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EXPLORING THE EFFECTS OF PLANT EXTRACTS, BIOLOGICAL AGENTS AND ESSENTIAL OILS ON THE NON-CHEMICAL MANAGEMENT OF BANANA LEAF SPOT DISEASE

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ABSTRACT

The present research aimed to effectively manage *Fusarium oxysporum*, the causal agent of banana leaf spot disease, through the utilization of diverse botanical extracts, essential oils, and biocontrol agents. Various concentrations (5%, 10%, and 15%) of botanical extracts, namely Garlic, Turmeric, Ginger, Neem, and Mint, were tested to assess their antifungal efficacy. Garlic demonstrated complete inhibition of radial growth (0.00 mm), with decreasing growth observed in Neem, Turmeric, Ginger, and the most extensive growth seen in Mint (32.83, 21.33, and 16.25 mm, respectively). Antifungal effects of specific essential oils (Clove, Tarpin, Castor, Bitter chamomile, Neem) were evaluated using the poisoned food technique at the mentioned concentrations. Clove exhibited complete growth inhibition (0.00 mm), followed by Tarpin, Castor, and Neem. Conversely, Bitter Chamomile oil promoted maximum growth (35.41, 30.41, and 25.50 mm). Control plates displayed growth of 90 mm. The study also assessed different *in vitro* biocontrol agents (*Trichoderma harzianum*, *Zasmidium anthuriicola*, *Penicillium sclerotiorum*, *Hypocrea lixii*, and *Chaetomium subaffine*) against the target pathogen. Among these, *Z. anthuriicola* (18.41 mm) prevented F. *oxysporum* growth most effectively, followed by *T. harzianum*, *P. sclerotiorum*, *C. subaffine*, and *H. lixii*. While managing this destructive disease remains a significant challenge, the eco-friendly strategies employed in the study demonstrated that Garlic extract, Clove oil, and the biocontrol agent *Z. anthuriicola* substantially hindered the mycelial growth of the target pathogen.

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INTRODUCTION

Banana is widely recognized as a main fruit that plays a vital role in the development of world economy (Chabi et al., 2018; Wang et al., 2023). It is cultivated in tropic and sub tropic regions of the world, about 135 countries producing bananas (Bubici et al., 2019). It is also Pakistan's main fruit with production of 154,800 tons and cultivated over the 34,800 hectares. The soil and climate favors its successful cultivations, mainly in the south of Pakistan in Sindh province. Banana frequently

used as a dollar fruit crop. Aside from that India, China, Brazil, Indonesia, Ecuador, Costa Rica, Mexico, Thailand and Colombia are the top banana-producers of the world (Junejo and Haq, 2014; Singh et al., 2016). It is rich source of several bioactive compounds, such as flavonoids, phenolic, amines, carotenoids, vitamin C, and vitamin E, which have antioxidant properties that includes a variety of human health benefits. Fungi, bacteria, and virus-induced diseases are key threatening factors in the productive quality of this crop, and almost all commercial banana cultivation is susceptible to such deadly pathogens (Raut and Ranade, 2004). Banana plants are facing threats to their nutritious quality due to diseases caused by infections from microorganisms such as fungi, viruses, bacteria, and nematodes. These infections affect various parts of the plant and result in significant yield losses, impacting both productivity and sustainable production.

Probably 39 fungal pathogens are affecting banana plant worldwide. Some fungal diseases which are reported worldwide are Septoria leaf spot, Cordana leaf spot, Brown spot, Panama disease, Sigatoka, Diamond spot, Cigar end rot, Anthracnose, Tip end rot, Root rot and Pitting disease (Jones and Daniells, 2019). Among them leaf spot disease of banana is the most important damaging disease (Arman et al., 2023). The symptomatology manifests as small lesions on the banana peel, disrupting the photosynthesis process and consequently leading to a decrease in the fruit's quality (Zakaria, 2023).

