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# The Effect of Packaging and Encapsulation Temperature of Red Chili Seeds with Trichoderma sp. on Viability and Inhibition of *Fusarium* sp.

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**Abstract**

**Background:** *Fusarium* sp. is one of the main threats to chili cultivation, thereby reducing its economic value. Therefore, the use of biological microorganisms, such as *Trichoderma* sp., is an environmentally friendly alternative for controlling *Fusarium* sp. Application techniques for Trichoderma sp. biological agents include seed coating or Encapsulation. However, ensuring the quality and effectiveness of encapsulated products during storage and distribution remains a concern. Thus, this study aims to examine the effects of packaging type and storage temperature on the viability and efficacy of Trichoderma sp. biological agents encapsulated in red chili seeds (*Capsicum annuum* L.) for inhibiting *Fusarium* sp. **Methods:** This study applied a completely randomized design (CRD) factorial with two factors, namely packaging material (aluminum foil and plastic) and storage temperature (5°C, 28°C, and 36°C), resulting in 7 treatments with three replications plus a control, resulting in a total of 21 experimental units. **Results:** Aluminum foil packaging stored at 28°C showed the highest viability of Trichoderma sp. and the most excellent antagonistic activity against *Fusarium* sp. **Conclusions:** These results provide a sustainable, environmentally friendly solution for biological seed storage.

***Keywords:*** Biological Agent; Encapsulation; *Fusarium* sp; Packaging; Storage Temperature; *Trichoderma* sp.



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## Introduction

Red chili (*Capsicum annuum* L.) is a major horticultural commodity in Indonesia with high economic value. Red chili production in East Java decreased by 125,403 quintals in 2022, influenced by climate change, water constraints, and pest and plant disease attacks (BPS, 2023). *Fusarium* wilt, caused by *Fusarium* sp., is a significant threat to red chili cultivation. This statement aligns with Hasanah et al. (2016), who reported that *Fusarium* sp. is a soil-borne pathogen that can cause crop failures of up to 50%. Most *Fusarium* genera are saprophytic and parasitic fungi. These fungi thrive in the vascular system of plants, causing plant death by producing toxins (Sastrahidayat, 2011). Conventional approaches to managing this disease often involve synthetic chemical fungicides. However, excessive use can have adverse effects, such as pathogen resistance, environmental degradation, and decreased soil fertility. Therefore, the use of biological agents such as Trichoderma sp. is a more environmentally friendly solution.

Trichoderma sp. can inhibit the growth of fungi that cause plant diseases, including those caused by *Fusarium* sp. (Purwantisari & Rini, 2009). One application of the Trichoderma sp. biological agent technique is through seed coating or Encapsulation. According to Yulia et al. (2019), Encapsulation is a technique for wrapping explants or seeds in a special coating that provides high durability and viability and can serve as a carrier for additives. This process uses three main ingredients: gum arabic as a coating, compost as a carrier, and Trichoderma sp. as the active ingredient. Gum arabic can protect volatile compounds from oxidation and maintain the viability of biological agents during storage (Kanakdande et al. 2007). Compost provides additional nutrients to both seeds and biological agents for a specific period. However, maintaining the quality and suitability of encapsulated products containing biological agents during distribution and storage, particularly regarding the viability of the biological agents and the encapsulated red chili seeds, remains a topic for further research. This management is crucial to ensure product safety, quality, and moisture retention, as well as to prevent product contamination.

High-quality packaging materials, such as aluminum foil and plastic, have low oxygen and water vapor permeability and high tensile strength or puncture resistance. Previous research has shown that aluminum foil and plastic packaging are more effective at maintaining the viability of biological agents and the physiological quality of seeds (Ratnawati et al., 2023; Richy, 2012). Meanwhile, temperature is a crucial factor that directly influences the development and growth of microorganisms. Trichoderma sp. fungi thrive at temperatures between 5°C and 36°C. However, research by Widiyanti et al. (2002) showed that Trichoderma sp. grew more rapidly in formulas stored at room temperature than in those stored at lower temperatures.

