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COMPARATIVE ANALYSIS OF CHEMICAL AND BIOLOGICAL CONTROL AGENTS EFFICACY IN MANAGING ALTERNARIA LEAF BLIGHT IN TURMERIC (*Curcuma longa* L.)

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ABSTRACT

Turmeric (*Curcuma longa* L., family Zingiberaceae), commonly known as Indian Saffron, is an economically important spice crop that is highly susceptible to several diseases under favourable environmental conditions. Among these, leaf blight caused by *Alternaria alternata* is one of the predominant diseases of turmeric leading to significant yield losses. The present investigation was carried out to test the in vitro efficacy of chlorothalonil and biocontrol agent, *Trichoderma harzianum* against the *Alternaria alternata*. The mycelial growth inhibition assays revealed that chlorothalonil was the most effective fungicide, with 200 ppm concentration exhibited the 70% inhibition of mycelial growth, corresponding to the LD_{70} value and outperforming other tested concentrations (25, 50, 500 and 1000 ppm). The biocontrol agent *Trichoderma harzianum* demonstrated rapid and aggressive growth, completely colonizing the culture plate within 6 days under monoculture conditions, compared with 9 days required by the pathogen. In dual culture assays, *T. harzianum* exhibited strong antagonistic activity, rapidly suppressing and completely restricting the growth of *A. alternata* within 4 days. These results showed the potential of chlorothalonil and *T. harzianum* as effective components of an integrated management strategy for turmeric leaf blight.
Keywords **:** *Alternaria alternata*, biocontrol, fungicides, *T. harzianum*, turmeric.

Introduction

Turmeric (*Curcuma longa* L.), a member of the family Zingiberaceae, is a perennial rhizomatous crop native to the Indian subcontinent and Southeast Asia. It has been cultivated for centuries and holds significant importance as a spice in culinary dishes and as a traditional medicinal plant. The key active compound in turmeric is curcumin, which is responsible for its distinct yellow colour and is largely responsible for its pharmacological properties. In culinary applications, turmeric is a common spice used in Indian, Middle Eastern, and Southeast Asian cuisines. It has a warm, bitter taste. Beyond its culinary uses, turmeric has gained popularity for its potential health benefits. It is known for its anti-inflammatory and antioxidant properties and has been studied for its potential role in treating various conditions, including arthritis, gastrointestinal issues, and dermatological problems.

Alternaria alternata is a necrotrophic fungal pathogen that infect a wide range of host plants, including turmeric. It is known to cause a disease commonly referred to as Alternaria leaf spot or blight in turmeric plants. The disease poses a serious threat to crop health and productivity. Infection is characterized by the development of dark brown to black necrotic lesions on foliage, typically shows concentric rings or lesions that impart a distinctive target like appearance. These lesions are surrounded by chlorotic halos, indicating localized tissue damage. As the disease progresses, the leaves may dry up, wither, and eventually lead to a decline in the plant's photosynthetic efficiency and rhizome yield.

Management of *Alternaria alternata* in turmeric relies on an integrated approach combining cultural, chemical and maintaining field hygiene. Adoption of good agricultural practices such as crop rotation, optimal plant spacing plays a crucial role in minimizing the primary inoculum and spread of the disease. Chemical control remains an important component of the disease management, particularly under conditions favourable to disease development. chlorothalonil is a broad-spectrum non-systemic fungicide that is commonly used in agriculture to control various fungal diseases, including *Alternaria alternata*, the causal agent of Alternaria leaf spot in turmeric.

Chlorothalonil primarily functions as a protective fungicide by forming a protective barrier on the surface of plant tissues. This prevents the germination of fungal spores and the penetration of the fungus into the plant cells. In addition to its preventive properties, chlorothalonil can also have curative effects. It inhibits the growth and development of the fungus by disrupting key biochemical processes within the fungal cells. It has a multi-site mode of action, meaning it affects various sites within the fungal cell. It can also have residual activity, remaining active on the plant surface for extended period of application, which enhances its protective efficacy and contributes to disease control in crop production.

