Dose range of *Trichoderma* pellets on suppressing *Synchytrium pogostemonis* caused budok disease in patchouli plants

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Abstract. Budok disease is a condition that often attacks patchouli plants, and can cause warts on leaves, swollen and thickened stems; and makes reddish-purple leaves look wrinkled and thick. Pesticides are typically the best solutions for disease control. Alternative treatments that can be used to treat this condition are biological control agents such as *Trichoderma* fungi. This fungus has been produced in pellet form and has been evaluated for its potential to control several important plant diseases. Three-week-old Patchouli seedlings were grown on polibag (5 kg). Application of the pellets were carried out by immersing it in the planting medium according to the dosage range tested. The doses tested were 0 g, 2.5 g, 5 g, 7.5 g and 10 g per polybag. The transmission of *S. pogostemonis* sap was done by using a mechanical method. The measurements of incubation period, leaf area and disease intensity were conducted at 120 days after planting. The result showed that *Trichoderma* pellets could suppress the development of *S. pogostemonis* in patchouli plants. When the dose was raised to 10 g, symptoms of budok disease appeared such as warts on leaves, wrinkled and thick leaves with purplish red colour, which eventually led to leaf malformation.

1. Introduction

Patchouli (*Pogostemon cablin*) is a plant native to the tropical regions of Asia. The species of family *Lamiaceae*, commonly called the "mint" or "deadnettle" family, is extensively cultivated in China, Indonesia, Cambodia, Myanmar, Vietnam and South America. Patchouli plants produce an essential oil that plays an important role as a source of foreign exchange through their contribution to the value of material exports.

Since 2010, patchouli production was 2,206 tons, but in 2005 it decreased to 1,986 tons and in 2016 decreased again to 1,954 tons [1]. As the largest patchouli oil producer, Aceh Province in Indonesia contributes to 70% of the national production, but until now the quality of the oil produced is quite low.
The decreasing in production was caused by various constraints and difficulties, especially pests and problems related to disease which can affect the development of patchouli production in Indonesia. The effects of budok disease on patchouli cultivation can be economically detrimental if no effort is made to try and control this issue. The loss of total production was caused by high levels of budok disease with a very severe category of (87.56%) [2]. Disease symptoms reported by patchouli farmers are the occurrences of swelling (scabies) or the formation of warts in the form of small lumps at the base of the stems and branches. Other reported symptoms include purplish red leaves accompanied by malformations as well as plants that fail to grow to their full size [3]. Various types of pesticides have been used as disease control, but currently it is not recommended due to several problems in the agro-ecosystem and risks to human health. Therefore, an alternative method of using biological agents is strongly recommended.

Many efforts have been made to control pathogens, one of which is by using Trichoderma spp fungi as biological agents. The use of biological agents Trichoderma sp. was shown to suppress budok disease 26% more effectively than fungicides with the active ingredient benomyl [4]. Trichoderma sp. was able to suppress and inhibit the growth of Fusarium fungi up to 56.07% through competition, parasitism and antibiosis mechanism. Many studies have been reported that Trichoderma harzianum is the best agent in biological control mechanisms. T. harzianum was able to survive and control growth space (high level of competition) when grown together with pathogens (Sclerotium sp. and Rigidoporus sp.) [5]. The use of T. harzianum as a biological control agent is still considered impractical in field applications because it requires a large number of workers and labor. The use of antagonistic agents as biological agents must be in the right formulation and with easily available ingredients [6]. Trichoderma sp. has been developed in the form of pellet formulations [7]. This pellet formulation is small in size so it is more practical, effective and efficient to carry and apply in the field [7]. Shelf-life tests for 4-week-old Trichoderma pellets were the best time and 30 °C was the best drying temperature for the viability of pellets and ability to inhibit the development of Fusarium oxysporum and Sclerotium rolfsii (in-vitro) [8]. The application of T. harzianum pellet 2.6 g / polybag was able to suppress Fusarium wilt on tomato seedlings up to 100% [9]. Until now, there is still limited information about the effects of Trichoderma pellets in suppressing budok disease, as well as the effective dosage on patchouli seedlings. Based on the description above, it is necessary to conduct research to find out whether Trichoderma pellets can reduce budok disease in patchouli plants.

