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Research Article

Influence of *Trichoderma harzianum* Alginate Pellet Storage Time on *Sclerotium rolfsii* Damping-Off of Chili

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Abstract

Background and Objective: *Sclerotium rolfsii* is a soil-borne pathogen that has a very wide host range, including chilli plants. This fungus causes damping-off and stems rot diseases. The objective of the research was to evaluate the effect of different storage times of *Trichoderma harzianum* alginate pellet with rice bran on its viability and its potential to control damping-off disease caused by *S. rolfsii* on chilli. **Materials and Methods:** The design used in this research was a Completely Random Design with seven treatments and four replications. The treatments were different storage times of alginate pellet *T. harzianum* (0, 2, 4, 6, 8, 10 and 12 weeks). Observation parameters were viability of alginate pellet, percentage of pre-emergence damping-off, percentage of post-emergence damping-off and number of *T. harzianum* propagules in soil. **Results:** The results showed that the different storage times of alginate pellet *T. harzianum* until 12 weeks did not affect its viability and inhibited the pre and post-emergence damping-off diseases. Also, the numbers of propagules of *T. harzianum* in the soil after 30 days of seeding at different treatments were still high and did not differ among them. **Conclusion:** The storage of *T. harzianum* alginate pellet for up to 12 weeks was still stable and able to suppress the development of *S. rolfsii* damping-off on chilli plant.

Key words: Pre-emergence damping-off disease, post-emergence damping-off disease, alginate pellet of *Trichoderma harzianum*

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The fungus *Sclerotium rolfsii* is a soil-borne pathogen that has a very wide host range, including chilli plants¹. This fungus attacks chilli plants ranging from the seedling phase, known as a damping-off disease to adult plants known as stem rot disease which causes death in a relatively short time. This fungus is difficult to control because it forms the sclerotium, a viable structure that can survive years in the soil without a host plant or in extreme environmental conditions^{1,2}.

The control efforts carried out are generally more emphasized on a chemical control using fungicides. The continuous use of fungicides can cause various negative impacts such as the death of non-target organisms, pathogen resistance and the emergence of residual effects. Therefore there is a need to find an environmentally friendly control such as the use of *Trichoderma harzianum* as biocontrol agents. The fungus *T. harzianum* is an antagonistic fungus with various antagonistic mechanisms such as parasitism, competition and antibiosis³ and it has been tested for its ability to suppress several soil-borne pathogens, including *S. rolfsii*⁴.

The introduction of the biological agent *T. harzianum* requires basic food to adapt to new environments. Besides that, the formulation is one of the main keys that determine the success of a biocontrol agent in controlling plant pathogens. One of the objectives of the biocontrol agent formulation is to stabilize microorganisms during production, distribution and storage⁵. Many research results showed that *T. harzianum* pellets can suppress the attack of various pathogenic fungi such as *Fusarium oxysporum* f. sp. *lycopersici* causing wilt disease on tomatoes⁶ and *Helminthosporium oryzae* causing brown spots on rice⁷.

This study aimed to determine the ability of *T. harzianum* alginate pellets with rice bran in suppressing *S. rolfsii* attacks on chilli plants and the effect of *T. harzianum* pellet storage time on *S. rolfsii* attacks.

MATERIALS AND METHODS

Research site: This research was carried out in the Phytopathology Laboratory and Green House, Agriculture Faculty, Andalas University from July to December, 2019. This study consisted of 2 stages, namely:

- Effect of the storage time of *T. harzianum* pellets on its viability
- Effect of the storage time of *T. harzianum* alginate pellets on *S. rolfsii* attacks on chilli plant

Effect of the storage time of alginate pellet formulation of *T. harzianum* on its viability:

The design used in this experiment was a Completely Randomized Design (CRD) with 7 treatments and 3 replications. The treatments consisted of several different storage times of alginate pellet formulation of *Trichoderma harzianum* with rice bran namely A (0 weeks), B (2 weeks), C (4 weeks), D (6 weeks), E (8 weeks), F (10 weeks) and G (12 weeks).

