**BIODIVERSITAS**
Volume 19, Number 3, May 2018
Pages: 974-977

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190328

# Short Communication: Viability and environmental effect to conidial germination of antagonistic fungi that potential as biological control of *Colletotrichum gloeosporoides* caused antracnose disease on chili

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Manuscript received: 12 March 2018. Revision accepted: 4 May 2018.

**Abstract.** *Authors. 2018. Short Communication: Viability and environmental effect to conidial germination of antagonistic fungi that potential as biological control of* Colletotrichum gloeosporoides *caused antracnose disease on chili. Biodiversitas 19: 974-977.* Some of antagonistic fungi from chili rhizosphere were potential to suppress the growth of *colletotrichum gloeosporoides* in vitro. This present study was aimed to observe the viability of antagonistic fungi and effect of temperature and ultraviolet light to its conidial germination. We targeted to obtain the isolate with the best viability and high resistance to temperature and ultraviolet changes. The experiment used a completely randomized design consisted of nine treatments and four replication. The treatment were nine of antagonistic fungi isolates from chili rhizosphere that consisted of two genera, *Trichoderma* and *Paecilomyces,* each comprised four isolates and unidentified isolate (X isolate). These four isolates were considered as replication for observation. The observed parameters observe included the width of colony, density of conidia, germination of conidia, effect of temperature and ultraviolet light changes to conidial germination. The results showed that antagonistic fungi from the genus *Trichoderma* had the best viability (the width of colony = 39.68-56.92 $cm^2$, density of conidia 40.50-57.50 x $10^9$ conidia/mL and germination of conidia > 80%) and highest resistance to temperature and ultraviolet changes compared with the other fungi used in this study.

**Keywords:** Antagonistic fungi, conidia, *Trichoderma*, temperature, ultraviolet light

## INTRODUCTION

The anthacnose disease caused by *Colletotrichum capsici* and *Colletotrichum gloesporoides* is one of the important and disastrous diseases in chili (Montri et al. 2009; Sharma and Kulshrestha 2015). This pathogen can infect the mature and immature fruit of pepper that caused the great loss of production (Robert et al, 2015). The *Colletotrichum* pathogen is difficult to control since it is seed-borne disease and it has high genetic diversity (Than et al. 2008)

Generally, farmers control the anthracnose disease by using fungicides. However, continue use of fungicides cause negative impacts on the environment and consumers. Thus, it is necessary to discover alternative controls to this pathogen that are environmentally friendly by using fungi that are antagonistic to pathogens as a biological control. According to Begum et al. (2008), some fungi and bacteria derived from soybean seeds have the potential to inhibit the growth of *C. truncatum* which causes anthracnose in soybean. Such isolates with high ability are *T virens* isolates UPM 23, *T. harzianum* isolates UPM 40 and *Pseudomonas aeruginosa* isolate 13B8. Ghosh and Chakraborty (2012) reported that five isolates of *T. viride* isolates were able to suppress the growth of *C. gleosporoides* fungi that caused anthracnose in *Roulvolfia serpentine* plants. Another study by Nurbailis and Martinius (2014) found that 9 of 52 fungi isolates originated from chili rhizosphere, potentially inhibited the growth of *C. gloeosporoides* that caused anthracnose in chili. Of the 9 isolates, 4 were *Trichoderma*, 4 were *Paecilomyces* isolates and one unidentified.

*Trichoderma* are free-living fungi, usually found in soil and root ecosystems. They are opportunistic, avirulent, plant symbionts, as well as being parasites to plant pathogen of fungi (Harman et al. 2004). In an artificial medium (Potato Dextrosa Agar) *Trichoderma* grow well within 3 days as fungal colony that can reach 9 cm diameter of petri dish (Shahid et al. 2013). *Trichoderma* spp. grow best in a temperature range of 25°C to 30°C (Singh et al. 2014).

*Paecilomyces* are cosmopolite fungi living in the soil, rotting plant debris and food product. Liang et al. (2005) reported that *Paecilomyces* spp. grow slowly with the growth rate ranged from 0.6-6.5 cm during 14 days. *Paecilomyces gunnii* have even much slower with the growth was only 2.2-2.8 cm on Czapek medium. Kiewnick (2006). *Paecilomyces lilacinus* can grow well with temperature between 24 and 30°C, but not above 36°C, the conidia germinated at temperature between 28 and 30°C.

