IN-VITRO COMPATIBILITY ASSESSMENT OF *Trichoderma harzianum* WITH CHEMICAL FUNGICIDES AND BOTANICAL EXTRACTS

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ABSTRACT

The threats of devastating soil-borne disease, limited availability of its management strategies, development of fungicide-resistant strains, outbreaks of new diseases, and growing concerns regarding nature and the environment have compelled us to use integrated disease management (IDM) strategies with appropriate biocontrol agents. *Trichoderma*, free-living fungi, are successful antagonists with promising biocontrol potentials and can be used with chemicals and botanicals in the IDM approach to control various plant pathogenic fungi. So, the present experiment was conducted to evaluate the compatibility of *Trichoderma harzianum* with chemical fungicides and botanicals in *in-vitro* using a poisoned food technique. The experiment was conducted in a completely randomized design with three replications for each treatment at the central laboratory of the Institute of Agriculture and Animal Science, Lamjung, Nepal, and data were taken at different time intervals and analyzed. For fungicides, the maximum compatibility was found in Copper oxychloride at 100 ppm, and the highest inhibition (100%) was observed in Carbendazim + Mancozeb, Carbendazim, and Hexaconazole even in lower concentration (100 ppm). For botanicals, *Azadirachta indica* and *Zingiber officinale* enhanced *T. harzianum*, and the highest compatibility was observed at 10% leaves extract of *Azadirachta indica* with a growth inhibition percentage of -5.43% (Day 5). Aqueous extracts of tested botanicals were found compatible with *T. harzianum*, except for the *Acorus calamus*, *Artemisia vulgaris*, and *Allium sativum*. In IDM practice, compatible fungicides and botanicals at recommended doses can be used with *T. harzianum*.

Keywords: Biocontrol, integrated disease management, poisoned food technique.

INTRODUCTION

With the advent of modern and intensive agriculture, the case of hazardous and unsystematic use of chemicals to control different soil and seed-borne disease has been pronounced frequently. Annually, around 3 to 4.6 million tons of pesticides are used (Pimentel *et al*., 2009; Zhang *et al*., 2011) and this had led to the development of chemical-resistant strains with declined soil antagonistic population making management of soil and seed-borne disease much challenging (Hobbelen *et al*., 2014; Meena *et al*., 2020). So, at present, even the chemicals don't produce satisfactory results against soil-borne pathogens; instead, the continuous application of chemicals will bring health and environmental hazard. *Trichoderma* is a free-living cosmopolitan fungus, commonly found in soil, plant root surface, and decaying wood (Kredics *et al*., 2014; Hermosa *et al*., 2012), and are extensively used for seed treatment, seed biopriming, seedling treatment, soil applications, and foliar applications (Benítez *et al*., 2004). *Trichoderma* spp. is the most exploited fungal biocontrol agent used for the control of various phytopathogens and is commercially marketed as a biopesticide,

biofertilizer, and bioremediation (Kumar *et al*., 2014). Earlier studies have shown that species in this genus possess multiple biocontrol mechanisms, namely competition of space and nutrients (Wells, 1988), mycoparasitism on pathogenic fungi (Lewis, 1989), production and excretion of metabolites (cell wall-degrading enzymes, antibiotics, siderophores) (Monte, 2001; Vinale *et al*., 2013), and induction of defense responses (local and systemic acquired resistance, induced systemic resistance). *Trichoderma* spp. produce non-volatile and volatile secondary metabolites capable of inhibiting the growth of pathogenic fungi; they also secrete different hydrolytic enzymes such as chitinases, proteases, cellulases, glucanases, and xylanases that degrade the cell wall of pathogenic fungi (Sood *et al*., 2020). Apart from pathogen's growth suppression, *Trichoderma* possesses a better capability to mobilize and absorb nutrients from the soil, promote growth and development, and improve yield and crop quality (Sood *et al*., 2020; Campos *et al*., 2020). Several authors have reported excellent control of phytopathogens by *Trichoderma* in pot experiments and greenhouse, but they fail to perform in the same way when taken in field conditions. *Trichoderma* being a biological organism, its biocontrol efficacy is affected by its shelf-life, ambient temperatures, soil pH, salinity, moisture content, competition, and disease pressure (Naeimi *et al*., 2020; Mukherjee *et al*., 2013). Korsten and Jeffries (2000) reported that the efficiency of biocontrol agents could be improved further when applied with the recommended fungicide at a lower concentration, and many earlier findings have reported that the combined use of *Trichoderma* with chemicals and botanicals provides better disease management (Mahesh *et al*., 2010). Nevertheless, several chemicals and botanicals harm the growth and colonization of *Trichoderma* (Sushir *et al*., 2015). As compared to other countries, pesticides use status in Nepal is low (0.396 kg a.i. per ha); however, the data on chemical pesticides used in agricultural commodities of Nepal, particularly in vegetable farming, suggest that there is the indiscriminate use of chemicals (Bhandari *et al*., 2018; Gyawali, 2018). This helps to develop resistance to pathogens and has a detrimental effect on the colonization of *Trichoderma*; so, an integrated approach of using *Trichoderma* with the recommended dose of chemicals and botanicals is at utmost necessary. Earlier works have examined *Trichoderma* sensitivity to biological agents, chemically active substances, and essential oils, but there is much less information on its compatibility with chemical fungicides and botanical extract. The botanicals used in this experiment are the most exploited ones in the context of Nepal with no recent work, so much information is still to be documented. The main aim of the present work is to evaluate the compatibility of *Trichoderma harzianum* against different concentrations of chemical fungicides and botanicals.

