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Biocontrol of Gray Mold in Tomato Fruits by *Trichoderma* sp.

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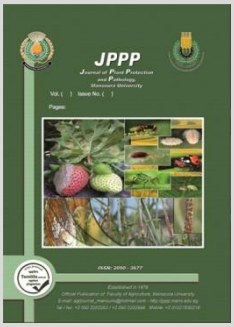

ABSTRACT

Gray mold is a serious rot in postharvest tomato fruits caused by the fungal pathogen *Botrytis cinerea*. *Botrytis* is the most prevalent postharvest fungi that cause major losses in fresh fruits, vegetables, and ornamentals as well as can infect more than 500 plant species. This investigation was designed to evaluate the effect of *Trichoderma* culture filtrate on the fungal pathogen in vitro and in vivo conditions. The culture filtrate significantly reduced *B. cinerea* growth by 94.6, and 73.7% at concentrations of 70, and 60%, respectively. Moreover, the culture filtrate of *T. reesei* at 70% concentration caused an enormous reduction in incidence and severity of the disease by 80.5, and 90.5% in comparison with the untreated group respectively. The secondary metabolites of several species of *Trichoderma* had great antifungal activity on the pathogen *B. cinerea*, thereby might be a promising and eco-friendly strategy for controlling gray mold in tomatoes and various postharvest fruits and vegetables.

Keywords: Postharvest diseases, Biocontrol, *Trichoderma*, Culture filtrate, Tomato

INTRODUCTION

The pathogen *Botrytis cinerea* is a necrotrophic fungal phytopathogen (Parnell *et al.* 2016). This fungus is responsible for grey mold in a very wide range of plant hosts, with approximately 500 plant species worldwide (Williamson *et al.* 2007; Nicot *et al.* 2016). This fungal pathogen can cause economic losses of 20-30% in the production of tomatoes, and up to 50-60% in the case of severe disease (Dean *et al.* 2012).

Applications of synthetic fungicides are the major strategy for the control of gray mold (Lu *et al.* 2016), but the extreme use of the fungicides causes the emergence of new resistant strains of fungi (Elsherbiny *et al.* 2017). Therefore, other alternative technologies to control gray mold in tomato fruits are required with their unique advantages including non-toxic, cost-effective, eco-friendly, and safe for human health such as plant extracts, essential oils, chemical additives, physical treatments, nanomaterials, and biocontrol agents (Sabzevari and Hofman 2022). Among different biological approaches, the use of fungal endophytes and their bioactive metabolites is a promising biocontrol tool against postharvest fungal pathogens (Peng *et al.* 2021). With this information, the objective of this study was to study the potential role of the culture filtrate of *Trichoderma* sp. as a biocontrol tool on the pathogen *B. cinerea* in tomato fruits.

MATERIALS AND METHODS

1. Pathogen

The fungus *Botrytis cinerea* was isolated from diseased tomato fruits with the typical symptoms of gray mold. The fungal growth was put over the surface of potato dextrose agar (PDA) and incubated at 22 °C. The identification of *B. cinerea* was confirmed based on the unique characteristics of cultures, and conidia according to Aktaruzzaman *et al.* (2017).

2. Biocontrol agent

The filamentous fungus *Trichoderma reesei* was isolated from the leaf and stem of various medicinal plants. The samples were washed in tap water. The leaf and stem were cut into small pieces (0.5 cm), and the surfaces were disinfected with NaOCl (1%) for 5 min. The samples were rinsed in sterile distilled water and dried. The samples were placed on PDA amended with streptomycin sulfate and incubated at 25 °C. The emergent colonies were identified based on culture morphology and spores (Rajani *et al.* 2021).

3. Preparation of culture filtrate

Five mm in diameter of *T. reesei* disks from active cultures were transferred into 200 mL of potato dextrose broth (PDB). At 25 °C in the dark, flasks were incubated for 20 d. The cultures were filtered, and the filtrate was centrifuged at 12000 × *g* for 20 min. The resulting filtrate was passed through GVWP membranes with a pore size of 0.22 µm.

