Mycological Spectrum (e-ISSN:3107-6203)
https://mycologicalspectrum.org/
Published by Association of Fungal Biologists, India

Research Article

Compatibility of *Trichoderma harzianum* with agrochemicals commonly used in vegetable crop production systems

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Abstract

An *in-vitro* investigation employing the poisoned food approach was carried out to test the compatibility of *Trichoderma harzianum* with different agrochemicals including 8 insecticides, 7 fungicides, 2 herbicides, 3 fertilizers, and 2 antibiotics at concentrations of 100 ppm, 500 ppm, 1000 ppm, and 2000 ppm respectively. The bioagent displayed good compatibility with Imidacloprid 17.8 % SL, Diafenthiuron 50% WP, Chlorantraniliprole 18.5% SC, Flubendiamide 20% WG, Azoxystrobin 23% SC, Deltamethrin 2.8% EC and Clothianidin 50% WDG at all four concentrations. Hexaconazole 5% EC, Propiconazole 25% EC, Tebuconazole 25.9% EC, Tebuconazole 50% + Trifloxistrobin 25 % WG, and Copper Oxychloride 50 % WP with *T. harzianum* showed incompatible reactions, respectively. Among the two herbicides tested, Glyphosate 41% SL was found to be compatible at all four different concentrations showing an inhibition percentage of less than 50% whereas Paraquat Dichloride 24% SL was found to be incompatible showing an inhibition percentage of 73.11% at 2000 ppm, and 50.85 % at 1000 ppm against *T. harzianum*. Similarly, among fertilizers, single super phosphate (SSP) showed the highest compatibility followed by muriate of potash (MOP) and Urea giving an inhibition percentage of 36.58 %, and 37.47 % at 500 ppm, and 100 % at 1000 and 2000 ppm, respectively. The antibiotics (Streptomycin and Gentamycin), used in the current investigation were found to be highly compatible with the fungal bioagent at all the given concentrations.

Keywords: Agrochemicals, Bioagent, Compatibility, Incompatibility, Poisoned food method, *Trichoderma harzianum*.

Citation: Bharadwaz P, Nath BC, Bhattacharyya PN, *et al.* (2026). Compatibility of *Trichoderma harzianum* with agrochemicals commonly used in vegetable crop production systems. *Mycological Spectrum 02(02):172-185*. https://doi.org/10.64993/MS.2.2.7

Received: 25 July 2026| **Accepted**: 25 September 2026 | **Published:** 04 October 2026.

Handling Editor: Dr. Shilpa Verekar | **Reviewers**: Dr. P.K. Sahu and Dr. S.K. Deshmukh

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1. Introduction

Vegetables are excellent sources of vitamins, minerals, carbohydrates, and other essential nutrients for a healthy diet. A meta-analysis of cohort studies involving 4,69,551 participants found that increased consumption of fruits and vegetables is associated with a lower risk of death from cardiovascular disease, with an average risk reduction of 4 % for each additional serving of fruits and vegetables per day (Wang et al., 2014). However, the improper and risky use of agrochemicals in various aspects of agriculture has resulted in serious contamination of food (Sarma et al. 2020; Bhattacharyya et al. 2026a). Thus, the awareness on risks associated to agrochemical residues in vegetable crops along with their associated environment and human health is increasing. To address these concerns, agriculture needs to adopt alternative methods for food production that can satisfy dietary needs. A novel technique is, thus, necessary to protect the seeds and seedlings from multiparous soil-borne infections and seed-borne inoculums, as well as other pests in the micro-biome.

Controlling major pests and diseases in agriculture including in vegetables using beneficial microbes offers a cost-effective, eco-friendly alternatives (Bhattacharyya et al. 2016) without the use of toxic chemicals, thereby proving effective for crop management (Bhattacharyya et al., 2018; Hanuman et al., 2018). Beneficial microbes help in inhibiting various phytopathogens, insect pests and improve plant growth by direct and indirect mechanisms (Bhattacharyya et al., 2012; Bora et al., 2015; Nath et al., 2016, Sarmah et al., 2020; Bhattacharyya et al., 2023; Bhattacharyya et al., 2024a; Bhattacharyya et al., 2024b; Bhattacharyya et al., 2024c; Bora et al., 2024; Bhattacharyya et al., 2026b). The biocontrol agents are effective individually, but their compatibility with agrochemicals such as fungicides, insecticides, and herbicides used in vegetable cultivation needs further exploration. The information on compatibility assists certain key information such as (i) whether the pesticide particles are useful when mixed with the biocontrol agents or not (ii) to reduce the incurred cost of a single spray (iii) to have proper knowledge regarding the detrimental effect of pesticide on durability and replication of microbial bioagents.