This statement indicates that this study should expand its analysis to determine the optimal combination of packaging types and storage temperatures across different conditions. These factors play a crucial role in determining the effectiveness and shelf life of biological agents on seeds. Therefore, this study aims to evaluate the effects of various packaging types (aluminum foil and plastic) and storage temperatures (low temperature 5°C, room temperature 28°C, and high temperature 36°C) on maintaining the viability of Trichoderma sp. and inhibiting *Fusarium* sp. The results of this study are expected to provide a more environmentally friendly, sustainable biological-agent-based seed storage solution. By understanding the optimal combination of packaging and storage temperatures, this study can also support productive red chili farming while reducing the negative environmental impact of chemical fungicides.

## Methods

### *Materials and Equipment*

This research study used the Lingga variety of red chili (*Capsicum annuum* L.) seeds to be encapsulated with the active ingredient Trichoderma sp. The Trichoderma sp. fungal isolate was obtained from the collection of Sarah Hikmah Marieska at the UPN "Veteran" East Java Agrotechnology Plant Health Laboratory and isolated from the rhizosphere soil in a lettuce nursery at Kaliandra Organic Farm, Pasuruan. The fungus was tested for its ability to inhibit the plant pathogen Rhizoctonia solani, which causes damping-off disease in lettuce. The isolate was used as the target pathogen in antagonism tests. *Fusarium* sp. was obtained from the collection of Lukmanul Hakim at the UPN "Veteran" East Java Agrotechnology Plant Health Laboratory, isolated from chili plants with *Fusarium* wilt symptoms. The encapsulation materials used included gum arabic as an adhesive and compost as a carrier, at a 1:1 (w/w) ratio: 5 grams of gum arabic dissolved in 100 ml of distilled water, and 5 grams of sterile, dry compost during the seed coating process. The packaging materials used in this study were:

Aluminum foil pouches (flat bags) measuring 6 cm long, 8 cm wide, and 100 µm thick, without clips. The plastic packaging used was marketed under the C-tik brand and

measured 6 cm long, 10 cm wide, and 30 µm thick. This plastic was equipped with rails or "clips" that could be opened and closed. This study used various storage devices, including a Toshiba GR-B28ISP two-door refrigerator for low-temperature storage (5°C), a tray for optimal/room-temperature storage (28°C), and a simple egg incubator for high-temperature storage (36°C).

### *Research Location and Sample*

This research was conducted from April to June 2024 at the Plant Health Laboratory and Microbiology Laboratory, Faculty of Agriculture, Veteran National Development University, East Java. Using an in vitro approach, this study employed a completely randomized design (CRD) with two factors. The first factor was storage temperature treatment: low temperature (5°C; R), room temperature (28°C; A), and high temperature (36°C; T). The second factor was packaging material, namely aluminum foil (F) and plastic packaging (L). The experimental sample combinations obtained were RF (low temperature 5°C + aluminum foil), RL (low temperature 5°C + plastic), AF (room temperature 28°C + aluminum foil), AL (room temperature 28°C + plastic), TF (high temperature 36°C + aluminum oil), and TL (high temperature 36°C + plastic). Thus, the treatment had seven treatment combinations, with three replications and a control, resulting in a total of 21 experimental units. This study used two different types of controls in several tests. The antagonist test used an independent isolate of *Fusarium* sp. as a control in the dual-culture method. Meanwhile, the colony diameter and spore density tests used chili seed encapsulation containing the active ingredient *Trichoderma* sp., stored at moderate temperatures without packaging.

### *Research Implementation*

The isolate rejuvenation procedure began with preparing the media and fungal isolates as the initial step in the research. Trichoderma sp. and *Fusarium* sp. isolates were rejuvenated on Potato Dextrose Agar (PDA) media to produce young isolates with optimal metabolic activity.

The seed encapsulation process began with the preparation of a binder solution by dissolving 5 g of gum arabic in 100 mL of distilled water, followed by homogenization with 10 mL of a Trichoderma sp. isolate suspension containing 10^6 spores/mL. Chili seeds were sprayed with the adhesive solution and suspension, followed by 5 grams of sterile compost. After coating, the seeds were filtered through a 200-mesh (75 µm) sieve, and the process was repeated until the seeds were evenly coated. The fully coated seeds were then air-dried. This process aligns with Sarah's (2023) research, which states that the encapsulated seeds are placed in a petri dish and air-dried for 30 minutes.