In recent years, numerous synthetic fungicides have been restricted or banned in several western countries due to growing concerns over their adverse environmental impacts and high acute toxicity. Despite these concerns, developing countries like India persist in their use, even as their harmful effects exists. The widespread and prolonged use of chemical fungicides has also led to the emergence of resistant strains of pathogenic microorganisms to further complicates the management of crop and agricultural plant diseases (Gaigole *et al*., 2011). At Present, synthetic fungicides remain the primary means of tackling plant diseases and microbial contamination in various agricultural commodities. However, the repetitive and indiscriminate application of these chemicals agents has given rise to health hazards in both animals and humans, owing to residual toxicity (Dohroo, 1990).

Trichoderma species have emerged as prominent biological control agents, have been extensively researched and implemented worldwide for the management of plant diseases. It has proven effective in managing plant diseases caused by various pathogens, including *Fusarium* spp. (John *et al*., 2010), *Alternaria* spp. (Arzanlou *et al*., 2013), *Pythium* spp. (Naseby *et al*., 2000), *Sclerotium* and *Rhizoctonia solani* (Seema and Devaki, 2012). The biocontrol potential of *Trichoderma* spp. Is mediated through a combination of direct and indirect antagonistic mechanisms, including mycoparasitism, antibiosis, competition for nutrients and space and induction of host plant resistance. The effectiveness of these mechanisms is influenced by multiple factors such as the specific *Trichoderma* strain, targeted pathogen, host plant characteristics and environmental conditions (Raut *et al*., 2014). Key to their antagonistic action against a broad spectrum of fungal pathogens are various fungal cell wall degrading enzymes, including chitinase and glucanase (Kucuk and Kivanc, 2008). The antagonistic potential of *Trichoderma harzianum* against numerous pathogenic fungi has been well documented by earlier researchers (Henis and Chet, 1975, Backman and Rodrigues-Kabana 1974, Hadar *et al*. 1979 and Elad *et al*., 1980).

Different *Trichoderma* spp. exhibited the capability to secrete around 40 different secondary metabolites, contributing to their mycoparasitic and antibiotic action. Most of the *Trichoderma* spp. are recognized for their production of volatile compounds, including acetaldehyde, ethylene, acetone and carbon dioxide (Siddiquee *et al*., 2012). In addition, several species produce antibiotics such as trichodermin (Godtfredsen and Vangedal 1964; Sharma *et al*., 2016), gliotoxin, viridin (Khan *et al*., 2004, Mukherjee *et al*., 2012, Vargas *et al*., 2014) and ergokonin (Malmierca *et al*., 2015, Rai *et al*., 2016) accountable for antifungal and antibacterial properties (Sivasithamparam and Ghisalberti 1998, Sharma *et al*., 2016). These biochemical attributes enhance the biocontrol efficacy of *Trichoderma* spp. through antibiosis, a key antagonistic mechanism mediated by the production of specific or non-specific metabolites, lytic enzymes, volatile compounds and other inhibitory substances. Consequently, *Trichoderma* Strains such as *T. harzianum*, *T. hamatum*, *T. asperellum* and *T. atroviride* are widely exploited in agriculture for the suppression of phytopathogens and as plant growth promoting agents.

By taking the considerations for economic importance of turmeric and increase incidence of leaf light, extensive reliance on synthetic fungicides, coupled with concerns over toxicity, environmental safety and resistance development which necessitates the exploration of ecofriendly activities. Therefore, present research has been carried out to evaluate the comparative efficacy of chemical fungicides and biocontrol agents against *A. alternata* aiming to support integrated and sustainable disease management in turmeric.

Materials and Methods

Design of the experiment

This experiment was conducted using complete randomized design (CRD) to evaluate the efficacy of chemical fungicide and biocontrol measures against turmeric leaf blight caused by *Alternaria alternata*. For chemical control, five different concentrations of fungicide chlorothalonil (25ppm, 50ppm, 200ppm, 500ppm and 1000ppm) were tested, while *Trichoderma harzinum* was used as a biocontrol agent. Each treatment was replicated three times. The antifungal activity was assessed *in vitro* using a poisoned food technique and treatments effects were quantified based on percentage mycelial growth inhibition. The experimentation was conducted at the Post Graduate Lab, Department of Plant Pathology, Punjab Agricultural University (PAU), Ludhiana.