MATERIALS AND METHODS

The experiment was conducted in the Plant Pathology laboratory of the Institute of Agriculture and Animal Science (IAAS), Lamjung in 2019. Six chemical fungicides and six botanical extracts were evaluated at three different concentrations for their compatibility study with *T. harzianum* using the poisoned food technique as mentioned by Nene & Thapliyal (1993). The pure culture of *T. harzianum* was obtained from Nepal Agricultural Research Council; fungicides were obtained from the local market, and plant extracts were prepared using different plants parts. The details of fungicides and plant extracts used in this experiment are shown in Table 1 and Table 2.

Phytoextracts used in the experiment were prepared similarly as by Ul-Haq *et al*. (2014). Healthy leaves, bulbs, and rhizomes of the plants were collected from their undisturbed habitat, and thoroughly washed in tap water followed by sterile distilled water. The wetted leaves were then air-dried in shade under natural conditions, and individual samples were grounded using mortar and pestle with the addition of distilled water (1:1 w/v).

Table 1. Chemical fungicides used in an experiment

Trade Name	Active Ingredient	Formulations	Mode of Action
Uthane M-45	Mancozeb	75%WP	Contact
Blutoxx	Copper oxychloride	50%WP	Contact
Saaf	Carbendazim + Mancozeb	12% + 63% WP	Systemic + Contact
Kriloxyl Gold	Metalaxyl + Mancozeb	8% + 64% WP	Systemic + Contact
Navistin	Carbendazim	50% WP	Systemic
Hexa	Hexaconazole	5% EC	Systemic

Table 2. Plant and plant parts used to make an aqueous solution in the experiment

Bojho	*Acorus calamus*	Rhizome
Neem	*Azadirachta indica*	Leaf
Garlic	*Allium sativum*	Bulb
Ginger	*Zingiber officinale*	Rhizome
Lantana	*Lantana camara*	Leaf
Titepati	*Artemisia vulgaris*	Leaf

The pulverized mass was squeezed through a clean and soft muslin cloth, and the extract obtained was taken in a beaker, boiled at 80°C for 10 minutes in a hot water bath, and again filtered through a double-layered muslin cloth. Subsequently, the extract was centrifuged at 4000 rpm for 5 minutes; the supernatant was filtered through Whatman's filter paper No. 1 under aseptic conditions, and this was taken as a 100% basic stock solution. The required amount of the filtrate was homogeneously mixed with PDA to obtain the desired concentration of 5%, 10%, and 15%. Likewise, for the evaluation of chemical fungicides, stock solutions of five fungicides were prepared by dissolving 1 gm of each fungicide in 10 ml of distilled water to make 75,000 ppm of Mancozeb 75% WP and Carbendazim 12% + Mancozeb 63% WP, 72,000 ppm of Metalaxyl 8% + Mancozeb 64% WP and 50,000 ppm of Copper oxychloride 50% WP and Carbenazim 50% WP. In the case of Hexaconazole, its available 5% EC solution was taken as the original stock solution. The required volume of chemicals was mixed in lukewarm molten PDA to prepare 100 ppm, 200 ppm, and 300 ppm of 60 ml amended media. Then, 20 ml of poisoned medium (either with fungicides or phytoextracts) was poured into each 90 mm sterilized petri plate and allowed to solidify. Using a sterile cork borer, mycelial discs (6 mm diameter) were cut from actively growing five-day-old pure cultures and placed in the middle of a petri dish containing amended PDA. Three replications were made for each treatment, and an unamended PDA media served as control. All the treated plates were incubated in a bacteriological incubator at 25±2˚C, and the measurement of radial growth (mm) was done using a vernier caliper scale (after 36, 48, 60, and 72 hours for fungicides-treated plates and after 3rd, 4th, 5th, and 6th day of incubation for plates treated with phytoextracts). *T. harzianum* compatibility with chemicals and botanicals was not conducted simultaneously but was conducted individually using the