The encapsulated and dried seeds are then packaged using various packaging materials, including aluminum foil and plastic. The aluminum foil pouches are sealed with a sealing machine to ensure safety and airtightness. The packaged seeds are stored at 5°C, 28°C, and 36°C for 1 month. After the storage period, the viability and antagonistic capabilities of the Trichoderma sp. isolates contained in the encapsulated seeds are tested.

### *Data Collection*

Data collection was conducted through four monthly observations, including testing the viability parameters of Trichoderma sp. and its ability to act as an antagonist against *Fusarium* sp.

In this study, the viability of Trichoderma sp. was determined based on indicators of propagule growth and reproduction, as indicated by colony diameter and spore density. Colony diameter was measured by counting the growth of Trichoderma sp. colonies in seed encapsulations individually for each experimental unit regrown in PDA media in a petri dish. Colony diameter was calculated from the marker point to the tip of

the fungal mycelium on the PDA media. The calculation of Trichoderma sp. colony diameter used the formula by Yetti et al. (2024), namely:

$$D = \frac{D1 + D2}{2}$$

Description:
D : Diameter
D1 : Horizontal side diameter D2 = Vertical side diameter
D : D1 + D2

Use a hemocytometer under a microscope and count five squares within a small box. This process is used to calculate spore density in a single-capsule formulation. The initial process involves grinding the seeds using a mortar, then suspending them in 9 ml of sterile distilled water and vortexing for 10 minutes (Wuryandari, 2004). The spore density on the hemocytometer is calculated using the formula provided by the Center for Seed and Plant Protection (2014) in Sara (2022), as follows:

$$S = \frac{X}{(L \; x \; h \; x \; d)} \; x \; 10^3$$

Description:
S : Spore density
X : Average number of conidia in boxes a, b, c, d, e
L : Area of counting box (0.0025 mm3 x 5)
h : Depth of counting field (0.1 mm)
d : Dilution factor
$10^3$ : Calculated volume of suspension (1 mL = 103 mm3)

Antagonistic ability, the antagonistic properties of Trichoderma sp. against *Fusarium* sp. were evaluated using the dual culture method on PDA media, by placing the encapsulated seeds containing the active ingredient Trichoderma sp. side by side with the *Fusarium* sp. isolate. The observed development was an interaction between the two fungi (Figure 1). The effectiveness of the inhibitory power was measured by the Percentage Inhibition of Radial Growth (PIRG), which was calculated based on the diameter area of the *Fusarium* sp. colony in the control culture (R1) and the diameter area of the *Fusarium* sp. colony that led to the Trichoderma sp. fungal colony (R2) using the formula (Dharmaputra et al. 1999):

$$PIRG = \frac{R1 - R2}{R1} \; x \; 100 \; \%$$

Description:
PIRG : Percentage of inhibition power
R1 : Area of diameter of *Fusarium* sp. colonies in the control culture
R2 : Area of diameter of *Fusarium* sp. colonies on the dual culture plate

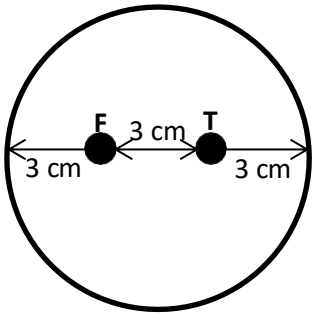


**Figure 1.** Dual Culture Calculation Method (Ningsih et al. 2016)

### *Data Analysis*

The results of the Trichoderma sp. viability test during Encapsulation and its ability to inhibit *Fusarium* sp. were analyzed using analysis of variance (ANOVA) using SPSS to explore the impact of treatments on the research parameters. If the data showed significant differences, further analysis was conducted using Duncan's Multiple Range Test (DMRT) at the 5% significance level to identify differences between treatments.

## Result

### *Characteristics of Trichoderma sp. Fungi*

Trichoderma sp., grown on Potato Dextrose Agar (PDA) for 7 days, as shown in Figure 2, exhibits flat, concentric colonies resembling mosquito coils, with a fibrous texture and smooth, wool-like edges. Initially, the colonies appear white, then change to light green in the center, then to dark green, and finally to white at the edges. This finding aligns with research conducted by Suanda (2019), which found that initially, the colonies are white, then change to light green in the center and then dark green, and have a circular shape with white edges.

