Evaluating the efficacy of *Trichoderma harzianum* and *Bacillus thuringiensis* on induce the plant growth and resistance of local variety patchouli under covered and uncovered seedlings methods

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**Abstract.** To increase plant resistance from an early age, it is necessary to introduce biological control agents from groups of fungi and bacteria. This study aims to determine the effect of *Trichoderma harzianum* and *Bacillus thuringiensis* Aceh isolates in increasing the superiority of Aceh patchouli plants that are resistant to pests and plant diseases. The study used non-factorial RAL method with cover and uncovered seedling methods. Both series were treated with the same biological control agent, the control without any treatment, the treatment of *T. harzianum* and *B. thuringiensis* while the observations were made when the seedling covered was opened. Observations included plant morphological characters, plant growth development and peroxidase enzymes. The results showed that morphologically the original patchouli growing in Lhoong district had similar morphological characters to the Lhokseumawe variety. The application of biological control agents of the *T. harzianum* and *B. thuringiensis* groups was more effective in increasing plant growth in the closed seedling treatment compared to the uncovered seedling. *T. harzianum* gave the best effect at a dose range of 1-1.5 while *B. thuringiensis* showed a better effect at a concentration of 10-15 ml. Both treatments increased the growth of patchouli seedlings as indicated by the better plant height and number of shoots. Furthermore, higher peroxidation enzymes were found in the closed seedling treatment with 1.5 g *T. harzianum* and 15 ml *B. thuringiensis*. The high peroxidase enzyme as an indicator of the more resistant plants have been induced to pests and diseases. From the screen house experiment, *T. harzianum* and *B. thuringiensis* were more efficient in inducing plant growth and disease resistance of local varieties of patchouli using the closed seedling method.
1. Introduction

Aceh Province is one of the centres for patchouli oil production in Indonesia, which until now has contributed significantly to meet the needs of patchouli oil in the country and exports. One of the districts in the centre of patchouli production in Aceh Province is Aceh Jaya District. The development of patchouli plants in Aceh Province in 2020, both in the central, provincial and district budgets, covers a total area of 95 ha with a seed requirement of 950,000 seeds. To meet the need for seeds in these development activities, an Assessment and Determination of Patchouli Seed Sources was carried out in Aceh Jaya District.

One of the obstacles faced in efforts to sustain the certified patchouli seed garden is the presence of pests and diseases. When the garden is attacked by pests or diseases, the certificate of the seed will be revoked. Therefore, it is necessary to make efforts to maintain the superiority of seeds, especially soil against pests and diseases from an early age by observing the growth process until there are symptoms of pest and disease attacks.

Teungoh Geunteut Village, Lhoong Aceh Besar District borders the Aceh Jaya district. Local patchouli varieties that have adapted for a long time are found in the village. This seed has an important role as a garden source for patchouli seeds because this variety is classified as a type that has been widely adapted and specific to local locations. Local varieties have genetic diversity that is still natural. One of the sources of genes used for plant improvement is to look for the remaining natural genetic diversity [1].

The use of microbial antagonist agents to control disease, one of which is *Trichoderma harzianum*. This fungus is a soil saprophytic fungus, free-living, and has a high interaction in the root system. *T. harzianum* is reported as a biological control agent with antagonistic mechanisms such as competition, mycoparasitism and antibiosis, besides that it can also produce toxins, enzymes, and is able to inhibit or degrade enzymes [2]; [3]. Meanwhile, from the group of bacteria, namely *Bacillus* sp., *Pseudomonas* sp., *Arthrobacter* sp., capable of dissolving bound P, and capable of producing growth hormone (IAA). PGPR bacteria can increase plant growth and crop production. PGPR bacteria can inhibit the growth of pathogens indirectly, through the synthesis of antibiotic compounds as biological control [4]. Another function of Biological Control Agents (BCA) of the two microbial groups is to stimulate plant resistance to pathogen attack. The two BCA s are thought to affect plant resistance and the superiority of patchouli seedlings as the main ingredient in Aceh patchouli propagation.