Preparation of growth media (Potato Dextrose Agar)

Potato Dextrose Agar (PDA) medium was prepared to support fungal growth using standard protocols. The PDA medium consisted of 250 grams of mashed potato extract, 20 grams of Dextrose and 20 grams of Agar-agar, dissolved in distilled water and adjusted to a final volume of 1000 ml (Figure 2a). The components were thoroughly mixed to obtain a homogeneous medium and sterilized by Autoclaving at 121°C and 15 psi pressure for 20-25 minutes. Once sterilized, 20 ml portions of the culture media were poured into each 100 x 15 mm petri plates under sterile laminar air flow conditions. The petri dishes were then partially covered with lid and left on the laminar air flow to cool and solidify at room temperature prior to use (Figure 2b).

Isolation and identification of the pathogen from the sample

The culture of *Alternaria alternata* used in the present study was isolated from naturally infected leaves of turmeric plants from fields of pathology Research Farm at Punjab Agricultural University, Ludhiana (Figure 1). Diseased leaf samples were excised along with adjacent healthy tissue using a sterile razor blade, surface sterilized in sodium hypochlorite solution for 1 min, and rinsed 3 times with sterile distilled water. The sterilized leaf bits were aseptically placed on potato dextrose agar medium (PDA) under laminar airflow conditions (Figure 3a). The plates were incubated at 25± 2°C for 7 days to allow fungal growth (Figure 3b). The emerging fungal colonies were identified as *A. alternata* based on morphological characteristics and standard taxonomic descriptors available in published literature (Figure 3c). The pathogen further purified using single spore isolation technique and stored the culture at 4°C and subculture as required. For pathogenicity testing, a conidial suspension ($1x10^6$ spores per ml) was prepared and artificially inoculated onto healthy turmeric plants. Typical leaf symptoms developed within 6-12 days after inoculation, and the pathogen was successfully reisolated from infected tissues on PDA, thereby fulfilling Koch's postulates.

In vitro evaluation of fungicide by poison food technique

The bio efficacy of the fungicide chlorothalonil will be tested against *Alternaria alternate* to determine its optimal application dose for effective pathogen suppression. The range of fungicide concentrations were assessed to quantify their inhibitory effects on pathogen growth which enable to identify minimum effective dose that achieves maximum control of the disease. This evaluation provides a scientific basis for dose standardization of chlorothalonil. This ensures the effective management of *A. alternate* while minimizing excessive fungicide dose.

Fungicidal stock solution

A stock solution of Chlorothalonil (2000 ppm) was prepared by dissolving 0.268 mg of Chlorothalonil wettable powder (WP) in 500 ml of sterile distilled water. The suspension was gently stirred to ensure complete homogenization. The prepared solution was stored in a sterile container covered with aluminium foil until further use.

Preparation of different treatments of fungicide

To facilitate a comparative evaluation of disease management efficacy, multiple concentrations of chlorothalonil were prepared and assessed. These graded doses were used to quantify the dose response relationship of fungicide which enable for the estimation of lethal dose causing 70% inhibition (LD_{70}) of the pathogen. Five different treatment concentrations were formulated as detailed below.