4. Effect on *Botrytis cinerea* growth

Five-mm mycelial disks of the pathogen were placed on the center of 90 mm PDA plates mixed with the culture filtrate of the biocontrol agent to prepare 0, 10, 20, 30, 40, 50, 60, and 70% concentrations. Three replicates were applied for each concentration. After 6 d at 25 °C, the diameter of *B. cinerea* growth was measured, and the inhibition was calculated as follows: Growth inhibition (%) = [(control growth - treatment growth) / control growth] × 100.

5. Effect of culture filtrate on gray mold in tomato fruit

Tomato fruits were washed under running water, sterilized in sodium hypochlorite (2% NaOCl) for 2 min, and then washed with sterile distilled water. The fruits were wounded (3 mm deep and 3 mm wide), and 20 µL of culture filtrate at 0, 40, 50, 60, and 70% concentrations were placed into each wound. After 2 h, the mycelial disks (5 mm diameter) of *B. cinerea* were added to the wounds. The treated fruits were set in sterilized plastic boxes in a humidified

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incubator at 25 °C for 6 d with 95-100 % relative humidity (RH). The assay was conducted twice, and three replicates were performed for each concentration, with 12 tomato fruits per replicate. The disease incidence was calculated based on the formula: Disease incidence (%) = [(rotten wounds / total wounds)] × 100. Disease severity (%) = [(lesion diameter of treatment / lesion diameter of control)] × 100.

6. Statistical analysis

The statistical analyses were conducted using SAS (version 9.1, USA) and subjected to a one-way analysis of variance (ANOVA). Tukey's test at $P < 0.05$ was applied to determine the significant differences between treatments.

RESULTS AND DISCUSSION

1. Antifungal activity

The growth of the pathogen *B. cinerea* was significantly ($P < 0.05$) inhibited by the *T. reesei* filtrate in a concentration-dependent manner (Fig. 1). The concentration of 70% caused a great decrease in *B. cinerea* growth by 94.6%. Also, 60% concentration of the filtrate inhibited the colony growth by 73.7%. By contrast, there is no significant difference ($P < 0.05$) between the concentrations of 10, 20, and 30%.

Several studies have evaluated the bioactivity of filtrates and metabolites of various species of *Trichoderma* against postharvest pathogens. For instance, the culture filtrate of *T. piluliferum* significantly inhibited *Colletotrichum musae* growth by 20% (Costa *et al.* 2021). Also, *T. asperellum* LQC96 was able to produce soluble compounds in culture media. These compounds highly decreased the colony diameter of *B. cinerea* in relation to the control group (Fujinawa *et al.* 2020). The culture filtrate of *T. harzianum* caused a considerable reduction in the mycelial growth of *A. alternata*, *A. brassicae*, and *A. solani* by 62.50, 60.00, and 48.28%, respectively, while *T. viride* filtrates recorded great inhibition against *A. alternata* (60.00%), *A. solani* (56.90%), *Fusarium solani* (56.90%), and *A. brassicae* (52.73%) (Meena *et al.* 2017).

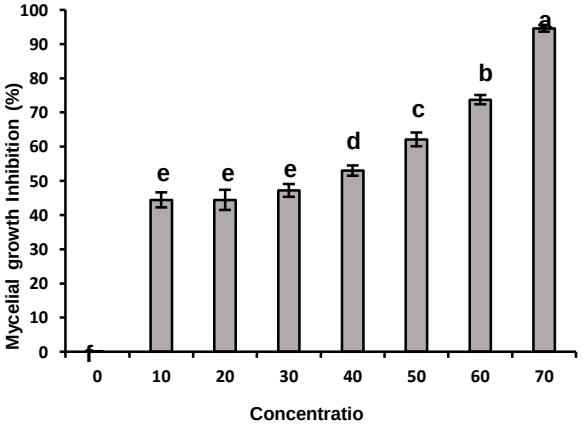

Fig. 1. Mycelial growth inhibition of *Botrytis cinerea* by culture filtrate of *Trichoderma reesei* at different concentrations. Results are expressed as average values ± standard error. The same letters mean insignificant differences between values at $P < 0.05$ with Tukey's test.