2. Materials and methods

2.1. T. harzianum propagation

Propagation of T. harzianum isolates was carried out aseptically in a laminar air flow cabinet. A small portion of fungi then was transferred to a petri dish containing Potato Dextrose Agar (PDA) media by using a scalpel knife and incubated at 25 °C for 5 days. T. harzianum isolates used in this study came from nutmeg plants which were part of the collections of the Plant Disease Laboratory, Faculty of Agriculture, Syiah Kuala University.

2.2. Formulation trichoderma pellet

Pellet formulation prepared by the method refer to Zikria [10] with modification. The basic ingredients used in making pellets were tofu and bran. Furthermore, the pellets were put into opaque paper and stored in an incubator at 30 °C for 48 hours. If the pellet water content had reached 12%, then the pellets were stored in a plastic valve and were deemed ready to be applied to the plant.

2.3. Applications of T. harzianum pellet on patchouli plant

Three-week-old patchouli plants were grown on polybag which contained 5 kg of soil. The application of pellet was carried out by immersing it into the planting medium according to the dosage tested. The doses tested were 0 g (Control), 2.5 g (D1), 5 g (D2), 7.5 g (D3) and 10 g (D4) per polybag.
2.4. **Transmission of sap to patchouli plants**
The transmission of *S. pogostemonis* sap was done by using mechanical method [11]. *S. pogostemonis* inoculum was prepared in the form of sap. Patchouli branches and leaves that showed symptoms of the disease were obtained from the village of Lhok Bengkuang, Tapak Tuan Subdistrict, South Aceh Regency. Samples were then washed, weighed as much as 10 g, cut into pieces and crushed using a blender by adding 100 ml of distilled water and finally filtered. Transmission of sap on healthy 3-week-old patchouli leaves was carried out by inoculating the unaffected young leaves. Before inoculation, the leaves’ surfaces were injured by using a pin, then the prepared sap was inoculated by spraying it on the leaves’ surfaces using a hand sprayer.

2.5. **Parameters observed**

2.5.1 **Morphology of patchouli plants.** The morphology of patchouli plants was observed by visually observing the tested patchouli plants. This morphology was observed in leaf shape, color, leaf size and stems.

2.5.2 **Incubation period (days).** Observation of incubation period was carried out from day 1 after inoculation until the appearance of the first symptoms.

2.5.3 **Plant leaf area (cm$^2$).** Observation of plant leaf area was measured by using Leaf Area Meter (LAM).

2.5.4 **Disease intensity (%).** Measurement of disease intensity was carried out at 120 days after planting or at the end of the study. The disease intensity was calculated using the formula referred to by Boggie and Person [12]

\[
\text{Disease intensity} = \frac{\sum (n \times v)}{N \times Z} \times 100\% 
\]

Information;

\( n = \) Number of plants for each attack category
\( v = \) Score for each attack category
\( N = \) Number of plants observed
\( Z = \) Score from the highest attack category

The category of budok disease intensity according to Kusnanta [13] is as follows:
0 = parts of plants attacked 0%, healthy
1 = parts of plants attacked 1-25%, mild attack
2 = parts of plants attacked 26-50%, medium attack
3 = parts of plants attacked 51-75%, severe attack
4 = parts of plants attacked > 75%, advance attack

2.6. **Statistical analysis**
Data was statistically analyzed for Analysis of Variance (ANOVA). Significant differences between mean values were determined using Least Significant Different (P=0.05).

3. **Results and discussions**

3.1. **Budok disease symptom**
Budok disease symptoms at 30 days after planting were observed. Results showed that the occurrence of budok disease was supported by the weather, due to rain which occurred several days after inoculation of the pathogens. The development of pathogens that cause budok disease (*S. pogostemonis*) is strongly influenced by environmental factors such as temperature, humidity, rainfall, chemical properties, soil
structure and fertility [14]. Other factors are the source of the inocula, the number of populations and also the age of the inocula. In this study, the morphological observations on patchouli plant showed that symptoms of budok disease started with the appearance of brownish purple spots on the leaf surface. During the next stage the leaves thickened and the thickness exceeded normal leaves. Leaf area shranked and became malformed, was oval shaped and slightly different from patchouli leaves in general. The color of the leaves was not green, but faded green and some were yellow. The disorder then continued to a more severe level of attack, which spread to other leaves in one stem. The newly formed leaves were small, stiff, thickened and purplish red. Although the symptoms of budok disease interfered with the growth of patchouli plants, these plants were still able to survive and took time to die. Symptoms that appeared were almost the same as the study results conducted by Wahyuno [15].

