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PRELIMINARY SELECTION AND EVALUATION OF FUNGICIDES AND NATURAL COMPOUNDS TO CONTROL GREY MOLD DISEASE OF ROSE CAUSED BY *BOTRYTIS CINEREA*

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ABSTRACT

Botrytis cinerea is a plant fungal pathogen causing the grey mold disease of rose (*Rosa indica* L.). Finding new and alternative environment-friendly control strategies than hazardous chemicals on different crop diseases is a crucial and healthy step to cope with the current challenges of climate change. Therefore, this study aimed to evaluate the efficacy of different botanical extracts and biocontrol agents (biopesticides) along with different fungicides against *B. cinerea* under *in-vitro* conditions. Three different concentrations i.e., 100, 200, and 300 ppm of five fungicides namely Acrobate, Melody, Cabrio top, Antracol, and Copper oxychloride, botanical extracts of eight plants Dhatura, Ginger, Aak, Neem and Onion, at three different doses of 5, 10, and 15%, and eleven biocontrol fungal agents were used as antagonistic under *in-vitro* on rose plants, The survey of disease incidence% of grey mold on the rose crop in the region shows that the Hyderabad region has a maximum (60%) disease incidence as compared to Tandojam region (40%). Among fungicides, the Cabrio top significantly reduced linear colony growth (31 mm) of *B. cinerea* at 300 ppm concentration. Among botanicals, extract of neem plant exhibited significantly lowest colony growth (23.33 mm) followed by the ginger plant (25 mm) and dhatura plant (26 mm). The higher concentration of fungicides and higher doses (15%) of botanicals extracts appeared significantly efficient to control the pathogen *B. cinerea*. Among biopesticides, *Fusarium solani* appeared prominent in reducing colony growth (25.16 mm) of the pathogen but the difference was not significant 300 with most of the tested biocontrol agents. The recommendation in this study is the high ability of botanical extracts and biocontrol agents in reducing the growth of grey mold, potentially considering using them instead of synthetic fungicides and more safety for the ecosystem. Keywords: *Trichoderma*, *Fusarium*, dhatura, botanical, biological control, synthetic fungicides.

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INTRODUCTION

Rose (*Rosa indica* L.) is considered one of the noble floriculture or garden crops since ancient civilizations and is cherished as an important part of social and cultural rituals and ceremonies (Debener and Linde, 2009). At present, it is used as a novel cut-flower plant species and cultivated extensively as an economic crop for different perfumery items, rose water, different medicinal and food items, and tonics. Rose has prime importance in Pakistan's floriculture industry. However, the rose industry is facing huge losses (around 30%) every year due to several pests and pathogen-associated diseases (Memon *et al.*, 2015). However, many plant pathogens such as viruses, bacteria, nematodes, and fungi infect the rose plant. The plant fungal pathogen included 48 fungal species reported as the cause of several diseases of rose plants worldwide (Azad and Shamsi, 2011; Haque *et al.*, 2013). In Pakistan, the major fungal diseases of the rose plant are black spot, *Alternaria* leaf spot, stem canker, *Cercospora* leaf spot and *Botrytis* blight (Islam *et al*. 2010). Among them, the grey mold disease of rose, caused by *Botrytis cinerea,* is an airborne pathogen involves in significant production losses of rose and other ornamental plant species (Jurick *et al.*, 2017; Ferrada *et al.*, 2016; Williamson *et al.*, 2007). Grey mold is ranked as the most serious diseases due to its huge cause of damage to rose production (Gangemi *et al.*, 2016). The grey mold disease in rose is a common problem in rose crop either in the greenhouse or under open field conditions which cause huge losses to the rose industry every year (Hennebert, 1973; Beever and Weeds, 2007; Elad *et al.*, 2007). *B. cinerea* [teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel] is a common and economically important pathogen of numerous greenhouse-grown ornamental crops (Daughtrey *et al.*, 1995).

Interestingly, *B. cinerea* is considered a lethal fungal pathogen for flowers of the rose plants during infection in the field. The symptoms of infection first appear as flecks or water-soaked spots on flower petals and then lesions appear on infected petals with wither and finally develop brown color symptoms. The development of disease is seemed to be increased in moist conditions (McNicol *et al.*, 1989).

