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# Soil-borne and compost-borne *Penicillium* sp. and *Gliocladium* spp. as potential microbial biocontrol agents for the suppression of anthracnose-induced decay on tomato fruits

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## Abstract

**Background:** Anthracnose is a soil-borne tomato (*Solanum lycopersicum* L.) disease caused by *Colletotrichum coccodes* and associated with early dying syndrome and postharvest fruits decays leading to significant yield losses. A biocontrol trial was conducted using *Penicillium* sp. and *Gliocladium* spp. isolates recovered from soil and compost for the evaluation of their antifungal potential against the target pathogen.

**Results:** Cell-free culture filtrates of the microbial agents tested at different concentrations displayed an important antifungal activity based on pathogen mycelial growth inhibition and spore germination suppression. The highest antifungal potential was induced by *Penicillium* sp. CH6, *G. catenulatum* Gc1 and *G. virens* Gv1 cell-free filtrates applied at 3 concentrations (10, 15 and 20% v/v). *C. coccodes* mycelial growth was significantly reduced by 16 to 84% with chloroform and ethyl acetate extracts of *Penicillium* sp. CH6 and *G. catenulatum* Gc1 isolates applied at the concentrations 1, 2.5 and 5% v/v. These treatments had induced an important morphological alteration to pathogen mycelium expressed mainly by a decrease in colony melanization. Testing on pathogen-inoculated fruits, treatment based on cell-free culture filtrates and organic extracts had significantly reduced anthracnose severity as compared to control. Rot lesion diameter was significantly decreased about 22% following treatments with *Penicillium* sp. CH6 and *G. catenulatum* Gc1 cell-free filtrates. All chloroform and ethyl acetate extracts of isolates tested had suppressed disease severity by 11 to 59% and 14 to 85%, respectively, as compared to the untreated control.

**Conclusions:** The results demonstrated the involvement of bioactive compounds in the cultural filtrates and organic extracts tested. These microbial agents may be explored as an alternative tool for the protection of tomato fruits from fungi-associated decays.

**Keywords:** Antifungal activity, Biological control, Culture filtrates, *Gliocladium* spp., Organic extracts, *Penicillium* sp., Tomato rot

## Background

Anthracnose is a soil-borne disease caused by various *Colletotrichum* species on a wide range of hosts estimated at more than 460 plant species such as pepper, pumpkin, papaya and tomato (*Solanum lycopersicum* L.) (Ridzuan et al. 2018). *C. coccodes* is one of the most widespread species in almost all tomato production areas and caused considerable economic losses

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(Gilardi et al. 2014) reaching up to 50% in some African countries (Ibrahim et al. 2011). As a hemibiotrophic pathogen, it is responsible of root rots and fruit decays in the field during rainy weather (Sanoubar and Barbanti 2017) and during the period of pre- or post-harvest ripening and storage (Sonawane and Shinde 2021). The colonization of unripe, living-host fruit tissue and fruit ripening occurs during the active necrotrophic stage (Alkan et al. 2009). This stage has led to the splashing of the spores of *C. coccodes* on the ripe or overripe fruit. Green fruit could be also infected and symptoms started to develop during tomatoes maturity (Sanoubar and Barbanti 2017). The typical lesions occurring on ripe fruits are black circular with concentric ring, black spores and depressed leading to reduced marketable fruit quality (Opeyemi et al. 2018). The development of these symptoms depends on the cultivation practices during harvest, transport, storage and marketing (Badawy and Rabea 2009) combined with the high levels of humidity and low temperatures. For disease control, chemical treatments in combination with radiation treatments, hot water soaking and refrigeration (Shu et al. 2017) were mainly applied at the postharvest phase due to their important satisfactory effect and their effective control on the prevention of the infection and the dispersal of the fungus (Huang et al. 2020). Unfortunately, the excessive and long-term use of several postharvest chemical fungicides has led to the development of resistant strains (Jaihan et al. 2018). Furthermore, this synthetic chemical fungicide applied to maintain fruit quality has easily created a serious environmental pollution and human health risk because of the difficult degradation and nontarget toxicity (Miftahurrohmat et al. 2021). These environmental concerns and the consumer awareness of the relationship between foods and health have actually contributed to the promotion of the organic tomato culture (Riahi et al. 2009). In this context, several alternative protection strategies including soil solarization, grafting, biofumigation, biological soil disinfestations, application of biocontrol agents and organic amendments were explored (Opeyemi et al. 2018). The biological control approach is a promising approach against plant diseases and more focused on the use of the different microorganisms isolated from various sources (Gu et al. 2020). *Gliocladium virens*, *G. catenulatum* (Cwalina-Ambroziak and Nowak 2012) and *Penicillium* sp. (Rahman et al. 2018) were widely explored by researchers due to their efficiency against various plant diseases (Atalla et al. 2020). Production of cell wall-degrading enzymes, mycoparasitism, competition for nutrients or space antibiotic production and the induction of

defense responses were the main reported mechanisms of action (Jinal and Amaresan 2020).

The aim of the present investigation was to evaluate the in vitro antifungal potential of the cell-free cultural filtrates and the organic extracts of various microbial agents belonging to *Penicillium* sp. and *Gliocladium* spp. against *C. coccodes* and their ability to suppress anthracnose development and severity on tomato fruits.

## Methods

### Fungal material

#### Pathogen isolation and culture

The soil-borne fungal pathogen (*C. coccodes*) used in the present study was isolated from tomato cv. Rio Grande fruits, showing typical anthracnose symptoms. Pathogen identification was carried out in the laboratory of Phytopathology of the Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem (CRRHAB), Tunisia, based on cultural and morphological traits. This pathogen was grown on potato dextrose agar (PDA) medium (Accumix™) amended with streptomycin sulfate (300 mg/l) (w/v) and incubated during 15 days at 25°C in the dark before use (Maatougui and Merzoug 1997).

#### Microbial biocontrol agents: isolation source and growth conditions

Eight isolates of *Penicillium* sp., one isolate of *Gliocladium virens* and one isolate of *G. catenulatum* were isolated from compost and tomato roots as detailed (Table 1). They were identified based on their morphological and cultural traits (Kubicek and Harman 1998) and stored in the culture collection of the laboratory of phytopathology of the Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem (CRRHAB), Tunisia. Their antagonistic potential has been previously proven on many phytopathogens

**Table 1** Origin of the different microbial agents used for tomato anthracnose biocontrol

| Isolate                        | Code | Isolation source |
|--------------------------------|------|------------------|
| <i>Penicillium</i> sp.         | CH5  | Compost          |
|                                | CH6  | Compost          |
|                                | CH7  | Compost          |
|                                | CH9  | Compost          |
|                                | CH11 | Compost          |
|                                | MC1  | Compost          |
|                                | MC3  | Compost          |
|                                | MC4  | Compost          |
| <i>Gliocladium virens</i>      | Gv1  | Tomato roots     |
| <i>Gliocladium catenulatum</i> | Gc1  | Tomato roots     |

(Daami-Remadi et al. 2012). They were cultivated on PDA medium and incubated at 25 °C in the dark during 7 days, as described by Comporta (1985), before being used for further assays and in vivo inoculation.