To prove the role of the two BCA microbes on patchouli seedlings, it is necessary to test especially on local superior varieties from the Lhoong Aceh Besar area by applying BCA *T. harzianum* and Bt in hatchery nurseries which aims to determine the effect of BCA Tichoderma and Bt in increasing the superior characteristics. Patchouli seeds one of which is pest resistant.

2. Materials and methods

Plant material local variety of patchouli and originally native in Lhoong village, that is widely planted in patchouli fields in Aceh Besar district, was used in this study. The patchouli seedlings were placed on screen house in field area. The seedling was planted on compost and soil medium at 1:2 composition then placed on polybag. Biological control agents of *T. harzianum* and *B. thuringiensis* inoculum were kindly provided by Plant disease Laboratory, Department of Plant Protection, Faculty of Agriculture, Syiah Kuala University, Indonesia. *T. harzianum* and *T. asperelum*, in the form of pellet, were prepared as described in [5] while the *B. thuringiensis* was cultured on golden snail extracts, were prepared as describe in [6], [7]. The plants were then inoculated with several dose *T. harzianum* (0; 0.5; 1 gr pellet Tichoderma) and several concentrations of *B. thuringiensis* (0; 10; 15 and 20 ml suspension of bacteria). Both the biological control agents *T. harzianum* and *B. thuringiensis* were applied at the leaf, steam of plants and at surface soil then covered with plastic cover.

Performance of Aceh patchouli, which is generally planted in Lhoong, Aceh Besar District observed separately to compare the character morphology of leaf, steam of plant. The observation of
plant growth, pest and disease symptoms, and peroxidase enzyme appeared 20 days post planted (dpp) or at which point the plastic cover was removed.

The test was carried out using a non-factorial randomized block design with 10 replications. The data on plant growth such as plant high statistically analysed for Analysis of Variance (ANOVA). Significant differences between mean values were determined using Least Significant Different (P=0.05).

3. Results and discussion

3.1. Performance of Aceh patchouli generally planted in Lhoong, Aceh Besar District.
Patchouli plant is a type of fibrous rooted plant, The leaves vary from round to oval and the stems are woody with diameter ranges from 10-20 mm. Multiple branching system and stratified around the intermediate trunk (3-5 branches per tier). After 6 months old plant, its height can reach 1 meter with a radius of branches about 60 cm wide. The qualitative characteristics of three varieties of aceh patchouli that can be distinguished by the colour of the base of stem. The tapaktuan variety, the colour of stem base is green with a little purple, the lhokseumawe variety is more purple and the sidikalang variety the most purple (Figure 1).

![Figure 1. Varieties of patchouli Aceh. Tapak Tuan (b) Lhokseumawe and (c) Sidikalang.](image)

Local patchouli variety from Lhoong village, Aceh Besar has been described, the results showed that morphologically the original patchouli growing in Lhoong district had similar morphological characters to the lhokseumawe variety (Figure 2).

![Figure 2. The morphology of the Aceh patchouli generally planted in Lhoong, Aceh.](image)

One of the good Aceh patchouli varieties is the Sidikalang patchouli which is resistant to nematode wilt disease and has wide adaptability [8]. Local varieties of patchouli cultivated in the village of Teungoh Genteut have almost the same morphological characteristics with patchouli from Aceh in
general, the difference the stem more purple and the diameter of about 0.4 cm larger. Characteristics in general were close to the lhokseumawe variety.

3.2. Plant height 30 DAP
Trichoderma harzianum can improve the growth and yield of various agricultural crops. The application of Trichoderma harzianum in increasing plant growth and decreasing inhibition of plant pathogens, such as fungi, bacteria and nematode have been determined by several reports [9]. Other side the endophytic bacteria have been isolated from root of higher plant in Aceh and identified as B. thuringiensis [10], this bacterium could inhibit nematode pathogen growth and fusarium fungi by particled, enzyme and mycotoxin mechanism [10,11].