Microscopic observations were carried out at 10×100 magnification, revealing green hyphae, greenish, globular (round) conidia in clusters on the surface of conidiophore cells, branched conidiophores, and long phialids (Figure 3). Microscopic appearance, according to research conducted by Anis and Adelia (2023), fungal isolates exhibited typical characteristics: branched conidiophores forming pyramids, short phialids tapering, sometimes thickening in the middle, and oval or oval-shaped conidia.

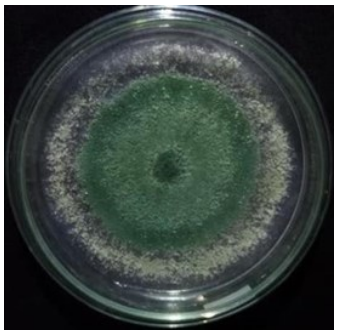

**Figure 2.** Macroscopic Morphology of Trichoderma sp. Colonies on PDA Media

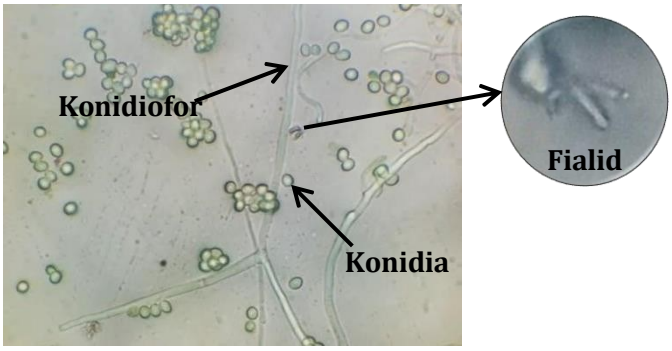


**Figure 3.** Microscopic Morphology of Trichoderma sp. (Magnification 10×100)

### *Characteristics of Fusarium sp. Fungi*

*Fusarium* sp. grown on 7-day-old PDA media, as seen in Figure 4, exhibits macroscopic characteristics of white, cotton-like colonies. The surface of the fungal colonies appears rough and fibrous, with a dark cream to orange color. *Fusarium* sp. isolates can exhibit a change in colony color from white to orange due to the formation of numerous sporodochia, which can significantly affect the appearance of the fungal colonies (Kaur et al., 2015 & Ram et al., 2021).

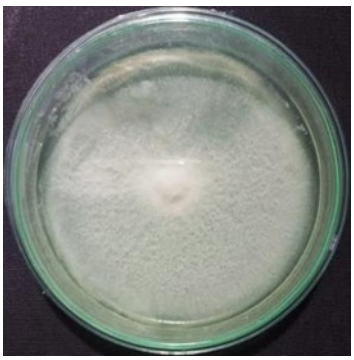

**Figure 4.** Macroscopic Morphology of *Fusarium* sp. Colonies on PDA Media

Microscopic observations of *Fusarium* sp. (Figure 5) showed the presence of oval-shaped microconidia with two septa and crescent-shaped microconidia with five septa. This observation is consistent with the research by Putra et al. (2020), which reported that *Fusarium* sp. macroconidia measured 22.13 to 26.29 µm in length and 3.78 to 4.74 µm in width, with 3-5 crescent-shaped septa. In contrast, microconidia measured 5.65 to 8.29 µm in length and 2.48 to 3.14 µm in width, consisting of 1-2 oval-shaped septa.