same pure culture grown at the same temperature. The formula given by Vincent (1947) was used to calculate growth inhibition percent (GIP) over control. The compatibility assessment of *T. harzianum* and chemical fungicide, was studied using the scale of the International Organisation for Biological Control (OILB) (Viñuela, 1993). This classification groups shows compatibility between microorganisms and chemical fungicides relying on the proportion of inhibition over control (<30%: harmless; 30–75%: slightly toxic; 75–90%: moderately toxic; >90%: toxic). Data were analyzed using analysis of variance with R-Stat (version 3.5.3). Mean comparison was done using Fisher-LSD test at 0.05 level of significance.

Growth inhibition percent (GIP) = [(C-T)/C] × 100, Where C = Mycelium growth of *T. harzianum* on the control plate; T= Mycelium growth of *T. harzianum* on treated plate

RESULTS AND DISCUSSION

In-vitro evaluation of *Trichoderma harzianum* with chemical fungicides

The data on *in-vitro* compatibility tests of six different chemical fungicides at three different concentrations: 100, 200, and 300 ppm with *T. harzianum* are depicted in Table 3 and the mycelial growth of *T. harzianum* after 72 hours of incubation is shown in Figure 1. The result revealed the highest compatibility of *T. harzianum* on Copper oxychloride at 100 ppm after 36, 48, 60, and 72 hours of incubation. Here, after 36 hours of incubation, the highest compatibility of Copper oxychloride 100 ppm with GIP of 1.75% was followed by 200 ppm of Mancozeb having GIP 16%. After 48 hours of incubation, the GIP of Copper oxychloride at 100 ppm was 1.42%, which was followed by 100 ppm of Mancozeb having GIP of 6.74%. Similarly, after 60 hours incubation, the GIP of Copper oxychloride was 0.71%, which was significantly at par (p<0.05) with 100 and 200 ppm of Mancozeb having GIP of 3.11% and 3.87%, respectively. After 72 hours of incubation, the GIP of Copper oxychloride 100 ppm i.e. 0.55% was statistically at par (p<0.05) with 100 and 200 ppm of Mancozeb having GIP of 0.74% and 3.77%, respectively. *T. harzianum* was highly sensitive to all the tested concentrations of Hexaconazole, Carbendazim and Carbendazim + Mancozeb, with 100% growth inhibition at all recorded incubation period. Compatibility decreased significantly (p<0.05) with the increase in concentration. The GIP of Mancozeb, Metalaxyl + Mancozeb, and Copper oxychloride decreased with an increase in the incubation period. Manadhar *et al*. (2020) also concluded that the GIP of *T. harzianum* decreased with a decrease in concentration and an increase in the incubation period. However, for Carbendazim, Hexaconazole, and Carbendazim + Mancozeb, there was 100% inhibition even with lower concentration (100 ppm) after 72 hours of incubation.

Table 3. Growth inhibition of *Trichoderma harzianum* at various concentrations of different chemical fungicides in *in-vitro* at different time intervals

Treatments	Conc (ppm)	Mycelial growth in Diameter (mm)				Growth Inhibition Percent (%)			
		36 hrs	48 hrs	60 hrs	72hrs	36 hrs	48 hrs	60 hrs	72 hrs
Mancozeb	100	57.36	71.84	78.60	83.38	16.00^{gh}	6.74^{g}	3.11^{fg}	0.74^{f}
	200	60.05	68.37	77.99	79.60	12.07^{h}	11.24^{fg}	3.87^{fg}	3.77^{ef}
	300	53.44	64.92	70.87	74.98	21.75^{fg}	15.72^{f}	12.65^{e}	10.73^{d}
Metalaxyl + Mancozeb	100	52.36	67.69	75.54	79.87	23.32^{f}	12.13^{f}	6.89^{f}	4.92^{e}
	200	45.62	57.23	68.88	72.68	33.20^{e}	25.71^{e}	15.10^{de}	13.48^{d}
	300	39.22	50.36	65.67	72.06	42.57^{d}	34.62^{d}	19.06^{d}	14.21^{d}