To control plant diseases, botanical extracts are being developed as alternatives to conventional fungicides. This is due to their relatively harmless nature, environmental friendliness, and safety for humans. These extracts also contribute to an increased shelf life of bananas, particularly during the green stage, across all tested cultivars. Among these extracts, S. *torvum* extract stands out, extending shelf life by 16-20 days in most cases. This duration of control surpasses that of benomyl (Bashir et al., 2020; Thangavelu et al., 2004). Essential oils, possessing a wide spectrum of antimicrobial activity, have been successfully applied to manage fungal and bacterial diseases in various fruits and vegetables. They hold potential as eco-friendly pesticides within integrated pest management programs (Bajpai et al., 2011; Shahzaman et al., 2017; Shahzaman et al., 2016; Sivakumar and Bautista-Baños, 2014).

Biocontrol, through the utilization of antagonistic agents (BCAs), has also demonstrated promise in countering destructive diseases like *Fusarium oxysporum*. This strategy is an important alternative for disease prevention (Ali et al., 2014; Bennett et al., 2011; Bubici et al., 2019; Pegg et al., 2019; Shakoor et al., 2015). The efficacy of biocontrol agents depends on a range of biological and physicochemical factors. These include the characteristics and form of the biocontrol agents, the impediments to initial adversary colonization, as well as subsequent variance considerations. To enhance the effectiveness of biocontrol, diverse approaches can be adopted. These encompass the utilization of banana plant endophytes such as BCAs (*Bacillus* spp.), the development of agents for water and nutrient retention, the application of suitable carriers for BCAs, the preservation of soil biodiversity, and the integrated management of *Fusarium* wilt and nematode diseases (Bajpai et al., 2011; Bibi et al., 2017; Gang et al., 2013; Hyder et al., 2020; Iqbal et al., 2022; Sivakumar and Bautista-Baños, 2014).

Given the challenges in managing this disease based on previous studies, our research aims to identify the most effective method for disease prevention.

MATERIALS AND METHODS

Pathogenic fungi

In the present study, pathogenic fungal isolates of *Fusarium oxysporum* were utilized. These isolates were obtained from the Mycology Laboratory, Department of Plant Pathology, Sindh Agriculture University Tandojam which had previously been confirmed as pathogenic. For the current research, these specific isolates were selected to investigate the impact of botanical extracts, bio-control agents, and essential oils under laboratory condition.

Preparation of plant extracts for phytochemical analysis

Plant materials, including *Azadirachta indica* (neem), *Curcuma longa* (Turmeric), *Mentha piperita* (mint), Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) leaves, were collected from healthy plants to ensure the quality of the samples. The plant leaves were washed and then air-dried at room temperature for 10 days. Afterward, they were finely pulverized using pestle and mortar separately. Specifically, 250 g of each plant material was soaked in 1000 ml of water in a conical flask. The flask was covered and placed on a shaker for 8 h to allow for efficient extraction. After the extraction period, the resulting mixtures were filtered using

Whattman No. 1 filter paper. The filtrates were then concentrated to dryness using a rotary evaporator. The dry extracts obtained after the process were stored at 4°C until they were ready for the subsequent trails (Kuberan et al., 2012).

Media preparation and amendment with plant extracts

The Potato Dextrose Agar (PDA) media was prepared with the addition of 250 mg of chloramphenicol to inhibit bacterial growth, followed by sterilization in an autoclave. Five milliliters of each extract concentration (5%, 10%, and 15%) were carefully placed into separate Petri dishes using a sterile pipette. Subsequently, 20 ml of PDA was added to each Petri dish, and the mixture was thoroughly agitated to ensure the even distribution of the extracts. For the controls, 5 ml of sterilized distilled water was used as an amendment.