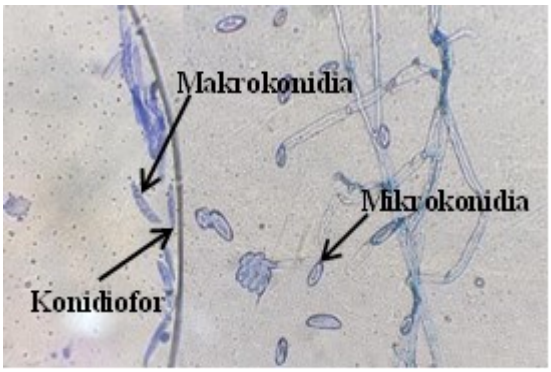


**Figure 5.** Microscopic Morphology of *Fusarium* sp. (Magnification 10×100)

### *Seed Encapsulation*

The shape and surface of the encapsulated and air-dried red chili seeds were observed. The encapsulated seeds retained their original shape. This change in shape is due to the addition of a coating material that completely coats the carrier and active ingredients on all seed surfaces. The seeds after coating were round and flat, with a slightly rough texture, black due to the compost, and twice the size and weight of the original seeds. The addition of Trichoderma sp., the active ingredient, did not affect the color or odor of the encapsulated seeds. Therefore, this technology is highly relevant to supporting sustainable agricultural systems. Seeds encapsulated with compost carrier, gum arabic adhesive, and the active ingredient Trichoderma sp. are shown in Figure 6.

**Figure 6.** Results of Red Chili Seed Encapsulation.

### *Trichoderma sp. Colony Diameter in Encapsulation*

In vitro testing results based on colony diameter measurements, using packaging and temperature as factors, showed significant effects for each week of observation. Further, Duncan's test results at the 5% level (Table 1) indicate a substantial difference between the treatments and the control (storage at moderate temperatures without packaging) in the first week. The control had a diameter of 6.33 cm, while the AL, RL, and RF treatments each had diameters of 6.7 cm, 6.43 cm, and 5.06 cm, respectively, indicating better results than the control. However, the average diameter varied from the second to fourth weeks, while AF consistently performed best across all observations, with the largest colony diameters. From the first to fourth weeks, the averages were 8.1 cm, 8.43 cm, 8.7 cm, and 8.9 cm, respectively.

The room-temperature treatment at 28°C with aluminum foil (AF) packaging resulted in larger colony diameters than the high-temperature treatment at 36°C with aluminum foil (TF) packaging. In second place was a room temperature of 28°C with plastic packaging (AL). Meanwhile, a high temperature of 36°C with aluminum foil packaging (TF) showed an average of 0 cm in the first to fourth weeks, indicating that Trichoderma sp. died. This finding is in line with research by Sulistiyono (2015), which highlighted temperature as an important factor in regulating growth, sporulation, and saprophytes' ability to produce non-volatile metabolites. These metabolites play a role in nutrient acquisition, competition for space, mycoparasitism, and extracellular enzyme production.

Observations showed that a room temperature of 28°C in the AF and AL treatments provided the best results in maintaining viability. Conversely, a high temperature of 36°C in the TF treatment followed by the TL treatment caused physiological stress in Trichoderma sp. This statement aligns with the research of Respati (2017) in Shabrina et al. (2023), which found that low temperatures can reduce enzyme activity, while high temperatures can cause enzyme denaturation and increase stress factors, resulting in suboptimal fungal growth. This indicates that mechanical protection from packaging and temperature control play significant roles in maintaining the effectiveness of Trichoderma sp. in controlling *Fusarium* sp., which is correlated with its colony growth. Larger colony diameters indicate better viability, which means Trichoderma sp. can produce more antimicrobial metabolites to inhibit the growth of *Fusarium* sp.

**Table 1.** Results of Trichoderma sp. Colony Diameter in Encapsulation

| Treatment | Colony Diameter (cm) of Trichoderma sp. during Encapsulation Observation (Week) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| RF | 6,43bc | 7,96c | 8,33c | 8,55c |
| RL | 6,7cd | 8,2c | 8,46cd | 8,53c |
| AF | 8,1e | 8,43c | 8,76e | 8,9e |
| AL | 7,5cd | 8,1c | 8,66de | 8,7cd |
| TF | 0a | 0a | 0a | 0a |
| TL | 5,06b | 5,7b | 5,93b | 6,1b |
| Control | 6,33bc | 8,28c | 8,7de | 8,7cd |

Note:

- The numbers in the same column show no significant difference at the 5% DMRT follow-up test level.
- RF (low temperature 5°C + aluminum foil), RL (low temperature 5°C + plastic), AF (room temperature 28°C + aluminum foil), AL (room temperature 28°C + plastic), TF (high temperature 36°C + aluminum oil), TL (high temperature 36°C + plastic)