Various control strategies are available to control this disease including fungicides, different environmentally friendly botanical extracts, and biological control agents. Natural compounds including botanical extracts have an antifungal influence on crop species (Jatoi *et al.*, 2020; Comans-Pérez *et al.*, 2021). Among them, the use of botanical extracts is eco-friendly management, safe, and more effective against Botrytis cinerea caused by the gray mold of Rose. Researchers are ever more turning their attention to natural products looking for new leads to increase better drugs against microbial infections. While the antimicrobial activity of botanical extracts has formed the basis of many applications in controlling diseases, food preservations, pharmaceuticals, medicines, and natural therapies. A lot of angiosperm plants are the storehouses of effective chemotherapeutants and results from biological transmission of these plants for a wide range of behavior proved that these botanical extracts can be used for treating diseases (Jatoi *et al.*, 2020). Biological control agents including many groups such as viruses, bacteria, nematodes, and fungi, as well as, insects, and mites have been proved to be effective against this disease (Hajek *et al.*, 2007; Sharma *et al.*, 2020). In nature, many types of fungi are useful in biological control comprising trichoderma, nonpathogenic fungi, nematophagous fungi, and entomopathogenic fungi that can be more antagonistic against other plant pathogens (Al-ani and Albaayit, 2018) On the other hand, different fungicides including Copper Oxychloride, Cabrio Top, Antracol, Melody duo, and Acrobate are being used against *B. cinerea* nowadays.

This study was designed to investigate the effects of various botanical extracts, different fungicides, and different biological agents against *B. cinerea*. The study can be utilized for the control of grey mold of rose under *in-vitro* and *in-vivo* conditions. The rose production of the country can be enhanced by using various management strategies against *B. cinerea* caused by gray mold in rose.

MATERIALS AND METHODS

Survey and sampling of rose fields

A comprehensive survey was conducted to estimate the disease incidence % of grey mold on rose from different locations of Tandojam and Hyderabad as the prevalence of the disease was obtained by applying the following formula;

$$\%\,Disease\ Incidence = \frac{No.of\ infected\ plants}{Total\ No.of\ Plants} \times 100$$

Isolation and identification of the causal fungus

The specimens collected during the survey were brought to the laboratory of the Mycology Department, Sindh Agriculture University, Tandojam, Pakistan. The identification of fungus was done as stated by (Jatoi *et al.*, 2020). The colony growth of the fungus was recognized based on their morphological characteristic as reported by (Nizamani *et al.*, 2020; Ahmed *et al.*, 2018).

Application of different control strategies against *Botrytis cinerea*

A comprehensive set of experiments were conducted using different control strategies like biocontrol agents, fungicides, and botanical extracts to examine their efficacy against *B. cinerea* under *in-vitro conditions*.

In-vitro* efficiency of different fungicides against *B. cinerea

The food poisoning methods to assess different synthetic fungicides used in this study include Copper Oxychloride, Cabrio Top, Antracol, Melody duo, and Acrobate at three concentrations (100, 200, and 300 ppm) against *B. cinerea*. A medium without any fungicide was considered as a control. The crisp PDA plates were added to different concentrations of fungicides inoculated with a 5 mm disk of 8-10 days aged pure culture of *B. cinerea*. Inoculated plates were then incubated at 25°C. Radial colony growth of the tested fungus was docket by drawing two perpendicular lines on the back of the petri plates crossing each other in the center of the plate. The data of colony growth was recorded with these lines in millimeters (mm) after every 24 hours until the plates were filled in any treatment.

In-vitro* efficiency of numerous anti-fungal botanical extracts against *B. cinerea

The efficacy of different botanical extracts e.g. Dhatura (*Dhatura metal),* ginger (*Zingiber officinale*), akk (*Calotropis procera*), neem (*Azadirachta indica*) and Onion (*Alium cepa*) were used at different doses i.e. 5%, 10% and 15% by using food poisoning method against *B. cinerea*. A medium without any botanical treatment was considered as a control. Followed by (Bhatti *et al.*, 2021).

In-vitro* efficiency of different biocontrol agents against *B. cinerea

Bio-control agents like *Trichoderma harzianum*, *Fusarium solani, Fusarium inconaton, Eupnecelium javeannicaum, Rithrinium, Colletotrichum gloeosporioides, Pestotiopsis humus, Neurospora,* and *Pestloliopmiss magnifere* were evaluated against *B. cinerea* in *in vitro* conditions. The dual culture technique was used as reported by Monte (2001) for this purpose.

Statistical analysis

The experiment was designed as CRD with four replications, data was recorded and expressed as Mean ± Standard deviation. ANOVA was performed using Statistics 8.1 software. The overall least significant differences ($p<0.05$) were calculated and used to detect significant differences among different treatments.

RESULTS

Disease incidence (%) of grey mold on rose in district Hyderabad (Pakistan)

The survey regarding grey mold disease incidence% on the rose crop was conducted in two locations i.e., Tandojam and Hyderabad of district Hyderabad. The results obtained showed significant differences in terms of disease incidence in these two regions (Figure 1). The region of Hyderabad appeared with a significant highest disease incidence (60%) as compared to Tandojam (40%).

