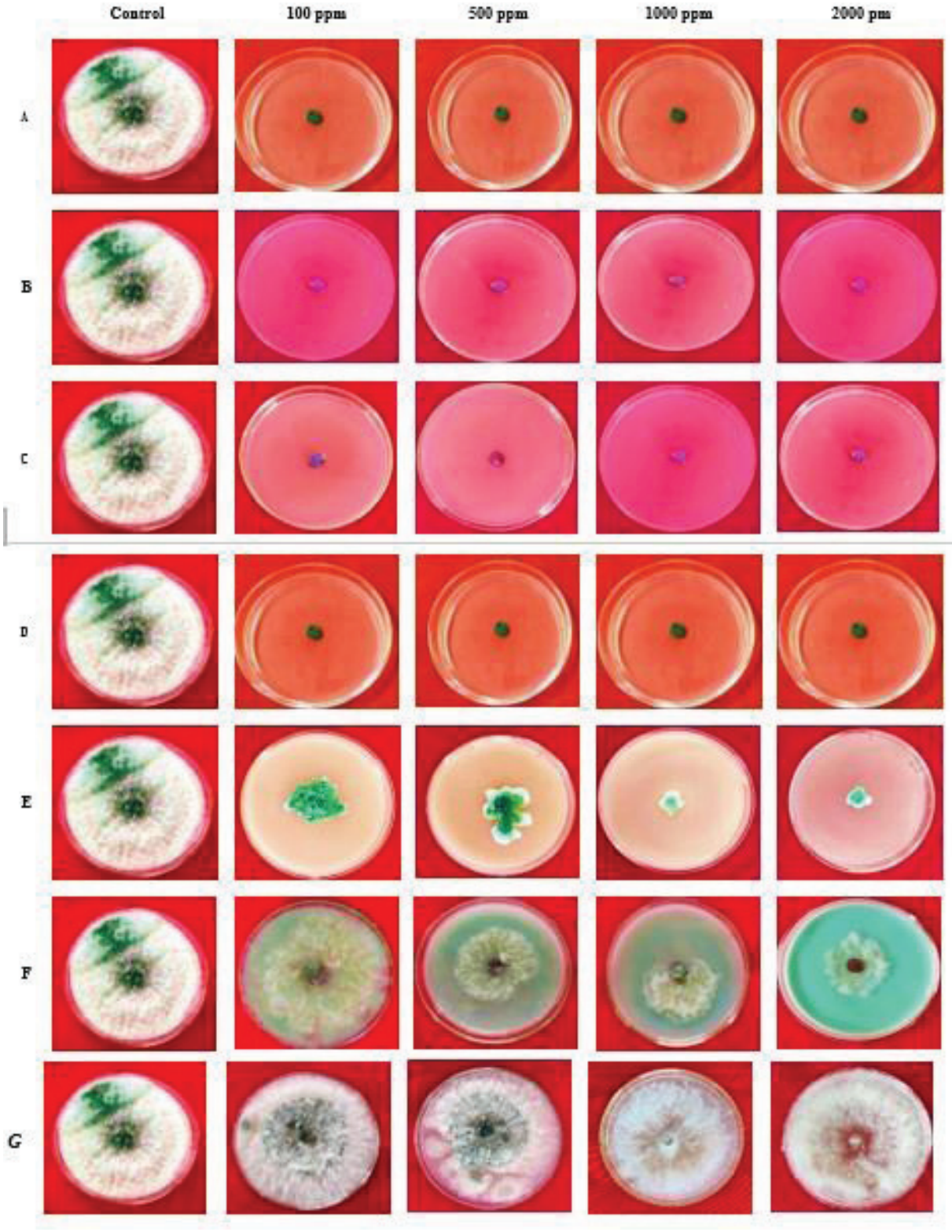

Fig. 1. (A- G). ***In-vitro*** **compatibility of** ***Trichoderma viride*** **with fungicides; (A) Hexaconazole +** ***T. viride*****; (B) Propiconazole +** ***T. viride*****; (C) Tebuconazole +** ***T. viride*****; (D) Tebuconazole 50% + Trifloxystrobin 25% +** ***T. viride*****; (E) Chlorothalonil +** ***T. viride;*** **(F) Copper Oxychloride +** ***T. viride*****; (G) Azoxystrobin +** ***T. viride***