The integration of fungicides, insecticides, and herbicides at prescribed levels alongside biocontrol agents has significantly enhanced disease and pest management strategies, in contrast to utilizing biocontrol agents or chemicals independently on separate platforms (Cerda et al., 2004). Various workers recommend the use efficiency of several microbial biocontrol agents *viz.*, *Trichoderma viride, T. harzianum, Bacillus subtilis, B. bassiana, Lecanicillium lecanii,* etc. against devastating pests and diseases.

Trichoderma harzianum is a prominent microbial bioinoculant, serving as a prominent, cost-effective and efficacious biocontrol agent and plant growth promoter due to its rapid growth and abilities to produce higher amounts of volatile, non-volatile compounds, and diverse antimicrobial compounds (Rahman et al., 2023; Laith et al., 2025). However, its effectiveness may be insufficient for severe pest infestations, necessitating the use of recommended doses of inorganic pesticides. A combined application of biopesticides and chemical pesticides may be beneficial (Dutta et al. 2016), making it crucial to understand the compatibility between these microorganisms and chemical pesticides. Most investigations on compatibility of

Trichoderma spp. with agrochemicals have concentrated on a few pesticides or a single category. Limited information exists on the compatibility of a wide range of vegetable production agrochemicals with *T. harzianum* under a uniform experimental technique. Thus, a characterised isolate of *T. harzianum* (Accession No. MZ836839) was tested for assessing the compatibility with 22 common agrochemicals, including the insecticides, fungicides, herbicides, fertilisers, and antibiotics. The findings assist to choose agrochemicals that can be used with *T. harzianum* in vegetable crop production system without affecting the bioagent.

2. Materials and methods

2.1 Collection and preservation of biocontrol agents and agrochemicals

Biocontrol agent, *T. harzianum* (Accession No. MZ836839) was collected from the Biocontrol laboratory of the Department of Plant Pathology, Assam Agricultural University (AAU),Jorhat to test their compatibility with agrochemicals. The biocontrol agent was grown on Potato Dextrose Agar (PDA) media and Trichoderma Specific Media (TSM) slants and incubated for five days at 28±1°C in a BOD incubator. The prepared slants were preserved in the refrigerator at 4°C for future use. A total of twenty-two commercial agrochemicals, comprising insecticides, fungicides, herbicides, fertilisers, and antibiotics were procured from certified agricultural input dealers in Jorhat, Assam, India. The names along with the board categories are mentioned in Table 1.

Table 1. List of agro chemicals used in the compatibility studies

Sl. No	Board Group	Chemical Name
01	Insecticide	Fenpropathrin 10% EC
02		Flubendiamide 20% WG
03		Thiamethoxam 25% WG
04		Diafenthiuron 50% WP
05		Deltamethrin 2.8 % EC
06		Clothianidin 50 % WDG
07		Chlorantraniliprole 18.5% SC
08		Imidacloprid 17.8 SL
09	Fungicide	Hexaconazole 5% EC
10		Propiconazole 25 % EC
11		Tebuconazole 25.9 % EC
12		Nativo (Tebuconazole 50 % + Trifloxystrobin 25 %)
13		Azoxystrobin 23 % SC
14		Chlorothalonil 75 % WP
15		Copper oxychloride 50% WP
16	Herbicide	Paraquat Dichloride 24% SL
17		Glyphosate 41 % SL
18	Fertilizers	Urea
19		MOP
20		SSP
21	Antibiotics	Streptomycin
22		Gentamycin

2.2 In-vitro compatibility of *T. harzianum* with various agrochemicals

Poison food technique was used to assess the compatibility of *T. harzianum* with various agrochemicals. PDA medium was prepared with double strength for carrying out the poison food technique. The media was then poisoned with different agrochemicals at different concentrations such as 2000, 1000, 500, and 100 ppm by dissolving them in sterilized distilled water (SDW). The prepared poisoned media were then poured into sterile Petri dishes. A 5-mm diameter disc of *T. harzianum* was cut out using a sterilized cork borer from the growing mycelium of 5 days old colony and was placed in the center of the Petri dishes with agrochemical poisoned media. Petri dishes with no agrochemicals in the medium served as control. For each treatment, three independent replications were maintained, and the 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 recorded for checking compatibility. The compatibility of agrochemicals with *T. harzianum* was evaluated by measuring the % inhibition of radial growth of mycelia. Agrochemicals inducing less than 50 % inhibition were considered as compatible, whereas those exhibiting 50 % or more inhibition were classified as incompatible with the bioagent.

