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Biological Control of Chili Anthracnose Disease using *Talaromyces flavus* Bodhi001

Thanaprasong Oiuphisittraiwat[1], Tida Dethoup[2], Arom Jantasorn[1]

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ABSTRACT

Background: Anthracnose disease of chili caused by *Colletotrichum* spp is one of the most destructive diseases affecting chili fruits in Thailand and significantly reduces fruit quality and chili production. Currently, this disease is managed primarily with synthetic fungicides that may affect public health and the environment adversely. Consequently, there is a need for biological management options. *In vitro* and *in vivo* evaluations of the antagonistic activity of *Talaromyces flavus* Bodhi001, *Talaromyces trachyspermus* Bodhi002, *Talaromyces flavus* Bodhi003 and *Neosartorya fischeri* Bodhi004 against *Colletotrichum capsici*, the causal agent of chili anthracnose disease, were conducted in the present study.

Methods: The activity of antagonistic fungi against *C. capsici* was determined using PDA plate by dual culture method. The spore suspensions of *C. capsici* and antagonistic fungi were prepared in sterile water and adjusted using a hemocytometer to achieve a final concentration of about 10^6 spores mL^{-1}.

Result: The most effective antagonistic strains were *T. flavus* Bodhi001 and *N. fischeri* Bodhi004, which inhibited the mycelial growth of *C. capsici* by 68.99% and 70.76%, respectively. Interestingly, the antagonistic *T. flavus* Bodhi001 strain was the most effective at reducing the severity of chili anthracnose *in vivo* by up to 80%. The biological control activity of *T. flavus* Bodhi001 was to produce antibiosis against *C. capsici*; therefore, testing can be recommended to confirm its field trial stability. The results indicate that the application of the antagonistic fungi *T. flavus* Bodhi001 may be quite effective in biological control of chili anthracnose.

Key words: Antagonistic activity, Biological control, Chili anthracnose disease, *Talaromyces flavus* bodhi001.

INTRODUCTION

Plant-pathogenic fungi species of *Colletotrichum* that cause anthracnose disease can limit both the quality and quantity of harvest yield losses on numerous tropical crops worldwide, such as bananas, (Zakaria *et al.,* 2009), tomatoes (Diao *et al.,* 2014), mangoes (Lu *et al.,* 2017; Li *et al.,* 2020) and chili peppers (Oo and Oh, 2019). In tropical and subtropical regions, including Thailand and Asia, chili (*Capsicum annuum* L.), a member of the Solanaceae family, is a vital crop (Ratanacherdchai *et al.,* 2007; Than *et al.,* 2008). During the growth phase of chili production, numerous plant diseases, including fungi, bacteria, viruses and nematodes, attack the crop. Anthracnose, which is caused by a fungus pathogen, is the most serious disease threat to chili production (Sharma *et al.,* 2005). The chili anthracnose disease in Thailand is mainly caused by *Colletotrichum gloeosporiodes* (Penz.) Sacc., *Colletotrichum capsici* (H.Syd.) E. Butl. and Bisby and *Colletotrichum acutatum*, which can cause severe losses at various stages of chili production, ranging from blossom stage to postharvest, causing 10-80% yield loss globally (Poonpolgul and Kumphai, 2007; Than *et al.,* 2008; Kommula *et al.,* 2017). Tiny black spots are commonly observed on the leaves, stems and both young and mature fruits as disease symptoms (Kim *et al.,* 1999). In general, the disease outbreak occurs during the rainy season or a long rainy period and it has reduced chili yields at both the young and mature fruit stages during field trials. In addition, farmers primarily apply synthetic fungicides as curative and preventative measures against chili anthracnose. Despite the effectiveness of synthetic fungicides against the anthracnose pathogen, their repeated and ongoing use raises concerns not only for their impact on human health, consumers and the environment, but also for the pathogen resistance that may result (Saxena *et al.,* 2016; Hawkins and Fraaije, 2018; Kongcharoen *et al.,* 2020). Consequently, the use of biological control agents (BCAs) and plant extracts is safe and eco-friendly methods of controlling plant pathogens have replaced the use of systemic fungicides (Jantasorn *et al.,* 2016a; Komhorm *et al.,* 2021).