In this study, two biological agents were used, T. harzianum and B thuringiensis. Both antagonistic agents have been analysed to increase the growth of patchouli seedlings during closed and uncovered seedling methods. The data showed that both bacteria and fungi had affected plant growth, but the control without any treatment, either in the cover or closed, showed a significant difference in plant height. Control plants had lower plant height than all treatments. The highest plant heights were found in T1S, T2S, (Trichoderma 0.5 and 1g/polybag) B1S and B2S (B. thuringiensis 10 and 15 ml/polybag) treatments. However, the lowest plants were found in the KT treatment, namely the open control. In this KT treatment the plants did not develop well and showed the lowest height with an average of 19.30 cm (Figure 3).

![Figure 3. Average patchouli plant height due to application of biological control agent Trichoderma and Bacillus on cover and uncover seedling methods.](image)

This proves that biological control agents really need certain conditions at the beginning of its development, moisture with sufficient water capacity preferable. Formulation of B. thuringiensis endophytic bacteria was able to increase soybean plant height [12]. The bacteria Arthrobacter sp. and B. thuringiensis produced IAA of 0.099 and 0.101, to increase the height of tomato plants [10]. B. thuringiensis was able to increase the height of chili plants up to 106.33 cm [13] giving Trichoderma sp. fungus can help stimulated the growth of tomato plant height, plants can grow well and lead to more optimal nutrient absorption.

3.3 Number of shoots at 30 DAP
The results of average number of shoots due to application of biological control agents under cover and uncover seedling methods can be seen on Figures 4.
Figure 4. Average number of patchouli shoots due to application of biological control agent Trichoderma and Bacillus on cover and uncover seedling methods.

*T. harzianum* and *B. thuringiensis* affected the number of shoots in the open nursery technique. Pseudomonas and Bacillus bacteria produce phytohormones or growth factors (growth regulators), this causes plants to produce hair roots in greater numbers so that it will increase the absorption surface of the roots to absorb nutrients. The phytohormones produced are indole acetic acid (IAA), cytokinins, gibberellins. Light is an absolute factor needed by plants to carry out the process of photosynthesis. In plants, the more light obtained from the environment will cause stunted growth of the plant itself because light will damage the work of the growth hormone (auxin) so that plants that get good light have shorter stems than plants that do not get light.

In addition, the cover and uncover seedling treated by Trichoderma and Bacillus had a very significant effect on the number of shoots of patchouli plants. The higher the dose of *Trichoderma* sp. given, the higher and more the number of plant shoots. That environmental conditions are very important for biological control of plant diseases [14]. Trichoderma isolates grew at temperatures ranging from 12 to 37°C, with maximum growth at 27°C [15]. *B. thuringiensis* chitinase may contribute to the biocontrol of *S. rolfsii* and other phytopathogenic fungi in soybean seeds in Integrated Pest Management programs [16]. When plant continue to growth, the number of shoots will be importance data to see the impact of biological control agents. In this study we finished for 2 months observation and could seeking the early growth of saddling.

3.4 Peroxidase content analysis

Observation of the peroxidase enzyme content was carried out at the end of the observation (30 DAP). to determine the induction ability of *T. harzianum* and *B. thuringiensis* in increasing the resistance of patchouli plants has been analysed. The results of the observation of the peroxidase enzyme content can be seen in Table 5.
The result showed peroxidase enzyme content in treatment application of *T. harzianum* and *B. thuringiensis* under cover gave higher number compare to the open one. Trichoderma which was applied to patchouli plants was then conditioned with high humidity in the absence of air causing the room temperature to decrease. This condition causes an increase in the growth of spores of fungi and bacteria.

4. Conclusions
The application of BCA of the *T. harzianum* and *B. thuringiensis* was more effective in the cover nursery method than in the open nursery method. BCA of the *T. harzianum* and *B. thuringiensis* groups gave the best impact in increasing the growth of patchouli seedlings as indicated by better plant height and number of leaves. Enzyme peroxidation was increased in BCA-applied plants with a sun cup nursery system.

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