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EFFECTIVENESS OF LETTUCE SEED ENCAPSULATION CONTAINING *Trichoderma* sp. IN CONTROL OF *DAMPING-OFF* DISEASE

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Abstract. *Utilization of the antagonist fungus Trichoderma sp. in suppressing damping-off disease caused by Rhizoctonia solani can be done in various ways. One of them is through coating the seeds or encapsulation. This study aims to determine the effectiveness of lettuce seeds encapsulation containing Trichoderma sp. in suppressing damping-off disease through storage time of up to 12 weeks. The results showed that the use of talc and kaolin as a material carrier in encapsulation with 1 week of storage had the highest germination rate of 96%, while the lowest germination was at 12 weeks of storage, which was only 0-5%. The use of talc carrier showed a low percentage of infected seedlings, starting from storage time of 0 to 8 weeks. At 8 weeks of storage, the provision of carrier material in the form of talc showed the lowest percentage of infected seedlings, which was 40% on the last day of observation and had the highest value of effectiveness in controlling Rhizoctonia solani damping-off disease, which was 60%. Thus, seed encapsulation using a talc carrier was the most effective in suppressing damping-off disease up to 8 weeks of storage.*
Keywords: *damping-off; rhizoctonia solani; seed encapsulation; trichoderma sp.*

1. Introduction

Organic vegetables are increasingly high in demand along with the increasing Indonesian awareness about the urge for healthy living. In its cultivation, lettuce also has several problems related to the presence of the plant-pest organism. Plant-pest organism attacks can occur in various phases of lettuce growth, one of which is in the seedling phase, which is commonly known as damping-off. Damping-off is a major fungal that limits agriculture production, causing disease in young seedlings in agricultural crops (Vanti *et al*., 2020). In general, many fungi and fungus-like species such as *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., and *Phytophthora* spp. have been reported as the most commonly considered pathogen as the causative agent of damping-off, the most important biotic stresses that weaken or damage seeds and seedlings of almost all species including fruit, vegetables, farm crops, ornamentals, and forestry crops. (Lamichhane *et al*., 2017).

The application of chemical products that are highly polluting to the environment and sometimes dangerous for health, has been traditionally related to one of the practice of intensive agriculture. A sustainable and eco-friendly control of diseases affecting the early stages of plant growth is essential, in order to guarantee consumer and environmental health (Bourguet and Guillemaud, 2016; Lamichhane *et al*., 2016; Toribio *et al*., 2021). In recent years, *Trichoderma* has acquired high importance because of its fungicidal and fertilizing Potential (Sood *et al*., 2020). According to Abbas *et al.* (2017), the mechanism by which *Trichoderma* strains displace

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phytopathogenic are essential of three types; direct competition for space or nutrients, production of antibiotic metabolites whether volatile or non-volatile nature and direct parasitism of certain species of *Trichoderma* on plant pathogenic fungi. Many studies reported that biological control with the genus *Trichoderma* proved effective in controlling *R. solani* which promotes plant growth and stimulates plant defense responses. The genus of Trichoderma contributes to a large number of its capabilities among different strains as multifunctional fungi that are found in a large variety of ecosystems. Typically, they are found from forest or agricultural soils (Zin & Badaluddin, 2020). In the preliminary in vitro dual culture test, isolates of *Trichoderma* sp. showed a great antagonism ability against the isolated pathogen *R. solani*.

The use of antagonistic fungi in suppressing and controlling disease can be done in various ways. The addition of antagonistic fungi is carried out to protect plants against pathogens. *Trichoderma* sp. as the active ingredient on seed coating can increase germination or seed viability. *Trichoderma* isolates have long been not only recognized as biological agents but also for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients. *Trichoderma* spp. proved their potential as bio-stimulant (Bhardwaj *et al*., 2014). The production of volatile organic compounds (VOCs) and diffusible molecules such as indole-3-acetic acid (IAA) utilized by *Trichoderma* spp. for the communication with plants. *Trichoderma* spp. are common inhabitants of the rhizosphere. (Contreras-Cornejo *et al*., 2022). Seed coating has been widely used in agriculture as an effective means to alleviate biotic and abiotic stresses, thus enhancing crop growth, yield, and health, in order to meet the needs of development of precision agriculture. To improve agricultural productivity via direct application to the rhizosphere and plant tissues, or seed inoculation, essential contributors is recognized with Plant growth promoting microorganisms (PGPM). (Ma, 2019). Seed encapsulation is a seed coating technique that can be applied with fungal spores and bacterial cells. This treatment was proven in the research of Laila *et al*. (2016), who suggested that seed coating with a combination of biological agents *Trichoderma* sp. and *Actinomycetes* sp. has a significant effect on inhibiting the growth of pathogens and has the highest inhibitory power. This study aims to examine the potential of *Trichoderma* sp. through the encapsulation of lettuce seeds and their effect on shelf life, control of damping-off of *Rhizoctoniasolani*, and determine the best carrier material in increasing the antagonistic ability of the fungus *Trichoderma* sp.

