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Application of trichoderma and aspergillus as biofertilizers in eco-friendly ratoon rice cultivation

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ABSTRACT
The study's goal is to find the best native fungus from rice husk waste so that a solid biofertilizer can be made with high-husk flour as a carrier material and an inert agent. This study was conducted on agricultural land in Seloliman Village, Trawas District, and Mojokerto Regency. Biofertilization and biological agent formulation activities carried out at Muhammadiyah University of Sidoarjo's Microbiology Laboratory aided the research. The experiment was conducted using a factorial randomized block design. The first factor consisted of three treatments: no fungi, *Trichoderma sp*., and *Aspergillus sp*. The second factor consists of soil treatment and apical treatment. The six treatment combinations were repeated four times (24 samples). The variables measured comprised plant height, number of panicles, weight of grain per plant, weight of 100 grams of grain, and the efficacy of biological agents in improving plant growth and productivity. All data underwent analysis of variety and then an HSD test at the 5% significance level to identify disparities among treatments. The study reveals that isolates Tc-013 and As-022 were identified as *Trichoderma esperellum* and *Aspergillus flavus* or *A. oryzae*, respectively. The application of *Trichoderma* and *Aspergillus* caused a decrease in the intensity of disease symptoms, reaching 64.7% and 37.3%, an increase in plant height and number of panicles, and an increase in the weight of 100 grains of 59.89 and 49.35%, respectively, as compared to the control treatment where the fungus was not applied.

Contribution/Originality: This research demonstrates the potential of a biologically active fungus, which has relatively never been used as a biofertiliser, to increase plant growth and production while maintaining the health of ratoon rice plants, particularly in areas where leaf necrosis pathogens are endemic.

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1.INTRODUCTION

The need for food for rice increases from year to year in line with the increasing population, while agricultural intensification is one approach to achieving food security that must be achieved and always maintained (Urfels et al., 2023).

Taking into consideration the efficient use of production time and resources, ratooning rice with one land processing and two planting periods is an alternative form of intensification. Ratoon rice cultivation has been developed in several countries and is considered to have higher resource use efficiency and less environmental impact compared to other rice growing systems (Firouzi, Nikkhah, & Aminpanah, 2018; Liu et al., 2019). Ratoon rice also shortens crop production time (Torres, Natividad, Quintana, & Henry, 2020). Apart from having advantages, this cultivation method also has disadvantages, including that the productivity of the ratoon phase planting is lower than the first phase (Yu et al., 2021).

However, in several tests, ratoon rice cultivation has the potential to contribute to the impact of global warming (Shen, Zhang, & Zhang, 2021). This is due to the fact that during its cultivation, it consistently depends on non-sustainable hydrocarbon-based production resources, particularly in the utilization of chemical fertilizers and pesticides. On the other hand, using production resources wisely, ratoon rice has the potential to overcome the impact of global warming (Yuan, Cassman, Huang, Peng, & Grassini, 2019) and can significantly reduce methane gas emissions and the carbon footprint of rice fields (Lin et al., 2022).

So far, various studies on rice ratoons that have been carried out relate to efforts to improve land use optimization techniques, reduce the potential impact of global warming, and develop strategies for dealing with pest disturbances and increasing soil fertility (Ding et al., 2022; Zheng et al., 2022). As is the case in rice cultivation practices in general, in rice rations, the choice of environmentally specific varieties is a necessity (Xu et al., 2022), which of course requires materials to support soil fertility and plant physiology.

Thus, the ratoon rice system has so far relied on the support of chemical fertilizers and pesticides, bearing in mind that certain diseases and pests are endemic in some areas (Ye et al., 2017; Zaidi, Mukhtar, & Mansoor, 2018). In line with the 2015 Paris Agreement commitment (Zhou et al., 2021), there is a demand for each country to reduce the use of fossil materials, including reducing and replacing the use of petroleum derivatives such as pesticides and fertilizers.

For this reason, alternative fertilizers and pesticides are needed in order to increase plant resistance and protection from biotic and abiotic stresses while being environmentally friendly. The use of biological agents that have the ability to biofertilize and act as biocontrol agents is a prospect for the search for wise alternatives to plant production resources. Trichoderma and Aspergillus are types of fungi that, when applied to plants, have the capacity to substitute for fertilizers and pesticides.