Efficacy of botanical extracts

The experiment followed a Completely Randomized Design with five replications. The radial growth rate method was used to assess the inhibitory effect of various plant extracts on *Fusarium oxysporum*. Petri plates containing PDA media were separately amended with 5%, 10%, and 15% concentrations of the plant extracts to test the inhibitory effect of plant extracts on the mycelial growth of *F. oxysporum*. Mycelial discs with a diameter of 3 mm were taken from a one-week-old pure culture of the pathogenic fungus, collected from the edge of a vigorously growing colony, and placed at the center of each Petri plate. The plates were then incubated at a constant temperature of 28 ± 2°C. The percentage inhibition of mycelial growth was calculated after seven days of inoculation using a formula suggested by Begum et al. (2010):

$$I = \frac{C - T}{C} \times 100$$

Where; I = Percentage inhibition, C = Radial growth in control, T =Radial growth in treatment.

Procurement of essential oils

High-quality essential oils, namely Tarpin, Neem oil, Castor oil, Bitter chamomile, and Clove oil, were obtained from a reputable market for the preparation of concentrations at 5%, 10%, and 15%, using 5% Tween 20.

Efficacy of different essential oils

To prepare the concentrations of essential oils, they were dissolved in 5% Tween 20 before being added to 20 ml of PDA medium. The mixture was then poured into Petri dishes and allowed to solidify. In each Petri dish, a mycelial plug measuring 0.5 cm in diameter was placed at the center. For the control groups, 5% Tween 20 was mixed with PDA without the essential oils. Both the experimental treatments and control groups were replicated five times, and the average measurements were calculated. To assess inhibition and efficacy, mycelial growth was monitored and measured as described previously.

Evaluation of selected bio-control agents against *Fusarium oxysporum*

Four commercial bioagents were utilized in these experiments, namely *Chaetomium subaffine*, *Trichoderma harzianum*, *Penicillium sclerotiorum*, *Zasmidium anthuriicola*, and *Hypocrea lixii*. The activity of the biocontrol agents against the development of pathogenic fungal colonies was evaluated using the dual culture technique on suitable culture media, specifically PDA. Sterile distilled water was employed as the control, with five replications, and the resulting measurements were averaged. Control sets were conducted concurrently without the use of the biocontrol agent formulations. Inhibition and efficacy were assessed following the methods previously described.

RESULTS AND DISCUSSION

Growth performance of *Fusarium oxysporum* in response to botanical extracts

The graphical results depicting the mycelial growth of *F. oxysporum* under different botanical extracts and dosages clarify that *F. oxysporum* completely failed to grow in Garlic extract (0 mm) at all tested dosages of 5%, 10%, and 15%, respectively. This was followed by Neem (23.66, 16.58, and 11.5 mm), Turmeric (29.58, 18.66, and 15.66 mm), and Ginger (27.58, 22.58, and 16.75 mm) at doses of 5%, 10%, and 15%, respectively (Figure 1). The maximum colony growth of *F. oxysporum* was observed under Mint (32.83, 21.33, and 16.25 mm) at doses of 5%, 10%, and 15%, respectively. On the control Petri plate, *F. oxysporum* exhibited mycelial growth of 90 mm.

Based on the means, Garlic extracts inhibited the growth of the pathogen completely. Neem extracts at a dose of 15% also effectively controlled the growth of *Fusarium* under *in vitro* conditions (Figure 2). Our study suggests that garlic extracts at 5%, 10%, and 15% were extremely effective in controlling the growth of the target pathogen. A similar study was conducted by (Daramola et al., 2023) where botanical extracts of garlic, tulsi,

neem, hena, and melia completely inhibited sporulation of *F. oxysporum*, *F. solani*, *Colletotrichum coccodes*, and *Alternaria solani*. Similar results were reported by previous researchers (Anuradha et al., 2023; Arman et al., 2023; de Alba et al., 2023; Ibiam et al., 2023; Xue et al., 2023) Neem extract and garlic exhibited better efficacy against *F. oxysporum* growth at concentrations of 5%, 10%, and 15%.