Copper Oxychloride	100	67.10	75.94	80.55	84.54	1.75^{i}	1.42^{h}	0.71^{g}	0.55^{f}
	200	28.30	35.01	41.77	44.30	58.55^{c}	54.44^{c}	48.51^{c}	47.27^{c}
	300	6.67	11.75	12.48	15.37	90.24^{b}	84.75^{b}	84.62^{b}	81.71^{b}
Carbendazim + Mancozeb	100	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
	200	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
	300	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
Carbendazim	100	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
	200	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
	300	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
Hexaconazole	100	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
	200	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
	300	0.00	0.00	0.00	0.00	100^{a}	100^{a}	100^{a}	100^{a}
Control		68.29	77.03	81.13	84.00	0.00	0.00	0.00	0.00
Mean						66.64	63.71	60.81	59.85
CV (%)						5.73	4.43	4.22	3.61
LSD (p<0.05)						6.32	4.67	4.25	3.58

Conc = Concentration, CV = Coefficient of variation, LSD = Least significant difference, Ppm = Parts per million, mm = Millimetre, Means followed by the same letter are not significantly different ($p<0.05$)

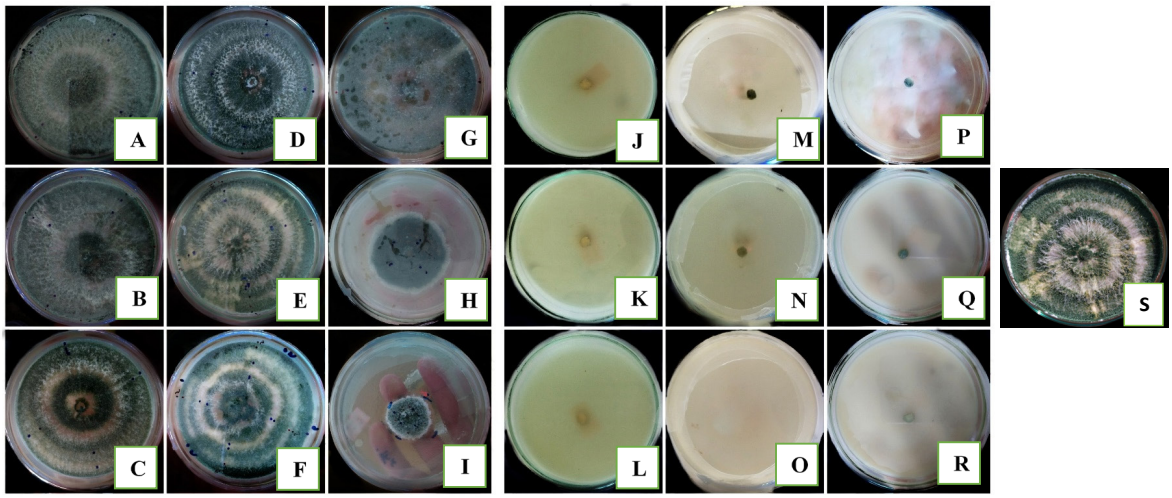

Figure 1. Mycelial growth diameter of *Trichoderma harzianum* after 60 hrs of inoculation in media amended with different concentration of various chemical fungicides- Mancozeb (A) 100 ppm, (B) 200 ppm, (C) 300 ppm; Metalaxyl + Mancozeb (D) 100 ppm, (E) 200 ppm, (F) 300 ppm; Copper oxychloride (G) 100 ppm, (H) 200 ppm, (I) 300 ppm; Carbendazim+Mancozeb (J) 100 ppm, (K) 200 ppm, (L) 300 ppm; Carbendazim (M) 100 ppm, (N) 200ppm, (O) 300 ppm; Hexaconazole (P) 100 ppm, (Q) 200 ppm, (R) 300 ppm, (S) Control