Figure 1. Budok disease symptom in patchouli plant.

3.2. Incubation period (days)
Observation of incubation period was carried out to determine the length of time between the time of inoculation and the appearance of the first symptom. Observation of the initial symptoms was characterized by the appearance of disease symptoms in the form of brownish purple spots on the leaf surface and thickening of the leaves exceeded normal leaves. Figure 2 shows that control treatment (D0) was not significantly different from treatment of T. harzianum pellet with doses of 2.5 g/ polybag (D1), 5 g/ polybag (D2) and 10 g/ polybag (D4) but very different from treatment of T. harzianum at dose of 7.5 g/ polybag (D3). The dose of T. harzianum pellets significantly affected the incubation period caused by S. pogostemonis on patchouli seedlings.

Statistically, the increasing of pellet dose applied to patchouli seedlings did not affect the incubation period of S. pogostemonis, but differences were seen in the mean incubation period. Control treatment (D0) had the shortest incubation period i.e. 4.75 days and treatment of T. harzianum pellets at a dose of 7.5 g/ polybag (D3) had the longest incubation period of 8 days.

The use of antagonistic fungi such as T. harzianum increased the resistance of patchouli plants. The plant protection mechanism by Trichoderma sp. not only attacks pathogens, but also involves the production of several secondary metabolites which function to increase plant growth and stimulate the plant’s own defense mechanisms [16]. Trichoderma can also be marketed as bio-pesticides, biofertilizers, growth enhancers and stimulants of the plant’s natural resistance. Trichoderma can be attributed not only to protecting plants but also to promote vegetative growth; as well as acts as soil amendment/ inoculant to improves nutrient content, decomposition and biodegradation [17].
### 3.3. Plant leaf area (cm²)

The results showed that pellet dose significantly affected the leaf area of patchouli plants at 120 days after planting observation or at the end of the study (Table 1).

**Table 1.** Leaf area of patchouli plants after treated with different dose of *T. harzianum* pellet.

| Symbol | Treatment   | Leaf area (cm²) |
|--------|-------------|-----------------|
| D0     | Control     | 3.18 (1.76)a    |
| D1     | 2.5 g/ polybag | 34.40 (5.79)b  |
| D2     | 5.0 g/ polybag | 43.13 (6.49)b  |
| D3     | 7.5 g/ polybag | 61.33 (7.75)bc |
| D4     | 10.0 g/ polybag | 35.45 (5.91)b  |

| BNT$_{0.05}$ | 1.49 |

**Description:** The numbers followed by letters that are not significantly different based on the BNT test at the level of 5%. The number inside () is the number of transformed √x.

The leaf area of patchouli plants at control (D0) was significantly different from all treatments. However, the treatment of *T. harzianum* pellet at a dose of 7.5 g/ polybag (D3) was not significantly different from dose of 2.5 g/ polybag (D1), 5 g/ polybag (D2) and 10 g/ polybag (D3). Results showed that the presence of *T. harzianum* pellets had a positive response on the patchouli leaf area while the untreated one showed low leaf area. The infected leaves were very visible from their small and thickened shape, rough surface and highly visible leaf bones. According to Nurhasanah [18], leaves damaged by *S. pogostemonis* infection will cause low leaf quality and a decrease in production of essential oils contained within. The conditions mentioned above are caused by the non-optimal physiological processes of the plant due to disruption of nutrients and water absorption which resulted in disruption of the photosynthesis process, which has an impact on non-optimal plant growth. In addition, there are three types of mechanisms possessed by antagonist agents against pathogens; namely competition, antibiosis, mycoparasitism, competing for nutrition, space and oxygen cause the pathogen to be depressed and eventually die [19]. *Trichoderma* also has another mechanism by producing chitinase enzymes to break down the cell wall of pathogen and change the cytoplasmic composition of fungal pathogenic cells [20].
3.4. Disease intensity (%)
Infected leaves were characterized by purple spots, thickened leaves, dwarf plants, malformations of new leaves and leaves that grew smaller than normal, in contrast to the normal patchouli leaves. Even some plants experienced molting and those left with dry twigs eventually died. Application of *T. harzianum* pellets at various doses tested had significant effects on the disease intensity in patchouli plants. The intensity of budok disease in patchouli plants treated with different doses of *T. harzianum* pellets at 120 days after planting (DAP) can be seen in Figure 3.