Trichoderma produces enzymatic extracellular compounds that are capable of degrading soil organic matter, which produces nutrients for plants, as well as compounds that act as regulators of plant growth (Amanullah & Khan, 2023; Mezadri et al., 2022; Sutarman, Setiorini, Li'aini, & Rahmat, 2022). With the chitinolytic enzymes it produces and the ability to compete in its niche, this fungus can inhibit and damage pathogenic fungal cells so that it can provide plant protection (Matas-Baca et al., 2022; Tjahjanti, Prihatiningrum, & Miftahurrohmat, 2022). Meanwhile, several species and strains of Aspergillus have many benefits in agriculture because these fungi are able to degrade soil organic matter (Hsieh, Kurzai, & Brock, 2017; Lopes et al., 2021), produce organic acids that can chelate metals from oxides (Klaic et al., 2018), increase the amount of dissolved phosphate in the soil (Klaic, Plotegher, Ribeiro, Zangirolami, & Farinas, 2017), and increase the biological oxidation of elemental sulfur (Majaron et al., 2020). Thus, these two types of fungi have the potential to act as biofertilizer biological agents that have the potential to increase plant growth and health.

So far, not much has been done on the utilization of these two biological agent fungi to help normal paddy rice plants, while ratoon rice research has only relied on the plant's ecophysiological response. This research integrates the function of nutritional support for rice plants through ratoon shoots and crowns and provides protection against various potential disturbances of indigenous diseases in lowland rice crops such as blast (*Pyricularia oryzae*), striped spot (Cercospora spp.), spot (*Helmintosporium*), and stem base rot (*Rhizoctonia* sp. and *Fusarium* sp.).

This research aims to determine the ability of the indigenous fungi *Trichoderma* sp. and *Aspergillus* sp. applied to shoots and shoots of rice cultivation during the rice ratoon period to provide support for the productivity and health protection of lowland rice.

2. METHOD

2.1. Identification of Biological Agents

This experiment used *Trichoderma* Tc-013 and *Aspergillus* As-22, two fungal isolates found by screening a group of indigenous isolates from lowland rice cultivation in Biting Hamlet, Seloliman Village, Trawas District, Mojokerto Regency. These are now in the collection of the Microbiology and Biotechnology Laboratory, Universitas Muhammadiyah Sidoarjo.

The two isolates were propagated in PDA-chloramphenicol media, incubated for 10 days, and observed macroscopically for the shape of the colonies. Furthermore, they were sampled from the culture dish and processed onto the surface of a glass object to observe the shape and dimensions of the hyphae and spores and identify them.

The mycelium from each isolate in a petri dish was taken as much as 50 mg and put in 200 µl dH_2O in a BashingBead™ tube, then deoxyribonucleic acid (DNA) isolation was carried out according to the standard procedure of Quick-DNA Fungal/Bacterial Miniprep Kit™ catalog number D6005. Then the samples were amplified using Forward Primer ITS 1 5'-TCC GTA GGT GAA CCT GCG G-'3 and Reverse Primer ITS 4 5' TCC TCC GCT TAT TGA TAT GC-3'. The cycle used was predenaturation at 95°C for 5 minutes, followed by denaturation at 95°C, annealing at 60°C and elongation at 72°C for 1 minute each. Final elongation (post-elongation) 72 °C, for 5 minutes. The cycle used is 40 cycles.

Sequencing of the polymerase chain reaction (PCR) DNA fragments was carried out using the Sanger sequencing method, with the PCR product sent to a commercial DNA sequencing service (1st Base; Singapore) using an ABI 3730XL sequencer machine.

Then the nucleotide arrangement obtained was compared to the gene bank using the Basic Local Alignment Search Tool (BLAST) program available at the National Center for Biotechnology Information (NCBI) (NCBI, 2022).

Homologous sequences obtained from the NCBI Gene Bank were reconstructed with MEGA 7 software (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) using the Neighbor-Joining method to produce a phylogenetic tree.

2.2. Biofertilizer Formulation

Colonies that have grown fill the petri dish for about 7-10 days, ready to be harvested and made as a suspension that was previously crushed using a blender. Suspensions containing isolates of biological agent fungi, each in the amount of one petri dish of the culture mixed with 250 ml of distilled water, were poured and mixed with 2,500 g of husk flour (40 mesh size) as a carrier agent until evenly distributed. After drying for 12-24 hours, a biofertilizer formula is formed. Prior to application, it is important to determine the active spore concentration of the fungus by the implementation of the serial dilution technique. The active population of spores is determined to be 10^6colony forming unit (CFU).gr^{-1}, if the calculation results exceed this amount, dilution will be carried out with the addition of sterile husk flour to reach an average population of 10^6 CFU.gr^{-1}.