Radial growth was recorded by measuring the radial growth of the colony in each treatment and the inhibition percentage of growth was calculated using the formula of 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)

2.3 Statistical analysis

The study was conducted using a Completely Randomised Design (CRD) with three replications. The recorded data underwent one-way analysis of variance (ANOVA) utilising OPSTAT statistical software. The means of treatment were analysed using the Critical Difference (CD) test at a significance level of 5% ($P \leq 0.05$).

3. Results and Discussion

3.1 In-vitro compatibility of *T. harzianum* with different insecticides

The highest inhibition was noted from Thiamethoxam 25% WG at 2000 ppm (53.92 %) followed by 1000 ppm (52.85 %) and Fenpropathrin 10% EC at 2000 ppm (50.52). However, at lower concentrations, both chemicals were found to be compatible with the fungus. All the other insecticides were compatible showing less than 50 % inhibition (Table 2). Thus, it can be confirmed that insecticides especially, Flubendiamide 20 % WG and Chlorantraniliprole 18.5% SC can be integrated with *T. harzianum*. Bharadwaz *et al.* (2023) mentioned that *T. viride* was generally compatible with most insecticides tested and highlighted the potential for integrating *T. viride* with selected insecticides in crop protection programs.

Table 2. *In-vitro* compatibility of *Trichoderma harzianum* with insecticides.

Insecticides	Concentrations (ppm)	Diameter of mycelia (cm)	Inhibition %
Imidacloprid 17.8 SL	100	9.00	0.00
	500	9.00	0.00
	1000	7.61	15.47
	2000	6.78	24.63
Thiamethoxam 25 % WG	100	7.32	18.66
	500	7.21	19.92
	1000	4.24	52.85
	2000	4.15	53.92
Deltamethrin 2.8 % EC	100	8.29	7.92
	500	7.49	16.81
	1000	6.40	28.88
	2000	5.82	35.30
Diafenthiuron 50 % WP	100	9.00	0.00
	500	9.00	0.00
	1000	7.65	15.00
	2000	7.07	21.41
Chlorantraniliprole 18.5 % SC	100	9.00	0.00
	500	9.00	0.00
	1000	9.00	0.00
	2000	9.00	0.00
Fenpropathrin10 % EC	100	9.00	0.00
	500	5.59	37.81
	1000	5.12	43.11
	2000	4.45	50.52
Flubendiamide 20 % WG	100	9.00	0.00
	500	9.00	0.00
	1000	9.00	0.00
	2000	9.00	0.00
Clothianidin 50 % WDG	100	9.00	0.00
	500	9.00	0.00
	1000	8.53	5.18
	2000	7.30	21.88
Control	control	9.00	0.00
	SE±(d)	0.18	
	C.D.@ 5%	0.36	

