



Ability of *Beauveria bassiana* to suppress *Colletotrichum truncatum* and increase the growth of chili plants (*Capsicum annuum*)

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Abstract

Anthrachnose is a disease caused by *Colletotrichum* sp., which can reduce the quality and yield of red chili by 45-60%. This study aimed to determine the ability of different *Beauveria bassiana* isolates to control anthracnose in chili. The research employed a Randomized Block Design (RBD) consisting of 6 treatments and 5 replications. The treatments included a control and 5 different *B. bassiana* isolates (PD114, TD312, WS, BbKo, KT2B21). The observed variables included incubation period, disease incidence and severity, plant height, number of leaves, flowering time, and chili fruit weight. The results showed that the application of different *B. bassiana* isolates have effectively inhibited the development of *Colletotrichum truncatum*, which caused anthracnose in chili. *B. bassiana* has demonstrated the ability to prolong the incubation period of *C. truncatum* (5.40 days) and reduce the percentage of disease incidence (16%). Furthermore, the application of *B. bassiana* increased the height of chili plants (40.40 cm) and the number of leaves (32 leaves), accelerated the flowering time (42.80 days), and boosted chili production (178.25 g). The WS and TD312 isolates revealed the most significant results. Although the severity of the disease was not impacted, the 2 isolates provided notable agronomic benefits to the chili plants.

Keywords: Endophytic fungi, Incidence, incubation, *Leptocorisa oratorius*, severity

Introduction

Chili (*Capsicum annuum* Linnaeus) is one of the important horticultural commodities in Indonesia has high economic value (Muljowati et al., 2021). One of the limiting factors of production is anthracnose, a disease caused by the fungus *Colletotrichum* sp. (Oo and Oh, 2016). Anthracnose

determines the production stability of chili and farmer income. Several species of *Colletotrichum* that have been reported to cause anthracnose include *C. truncatum*, *C. siamense*, and *C. scovillei* (Cannon et al., 2012; Gao et al., 2020; Gao et al., 2021), *C. gloeosporioides* (Than et al., 2008; Weir et al., 2012), *C. acutatum* (Widodo and Hidayat

2018), *C. orchidearum*, *C. magnum*, *C. spaethianum*, and *C. truncatum* complex (Damm et al., 2012; Damm et al., 2019).

Symptoms of anthracnose in chili fruit manifest as blackish-brown and dry spots on the surface, which continue to soft rot (Islam et al., 2020). These blackish-brown spots are the fruiting bodies of the *Colletotrichum* sp. caused by setae that form on the acervulus. Moreover, the growth of *Colletotrichum* sp. on chili seeds can result in failed germination and wilting (Oo and Oh, 2016).

Controlling using endophytic fungi is one of the alternatives to reduce pathogen attacks (Rachmawati et al., 2016). Endophytic fungi protect plants through direct and indirect mechanisms. Endophytic fungi have a mutualistic relationship with their host plants (Oteino et al., 2015), such as increase plant growth (Oteino et al., 2015), inhibit pathogens (Bolívar-Anillo et al., 2020), remove soil pollutants (Akram et al., 2023), and increase tolerance to abiotic stresses such as salinity, drought, and extreme temperatures (Abdelaal et al., 2021); (Alkahtani et al., 2020).

Beauveria bassiana fungus can control several species of insect pests and plant pathogens (Gothandapani et al., 2015; Yanti et al., 2019), such as *Alternaria alternata* on tomatoes (Sinno et al., 2021) and *Rhizoctonia Solani* (Azadi et al., 2016). Endophytic fungi, such as *B. bassiana*, have been reported to be able to control *Botrytis cinerea* in chili and tomato plants (Barra-Bucarei et al., 2020). Endophytic fungi isolated from shallots can inhibit the growth of *C. gloeosporioides* mycelium with an inhibition percentage of 10.77 - 55.92%. The isolate of S2D11 is the most effective in inhibiting the growth of *C. gloeosporioides* (Trizelia et al., 2023). However, its specific effectiveness in suppressing *Colletotrichum* in chili plants remains largely unexplored, particularly its impact on anthracnose and plant growth.

Regarding the ability of *B. bassiana* to control various pathogens, this study aimed to determine the ability of different *Beauveria bassiana* isolates to control anthracnose in chili and its effect on the growth of chili plants.

