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Application of Biofertilizing Agents and Entomopathogenic Fungi in Lowland Rice

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Abstract. Protection of rice plant health that is environmentally friendly and free of toxic synthetic chemicals requires the availability of effective biological agents in biocontrol and biofertilization. This research aims to determine the effect of *Trichoderma asperellum* and entomopathogenic fungi on growth, pest attack index, and lowland rice production in stem borer endemic land. The experiment was arranged in a split plot design with the main plot being Trichoderma applications consisting of without and with *Trichoderma asperellum*, while the sub plots were applications of entomopathogenic fungi consisting of *Beauveria bassiana*, *Metarhizium anisopliae*, and without entomopathogens. The experiment was repeated four times. Data were analyzed using ANOVA and HSD test at the 5% level. The interaction of these two factors has a significant effect on plant response. Combination application of *Trichoderma esperellum* with *Beauveria bassiana* and *Metarhizium anisopliae* increased the maximum number of tillers by 39.53% and 46.51% respectively, reduced the intensity of the rice white stemborer attack symptoms by 39.53-46.51%, increased the maximum number of panicles by 15.76% and 13.34%, grain weight per hill 21.66% and 17.33%. *Trichoderma asperellum* and entomopathogens can be used to protect rice plants in areas endemic to stem borer pests.

1 Introduction

Food security has been at risk from a number of disruptions to the cultivation of food crops. A decrease in harvested area of 141.95 thousand hectares, or 1.33 percent, was recorded due to disruptions. This decrease was compared to the rice harvested area in 2020, which totalled 10.66 million hectares, indicating a decline in food security [1]. The presence of pests is mentioned as one of the factors contributing to the worsening production of rice (*Oryza sativa L.*).

The utilization of chemical pesticides to counteract pest attacks and chemical fertilizers to enhance plant resilience to environmental stress, whether biotic or abiotic, has had damaging consequences. Such consequences encompass the formation of plant resistance to pests [2], the suppression of non-target organisms that serve a beneficial purpose to plants [3], and the hampering of the physical and biological traits of the soil that ultimately results in decreased land productivity [4]. Moreover, the effects of chemical pesticides are detrimental to agricultural ecosystems, leading to damage and instability [5], environmental

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pollution [6], and potential threats to human health [7]. Additionally, the reliance on synthetic chemicals across all cultivation practices exacerbates the challenges faced in meeting joint carbon emission targets agreed upon by all stakeholders [8].

One of the superior substitutes for the use of synthetic chemicals is the utilization of biological agents from the fungi division, which includes those that serve as active ingredients in biological fertilizers and those that serve as active ingredients in insecticides. Trichoderma fungi exhibit a biocontrol function against various pathogens [9-10], and are also capable of promoting plant growth whilst enhancing the plant's resistance to biotic stress [11-13]. Entomopathogenic fungi, for example *Beauveria bassiana*, are capable of producing toxins that can damage host cells and ultimately prove fatal to insects [14]. Multiple studies have demonstrated its effectiveness in controlling armyworm (Lepidoptera) [15], beetles (Coleoptera) [16-17], and flies (Diptera) [18-19]. Similarly, *Metarhizium anisopliae* has also been shown to be an effective method of controlling various insect pests [20-22].

Both forms of entomopathogens demonstrate efficacy in managing the larval stage of leaf and stem borers in plants by direct contact of spores or germ tubes with the insect body's cuticle [23]. Multiple studies have been conducted indicating the fungus's performance after contact with host body cells [24-25]. The efficacy of applying this fungus to larvae that have infested plant tissue remains unknown, including against white rice stem borer (sundep) pests that grind the inner plant tissue of rice stems during their attack phase. Young Lepidoptera larvae that bore into rice stems during the seedling phase cause white symptoms on the leaf sheaths, while during the generative phase, they produce white on the panicles and empty grains [26]. On the contrary, it is believed that both *B. bassiana* and *M. anisopliae* possess the capability to generate substances that aid in the growth of plants [27-28].

Given the capacity of these two entomopathogenic fungi to enhance plant fertility, it is imperative to assess their efficacy in strengthening the resistance of rice crops against infestation. At present, *Trichoderma* is extensively used as a biofertilizer. The potential biofertilizer capabilities of the two entomopathogenic fungi must undergo evaluation and be compared to those of Trichoderma. This will facilitate future acquisition of alternative biofertilizer agents, which demonstrate the ability to enhance plant resistance to stem borer infestation.