3.2 In-vitro compatibility of *Trichderma harzianum* with different fungicides

A total of 7 fungicides were used for this experiment, out of which, Azoxystrobin 23 % SC was found to be highly compatible showing inhibition rate of 0 %, 0 %, 4.96 % and 10.41 % at 100, 500, 1000 and 2000 ppm respectively and the triazole fungicides showed maximum inhibition of 100 % whereas combination of Tebuconazole 50 % + Trifloxystrobin 25 % showed an inhibition percentage of more than 50 % (Table 3). Azoxystrobin was compatible with *T. harzianum* (Pandey *et al*., 2006) at different dosages. Azoxystrobin, a strobilurin fungicide, blocks electron transport system (ETS) at the cytochrome bc1 complex to decrease mitochondrial respiration. These current findings indicate the inherent resistance of *T. harzianum* to azoxystrobin or its ability to maintain respiratory function at the tested doses. Earlier compatibility research suggests azoxystrobin can be employed in *Trichoderma*-centric biocontrol. Conversely, Triazole fungicides (hexaconazole, propiconazole, and tebuconazole) suppress *T. harzianum* completely, likely due to their mode of action (Sarkar *et al*., 2010; Singh *et al.,* 2021). Triazoles impede sterol biosynthesis (Rather *et al*., 2022), an essential ergosterol enzyme. Ergosterol is essential to fungal cell membranes, affecting membrane stability, hyphal extension, and proliferation. As a fungus, *T. harzianum* is highly susceptible to fungicides that block ergosterol formation, suppressing mycelial growth *in-vitro*. The findings also align with Mareeswaran and Asir (2016) and Singh and Singh (2009), who studied the effects of various agrochemicals Propargite, Glyphosate, Ammonium salt of glyphosate, Propiconazole, Hexaconazole, Carbendazim combined with Mancozeb on fungal bioagents. They recorded that Ammonium salt of glyphosate was particularly incompatible with *T. harzianum*. The reference to the work done by Gangwar *et al*. (2013a) can also be taken where they studied the nature of chemicals when mixed with bioagents including *T. harzianum* in *in-vitro* conditions and found that Carbendazim, Copper Oxychloride, Streptocycline, Monocrotophos, Quinalphos, Pendamethalin and butachlor showed compatibility at 200-1000 ppm but Mancozeb was found to be incompatible even at lower concentrations. The growth media's compatibility with pesticides indicates the potential use of microbial bioagents for bioremediation of pesticide-contaminated soil. However, chemical toxicity at elevated concentrations restricts the growth of fungal mycelium.

Table 3. *In-vitro* compatibility of *T. harzianum* with fungicides.

Fungicides	Concentrations (ppm)	Diameter of mycelia (cm)	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	0.000	100.00
Tebuconazole 50%+ Trifloxistrobin 25% WG	100	2.90	67.74
	500	2.83	68.52
	1000	1.85	79.44
	2000	1.47	83.66
Azoxystrobin 23% SC	100	9.00	0.00
	500	9.00	0.00
	1000	8.55	4.96
	2000	8.06	10.41
Chlorothalonil 75% WP	100	3.65	59.47
	500	3.48	61.30
	1000	0.00	100.00
	2000	0.00	100.00
COC 50% WP	100	5.27	41.47
	500	3.46	61.55
	1000	3.36	62.66
	2000	3.43	61.88
Control	Control	9.00	0.00
	SE±(d)	0.15	
	C.D. @ 5%	0.29	

3.3 In-vitro compatibility of *T. harzianum* with different herbicides

Among the two herbicides tested, Glyphosate 41 % SL was found to be compatible with *T. harzianum* at all the four concentrations showing an inhibition percentage of less than 50 % and, thus, can be used in association with *T. harzianum*, showing 18-40 % inhibition rate at a maximum concentration of 100 ppm, 500 ppm, 1000 ppm, and 2000 ppm respectively. However, Paraquat dichloride 24 % SL showed the highest inhibition of 73.11 % at 2000 ppm (Figure 1 and Figure 2). Glyphosate mostly blocks the shikimate pathway (Orcaray *et al.*, 2010), hence its effect on fungal proliferation varies by alterations in species and concentration. The low inhibition in this study suggests that *T. harzianum* is more resistant to glyphosate at the dosages tested. However, physiological and molecular studies are required to understand the cause and concerns. The result was found in accordance with the works of Dutta et al. (2016), who did a detailed study and were able to conclude that herbicides at lower doses can be incorporated with the fungal bioagents. The compatibility of herbicides

needs to be addressed at the field level so that they can be combined successfully with the bioagents as they have the ability to inhibit the toxicants produced by the herbicidal chemicals and spread their mycelia mat all around the Petri dish of 90 mm diameter.