2. Methods

The research was conducted from September 2021 to January 2022 at the Plant Health Laboratory of the Faculty of Agriculture, National Development University of East Java "Veteran" and in Sedati Subdistrict, Sidoarjo. The study used a Complete Factorial Random Design (RALF) with 2 factors, namely the type of carrier material (talc, kaolin, and nano biochar), and the length of storage time (0 weeks, 1 week, 4 weeks, 8 weeks, 12 weeks). The results obtained are analyzed using ANOVA (Analysis of Variance). If the conclusion is obtained with the condition that F calculated > F Table 5% then, the average difference between treatments is tested using the DMRT (Duncan Multiple Range Test) at the level of 5%.

2.1 Isolation and Propagation of *Rhizoctonia solani* Pathogens

The isolates of the fungus *Rhizoctoniasolani* used in this study were obtained from the collection of the Plant Health Clinic, Faculty of Agriculture, Padjadjaran University. Isolates of the pathogenic fungus *R. solani* were cultured on rice media by soaking the rice overnight, then putting it in 100 g of heat-resistant plastic and sterilizing it using an autoclave (121^{o}C, 1 atm) for 15 minutes. A total of 3 pieces of *R. solani* culture (0.8 cm in diameter) were inoculated into cold sterile media and then incubated for 2 weeks. This method is adapted from Herawati and Istifadah (2019).

2.2 Isolation and Propagation of Antagonist Fungus *Trichoderma* sp.

Trichoderma sp. isolate used in this study was obtained from the rhizosphere soil in the lettuce nursery area of Kaliandra Organic Farm, Pasuruan. The isolation was carried out by doing a serial dilution of the soil until 10^{-7}, then 100µl of dilution was inoculated on Potato Dextrose Agar (PDA) media. The isolates were isolated on PDA media. *Trichoderma* sp. which will be used for capsule formulation were cultured on PDA slant media and incubated for 7 days before use. *Trichoderma* was harvested from PDA media and diluted to a conidia density of 2.57 x 10^{8}.

2.3 Seed Encapsulation

The encapsulation material used consists of three categories, namely; 1) adhesive (binder) in the form of glucose as much as 5 g, 2) carrier or filler (bulking agent) in the form of nano biochar, talc, kaolin, and CaCo3 each as much as 20 g and has been sterilized using an oven, 3) *Trichoderma* sp. inoculum suspension as an active ingredient with conidia density 2.57 x 10^{8}. A total of 1 g of lettuce seeds were put into an encapsulation bath, then sprayed with adhesive solution 5 times (± 2 ml), then 1 g of dry matter was added. After 1 min, the cycle was stopped, then the coated seeds were filtered through a 200-mesh (75µ) sieve. The treatment consisted of 3 carriers, namely talc (PT), kaolin (PK), and biochar (PB). The second factor is 5 storage times, namely 0 weeks (S0), 1 week (S1), 4 weeks (S4), 8 weeks (S8), and 12 weeks (S12).

