Vol. 13, No. 3, pp. 747-759, August 2025 DOI: https://doi.org/10.23960/jat.v13i3.10720

Jurnal Agrotek Tropika

P-ISSN: 2337-4993
E-ISSN: 2620-3138

Journal homepage: https://jurnal.fp.unila.ac.id/index.php/JA

POTENSI *TRICHODERMA* SEBAGAI BAHAN PENGAWET UMBI DAN BIOFUNGISIDA BENIH BAWANG MERAH

POTENTIAL OF TRICHODERMA AS A PRESERVATIVE FOR SHALLOT BULB AND BIOFUNGICIDE

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ARTICLE HISTORY:
Received: 3 May 2025
Peer Review: 21 July 2025
Accepted: 7 August 2025

KEYWORDS:
Allelochemicals, echinochloa crus-galli, sweet potato tuber skin extract

ABSTRACT

This study aims to identify the fungal species responsible for shallot bulb (Allium cepa) rot during storage and to evaluate the efficacy of Trichoderma esperellum in preserving bulbs and safeguarding shallot seeds from these rotting pathogens. The first phase involved isolating the pathogenic fungi and confirming their role via Koch's postulates. Subsequently, an in vitro inhibition assay was conducted to assess interactions between Trichoderma and the pathogenic fungi, followed by experiments on bulb preservation and tests of seed protection. Results revealed that Penicillium sp. was the causative agent of rot. In vitro, T. esperellum inhibited pathogen colony growth by 61.9 ± 1.2% at 96 hours and stimulated its own colony growth by 172.1 ± 10.8% at 48 hours post-inoculation. When applied in storage, suspensions and filtrates of T. esperellum reduced bulb rot by 71.5% and 67.9%, respectively, and enhanced germination rates by 127.8% and 122.2% compared to untreated controls. These findings demonstrate that Trichoderma filtrate holds strong potential as an active ingredient in biopesticides for bulb preservation and seed protection in shallots.

KATA KUNCI:
Alelokimia, echinochloa crus-galli, ekstrak kulit umbi ubi jalar

ABSTRAK

Penelitian ini bertujuan untuk menentukan fungi penyebab busuk umbi bawang merah (*Allium cepa*) di penyimpanan serta mengukur kemampuan *Trichoderma esperellum* dalam mengawetkan umbi dan memberi perlindungan benih bawang merah dari serangan patogen pembusuk. Tahap awal adalah memastikan fungi patogen yang sudah diisolasi dengan Postulat Koch. Tahap berikutnya adalah uji daya hambat diantara *Trichoderma* dan fungi patogen, pengawetan siung, dan uji kemampuan perlindungan benih bawang merah. Hasil penelitian menunjukkan penyebab busuk adalah *Penicilium* sp. Dalam uji *in vitro T. esperellum* mampu menghambat pertumbuhan koloni patogen mencapai 61,9±1,2% dan meningkatkan pertumbuhan koloninya sendiri 172,1±10,8%, masing-masing pada 96 dan 48 jam setelah inokulasi. Pemberian suspense dan filtrat *T. esperellum* masing-masing menekan pembusukan umbi hingga 71,5% an 67,9% di penyimpanan serta meningkatkan perkecambahan 127,8% dan 122,2% dibandingkan tanpa *Trichoderma*. Pemanfaatan filtrat *Trichoderma* dapat dimanfaatkan sebagai bahan aktif biopestisida bagi pengawetan siung dan perlindungan bibit bawang merah.

1. INTRODUCTION

Shallots (*Allium cepa* L.) are the second most important vegetable crop due to their significant economic value, health benefits, and as an important functional food, as an ingredient in various cuisines around the world (Ricciardi *et al*., 2020). Shallot peels extract has been shown to contain 44 bioactive compounds that are beneficial for health (Zhang *et al*., 2023). Thus, the production and consumption of shallots have become a concern for producers and researchers so that they can contribute to meeting human needs, including strengthening Indonesia's national food security.

