

Development of *Trichoderma* Formulation and Application to Control Durian Anthracnose Disease

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Abstract

Anthracnose is a plant fungal disease that damages both quantitative and qualitative crop yields. Biological control of plant disease is an alternative method enabling reduced use of chemicals. The objective of this research was to develop a formulation of *Trichoderma* for controlling plant anthracnose disease. The efficacy of *T. asperellum* MSU007 in controlling *Colletotrichum* sp. by dual culture technique showed that *T. asperellum* MSU007 was able to inhibit the mycelium growth of *Colletotrichum* sp. with 50.76 - 74.85 % inhibition. Three formulations of *T. asperellum* MSU007 were developed. The viability of *T. asperellum* MSU007 in the formulation after drying at 45 °C showed that the viabilities of formulation 2 and 3 were not significantly different at 5.26×10^8 and 5.30×10^8 cfu/g, respectively, while that of formulation 1, the viability of *T. asperellum* MSU007 was reduced to 3.73×10^6 cfu/g. After storage at room temperature for 30 days, formulations 2 and 3 did not result in significantly different viability of *T. asperellum* MSU007 at 4.40×10^8 and 4.90×10^8 cfu/g, respectively, and after 90 days of storage at room temperature, it was found that only formulation 3 did not decrease *T. asperellum* MSU007 viability at 4.33×10^8 cfu/g. The efficacy of *Trichoderma* bioproduct in inhibiting anthracnose pathogens on durian leaves was tested. It was found that spraying formulation 3 after inoculation of *Colletotrichum* sp. resulted in the least anthracnose severity at 15.67 ± 4.04 %, which was significantly different from other treatments including the use of carbendazim. However, it is necessary to further study the efficacy of *Trichoderma* formulation in the inhibition of durian anthracnose pathogens in field conditions in order to be able to use this bioproduct in future disease control.

Keywords: Bioproduct, *Colletotrichum* sp., Durian anthracnose, Powder formulation, *Trichoderma asperellum* MSU007

Introduction

Anthracnose is one of the most serious diseases of many plant species in many areas where crops are grown. It is common on leaves of seedlings and causes a dieback of twigs. Anthracnose is caused by fungi in the genus *Colletotrichum*. Durian leaf anthracnose disease is caused by *Colletotricum gleosporoides* in association with meteorological nutritional and environmental factors [1,2]. This disease occurs on durian leaves as discrete, round, necrotic leaf spots and irregularly shaped necrotic patches, usually near the margin of the leaf tip. The leaf spots form commonly towards the margins and are tan to dark brown in color, often with a darker border. Infection of young leaf flushes may occur when their emergence coincides with rainy weather. These infections often show up as lesions along the margins of the young bronze or pale green leaves, in which case they are semi-circular in shape. In very humid weather new twigs may show a dark affected area from the tip backwards, sometimes with defoliation of the young shoots. In serious cases of durian anthracnose, the leaf loses the photosynthetic area and finally falls off. The tree will lose strength, affecting the growth and development of the tree [3]. The recommended method to control anthracnose disease in the nursery is to apply foliar sprays. Spraying fungicides such as benomyl, thiophanate methyl or carbendazim with the combination of chlorothalonil, propineb, menthiram, mancozeb and maneb will be suitable. Four fungicides have been traditionally used in durian orchards; carbendazim, trifloxystrobin, prochloraz and mancozeb. There are 3 other fungicides (pyraclostrobin, difenoconazole and hexaconazole) that leave less toxic residue than the first 4 types [2-4].

Chemically synthesized pesticides have been used for several years to protect crops from disease. In recent years, environmental pollution due to excessive use of such synthetic chemicals has become a major environmental and social problem. At the same time, increasing production to obtain high yields from limited cultivated areas is an important aspect in agriculture. With such a background, the development of microbial pesticides using microorganisms present in nature is accepted to reduce environmental pollution, and the risk of chemical residue build-up in the food chain while maintaining efficient agricultural production [5-7]. A widely used organism in the biocontrol of plant diseases is *Trichoderma*. The biological activities of species of *Trichoderma*, which are commercially used as biocontrol agents against plant fungal pathogens, are based on different mechanisms, such as the production of antifungal metabolites, mycoparasitism and competition for space and nutrients [8]. *Trichoderma*-based biocontrol agents also possess the ability to promote plant growth and soil remediation activity. Their capability to synthesize different antagonistic compounds and micro-nutrients enhance their biocontrol activity [9].