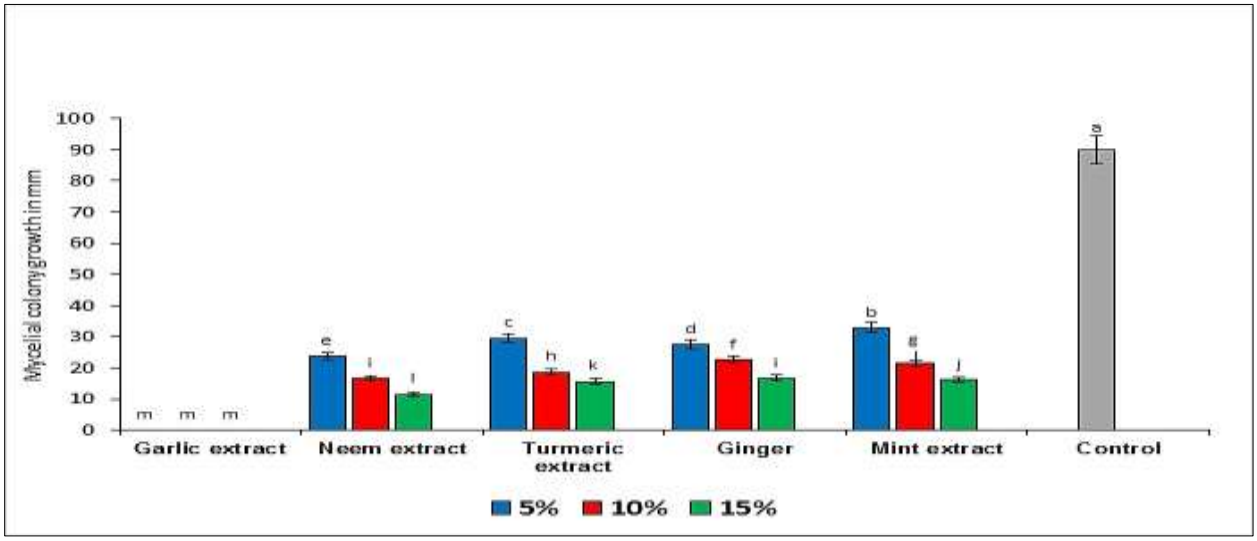

Figure 1: Effect of different concentrations of botanical extracts on the mycelial growth of *Fusarium oxysporum*. Significant differences using student's t-test (P<0.05, P<0.01). Error bar represent the SD of the average from three different biological replicates.

Figure 2: Linear colony growth (mm) of *Fusarium oxysporum* at various concentrations of botanical extracts in comparison with control.

The concluding results of the current investigation align with the research of (Ahamed et al., 2017) who evaluated the effects of extracts from five medicinal plants against *F. oxysporum in vitro* and found that *A. indica* exhibited the best performance at higher concentrations.

Effect of different essential oils on the growth of *Fusarium oxysporum*

To determine the effects of different essential oils on the radial growth of *F. oxysporum* at various dosages, the results indicated that *F. oxysporum* completely ceased to grow when exposed to Clove oil (0 mm) at all tested concentrations. In comparison, Tarpin oil (14.75, 10.58, and 7.75 mm), Castor oil (35.75, 28.66, and 24.66 mm), and Neem oil (32.75, 29.75, and 26.75 mm) at dosages of 5%, 10%, and 15%, respectively, exhibited growth inhibition effects (Figure 3). Conversely, Bitter Chamomile oil (35.41, 30.41, and 25.5 mm) at dosages of 5%, 10%, and 15%, respectively, negatively influenced the growth of *F. oxysporum*. In the control condition, the pathogen grew vigorously, reaching 90 mm.