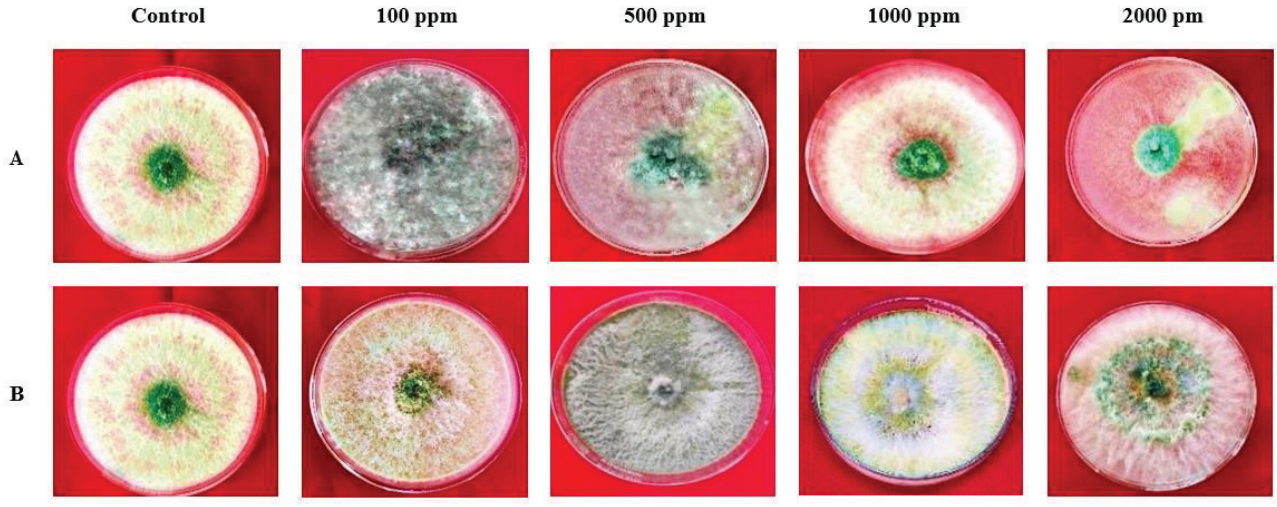

Fig. 2. (A-B). *In-vitro* compatibility of *Trichoderma viride* with antibiotics; (A) Streptomycin + *T. viride*; (B) Gentamycin + *T. viride*

The fungicides can restrict the growth of the mycelium of the fungus completely. This inhibitory effect may be due to the toxicity of the chemicals on the *Trichoderma* spores and cells leading to destruction of the hyphae. The mycelium is not able to grow profusely, and the infection peg is not built up to cause infection. In the case of the beneficial bioagents including *Trichoderma* spp. due to the fungicidal effect, their antagonistic effect against the pathogenic fungus reduces drastically, and thus the combination of fungal bioagent with fungicides is not recommended at all in IPM. The result was found to be in co-relation with the works done by Dutta *et al.* (2016) did a detailed study and were able to conclude that herbicides (atrazine 50 WP, glyphosate 41% SL and paraquat dichloride 24% SL) at lower doses can be incorporated with the fungal bio-agents. The result is in line with Pinnamaneni *et al.* (2010) who did a detailed study on the compatibility of *T. viride* with commonly used pesticides including fertilizers and found that SSP was the most compatible one followed by MOP and then urea. Kumar *et al.* (2017) also studied the compatibility of certain fertilizers against *T. viride* and found their compatibility with the bio-agent. Dutta *et al*. (2016) also did an assessment of certain agrochemicals including SSP, MOP, and urea with microbial biopesticides that are most used in tea of Assam condition basically in Northeast India and found that the fertilizers were compatible at respective concentrations. The results are coinciding with the work done by Gangwar *et al.* (2013)who conducted an experiment to find out the compatibility of fungal bioagents against the most used agrochemicals including streptomycin, and gentamycin where they found the high compatibility of antibiotics at 250, 500, 1000 and 2000 ppm showing maximum radial growth of 66.5 mm to 85.00 mm. Also, similar results were found by Mishra *et al.* (2019) against the compatibility of bioagents with the chemical pesticides including- streptocycline, streptomycin, carbendazim (50% WP), propiconazole (25% EC), tridemorph (BO% EC), hexaconazole (5% EC), chlorothalonil (75% WP) and mancozeb (75% WP) and found that the fungal BCAs were compatible with the antibiotics at all the respective concentrations.

CONCLUSION

The present result indicates that the combined application of compatible bioagents and agrochemicals have showed efficacy in plant protection. The approach eventually explores the for the reduced need of chemical pesticides in agriculture, thus, assists to maintain environmental integrity and safety. Incorporation of microbial biopesticides with recommended doses of PPFs is an effective strategy for adopting suitable IPM modules under climate-smart agriculture for improved plant growth and yield, and plant protection. Development of induced systemic resistance (ISR) in plants is another issue that gets strengthened due to repeated uses of efficacious microbials with potent mode of action. Proper screening and characterization of microbial bioagents with the advent of latest molecular tools and techniques would facilitate their application in sustainable agriculture. Multilocational field trials to test the efficacy of desirable bioagents needs to be made for adoption of suitable IDM under field situations.

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