2.3. Field Efficacy Test

The rice fields that have been harvested are irrigated for approximately 3 days, and then the rice stalks remaining from harvesting are cut with the aim of growing new rice shoots. The cutting size is about 3-5 cm. Biofertilizer formula is given as a soil treatment, which is considered fertilization, carried out a week after harvesting the first stage of rice plants until the ratoon plants are irrigated, and as an apical treatment after the plants have been watered until the panicles begin to fill. The treatments in this experiment were as follows: (i) without biofertilizer application but using conventional chemical fertilizers, (ii) Trichoderma biofertilizer application, and (iii) Aspergillus biofertilizer application. This experiment was repeated seven times. Each experimental unit is a plot measuring 2x5 m^2. The determination of plot boundaries for each experimental unit is carried out after harvest. As a soil treatment, each biofertilizer is given to the soil around the plant roots at a dose of 200 grams (husk flour formula containing 10^6 CFU.g^{-1} active spores of biological agents) per plant. Apical treatment is applied to the canopy using 200 g of biofertilizer (a husk powder formula containing 10^6 CFU.g^{-1} active spores of biological agents) dissolved in 2,000 ml of neutral water as a suspension which will be sprayed eight times during the growth and filling of the rice grains at intervals of one week. Soil treatment is given before planting or a week after harvest. Next, the plant height, number of panicles per plant, harvest weight per plant, and weight of 100 grains were observed. Since spots caused by *Cercosspora oryzae* and *Helmintosporium oryzae* are common on the land that was used, itt was tested how applying biofertilizer might affect the plants' ability to fight these pathogens. Assessment of plant health is carried out at the beginning of the generative phase, or between 42 and 70 days after planting (DAP). The criteria used to determine the intensity of attack symptoms are as shown in Table 1.

Table 1. Criteria for symptoms of endemic pathogens in rice plants.

Score	Criteria for attack symptom
0	No damage occurred
1	As many as 1-25% of the leaves have striped and spotted spots on the leaves
2	As many as 25-50% of the leaves have striped and dotted spots on the leaves or 25% have wide spots covering each leaf
3	As many as 50-75% of the leaves have striped and dotted spots on the leaves or 50% of the spots expand to cover each leaf or 25% of the leaves die
4	More than 75% of the leaves have striped and dotted spots on the leaves or 75% of the spots have widened to cover each leaf or 50% of the leaves have died

2.4. Data Analysis

Field experiment data were analyzed using analysis of variance (ANOVA) at the 5% level, followed by the honestly significant diffirence (HSD) test at the 5% level to determine differences between treatments.

3. RESULTS AND DISCUSSION

3.1. Identification Results

The results of macroscopic observations of the shape and color of the colonies of the two biological agents, fungi, as well as microscopic observations showing woven hyphae and spores, are shown in Figure 1. The green color on the colony of isolate Tc-013 is typical of *Trichoderma*, with branched hyphae and conidiospores, each with a diameter of 2.56±0.39 µm and 2.68±0.45 µm. Meanwhile, the As-022 colony appeared brown-black with branched hyphae measuring 4.72±0.63 µm and an average spore diameter of 2.74±0.15 µm.