One approach includes the use of biological control agents and their products as alternatives to synthetic agrochemicals. Many strains of *Trichoderma* spp. are widely studied fungi and are among the most commonly used microbial biological control agents in agriculture. They are presently marketed as bio-pesticides, biofertilizers, growth enhancers and stimulants of natural resistance. The efficacy of those fungi can be attributed to their ability to protect plants, enhance vegetative growth and contain pathogen populations under numerous agricultural conditions, as well as acting as soil amendments for improvement of nutrient ability, decomposition and biodegradation. The viable fungal spores are incorporated in various formulations, both traditional and innovative, for applications as foliar sprays, pre-planting applications to seeds or propagation material, post-pruning treatments, incorporation in the soil during seeding or transplant, watering by irrigation or are applied as a root drench or dip. The most common formulation is a wettable powder, followed by granules [10]. The commercial success of products containing these fungal biocontrol agents can also be attributed to the large volume of viable propagules that can be produced rapidly and readily on numerous substrates at a low cost in diverse industrial fermentation systems [11]. The living microorganisms can be incorporated into various formulations as conidia suspensions, in liquid culture filtrates, and can be integrated with various inert components and stored for months without losing efficacy [12]. The products are used in the greenhouse, nursery, field and orchards as well as in hydroponics. *Trichoderma*-based preparations are commercialized worldwide and used for crop protection from various plant pathogens or to increase plant growth and productivity in a variety of fields, greenhouses, nurseries, horticultural, fruits, trees and ornamental crops [13,14]. In general, products formed from solid or semi solid-state fermentation do not require sophisticated formulation procedures prior to use. For example, grain or other types of organic matter upon which *Trichoderma* is grown are simply dried, ground and added to the area to be treated. Biomass produced in liquid fermentation can either be separated from medium and concentrated, or entire biomass with medium can be incorporated into dusts, granules, pellets, wettable powders or emulsifiable liquids. The carrier material may be inert or food based or a combination of both. *Trichoderma* spp. can be formulated as pellets, dusts, powders and fluid drill gels [15]. The various types of *Trichoderma* formulations used in the biological control of crop diseases include talc-based formulation, *Trichoderma* is grown in liquid medium and mixed with talc powder in the ratio of 1:2 and dried to 8 % moisture under shade. The talc formulations of *Trichoderma* have a shelf life of 3 - 4 months [16]. Therefore, this research aims to develop a powder formulation of *Trichoderma* and further application of *Trichoderma* bioproduct for the control of anthracnose disease of durian.

Materials and methods

Microorganisms

In 2021, 6 isolates of *Colletotrichum* spp., the causative agent of durian anthracnose were collected from Sisaket province, the northeastern Thailand. The antagonistic fungus (*Trichoderma asperellum* MSU007) was obtained by the Microbiology Laboratory, Department of Biology, Faculty of Science, Mahasarakham University. All fungal isolates were stored in 20 % glycerol at -20 °C until use.

Efficacy of *Trichoderma asperellum* MSU007 to control durian anthracnose pathogen

Trichoderma asperellum MSU007 was *in vitro* tested for antagonistic activity against *Colletotrichum* spp. isolates using the dual culture technique. The experiments were conducted by placing a 0.8 cm diameter disc of 7 days old *T. asperellum* MSU007 on a Potato dextrose agar (PDA) plate and then placing a 0.8 cm diameter disc of each *Colletotrichum* isolate. The discs were placed at 1.5 cm distance from the edge, so they opposed each other on the same diagonal line. Incubation was at 28 °C, 5 replications were used for each *Colletotrichum* isolate and daily measurements of the radial distance of *Colletotrichum* sp. and *T.*

asperellum MSU007 were made. The percent inhibition rate growth (PIRG) was calculated following Sutthisa [17].

$$\text{PIRG} = [(R_C - R_T)/R_C] \times 100$$

where, R_C The mean radius of the pathogenic fungi growing on control plate, and R_T The mean radius of the pathogenic fungi growing on treatment plate.