Poison Food Technique Assay

The antifungal activity of chlorothalonil was evaluated using the poisoned food technique, following the methodologies outlined by Shrestha and Tiwari (2010), Zaker *et al*. (2016), and Mohammad *et al.* (2016) with minor modifications. Petri plates were prepared by pouring Chlorothalonil stock solution at concentrations of 2000 ppm into molten potato dextrose agar (PDA) to obtain the required treatment concentrations. Specified volumes (0.25, 0.5, 1.0, 2.0, 5.0, and 10.00 ml) fungicide solution was added to PDA, thoroughly mixed to ensure homogeneity and allowed to solidify under aseptic conditions. Plates

containing chlorothalonil served as positive controls, while with normal saline solution (NSS) without fungicide as negative controls. Mycelial discs (5mm diameter) were excised from peripheral position of 7-day-old fungal cultures using a sterile cork borer. A single disc was placed at the centre in the inverted position to make direct contact between the mycelium and the surface of the agar of each petri plate with different treatments. The plates were sealed with parafilm and then incubated at room temperature for 7 days. Each treatment replicated three times. Radial mycelial growth was measured in centimetres at regular intervals until the fungus completely colonized the control plates.

$$C_1V_1 = C_2V_2$$

Where,
C_1 is the stock solution concentration,
V_1 is the volume of stock solution needed,
C_2 is the desired treatment concentration
V_2 is the final volume of the treatment solution.

Percent mortality

The efficacy of different doses of chlorothalonil will be calculate by using Abbot's formula:

$$Percent\ mortality\ (\%)\ \frac{dc - dt}{dc}\ X\ 100$$

Where:
dc- Average diameter of the colony in the control set
dt - Average diameter of the colony in the treatment set

In vitro evaluation of Biocontrol agent by dual culture method

The In-vitro antagonistic potential of *Trichoderma harzianum* against Alternaria blight of turmeric *A. alternata*, was evaluated under controlled laboratory conditions. Fresh cultures of *T. harzianum* were obtained by subculturing from old stocks to establish actively growing lawn cultures. Colonies exhibiting characteristic whitish green to green, resembling typical *Trichoderma*, were cultured on PDA and maintained at 4°C for subsequent experimental use. The antagonistic interaction between *T. harzianum* and *A. alternate* was assessed using the dual culture technique on PDA medium. Mycelial discs (5mm diameter) excised from 7-10 day cultures of A. alternate were placed approximately 1 cm from the edge of the petri plate. A similar sized disc of *T. harzianum* was positioned on the opposite side of the same plate. Each treatment was replicated twice and incubated at 27±2°C. observations on radial colony growth and percentage inhibition were recorded at 6 and 9 days after inoculation. The percent inhibition of mycelial growth relative to the control was calculated using the formula described by Vincent (1947).

$$Inhibition\ of\ mycial\ growth\ (\%)\ \frac{dc - dt}{dc}\ X\ 100$$

Where:

dc- Average diameter of the colony in the control set
dt - Average diameter of the colony in the treatment set

Results and Discussion

In vitro evaluation of fungicide by poison food technique

The in vitro antifungal efficacy of chlorothalonil against *A. alternata* was evaluated using the poison food technique and results are presented in Table 1, Figure 4. A clear dose dependent response was observed among different tested concentrations. The results showed that the fungicide *i.e.,* chlorothalonil at 200 ppm was significantly most efficient in poison food technique when compared with lower (25 ppm and 50 ppm) and higher (500 ppm and 1000 ppm) concentrations against *A. alternata*. These findings suggest that an intermediate fungicide concentration provide maximum antifungal activity, which highlights the importance of dose optimization for effective disease management (Table 2).

The graphical analysis of dose response data revealed that the lethal dose causing 70% inhibition of *A. alternata* was achieved at a chlorothalonil concentration of 200 ppm and the most probable line was also observed to be aligning with the plot. The plot results for the chemical control experiment against *A. alternata* with the help of fungicide chlorothalonil is presented below graph Figure 5. The present results are consistent with earlier reports demonstrating the efficacy of fungicides against Alternaria species. Hariprasad *et al*. (2017) reported a mean mycelial growth inhibition of 78.83 % in *Alternaria tenuissima* with Carboxin + Thiram. Similarly, Mohan *et al*. (2018) observed complete mycelial growth inhibition in *Alternaria macrospora* when treated with Carbendazim + Mancozeb and copper oxychloride resulted in 75.44% growth inhibition.