2.4 Seed Germination Test

The germination test was carried out on whatman filter paper that had been moistened with sterile distilled water and placed in a 9 cm diameter petri dish. Seeds that have been treated with encapsulation and controls are placed in petri dishes containing moistened filter papers. The seeds were then observed for germination for 7 days. Observations were made to determine the percentage of seed germination or viability. The percentage of seed germination (GR) is calculated using the following Equation 1.

$$GR\ (\%) = \frac{\sum Germinated\ seeds}{\sum Tested\ seeds}\ x\ 100\% \quad (1)$$

2.5 Effect of Encapsulation on *Damping-off*

Seeds are first sown in a nursery tray containing sterile planting media until it is ± 30 days old. Subsequently, the seedlings were planted in 20 x 20 x 5 cm polybags containing sterile soil and compost in a 1:1 ratio. *Rhizoctoniasolani* culture on rice media was placed in each planting hole as much as 0.5 g per hole. The percentage of infected seedlings was calculated based on the number of infected seedlings and symptoms that appeared after the seedlings were 17 days after planting (DAP) or 3 days after transplanting and pathogen inoculation. Observational data is calculated using the following Equation 2 (Nurlela *et al*., 2016).

$$\text{Percentage of Infected Seedlings (K)} = \frac{n\ (Total\ Infected\ Seedlings)}{N\ (Total\ Seedlings)}\ x\ 100\% \quad (2)$$

The effectiveness of control on damping-off disease was obtained from the calculation of the effectiveness of the control Equation 3 for the control of damping-off disease (Gusnawaty, 2011).

$$Effectiveness = \frac{\%\ damping\ off\ in\ control - \%\ damping\ off\ in\ Treatment}{\%\ damping\ off\ in\ control}\ x\ 100\% \quad (3)$$

3. Results and Discussion

3.1 Seed Germination Test Results

The results of the germination test showed that all treatments had a significantly different effect on increasing seed germination. Successful pelleting of seeds requires screening and selecting the most appropriate inert filler materials that are compatible with seed survival and germination (Kangsopa *et al.*, 2018). The highest seed germination from the first day of observation until day 7 of observation was namely PTS1 and PKS1 treatments. Then followed by PTS0, which was 85% on the 1st day and 95% on the 7th day. The lowest germination was at 12 weeks of storage, where seed germination decreased to only 0 – 5% from day 1 to day 7. This is reinforced by research conducted by Putri and Majid (2019), that there is an interaction between the carrier material and storage time in the provision of seed-coating on peanut seeds. The kaolin carrier treatment with 7 days of storage showed the best results on dry weight and seed viability compared to other treatments and was significantly different from the control. The results of the lettuce seed encapsulation germination test are shown in Table 1.

Table 1. Lettuce Seed Encapsulation Germination

Treatment		Seed Germination (%)
Control		73 bc
0 Week	Talc	95 e
	Kaolin	93 e
	Biochar	68 b
1 Week	Talc	96 e
	Kaolin	96 e
	Biochar	73 bc
4 Weeks	Talc	88 cde
	Kaolin	87 cde
	Biochar	77 bcd
8 Weeks	Talc	88 cde
	Kaolin	92 de
	Biochar	93 e
12 Weeks	Talc	0 a
	Kaolin	0 a
	Biochar	5 a

The numbers in the same column followed by unequal letters were significantly different at the 5% DMRT test level.

The highest seed germination on the 7th day of observation was pts1 and PKS1 treatment by 92-93% and 96%, while the lowest germination rate is in the treatment of PTS12, PKS12 and PBS12. Where seed germination is only 0 – 5%. The increase in seed germination is suspected due to the addition of encapsulation carriers in the form of dry ingredients and active ingredients in the form of *Trichoderma* sp. The addition of carrier materials of talc and kaolin is thought to be able to assist in the seed imbibition process, so the water could stick to the carrier material. In addition to the effect of the dry carrier, the active ingredient *Trichoderma* sp. has given effect on seed germination. This is following what was stated by Putri and Majid (2019), that the addition of a biocontrol agent in the form of *T. harzianum* in the coating material affects the percentage of germination, compared to uncoated seeds. This is due to the role of coating materials and *T. harzianum* which is capable of producing indole-3 acetic acid (IAA) and gibberellins. According to Miransari and Smith (2014), gibberellins can stimulate the synthesis of a-amylase which can trigger germination and IAA can stimulate the formation and elongation of roots during the germination process.