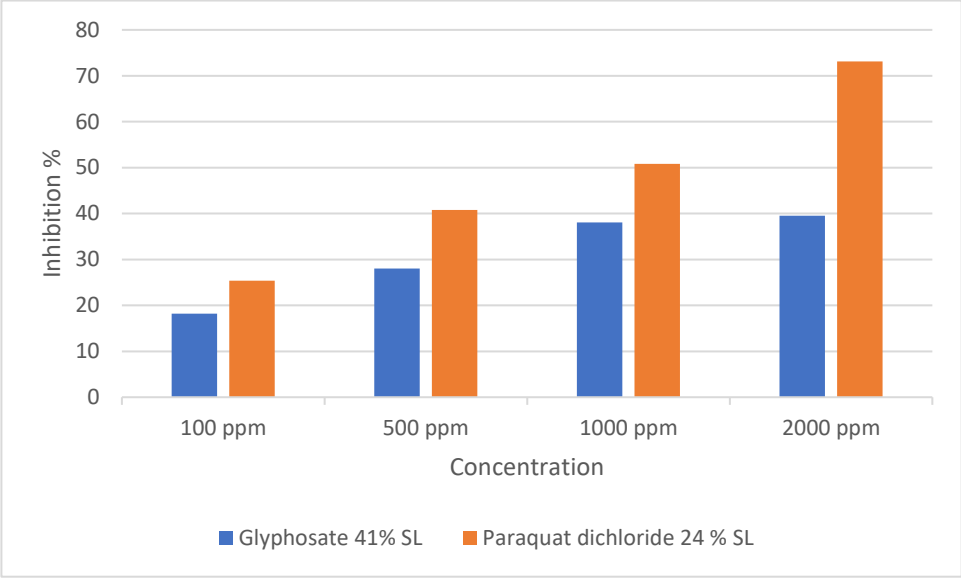

Figure 1. *In-vitro* compatibility of *T. harzianum* with herbicides.

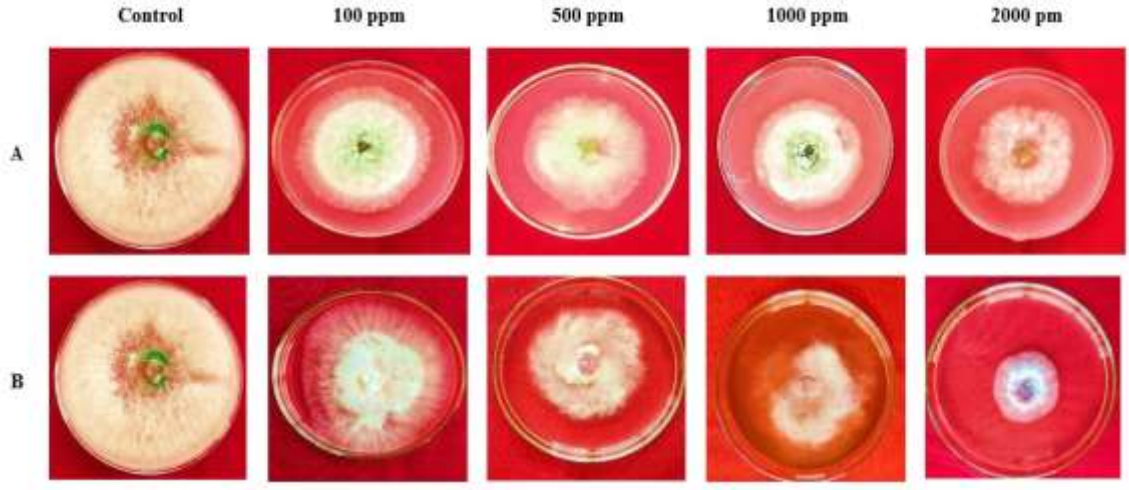

Figure 2. *In-vitro* compatibility of *T. harzianum* with herbicides. (A) Glyphosate + *T. harzianum;* (B) Paraquat dichloride + *T. harzianum*.

3.4 In-vitro compatibility of *T. harzianum* with different fertilizers

Out of the three fertilizers used for the study, SSP showed the highest compatibility showing 0 % inhibition and maximum mycelia growth of 9 cm in diameter. However, Urea and MOP showed an inhibition percentage of 100 % at 1000 and 2000 ppm respectively. But at 100 ppm and 500 ppm, the % inhibition was less than 50 % (Figure 3 and Figure 4). The findings are consistent with Shylaja *et al.* (2012), who too reported the incompatibility of *T. harzianum* with SSP and calcium ammonium nitrate at higher concentrations. Similarly, they also reported the compatibility of *T. harzianum* with Urea, SSP, and MOP. Mareeswaran and Asir (2016) studied the compatibility of agrochemicals Propargite, Glyphosate, Ammonium salt of glyphosate, Propiconazole, Hexaconazole, Combination of Carbendazim and Mancozeb with fungal bioagents and reported the compatibility of fertilizers including propargite and glyphosate with bioagents. Determining the compatibility of fertilizers with bioagents can reduce the use of toxic chemicals, minimize environmental pollution, lower costs, and enhance soil fertility by providing nitrogen and other essential nutrients, along with the advantages of synthetic fertilizers that cannot be achieved when used alone.