Conidial germination of various antagonistic fungi is strongly influenced by changes in temperature and ultraviolet light exposure. According to Poosapati et al. (2014) conidia of *Trichoderma* spp from different agro climatic zone of India reduced their ability to germinate at

temperature 37°C except for *T. asperellum*-TaDOR673. This fungi isolate was highly tolerant at high temperature 52°C, and was still able to produce fairly dense conidial and higher conidial germination compared to other isolate**.** The result of screened in vitro and in vivo indicated *T. asperellum*-TaDOR673 effectively for controlling the collar rot disease caused by *Sclerotium rolfsii* in groundnut.

Gupta and Sharma (2013), reported that generally *T. harzianum* can grow well at 25-30°C on Potato Dextrosa Agar. The growth will be reduced when the temperature increase above 37°C and no growth was observed at 45°C six days after inoculation. Jamali and Banihashemi (2012) stated that there is a species of *Paecilomyces* fungi that is thermophilic, and is still able to grow well above 50°C.

Despite of temperature, ultraviolet light also influence the germination of conidia. Menetrez et al. (2010) reported that the ultraviolet radiation could cause cell mortality and genetic mutation when the fungi are exposed under ultraviolet light within a certain period. Asthana and Tuveson (1992) stated that the ultraviolet-B irradiation components on the sun could damage protein and DNA of the pathogenic fungi, thus affecting the physiological activity and metabolic response. Rodrigues et al. (2016) reported that 5 minutes exposure to ultraviolet can reduce conidial germination of of *Beauveria bassiana* from 95% to 52% and from 96% to 54% for *M. anisopliae.*

The objective of this study was to determine the viability and the effect of temperature and ultraviolet light towards conidial germination of antagonistic fungi indigenous to chili rhizosphere. This fungi has the potential in suppressing the growth of *C. gloeosporiodies* that caused anthracnose disease on chili

## MATERIALS AND METHODS

### Design

The study involved two objectives, i.e. testing the viability of antagonistic fungi that have the potential for inhibiting the growth of *C. gloeosporoides* that causes anthracnose disease in chili; and to assess the effect of temperature and ultraviolet rays on the conidial germination of this antagonistic fungi. This study used Completely Randomized Design (CRD) with 9 treatments and 4 replications. The treatments were various isolates of antagonistic fungi: *Trichoderma* spp. (sp.1, sp.2, sp.3 and sp.4) *Paecilomyces* spp. (sp.1, sp.2, sp.3 and sp.4) and isolate X. Each of isolate made four replication for observation.

### Rejuvenation and propagation of antagonistic fungi isolate

Rejuvenation of antagonistic fungal isolates was performed by reproducing the fungal isolates on PDA medium. The reproduced antagonistic fungi in the PDA medium were extracted using a 0.5 cm diameter borer core and transferred into a Petri dish which contains a PDA medium, then incubated for 7 days.

### The width of colony

The coverage test of colonial growth was performed by extracting a piece of Fungi mycelium which has been reproduced in PDA medium using 0.5 cm diameter borer corer and then grown into Petri dish which contained PDA medium and incubated at room temperature. The each Fungi colonial coverage was measured from day 2 after inoculation until the culture was 14 days old.

### Conidial germination test

Conidial germination test was performed by preparing a 1 $cm^2$ size and 2 mm thick of PDA medium that was located on a sterile object glass. 10 µl of conidial suspension containing $10^6$ conidia/mL was dripped. The object glass was inserted into a sterile Petri dish containing a moistened filter paper and incubated at room temperature for 24 hours.

### Conidial density

Conidial density of each Antagonistic fungal isolate was performed by preparing a conidial suspension with concentration of $10^6$ conidia/mL. 0.1 mL, each isolate was inserted in a Petri dish containing PDA media and incubated at 28°C. After 14 days, the cultures were inserted into Erlenmeyer and added with 50 mL of sterile distilled water. The cultures were then homogenized in a shaker for 5 minutes and filtered using Whatman-paper no. 4. The filtered conidias were put into a test tube and added with 9 mL sterile distilled water and diluted to $10^{-4}$. The conidial concentration of the suspension was calculated using a haemocytometer.

### Conidial sensitivity to temperature

The sensitivity test of conidial fungal isolate towards the temperature change was performed by extracting a conidial suspension from each antagonistic fungal isolate at a concentration of $10^6$ conidia/mL, and then inserted into a 5 mL reaction tube. Each isolate was incubated in a water bath using different temperatures (10°, 30°, and 50°C;) and a control for 30 minutes. Calculation of the conidial germination was performed using the same method as germination test of conidial antagonistic fungi.

### Conidial sensitivity to ultraviolet light

Sensitivity test of conidial antagonistic fungi against UV light was performed using: pure culture aged 14 days in a petri dish that was placed on open space to be exposed to direct sunlight. The exposure to the UV light was performed for 0, 30, and 60 minutes. The germination of conidial antagonistic aungi was calculated using the same method as germination test experiment.