### Plant material

The experiment was conducted in vivo using the widely cultivated Tunisian tomato cultivar Rio Grande. Tomato fruits were selected according to their maturity, firmness, consistency and the absence of any apparent rot symptoms on their surfaces. They were surface-disinfected with 10% sodium hypochlorite for 5 min, then rinsed with sterile distilled water (SDW) and air-dried before use.

### Assessment of the antifungal potential of the microbial agents tested

#### In vitro potential of cell-free culture filtrates

The different microbial agents were transferred from Petri plates to 200-ml Erlenmeyer flasks containing 100 ml of potato dextrose broth (PDB) medium (Accumix™). These liquid cultures were then incubated at room temperature on a rotary shaker at 150 rpm/min during 15 days (Dennis and Webster 1971). They were centrifuged thrice at 10,000 rpm for 10 min. Supernatant fluids were sterilized by filtration through 0.22-μm millipore size filter (Lepengue et al. 2009). The extracted filtrates were aseptically added to molten PDA medium cooled at 45 °C and supplemented with Streptomycin sulfate (300 mg/l) at the concentrations (10%, v/v) and poured into Petri plates. After solidification of the mixture, three 6-mm-diameter mycelial-agar plugs, removed from *C. coccodes* cultures, were placed equidistantly in each Petri plate. Fungal cultures were incubated at 25 °C for 7 days. The effect of the tested culture filtrates on pathogen growth was estimated based on colony diameter compared to the untreated control. The mycelial growth inhibition rate (%) was calculated using the following formula:

$$I\% = \left[ \frac{(C1 - C2)}{C1} \right] * 100$$

where C1: mean colony diameter in control plates and C2: mean colony diameter in filtrate-amended plates.

The most active cell-free cultural filtrates of the tested biocontrol strains in reducing *C. coccodes* spore germination and mycelial growth were further screened at three concentrations (10, 15 and 20%, v/v) and amended to PDA medium as described above. The solidified agar plates were inoculated by triplicate mycelial agar plugs (5 mm in diameter) and incubated at 25 °C for 7 days. Plates without microbial filtrates served as control. The pathogen colony diameter was measured and the

percentage of radial growth inhibition was calculated as described above.

#### In vitro potential of organic extracts

The liquid–liquid extraction of the antifungal metabolites from the microbial cell-free cultural filtrates was carried out only for those showing an interesting antifungal potential against the target pathogen. Two organic solvents were used, i.e., chloroform and ethyl acetate. Ten milliliters from each filtrate, which prepared as described above, were placed in a separating funnel before adding carefully 10 ml of the extraction solvent (Fu et al. 2011). This mixture was reversed several times with degassing from time to time and left to settle (open cap) in order to reach the equilibrium between the two phases. The organic phases of chloroform (the lower phase for extraction) and ethyl acetate (the upper one) were collected and the aqueous phase was replaced in the funnel. This process was repeated 2 other times by adding 10 ml of solvent for each step. The solvent was evaporated using a rotary evaporator at 90 °C with a slight rotation at 150 rpm (Zain et al. 2011). Each obtained organic phase extract was dissolved into 2 ml of methanol for further antifungal potential analyses against *C. coccodes* (You et al. 2009). This different extract was tested at three concentrations (1, 2.5, and 5% (v/v)) on PDA supplemented with streptomycin sulfate (300 mg/ml) (Smaoui, 2010). After solidification of the mixture, 3 agar plugs of the pathogen (6 mm in diameter) were equidistantly placed in the Petri plate. Fungal colony diameter was measured after 7 days of incubation at 25 °C.

#### Disease suppressive potential of culture filtrates and organic extracts

Cell-free cultural filtrates and organic extracts of the microbial agents tested, which prepared as described above, were applied to disinfected tomato fruits by injecting 100 μl in each wound (diameter 6 mm). Two hours post-treatment, an agar plug colonized by *C. coccodes* was placed within each wound. The positive control was inoculated with the pathogen and treated with a same volume of sterile distilled water only and the negative control was inoculated with un-colonized agar plug and treated similarly using sterile distilled water. All tomato fruits were incubated at 25 °C for 5 days in plastic boxes to maintain a high relative humidity (Mónaco et al. 2009). The diameter of the lesion developing around inoculated wounds was measured.

#### Statistical analysis

All measured parameters were analyzed using the software SPSS 16.0 (Statistical Package for the Social Sciences) using the general linear models (GLM). The

in vitro effect of culture filtrates and organic extract of the microbial agents tested was analyzed according to a completely randomized design with two factors (culture filtrates or organic extracts tested and concentrations used). Three replications were used for each individual treatment and the whole experiment was repeated twice. For all in vivo trials, each individual treatment was replicated 5 times (five fruits) and the whole experiment was repeated twice. The analyses were conducted according to a completely randomized design when the culture filtrates or the organic extracts tested were the only fixed factor. All data were analyzed using a standard analysis of variance (ANOVA) with interactions. Means comparisons were separated using Student–Newman–Keuls (SNK) and/or LSD test (at  $P \leq 0.05$ ).

## Results

### Characteristics of *Colletotrichum coccodes* isolate

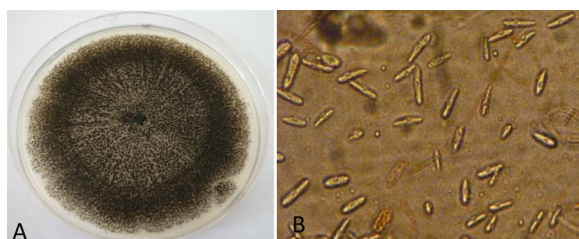
After 7 days of incubation on PDA at 25 °C, *C. coccodes* isolate obtained from tomato fruit with typical anthracnose symptoms produced sparse white to grey aerial mycelium with abundant black, spherical microsclerotia (av. 2594 µm) that are distributed evenly over the agar surface and ordered in concentric rings. Conidia were hyaline, aseptate and cylindrical 16–26 × 3–5 µm, with tapered ends (Fig. 1).