[1]Innovative Agriculture Major, Bodhivijjalaya College, Srinakharinwirot University, Ongkharak, Nakhon-Nayok 26120, Thailand.
[2]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

Corresponding Author: Arom Jantasorn, Innovative Agriculture Major, Bodhivijjalaya College, Srinakharinwirot University, Ongkharak, Nakhon-Nayok 26120, Thailand.
Email: aromj@g.swu.ac.th

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Currently, biological management strategies for plant disease control are highly recommended for disease management. Several BCAs and plant extracts have been developed as commercial biocontrol agent products to manage plant diseases, including *Trichoderma* spp., which are well-known as effective biocontrol agents for many economic crop diseases (Swain *et al.,* 2018) and *T. asperellum*, which exhibits strong potential to control rice diseases (Charoenrak and Chamswang, 2015). However, *Trichoderma harzianum* has been used to control *Colletotrichum capsici*, the fungus that causes chili pepper anthracnose (Ekefan *et al.,* 2009). Interestingly, Imtiaj and Lee (2007) reported that *Cordyceps sobolifera*, an entomopathogenic fungus, exhibits potent antagonistic activity against *C. gloeosporiodes* and *C. miyabeanus*. Similarly, *Ophiocordyceps sobolifera* is a potent antagonist against anthracnose disease caused by *Colletotrichum* spp. (Jaihan *et al.,* 2016). In addition, *Talaromyces* species have demonstrated potential antagonistic activity against plant pathogens in numerous crops via mycoparasitism, antibiosis and space and nutrient competition. *Talaromyces* spp. have recently been identified as a potential antagonist against crop diseases worldwide, such as rice disease (Dethoup *et al.,* 2018), vascular wilt diseases in potatoes and tomatoes (Naraghi *et al.,* 2012; Bahramian *et al.,* 2016) and mango fruit rot (Suasa-ard *et al.,* 2019). In our previous study, we investigated biological approaches to plant disease control (Jantasorn *et al.,* 2016b; Jantasorn *et al.,* 2016c; Jantasorn *et al.,* 2017) and found that *Talaromyces flavus* Bodhi001 suppressed the development of black spot disease and reduced disease incidence by up to 32.56% under greenhouse conditions (Komhorm *et al.,* 2021). Therefore, the objectives of this study were as follows: (a) to evaluate the antagonistic activity of *Talaromyces flavus* (Klöcker) Stolk and Samson Bodhi001, *Talaromyces trachyspermus* (Shear) Stolk and Samson Bodhi002, *Talaromyces flavus* (Klöcker) Stolk and Samson Bodhi003 and *Neosartorya fischeri* (Wehmer) Malloch and Cain Bodhi004 against chili anthracnose disease *in vitro* and (b) to test their potential use against *Colletotrichum capsici in vivo*.

MATERIALS AND METHODS

Isolation of antagonistic fungi

The antagonistic fungi were isolated from soils collected from a riparian forest at College of Creative Agriculture for Society, Srinakharinwirot University Sakaeo Campus, Thailand, using the serial dilution and spread plate method on warm glucose ammonium nitrate agar containing 0.05% streptomycin sulfate, as described previously by Jantasorn *et al.* (2016b). The medium-grown mycelium was subcultured on potato dextrose agar (PDA), incubated at room temperature (28±3°C) and maintained on slant PDA at 4°C for further strain identification. The identification of antagonistic fungi was based on a molecular technique utilizing internal transcribed spacer (ITS) regions (Omid *et al.*, 2017) and a morphology characterization technique.

Fungal pathogen

Colletotrichum capsici, the chili anthracnose pathogen examined in this study, was isolated from chili fruits exhibiting anthracnose symptoms as black spots using the tissue transplanting technique described by Yadav *et al.* (2021). After 7 days of the incubation period, the newly growing mycelium was subcultured on PDA medium at 28±3°C. Under microscopic observation, the pathogen was identified based on conidial morphological and cultural characteristics. The chili fruits were tested for pathogenicity using Koch's postulates prior to their use in this study.

Spore suspension preparations

Colletotrichum capsici and antagonistic fungi, *T. flavus* Bodhi001, *T. trachyspermus* Bodhi002, *T. flavus* Bodhi003 and *N. fischeri* Bodhi004, were cultured separately on PDA medium at 28±3°C for 14 days. The spore suspensions were prepared in sterile water. Then, 10 mL of sterile water was poured onto a culture plate and a sterile loop was used to scrape the spores from the mycelium. The mycelial fragment of each fungus was extracted from the spore suspensions using three layers of sterile cheesecloth under aseptic conditions. The spore suspensions were diluted with sterile water and adjusted using a hemocytometer to achieve a final concentration of 10^6 spores mL^{-1}.