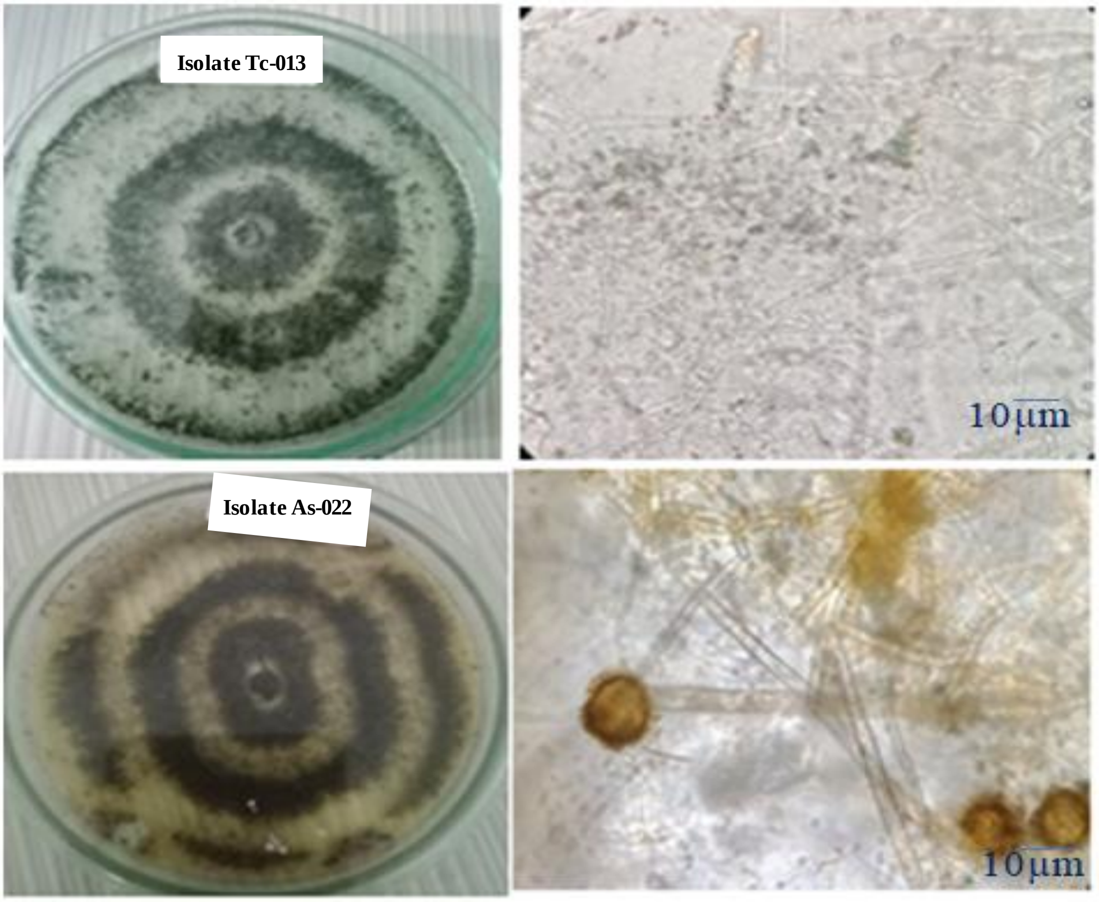

Figure 1. Morphology of Trichoderma sp. Tc-013 (top) and Aspergillus sp. As-022 (bottom) is used as a biological agent.

The nuleotide sequencing results of the two fungal isolates, each of which totaled 579 nucleosides for Tc-013 and 551 nucleosides for As-022, are presented in Figure 2.

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ACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCAAT
GTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGG
TGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCA
AACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTG
AGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTT
GGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACAT
TGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCAT
TTCAACCCTCGAACCCCTCCGGGGGATCGGCGTTGGGGATCGGG
ACCCCTCACACGGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGC
CGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGC
GCGGCGCGTCCACGTCCGTAAAACACCCAACTTTCTGAAATGTTG
ACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCA
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GTTCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCTTAG
TTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCA
GCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGA
TCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACT
TTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAG
CGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGAATCAT
CGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGG
CATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGT
GTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAA
GGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTT
TGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAA
TCAATCTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGC
TGAACTTAAGCATATCAATAAGCGGAGG
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Figure 2. Nucleoside sequence of DNA sequences of Tc-013 (top) and As-022 isolates (bottom).

In the process of matching nucleotide arrangements to DNA sequences, according to Brock, Döring, and Bidartondo (2009), a species is said to be the same if the ITS homology of the organism's rDNA sequence has a similarity of 97% (Sutarman, 2022). Thus, when matching with collections contained in BLAST, similarities below 97% are ignored, and priority is given to those with 100% similarity.

The BLAST search results from 2022 show that the Tc-013 sequences is 100% identical to the sequence from Trichoderma asperellum (Sequence ID: MH56933331.1) (NCBI, 2022). Meanwhile, isolate As-022 is similar to Aspergilluysoryzae (Sequence ID: MH56933331.1), and Asspergillus flavus (Sequence ID: KX067855.1) with 100% similarity at 557 nucleotide sequence length. Reconstruction results using MEGA software (Kumar et al., 2018) with Neighbor-Joining method obtained a phylogenetic tree as shown in Figure 3.