The results revealed that the mycelial growth of *T. harzianum* was influenced by the various dose of each fungicide compared with control. According to the OILB scale, the compatibility of the six fungicides tested, Mancozeb was harmless at all the concentrations with growth inhibition percent <30% throughout the experiment. Metalaxyl + Mancozeb at 100ppm was harmless; however, its 200 and 300ppm were slightly toxic at the beginning of the experiment but later turned harmless with time. Copper oxychloride 100ppm was harmless,

200ppm was slightly toxic, and 300ppm was moderately toxic throughout the experiment. Three fungicides (Carbendazim + Mancozeb, Carbendazim and Hexaconazole) were toxic at all concentrations throughout the experiment. Harmless and slightly toxic fungicides can be used in IDM; however, particular fungicides can be harmless at lower concentrations and toxic at higher concentrations, so understanding concentration is critical. Similar work was carried out by Bagwan (2010), who also found that Mancozeb (0.2%) and Copper oxychloride (0.2%) were compatible and comparatively safer to *T. harzianum*. Bheemaraya *et al*. (2012) also reported Metalaxyl-M + Mancozeb and Mancozeb were compatible with *Trichoderma* spp., while Carbendazim completely inhibited radial mycelial growth. Manadhar *et al*. (2020) also reported a high inhibitory effect of Carbendazim, Hexaconazole, and Carbendazim + Mancozeb even at low concentrations (<100ppm). The high inhibition of Carbendazim is due to its effect on DNA synthesis that blocks nuclear division; it binds with the β-tubulin of fungal pathogens, causes inhibition of microtubule assembly, ultimately hinders cell division, and may lead to cell death; similarly, for Hexaconazole, there is the presence of systematic demethylation inhibitors, primarily acting on the vegetative stage of fungi that hinder the mycelial development either inside or on the surface of the host plant (Clemons & Sisler, 1971; Khalfallah *et al*., 1998). Saaf also possesses higher mycelium inhibition, as it is the mixture of Carbendzim and Mancozeb and has a collective effect of systemic and contact fungicides.

***In-vitro* evaluation of *Trichoderma harzianum* with botanical extracts**

The data presented in Table 4 are supported by Figure 2, which show that among botanicals, *A. calamus* showed almost complete suppression of mycelial growth of *T. harzianum*, revealing a GIP ranging from 93.53% to 99.27%, whereas the highest compatibility was observed in leaves extract of *A. indica* with a GIP ranging from -5.43% to 3.57%. On the 3rd day of incubation, the highest compatibility was observed on the *Z. officinale* at 5% with a GIP of -4.97%, which was statistically at par (p<0.05) with 5% and 10% of *A. indica*. On the 4th day of incubation, the highest compatibility was observed on *A. indica* at 10% with a GIP of -4.02%, which was statistically at par (p<0.05) with its 5% and 15% concentrations, and also with 5%, 10%, and 15% *Z. officinale* concentrations. Similarly, on the 5th day of the experiment, the highest compatibility was observed on *A. indica* at 10% with a GIP of -5.43%, which was not statistically different (p<0.05) from its 5% and 15% concentrations, as well as from *Z. officinale* at 5%, 10%, and 15% concentrations. And on the 6th day of incubation, the highest mycelial growth (82.56mm) was observed on *A. indica* at 10% with a GIP of -5.02%, which was statistically at par (p<0.05) with its 5% concentration, as well as with 5%, 10% and 15% concentrations of *Z. officinale*. Compatibility decreased significantly (p<0.05) with the increase in concentration. In Table 4, the GIP of *A. vulgaris*, *A. sativum*, *and L. camara* decreased with an increase in incubation period, but for *A. calamus* the GIP increased with an increase in incubation period. However, for *A. indica* and *Z. officinale*, the GIP was variable with time. The decreased GIP of these botanicals extracts to time may be due to the increased efficacy of *T. harzianum* to neutralize the active compounds present in botanicals.