Study of physical factors affecting growth of *Trichoderma asperellum* MSU007

Determination of growth pH

The experiment was conducted by transferring a 0.8 cm diameter disc of 7 days old *T. asperellum* MSU007 onto the center of PDA plates that adjusted the pH to 4, 5, 6, 7 and 8. Five replicates of each experiment were incubated at 28 °C for 7 days and the results were observed by measuring the diameter of the fungal colonies.

Determination of growth temperature

The experiment was conducted by transferring a 0.8 cm diameter disc of 7 days old *T. asperellum* MSU007 onto the center of the PDA plate. Five replicates of each experiment were performed and incubated at 4, 20, 28 and 37 °C for 7 days. Then, the colony diameter of the fungus was measured.

Preparation of *Trichoderma asperellum* MSU007 formulation

The *T. asperellum* MSU007 formulation was developed by preparing a buffer solution containing 0.2 % carboxyl methyl cellulose (CMC), 0.5 % chitosan and 1.0 % Tween 80. The *T. asperellum* MSU007 conidial suspension (1×10^{10} conidia/mL) was mixed with buffer solution and 100 g carrier (talcum for formulation 1, corn starch for formulation 2 and tapioca starch for formulation 3). Each formula contained components as shown in **Table 1**. The *T. asperellum* MSU007 formulations were dried at 45 °C for 4 days and ground to a fine powder and stored at room temperature [18].

Table 1 Component of *Trichoderma asperellum* MSU007 formulation.

| Component | Formulation 1 | Formulation 2 | Formulation 3 |
|--|---------------|---------------|---------------|
| Carboxyl methyl cellulose (CMC) | 0.2 % | 0.2 % | 0.2 % |
| Chitosan | 0.5 % | 0.5 % | 0.5 % |
| Tween 80 | 1 % | 1 % | 1 % |
| Talcum | 88.3 % | - | - |
| Corn starch | - | 88.3 % | - |
| Tapioca starch | - | - | 88.3 % |
| 10^{10} conidia/mL <i>T. asperellum</i> MSU007 | 10 % | 10 % | 10 % |

Determination of pH

One g of each *T. asperellum* MSU007 formulation was suspended in 99 mL dH₂O. After standing the suspension for 15 min, the pH was measured 3 times with a pH meter.

Determination of moisture content

One g of *T. asperellum* MSU007 formulation was placed in an aluminum foil container and dried at 105 °C in the hot air oven for 3 h. The sample was immediately transferred to a desiccator and allowed to cool down and the weight was recorded according to Sawatphanit *et al.* [19]. Moisture content (%) was calculated using the formula:

$$\frac{(W_2 - W_1) - (W_3 - W_1)}{(W_2 - W_1)} \times 100.$$

where, W_1 is the weight of an aluminum foil container; W_2 is the weight of the formulation in aluminum foil before drying; W_3 is the weight of the formulation in aluminum foil after drying.

Evaluation of viability of *Trichoderma asperellum* MSU007 in a powder formulation

The 3 formulations were stored in aluminum foil bags and maintained at room temperature for 0, 30 and 90 days. Following the storage period, 1 g of each powdered formulation was collected for assessing

the viability of *T. asperellum* MSU007. This assessment was conducted using the serial dilution and plate count techniques, with the fungal colonies grown on PDA plates and the process repeated 3 times for replication.

Efficiency of *T. asperellum* MSU007 formulation to control durian anthracnose disease

Durian leaves (*Durio zibethinus* Murr. cv. Monthong) were sterilized and then inoculated with *Colletotrichum* sp. conidia suspension (10^6 conidia/mL) by the spraying method and then incubated in a humidified box at room temperature for 24 h before spraying treatments. The experiment was performed in a completely randomized design (CRD) with 5 replications. Foliar spraying treatments were as follows: 1) Formulation 1 (80 g/20 L), 2) Formulation 2 (80 g/20 L), 3) Formulation 3 (80 g/20 L), 4) Carbendazim (30 g/20L) and 5) dH₂O as control. Observation of disease incidence and disease severity index modified from the formula of Dembele *et al.* [20] were recorded as follows: 0 = no symptoms, 1 = 1 - 10 %, 2 = 10 - 25 %, 3 = 25 - 50 %, 4 = 50 - 75 % and 5 > 75 % of infected leaves area, respectively.

Statistical analysis of data

All the data were subject to analysis of variance (ANOVA) and treatment mean comparisons were performed using least significant differences at $p = 0.05$ by (LSD).