## RESULTS AND DISCUSSION

### Viability of antagonistic fungi

The viability of various antagonistic fungi used in this study varied significantly between isolates, while conidial germination did not vary significantly between isolates (Table 1).

The largest area of antagonistic fungal colonies was found in *Trichoderma* sp.2 isolate, with colony area of 56.92 $cm^2$ and the smallest was found in *Paecilomyces* sp.1 isolate with 11.78 cm2 colony area (Table 1). The highest density of conidial was found in the *Trichoderma* sp.3 isolate as 57.5 x 109 conidia/mL and the smallest was found in *Paecilomyces* sp.4 as 26.50 x 109 conidia/mL. Isolate X did not form conidial (Table 1). In general, conidial germination of various antagonistic fungi was relatively high, as more than 80% and did not vary significantly between isolates.

**Temperature effect to germination of antagonistic fungal conidia**

The effect of temperature change towards germination of conidial of antagonistic fungi varied significantly between isolates (Table 2). For some isolates such as *Paecilomyces* sp.4, *Paecilomyces* sp.1, *Trichoderma* sp.4, the germination of conidial at 10°C survived above 60%, while isolates *Paecilomyces* sp.2, *Trichoderma* sp.3, *Trichoderma* sp.2 and *Paecilomyces* sp.3 only survived for 40-60%. At a temperature of 30°C, conidial germination of indigenous rhizosphere antagonistic fungi was normal and the lowest conidial germination was found in *Paecilomyces* sp.1 isolate (72%). In general, at a temperature of 50°C, the germination of conidial of antagonistic fungal was decreases. Two *Trichoderma* isolates which were still able to germinate well were *Trichoderma* sp.1 (98.25%) and *Trichoderma* sp.2 (79.50%) (Table 2).

**The effect of ultraviolet light towards the germination of antagonistic fungal conidia**

The duration of UV light exposure to the antagonistic fungi tested in this study had significantly different effects on germination of the conidial (Table 3). The highest germination of conidial was observed at 30 minutes UV exposure was recorded in *Trichoderma* sp.2 isolate (94.5%), and the lowest was in *Paecilomyces* sp.2 isolate (24%). Generally the germination of the conidia was decreased at 60 min UV exposure except for *Trichoderma* sp.2 was still germinated highly (84.25%).

**Table 1.** The widht of colony, conidial density and germination of several antagonistic fungi from chili rhizosphere

| Antagonistic fungi isolates | Viability of several antagonistic fungi | | |
|---|---|---|---|
| | Coloni coverege ($cm^2$) | Density of conidia ($x10^9$ con./mL) | Germination of conidia (%) |
| *Trichoderma* sp.3 | 56.92 a | 57.50 a | 86.50 a |
| *Trichoderma* sp.1 | 56.77 a | 40.50 abc | 90.50 a |
| *Trichoderma* sp.4 | 48.77 ab | 52.00 ab | 100.00 a |
| *Trichoderma* sp.2 | 39.68 bc | 44.50 abc | 100.00 a |
| *Paecilomyces* sp.2 | 38.69 bc | 36.75 abc | 100.00 a |
| Isolate X | 36.83 bc | - | - |
| *Paecilomyces* sp.3 | 28.87 c | 27.00 bc | 100.00 a |
| *Paecilomyces* sp.4 | 25.46 cd | 26.50 c | 100.00 a |
| Paecilomyces sp 1 | 11.78 d | 28.50 bc | 100.00 a |

Note: The figures in the column followed by the same lowercase letter are not significantly different according to DNMRT 5%

**Table 2.** Effect of temperature to germination of antagonistic fungal conidia

| Antagonistic fungi isolates | Percentage of germination of conidia (%) at various temperature (°C) | | | |
|---|---|---|---|---|
| | Room temp. (27) | 10 | 30 | 50 |
| *Trichoderma* sp.4 | 100 a | 76.75 a | 100 a | 58.50 abc |
| *Trichoderma* sp.2 | 100 a | 60.00 ab | 96.75 a | 79.50 abc |
| *Pecilomyces* sp.4 | 100 a | 40.50 b | 95.00 a | 46.75 bc |
| *Paecilomyces* sp.2 | 100 a | 56.25 ab | 100 a | 59.00 abc |
| *Paecilomyces* sp.1 | 100 a | 55.50 ab | 73.25 b | 38.75 c |
| *Paecilomyces* sp.3 | 100 a | 49.25 ab | 100 a | 47.75 bc |
| *Trichoderma* sp.1 | 90.5 a | 65.00 ab | 97.50 a | 98.25 a |
| *Trichoderma* sp.3 | 86.5 a | 65.00 ab | 100 a | 81.50 ab |
| Isolat X | - | - | - | - |