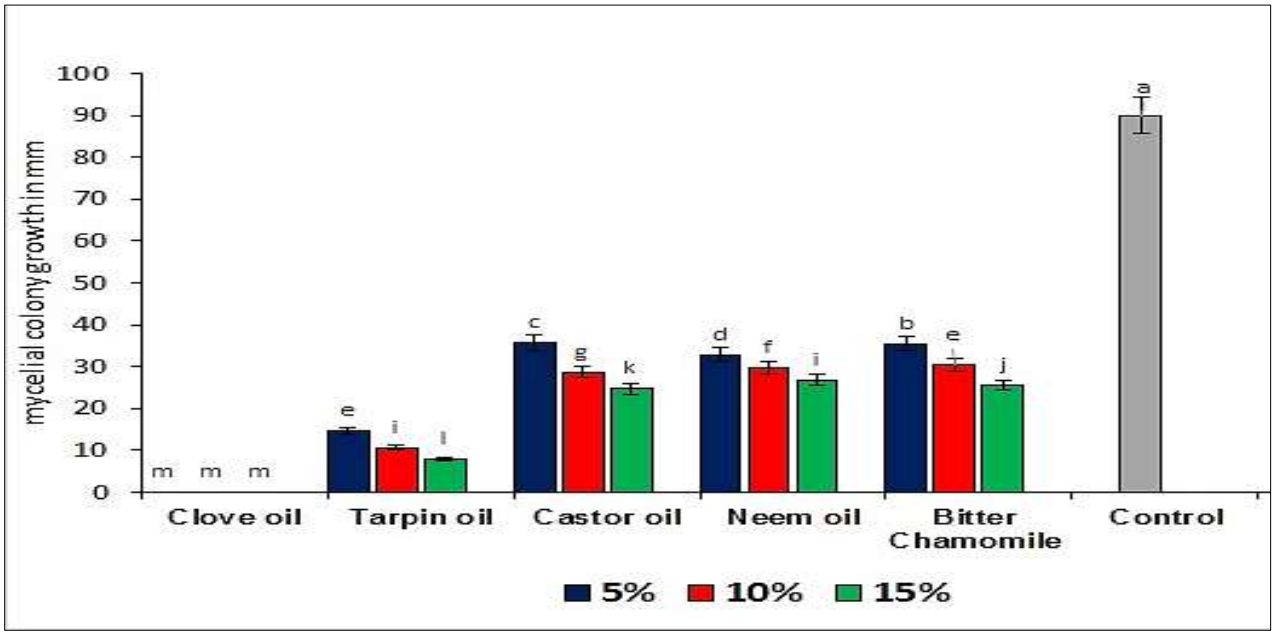

Figure 3: Effect of different essential oils on the mycelial growth of *Fusarium oxysporum*. Statistical significant differences using student's t-test ($P<0.05$, $P<0.01$). Error bar represent the SD of the average from three different biological replicates.

Based on the analyzed data, Clove oil exerted positive control over the disease, effectively stopping the growth of the pathogen under *in vitro* conditions (Figure 4). Among the essential oils, Clove oil demonstrated the greatest effectiveness at concentrations of 5%, 10%, and 15% against the vegetative growth of *F. oxysporum*. These findings align with the research conducted by (Kong et al., 2023), revealing the significant impact of Clove essential oil (*S. aromaticum*) on the vegetative development of *F. oxysporum*. Furthermore, a report published by (Kassem et al., 2023) indicated that cloves are capable of inhibiting mycelium expansion in *F. moniliforme*, *F. oxysporum*, *B. theobromae*, *F. solani*, *R. solani*, and *T. paradoxa*, suggesting the effectiveness of certain plant extracts and natural oils. (Ghosh et al., 2023) found that clove oil was particularly effective in inhibiting conidial germination, hyphal growth, and sporulation of *F. oxysporum* under in-vitro conditions.

In the context of plant diseases, the use of clove oil has yielded positive outcomes. For instance, the application of aqueous emulsions of clove oil effectively reduced tomato *Fusarium* wilt disease in soil mixtures. Studies have also proposed that combined treatment with clove oil, thyme oil, and rosemary oil can effectively reduce *Fusarium* wilt in tomatoes. (Yang et al., 2023) described how clove essential oil played a pivotal role in achieving complete inhibition of the tested pathogen in all the treatments, showcasing its significant biological function.

Concerning the concentration-dependent effects of clove oil on *F. oxysporum*, treated samples exhibited increased habitat and biological activity, with varying concentrations. Mycelial growth at a concentration of 100 ppm measured 5.6 cm, while growth marginally decreased to 0.44 cm at 400 ppm. Complete inhibition of *F. oxysporum* was observed at 500 ppm, representing the highest inhibition ratio among the tested essential oils compared to the progression of the corresponding pathogen under control conditions (7.72 cm).