Methods

The research was conducted at the Biological Control and Phytopathology Laboratories of the Department of Plant Protection and continued at the Wirehouse of the Faculty of Agriculture, Universitas Andalas, Padang, Indonesia, from November 2023 to April 2024.

Methods

The study used a randomized block design involving 6 treatments and 5 replications. The treatments included 5 isolates of *B. bassiana*, include WS (*B. bassiana* from *Leptocorisa oratorius*), PD114 (*B. bassiana*, an endophyte in chili leaves), TD312 (*B. bassiana* endophytes in wheat), BbKo (*B. bassiana* endophytes in coffee leaves), and KT2B21 (*B. bassiana* endophytes in cocoa leaves). The *B. bassiana* isolates is a collection of the Biological Control Laboratory, Faculty of Agriculture, Universitas Andalas.

Providing Chili Seeds

Local chili seeds from Pauh District, Padang City, West Sumatra, were observed to be affected by anthracnose. Seeds obtained from the middle part of the fruit were air-dried at room temperature (20°-25°C) for two days. Subsequently, they were cut into three parts, with the middle part selected for further use. Before treatment, seeds were soaked in 70% alcohol for one minute and rinsed three times with distilled water. After cleaning, seeds were air-dried in a laminar airflow cabinet for 60 minutes. For the propagation of *B. bassiana*, fungal isolates were derived from various parts of chili plants and wheat stems. Five *B. bassiana* isolates—

WS, PD114, TD312, BbKo, and KT2B21—were used in the study.

Propagation of *Beauveria bassiana*

The *B. bassiana* isolates used were obtained from different parts of chili plants

(stems, leaves, roots) and wheat stems. A total of 5 isolates of *B. bassiana* were utilized, namely WS, PD114, TD312, BbKo, and KT2B21. All isolates were cultured using SDAY (Sabouraud Dextrose Agar with Extract) media and then incubated for 21 days (Figure 1).

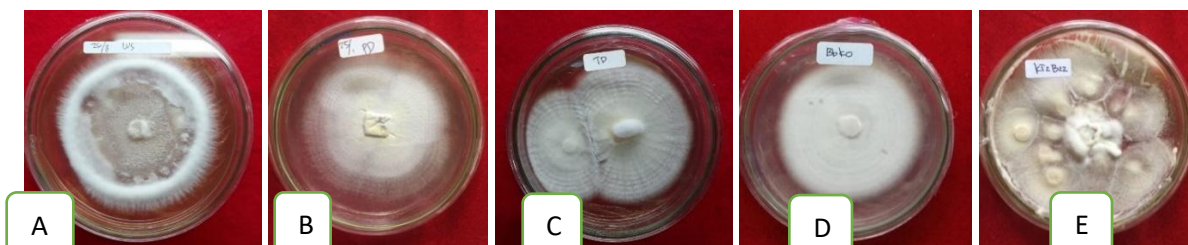


Figure 1. *B. Bassiana* culture macroscopically: A). *B. bassiana* WS, B). *B. bassiana* PD114, C). *B. bassiana* TD312, D). *B. bassiana* BbKo, E). *B. bassiana* KT2B21

Preparation of *B.bassiana*

To prepare the conidia suspension of *B. bassiana*, 10 ml of distilled water and three drops of Tween 80 were added to the *B. bassiana* isolate in a 3-week-old petri dish. The conidia were collected using a fine brush, transferred into a test tube, and subjected to a serial dilution of 10^{-3} , resulting in a 10^8 conidia/ml concentration. The conidia density was determined using a hemocytometer (Improved Neubauer).

Application of *B. bassiana* on Chili Seeds

To apply *B. bassiana* on chili seeds, the seeds were sterilized by soaking them in distilled water, 1% NaOCl, and then again in distilled water for 1 minute. Subsequently, the seeds were soaked in the *B. bassiana* suspension for 9 hours. Afterwards, the chili seeds were planted in a potray at a depth of 0.5 cm, with 2 seeds planted in each hole.

Preparation of Planting Medium

The planting medium consisted of a mixture of soil and manure in a 2:1 volume ratio. The soil and manure were sterilized using the Tyndalization method, which involved exposing them to hot steam at 100°C for 1 hour, repeated three times with 24-hour intervals. After sterilization, the medium was

left for 24 hours. Subsequently, 10 grams of the medium was placed in pottrays for sowing, and 10 kg was used for planting in polybags.