2. Tomato fruits assay

The disease incidence of the disease on tomato fruits was significantly ($P < 0.05$) inhibited by *T. reesei* filtrate (Fig. 2A). In particular, the disease incidence was dramatically decreased by 80.5% compared to the control ($P < 0.05$) when using the concentration of 70%. Moreover, the concentration of 70% showed an immense reduction in the disease severity of grey mold on tomato fruits by 90.5% in comparison with the control group (Fig. 2B). Likewise, the grey mold severity was reduced by 74.9% on tomato fruits inoculated with *B. cinerea* when applied 60% concentration of culture filtrate.

These results agree with those found by Batta (2007) who reported that the treatments by *T. harzianum* were significantly reduced ($P \leq 0.05$) the lesion diameters of *B. cinerea* on wounded fruit of the strawberry, grape, kiwi, and pear fruit. In a similar study, *T. viride* and *T. longibrachiatum* caused a remarkable reduction in lesion diameter on papaya fruit inoculated with *Colletotrichum gloeosporioides*, the causal agent of anthracnose (Valenzuela *et al.* 2015). In this context, the application of *T. piluliferum* filtrate on banana fruits decreased the anthracnose decay caused by *C. musae* compared with the fungicide Imazalil (Costa et al. 2021).

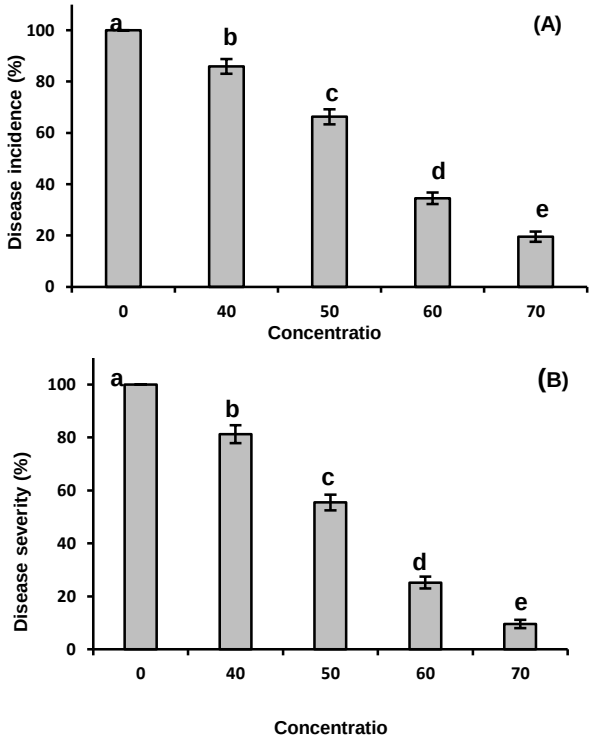

Fig. 2. Disease incidence of gray mold (A), and disease severity (B) in tomato fruits treated with culture filtrate of *Trichoderma reesei* and then inoculated with *Botrytis cinerea*. Results are expressed as average values ± standard error. The same letters mean insignificant differences between values at $P < 0.05$ with Tukey's test.

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المكافحة الحيوية للعفن الرمادي في ثمار الطماطم باستخدام *Trichoderma*

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العفن الرمادي المتسبب عن فطر *Botrytis cinerea* هو مرض خطير في ثمار الطماطم بعد الحصاد. كان الهدف من هذا البحث هو دراسة الفعالية المضادة للفطريات لراشح مزارع فطر *Trichoderma* على المسبب المرضى فى المعمل وعلى الثمار. وقد أدى راشح المزارع إلى تثبيط نمو فطر *B. cinerea* بنسبة 94.6 و73.7٪ وذلك عند استخدام بتركيزات 70 و60٪ على التوالي. علاوة على ذلك، أدى راشح مزارع فطر *T. reesei* بتركيز 70٪ إلى انخفاض هائل في كل من الإصابة بالمرض وشدة المرض بنسبة 80.5 و90.5٪ على التوالي. وأشارت النتائج إلى أن راشح مزارع أنواع فطر *Trichoderma* كان له نشاطاً مضاداً على المسبب المرضى *B. cinerea*، وبالتالي قد يكون استخدامه بمثابة استراتيجية واعدة لمكافحة العفن الرمادي في الطماطم وفي مختلف أنواع الفواكه والخضروات فى مرحلة ما بعد الحصاد.