Over the past five years (2000-2024), Indonesian shallot production has ranged from 2,004,000 to 2,085,000 tons per year, representing a 5.06% increase compared to 2023 (BPS, 2025). However, production experience downward trend in the 2019-2023 period, with a decline rate of 15,48% (BPS, 2023). Meanwhile, other data indicate that 22,000 to 29,000 tons of shallots are wasted annually (Secretariate General - Ministry of Agriculture Republic of Indonesia, 2023). A decline in production and the disposal of products as waste are indicators of post-harvest disturbances caused by pathogenic organisms and rot. *Fusarium oxysporum* is a cosmopolitan fungus that usually damages shallot bulbs (Liu *et al*., 2022), in addition to the *Penicillium* and *Botrytis* species which also have the potential to disrupt growth and cause crop failure (Xu *et al*., 2023).

So far, efforts to prevent damage and loss of yields have always used toxic synthetic chemical pesticides starting from seed preparation to planting. Such actions are in clear violation of the commitments set forth in the 2015 Paris Agreement (Zhou *et al*., 2021), besides giving rise to the risk of agro-ecosystem degradation in the form of biotic and abiotic stress on cultivated plants (Venäläinen *et al*., 2020). Meanwhile, there are no preservatives or protectants that can protect tubers from damage caused by organisms carried from the field during harvest and post-harvest storage.

Currently, available preservatives for shallots rely on toxic pesticides that pose risks to both human health and the environment (Bellisai *et al*., 2023) and are only used as seed treatment for planting needs in the field. On the other hand, to preserve tubers of food ingredients that are ready to use and safe when consumed are not yet available. To achieve this, it is necessary to find alternative materials that serve the purposes of seed treatment and food preservation, while being environmentally friendly when used on seeds and safe for human consumption. To prepare materials for seed treatment and bulb preservation that are environmentally friendly and serve as alternatives to toxic synthetic chemicals, extracellular compounds produced by biological agents are used, as they are known to effectively inhibit pathogens that damage these materials. One organism that meets these criteria is the fungus *Trichoderma*. This fungus is able to produce various extracellular compounds in the form of enzymatic compounds and useful secondary metabolites such as alkaloids, glycosides, tannins, terpenoids, steroids, flavonoids, quinones, and saponins (Lavicoli *et al*., 2017) which can inhibit pathogens that cause rot (Li *et al*., 2019; Zhang *et al*., 2021).

Trichoderma have been widely used as active ingredients in biopesticides and biofertilizers due to their positive effects in inducing plant resistance to pathogens (Ostadi *et al*., 2023), thereby reducing disease incidence (Singh *et al*., 2021) and enhancing nutrient absorption and plant growth (Kazerooni *et al*., 2022). Several extracellular compounds produced by *Trichoderma* have been shown to effectively control *Fusarium* (Farihadina & Sutarman, 2022) and other rot-causing agent, making *Trichoderma* a powerful and effective biocontrol agent (Woo *et al*., 2023). The application of extracellular compound filtrate to seeds as a seed treatment, in addition to directly protecting the seeds from dumping off pathogen infection, can also act as a fertilizer that can encourage plant growth (Abdelrhim *et al*., 2023; Liu *et al*., 2022) and induce an increase in the biological activity of quality soil at the start of planting (Al-Shuaibi *et al*., 2024; Kabir *et al*., 2023).

Onion bulbs can also be infected with *Aspergillus niger* and *Penicillium* species, which produce mycotoxins and cause bulb rot (Dimant & Degani, 2023; Silva *et al*., 2021); this is also found in

storage. However, this pathogenic fungus can be suppressed by *Trichoderma* which has been proven to produce extracellular compounds including the chitinase which can damage the cell walls of pathogenic fungi (He *et al*., 2019; Morán-Diez *et al*., 2021). Therefore, enzymatic substances and various inhibitory compounds can be isolated and effectively used for bulb preservation and seed protection in shallot.