Inoculation of *Colletotrichum truncatum*

To prepare the conidial suspension, 10 ml of distilled water was added to a 3-week-old culture of the *C. truncatum* isolate in a petri dish. The conidia were collected using a fine brush and then transferred to a test tube. A serial dilution achieved a concentration of 10^6 conidia/ml. The conidial density was determined using hemocytometer. Inoculation was performed by spraying 10 ml of the fungal suspension onto each chili plant, covering the entire surface. Following inoculation, each plant was enclosed in transparent plastic for 2 days. After this period, the plastic cover was removed (Dzung et al., 2017).

Variables Observed

Incubation period (day)

The incubation period was observed during the first inoculation until symptoms appeared on the chili plants.

Disease incidence and severity

The development of the disease was monitored every 7 days following the initial

appearance of anthracnose symptoms on the chili fruit up to 110 days after planting. Disease incidence was determined using the formula:

$$I = \frac{n}{N} \times 100 \dots\dots\dots (1)$$

Notes: = Disease incidence (%), n = Number of affected plants, and N = total number of plants observed.

Disease severity was determined by observing the chili fruit with anthracnose symptoms. Observations were carried out every week after symptoms appeared until 110 days after planting. The percentage of disease severity can be calculated using the formula:

$$S = \frac{\sum nxv}{ZxN} \times 100 \dots\dots\dots (2)$$

S = disease severity, n = number of plants at each scoring, v = disease attack scale value for each individual plant, Z = highest damage score, N = number of plants observed

The damage score for anthracnose was based on the scale of damage to plants affected by the disease (herwidyarti et al., 2013). the damage scores: 0 = no damage, 1 = spots covering an area of 1 - 20%, 2 = spots covering an area of 21 - 40%, 3 = spots covering an area of 41 - 60%, 4 = spots covering an area of > 60%

Plant height (cm)

Observations of plant height were carried out once a week by measuring the plant from the base of the stem to the longest leaf. Plant height was measured when the plant is 1 - 8 weeks old after planting.

Number of leaves

The number of leaves was calculated together with the height of the plant. Leaf counting was performed manually by counting all fully developed leaves on each plant. Only healthy, fully expanded leaves were included in the count.

Flowering time (day)

Observations of flower time per sample in the treatment and control were carried out when the first flowers appear, namely when the plant has entered the flowering process.

Fruit weight (g)

Observations of fruit weight per plant were carried out by weighing the fruit from the first to the last harvest. Fruit weight was measured when the chili plant reached fully ripe fruit with a red color. The total of fruit weight per plant was obtained by adding the fruit weight for each harvest dividing it by the number of plants.

Data Analysis

Data were analyzed using analysis of variance (ANOVA) and LSD test at a level of 5%, using statistical software 8.

Results

Incubation Period (day)

The application of *B. bassiana* on chili plants from various isolates has been observed to prolong the incubation period of *C. truncatum*. Notably, the treatment of KT2B21 isolate resulted in the longest incubation period (5.4 days), significantly different from the treatment of PD114 isolate (3.80 days) and comparable to the treatment of other isolates. These findings indicate that *B. bassiana* can delay the incubation period of *C. truncatum* (Table 1).

Table 1. The incubation period of *Colletotrichum truncatum* treated with *B. bassiana* from different isolate sources

<i>B. bassiana</i> isolates	Incubation period (day)	
<i>B. bassiana</i> KT2B21	5.40 ± 0.68	a
<i>B. bassiana</i> WS	5.00 ± 0.54	ab
<i>B. bassiana</i> TD312	5.00 ± 0.70	ab
<i>B. bassiana</i> BbKo	4.60 ± 0.44	ab
<i>B. bassiana</i> PD114	3.80 ± 0.37	bc
Control	2.83 ± 0.17	c

*Numbers in the same column followed by different letters indicate significant differences based on a LSD (5%)

The early symptoms of the disease are blackish-brown spots on the surface of the fruit. These spots will enlarge, causing a wrinkled indentation and eventually drying out. If left untreated, the blackish-brown spots could develop into soft rot symptoms in red chili plants. Applying *B. bassiana* on chili plants can help delay the appearance of anthracnose disease symptoms caused by *C. truncatum* (Figure 2).