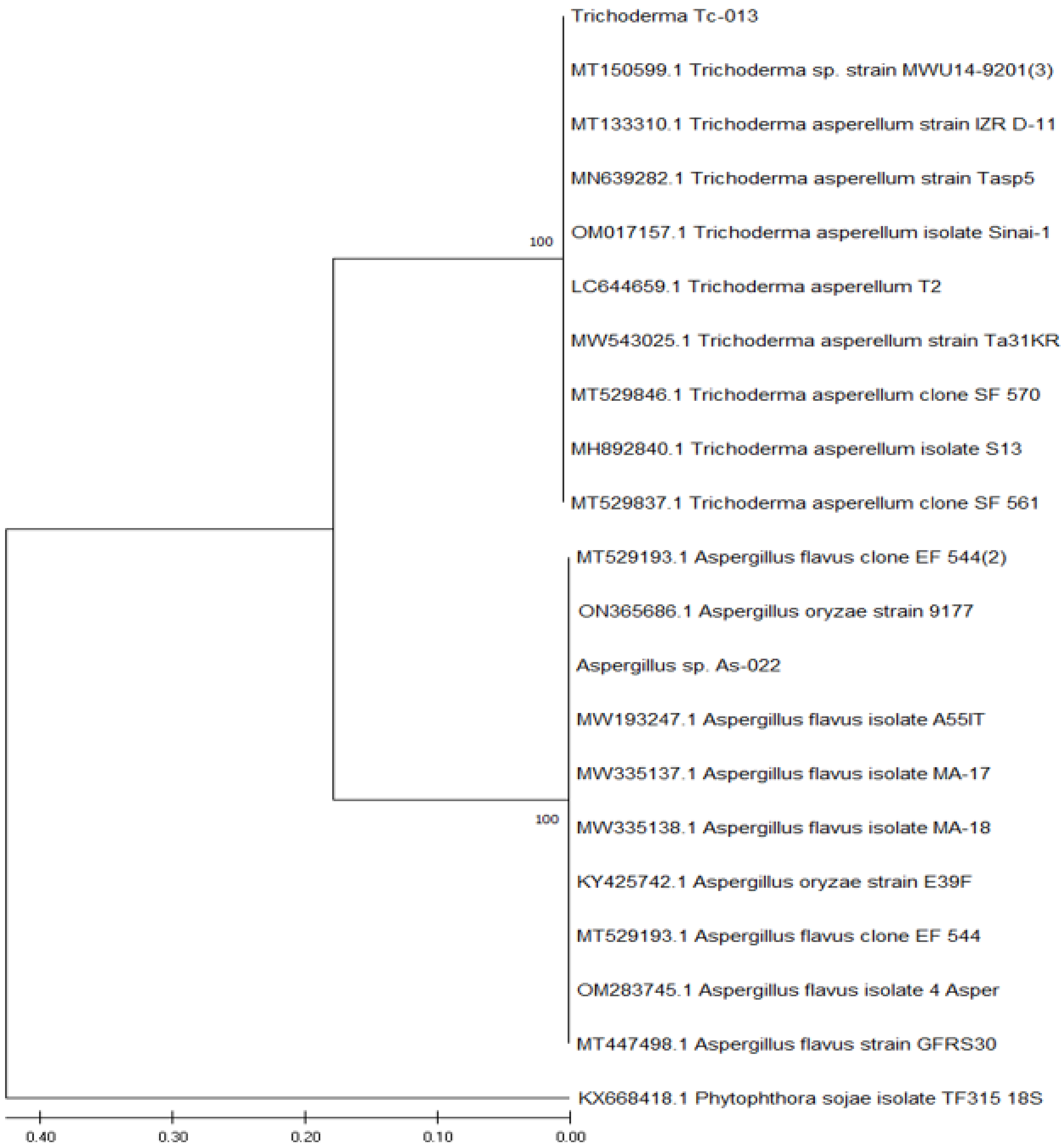

Figure 3. Filogegentik isolate Tc-013 dan As-022.

3.2. Field Test Results

3.2.1. Plant Growth

The response of ratoon rice terms of grain weight and 100 grain weight per plant showed that biofertilizer made the average higher than when no biofertilizer was used Figures 4 and 5. Table 2 shows the idex of necrotic disease symptoms on leaves and how much the symptoms got better when biofertilizer was used compared to when biofertilizer wasn't used.

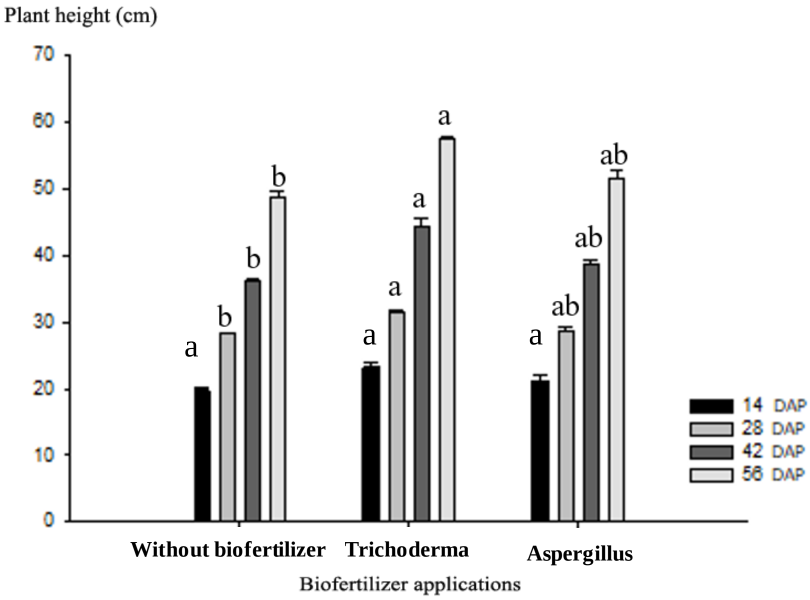

Figure 4. The average effect of biofertilizer application on plant height 14-56 HSP.
Note: Different letters in the column indicating the same observation time indicate differences in the effect of biofertilizer application on the 5% HSD test.