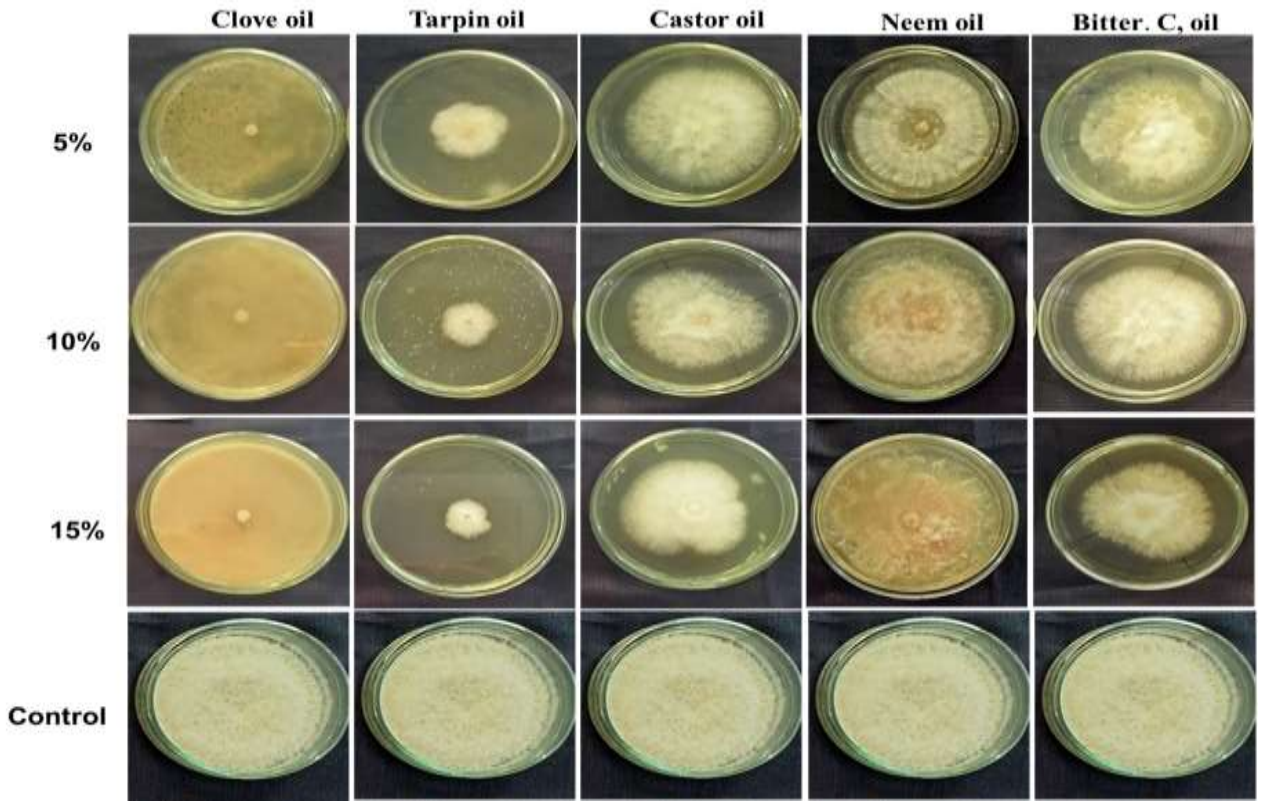

Figure 4: Colony growth (mm) of *Fusarium oxysporum* at various concentrations of essential oils in comparison with control.

Evaluation of selected bio-control agents against *Fusarium oxysporum*

The current results demonstrate that *F. oxysporum* exhibited the least mycelial development when exposed to *Zasmidium anthuriicola* (18.41 mm), whereas *Trichoderma harzianum* (20.00 mm), *Penicillium sclerotiorum* (21.41 mm), *Chaetomium subaffine* (29.50 mm), and *Hypocrea lixii* (34.00 mm) inhibited the growth of the targeted isolate (Figure 5, 6).