### Antifungal potential of the microbial agents tested

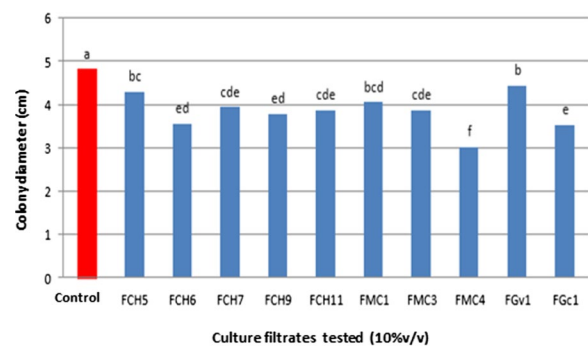
ANOVA analysis revealed significant variation (at  $P \leq 0.05$ ) in the colony diameter depending on the bio-control isolates tested and the concentration applied and this for the cell-free cultural filtrates and organic extracts trials. Also, a significant variation in the fruit lesion dimension effect was detected depending on treatments tested (filtrates or organic extracts).

### Antifungal potential of cell-free culture filtrates of the tested microbial agents

Data given in Figs. 2, 3 showed that cultural filtrates from *Penicillium* sp. (MC4 and CH6 isolates), and *G.*



**Fig. 1** Colony appearance of *Colletotrichum coccodes* isolate grown on potato dextrose agar at 25 °C for 10 days (a) and its cylindrical conidia with round ends (× 400) (b)

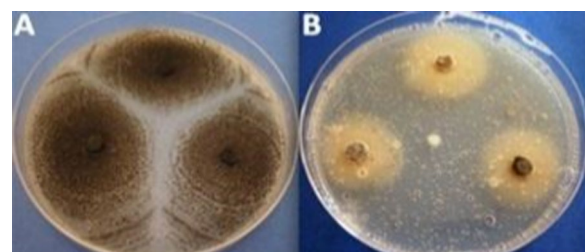


**Fig. 2** Antifungal potential of cell-free cultural filtrates of *Penicillium* sp. and *Gliocladium* spp. tested at 10% v/v against *Colletotrichum coccodes* mycelial growth recorded after 9 days of incubation at 25 °C, compared to the untreated control. FCH5, FCH6, FCH7, FCH9, FCH11, FMC1, FMC3 and FMC4: Culture filtrates from *Penicillium* sp. cultures of CH5, CH6, CH7, CH9, CH11, MC1, MC3 and MC4 isolates; FGv1: Cultural filtrate of *Gliocladium virens* (Gv1 isolate); FGc1: Cultural filtrate of *G. catenulatum* (Gc1 isolate). Bars sharing the same letters are not significantly different according to the Student–Newman–Keuls tests at  $P \leq 0.05$

*catenulatum* (Gc1) applied at 10% had reduced *C. coccodes* mycelial growth by 37, 27 and 26%, respectively, as compared to the untreated control. The remaining isolates exhibited different inhibition rates ranging from 8 to 21% compared to control. Significant reductions in pathogen spore germination and mycelial melanization were mainly noted following treatments with cell-free culture filtrates from *Penicillium* sp. (CH5 and CH6), *G. catenulatum* (Gc1) and *G. virens* (Gv1) isolates.

### Comparative efficiency of cultural filtrates tested depending on concentrations used

Cultural filtrates of four microbial agents (*Penicillium* sp. CH5 and CH6, *G. catenulatum* (Gc1) and *G. virens* (Gv1)) showed a significant inhibitory effect and fixed



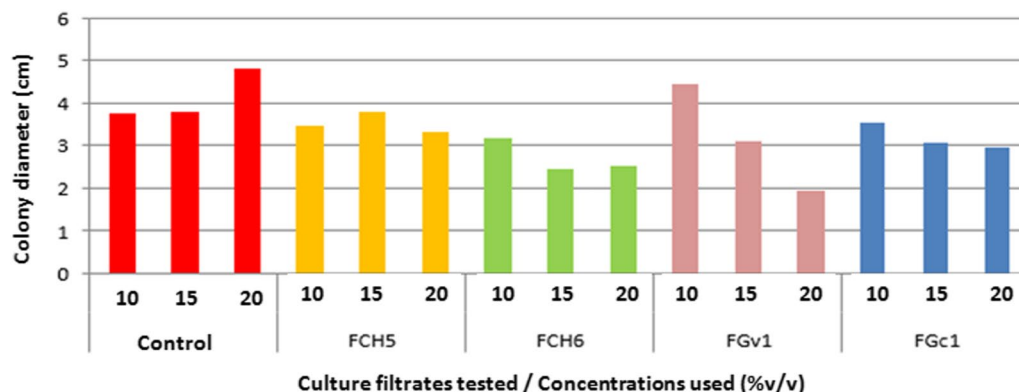
**Fig. 3** Reduced growth and altered mycelial density and melanization observed after 9 days of incubation at 25 °C on *Colletotrichum coccodes* colonies (b) grown on PDA medium supplemented with *Penicillium* sp. cell-free filtrate (CH6) as compared to the untreated controls (a)



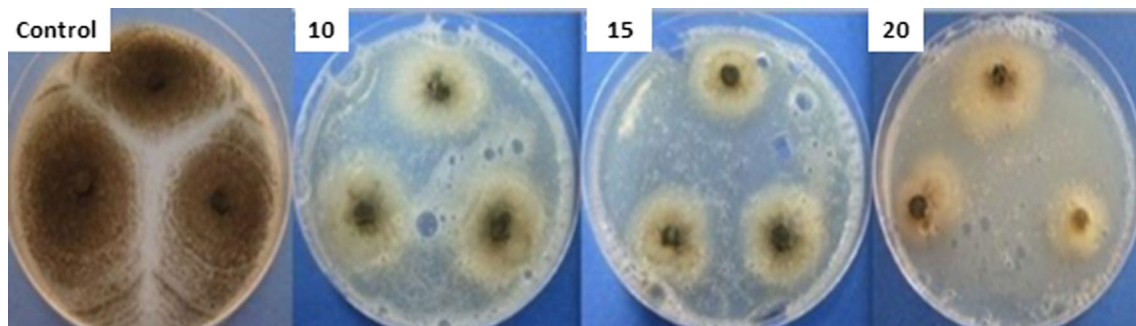
at 25% over control, were tested at different concentrations (10, 15 and 20% v/v). ANOVA analysis showed that the diameter of *C. coccodes* colonies significantly varied (at  $P \leq 0.05$ ) depending on filtrates tested and concentrations used (Fig. 4). In fact, pathogen mycelial growth was inhibited by 7, 8 and 15%, compared to the untreated control, when grown on PDA medium amended with 10% v/v of the cultural filtrates of *G. catenulatum* Gc1, and *Penicillium* sp. CH5 and CH6 isolates, respectively. When applied at 15% v/v, treatments with *Penicillium* sp. CH6, *G. catenulatum* Gc1 and *G. virens* Gv1 cultural filtrates had suppressed the in vitro pathogen growth by 19 to 36% over control, respectively. The highest inhibition rates, ranging from 31 to 60%, were recorded using filtrates at 20% v/v. The increase of concentration of filtrates for the CH5 of *Penicillium* sp. (Fig. 5) and Gv1 of *G. virens* was associated to an important reduction in spore germination and mycelium melanization of *C. coccodes* by 80 to 95% as compared to control.