Results and discussion

Efficacy of *Trichoderma asperellum* MSU007 to control durian anthracnose pathogen

The efficacy of *T. asperellum* MSU007 in controlling *Colletotrichum* sp., a causative agent of durian anthracnose pathogen, by dual culture technique was recorded 7 days after inoculation. The results revealed that *T. asperellum* MSU007 inhibited the mycelial growth of the pathogen, achieving a percent inhibition rate growth ranging from 50.76 ± 3.47 to 74.85 ± 2.50 % (Table 2, Figure 1). These findings align with previous research by Cruz-Quiroz *et al.* [21], who evaluated the potential of *Trichoderma* strains against *Colletotrichum gloeosporioides* and reported a maximum inhibition percentage of 22.5 %. Additionally, Rana *et al.* [22] conducted a laboratory investigation on various *Trichoderma* species and found that all 5 species effectively inhibited the mycelial growth of *Colletotrichum falcatum*. Notably, *Trichoderma harzianum* demonstrated the highest growth inhibition percentage (84.4 %) in the dual culture setup, while *T. koningii* exhibited the lowest inhibition percentage (76.2 %). Moreover, Shovan *et al.* [23] collected 20 isolates of *T. harzianum* from the rhizosphere and rhizoplane of different crops and assessed their antagonistic effects against *Colletotrichum dematium* using the dual plate culture technique. The screened *Trichoderma* isolates exhibited substantial variability in antagonism, with reductions in radial growth of *C. dematium* ranging from 50.93 to 89.44 %. Among the promising antagonists, the T3 isolate of *T. harzianum* displayed the highest inhibition percentage, suppressing the radial growth of *C. dematium* by 89.44 %. *T. asperellum* TC01 was able to reduce the disease severity of *C. gloeosporioides* C62, the anthracnose of the tea plant (*Camellia sinensis*) [24]. The *in vitro* inhibiting *Colletotrichum* sp., the chili anthracnose was conducted and resulted in the reduction of the intensity of symptoms. The disease suppression showed an increase in fresh fruit weight of chili up to more than 80 % [25]. Other 2 antagonists, *T. asperellum* and *T. harzianum* found to be the potential biocontrol agents against *C. truncatum* causing anthracnose disease in chili. Those antagonistic fungi showed the ability to colonize the roots of chili seedlings and the promotion of various plant growth parameters [26].

Table 2 Efficacy of *Trichoderma asperellum* MSU007 to control *Colletotrichum* spp., a causative agent of anthracnose disease.

| Isolates | Percent inhibition rate growth(%) ^{1/} |
|------------------------------------|---|
| <i>Colletotrichum</i> sp. MSUC-01 | 74.85 ± 2.50^a |
| <i>Colletotrichum</i> sp. MSUUN-01 | 50.83 ± 3.82^d |
| <i>Colletotrichum</i> sp. MSUF-01 | 69.14 ± 2.14^b |
| <i>Colletotrichum</i> sp. SSKF-01 | 61.06 ± 2.79^c |
| <i>Colletotrichum</i> sp. SSKUN-01 | 50.76 ± 3.47^d |
| <i>Colletotrichum</i> sp. SSKUN-02 | 62.00 ± 2.00^c |

^{1/} Different superscripts within the same column indicated significant differences ($p < 0.05$), $n = 5$.