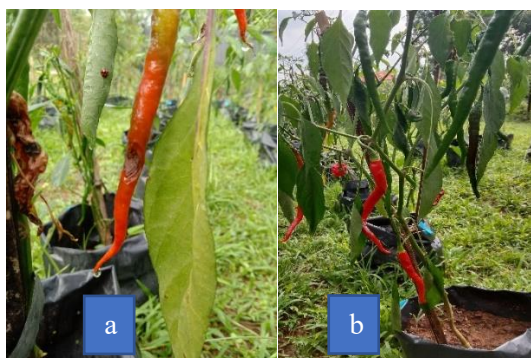


Figure 2. Symptoms of anthracnose in chili fruit, a). with anthracnose, b). Healthy fruit

Disease incidence and severity

The application of *B. bassiana* on chili with different isolates has affected the incidence of the disease but has not affected the severity of anthracnose caused by *C. truncatum* attack.

In the study, it was observed that the treatment without *B. bassiana* had the highest disease incidence (76.00%). However, the application of *B. bassiana* isolates led to a reduction in disease incidence. Notably, isolates from TD312, PD114, and WS reduced disease incidence at 28.00%, 20.00%, and 16.00%, respectively, including BbKo application. However, *B. bassiana* isolates did not reduce disease severity (Table 2, Figure 3).

Table 2. Incidence and severity of anthracnose after application of different isolates of *B. bassiana*

<i>B. bassiana</i> isolates	Incidence $\pm$ SE (%)	Severity $\pm$ SE (%)
Control	76.00 $\pm$ 4.00 a	63.54 $\pm$ 7.61 a
<i>B. bassiana</i> KT2B21	60.00 $\pm$ 6.32 ab	55.00 $\pm$ 9.57 a
<i>B. bassiana</i> BbKo	52.00 $\pm$ 10.10 b	46.80 $\pm$ 6.83 a
<i>B. bassiana</i> TD312	28.00 $\pm$ 6.32 c	51.60 $\pm$ 5.47 a
<i>B. bassiana</i> PD114	20.00 $\pm$ 4.89 c	45.20 $\pm$ 4.26 a
<i>B. bassiana</i> WS	16.00 $\pm$ 4.00 c	46.60 $\pm$ 4.23 a

*Numbers in the same column followed by different letters indicate significant differences based on a LSD (5%)



Figure 3. Growth of chili plants after application by different *B. bassiana* isolates: a) TD312 isolate, b) KT2B21 isolate, c) PD114 isolate, d) WS isolate, e) BbKo isolate, f) Control

Plant Height (cm)

The treatment by *B. bassiana* with WS and TD312 isolates affects the height of chili significantly. The TD312 isolate also increased an average height of 35.80 cm. Conversely, PD114, BbKo, and KT2B21 isolates did not significantly increase plant height (Table 3).

Table 3. Height of chili plants after application of different *B. bassiana* isolates

<i>B. bassiana</i> isolates	Height (cm) $\pm$ SE
<i>B. bassiana</i> WS	40.40 $\pm$ 2.22 a
<i>B. bassiana</i> TD312	35.80 $\pm$ 0.91 ab
<i>B. bassiana</i> PD114	31.00 $\pm$ 1.68 bc
<i>B. bassiana</i> BbKo	30.00 $\pm$ 1.83 bc
<i>B. bassiana</i> KT2B21	25.00 $\pm$ 3.61 c
Control	24.95 $\pm$ 0.83 c

*Numbers in the same column followed by different letters indicate significant differences based on a LSD (5%)

Number of Leaves

Applying *B. bassiana* on chili plants with different isolates has impacted the number of leaves. Generally, chili plants treated with *B. bassiana* isolates exhibited a significantly higher number of leaves in the WS, TD312, and KT2B21 isolate treatments. The BbKo isolate resulted in the lowest number of leaves, which was not significantly different from the control (Table 4).

Table 4. Number of leaves of chili plants after application of different *B. bassiana* isolates

<i>B. bassiana</i> isolates	Leaves (leaf) $\pm$ SE
<i>B. bassiana</i> WS	32.00 $\pm$ 3.39 a
<i>B. bassiana</i> TD312	27.00 $\pm$ 1.08 ab
<i>B. bassiana</i> KT2B21	26.80 $\pm$ 1.38 ab
<i>B. bassiana</i> PD114	25.60 $\pm$ 2.83 bc
<i>B. bassiana</i> BbKo	19.60 $\pm$ 1.02 cd
Control	17.77 $\pm$ 0.63 d

*Numbers in the same column followed by different letters indicate significant differences based on a LSD (5%)

Flowering Time (day)

Applying WS and TD312 *B. bassiana* isolates accelerated flowering times in chili plants (42.80 and 43.40 days, respectively).