By cultivating this fungal biocontrol agent on a suitable medium and collecting the filtrate from the medium where its colonies grow, the following materials can be obtained: (i) an alternative to toxic synthetic chemical pesticides for seed treatment, supporting seed and seedling care and protection, and (ii) natural preservatives for shallot cloves and bulbs, contributing to food storage and human health. However, specifically for seed treatment purposes, *Trichoderma* propagule suspension from multiplication results in growth media without filtration can be used, considering that *Trichoderma* spores will germinate and grow to protect shallot seedlings.

The novelty of this research is the study of the integration between (i) the product of the activity of biological agent fungi which is projected as an alternative pesticide for seed treatment to protect seeds and seedlings and during the early growth of shallot plants from disturbances and attacks by dangerous pathogens that can kill plants, and (ii) products that can be used to preserve shallot cloves as food in storage for direct consumption purposes. These two functions are combined in one material which can later be continuously developed through research and implementation to create products that can be used to support food security but are environmentally friendly.

This study aims to identify the fungi responsible for rotting shallot bulbs during storage and to evaluate the effectiveness of T. asperellum, a potential biopesticide agent, in inhibiting pathogenic fungi and preserving the bulbs for both storage and seed use.

2. MATERIALS AND METHODS

2.1 Pathogen Identification and Kock's Postulates

Shallot bulbs showing rot symptoms were sampled by cutting the affected tissue into pieces approximately 5 mm in size. To eliminate contaminating microbes not related to the rot-causing organisms under investigation, the samples were immersed in 50% alcohol for 30 seconds and rinsed twice with distilled water. After draining, the samples were placed in the middle of Potato dextrose agar-chloramphenicole (PDA-c) media in a petri dish. After four days of incubation, hyphal threads were sampled using the tip of a sterile inoculating needle and smeared onto the surface of PDA-C medium. At the end of the 12-day incubation period, the hyphae of the pure rotting pathogenic fungal colony that had grown perfectly were blended to produce a suspension containing hyphal pieces and spores. Meanwhile, fresh shallot cloves without rot symptoms were rinsed, drained, and surface-sterilized in 50% alcohol for 30 seconds. They were then ready to be used for the Koch's postulate test. The surface of the shallot cloves was sprayed with a suspension of pathogenic fungi evenly and incubated for seven days. Bulbs that showed rot symptoms similar to the rot symptoms at the beginning of the observation were cut into the tissue and inoculated onto the surface of the PDA-c medium. Furthermore, the appearance of the pathogenic fungi was observed macroscopically and microscopically to determine the type of isolate of the pathogenic fungi that caused rot in the shallot cloves.

2.2 Inhibition Test (In Vitro)

The initial stage was an antagonism test, which measured the inhibitory effect of *Trichoderma* biological control fungi on pathogenic fungi, as well as the potential inhibition of *Trichoderma* by the pathogens. In this experiment, *T. esperellum* Tc-Jjr-02, collection of the Microbiology and

Biotechnology Laboratory of the Muhammadiyah University of Sidoarjo (UMSIDA), was used. From each colony of *Trichoderma* isolates and pathogenic fungi isolates aged 12 weeks, samples were taken and placed on PDA-c media facing each other in one dish (dual culture) or individually (mono culture) in different dishes (Sutarman et al., 2021). The increase in the growth of the colony radius of each isolate was measured and compared with the length of the growth of the colony radius of each isolate grown individually. The test was carried out for 96 hours and was observed every 24 hours.

The next stage was the passive inhibition test, conducted by growing *Penicillium* propagules using a method similar to the active inhibition test. However, the PDA-C medium used in this stage contained *Trichoderma* filtrate at concentrations of 2% and 5%, with 0% serving as the control.

2.3 Tubers Preservation and Germination Test

The enrichment of the biological agent *Trichoderma asperellum* Tc-Jjr-02 was carried out by culturing it on PDA-C medium for 14 days. After incubation, the culture was harvested, yielding a total of 10^7 CFU/mL. This suspension was then used to prepare both the spore solution and the total filtrate containing *Trichoderma*'s extracellular compounds.