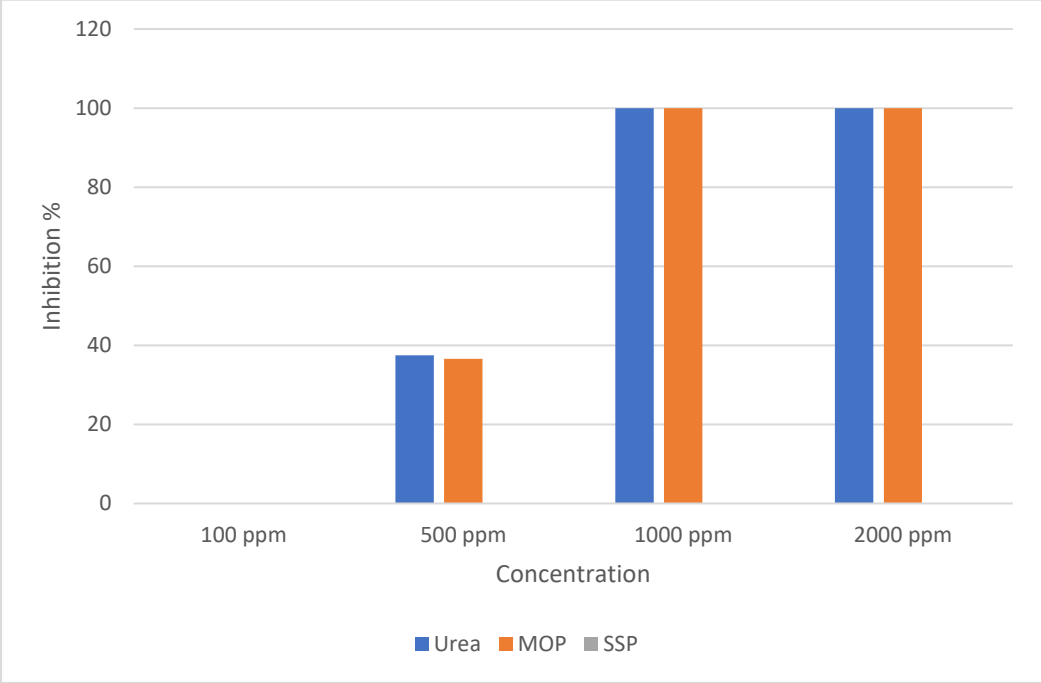

Figure 3. *In-vitro* compatibility of *T. harzianum* with fertilizers.