In-vitro* efficiency of different fungicides on the linear colony growth of *B. cinerea

The data presented in Figure 2 showed a significant reduction in linear colony growth of *B. cinerea* among all tested fungicides as compared to the control as the higher concentration (300 ppm) exhibited significantly reduced linear colony growth of *B. cinerea,* followed by 200 and 100 ppm in all tested fungicides (Figure 3). Specifically, Cabrio top fungicide appeared with significantly reduced linear colony growth (31 mm) of *B. cinerea* followed by Acrobate MZ (33.33mm) and Antracol (35mm) at 300 ppm concentration. Indeed, control appeared with significantly in the highest linear colony growth of 90mm. For testing fungicides, Melody duo at 100 ppm concentration appeared with significant highest linear colony growth of 62.66 mm of *B. cinerea*.

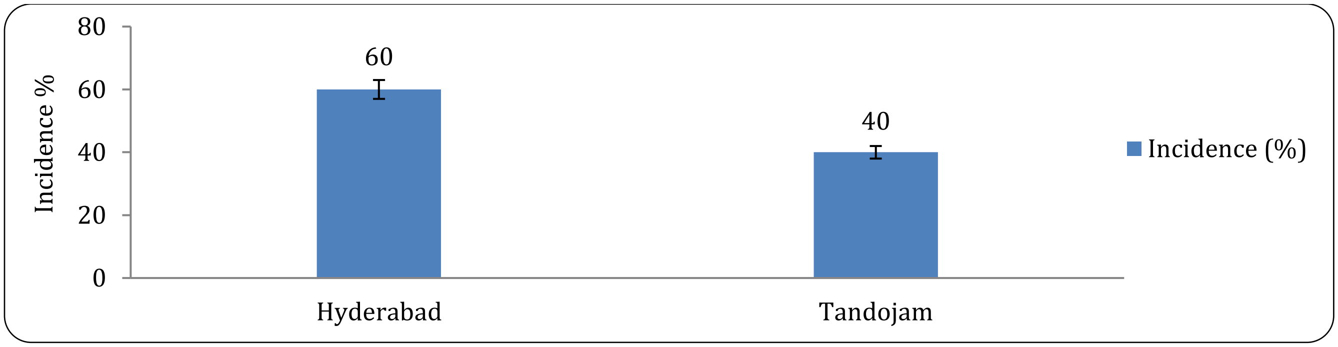


Figure 1. Grey mold disease incidence (%) on the rose crop in Tandojam and Hyderabad, Sindh, Pakistan.