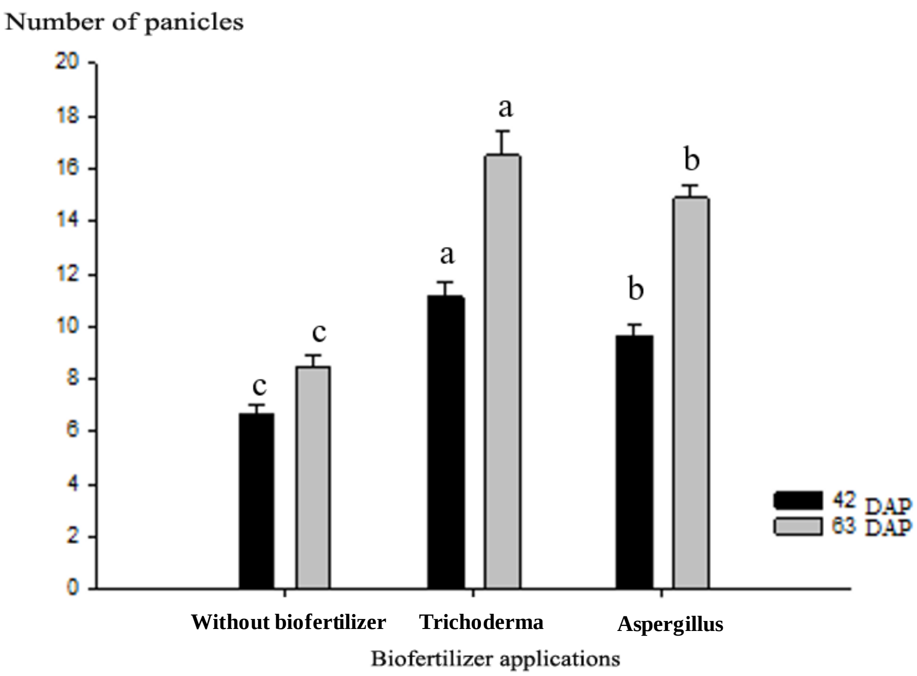

Figure 5. The average effect of biofertilizer application on the number of panicles per plant was 42 and 63 DAP.
Note: Different letters in the column indicating the same observation time indicate differences in the effect of biofertilizer application on the HSD test at 5% level.

Table 2. Effect of biofertilizer application on the intensity of leaf necrotic spot disease symptoms of ratoon rice variety IR 64 and its reduction compared to no biofertilizer at 42 and 70 DAP.

Treatments	42 DAP		70 DAP	
	Intensity of disease symptoms (%)	Reduction in the intensity of disease symptoms (%)	Intensity of disease symptoms (%)	Decreased intensity of disease symptoms (%)
No biofrtilizer	22.77±0.63	-	35.27	-
Trichoderma	8.04±0.77	64.7	14.29	59.5
Aspergillus	14.29±1.17	37.3	23.66	32.9

3.2.2. Plant Production and Biological Age Performance

The response of ratoon rice in terms of grain weight and 100 grain weight per plant showed that the application of biofertilizer made the average higher than when no biofertilizer was used (Figures 6 and 7).

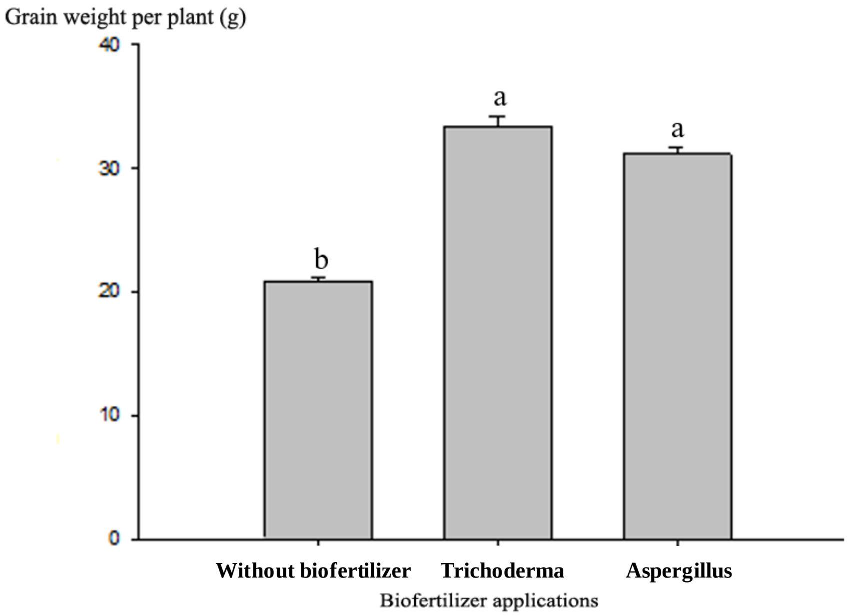

Figure 6. The average effect of biofertilizer application on grain weight per plant.

Note: Different letters in the column indicate differences in the effect of biofertilizer application on the HSD test at 5% level .