#### Antifungal potential of ethyl acetate extracts of microbial agents tested

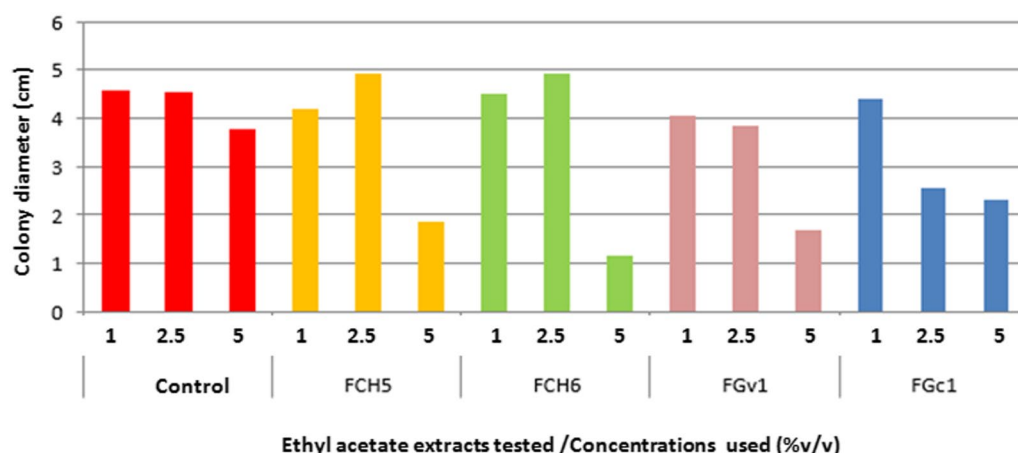
The diameters of *C. coccodes* colonies formed after 9 days incubation at 25 °C, varied significantly depending on the tested extracts, the concentrations used, and their interactions (Fig. 6). Treating at 1% v/v, ethyl acetate extracts of all microbial agents, except that from *Penicillium* sp. CH6, had inhibited pathogen growth by 4 to 12% than the untreated control. However, at the 2.5% v/v, only *G. virens* Gv1 and *G. catenulatum* Gc1 extracts had significantly suppressed pathogen growth by 16 and 44% over control, respectively. More interestingly, the effectiveness of extracts tested applied at 5% v/v had raised to 39–70% for all tested microbial agents with an important alteration of morphological traits on *C. coccodes* colonies (Figs. 7, 8). Significant reductions in mycelial melanization and spore germination were also noted using extracts at 2.5 to 5% v/v leading to 85% and 95% decrease as compared to control, respectively.



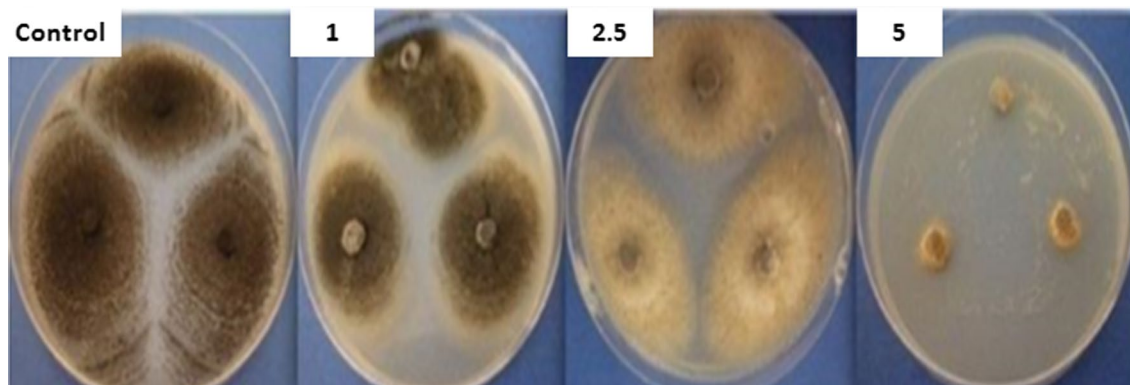
**Fig. 4** Effect of three concentrations of cell-free cultural filtrates of the tested microbial agents against *Colletotrichum coccodes* mycelial growth noted after 9 days of incubation at 25 °C. FCH5 and FCH6: Culture filtrates of *Penicillium* sp. CH5 and CH6 isolates; FGv1: Cultural filtrate of *Gliocladium virens* Gv1; FGc1: Culture filtrate of *G. catenulatum* Gc1. LSD (Cultural filtrates tested × Concentrations used) = 0.45 cm at  $P \leq 0.05$



**Fig. 5** Radial growth inhibition and alteration of morphological traits of *Colletotrichum coccodes* colonies when grown on PDA medium amended with different concentrations (% v/v) of *Penicillium* sp. CH5 cell-free filtrates as compared to the untreated controls



**Fig. 6** Effect of three concentrations of ethyl acetate extracts from the tested microbial agents against *Colletotrichum coccodes* mycelial growth recorded after 9 days incubation at 25 °C. ECH5 and ECH6: Ethyl acetate extracts of *Penicillium* sp. CH5 and CH6 isolates; EGv1: Ethyl acetate extracts of *Gliocladium virens* Gv1; EGc1: Ethyl acetate extracts of *G. catenulatum* Gc1. LSD (Ethyl acetate extracts tested  $\times$  Concentrations used) = 0.28 cm at  $P \leq 0.05$



**Fig. 7** Radial growth inhibition and alteration of morphological traits of *Colletotrichum coccodes* colonies when grown on PDA medium amended with different concentrations (% v/v) of *Penicillium* sp. CH6 ethyl acetate extract as compared to the untreated controls

