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Effect of Ectomycorrhizal Fungi and *Trichoderma harzianum* on the Clove (*Syzygium aromaticum* L.) Seedlings Performances

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Abstract: This study aims to find out the interaction effect of application combination of ectomycorrhizal fungi and Tc-Jjr-02 *Trichoderma harzianum* isolate on initial growth of clove seed (*Syzygium aromaticum* L.). The experiment was arranged factorially by using Completely Randomized Design; first factor: application of ectomycorrhizal fungi (without and with ectomycorrhiza), while the second factor: the application of *Trichoderma* fungi (without and with *T. harzianum*), the experiment was repeated 4 times. The variables observed were: number of leaves, leaf area, stem diameter, root length, root weight, wet weight and dried straw, and intensity of mycorrhiza infection. Data analysis using 5% ANNOVA continued with HSD test. The results showed that there was a significant interaction effect between the combination of ectomycorrhizal fungi and *Trichoderma* application on the number and extent of leaf and the intensity of root infection of clove seedlings. The combination of ectomycorrhizal fungi and *T. harzianum* isolates can be used for the production of healthy clove seedlings.

1 INTRODUCTION

Clove is one of the most popular crops in the world and has high economic value. Meanwhile, so far many clove plants in Indonesia are relatively old and require regeneration. Clove plant regeneration is usually constrained by drought stress and low soil fertility in addition to the threat of plant disease pathogens.

The utilization of mycorrhizal fungi for the development of dryland crops has been widely applied to improve soil fertility and plant growth (Barea *et al.*, 2011; Richardson *et al.*, 2011; Neumann & Matzner, 2005), but specifically on the preparation of seedlings such as cloves relatively have not received adequate attention, especially related to the possibility of creating mutualism symbiosis between rooting and ectomycorrhizal fungi. Ectomycorrhizal fungi help improve plant resistance and absorption of plant nutrients (Steiner *et al.*, 2007).

The attention for protection and maintenance of rooting hygiene based on utilization of ectomycorrhizal fungi as a symbionary would be insufficient for plant growth guidance after planting in the field. It would be required a soil growing media quality and health improvement. The application of *Trichoderma* as planting medium is expected not only

to play a role in the bio-fertilization process and to help increase plant growth (Buysens *et al.*, 2016; Yousef, Tartoura & Abdelraouf, 2016), producing secondary metabolites (Vinale *et al.*, 2008) and phytohormone (Gravel, Antoun & Tweddell, 2007; Chowdappa *et al.*, 2007), but also provide protection for plant health. *Trichoderma* suppresses disease-causing pathogens (Srivastava *et al.*, 2010; Legaya *et al.*, 2016).

Responding to the challenge of rejuvenation of clove plantation in marginal dry land, it is necessary to examine the combination of *Trichoderma* application that plays role in rhizosphere and the application of ectomycorrhizal fungi that plays role in rizosflein and rizosphere in giving effect to the growing ability especially at seedlings and young clove even though live in limited environment.

This study aims to find out the effect of combination application of ectomycorrhizal fungi and *Trichoderma harzianum* against clove seedling growth.

2 MATERIALS AND METHODE

This research was conducted in Laboratory of Microbiology and Green House of Faculty of Agriculture, Campus 2 Gelam, Candi, Sidoarjo at altitude \pm 7 m asl. The average temperature is 27-35°C and the moisture averages 50-60%. The study took place from January to April 2016.

The ectomycorrhizal fungi which isolated from roots of clove plant in Damas village, Watulimo-Trenggalek were grown in PDA-chlramphenicol (Vargas Gil, Pastorb & Marcha, 2009) medium for 8 days to be used in this experiment. Soil was taken from same location of fungi s then sterilized in an autoclave at 121°C 1 atm. Fungi cultures were harvested and diluted with distilled water to obtain a suspension containing 10^8 spores per ml. The suspension is poured and stirred evenly with a sterile soil medium in moisture conditions, and then used for the germination of clove seeds. Two weeks after seed inoculation with ectomycorrhizal fungi, we cultivating Tc-Jjr-02 *Trichoderma harzianum* isolate (collection of Microbiology Laboratory of Agriculture Faculty of Universitas Muhammadiyah Sidoarjo) at the same medium and incubation time. After 10 days of incubation, the culture was harvested and it produced a suspension containing 1.49×10^6 cfu of spores per ml. Furthermore, the suspension is poured into soil medium and sterile compost (3: 1) so we obtained planting medium containing *T. harzianum* 0.831 $\times 10^7$ cfu/ml spores per gram of soil growing media; this is we called as giving ectomycorrhizal treatment. For the treatment without mycorrhiza, both the seedling media and planting medium only given distilled water. Approximately 4 weeks after inoculation, clove seeds and young clove root have been infected, then the clove seedlings are transferred into planting medium and incubated for eight weeks. This factorial experiment comprises the *Trichoderma* application factor which consist without and with *Trichoderma* and ectomycorrhizal application factor which consist without and with ectomycorrhizal. The experiment was repeated four times to obtain 16 experimental units. We observed the plant height and diameter every week; while, the intensity of mycorrhiza infection (Garcia-Gonzales *et al.*, 2005), wet weight and dry weight of stover were observed at the end of experiment or eight weeks after planting (WAP). Data were analysed by multifactor analysis followed by 5% HSD test.