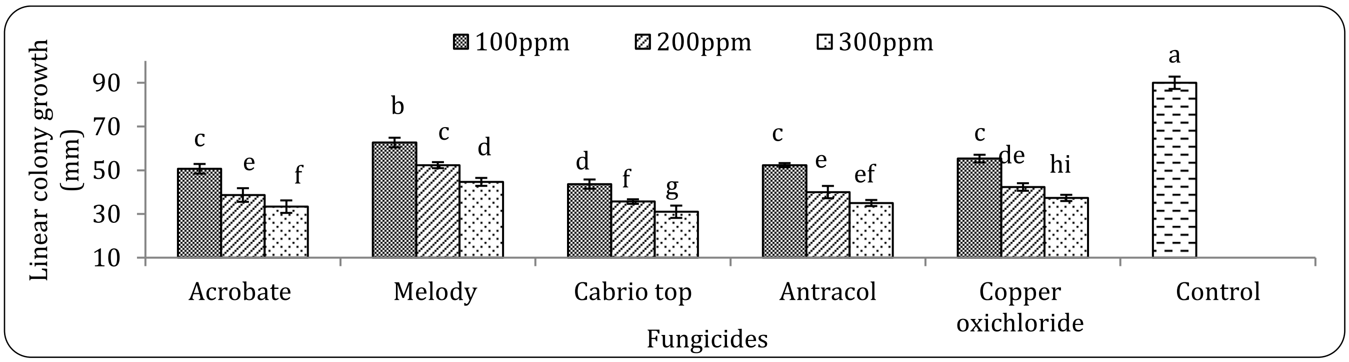
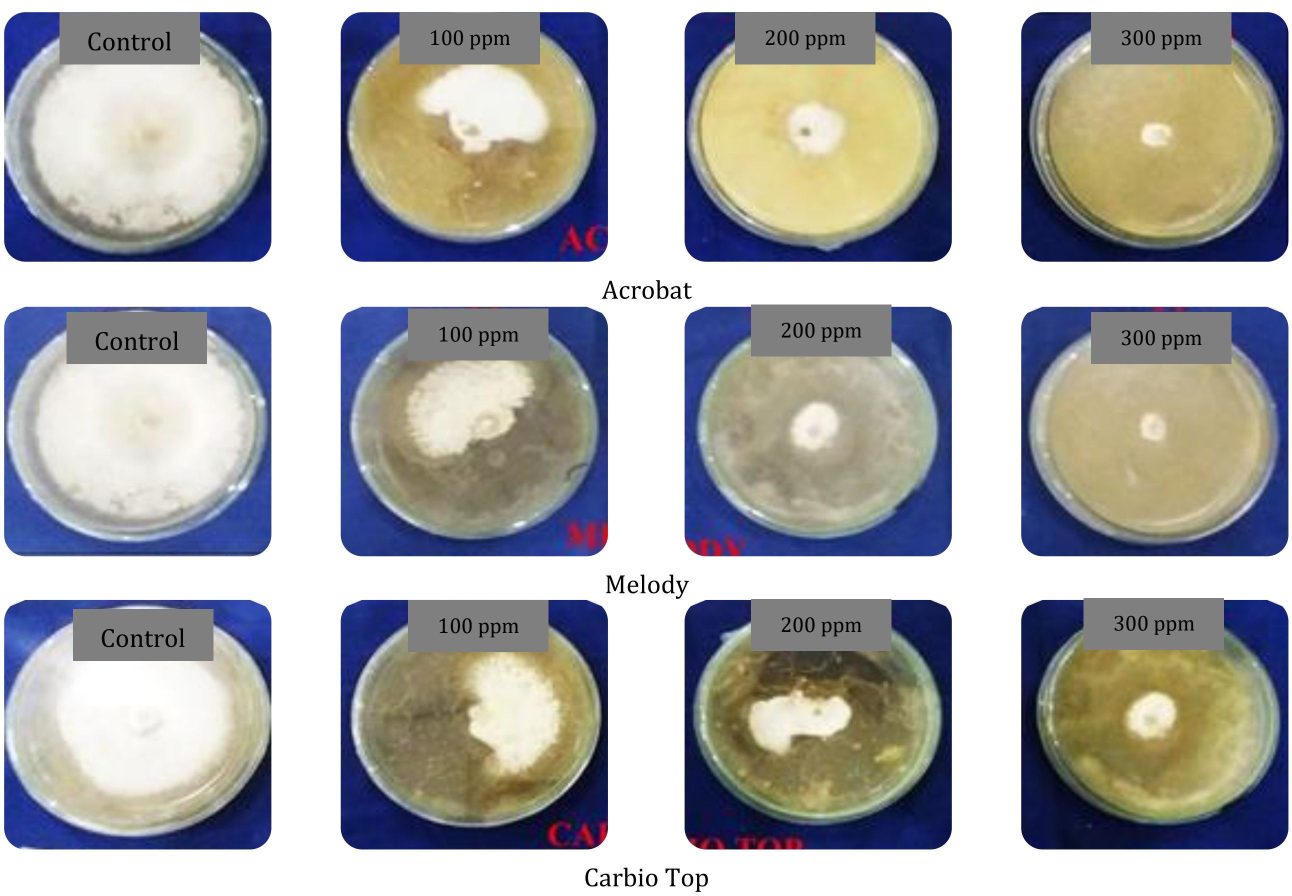


Figure 2. Effect of different fungicides on linear colony growth of *B. cinerea* under *in-vitro* conditions.