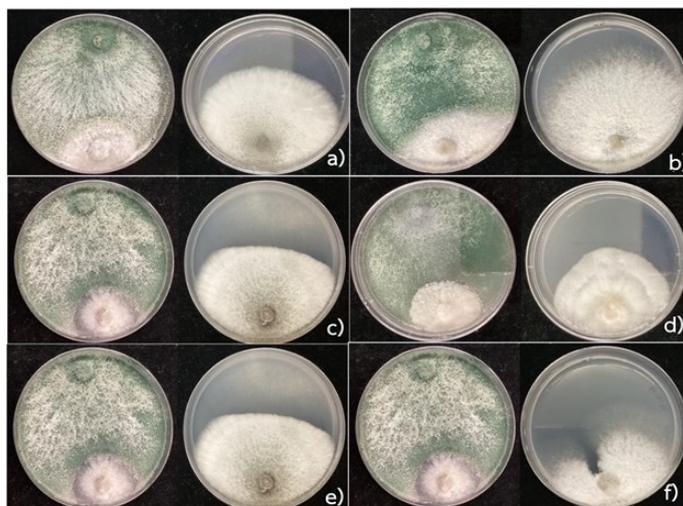


Figure 1 Efficacy of *T. asperellum* MSU007 in controlling anthracnose pathogen by dual culture technique, 7 days after inoculation; a) *Colletotrichum* sp. MSUC-01, b) *Colletotrichum* sp. MSUUN-01, c) *Colletotrichum* sp. MSUF-01, d) *Colletotrichum* sp. SSKF-01, e) *Colletotrichum* sp. SSKUN-01, and f) *Colletotrichum* sp. SSKUN-02.

Study of physical factors affecting growth of *Trichoderma asperellum* MSU007

Determination of pH

The pH conditions suitable for the growth of *T. asperellum* MSU007 were tested by adjusting the pH of the agar medium to 4, 5, 6, 7 and 8, and incubating at 28 °C for 7 days. It was found that the colony diameter of *T. asperellum* MSU007 after all treatments was ranged from 81.58 to 83.18 mm, which was not significantly different statistically (**Table 3**). This is consistent with the results of Singh *et al.* [27] who determined the optimal parameters for the biomass production of *Trichoderma*. A significant difference in the biomass production was recorded among the species at tested pH levels (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0). The most favorable pH ranged between 5.5 and 7.5 in which total dry weight of mycelium varied between 1.41 and 1.35 g. Another study by Kolli *et al.* [28] examined 26 isolates of *Trichoderma* for their pH tolerance and biomass production. It was found that different isolates of same species varied in biomass production at tested pH levels including 4.5, 5.5, 6.5 and 7.5. Sixteen isolates showed highest biomass at pH 7.5 and none at pH 4.5. Among all tested isolates *T. reesei* and *T. longibrachiatum* were less affected by the changes in pH.

Table 3 Study of pH conditions suitable for the growth of *T. asperellum* MSU007.

| pH | Colony diameter (mm) ^{ns} |
|----|------------------------------------|
| 4 | 81.58 ± 1.44 |
| 5 | 82.92 ± 0.92 |
| 6 | 83.18 ± 0.65 |
| 7 | 82.32 ± 1.20 |
| 8 | 81.66 ± 2.03 |

^{ns} not significant

Determination of temperature

The optimum temperature for the growth of *T. asperellum* MSU007 was tested by incubating at 4, 20, 28 and 37 °C. After 7 days of incubation, it was found that incubation at 28 °C resulted in the highest growth of *T. asperellum* MSU007 with a diameter of 84 mm, which was significantly different from the other conditions. *T. asperellum* MSU007 did not grow at 4 °C (**Table 4**). Petrisor *et al.* [29] studied the effect of temperature and pH on the radial growth rate and biomass yield of 2 strains of *Trichoderma* (Td85 and Td50). Four incubation temperatures (15, 25, 30 and 35 °C) and 4 values of pH (4.5, 5.5, 7.5 and 8.5) were tested. A significant difference in biomass production was recorded between strains and among the

pH levels tested. The most favorable pH range was between 4.5 and 5.5 in which biomass yield varied between 1.5 and 2.46 g. The fungus studied tolerated a different range of pH levels, but growth was reduced on alkaline media between 7.5 and 8.5. Both strains grew better at 25 - 30 °C but very slowly at 15 °C.

Table 4 Study of optimum temperature for growth of *T. asperellum* MSU007.

| Temperature (°C) | Colony diameter (mm) ^{1/} |
|------------------|------------------------------------|
| 4 | 0 ± 0.00 ^d |
| 20 | 71.6 ± 4.56 ^b |
| 28 | 84.0 ± 0.00 ^a |
| 37 | 15.0 ± 7.02 ^c |

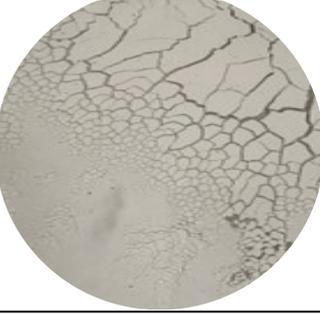
^{1/} Different superscripts within the same column indicated significant differences ($p < 0.05$), n = 5.