### *Trichoderma sp. Spore Density in Encapsulation*

In vitro testing results based on spore density calculations indicate that temperature consistently affects the spore density of Trichoderma sp. during seed encapsulation. In contrast, packaging alone does not significantly affect it. However, when combined, the

interaction between the two shows a significant effect. This suggests that the combined treatment interaction can yield different results than the effects of temperature or packaging alone. This interaction can be explained by the packaging's protection or modification of the microenvironment, thereby affecting the environment surrounding the spores.

The results of the 5% Duncan test (Table 2) indicate that a high temperature of 36°C, both in aluminum foil (TF) and plastic (TL) packaging, consistently yielded the lowest spore density over the four weeks of observation compared to the other treatments. Conversely, a low temperature of 5°C in aluminum foil (RF) and plastic (RL) packaging showed a fairly stable spore density, close to the control level, though slightly lower. At room temperature (28°C) in aluminum foil (AF) packaging, the fourth week produced the highest spore density, 5.77×108. followed by AL (room temperature + plastic) with a value of $5.23\times10^{8}$.

Observations of spore density indicate that temperature is the dominant factor in increasing spore density. In contrast, packaging type is less influential, although the interaction between the two shows significant synergistic potential for optimizing spore growth. According to Ou (1985) and Patel et al. (2022), temperature and humidity are important environmental factors supporting the survival and developmental activity of germinating conidia during storage. The highest yields were obtained at room temperature. This finding indicates that room temperature can significantly increase microorganism metabolic activity and spore formation.

High temperatures yield the lowest spore density values, leading to reduced spore viability; however, protection from aluminum foil and plastic still provides protection. This phenomenon can be attributed to damage to the spore structure and to accelerated oxidation processes despite physical security provided by the packaging. Extreme temperatures, both high (36°C) and low (5°C), reduce spore density and increase the risk of physiological damage, such as dehydration and dislocation of the polar capsule. This ultimately results in lysis (loss of contents) and spore wall rupture or disintegration. Research by Mahasri et al. (2019) and Li and Shi (2014) supports this finding, stating that suboptimal temperatures cause spores to harden, shrink, or freeze due to dehydration and lack of moisture.

**Table 2.** Results of Trichoderma sp. Spore Density in Encapsulation

| Treatment | Spore Density (108 spores/ml) of Trichoderma sp. during Encapsulation Observation (Week) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| RF | 2,6c | 3,32c | 4,28d | 4,73cd |
| RL | 2,63c | 3,28c | 4,3d | 4,7cd |
| AF | 4,17d | 4,88d | 5,23e | 5,77e |
| AL | 3,73d | 4,65d | 4,99e | 5,23de |
| TF | 0,42a | 0,47a | 0,52a | 0,57a |
| TL | 1,46b | 1,75b | 1,81b | 1,91b |
| Kontrol | 2,48c | 2,97c | 3,37c | 4,19c |

Note:
- The numbers in the same column show no significant difference at the 5% DMRT follow-up test level.
- RF (low temperature 5°C + aluminum foil), RL (low temperature 5°C + plastic), AF (room temperature 28°C + aluminum foil), AL (room temperature 28°C + plastic), TF (high temperature 36°C + aluminum oil), TL (high temperature 36°C + plastic)

### *Inhibitory ability of Trichoderma sp. against Fusarium sp.*

In-vitro testing based on research shows that temperature and packaging factors, both individually and interactively, affect the antagonistic ability of Trichoderma sp. to inhibit *Fusarium* sp., with different contributions. This statement indicates that both

factors not only influence antagonism individually but also have a synergistic effect on the effectiveness of Trichoderma sp. The results of the 5% Duncan's follow-up test showed significant differences in the effects of temperature, packaging, and their interaction on the antagonistic ability of Trichoderma sp. against *Fusarium* sp., with a consistent increasing pattern across observations (Table 3). Observations from the first to the fourth week showed that the control had the highest value among the treatments, with average values of 64.46%, 76.08%, 84.67%, and 95.10%, respectively. The highest value in the control was due to the calculation of the diameter-area of *Fusarium* colonies inoculated on PDA media individually, which served as the reference in the antagonistic properties test.