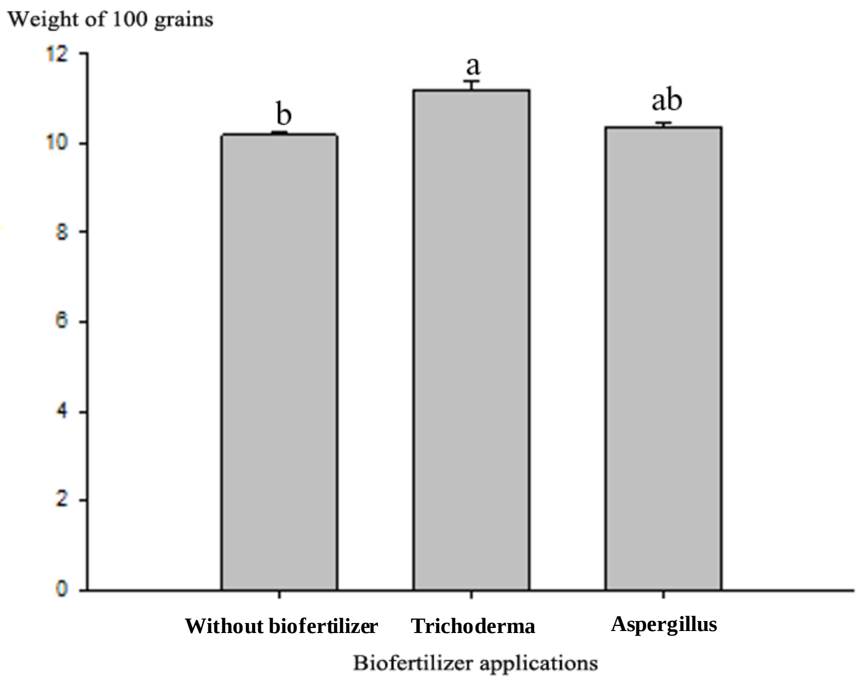

Figure 7. The average effect of biofertilizer application on the weight of 100 grains of grain per plant.

Note: Different letters in the column indicate differences in the effect of biofertilizer application on the HSD test at 5% level.

Application of biofertilizer with Trichoderma and Aspergillus fungi can increase plant height, number of panicles, grain weight per plant, and weight of 100 ratoon rice plants compared to treatments not applied (control) (Table 3).

Table 3. Performance of biofertilizer biological agents on increasing growth response and production of rice varieties IR 64 ratoon model.

Agenhayati biofertilizer	Plant response to performance of biofertilizer biological agents (%)*			
	Biofertilizer biological agent Increase in average plant height 56 DAP	Increase in average number of panicles	Increase in average grain weight per plant	Increase in average weight of 100 grains
Trichoderma	17.94	94.12	9.98	59.89
Aspergillus	5.69	75.00	1.77	49.35

Note: * Increased growth response and plant production compared to control (without biofertilizer).

3.3. Discussion

Isolate Tc-013 has similarities in colony appearance, hyphal branching morphology, and hyphal and spore diameter dimensions to *Trichoderma esperellum* (Sutarman, 2022; Sutarman, Jalaluddin, Li'aini, & Prihatiningrum, 2021). BLAST search results (NCBI, 2022) showed 100% similarity to *T. asperellum* (Sequence ID: MT102403.1). Isolate Tc-013 also has similarities with isolate RM-28, whose data is stored at NCBI with the additional number MK092975 and identified as *T. Asperellum* (Anam, Reddy, & Ahn, 2019), and isolate T1 (accession numbers GenBank LC158827, KU497722, and KU497723) (Baiyee, Ito, & Sunpapao, 2019), andisolate TC01 (GenBank accession numbers MH752042 and MN813963) (Shang, Liu, & Xu, 2020). The shape and dimensions of the conidispores and chlamydospores are also similar to those of the endophytes of *T. asperellum* VM 100 (KY412854) (Leylaie & Zafari, 2018) and *T. asperellum* isolate GDFS1009 (Karuppiah, Sun, Li, Vallikkannu, & Chen, 2019) and isolates Ta1 and Ta2 (Hewedy et al., 2020).

Morphologically, isolate As-022 cannot be differentiated from several isolates found in Indonesia and from various other countries. As-022 has sequences that are similar to many Aspergillus variants, which were shown phylogenetically with primers ITS-1 and ITS-2 with identical levels of up to 100% as *A. oryzae* (KY655350.1) (Devi & Joshi, 2015) and as *A. Flavus* (Alshehri & Palanisamy, 2020). For the final determination of these two naming alternatives, in-depth research is needed regarding their physiological performance and the metabolites they produce.

When Trichoderma and Aspergillus biofertilizers were used, the plants grew taller, had more panicles, and produced more grain. The 100 ratoon rice plants also gained weight (Table 1). This shows that both Tc-013 and As-022 isolates have demonstrated their ability as biological agents that act as biofertilizers.