On the other hand, KT2B21, PD114, and BbKo isolates showed no significant difference. These findings meant WS and TD312 isolates were effective in accelerating the flowering time compared to the other isolates (Table 5).

Table 5. Time of chili plants flowering after application of different *B. bassiana* isolates

<i>B. bassiana</i> isolates	Flower appearance (day) $\pm$ SE
<i>B. bassiana</i> WS	42.80 $\pm$ 0.24 b
<i>B. bassiana</i> TD312	43.40 $\pm$ 1.08 b
<i>B. bassiana</i> KT2B21	52.20 $\pm$ 1.38 a
<i>B. bassiana</i> PD114	50.00 $\pm$ 2.38 a
<i>B. bassiana</i> BbKo	50.80 $\pm$ 2.92 a
Control	54.13 $\pm$ 2.45 a

*Numbers in the same column followed by different letters indicate significant differences based on a LSD (5%)

Fruit Weight (g)

Applying WS and TD312 *B. bassiana* isolates resulted in the highest chili fruit weights (171.50 and 178.25 g, respectively) and significantly differed from the other treatments. This suggests that WS and TD312 isolates were the most effective in increasing chili fruit weight. In contrast, the KT2B21, PD114, and BbKo isolates led to lower fruit weights, ranging from 66.25-93.50 g (Table 6).

Table 6. Chili fruit weight after application of different *B. bassiana* isolates

<i>B. bassiana</i> isolates	Weight (g)
<i>B. bassiana</i> WS	171.50 $\pm$ 50.7 a
<i>B. bassiana</i> TD312	178.25 $\pm$ 47.6 a
<i>B. bassiana</i> KT2B21	66.25 $\pm$ 16.2 b
<i>B. bassiana</i> PD114	72.00 $\pm$ 11.3 b
<i>B. bassiana</i> BbKo	87.50 $\pm$ 25.0 b
Kontrol	93.50 $\pm$ 15.1 b

*Numbers in the same column followed by different letters indicate significant differences based on a LSD (5%)

Discussion

The application of different isolates of *B. bassiana* in controlling *C. truncatum* in chili plants affects its incubation period, severity,

and incidence of anthracnose. This showed that applying different isolates of *B. bassiana* can inhibit anthracnose development in chili plants. Treatment of different isolates of *B. bassiana* isolates can extend the incubation period of anthracnose. Treatment of KT2B21 *B. bassiana* isolate can extend the incubation period almost twice the normal (Table 1). This delay occurs due to *B. bassiana* extending the incubation period of *C. truncatum*, which is the period between initial infection and the appearance of disease symptoms in plants. By extending the incubation period, the damage to chili plants appears more slowly. Plants have more time to develop naturally. The level of damage caused by *C. truncatum* was lower than that of plants not treated with *B. bassiana* isolates.

Symptoms of *C. truncatum* attack on chili plants appeared on the 3rd day after inoculation in the control treatment (Table 2). The lower incidence of disease compared to the control was due to the ability of *B. bassiana* to induce plants to produce phenolic compounds. Phenolic compounds will form the most important defense reaction against pathogens. If phenolic compounds are released quickly, they will effectively prevent pathogen penetration, but if phenolic compounds are released slowly, this can cause the spread of disease and the development of symptoms to be faster (Azadi et al., 2016). Harman et al. (2004) also stated that the growth stimulation activity by entomopathogenic fungi is the same as that of plants treated with rhizobacteria. Meanwhile, according to Ownley et al. (2010), *B. bassiana* is one example of a fungus that induces resistance in plants.