Evaluation of antagonistic behavior

A dual culture method was used to determine the activity of antagonistic fungi against *C. capsici* on the PDA plate. *C. capsici* and antagonistic fungi were cultured on PDA medium for 7 days at 28±3°C. Then, mycelial plugs of four antagonistic fungi and *C. capsici* with a diameter of 0.5 cm were created using a sterile cork borer obtained from 7-day-old active mycelium. The mycelial plugs of the four antagonistic fungi and *C. capsici* were placed 2 cm away from the edge of the 9 cm diameter plate on the opposite side and incubated at 28±3°C for 14 days. The *C. capsici*-inoculated PDA medium served as a control and was placed on a separate plate. Five replications and four repetitions of the experiment were carried out. The percent inhibition of radial growth (%PIRG) was calculated using the following formula:

$$\frac{R1 - R2}{R1} \times 100$$

Where;

R1= The radial growth of *C. capsici* in the control treatment.
R2= The radial growth of *C. capsici* in the dual culture test.

The effectiveness of antagonistic fungi against *C. capsici* on chili fruits, determined using a detached fruit assay

The mature fruits of the chili variety Jinda were purchased at a market in Bangkok, Thailand. Similar-sized and ripe chili fruits were selected, washed with tap water to remove dust and surface contaminations, rinsed with sterile distilled water and wiped with sterile tissue paper. The surface of chili fruits was disinfected by soaking them in 70% (v/v) ethanol for 2 min, then rinsing them three times with sterile distilled water and wiping them with sterile tissue paper before drying them in a flow cabinet. Chili fruits were wounded using sterile 200 μL tips (2 mm in diameter, one wound per chili fruit). Chili fruits were then soaked separately in a spore suspension of antagonistic *T. flavus* Bodhi001, *T. trachyspermus* Bodhi002, *T. flavus* Bodhi003 and *N. fischeri* Bodhi004 at 10^6 spores mL^{-1} for 5 min and placed in 15 × 2 × 25 cm (length × width × height) plastic boxes containing 10 fruits per box. The center of the treated chili fruits was inoculated with a mycelial plug of *C. capsici*. The control treatment consisted of chili fruits inoculated with a *C. capsici* mycelial plug and soaked in distilled water. The experiments were conducted with 40 replicates (40 chili fruits per treatment and one wound per fruit) and repeated three times. Regarding the boxes containing treated chili fruits, they were kept moist with wet paper for 5 days at room temperature. After 5 days of inoculation, the length of lesions on treated fruits was measured and recorded. Using the following formula, the severity of the disease was calculated as a percentage of the length of lesions on the treated fruits compared to the control treatment:

$$\%\ \text{Disease severity} = \frac{A - B}{A} \times 100$$

Where;

A= The average length of chili fruit lesions in the control treatment.

B= The average length of chili fruit lesions in the antagonistic fungi treatment.

Statistical analysis

The data in this experiment were analyzed using a one-way analysis of variance (ANOVA) and the means were compared using the least significant difference (LSD) with a 95% level of statistical significance ($p<0.05$). The statistical analysis was conducted with the aid of the statistical program 122 Statistix8 (Analytical Software, SXW, Tallahassee, FL, USA). At least three replications were utilized to calculate means and standard deviations.

RESULTS AND DISCUSSION

Confirmation of antagonistic activity against the chili anthracnose disease

Four antagonistic fungi exhibited promising antagonistic activity against *C. capsici*. *T. flavus* Bodhi001 and *N. fischeri* Bodhi004 inhibited the mycelial growth of *C. capsici* by 68.99 and 70.76%, respectively, compared to the growth of *C. capsici* alone in the control treatment (Fig 1 and 2). The biocontrol activities of *T. flavus* Bodhi001 and *N. fischeri* Bodhi004 included antibiosis production and nutrient and space competitions (Fig 2). *T. trachyspermus* Bodhi002 and *T. flavus* Bodhi003 inhibited the mycelial growth of the pathogen by 59.84 and 60.38%, respectively, in a dual culture test (Fig 1). During the confrontation between *T. flavus* Bodhi001 and *C. capsici*, antibiosis mechanisms with a distinct inhibition zone measuring between 0.6 and 0.7 cm in width were observed.