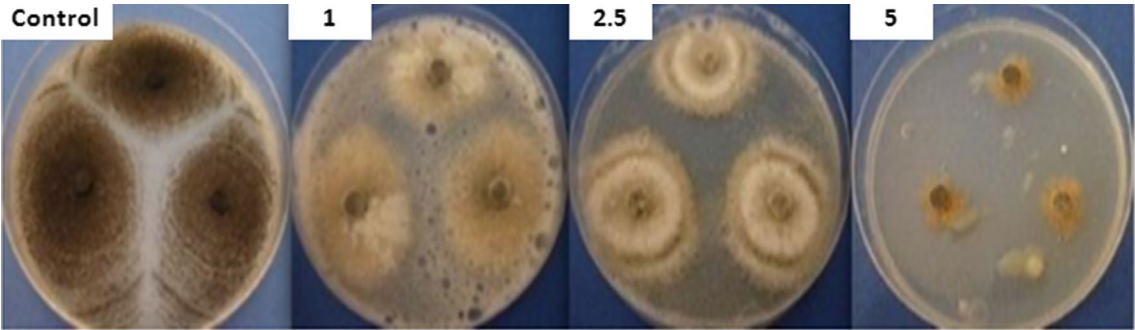
#### Antifungal potential of chloroform extracts of microbial agents tested

ANOVA analysis revealed that the diameter of pathogen colonies significantly varied (at  $P \leq 0.05$ ) depending on chloroform extracts tested, concentrations added and their interactions. In fact, *C. coccodes* colonies grown on PDA medium amended with *Penicillium* sp. (CH5 and CH6 isolates) and *G. virens* Gv1 chloroform extracts, and treated at 1% v/v, showed 8 to 24% decrease in their radial growth as compared to controls. At 2.5% v/v, pathogen growth was inhibited by 24 and 19% using *Penicillium* sp. CH5 and *G. catenulatum* Gc1 chloroform extracts. However, pathogen inhibition rate was raised to 84, 58, 44 and 43% when Gv1 (*G. virens*), Gc1 (*G. catenulatum*), CH6 and CH5 of *Penicillium* sp. chloroform extracts were

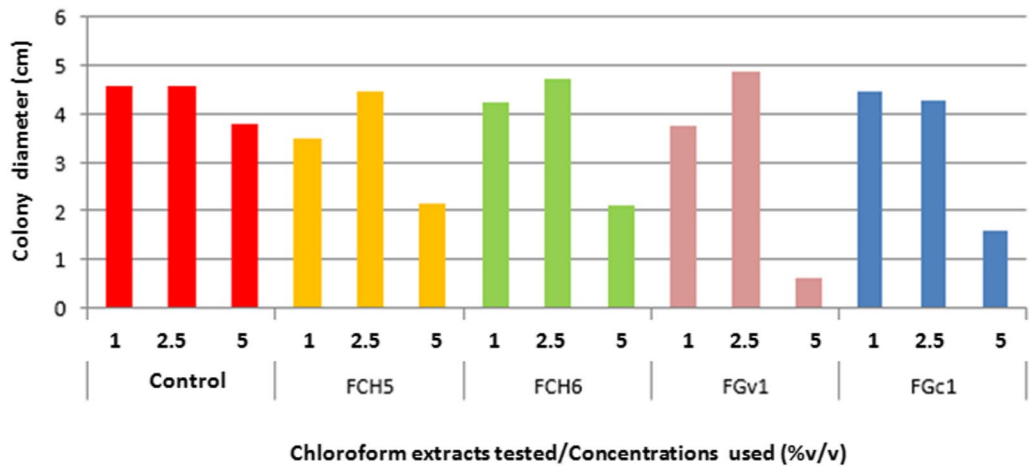
applied at 5% v/v, respectively. This last concentration was highly effective in inhibiting the spore germination by 100% and in altering the morphological traits of *C. coccodes* colonies as compared to control (Figs. 9, 10, 11).

#### Suppression of anthracnose-induced fruit decay using microbial cell-free filtrates

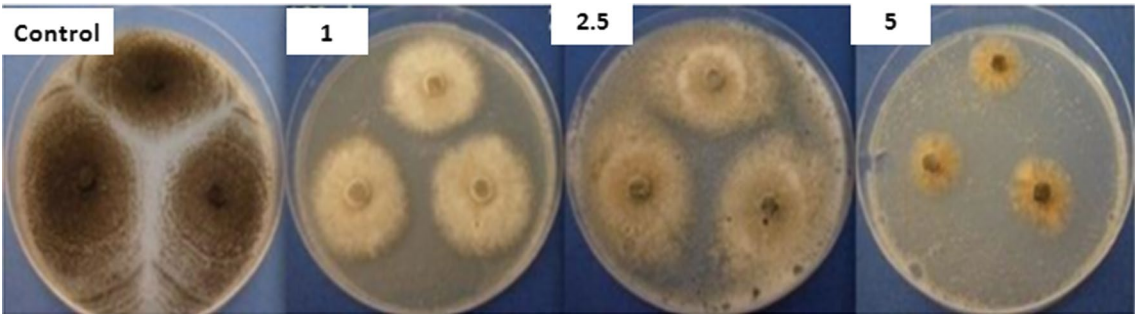
The cell-free cultural filtrates of the microbial agents tested were treated on tomato fruits in order to check their ability to suppress anthracnose decay severity. ANOVA analysis revealed that the external diameter of the rot developed within treated and inoculated wounds significantly varied (at  $P \leq 0.05$ ) depending on treatments tested. Data given in Fig. 12 showed that disease severity was significantly lowered by 22 to 25% versus control



**Fig. 8** Radial growth inhibition and alteration of morphological traits of *Colletotrichum coccodes* colonies when grown on PDA medium amended with different concentrations (% v/v) of *Gliocladium virens* Gv1 ethyl acetate extract as compared to the untreated controls