Various evidence has been shown by the ability of Trichoderma sp. as a biofertilizer, which is able to increase biological activity around the rhizosphere of plants before the soil surface under watery conditions. However, it appears that the role of Trichoderma, which is applied through spraying the plant canopy, makes a significant contribution to helping plant growth and improving the soil structure around plant roots by decomposing organic substances contained in the soil. Many organic substances are available in the rhizosphere. With the application of the fungus *Trichoderma* sp., the organic material will be decomposed and converted into ions that can be absorbed and utilized by plants. In addition, this fungus acts as a mycoparasite against pathogenic fungi and also produces metabolites that act as growth hormones for plants (Vinale et al., 2014) so that it can induce disease resistance (He et al., 2019). Trichoderma degrades organic matter to produce nutrients and increases plant resistance to abiotic environmental stress (Sachdev, Singh, & Singh, 2018).

Aspergillus sp. has the ability to fix nitrogen in the soil, thus helping the plantsmeet their nitrogen needs. Such a function benefits the growth of the plants (Dutta & Das, 2017). *Aspergillus* sp. plays a significant role in decomposition, bioremediation, and biocontrol, being used to synthesize organic acids, enzymes, and secondary metabolites (Kagot, Okoth, De Boevre, & De Saeger, 2019). Numerous studies provide evidence for the effectiveness, quantity, and efficiency of Aspergillus' extracellular hydrolytic enzymes (Brown et al., 2016). Several Aspergillus species' genomes exhibit the genetic expression of cellulase and hemicellulase enzymes' ability to operate proficiently (Cong et al., 2017; De Gouvêa et al., 2018) indicating the organisms' potential as bio-fertilizer agents. This fungus also produces cellulase and hemicellulase (Midorikawa et al., 2018). *Aspergillus* can survive in poor temperature and humidity conditions, has high adaptability to substrate complexity, produces various useful secondary metabolites, and produces various types of enzymes that degrade various polysaccharides and proteins (Flores-Gallegos, Veana-Hernandez, Michel-Michel, Lara-Victoriano, & Rodríguez-Herrera, 2016) and other lignocellulose degrading enzymes (Monclaro et al., 2020), as well as describe the complex structure of lignocellulosic biomass by releasing monomer sugars (Dimarogona, 2016) as an energy source. Therefore, this fungus shows promise for breaking down organic matter and may have practical applications in the development of biological fertilisers.

Aspergillus is generally capable of producing volatile organic compounds (VOCs), including various acid molecules, alcohols, aldehydes, aromatics, ketones, terpenes, thiols, and their derivatives (Wang et al., 2021). Even from the bioconversion process of organic materials, compounds can be produced that are capable of promoting plant growth and acting as a signal of spore germination so as to guarantee positive interactions in their ecology (Lemfack, Nickel, Dunkel, Preissner, & Piechulla, 2014).

The characteristics of the two fungal isolates showed their ability to support plant growth (Table 2), especially through the application of plant canopy spraying. The index of attack symptoms was much lower (Table 1) in the application of biological agents. Meanwhile, the ability to reduce the intensity of attack symptoms in the Trichoderma application was much greater than the Aspergillus application. This is possible because there is a lot of research evidence showing the strength of this fungus as a biocontrol agent. The activity of volatile metabolites produced by *T. eseprellum* is able to inhibit *F. Oxysporum* (Tao et al., 2020) in addition to supporting plant growth (Al-Askar, Saber, Ghoneem, Hafez, & Ibrahim, 2021) considering that T. esperellum is also capable of producing auxin (Wang et al., 2020). In this study, the application of these two biological agents, fungal isolates, increased the grain weight and weight of 100 grain grains, respectively, 75-94.12 and 49.35-58.89% (Table 2). This provides a projection to increase production potential equivalent to planting rice twice. In general, ratoon rice can produce 50% of the first harvest (Oda, Nguyen, & Huynh, 2019). In addition, ratoon rice is a wise choice in order to help reduce pressure on the environment due to conventional rice cultivation while maintaining food security (Jiang et al., 2021; Yang et al., 2022).

4. CONCLUSION

The biological agent fungus isolates Tc-013 and As-022 were each identified based on molecular markers as *Trichoderma esperellum* and *Aspergillus flavu,s* or *A.oryzae*. Application of Trichoderma and Aspergillus formulated as biofetilizers increased plant height by 17.94 and 5.69%, respectively, 56 days after planting (DAP), increased the number of panicles by 94.12 and 75.00%, respectively, and reduced the intensity of attack symptoms by 64.7% and 37.3% at 42 DAP and 59.5% and 32.9% at 70 DAP. These two biological agents were able to increase the weight of first-plant grain by 9.98 and 1.77%, respectively, and increase the weight of 100 grains by 59.89 and 49.35%, respectively. Biofertilizer with the active ingredients Trichoderma isolate Tc-013 and Aspergillus As-022 has great potential to be applied to wetland plants as ratoon rice to increase growth and provide protection for plant health.

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Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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