Colletotrichum sp. requires time to penetrate and cause plant disease (Darnetty et al., 2022). Initial symptoms such as discoloration and blackened depressions in chilies are observed around the third day after

inoculation (Figure 2). The protective mechanism of *B. bassiana* as an endophytic fungus in host plants involves competition, parasitism, or antibiotic production, including the synthesis of enzymes, secondary metabolites, and volatile compounds that induce resistance and inhibit the growth of plant disease-causing agents (Lopez et al., 2014; Rivas-Franco et al., 2020; Bamisile et al., 2021). Additionally, *B. bassiana* secretes various enzymes, such as chitinase, protease, amylase, and lipase, which degrade the cell wall layer of pathogenic fungi (Bayu et al., 2021). *B. bassiana* has been found to inhibit ZYMV virus disease in Cucurbitaceae plants (Jaber and Salem, 2014).

Moreover, studies have demonstrated *B. Bassiana* ability to reduce the *Rhizoctonia solani* pathogen in tomato plants (Azadi et al., 2016) and suppress the development of *Botrytis cinerea* in chili and tomato (Barra-Bucarei et al., 2020). *B. bassiana* also produces metabolites that inhibit the growth of pathogenic fungi (Suryanarayanan et al., 2009; Sukapiring et al., 2022). *B. bassiana* also can control plant pathogens (Sinno et al., 2021).

Furthermore, aside from its ability to suppress various plant pathogens, the use of *B. bassiana* on chili plants has been found to promote plant growth. The WS and TD312 *B. bassiana* isolates increased the height of chili plants (40.40 cm and 35.8 cm, respectively) (Table 3). *B. bassiana* produces growth stimulants such as gibberellins, auxins, and cytokinins, which impact the growth of both plumules and radicles (Dai et al., 2008). Applying *B. bassiana* can enhance chili plants' height and leaves number (Bayu et al., 2021).

Application of *B. bassiana* through seed soaking can affect the content of compounds in plants such as the discovery of chemical compounds of the fatty acid group, namely Hexadecanoic acid, methyl ester (CAS) Methyl

palmitate Uniphat A60, n-Hexadecanoic acid Hexadecanoic acid Palmitic acid, 9-Octadecenoic acid, methyl ester, (E)-Elaidic acid, methyl ester, 9-Octadecenoic acid (Z)- (CAS) Oleic acid Red oil Oelsauere, Octadecanoic acid Stearic acid n-Octadecanoic acid Humko, these compounds are primary metabolite compounds used as energy for growth (Saragih et al., 2020).

Applying *B. bassiana* also leads to flower appearance in chili plants (Table 5). The faster flower appearance will increase the production of growth-promoting metabolites (Dai et al., 2008). Additionally, inoculation of *B. bassiana* increases seedling emergence, plant height, number of leaves, and fresh root weight in shallots (Yuliana et al., 2019). *B. bassiana* can develop systemically in all rice plant tissues (Hendra et al., 2022); (Liswandi et al., 2023), and it can colonize seeds and chili plant seedlings through the seed-soaking method (Saragih et al., 2020; Akbar et al., 2022).

The PD114 and TD312 *B. bassiana* isolates have shown greater efficacy in suppressing seed-borne pathogens and promoting the growth of chili seedlings (Cyntia, 2020). Fatty acid compounds, commonly present in plants, serve as energy sources for cells, reinforce cell membranes, and provide protection against drought, mineral loss, and pathogens (De Carvalho and Caramujo, 2018); (Trenkamp et al., 2004); (Al-Khayri et al., 2023). Additionally, the application of *B. bassiana* through seed soaking has been observed to enhance the germination and growth of chili plants by establishing a presence in the roots, stems, and leaves (Saragih et al., 2020).

Conclusion

The results showed that *B. bassiana* significantly suppressed *C. truncatum*, prolonging the incubation period (5.40 days)

and reducing disease incidence (16%). Although disease severity was not significantly affected, the application of *B. bassiana* notably enhanced plant growth, increasing plant height (40.40 cm), number of leaves (32 leaves), and accelerating flowering (42.80 days), boosted chili production (178.25 g per plant). Among the tested isolates, *B. bassiana* WS and *B. bassiana* TD312 exhibited the most promising effects, indicating their strong potential for integrated disease management (IDM) and sustainable chili production. This study highlights the dual role of *B. bassiana* as a biocontrol agent and a plant growth promoter, underscoring its potential as an eco-friendly alternative.

Declaration

Author contribution

Dini Puspita Yanty is the main contributor, Trizelia is corresponding author, Darnetty and Jumsu Trisno are co-author for this paper. All authors read and approved the final paper.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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