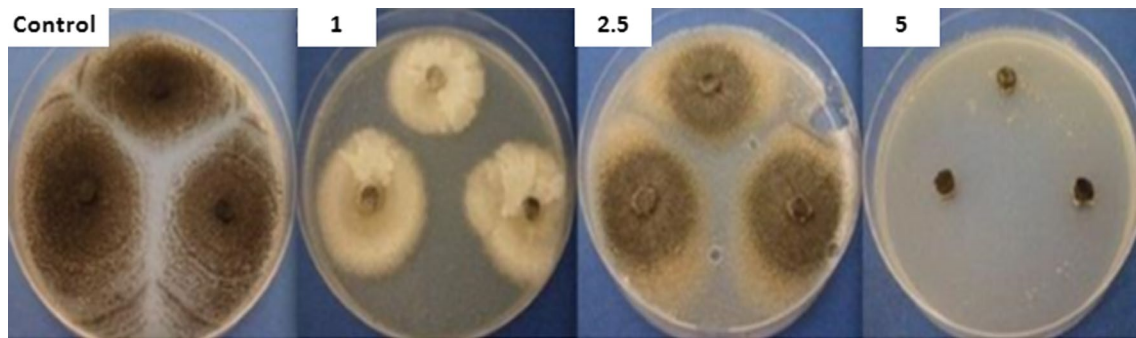


**Fig.9** Effect of three concentrations of chloroform extracts of microbial agents tested against *Colletotrichum coccodes* mycelial growth recorded after 9 days incubation at 25 °C. ECH5 and ECH6: Chloroform extracts of *Penicillium* sp. CH5 and CH6 isolates; EGv1: Chloroform extract of *Gliocladium virens*Gv1; EGc1: Chloroform extract of *G. catenulatum* Gc1. LSD (Chloroform extracts tested × Concentrations used)=0.29 cm at  $P\leq0.05$

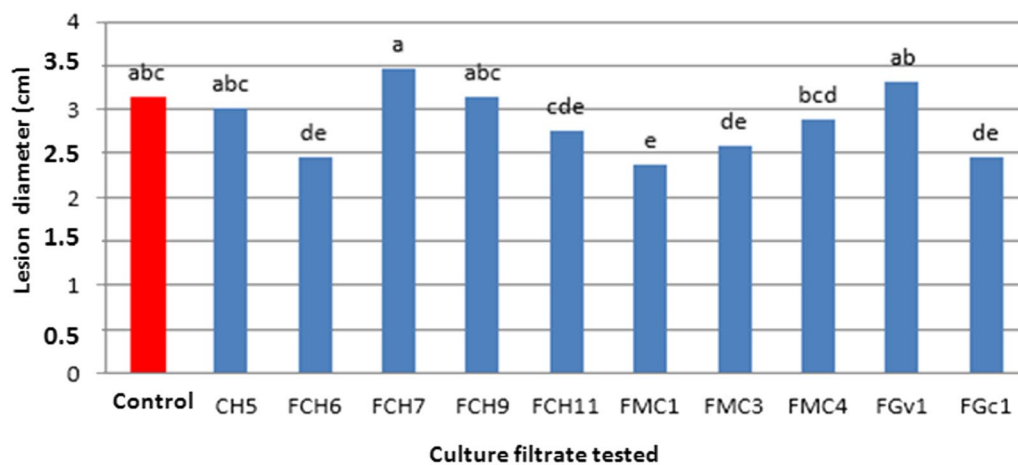


**Fig. 10** Radial growth inhibition and alteration of morphological traits of *Colletotrichum coccodes* colonies when grown on PDA medium amended with different concentrations (% v/v) of *Penicillium* sp. CH5 chloroform extract as compared to the controls

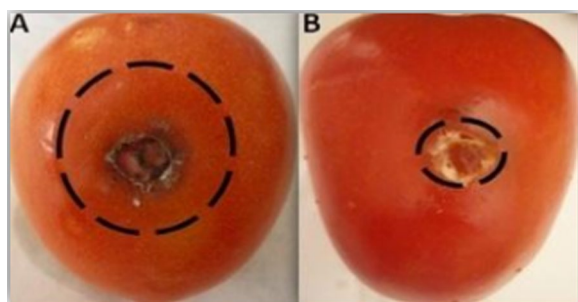
following fruit treatments with cell-free filtrates from *Penicillium* sp. (MC1, CH6) and *G. catenulatum* (Gc1) cultures but the decreases induced by the other tested treatments were not significant as compared to control (Fig. 13).



**Fig. 11** Radial growth inhibition and alteration of morphological traits of *Colletotrichum coccodes* colonies when grown on PDA medium amended with different concentrations (% v/v) *Gliocladium virens* Gv1 chloroform extract as compared to the controls



**Fig. 12** Effect of various microbial cell-free cultural filtrates on anthracnose-induced decay severity on tomato fruits recorded after 7 days of incubation at 25 °C compared to the inoculated and untreated control. FCH5, FCH6, FCH7, FCH9, FCH11, FMC1, FMC3 et FMC4: Culture filtrates of *Penicillium* sp. (isolates CH5, CH6, CH7, CH9, CH11, MC1, MC3 and MC4); FGv1: Culture filtrate of *Gliocladium virens* Gv1; FGc1: Cultural filtrate of *G. catenulatum* Gc1. Bars sharing the same letter are not significantly different according to the test Student–Newman–Keuls at  $P \leq 0.05$

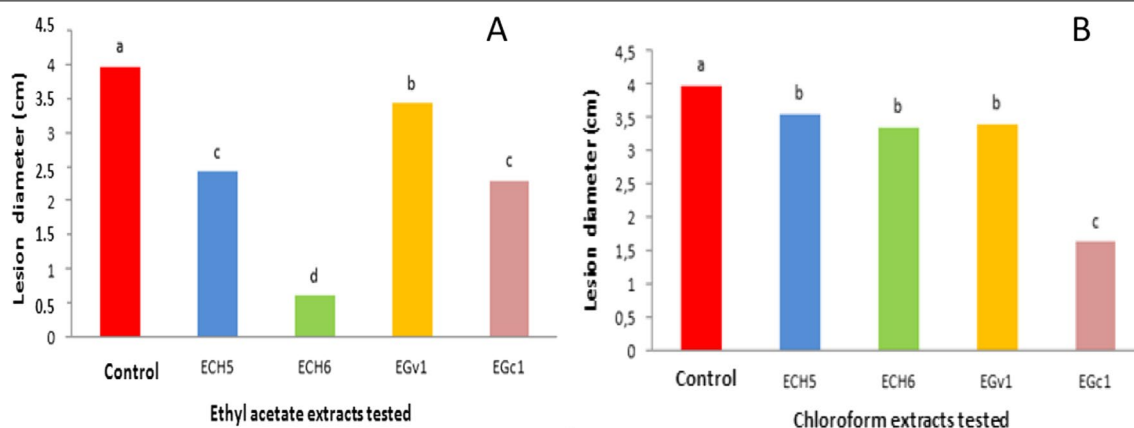


**Fig. 13** Suppression of anthracnose-induced decay using the cultural filtrate of *Penicillium* sp. MC1 on tomato fruits (B) as compared to pathogen-inoculated and untreated control (A) recorded after 7 days of incubation at 25 °C. The black dots are added to delimit the dimensions of the anthracnose-induced decay on tomato fruits