**Figure 7**. Clear Zone in Antagonist Test

The results across all storage temperature treatments and packaging types demonstrated a mechanism of encounter between two colonies, characterized by faster growth of Trichoderma sp. than that of the pathogen (Figure 7). This inhibition is due to secondary metabolites produced by the antagonistic fungus, which prevent the growth of *Fusarium* sp. Consequently, *Fusarium* sp. colonies cannot grow larger than Trichoderma sp. This indicates that the presence of Trichoderma sp. in seed encapsulation can effectively inhibit pathogen growth, except under unfavorable conditions, such as high temperatures, which cause damage to the microorganisms.

The encounter between two colonies in the inhibition test parameters, when observed under a microscope, indicates abnormal *Fusarium* sp. hyphae, characterized by swollen hyphae and the formation of chlamydiospores as a defensive structure (Figure 8). The abnormality of *Fusarium* sp. hyphae is a consequence of the antagonistic mechanism of Trichoderma sp. This is supported by the statement by Agustin et al. (2023) that *Fusarium* sp. hyphae can inhibit pathogen growth. Under the influence of antagonistic activity, abnormal growth occurs, including lysis, curling, swelling, twisting, rolling, and the formation of chlamydiospores. Chlamydiospores are produced when the fungus feels stressed, triggering a pathogenic defense that produces single-celled, thick-walled, asexual spores resistant to adverse conditions.

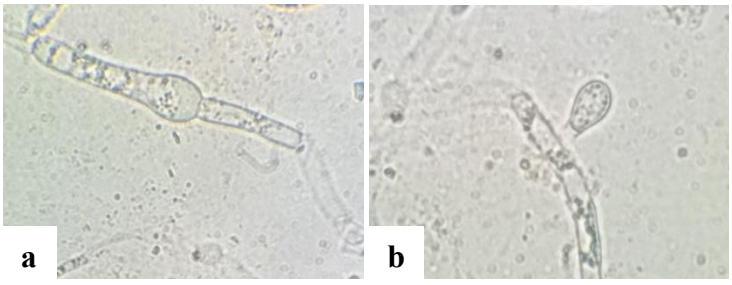


**Figure 8.** Abnormalities of *Fusarium* sp. Hyphae a.) Swollen Hyphae and b.) Chlamydiospores (Magnification 10×100)

**Table 3.** Results of the Antagonistic Ability of Trichoderma sp. in Encapsulation

| Treatments | Antagonist Test (%) of Trichoderma sp. in Encapsulation Observation (Sunday) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| RF | 42,92b | 44,88b | 48,46b | 49,97b |
| RL | 43,21b | 45,29b | 47,04b | 49,28b |
| AF | 49,38c | 50,21c | 57,71c | 61,56c |
| AL | 47,30c | 48,03c | 56,04c | 59,09c |
| TF | 26,04a | 28,33a | 36,46a | 39,22a |
| TL | 40,05b | 43,26b | 45,46b | 47,28b |
| Kontrol | 0a | 0a | 0a | 0a |

Note:

- The numbers in the same column show no significant difference at the 5% DMRT follow-up test level.
- RF (low temperature 5°C + aluminum foil), RL (low temperature 5°C + plastic), AF (room temperature 28°C + aluminum foil), AL (room temperature 28°C + plastic), TF (high temperature 36°C + aluminum oil), TL (high temperature 36°C + plastic)

## Conclusions

The combination of aluminum foil packaging and a temperature of 28°C was the best treatment for maintaining Trichoderma sp. viability, seed germination, and antagonistic ability against *Fusarium* sp. These findings support the initial hypothesis and provide an innovative solution for more sustainable biological-agent-based seed storage management.

## Acknowledgements

The combination of aluminum foil packaging and a temperature of 28°C was the best treatment for maintaining Trichoderma sp. viability, seed germination, and antagonistic ability against *Fusarium* sp. These findings support the initial hypothesis and provide an innovative solution for more sustainable biological-agent-based seed storage management.

## Declaration statement

The authors reported no potential conflict of interest.

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