Effect of antagonistic activity on *C. capsici* severity, determined using a detached fruit assay

On chili fruits inoculated with 10^6 spores mL^{-1}, the effect of antagonistic fungi *T. flavus* Bodhi001, *T. trachyspermus* Bodhi002, *T. flavus* Bodhi003 and *N. fischeri* Bodhi004 on controlling chili anthracnose *in vivo* was evaluated. Compared to the control treatment, chili fruit treated with a spore suspension of four antagonistic fungi suppressed anthracnose disease severity significantly. Chili fruits treated with *T. flavus* Bodhi001 demonstrated the greatest reduction in disease severity (0.98%) and anthracnose disease (80%)

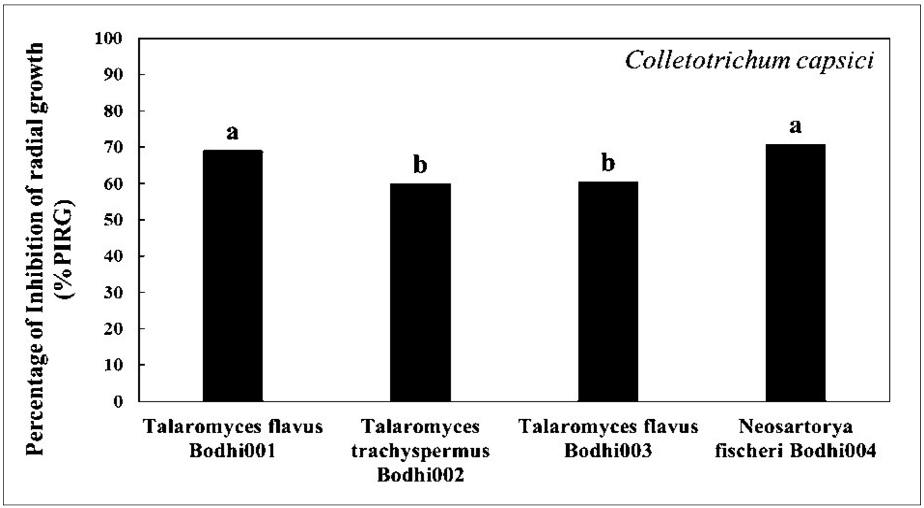

Fig 1: Biological control of four antagonistic fungi against *Colletotrichum capsici*, the pathogen responsible for chili anthracnose, using an *in vitro* dual culture test.

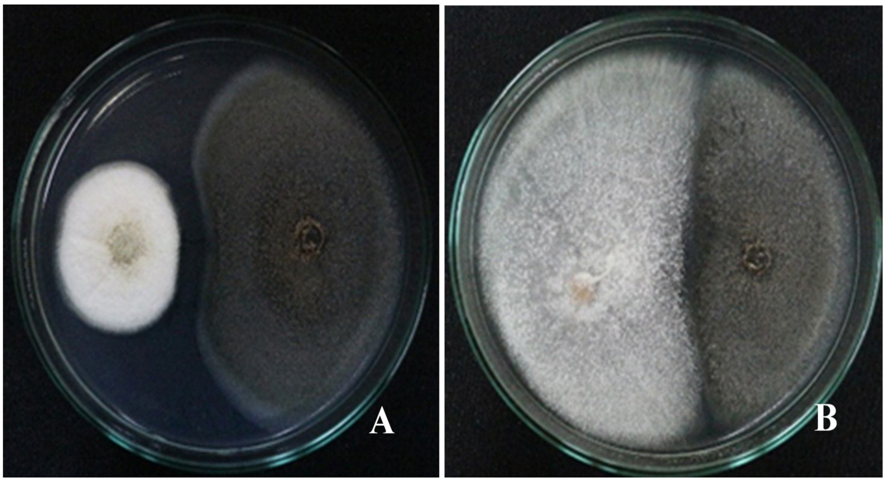

Fig 2: Effect of antifungal activities of antagonistic fungi isolate (left). *Talaromyces flavus* Bodhi001 (A) and *Neosartorya fischeri* Bodhi004 (B) against *Colletotrichum capsici*, the pathogen responsible for chili anthracnose (right), using a dual culture method.