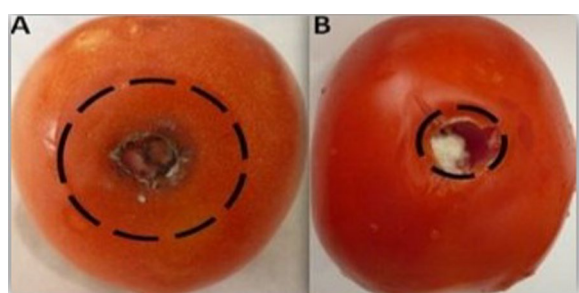
#### Suppression of anthracnose-induced fruit decay using microbial organic extracts

Organic extracts of the four most effective microbial cell-free filtrates were further screened for their capacity to suppress *C. coccodes* development on tomato fruits. The diameter of rot lesion, which induced after 7 days of incubation at 25 °C, varied significantly (at  $P \leq 0.05$ ) depending on extracts tested. In fact, anthracnose-induced decay severity was significantly reduced by 14 to 85% using ethyl acetate extracts from *Penicillium* sp. CH5 and CH6 isolates, *G. catenulatum* Gc1, and *G. virens* Gv1 (Fig. 14a, 15). However, the inhibition rates which were recorded following treatments with chloroform extracts ranged between 11 and 59% (Figs. 14b, 16).

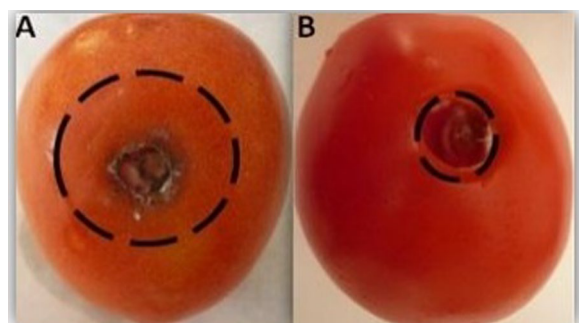




**Fig. 14** Effect of the ethyl acetate (a) and chloroform (b) extracts of the microbial agents tested on the severity of anthracnose-induced decay on tomato fruits noted 7 days of incubation at 25 °C as compared to pathogen-inoculated and untreated control. ECH5 and ECH6: Ethyl acetate and chloroform extracts of *Penicillium* sp. isolates CH5 and CH6; EGv1: Ethyl acetate and chloroform extract of *Gliocladium virens* Gv1; EGc1: Ethyl acetate and chloroform extract of *G. catenulatum* Gc1. Bars sharing the same letter are not significantly different according to the Student–Newman–Keuls test at  $P \leq 0.05$



**Fig. 15** Suppression of anthracnose-induced decay using the ethyl acetate extract of *Gliocladium catenulatum* Gc1 on tomato fruits (b) as compared to pathogen-inoculated and untreated control (a) recorded after 7 days of incubation at 25 °C. The black dots are added to delimit the dimensions of anthracnose-induced decay on tomato fruits



**Fig. 16** Suppression of anthracnose-induced decay using the chloroform extract of *Gliocladium catenulatum* Gc1 on tomato fruits (b) as compared to pathogen-inoculated and untreated control (a) recorded after 7 days of incubation at 25 °C. The black dots are added to delimit the dimensions of anthracnose on tomato fruits

## Discussion

Inhibitory effect of biocontrol agents tested was observed using the cultural filtrate and the different organic extracts. Similar trend was recorded with the cultural filtrate from *T. harzianum* against *C. coccodes* growth (Maurya et al. 2008). Bridžiuvienė and Repečkienė (2009) also demonstrated the antifungal potential of *T. virens* and *Penicillium* sp. cultural filtrates toward various *Colletotrichum* species. The cell-wall-degrading enzymes (CWDEs), such as chitinase and  $\beta$ -1,3-glucanase, released in the cell-free cultural filtrates were responsible of the inhibition of *C. gloeosporioides* (Ruangwong et al. 2021). The cultural filtrate of *T. viride* and *Pseudomonas fluorescens* were very effective in inhibiting the mycelial growth of *C. capsici*, the causal agent of anthracnose on chili (Sonawane and Shinde 2021). Okhovvat (1997) also highlighted the antifungal potential of *T. viride* and *T. harzianum* cultural filtrates against *C. coccodes* growth which ranged between 18 and 55% when used at 3.5 to 5% v/v, respectively. Also, some bacterial cell-free culture filtrates such as those of *Bacillus amylolique faciens* displayed antifungal activity against several phytopathogenic fungi including *C. dematium* where this effect was more attributed to antibiotics released in these filtrates (Yoshida et al. 2002). Similarly, the application of the cultural filtrate of the novel endophytic bacterium, *Stenotrophomonas maltophilia* at 1 ml has suppressed *C. nymphaeae* mycelial growth (Alijani et al. 2020). Likewise, the sterile cultural filtrate of *B. subtilis* has shown an important inhibitory effect toward various fungal phytopathogens including *C. gloeosporioides* (Huang et al. 2020). Moreover, these findings of the present study are in agreement with Shu et al. (2017) results where similar inhibitory effects of *Streptomyces katrae*

cell-free culture filtrates were recorded against *C. musae* conidial germination. The treatment of the cultural filtrate of the entomopathogenic fungus (genus *Cordyceps*) has also reduced under in vitro conditions the conidial germination of all *Colletotrichum* spp. isolates tested (Jaihan et al. 2016). The hyphal destruction and the inhibition of conidial germination were induced by the cultural filtrate of *Streptomyces deccanensis* on *C. gloeosporioides* (Gu et al. 2020). A positive correlation between the levels of the culture filtrate efficacy and the increase in their concentrations was recorded. Therefore, a similar variations in the inhibitory effects depending on concentrations were reported in the case of *B. licheniformis* filtrates against *C. gloeosporioides* and treated at different dilutions (10, 100, 200, 400, 800 and 1000 times) when the highest antifungal potential was noted at the dilution 200 (He et al. 2009). Moreover, *Serratia marcescens* cultural filtrates used at 40 and 50% v/v had completely limited *C. lindemuthianum* mycelial growth, mycelial dry weight and conidial germination (Papitha et al. 2020). Similarly, *T. viride*, *T. harzianum*, *T. reesei* and *T. saturnisporum* filtrates, which were used at 0, 5, 10, 25 and 50% v/v, had suppressed *C. capsici* growth by 21 to 68% over control when the highest antifungal potential was recorded at the concentration 50% v/v (Ajith and Lakshmidhevi 2010). An interesting efficiency was also noted using the cultural filtrates of 19 isolates of *T. asperellum* and one isolate of *T. harzianum* at 10 and 25% v/v against *C. graminicola* mycelial growth. This highest growth inhibiting effect, which estimated at 68.18 and 80.68% over control, was noted using *T. asperellum* at 10 and 25% v/v, respectively (Manzar and Singh 2020). However, Padder and Sharma (2011) study as a significant decrease in *C. lindemuthianum* spore germination was noted using *T. harzianum*, *T. viride*, *G. virens* and *T. hamatum* cultural filtrates at 25 and 50% v/v. The serial dilutions of *T. viride* at 5, 10, 15 and 20% v/v had also inhibited *C. gloeosporioides* mycelial growth and spore germination with the highest inhibitory effect, by 90.90% over control, was noted at 20% v/v (Yasmin and Shamsi 2019). Similar inhibitory effect was obtained with the different organic extracts of the bio-control agents tested on the present study with the serial dilutions. Similar findings were noted by Pornsuriya et al. (2010) using ethyl acetate, n-hexanoic and methanolic extracts of *G. catenulatum* and *T. harzianum* isolates for the control of *Pythium aphanidermatum*, the causal agent of pine apple root rot. These authors also reported the effect of ethyl acetate extracts from isolates of *G. virens* and *Penicillium* spp. on *P. aphanidermatum* at 0, 10, 50, 100, 500 and 1000 µg/ml.