From the above experiment, *A. indica* and *Z. officinale* at all concentrations are compatible with a noticeable growth-enhancing effect on *T. harzianum*. *A. sativum* 5% and *L. camara* 5% and 10% extracts are also compatible revealing lower GIP. In opposite to this, all concentrations of *A. calamus* and *A. vulgaris, A. sativum* (10% and 15%), *L. camara*

15% have significant growth inhibition of *T. harzianum*; similar findings were observed by Bagwan (2010), who also found that neem leaf extract 10% and neem oil 5% enhanced the growth of *T. harzianum*. But to date, no *calamus* and *A. vulgaris, A. sativum* (10% and 15%), *L. camara* 15% have significant growth inhibition of *T. harzianum*; similar findings were observed by Bagwan (2010), who also found that neem leaf extract 10% and neem oil 5% enhanced the growth of *T. harzianum*. But to date, no earlier reports have yet reported the growth-enhancing effect of *Z. officinale* and *A. sativum* on *T. harzianum*. Sharma and Chandel (2016) reported that *A. sativum* is not compatible with *T. harzianum*, but they had only tested on a higher concentration of 15% and 30%. The inhibition of *A. sativum* is due to the presence of the bioactive compound allicin which has antibacterial and anti-fungal properties (Block, 1985). Ayodele *et al*. (2018) also reported that an aqueous extract of *Z. officinale* exhibited no antifungal properties against *T. harzianum*. The principal antifungal compound found in *A. calamus* is β-asarone, and its antifungal mode of action could be due to the inhibition of ergosterol biosynthesis (Karwowska *et al*., 1997; Rajput & Karuppayil, 2013). The phytochemical analysis of leaf extract of *L. camara* showed the presence of different secondary metabolites, like alkaloids, glycoside, 5-Heptenoic acid, trepenoids, saponin, tannins and the presence of these compounds are responsible for its antifungal activity (Bashir *et al.,* 2018).The antifungal activity of different botanicals varies with the amount of active antifungal chemicals they possess; different mechanisms of action have been reported to describe their antifungal potential: ruptured cell wall and membrane disruption, chitin synthesis inhibition, accumulation of reactive oxygen species, mitochondrial dysfunction, and some specific enzyme activities inhibition (Marei *et al*., 2012; Nazzaro *et al*., 2017).

Table 4. Growth inhibition of *Trichoderma harzianum* at various concentrations of different botanical extracts in *in-vitro* at different time intervals

Botanical Extracts	Conc (ppm)	Mycelial Diameter (mm)				Growth Inhibition Percent (%)			
		Day 3	Day 4	Day 5	Day 6	Day 3	Day 4	Day 5	Day 6
Acorus calamus	5	4.71	4.71	4.71	4.71	93.53^{b}	93.90^{b}	93.99^{b}	94.01^{b}
	10	1.61	1.61	1.61	1.61	97.79^{ab}	97.91^{ab}	97.94^{ab}	97.95^{ab}
	15	0.58	0.58	0.58	0.58	99.21^{a}	99.25^{a}	99.26^{a}	99.27^{a}
Artemisia vulgaris	5	33.55	36.66	39.95	52.89	53.88^{f}	52.52^{e}	48.99^{d}	32.71^{ef}
	10	26.48	34.84	41.80	51.09	63.59^{e}	54.88^{e}	46.62^{de}	35.01^{e}
	15	16.12	18.76	22.75	26.81	77.85^{d}	75.71^{d}	70.95^{c}	65.90^{c}
Allium sativum	5	46.23	70.82	77.19	82.18	36.46^{h}	8.29^{g}	1.43^{gh}	-4.54^{jk}
	10	10.45	19.49	38.29	58.59	85.63^{c}	74.76^{d}	51.10^{d}	25.47^{g}
	15	9.02	13.56	26.51	38.50	87.61^{c}	82.44^{c}	66.15^{c}	51.02^{d}
Lantana camara	5	57.39	70.37	75.33	75.99	21.10^{i}	8.87^{g}	3.80^{g}	3.33^{i}
	10	38.20	46.78	52.48	66.90	47.48^{g}	39.41^{f}	32.98^{f}	14.90^{h}
	15	33.58	38.24	44.03	56.06	53.84^{f}	50.47^{e}	43.77^{e}	28.69^{fg}

Zingiber officinale	5	76.36	79.02	81.21	81.21	-4.97^{k}	-2.34^{h}	-3.71^{i}	-3.31^{jk}
	10	68.08	79.66	81.28	81.31	6.51^{j}	-3.16^{h}	-3.80^{i}	-3.43^{jk}
	15	67.22	79.24	80.75	80.75	7.60^{j}	-2.62^{h}	-3.12^{hi}	-2.72^{jk}
Azadirachta indica	5	76.25	78.44	81.97	81.97	-4.82^{k}	-1.58^{h}	-4.69^{i}	-4.28^{jk}
	10	74.79	80.32	82.56	82.56	-2.82^{k}	-4.02^{h}	-5.43^{i}	-5.02^{k}
	15	70.15	76.56	78.62	79.14	3.57^{j}	0.85^{h}	-0.40^{ghi}	-0.67^{ij}
Control		72.74	77.22	78.30	78.60	0.00	0.00	0.00	0.00
Mean						45.72	40.31	35.32	29.13
CV (%)						6.28	7.46	8.73	8.54
LSD(p<0.05)						4.75	4.98	5.11	4.12