Against the mycelial growth of the targeted pathogen, the bio-control agents *Z. anthuriicola* and *T. harzianum* demonstrated improved efficacy. These suggested results from the current study align with the research findings of E Arman et al., 2023 where the antifungal activity of *Trichoderma* spp. against various plant pathogenic fungi, such as *Alternaria*, *Fusarium*, and *Colletotrichum*, was found to be the most effective. In the experimental treatments, these agents achieved 100% control over the growth of *Alternaria*, *Colletotrichum*, and *Fusarium* within 7 days of incubation. The same *Trichoderma* bio-control agent was studied by (Mishra et al., 2023), wherein it exhibited inhibitory effects on the growth of fungal pathogens.

Another study conducted by Mishra et al. (2023) focused on *T. harzianum*, revealing a maximum inhibition ratio of 82.65% through mutual cultivation. In a report published by (Paghaleh et al., 2023), *Trichoderma* isolates were investigated, and *T. koningii* was identified as the most effective isolate in reducing the vegetative growth of *Verticillium*, the pathogen responsible for pistachio wilt disease. Findings from the research study by (Ishaq et al., 2023) suggested that *F. oxysporum*

strains isolated from various fields in Iran exhibited high pathogenicity. The bio-control agent *T. harzianum* exhibited significant effects in inhibiting wilt-causing fungi in brinjal under *in vitro* conditions, while also promoting plant growth. The existing literature underscores the capability of bio-control *Trichoderma* to suppress the mycelial growth of fungal pathogens. These bio-control agents should be employed to protect crops from diverse fungal infections and the detrimental effects of chemical agents.

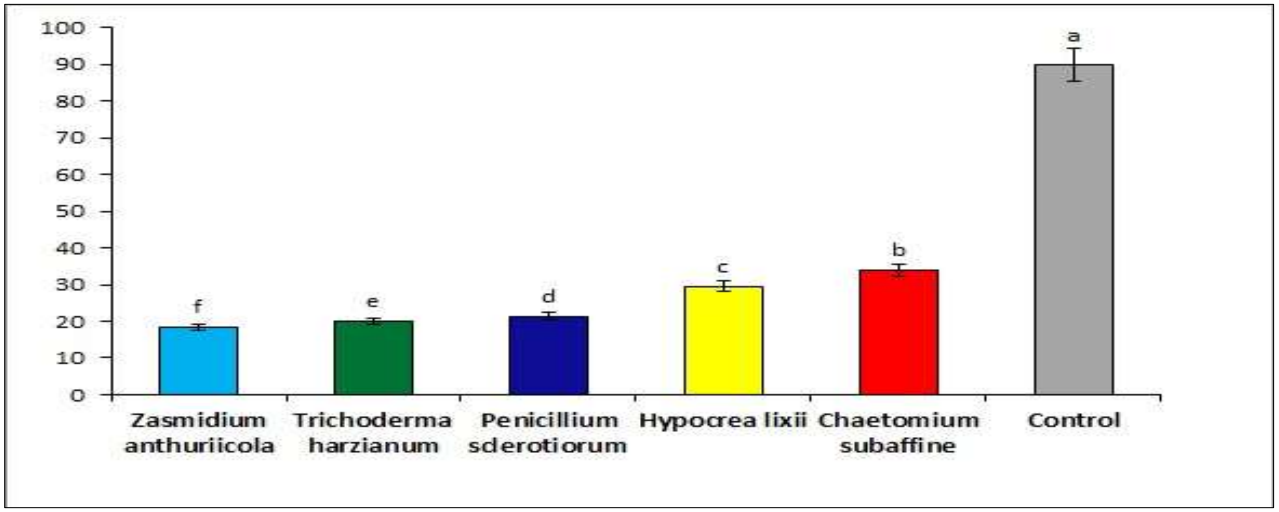

Figure 5: Screening of different bio-control agents against the mycelial growth of *Fusarium oxysporum*. Statistical significant differences using student's t-test ($P<0.05$, $P<0.01$). Error bar represent the SD of the average from three different biological replicates.