However, several previous studies reported the effectiveness of microbial culture filtrates against postharvest pathogens as was the case of Palaniyandi et al. (2011)

study who found that *Streptomyces* filtrates had reduced anthracnose incidence and severity under controlled conditions by 85–88% over control. Nevertheless, some filtrates have reduced anthracnose severity and pathogen sporulation at the inoculation site as Bosah et al. (2010) whom showed the ability of *Penicillium* sp. isolates to reduce *Sclerotium* sp. mycelial development through the production of antibiotics. This reduction of fruit infection levels was also recorded using cell-free filtrates from *T. viridae*, *T. pseudokoningii* and *T. harzianum* cultures against *C. capsici* development (Rahman et al. 2018). In addition, the application of *T. harzianum* culture filtrate under greenhouse conditions has reduced greatly disease severity associated to *C. sublineolum* infection and has also concomitantly promoted sorghum growth (Saber et al. 2017). Recently, Ruangwong et al. (2021) demonstrated the effectiveness of *T. koningiopsis* culture filtrate in controlling *C. gloeosporioides* on chili pepper and recorded no disease symptoms following treatments. Similar decreases in disease lesion and severity on chili pepper (*C. gloeosporioides*) were also observed by Jaihan et al. (2016) using the culture filtrate of the entomopathogenic fungus *Ophiocordyceps sobolifera*. Similar disease suppressive effects have been obtained by Ara et al. (2012) using 50 µl of methanolic extracts of the actinomycete bacterium (*Streptomyces* sp.) for the control of *C. musae* on banana fruits. The n-butanol extracted of the chitinolytic bacterium *Paenibacillus ehimensis* has also completely inhibited the spore germination of the phytopathogen *C. gloeosporioides* on pepper fruits (Anees et al. 2019). Similarly, the ethanol extracts of *O. sobolifera* applied at 50% (v/v) on chili pepper have also reduced anthracnose severity and symptom appearance associated to *C. capsici* and *C. gloeosporioides* infections under pot conditions (Jaihan et al. 2018). The ethyl acetate extract of the endophytic fungus *Epicoccum dendrobii* has eventually suppressed *C. gloeosporioides* development on detached leaves of Chinese fir (Bian et al. 2021).

## Conclusions

The present study demonstrated the antifungal activity of cell-free cultural filtrates and organic extracts of microbial agents (*Penicillium* sp. and *Gliocladium* spp.) tested against the in vitro and the in vivo growth of *C. coccodes*. The most effective bio-agents in controlling *C. coccodes* pathogen mycelial growth and anthracnose severity on tomato fruits were CH6 of *Penicillium* sp. and Gc1 of *G. catenulatum*. Further experiments are needed for the identification of the nature of chemical compounds involved in the recorded antifungal activity by the high-performance liquid chromatography (HPLCMAS) for the corresponding solvent extract.

## Abbreviations

*C. coccodes*: *Colletotrichum coccodes*; *C. capsici*: *Colletotrichum capsici*; *C. dematium*: *Colletotrichum dematium*; *C. nymphaeae*: *Colletotrichum nymphaeae*; *C. graminicola*: *Colletotrichum graminicola*; *C. lindemuthianum*: *Colletotrichum lindemuthianum*; *C. acutatum*: *Colletotrichum acutatum*; *C. sublineolum*: *Colletotrichum sublineolum*; *C. musae*: *Colletotrichum musae*; *C. lindemuthianum*: *Colletotrichum lindemuthianum*; *C. gloeosporioides*: *Colletotrichum gloeosporioides*; *G. catenulatum*: *Gliocladium catenulatum*; *G. virens*: *Gliocladium virens*; *PDA*: Potato dextrose agar; *PDB*: Potato dextrose broth; *T. harzianum*: *Trichoderma harzianum*; *T. virens*: *Trichoderma virens*; *T. viride*: *Trichoderma viride*; *T. reesei*: *Trichoderma reesei*; *T. saturnisporum*: *Trichoderma saturnisporum*; *T. hamatum*: *Trichoderma hamatum*; *T. atroviride*: *Trichoderma atroviride*; *T. asperellum*: *Trichoderma asperellum*; *T. pseudokon*: *ingii*: *Trichoderma pseudokoningii*; *T. koningiopsis*: *Trichoderma koningiopsis*; *B. subtilis*: *Bacillus subtilis*; *B. licheniformis*: *Bacillus licheniformis*; *O. sobolifera*: *Ophiocordyceps sobolifera*.

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## Authors' contributions

The concept and design of the experiments were prepared by all authors. MH carried out the experiment, collected the data, performed the analysis and wrote the paper. RAB and HJK coordinated the laboratory works and helped in the writing paper. MDR supervised the results analysis and corrected the manuscript draft. All authors have read and approved the manuscript.

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## Availability of data and materials

All data generated and/or analyzed during the present study are available in the manuscript, and the corresponding author has no objection to the availability of data and materials.

## Declarations

### Ethics Approval and Consent to participate

The study was conducted on plant-pathogen fungus and beneficial Fungus that are abundant in the environment and does not require ethical approval.

### Consent for publication

Not Applicable.

### Competing interests

All authors declare that they have no conflict of interest.

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