Conc = Concentration, CV = Coefficient of variation, LSD = Least significant difference, mm = Millimetre, means followed by the same letter are not significantly different (p<0.05)

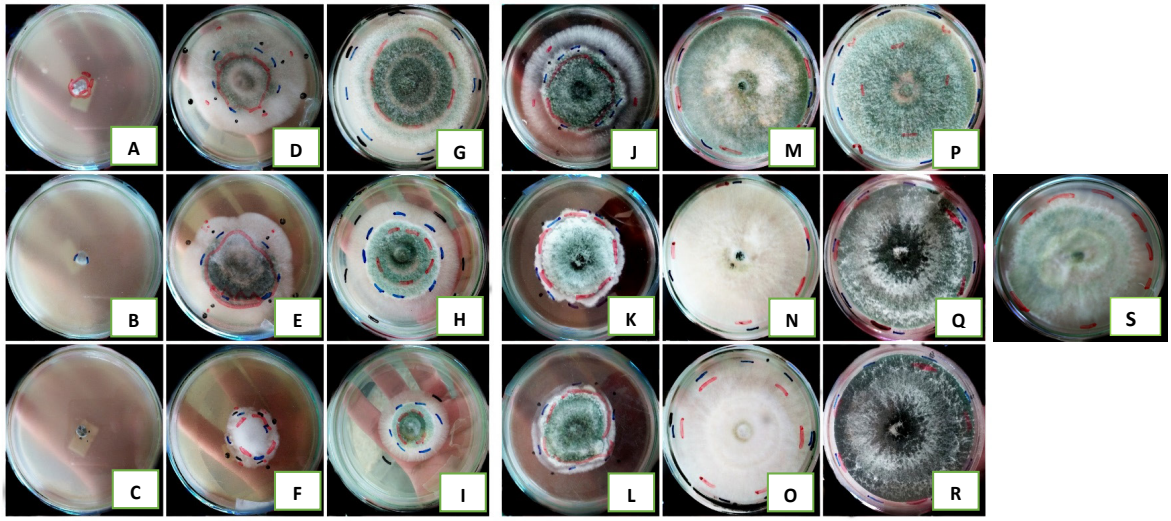

Figure 2. Mycelial growth of *Trichoderma harzianum* after 6th day in media amended with different concentration of various botanical- *Acorus calamus* (A) 5%, (B) 10%, (C) 15%, *Artemisia vulgaris* (D) 5%, (E) 10%, (F) 15%, *Allium sativum* (G) 5%, (H) 10%, (I) 15%, *Lantana camara* (J) 5%, (K) 10%, (L) 15%, *Zingiber officinale* (M) 5%, (N) 10%, (O) 15%, *Azadirachta indica* (P) 5%, (Q) 10%, (R) 15%, (S) Control

Linear and quadratic relationship between concentrations of different chemicals and plant extract and the mycelial growth diameter on different days after incubation is given in the supplementary file.

CONCLUSION

The experiment concluded that 100 ppm of Copper oxychloride and 100, 200 and 300 ppm of Mancozeb and Metalaxyl + Mancozeb are compatible with *T. harzianum*. Among the botanicals, all the tested concentrations of *A. indica* and *Z. officinale,* 5% and 10% *L. camara,* and 5% *A. sativum* are compatible with *T. harzianum*. Furthermore, *A. indica* and *Z officinale*

promoted the growth of *T. harzianum.* The compatibility of chemical fungicides significantly decreased with an increase in concentration, so in an integrated approach, the appropriate concentration of chemical fungicides must be used; and farmers are recommended to use botanicals having enhancing effect on the growth of *T. harzianum.* Integration of compatible chemicals and botanicals with *T. harzianum* provides better disease management in the long run and is environmentally friendly. However, to find the effectiveness of these integrated approaches in controlling diseases, a field trial with a combination of chemicals, botanicals, and *T. harzianum* is necessary to carry out.

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