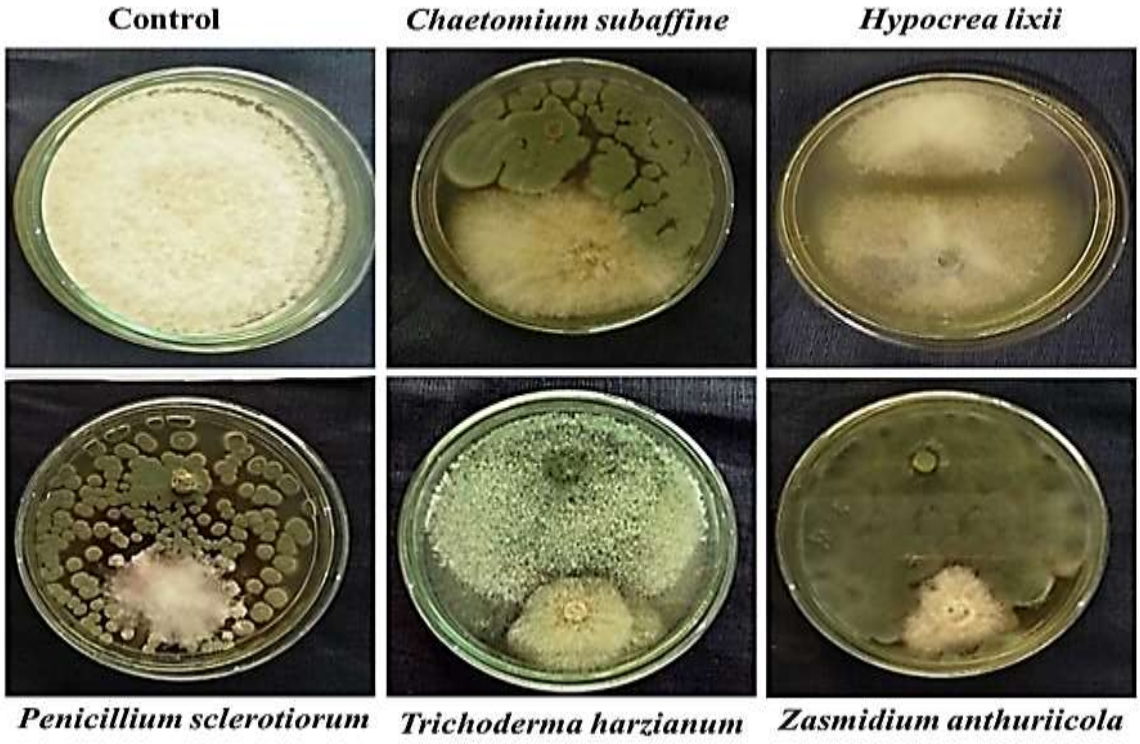

Figure 6: Mycelial growth (mm) of *Fusarium oxysporum* against different bio-control agents in comparison with control.

CONCLUSIONS

Based on the current findings, garlic extract was observed to have an influence on the growth of the tested pathogen. Clove oil, as an essential oil, also demonstrated better performance in controlling mycelial growth. Furthermore, the potent biocontrol agent *Zasmidium anthuriicola* significantly inhibited the mycelial colony growth of the target pathogen.

AUTHORS' CONTRIBUTIONS

MSAT and MAA conceptualized the study; MAA, GHJ, GBP, GHJ curated the data; GBP, FUR, JUDH, SS, MMQ done the formal analysis; MSAT made investigations; MAA, GHJ designed the methodology; MSAT, MAA, G.H.J. and FUR wrote, reviewed and edited the manuscript; MSAT, MAA, GHJ proofread the manuscript; All authors have read and agreed to the published version of the manuscript.

CONFLICT Of INTEREST

The authors declare no conflict of interest.

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