Note: The figures in the column followed by the same lowercase letter are not significantly different according to DNMRT 5%

**Table 3.** Effect of ultraviolet light to germination of antagonistic fungal conidia

| Antagonistic fungi isolates | Percentage of germination of conidia (%) at different duration of UV light exposure (minute) | | |
|---|---|---|---|
| | 0 | 30 | 60 |
| *Trichoderma* sp.4 | 100.00 a | 81.25 a | 69.50 ab |
| *Trichoderma* sp.2 | 100.00 a | 94.50 a | 84.25 a |
| *Pecilomyces* sp.4 | 100.00 a | 24.00 c | 73.00 a |
| *Paecilomyces* sp.2 | 100.00 a | 76.00 a | 15.00 b |
| *Paecilomyces* sp.1 | 100.00 a | 37.50 bc | 56.00 ab |
| *Paecilomyces* sp.3 | 100.00 a | 42.00 bc | 37.25 ab |
| *Trichoderma* sp.1 | 90.50 a | 62.75 ab | 53.25 ab |
| *Trichoderma* sp.3 | 86.50 a | 78.50 a | 75.00 a |
| Isolat X | - | - | - |

Note: The figures in the column followed by the same lowercase letter are not significantly different according to DNMRT 5%

## Discussion

The *Trichoderma* genus exhibits faster growth and higher conidial density than the *Paecilomyces* genus. This genus is one of the soil fungi that has a variety of habitats and is highly competitive against other saprophytic fungi. According to Harman et al. (2004), *Trichoderma* are cosmopolite, usually found in soil and root ecosystems. They are opportunistic, avirulent, plant symbionts, as well as being parasites to plant pathogen of fungi. On the second day after incubation , the colour of *Trichoderma* colony on PDA medium was white because of its mycelium. On the third day, then the colour was turning to green, due to the formation of conidial. According to Shahid et al. (2013), the growth of *Trichoderma* fungal colony isolates can fill the 9 cm diameter petri dish within 3 days. This genus produced conidial that are green, green, greenish yellow and dark green and concentric conidia.

The *Paecilomyces* is a genus of fungi that has slow growth rate and low conidial density. The *Paecilomyces* genus growth on PDA medium can fill the 9 cm diameter petri dish on 17th day and produced low conidial density. According to Liang et al. (2005) the growth of *Paecilomyces* spp. fungi ranged from 0.6-6.5 cm for 14 days, even *P. gunnii* growth was very slow accounted only for 2.2-2.8 cm on Czapek Agar medium.

In general, the germination of antagonistic conidial fungi at room temperature 30°C was quite high with more than 80%. High germination is essential for the growth and development of fungi as a biological agent. Shahid et al. (2013) reported that *Trichoderma* grow optimally and produces a high conidial at 25-30°C. *Trichoderma* sp.1 and *Trichoderma* sp.3 isolates were able to germinate well at 50°C with conidial germination of 98.25% and 81.50% respectively. This indicates that *Trichoderma* was able to germinate above the optimum temperature. The results of research by Poosapati et al. (2014) showed that the *Trichoderma asperellum* TaDOR673 fungi isolate was highly tolerant to 52°C high temperature, and the fungi was still able to produce fairly dense conidial and higher conidial germination compared to other isolates.

One of *Paecilomyces* isolates that were capable of germinating at temperature above 50°C was *Paecilomyces* sp.1 isolate, thus resistant to high temperatures. Jamali and Banihashemi (2012) stated that *P. crustaceus* and *P. variotii* are thermophilic fungi that could grow well at high temperatures of 50°C and 60°C.

The exposure towards ultraviolet irradiation on the indigenous rhizosphere antagonistic fungi has an effect to its conidial germination. The longer the irradiation time would decreased the conidial germination. The decrease in germination may caused by the conidial cell damage. According to Menetrez et al. (2010), the UV radiation could cause cell mortality and genetic mutation when the fungi are exposed under UV light within a certain period of time. According to Asthana and Tuveson (1992) the UV-B irradiation components on the sun could lead into protein and DNA damage of the pathogenic fungi, thus affecting the physiological activity and metabolic response of the organism.

The antagonistic fungi from the genus *Trichoderma* genus had the best viability (the width of colony = 39.68-56.92 $cm^2$, density of conidia 40.50-57.50 x $10^9$ conidia/mL and germination of conidia > 80%) and highest resistance to temperature and ultraviolet changes compared with the other fungi used in this study. The *Trichoderma* isolates potential to aplicate to chili fruit for controlling antracnose disease.

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