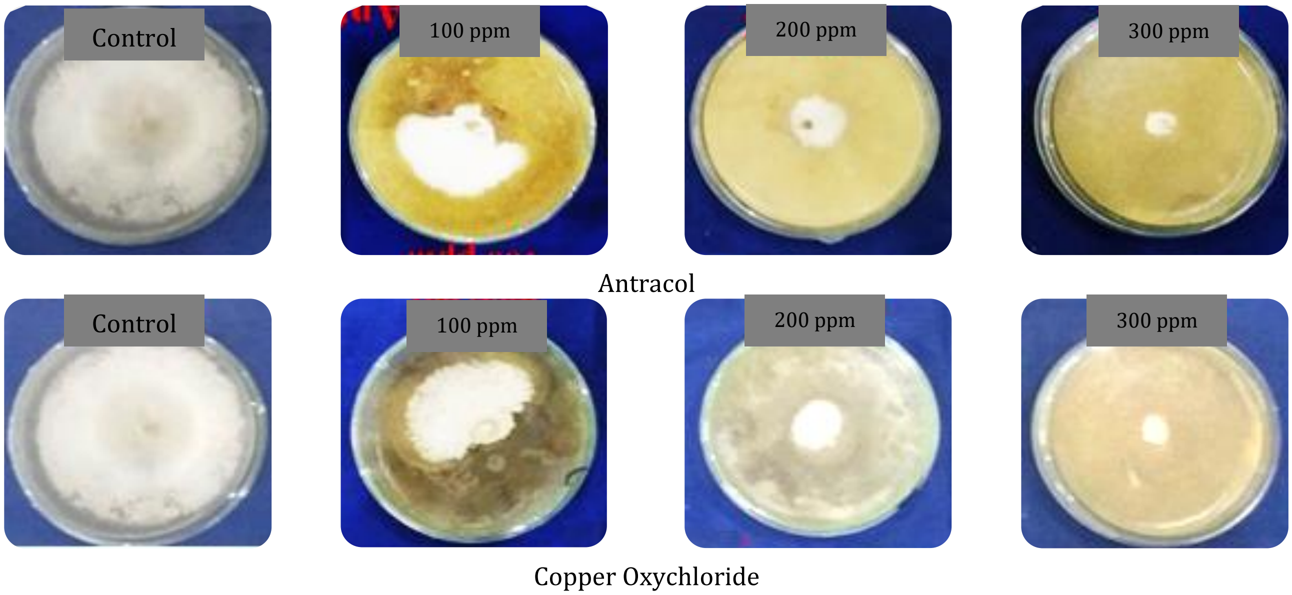


Figure 3. Linear colony growth of fungus *B. cinerea* as affected by different fungicides.

In-vitro* effect of different botanical extracts on linear colony growth of *B. cinerea

Similar to fungicides, all tested botanical extracts significantly reduced linear colony growth of *B. cinerea* than control (Figure 4). Likewise, the higher the doses the higher the efficiency of botanical extracts was (Figure 5). As, the higher doses i.e. 15% resulted in significantly reduced linear colony growth of *B. cinerea* followed by 10% and 5% in all tested botanical extracts. The neem extract significantly reduced linear colony growth of *B. cinerea* (23.33 mm) followed by Ginger (25mm) and Dhatura (26mm) at a 15% dose. As expected, the significant highest linear colony growth of 90mm was observed under control. The testing of botanical extracts, Akk at a 5% dose appeared with maximum linear colony growth of *B. cinerea.*

In-vitro* effect of biocontrol agents against colony growth of *B. cinerea

The data presented in Figure 6 revealed the significant efficiency of all tested biocontrol agents against *B. cinerea* as compared to control. However, there were not much significant differences in terms of reduction of linear colony growth of *B. cinerea* among the tested biocontrol agents. Though *Fusarium solani* appeared with the minimum colony growth of *B. cinerea* (25.16mm) but the reduction was not significantly different from the rest of the tested biocontrol agents except *Calletro* and *Pestloliopmiss magnifere* (35.66 mm).

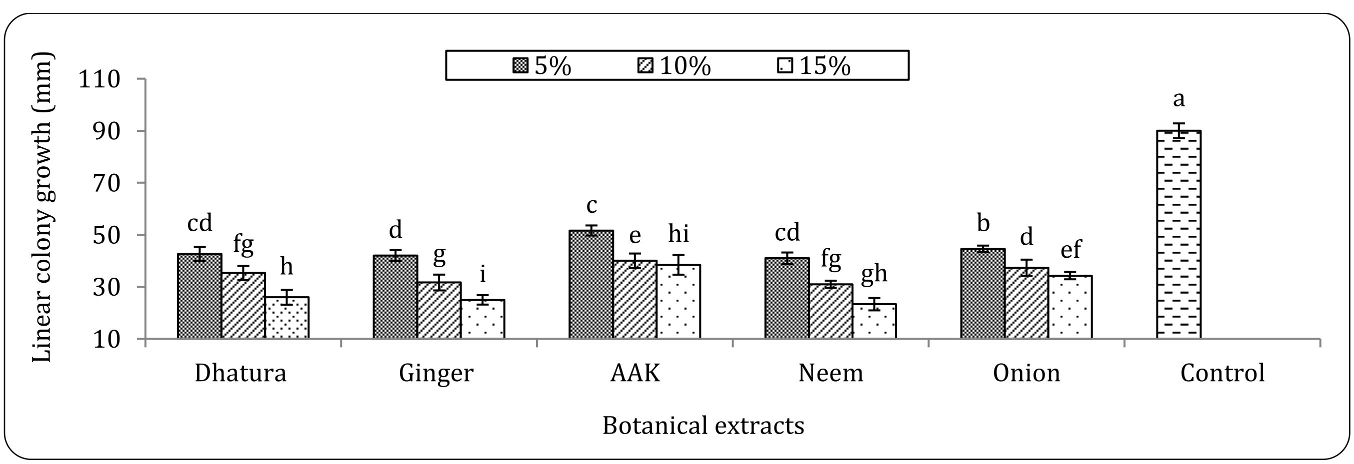


Figure 4. Effect of different botanical extracts on linear colony growth of *B. cinerea* under *in-vitro* conditions.