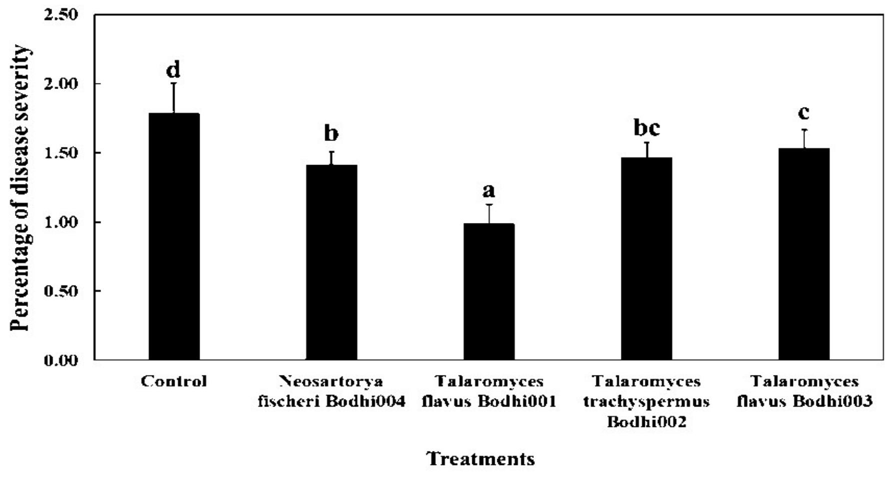

Fig 3: *In vivo* Determination of the effect of antagonistic fungi on the Chili anthracnose disease using a detached chili fruit assay. The mean values are presented alongside their standard deviation (±). Using the least significant difference (LSD) test at $p<0.05$ and n=40, the means following the same letter in each column are not significantly different.

when compared to other antagonistic strains and the control treatment (Fig 3 and 4). However, fruits treated with *N. fischeri* Bodhi004, *T. trachyspermus* Bodhi002 and *T. flavus* Bodhi003 reduced the severity of anthracnose by 1.41, 1.46 and 1.53%, respectively (Fig 3). In addition, chili fruits treated with distilled water exhibited a pronounced anthracnose symptom (Fig 4).

Biological approaches to plant disease management, including biological control agents and plant extracts, have been isolated and evaluated (Poveda, 2021). The antagonistic microorganisms combat plant pathogens through multiple mechanisms, including parasitism, induction of host resistance, antibiosis and space and nutrient competition. This study revealed that *T. flavus* Bodhi001, *T. trachyspermus* Bodhi002, *T. flavus* Bodhi003 and *N. fischeri* Bodhi004 inhibited the mycelial growth of *C. capsici in vitro* by more than 50%. *In vitro* antibiosis produced by *T. flavus* Bodhi001 inhibited the mycelial growth of *C. capsici* significantly (Fig 2). Similar to previous reports, our findings showed that *Talaromyces* species exert antagonistic mechanisms against plant pathogens by producing antibiosis (Dethoup *et al.,* 2018; Suasa-ard *et.al.,* 2019; Komhorm *et al.,* 2021). In a dual culture test, *T. flavus* Bodhi001 inhibited the mycelial growth of *A. brassicicola* by 64% and formed an inhibition zone between 0.8 and 0.9 cm wide. Under greenhouse conditions, the spore suspension containing 10^6 spores mL^{-1} of this antagonistic fungus inhibited the development of black spot disease in Chinese kale by up to 32.56% (Komhorm *et al.,* 2021). Jantasorn *el al.* (2016c) also reported that the crude extract of *T. flavus*

Fig 4: The effect of four antagonistic fungi on *Colletotrichum capsici*, the pathogen responsible for Chili anthracnose. Treated with distilled water as the control (A); *Neosartorya fischeri* Bodhi004 (B); *Talaromyces flavus* Bodhi001 (C); *Talaromyces trachyspermus* Bodhi002 (D); *Talaromyces flavus* Bodhi003 (E) at 10^6 spores mL^{-1}.

Bodhi001 completely inhibited the radial growth of *Phytophthora palmivora*, *Pyricularia oryzae*, *Sclerotium rolfsii* and *Lasiodiplodia theobromae*, including *C. capsici* and *C. gloeosporioides*, which cause chili anthracnose. The results of the current and previous studies indicate that *T. flavus* Bodhi001 has effective antagonistic activity against phytopathogenic fungi that cause numerous economic crop diseases by producing antibiosis.