Preparation of *Trichoderma asperellum* MSU007 formulation

Formulation development of powdered *T. asperellum* MSU007 fungi

T. asperellum MSU007 underwent a drying process at 45 °C for a duration of 4 days. The resulting powdered formulations exhibited varied colors and textures based on the type of carrier used. Notably, Formulation 1 displayed a pH of 8.51 along with a moisture content of 2 %. Meanwhile, Formulation 2 showed a pH of 5.82 accompanied by a moisture content of 6 %, and Formulation 3 had a pH of 5.77 with a moisture content of 4 % (Table 5).

Table 5 Characteristics of powdered formulation of *T. asperellum* MSU007.

| Formulation | Characters (after 45 °C/4 day drying) | pH | Moisture content (%) |
|-------------|---|-------------|-------------------------|
| 1 | White fine and smooth powder  | 8.51 ± 0.01 | 2.0 ± 0.1 |
| 2 | White fine powder but not smooth  | 5.82 ± 0.02 | 6.0 ± 0.5 |
| 3 | Creamy and silky fine powder  | 5.77 ± 0.02 | 4.0 ± 0.2 |

Evaluation of viability of *Trichoderma asperellum* MSU007 in a powder formulation.

After drying at 45 °C, the viabilities of *T. asperellum* MSU007 in formulations 2 and 3 were not significantly different at 5.26×10^8 and 5.30×10^8 conidia/g, respectively. Formulation 1 decreased viability of *T. asperellum* MSU007 to 3.73×10^6 cfu/g.

The viabilities of *T. asperellum* MSU007 after storage at room temperature for 30 days in formulation 2 and 3 were not significantly different at 4.40×10^8 and 4.90×10^8 cfu/g, respectively. The viability of formulation 1 was reduced to 2.43×10^6 cfu/g.

The evaluation of the viability of *T. asperellum* MSU007 in different formulations after 90-days storage period at room temperature. The study found that only formulation 3 maintained viability without a significant decrease, exhibiting a viability of 4.33×10^8 conidia/g. Formulations 1 and 2 showed no significant difference in survival, with viabilities of 1.93×10^6 and 2.47×10^7 conidia/g, respectively (Table 6). The *Trichoderma* formulations were assessed against specific criteria, including the requirement for a minimum of 2×10^6 colony-forming units (CFU) per mL or g on a selective medium and the need for the moisture content to remain below 8 % in dry fungal formulations [30]. The extended shelf life of a formulated biocontrol agent greatly influences successful marketing. The viability of *Trichoderma* in coffee husk formulation exceeded 18 months, while talcum, peat, lignite and kaolin-based formulations had a shelf life of 3 - 4 months. Viable propagules of *Trichoderma* in talcum formulation decreased by 50 % after 120 days of storage [31]. Sriram *et al.* [32] attempted to enhance the shelf life of talcum formulations by incorporating various ingredients (chitin and glycerol) in the production medium and subjecting them to a heating shock at the end of the logarithmic phase during fermentation. This approach successfully extended the shelf life of the talcum formulation to 1 year.

Table 6 Viability of *T. asperellum* MSU007 in powdered formulation after storage at room temperature.

| Formulation | Viability of <i>T. asperellum</i> MSU007 (cfu/g) ^{1/} | | |
|-------------|--|----------------------|----------------------|
| | 0 days | 30 days | 90 days |
| 1 | 3.73×10^6 b | 2.43×10^6 b | 1.93×10^6 b |
| 2 | 5.26×10^8 a | 4.40×10^8 a | 2.47×10^7 b |
| 3 | 5.30×10^8 a | 4.90×10^8 a | 4.33×10^8 a |

^{1/} Different superscripts within the same column indicated significant differences ($p < 0.05$), n = 3.

Efficiency of *T. asperellum* MSU007 formulation to control durian anthracnose disease

Testing the efficacy of *Trichoderma* biopesticides in inhibiting anthracnose pathogens on durian leaves was done by spraying the conidia suspension of *Colletotrichum* sp. at a density of 10^6 conidia/mL on durian leaves for 24 h. After that, each of the 3 formulations of *T. asperellum* MSU007 (80 g/20 L) was subsequently sprayed on those durian leaves with the comparison with carbendazim (30 g/ 20 L) and sterile distilled water. The results showed that spraying with formulation 3 resulted in the lowest disease index and least anthracnose severity at 15.67 ± 4.04 %, which was significantly different from other treatments, including the use of the carbendazim (Table 7).