Preparation of alginate pellet formulation of *T. harzianum*:

The rice bran was mashed with a coffee grinder and the suspension was made by mixing 63 g of rice bran with 450 mL of sterile distilled water. Meanwhile, alginate suspension was prepared by blending 9 g of alginate with 450 mL of sterile distilled water for 1 min. Both suspensions were sterilized by using an autoclave for 20 min at 121°C, mixed and added 150 mL *T. harzianum* suspension (10⁶ conidia mL⁻¹). The mixture was shaken for 5 min. Furthermore, this suspension mixture was added dropwise to 0.2 M CaCl₂. After 20 min the pellets are collected on 1 mm pore size plastic mesh and washed with running water and dried for 24 hrs at room temperature⁸.

Viability of *T. harzianum* on alginate pellets:

Twenty-five pellets were arranged on Petri dishes (9 mm) containing PDA and each treatment consisted of 3 Petri plates and then incubated for 1 week. Observation of the viability of *T. harzianum* in alginate pellets was started on the second to the eighth day after incubation.

Effect of storage time of alginate pellet formulation of *T. harzianum* on *S. rolfsii* attack:

The design used in this study was a Completely Randomized Design (CRD) with 8 treatments and 3 replications. The treatments consisted of several different storage times for *T. harzianum* pellets, namely A (without pellet), B (without storage), C (2 weeks), D (4 weeks), E (6 weeks), F (8 weeks), G (10 weeks) and H (12 weeks).

Isolation and identification of *S. rolfsii*:

S. rolfsii isolate was isolated from infected chilli plants. Isolation was carried out using a single spore method. After obtaining a pure culture it was identified using the literature by Watanabe⁹.

Propagation of *S. rolfsii*:

S. rolfsii were cultured massively in Corn Meal Sand (CMS) medium for 10 days by placing the medium in 100 g of heat-resistant plastic bags and giving the bag a plastic pipe ring and gagging it with cotton. Then it was

sterilized in an autoclave for 20 min at a temperature of 121°C for 2 consecutive days. After sterilization, 1 cm² of *S. rolfsii* (7 days) on PDA medium was placed in plastic bags.

Seeding of chilli seeds and Infestation of *S. rolfsii*: The seeding medium used was a mixture of soil and chicken manure (1:1 v/v), which has been sterilized by the Tyndallization method. The medium was placed in a seedbed (15×15×45 cm) as much as 4 kg per seedbed. Then seeds were seeded as much as 75 seed/seedbed.

Infestation of *S. rolfsii* was carried out 2 weeks before seeding by mixing 30 g of *S. rolfsii* in a CMS substrate with 1 kg of soil.

Introduction of *T. harzianum* alginate pellet: The introduction of *T. harzianum* alginate pellets from various storage periods (according to treatment) was carried out one week before seeding as much as 5 g kg⁻¹ soil by mixing *T. harzianum* pellets with soil.

Observation parameters

Percentage of infected seeds before emerged to the soil surface (pre-emergence damping-off): This observation was carried out 30 days after seeding. The percentage inhibition of pre-emergence damping-off was calculated using the following formula by Kumar *et al.*¹⁰:

$$P (\%) = \frac{\text{PreC} - \text{PreT}}{\text{PreT}} \times 100$$

where, P is the Percentage inhibition, PreC is the Percentage of pre-emergence damping-off in control and PreT is the Percentage of pre-emergence damping-off in treatments.

Percentage of infected seeds after emerged to the soil surface (post-emergence damping-off): This observation was carried out 30 days after seeding. The percentage inhibition of post-emergence damping-off was calculated using the following Eq:

$$P (\%) = \frac{\text{PoC} - \text{PoT}}{\text{PoT}} \times 100$$

where, P is the Percentage inhibition, PoC is the Percentage of pre-emergence damping-off in control and PoT is the Percentage of pre-emergence damping-off in treatments.