***In vitro* evaluation of Biocontrol agent by dual culture method**

The results of the biological control assay evaluating the antagonistic potential *T. harzianum* against *A. alternata* are mentioned in Table 3. The biocontrol agent *T. harzianum* exhibited significant inhibitory effect on the pathogen compared with the untreated control. The inoculation and infection rate of the pathogen (*A. alternata*) and the biocontrol agent (*T. harzianum)* individually and in combination are represented in Figure 6, 7. The colony growth pattern of both the pathogen and the biocontrol agent exhibited

competitive interaction, with neither organism completely over growing or displacing the other once establishment had occurred. The spatial expansion of each colony remained largely confined to its respective zone, indicating minimal interference during early establishment. This growth behaviour suggests a competitive mode of interaction rather than direct mycoparasitism, with both microorganisms maintaining distinct growth fronts throughout the observation period.

Their findings are agreement with the results of Jakatimath *et al*. (2017) reported that *Trichoderma spp*. was most effective against *Alternaria alternata* than *Pseudomonas fluoroscens* and *Bacillus subtilis*. Chaitanya *et al*. (2018) demonstrated that the beneficial fungus *T. harzianum* can effectively suppress the harmful fungus *A. alternata* in controlled lab settings. Using the dual culture plate technique, they recorded a mycelial growth inhibition of 60.71%, indicating the efficacy of *T. harzianum* as a promising biocontrol agent for the management of Alternaria diseases.

Conclusion

Among the chemical treatments evaluated, chlorothalonil at 200ppm resulted in 70% mycelial growth inhibition of *A. alternata*, which represents the LD_{70} and confirming its superior efficacy relative other tested concentrations. In biological control assays, *T. harzianum* exhibited rapid colonization, completely covering the culture plate within 6 days, whereas the pathpgen required 9 days. In dual culture assays, *T. harzianum* outcompeted the pathogen, rapidly restricting its growth and achieving complete suppression of the *A. alternata* colony within 4 days. These results collectively demonstrate the effectiveness of chlorothalonil at a standardized dose and highlight the strong antagonistic potential of *T. harzianum* for the management of Alternaria blight.

Table 1 : Composition of working solutions for different treatments of chlorothalonil

S.No.	Final conc. (ppm)	Stock sol. (ml)	Distilled Water (ml)	2x PDA (ml)
1.	25	0.25	9.75	10
2.	50	05	9.5	10
3.	200	2	8	10
4.	500	5	5	10
5.	1000	10	10	10

Table 2 : Percent inhibition of *A. alternata* with chlorothalonil

S.No.	Fungicidal Dose (ppm)	Percent inhibition (%)
1.	25 ppm	68.75
2.	50 ppm	68.75
3.	200 ppm	70.62
4.	500 ppm	81.25
5.	1000 ppm	80.62

Table 3 : Antifungal activities of *T. harzianum* against *A. alternata*.

Colony description	Individual colony (cm)		Biocontrol (cm)	
Days	***A. alternata***	***T. harzianum***	***A. alternata***	***T. harzianum***
1	0.5	0.76	0.5	0.93
2	1	2.47	1.22	2.12
3	1.7	4.19	1.86	3.74
4	3.7	5.82	2.53	4.82
5	4.4	7.45	2.53	5.63
6	5	8	2.53	5.91
7	6.2	8	2.53	5.91
8	7.1	8	2.53	5.91
9	8	8	2.53	5.91

Fig. 1 : Diseased leaf sample of leaf blight of turmeric (*A. alternata*)

Fig. 2a : Liquid Potato Dextrose Media (PDA) conical flask b)