The *Trichoderma* culture from one PDA-C plate was blended with water for 5 minutes to produce a 200 mL suspension. The resulting coarse suspension was diluted 1:1,000 (10^3) to obtain a *Trichoderma* suspension with an active spore density of 10^6 CFU/mL. The results of the enrichment of fungal propagules of biological agents through the bioreactor were filtered using a sterile membrane with 0.22 μm pores. The filtration results were ready to be used for preservation after being diluted 20 times with distilled water so that the filtrate concentration became 5%. The filtrate obtained was free of fungal propagules in the form of spores or hyphae and even free from contaminants including from the type of *Trichoderma* itself. Propagation of onion bulb rot pathogens on PDA-c media for use in onion clove preservation experiments. After a 14-day incubation period, both isolates were ready for in vivo testing.

Preservation. Onion cloves collected one week after harvest were surface-sterilized by immersing them in 50% alcohol with gentle stirring for 30 seconds, followed by three rinses in sterile water with stirring, each lasting 30 seconds. Meanwhile, a suspension of the filtration results of pure *Trichoderma* culture was prepared with a sterile membrane with pores of 0.22 μm and without filtration was diluted 20 times by adding distilled water. Five drained onion seeds were placed on a sterile tray and sprayed with a pathogen suspension containing 10^6 CFU/mL of active spores. After incubation for 12 hours, spraying of the *Trichoderma* suspension with or without filtration was repeated five times each, as well as without suspension and *Trichoderma* filtrate. For the suspension without filtration, the spores of this biological agent have been homogenized at a density level of 10^6 CFU.mL^{-1}. Considering the emergence of post-inoculation symptoms of about 10 days (Živković *et al*., 2021), the incubation period was determined to be 14 days. The shallot bulbs that had been inoculated according to the treatment were placed in a sterilized room/cover. The intensity of the symptoms of pathogen infection was measured 14 days after inoculation calculated using formula (1) which is based on the symptom criteria as stated in Table 1.

$$\text{Intensity of symptoms: } Is = \sum \frac{\sum_{i=l}^{k=4}(i.ni)}{N \,.\, k} \times 100 \quad \text{..... (1)}$$

With the provisions i = the score value of the symptoms of red onion clove infection as stated in Table 1, ni = the number of cloves with the i-th score, N = the number of cloves observed in each experimental unit and k = the highest symptom score.

Table 1. Score and category of symptoms of rotting shallot cloves infected with *Penicillium* sp.

Score	Symptom criteria
0	No symptoms of infection (healthy)
1	1-20% of a tuber shows symptoms of infection/rot (mild symptoms)
2	20-50% of one tuber shows symptoms of infection/rot (moderate symptoms)
3	>50% of one tuber has symptoms of infection/rot (severe symptoms)

The three types of treatments were arranged in a Completely Randomized Design (CRD). The observation data were analyzed using ANOVA at a 5% level followed by the HSD Test at a 5% level to determine the differences between treatments.

Germination. The method is the same as in preservation, but in one container, 50 healthy shallots that have not been contaminated or do not show symptoms of pathogen infection and have been incubated for 10 weeks in storage are placed. Each treatment is repeated three times and the percentage of germination is calculated.

3. RESULT AND DISCUSSION

3.1 Results of Pathogen Identification and Koch's Postulates

The identified pathogenic fungi that consistently infected shallot bulb exhibited macroscopic and microscopic characteristics, as shown in Figure 1. The colony on PDA-c media appears as a white hyphae weave. The hyphae are septate with a diameter of 2.87 ± 0.29 µm hyaline, while the spores are hyaline with a size ranging from 3.1 ± 0.32 µm. Based on both macroscopic and microscopic morphological characteristics, the pathogenic fungus responsible for the rotting of shallot cloves in storage was identified as *Penicillium* sp. Soil-borne fungi that have also been determined as *Penicillium* species have a similar colony growth pattern with a black base but on the white surface with an average hyphae diameter of 2.53 ± 0.28 µm and conidispores of 2.58 ± 0.13 µm (Yuliantoro *et al*., 2023).