Amount of *T. harzianum* propagules in the soil: The amount of *T. harzianum* propagules in the soil was counted 30 days after seeding.

RESULTS

***T. harzianum* viability on alginate pellets:** Different storage times of alginate pellet formulation of *T. harzianum* with rice bran did not affect on viability of *T. harzianum*. The viability of *T. harzianum* in all treatments of *T. harzianum* pellet storage up to 12 weeks were 100% as showed in Table 1.

***T. harzianum* effect on alginate pellets on pre-emergence damping-off disease:** Application of *T. harzianum* alginate pellets at all different storage times significantly reduced the number of infected seeds before they emerge to the soil surface (pre-emergence damping-off). Percentage of infected seeds on control (without *T. harzianum* alginate pellet) was 13.66% and significantly different from other treatments. Statistically, there was no effect of pellet storage time on the number of infected seeds before they emerge to the soil surface. Percentage inhibition of pre-emergence damping-off ranged from 70.71-92.69% in Table 2.

***T. harzianum* effect on alginate pellets on post-emergence damping-off disease:** The result of this study also indicated that application of *T. harzianum* alginate pellets at different storage times significantly reduced the number of infected seeds after they emerged to the soil surface (post-emergence

Table 1: Viability of *T. harzianum* alginate pellet on medium PDA

Treatments (storage time of pellet)	Viability <i>T. harzianum</i> (%)
Control (without storage)	100
2 weeks	100
4 weeks	100
6 weeks	100
8 weeks	100
10 weeks	100
12 weeks	100

Table 2: Percentage of infected seeds before emerged to the soil surface (pre-emergence damping off)

Treatments (storage time of pellet)	Pre-emergence damping-off (%)	Inhibition (%)
Without pellet	13.66 ^a	-
Without storage time	3.33 ^b	75.62
2 weeks	2.00 ^b	85.36
4 weeks	1.00 ^b	92.69
6 weeks	3.33 ^b	75.62
8 weeks	3.66 ^b	72.20
10 weeks	4.00 ^b	70.71
12 weeks	3.00 ^b	78.04

Numbers within the same column followed by the same letters are not significantly different (p = 0.05)

Table 3: Percentage of infected seeds after emerged to the soil surface (post-emergence damping-off)

Treatments (storage time of pellet)	Post-emergence damping-off (%)	Inhibition (%)
Without pellet	24.66 ^a	-
Without storage time	0.66 ^b	97.32
2 weeks	2.00 ^b	89.21
4 weeks	6.00 ^b	76.56
8 weeks	1.66 ^b	93.27
10 weeks	2.66 ^b	89.21
12 weeks	2.33 ^b	90.55
12 weeks	3.33 ^b	86.50

Numbers within the same column followed by the same letters are not significantly different ($p = 0.05$)

Table 4: Number of *T. harzianum* propagules in the soil 30 days after seeding

Treatments (storage time of pellet)	Number of <i>T. harzianum</i> propagules/gram soil
Without pellet	7.20×10^{2a}
Without storage time	1.33×10^{6b}
2 weeks	0.85×10^{6b}
4 weeks	0.80×10^{6b}
6 weeks	0.52×10^{6b}
8 weeks	1.05×10^{6b}
10 weeks	0.90×10^{6b}
12 weeks	0.40×10^{6b}

Numbers within the same column followed by the same letters are not significantly different ($p = 0.05$)

damping-off). Percentage of post-emergence damping-off on control was 24.66% while those of other treatments were ranged from 1-4%. Statistically, there was no effect of pellet storage times on the number of infected seeds after they emerged from the soil surface. Percentage inhibition of post-emergence damping-off was ranged from 76.56-97.32 in Table 3.