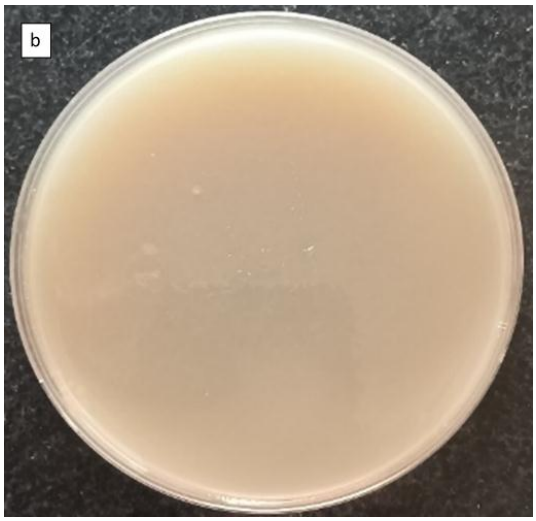

Fig. 2b : Solidified PDA media in petri plate

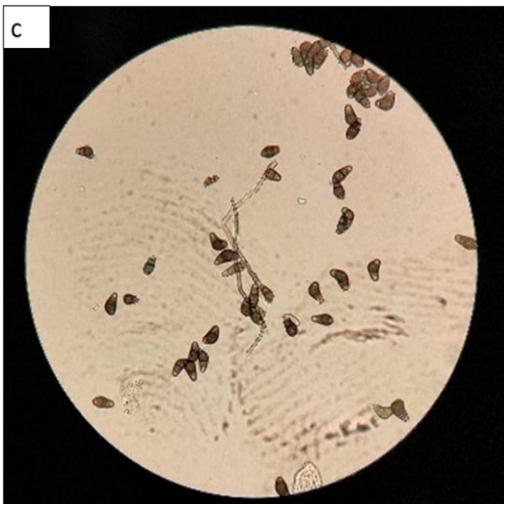

Fig. 3c : Identification pathogen under light microscope

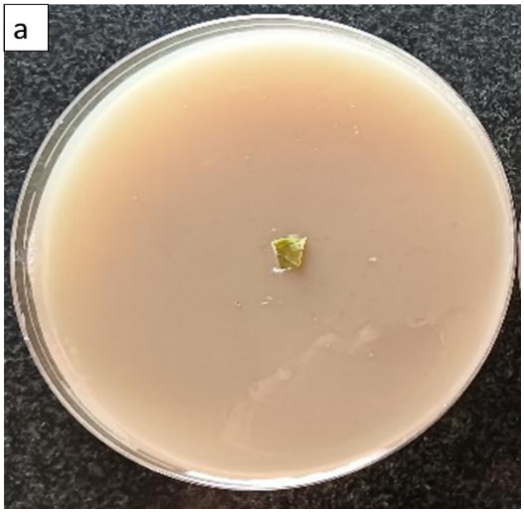

Fig. 3a : Sample inoculated on PDA media under aseptic condition

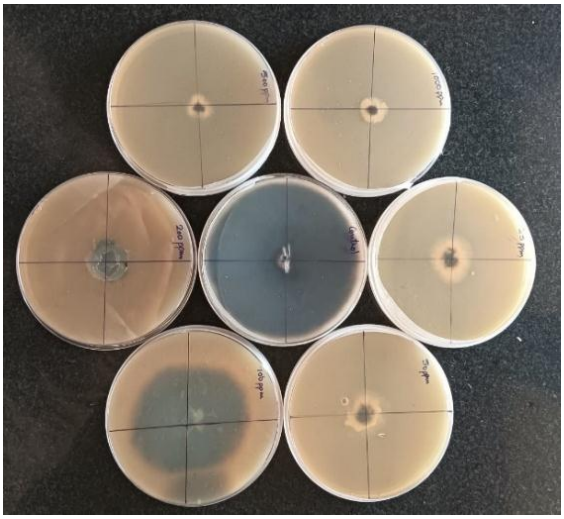

Fig. 4 : Different fungicidal treatments along with control

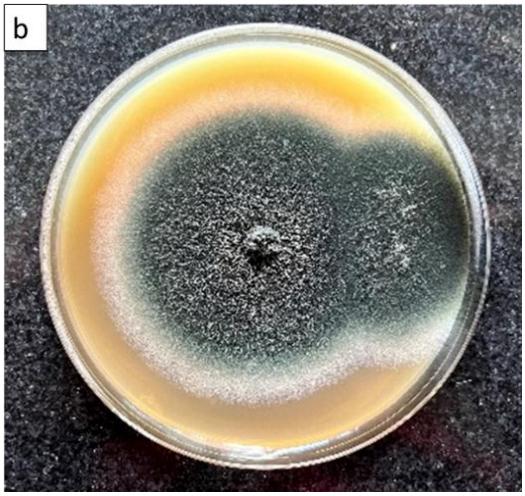

Fig. 3b : Growth of pathogen on PDA media

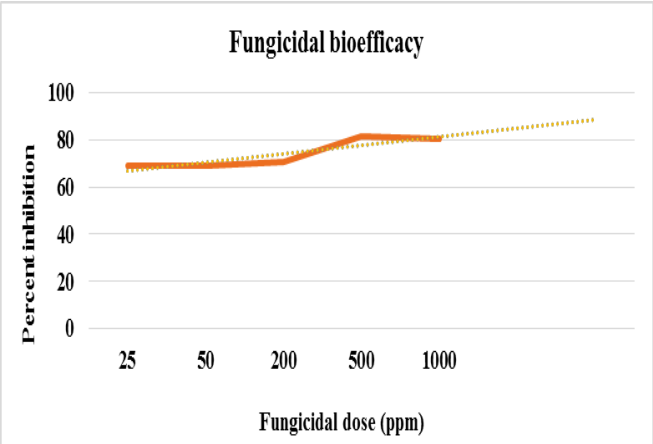