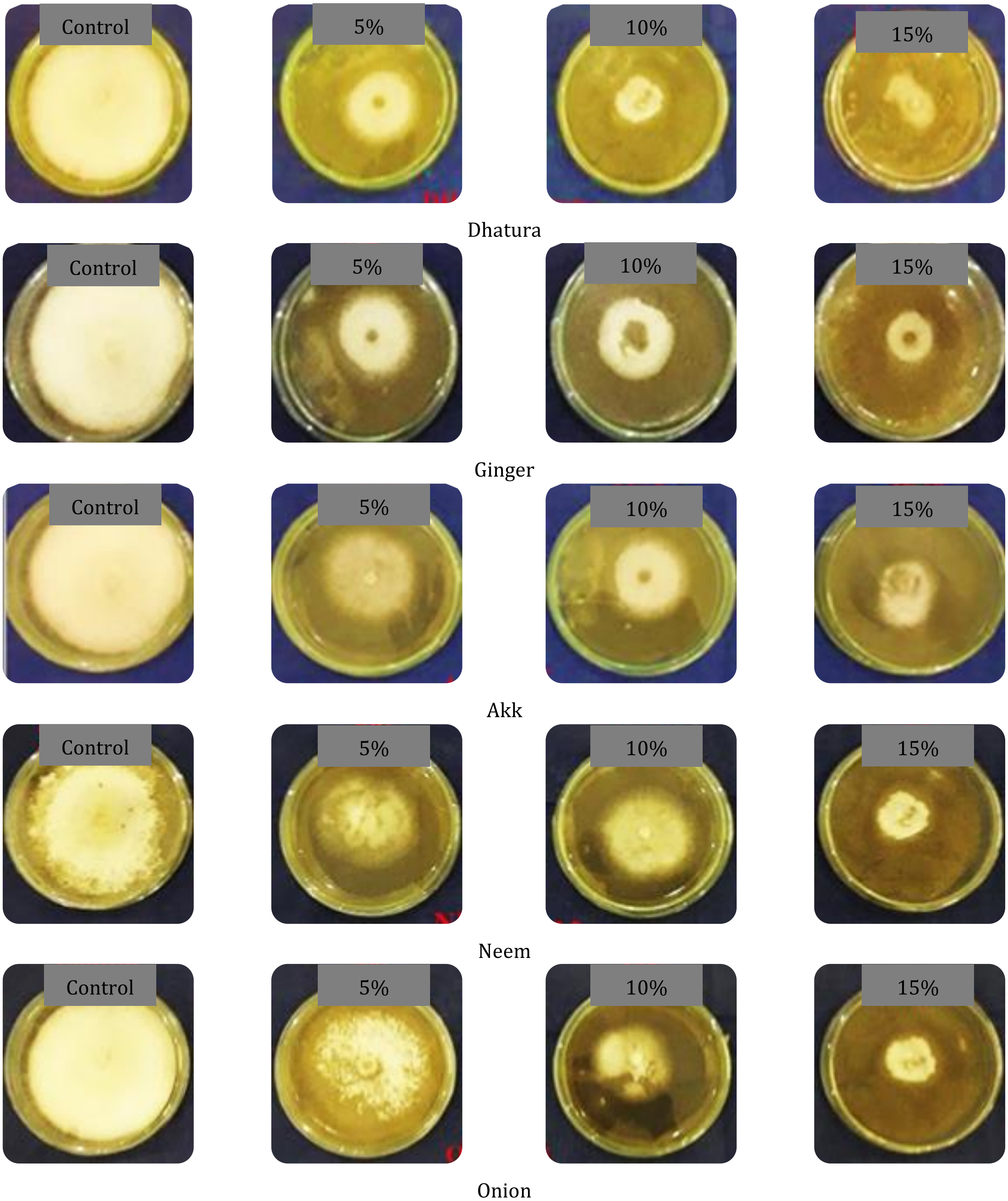


Figure 5. Linear colony growth of fungus as affected by different botanicals.

Comparative study of different Fungicides, Botanicals, and Bio-agents against *B. cinerea* under in-vitro conditions

THE different Fungicides, Botanicals, and Bio-agents against *B. cinerea* under in-vitro conditions, (Figure 7) were tested for their comparative performance against *B. cinerea* causal agent grey mold of rose. The results of the present study show that there was a highly significant effect of all doses of Bio-agents tested for the mycelial colony growth of fungus under *in-vitro* conditions (p<0.05). Among all treatments, Bio-agents were found highly effective followed by Botanicals, which were found mildly effective, while the effects of fungicides were lowest as compared to Botanicals and Bio-agents. The control treatment showed no effect against *B. cinerea* causal agent grey mold of rose.