Table 7 Efficacy of *T. asperellum* MSU007 formulation for controlling anthracnose on durian leaves.

| Treatment | Disease index | Disease severity (%) ^{1/} |
|-------------------|---------------|------------------------------------|
| Formulation 1 | 3 | 38.33 ± 2.89 ^b |
| Formulation 2 | 3 | 35.00 ± 5.00 ^b |
| Formulation 3 | 2 | 15.67 ± 4.04 ^c |
| Carbendazim | 3 | 31.67 ± 2.89 ^b |
| dH ₂ O | 5 | 80.00 ± 5.00 ^a |

^{1/} Different superscripts within the same column indicated significant differences ($p < 0.05$), n = 5.

In this study, *T. asperellum* MSU007 was subjected to different pH levels on agar medium, ranging from 4 to 8. It was obtained that the colony diameter remained consistently at approximately 81.58 to 83.18 mm, indicating that pH had no significant effect on its growth. This antagonistic fungus thrived best at 28 °C, exhibiting a diameter of 84 mm after 7 days, which significantly outperformed growth under other temperature conditions. The fungal formulation underwent a drying process at 45 °C for 4 days, leading to the development of powdered formulations with diverse colors and textures. These variations were influenced by the type of carrier used, as well as fluctuations in pH levels and moisture content. After a 90 day storage period at room temperature, the viability of the fungus was evaluated. Remarkably, only formulation 3 maintained high viability with 4.33×10^8 conidia/g, while formulations 1 and 2 exhibited lower viabilities. Finally, the efficacy of these formulations was tested for controlling anthracnose pathogens on durian leaves, and formulation 3 yielded the best results, significantly reducing disease severity.

Some isolates of *Trichoderma* sp. have been reported as effective antagonists in controlling anthracnose fungal disease. *T. harzianum* isolate T-39 was obtained to be effective in controlling anthracnose (*Colletotrichum acutatum*) in strawberry, under controlled and greenhouse conditions [33]. Three *Trichoderma* strains (T-39, T-161 and T-166) were evaluated in large-scale experiments using different timing applications and dosage rates for the reduction of strawberry anthracnose. All possible combinations of single, double or triple mixtures of *Trichoderma* strains, applied at 0.4 and 0.8 % concentrations, and at 7- or 10-day intervals, resulted in the reduction of anthracnose severity. The higher concentration (0.8 %) was superior in control whether used with single isolates or as a result of combined application of 2 isolates, each at 0.4 % [33]. Singh *et al.* [7] studied the effect of different agrowastes on the population density and shelf life of *T. harzianum* and indicated that all the substrates showed effective propagation of *T. harzianum*. However, the population counting after 30 days was maximum in the use of tea leaves (8×10^8 cfu/g) and shelf life was found to be maximum (2.9×10^5 cfu/g after 210 days) in wheat bran-sawdust. The application of these formulations on chickpea and groundnut plants significantly reduced the percent mortality due to the chickpea wilt complex and groundnut collar rot diseases. Wijesinghe *et al.* [34] developed a formulation of *T. asperellum* to control black rot disease on pineapple caused by *Thielaviopsis paradoxa*. The highest antagonistic activity was achieved when the concentration of *T. asperellum* conidia was 1×10^7 conidia/mL. The highest biomass and number of cfu/mL of the *T. asperellum* peaked at 144 h after incubation in a yeast waste residue medium. Complete mycelial growth inhibition occurred at concentrations above 52,600 mg/mL.

Conclusions

T. asperellum MSU007 was effective in controlling various isolates of *Colletotrichum* sp., the durian anthracnose, both *in vitro* and *in vivo*. Three formulations of *T. asperellum* MSU007 were developed. Formulation 3 which used Tapioca starch as a carrier was the most effective in controlling anthracnose on durian leaves with disease severity decreased at 15.67 ± 4.04 %. This formulation expressed a high potential to be used as an alternative biofungicide with a convenient preparation. However, it is necessary to further study the efficacy of MSU007 formulation in the inhibition of durian anthracnose pathogens in field conditions in order to be able to use this bioproduct in future fungal disease control. This antagonistic fungus might be an alternative eco-friendly method for controlling plant pathogens.

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