Observations of *Penicillium* fungus that caused rot in legumes showed an average conidial size of 3.8 x 3.2 µm, was identified as *P. expansum* (Lee *et al*., 2022), Meanwhile, a similar species found in horticultural plants, *Polygonatum odoratum,* is also known as *P. expansum* (Kim *et al*., 2022). According to findings by other researchers, a consortium of morphologically similar pathogenic fungi—particularly based on symptom onset around 10 days after infection, symptom development, and spore size—was identified as as *Penicilium* spp. (Wang *et al*., 2023). Similarities compared to the findings of other researchers in morphology are not enough to determine to the species level. Further research is needed, especially determination based on molecular markers (Stošić *et al*., 2025) by comparing the observed fungal nucleotide sequences with gene bank archives through the use of BLAST (https://www.ncbi.nlm.nih.gov/) (NCBI, 2025) and we can also see the kinship with other fungi (Zhang *et al*., 2020). After passing a series of Koch postulate, this fungus consistently causes infections with symptoms similar to the initial condition.

3.2 Inhibition Test Results

The results of the in vitro active inhibition test on PDA-C medium, conducted over a 96-hour incubation period after inoculation (HAI), are presented in Tables 2 and 3.

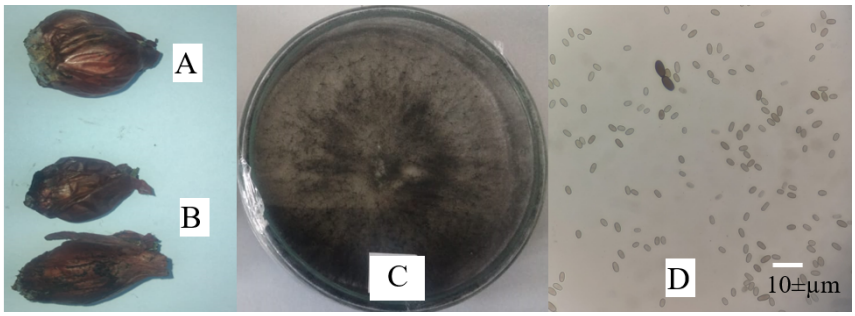

Figure 1. Infected shallot bulbs before (A) and after (B) Koch's Postulates, appearance of colonies (C) and conidiospores (D) of pathogenic fungi.

Table 2. Average radius of growth of *Penicilium* sp. colonies (cm) and % growth inhibition by *T. esperelum* at 24-96 HAI

Growth model	24 HAI	48 HAI	72 HAI	96 HAI
Dual culture	1.42±0.07	3.03±0.47	3.33±0.48	3.80±0.12
Mono culture	1.47±0.06	4.77±0.35	7.70±0.25	9.97±0.03
Inhibition (%)	3.40	36.41	56.70	61.92

Table 3. Average radius of *T. esperelum* colony growth (cm) and % growth inhibition by *Penicilium* sp. at 24-96 HAI

Growth model	24 HAI	48 HAI	72 HAI	96 HAI
Dual culture	1.50±0.03	5.53±0.22	6.23±0.12	6.73±0.12
Mono culture	1.42±0.04	2.03±0.07	5.93±0.19	9.93±0.03
Inhibition (%)	-2.3	-172.1	-5.1	2.2

Direct inhibition of *T. esperelum* against the pathogenic fungi that cause onion bulb rot began at 48 HAI with an inhibition rate of 36.41% and continued to increase to 61.92% at 96 HAI. Meanwhile, inhibition of *Penicillium* against *T. esperellum* began to occur at 96 HAI. Starting at 48 HAI, the activity of this pathogenic fungus actually accelerated the growth of *Trichoderma* colonies with an inhibition percentage of (-) 172.1% (Table 3). This is in line with the characteristics of *Trichoderma* which excels in competition for space and resources (Natsiopoulos *et al*., 2024). Various extracellular compounds which are bioactive secondary metabolites are produced by *Trichoderma* (Tian *et al*., 2024) potentially inhibit the growth of pathogenic fungal colonies including *Pencillium*. Meanwhile, this pathogenic fungus produces compounds such as organic acids, polygalacturonase enzymes involved in the breakdown of cell walls, solistatinol and viridicatin, and polyketide derivative compounds (Moraes Bazioli *et al*., 2019; Shah *et al*., 2023), in addition to producing mycotoxins (Xu *et al*., 2023) which can suppress *Trichoderma*. On the other hand, various compounds can be utilized by *Trichoderma* while encouraging its growth (Singh *et al*., 2024). Between these two types of fungi have shown interactions with different response characteristics. The inhibition of *Penicillium* sp. colony growth passively by *Trichoderma* filtrate is presented in Table 4.