![Figure 3. Disease intensity in patchouli plants treated with different doses of *T. harzianum* pellet at 120 days after planting (DAP).](image)

The disease intensity in Control was 77.63% and was the heaviest compared to other treatments. Control was not treated with *T. harzianum* pellets but only inoculated with the *S. pogostemonis* pathogen. *T. harzianum* pellets at doses of 2.5 g/ polybag (D1), 5 g/ polybag (D2), 7.5 g/ polybag (D3) and 10 g/ polybag (D4) showed lower disease intensity at 26.12%, 14.48%, 6.11% and 11.81% respectively. Disease intensity in the control treatment was categorized as a severe attack, while *T. harzianum* pellets at 0.5 g/ polybag (D1) were categorized as medium attacks, then at 5 g/ polybag (D2), 7.5 g/ polybag (D3) and 10 g/ polybag (D4) were categorized as mild attacks. It was suspected that *T. harzianum* has the ability to inhibit and suppress the development of *S. pogostemonis* with various antagonistic mechanisms, such as competition, antibiosis and mycoparasites.

This low disease intensity might also be caused by several different factors. According to Siboe [14] and Abdullah et al., [21] the pathogenicity of a *S. pogostemonis* is influenced by internal factors such as the age and physical condition of the pathogen itself as well as external factors such as climate and environmental conditions. The age of *S. pogostemonis* inoculums used in this study was more than 24 hours after being taken from its place, which was 72 hours. This might be the reason why all treatments showed the low disease intensity. Idris and Nasrun [3] inoculated *S. pogostemonis* on plants with inoculum ages of 1, 24, 48, 60 and 72 hours. The highest disease intensity was found at 1 hour-aged of inoculum which was 90.24% and 24 hours-aged of inoculum at 87.45%. Meanwhile, the use of inoculums material that was more than 24 hours showed lower pathogenicity. According to Dayal [22], *S. pogostemonis* pathogenicity may decrease because in its development this fungus produces active zoospores and resting spores that depended on environmental and plant conditions [23].

External factors of environmental conditions such as climate also affect the development of pathogens. The climate aspect that is thought to affect the distribution and development of *S. pogostemonis* the most is rainfall. Disease development is relatively faster in the rainy season than in the dry season. This study was conducted from April to July with relatively low rainfall. The same result
was found by Nurmansyah [2], patchouli plants that were inoculated with *S. pogostemonis* pathogens in June had a disease intensity of 1.6%, in July it reached 4.7% and August reached 32.1%. High rainfall suitable for the growth of *S. pogostemonis* pathogens were during the periods of August-October.

4. Conclusions
The application of *Trichoderma harzianum* pellets at the dose of 7.5 g/ polybag was found to suppress the development of *Synchytrium pogostemonis* in patchouli seedlings. When *T. harzianum* pellets were applied to more than 7.5 g/ polybag, it was shown to have a negative impact on the plant. The dose of 7.5 g/ polybag is the effective dose for controlling budok disease caused by *Synchytrium pogostemonis*.

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**Acknowledgements**

This work was supported and funded by PSN, Penelitian Strategis Nasional Institusi, Ministry of Research, Technology and Higher Education No. 99/UN 11.2/PP/SP3/2018. The author would like to thank Atsiri Research Centre of Syiah Kuala University for dissemination of *Trichoderma* pellet product supported and Yusmaini as Laboratory Assistant for helping this research.