Fig. 5 : Different fungicidal dose (ppm) and inhibition rate (percent)

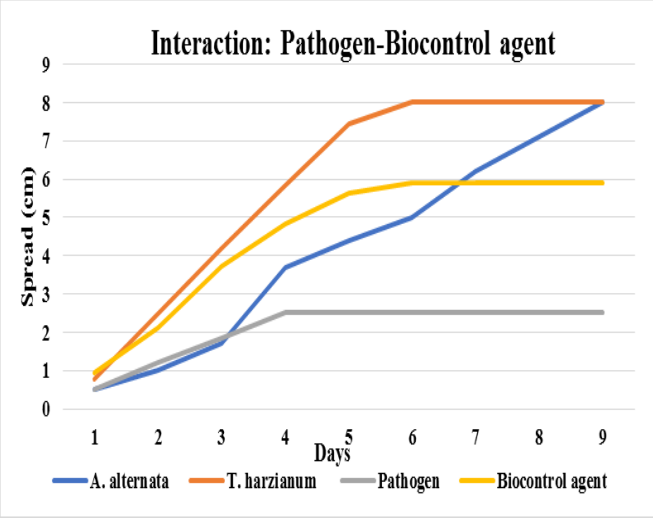

Fig. 6 : Showing the comparison of *T. harzianum* and *A. alternata*

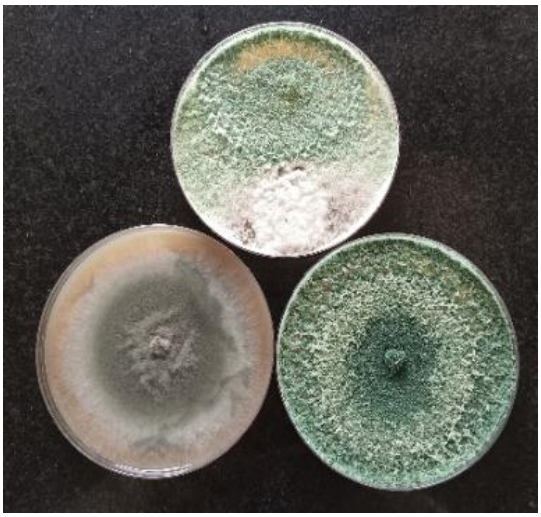

Fig. 7 : Treatment of *T. harzianum* + *A. alternata*, *T. harzianum (control) and A. alternata (control)*

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Conflict of interest

The authors declare that they have no conflicts of interest

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