Number of *T. harzianum* propagules in the soil:

Application of *T. harzianum* alginate pellet was able to increase the number of *T. harzianum* propagules in the soil observed 30 days after application. The difference in pellet storage times did not show a statistically significant effect on the number of propagules formed in Table 4.

DISCUSSION

The results of this study showed that the storage time of alginate pellet of *T. harzianum* up to 12 weeks did not affect the viability of *T. harzianum* as shown in Table 1. This happened because *T. harzianum* was immobilized in certain carriers, alginate and rice bran that made its shelf life increase. This is following what was suggested by Harman¹¹ that *Trichoderma* culture should be entrapment in certain carriers and should be prepared as formulations for easy application and storage. The results of research by Bhai⁴ also showed

that the conidial and mycelial stages in encapsulated form remained viable for more than six years at room temperature.

Application of alginate pellet of *T. harzianum* at different storage times could suppress the pre and post-emergence damping-off diseases however the different storage times of pellets did not show the different ability in suppressing the disease. The ability to suppress the diseases because *T. harzianum* is an antagonistic fungus with various antagonistic mechanisms such as parasitism, competition and antibiosis¹². *Trichoderma* strains can produce extracellular enzymes and antifungal antibiotics and they can also be competitors to plant pathogenic fungi, stimulating plant development and inducing resistance¹³. Mayurama *et al.*⁸ also stated that the *T. harzianum* encapsulation showed an improvement in the chitinolytic and cellulosic activity. Research results by Kucuk and Kivanc¹³ showed that alginate encapsulation of *Trichoderma* successfully improved chitinase and -1, 3-glucanase production *in vitro* for a prolonged time even at higher temperature (40°C). Attitalla and Salleh¹⁴ also stated that the immobilized *Trichoderma* improved chitinase production compared with free spore suspension especially when chitin was used as a carbon source. Alginate encapsulated *Trichoderma* did not only prolong the metabolic activity of the entrapped organism but also it promoted the slow release of microbial spores into the medium for successful enzyme production¹⁵.

The ability of *T. harzianum* alginate pellets to suppress the attack of plant pathogenic fungi has been shown by many researchers such as *Rhizoctonia solani* on beet seed¹⁶ and clonal propagation of *Eucalyptus*¹⁷, *Sclerotinia sclerotiorum* on potato¹⁸, *Fusarium oxysporum* f. sp. *lycopersici* on tomatoes⁶ and *Helminthosporium oryzae* on rice¹⁴. The result of research by Kucuk and Kivanc¹³ also showed that encapsulated fungus was able to provide greater control of *S. sclerotiorum* *in vitro* test.

Differences in the duration of storage of *T. harzianum* pellets did not affect the suppression of the *S. rolfsii* attack on chilli. This happened because the *T. harzianum* pellets stored for up to 12 weeks were still stable as evidenced by their viabilities that were not different as shown in Table 1. This was also supported by the number of *T. harzianum* propagules in the soil 30 days after the introduction of the pellets did not show a significant difference among treatments.

CONCLUSION

The viability of the *Trichoderma harzianum* alginate pellet was stable until 12 weeks of storage. Application of *T. harzianum* alginate pellet at different storage times as

much as 5 g kg⁻¹ of soil reduced Pre-emergence damping-off and Post-emergence damping-off diseases by *S. rolfisii* on chilli plants with percentage inhibition above 70 and 76%, respectively. Storage of *T. harzianum* alginate pellets at different times did not affect their effectiveness in reducing *S. rolfisii* attacks on chilli plants. Also, the different storage times of *T. harzianum* alginate pellets did not affect the number of *T. harzianum* propagules in the soil 30 days after the introduction of the pellet.

SIGNIFICANCE STATEMENT

This study discovered the ability of *Trichoderma harzianum* alginate pellet with rice bran at different storage times up to 12 weeks in reducing damping-off on chilli that can be beneficial for alternative control damping-off. Storage of *T. harzianum* pellet up to 12 weeks did not affect its ability in reducing the damping off disease.

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