Treatment with *T. flavus* Bodhi001 inhibits the development of the anthracnose disease, as determined by the detached fruit assay. Chili fruits treated with this antagonistic fungus demonstrated a 0.98% reduction in lesion length and disease severity of *C. capsici*, as well as an 80% reduction in anthracnose disease (Fig 3 and 4). These results indicate that *T. flavus* Bodhi001 suppressed the severity of the disease more effectively than other antagonistic fungi examined in the current study. Our results also revealed that *T. flavus* Bodhi003 and *T. trachyspermus* Bodhi002 had the lowest antagonistic activity against *C. capsici*, the pathogen responsible for chili anthracnose disease, compared to other strains tested *in vitro* and *in vivo* in this study. In addition, it has been demonstrated that the antifungal effect of *Talaromyces* and *Neosartorya* species against plant pathogens is dependent on the conditions of the plant materials and experimental methods. For example, *in vitro* tests revealed that *N. fischeri* Bodhi004, *T. trachyspermus* Bodhi002 and *T. flavus* Bodhi003 effectively inhibited the mycelial growth of *C. capsici*. However, *in vivo* testing revealed that these three antagonistic fungi had a minimal effect on anthracnose disease severity. These findings imply that antagonistic strains and species isolated from different habitats can produce a variety of bioactive compounds (Suay *et al.,* 2000). Our study confirmed the efficacy of the *T. flavus* Bodhi001 isolate against *C. capsici*, the causal agent of chili anthracnose, both *in vitro* and *in vivo*. In addition, there are few reports of *Talaromyces* species demonstrating a potent effect on preventing the development of chili anthracnose. *Talaromyces* species demonstrated significant antagonistic activity against plant diseases in numerous economic crops, including vascular wilt disease of potato and tomato (Naraghi *et al.,* 2012; Bahramian *et al.,* 2016), rice disease (Dethoup *et al.,* 2018; Dethoup *et al.,* 2022), Chinese kale black spot disease (Komhorm *et al.,* 2021) and *Lasiodiplodia theobromae*, which causes mango fruit rot (Suasa-ard *et al.,* 2019). Many other studies have reported the *in vitro* and *in vivo* effectiveness of antagonistic fungi, *Trichoderma* species, against chili anthracnose disease (Ruangwong *et al.,* 2021; Yadav *et al.,* 2021). According to Jaihan *et al.* (2016), the entomopathogenic fungi strain *Ophiocordyceps sobolifera* inhibited the mycelial growth and conidial formation of *Colletotrichum* spp. *in vitro*. Similarly, the mushroom culture filtrate, *Clitocybe nuda* (LA82), effectively reduced the severity of anthracnose disease (Chen and Huang, 2010). Suprapta (2022) found that the formulation of *Paenibacillus polymyxa* C1 effectively controlled the anthracnose disease under field conditions.

Our results revealed that the antagonistic fungi *T. flavus* Bodhi001 isolated from riparian forest soils exhibited the greatest biocontrol effect in controlling chili anthracnose disease *in vivo* and significantly inhibited the mycelial growth of *C. capsici in vitro*. It has been demonstrated that their antagonistic mechanism prevents plant pathogen infection by producing antibiosis, thereby reducing the severity of chili anthracnose. However, there are no reports of human or environmental toxicity associated with the use of spore suspensions of *Talaromyces* species for the control of plant diseases. This study demonstrated that *T. flavus* Bodhi001 is a promising biocontrol agent for chili anthracnose disease caused by *C. capsici* and could be developed as an alternative to synthetic fungicides for disease management in organic and sustainable cropping systems.

CONCLUSION

The results of this study confirmed the biocontrol potential of *T. flavus* Bodhi001 against *C. capsici*, the pathogen responsible for chili anthracnose. By producing antibiosis in dual culture tests, this strain has demonstrated its high antagonistic activity. *T. flavus* Bodhi001 exhibited the most potent antagonistic activity against the chili anthracnose disease *in vivo*. These results indicate that *T. flavus* Bodhi001 has the potential to be developed as a biological control agent for chili anthracnose disease management. However, additional research is necessary to investigate the efficacy of *T. flavus* Bodhi001 under field conditions.

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Conflict interests

The authors declare that they have no conflict of interest.

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