At 48 HAI *Trichoderma* has inhibited colony growth by 36.4% (Table 2), while utilizing resources to meet its life needs in growing rapidly (Singh et al., 2024; Zhang et al., 2021) and responding to changes in substances including antimycotics that arise due to the activity of pathogenic fungi (Thambugala et al., 2020). This condition further proves that *Trichoderma* is effective in inhibiting and controlling pathogenic fungi. *Trichoderma* filtrate 2% was able to inhibit 54.65% at 24 HAI; while at the same time the inhibition reached 58.14% at a filtrate concentration of 5% (Table 4).

Table 4. Average radius of *Penicilium* sp. colony growth (cm) and percentage of growth inhibition by *T. esperelum* filtrate at 24-96 HAI

Trichoderma Filtrate		Observation time			
		24 HAI	48 HAI	72 HAI	96 HAI
Concentration	0%	2.87±0.13	3.50±0.19	4.33±0.21	4.57±0.06
Concentration	2%	1.30±0.10	1.83±0.08	2.20±0.10	2.40±0.10
Inhibition (%)		54.65	47.62	49.23	47.45
Concentration	5%	1.20±1.00	1.60±1.83	1.80±2.10	2.00±2.20
Inhibition (%)		58,14	54,29	58,46	56,20

Table 5. The average effect of *Trichoderma*-based preservatives on the intensity of stem rot symptoms in red onions inoculated with *Penicilium* sp. at 10 days after inoculation (DAI)

Treatment	Average	Δ (%)
Without *Trichoderma*	51.87±5.78 a	-
Trichoderma Suspension	14.80±5.73 b	71.5
Trichoderma Filtrate	16.65±6.08 b	67.9
HSD 5%	8.67	

Note: Numbers followed by different letters indicate differences in effect at the 5% HSD test level; Δ is the percentage reduction in rot symptoms against the "without *Trichoderma*".

3.3 Preservation and Germination

The provision of preservatives in the form of suspension and filtrate of *T. eseprellum* applied to shallots that have been inoculated with *Penicilium* sp. showed a very significant effect (p<0.01). The average effect of different *Trichoderma* preservatives and the decrease in the intensity of rot symptoms by administering a suspension containing *Trichoderma* propagules and its filtrate are presented in Table 5.

Trichoderma suspension application was able to suppress the growth of onion bulb rot symptoms at 10 DAI by 71.5% compared to the control (without *Trichoderma*). Meanwhile, the filtrate from the suspension that was free of *Trichoderma* propagules could suppress up to 67.9%. Without *Trichoderma*-based substances, tuber rot symptoms reached an average of 51.87±5.78 which is in the severe category and tubers can no longer be used as food or seeds.

Based on the germination test, the average germination was obtained as shown in Figure 2 with a representation of the appearance of sprouts that grew in the three types of treatments with an incubation period of 10 days (Figure 2). During the 10-day incubation period, the germination rate of onion bulbs only reached 37.5±63% with conditions as shown in Figure 3 (A). In the sprout section, there was a pathogen infection that would threaten the death of the sprouts in the next growth phase. Meanwhile, both in the administration of *Trichoderma* suspension and filtrate (Figure 3 B and C), 85.4±9.6% and 83.3±7.2% showed black spots and mycelium of pathogenic fungi, respectively. This is because *Penicillium*, which was applied before *Trichoderma* application, is a fungus that damages plant harvest products (Luciano-Rosario *et al*., 2020) and utilizes sucrose and other organic materials as energy sources (Li *et al*., 2024). The distinctive characteristics of this pathogenic fungus can cause serious attacks on tubers (Park *et al*., 2021). Considering that all seeds had been inoculated with pathogens, good seedling growth proved that the extracellular compounds produced by this biological agent fungus in both the Trichoderma Suspension and Trichoderma Filtrate treatments in this experiment had stimulated seedling growth and triggered plant defense responses to biotic stress (Ortiz Vásquez *et al*., 2025; Sutarman *et al*., 2022) as well as supporting growth and playing an important role in controlling pathogens (Sutarman *et al*., 2023; Yao *et al*., 2023).