3.2 Percentage of Infected Seedlings

Based on the observation, the encapsulation treatment had a lower percentage of damping-off than the control. This observation was done from 3 days after inoculation until the 7th day after inoculation. Carrier materials that have a low average percentage of infected seeds are talc and kaolin carriers. The provision of talc-carrying material shows a low percentage of infested seedlings, namely at 8 weeks storage time, which is 40% on the last day of observation. Meanwhile, the kaolin-carrying material provides the lowest percentage of infested seedlings at a 12-week storage time of 60% on the last day of observation.

Mycoparasitism, antibiosis, and competition for nutrients or space among others which may operate independently or together to suppress plant pathogens are the main three mechanisms of biocontrol of Trichoderma spp. are (Mukhopadhyay & Kumar, 2020). The use of active ingredients in the form of *Trichoderma* sp. is thought to be able to reduce the percentage of seedlings attacked by the mechanism of Trichoderma sp. in the form of direct competition for space and nutrients and direct parasitism. This is reinforced by research conducted by Sinha *et al.* (2018), where wilt disease significantly reduced by 87.5% with a 1% talc-based formulation of Trichoderma harzianum and *T. viride* on seed treatment. Maximum germination along with increases in plant height, root length, and yield are also recorded by the 1% talc-based formulation of *T. harzianum*. The results of the percentage of infected seedlings are shown in Figure 1.