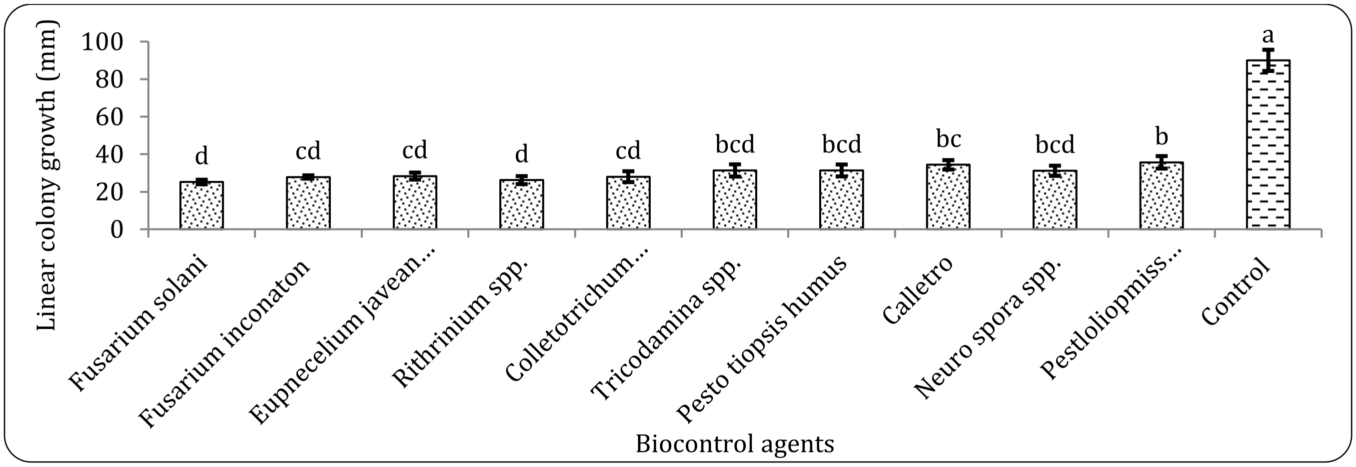


Figure 6. Effect of different biocontrol agents on linear colony growth of *Botrytis cinerea* under in-vitro conditions.

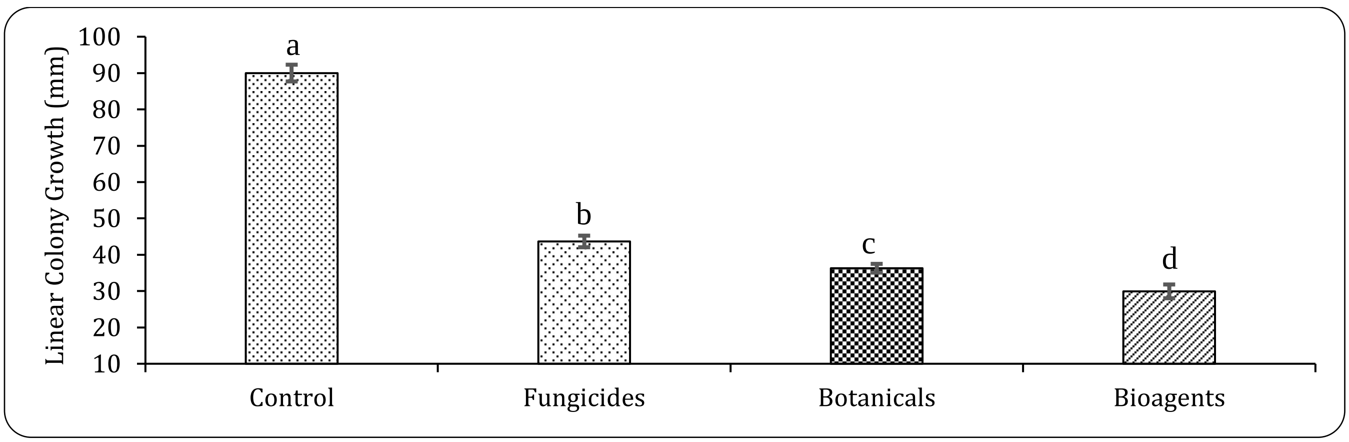


Figure 7. Comparative study of different Fungicides, Botanicals, and Bioagents against *B. cinerea* under in-vitro conditions.

DISCUSSION

The grey mold disease is distractive plant disease on roses that is caused by *Botrytis cinerea*. In this study, we estimated the disease incidence between 40% and 60% in different locations of the Tandojam and Hyderabad regions, respectively. The application of the chemical fungicide 'Cabrio Top' appeared as significantly effective to control the linear colony growth of *B. cinerea* at 300 ppm. Many fungicides showed high efficacy in inhibition of growth mycelium for 100% such as Cabrio Top (Pyraclostrobin+ Metiram) (Ferrada *et al.*, 2016; Hao *et al.*, 2017) reported that the growth of the targeted fungus was significantly reduced by the following fungicides propiconazole, carbendazim, hexaconazole, difenoconazole, and thiophanate methyl. They stated that chemical fungicides reduced fungus growth significantly (*p<0.05*) better than botanicals extracts; however, botanical extracts also showed better response as compared to the control treatment. The effectiveness of botanical extracts in post-harvest storage of marigold can be used at commercial scale.