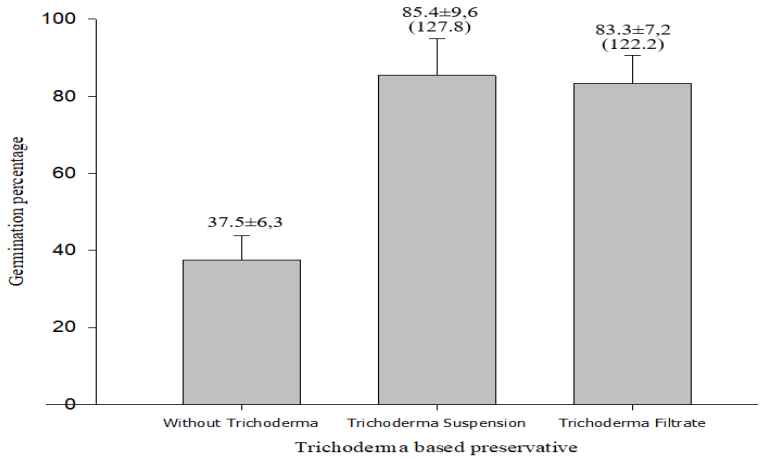

Figure 2. Average percentage of germination of shallot bulbs given *Trichoderma*-based materials at 10 days after germination. The numbers in brackets indicate the percentage increase in germination compared to without *Trichoderma*.

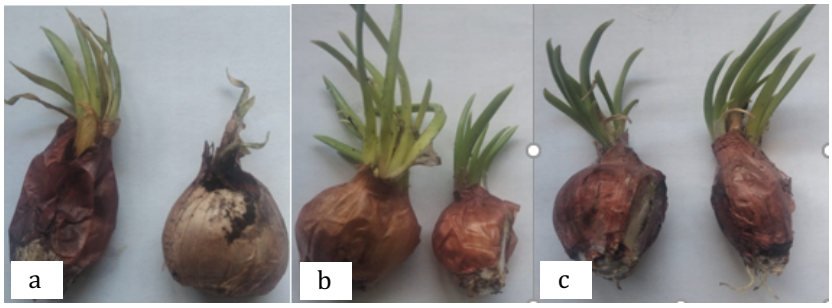

Figure 3. Appearance of sprouts in treatments without *Trichoderma* (A) and with *Trichoderma* suspension (B) and filtrate (C).

4. CONCLUSION

Tichoderma esperellum collection of the UMSIDA Microbiology Lab in a dual culture in-vitro test was able to inhibit the growth of pathogen colonies by 61.9% and increase the growth of its own colonies by -172.1% respectively at 96 and 48 hours after inoculation. Application of *T. esperellum* suspension and filtrate respectively suppressed bulb rot by 71.5 and 67.9% in storage and increased germination by 127.8 and 122.2% compared to without *Trichoderma*. The use of *Trichoderma* filtrate has the potential as an active ingredient in biopesticides for the preservation of cloves and protection of shallot seeds.

5. ACKNOWLEDGEMENT

Thanks are extended to the Directorate of Higher Education of the Muhammadiyah Central Board for the Funding of the Muhammadiyah National Research Grant Batch VIII in 2024 with contract Number: 0258.603/I.3/D/2025 and the Muhammadiyah University of Sidoarjo for the support of the necessary research facilities.

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