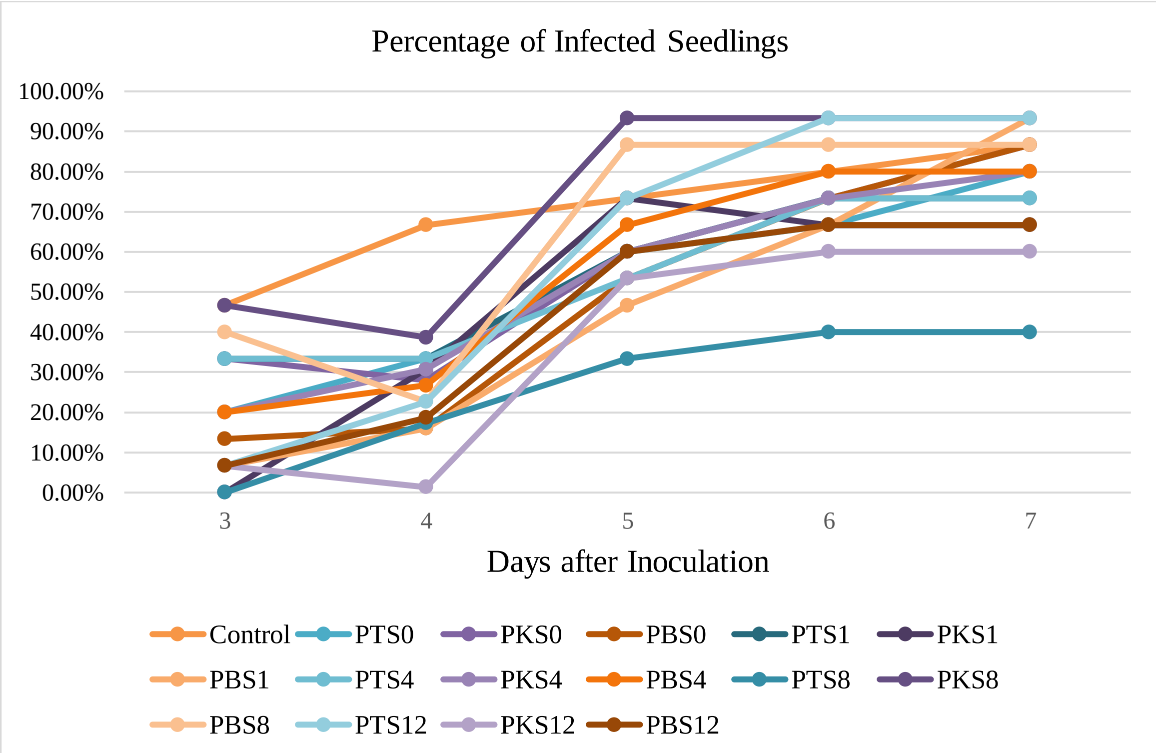

Figure 1. Percentage of Infected Seedlings

3.3 Effectiveness of Control

Based on observations, the control effectiveness at 3 DAI tends to be high, the highest control are on PTS8 and PKS1 treatments, in which the control effectiveness reaches 100%, while the lowest is in the PKS8 treatment. The percentage of control effectiveness tends to decrease with increasing days. However, based on the graph, some treatments tend to constantly show a higher percentage of effectiveness than other treatments. At 5 HSI, the highest percentage of effectiveness was in the PTS8 and PBS0 treatments, which was 66.70%. Meanwhile, on the 6th and 7th days, the treatment with higher control effectiveness than the other treatments were the PTS8 treatment. This is reinforced by research conducted by (Moka *et al.*, 2021) The Talc formulations of the Trichoderma isolate significantly reduced the damping-off disease in onion and enhanced the seed germination and yield of onion in field trials. This is indicated by one of the Trichoderma isolate (Tr55) showed the greatest reduction in the incidence of pre and post-emergence damping-off when applied in seed+soil treatments. Broad-spectrum antagonistic activity and production of the lytic enzyme chitinase are biocontrol traits that also exhibited by the isolate. The results of the effectiveness of the control are shown in Figure 2.

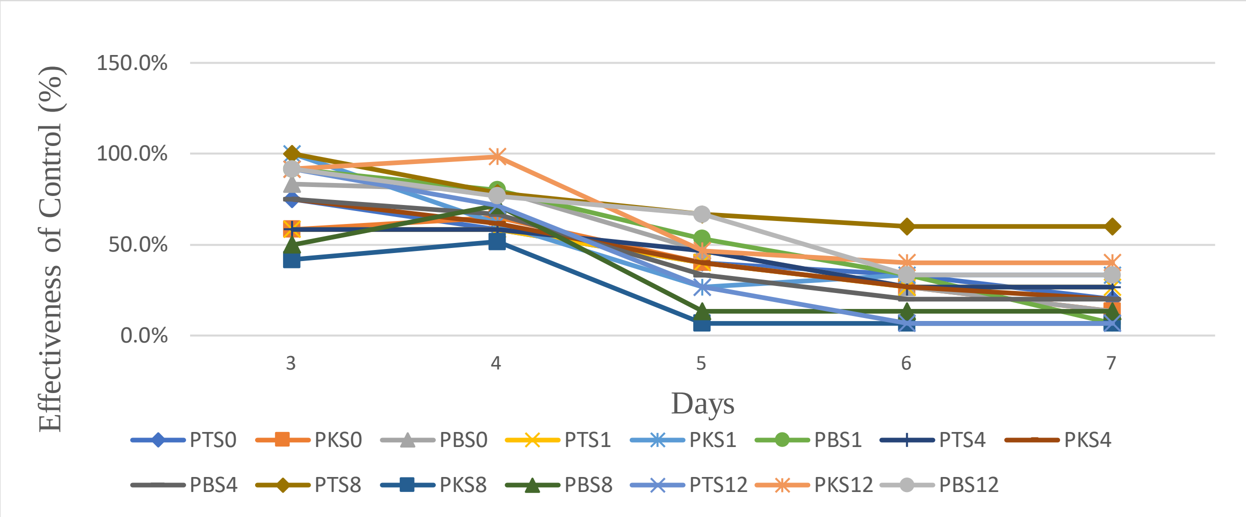

Figure 2. Effectiveness of control of lettuce seeds encapsulation

1. Conclusions

Lettuce seed encapsulation was able to increase the viability or germination of the seeds and the effectiveness of seedling fall control up to 8 weeks of storage. The use of carriers of talc and kaolin in encapsulation with 1 week of storage had the highest germination rate of 96%. The use of talc carrier showed a low percentage of infected seedlings, starting from storage time to 8 weeks. At 8 weeks of storage, the provision of carrier material in the form of talc showed the lowest percentage of infected seedlings, which was 40% on the last day of observation and had the highest value of effectiveness in controlling seedling fall disease, which was 60%. Based on the results of this study, Encapsulation of lettuce seeds with talc carrying material is quite effective (60%) controlling damping-off until the 8th week of storage. However, it is necessary to conduct further research on the production, storage, and selection of materials used for encapsulation of lettuce seeds to obtain a better and more effective product in suppressing damping-off disease.

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