3 RESULTS AND DISCUSSION

Variance analysis result (F test 5% real level) from treatment combination give a significant interaction effect to leaf number and leaf surface area, but its effect is not significant to the height and diameter of seed stem. The treatment mean rate on growth

parameters of clove seedling section is shown in Table 1. Treatment without *Trichoderma* application in non-camicidal seedlings (T0M0) shows the height, leaf number, leaf area, and stem diameter not unlike the application of *Trichoderma* and ectomycorrhiza (T1M1). From this result, we can see that the positive effect in increasing plant growth from the application of both bio-fertilizer agent has not been visible until 7 HST.

Table 1: The mean rate of growth parameter on crown clove seedling^{*)}

Treat-meant	Height (cm)	Leaf number	Leaf area (cm ²)	Stem diameter (cm)
T0M0	6.15	5.25 c	21.35 c	0.22
T0M1	7.03	4.00 ab	11.58 ab	0.21
T1M0	4.78	3.25 a	6.20 a	0.16
T1M1	6.73	4.50 bc	14.73 b	0.23
HSD 5%		0.99	5.42	

^{*)} T0 = without *Trichoderma*, T1 = with *Trichoderma*, M0 = without mycorrhiza, M1 = with mycorrhiza; the numbers followed by same letter in same column show no significant difference in 5% HSD test.

Except for the intensity of mycorrhiza infection, the interaction effect of treatment combination on root performance of clove seed is not real. The mean root roots, root weights, and intensity of mycorrhiza infections are presented in Table 2.

Table2: The mean rate of rooting clove seedling performance^{*)}

Treatment	Root length (cm)	Root weight (gr)	Intensity of mycorrhiza infection (%)
T0M0	10.08	0.20	0.00 a
T0M1	9.85	0.18	58.75 b
T1M0	5.78	0.18	0.00 a
T1M1	7.63	0.14	63.13 b
HSD 5%			38.43

^{*)} T0 = without *Trichoderma*, T1 = with *Trichoderma*, M0 = without mycorrhiza, M1 = with mycorrhiza; the numbers followed by same letter in same column show no significant difference in 5% HSD test.

Root length and root weight in control treatment (T0M0) showed the highest results compared to treatment of *Trichoderma* and ectomycorrhiza (T1M1) applications. This indicates that the activity of *Trichoderma* fungi and ectomycorrhizal fungus suppress the root growth. This difference is possible, given that the mycorrhiza fungi exhaust the energy sourced from the plant root tissue (Heinemeyer *et al.*, 2005; Talbot, Allison & Treseder, 2005; Tomè, Tagliavini & Scandellari, 2005), in addition, it might possible, there is an activity of hydrolytic extracellular enzyme which produced by fungi (Read & Perez-

Moreno, 2005; Geissler & Horwath, 2005) that might disrupt the cell wall of roots. Nevertheless, if we looking at the intensity of mycorrhiza infection with an average of over 60% showing that mycorrhiza seeds with media which enriched by *Trichoderma* has created a high growth potential on next growth phase especially when planted in field. The intensity of mycorrhiza infection will be positively correlated with the number of potential inoculums of mycorrhiza fungi in the soil which will further improve soil fertility (Driver, Holben & Rillig, 2005; Bedini *et al.*, 2007).

4 CONCLUSION

The application combination of ectomycorrhizal fungi which originating from root system of clove tree and Tc-JJr-02 *Trichoderma harzianum* isolate gave a significant interaction effect on the increasing of leaf number and leaf surface area and increasing the intensity of mycorrhiza infection. The implication of this research is that the combination of ectomycorrhizal fungi and *T. harzianum* isolates can be used for the production of healthy clove seedlings

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