Among the botanical extracts, the extraction of leaf Neem, ginger, and *dhatura* showed better efficiency against linear colony growth as compared to onion, and akk (Moslem and El-Kholie, 2009) mentioned to potential using the aqueous extract for Neem in inhibiting the growth of many plant pathogens. Leaf Neem extract contained many antifungal compounds having an effective role against several plant pathogens. (Singh *et al.*, 1980) detected the potential of aqueous extract of leaf Neem inhibited spore germination of fungi. (Masum *et al.*, 2009), found a 90% reduction of two fungi including *Bipolaris sorghicola* and *Curvularia lunata* as seed-borne infections to Sorghum. However, (Dabur *et al.*, 2004; Dabur *et al.*, 2005) discovered a novel antifungal (Alkaloid compound) 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate in the extraction of leaf *Datura metel*. The antifungal activity in the aqueous extract of *D. metel* showed high inhibition for the fungus growth of *Alternaria carthami.* (Ranaware *et al.*, 2010; Naik *et al.*) mentioned the efficacy of aqueous extracts of two plants such as Neem and ginger in high inhibiting the growth of plant fungus pathogen *Alternaria solani*.

On the other hand, all biocontrol agents of fungi under *in-vitro* conditions showed efficiency in reducing the linear colony growth of *B. cinerea* against the grey mold of rose. Two fungi as *F. solani* and *Rithrinium* sp. showed more antagonistic against *B. cinerea* as compared to other fungi. In general, all fungal agents showed the antagonistic ability against the plant pathogen *B. cinerea*. Some species of fungi have several mechanisms potential that influences the plant fungal pathogen (Al-Ani and Salleh, 2010; Al-Ani, 2019b). Many endophytic fungi are isolated from various plants (Al-Ani 2019 a,b,c; Sharma et al. 2020)(Al-Ani, 2019a; LKT, 2019) including a lot of species between nonpathogenic fungi such as *Fusarium* (Waweru *et al.*, 2014; Al-Ani and Furtado, 2020) and useful fungi such as *Trichoderma.* (Al-Ani and Mohammed, 2020; Al-Ani *et al.*, 2013; Al-Ani, 2017) mentioned the ability of endophytic *F. solani* in control of *F. oxysporum* f. sp. *cubense* tropical race 4 (*Foc*TR4). The efficacy of fungal biocontrol agents in antagonistic, the plant fungal pathogen under *in-vitro* is coming from two important mechanisms. Two mechanisms are used during fungal biocontrol agents attacking another fungus including (1) mycoparasitism (Al-Ani 2018 b), and (2) producing compounds as the volatile (Al-ani and Albaayit, 2018), and non–volatile. A similar kind of result was reported by (Talibi *et al.*, 2014). Scordino *et al.,* (2008) evaluated *Penicillium* species for the control of black rot disease. Indeed, The role of several useful fungi and the utilization for the microbial technique are more helpful in controlling plant fungal pathogens as an alternative of chemical fungicides. (Al-Ani *et al.*, 2020; Jatoi *et al.*, 2020) mentioned controlling plant fungal pathogens by using the botanicals extracts, biocontrol agents, and fungicides as integrated management for plant fungal pathogens.

As the chemical fungicides are much exclusive and have health hazardous effects on human health and the environment, so different alternatives of chemical fungicides were tried to control the *B. cinerea*. For this purpose, we have used different treatments under *in-vitro* conditions for their efficacy against *B. cinerea* at a different doses. Among them, the Bioagents were found highly effective in reducing the linear growth of fungus followed by botanicals at the same time. The fungicides were found less effective in reducing the linear growth of *B. cinerea*. Our results are in accordance with (Thambugala *et al.*, 2020) who obtained the good inhibitory effect of *Bio-agents.*

CONCLUSIONS

This study showed the possible management of *B. cinerea* by different methods. A chemical fungicide Melody duo, a botanical extraction of akk, and two fungi as *F. solani* and *Arthrinium* sp. are more efficient in controlling *B. cinerea*. These three factors can be utilized in the program of Integrated Pest Management (IPM) to control *B. cinerea* of grey mold in roses. The potential of two factors *Calotropis procera* and two biocontrol fungi can be used against *B. cinerea* in reducing the residue of chemical synthetic fungicides. The utilization of these two factors will save the ecosystem from pollution by chemical material of fungicides.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.