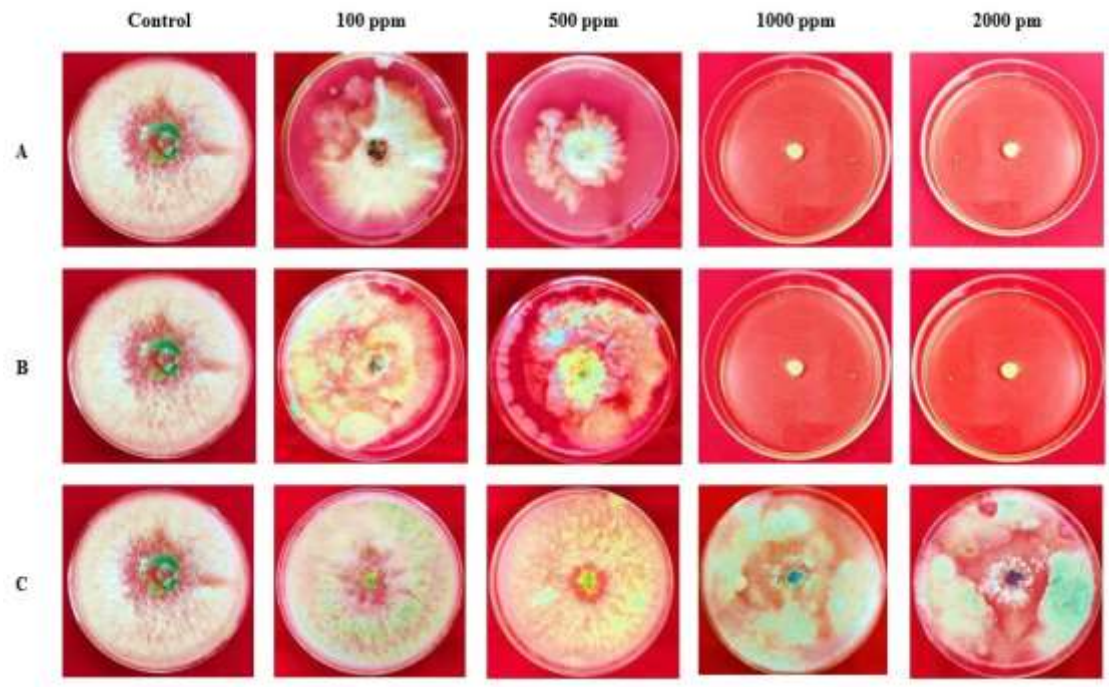

Figure 4. *In-vitro* compatibility of *T. harzianum* with fertilizers. (A) Urea + *T. harzianum*; (B) MOP + *T. harzianum*; (C) SSP + *T. harzianum*.

3.5 In-vitro compatibility of *T. harzianum* with different antibiotics

The antibiotics, Streptomycin and Gentamycin used in the current investigations were found to be highly compatible with the fungal bioagent at all the given concentrations i.e., 100 ppm, 500 ppm, 1000 ppm, and 2000 ppm. With *T. harzianum*, streptomycin showed an inhibition rate of 0 %, 0.74 %, 4.07 % and 3.66 % at 100, 500, 1000 and 2000 ppm. On the other hand, gentamycin gave an inhibition rate of 0 %, 1.18 %, 5.00 %, and 6.55 % at 100, 500, 1000, and 2000 ppm respectively (Figure 5). The results coincide to Gangwar et al. (2013a and 2013b), who conducted an experiment to find out the compatibility of fungal and bacterial bioagents against the commonly 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.

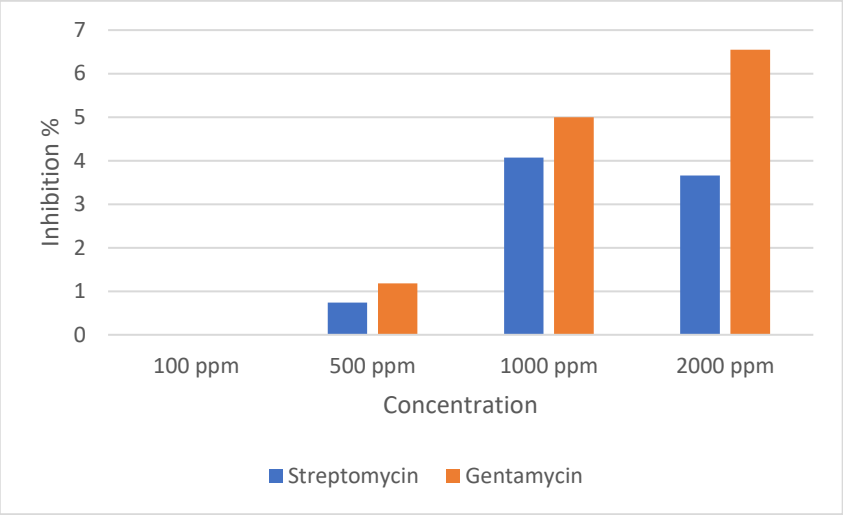

Figure 5. *In-vitro* compatibility of *T. harzianum* with antibiotics.

4. Conclusions

The current investigation demonstrates that the compatibility of *T. harzianum* varied considerably among the agrochemicals evaluated. Most pesticides, glyphosate, certain fertilisers, and the evaluated antibiotics were compatible with the bioagent, but triazole fungicides and several other fungicides significantly suppressed its growth *in-vitro*. These results provide valuable insights for choosing agrochemicals that can be used with *T. harzianum* in vegetable cultivation systems. However, further research in greenhouse and field conditions are necessary to corroborate these laboratory findings prior to endorsing their integrated use.

5. Acknowledgements

Authors are thankful to the Director of Research (Agri.), and the Director of Post Graduate Studies, AAU, Jorhat, Assam, India, for providing all necessary facilities and support for the successful completion of the study. The corresponding author is also thankful to the Department of Biotechnology, Govt. of India for financial support (BT/NER/143/SP42744/2021; Dated 01/10/2021).

6. Author's Contribution

Conceptualization and designing of the research work (BCN, PB); Execution of experiments (PB, RC, SS, MR, MER); Analysis of data and interpretation (PB, BCN, PNB, LH, POB, AK); Preparation and revision of the manuscript (PB, BCN, PNB, RNB, LH, AM, SK, DT).

7. Funding

No funding was received for conducting this study.

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