This research aims to investigate the impact of biological agents, as well as the potential interactions between entomopathogenic fungi and *Trichoderma*, on the growth and resistance of rice paddy against stem borer attack. The study will explore the extent to which these agents can maintain growth ability and reduce the severity of symptoms caused by the pest. Furthermore, the investigation aims to provide insights into the mechanisms underlying the observed effects and their implications for pest management in rice cultivation.

2 Method

2.1 Trial preparation

Biological agent fungi isolates comprising *Trichoderma asperellum* Tc-011, *Beauveria bassiana* Bs-07, and *Metarhizium anipsoliae* Ms-09 were obtained from the Laboratory of Microbiology and Biotechnology at the University of Muhammadiyah Sidoarjo (LMB-UMSIDA). These isolates were cultured on PDA media for 12 days. Each of the three samples was collected, then combined with distilled water in a 1,000 ml beaker using a blender until the volume reached 500 ml. The resulting mixture was a suspension containing the propagules of each biological agent and was added to the water once again with thorough stirring until the beaker was nearly full. The mixture was poured into a tray filled with 5,000 g of coarse husk flour (40 mesh) and stirred until it became entirely even, forming a solid biological agent formula in the form of flour. Following a 14-day incubation period, the dilution method [29] was employed to conduct population measurements of the biological agents, revealing that the spore densities of the three biological agents were 10^5 CFU.g^{-1} when utilized as treatments in this experiment.

Soil for seedbeds and rice paddies is prepared using a tractor engine to create the required porosity and drainage for optimum growth of rice plants. In addition, M70D rice variety has undergone germination tests achieving an impressive success rate of 98-100%.

2.2 Efficacy trial

The study was conducted in the agricultural land of Ngengkreng Hamlet, Sewor Village, Sukorame District, Lamongan Regency. White rice stem borer (*Scirpophaga* sp.) infestations often occur in this village and are known to attack plants in the vegetative growth phase. The experiment began in a nursery that was set up as a split plot design. The main plot involved Trichoderma applications, including both with and without *Trichoderma*, while the subplots were entomopathogenic fungi applications consisting of treatments without entomopathogens, with *B. bassiana*, and with *M. anisopliae*. The experiment was conducted four times. In the nursery phase, each experimental unit consisted of seedling beds measuring 1 m x 4 m. Each primary plot is encircled by drainage channels to divert water and prevent it from flowing into other plots. For each Trichoderma-treated plot, a solid formulation consisting of 2,000 g of husk flour was mixed two weeks before seeding the beds or applied at a rate of 500 g/m^2. The control plot was solely supplied with husk flour at the same dosage. Entomopathogenic fungi were applied by soaking 400 grams of flour in 10 litres of sterile water for 12 hours. After even stirring, the filtrate was sprayed one week after stocking. For the control treatment, sprouts and seedlings were sprayed only with a suspension obtained by sieving husk flour without entomopathogenic fungal inoculants. The suspension was incubated for 6 hours before spraying. Spraying was carried out up to four times, with a 7-day interval between each round. To prevent the spraying droplets from landing on other plots, a plastic screen was used to cover each treated plot during spraying. The same measure was employed during experiments conducted in paddy fields.

During the field trials, a comparable process was implemented to that of the nursery beds. Trichoderma flour formula was administered to each main plot, measuring 2 m x 10 m, at a dosage of 500 g/m^2. Rice husk flour was only applied once during tillage or two weeks prior to plantation. Drainage was set up surrounding each main plot to redirect rainwater that came into contact with the treated area, preventing flow into other plots. Seedlings at a height of approximately 25 cm, possessing 5-6 leaves and a large, sturdy rootstock, and devoid of visible disease or pest infestations, are placed 25 cm apart when planted. Furthermore, entomopathogenic fungi are applied during initial planting and every two weeks thereafter until maximum tiller growth. In each plot of the experimental unit, the plant canopy is sprayed in the evening with a filtered suspension composed of 2,000 g of dissolved fungi in 40 litres of water and allowed to soak for six hours.

2.3 Variable observation and statistical analysis

The observed vegetative growth was monitored by the biweekly tiller production starting at day 14 post-planting. The treatment that did not receive applications of *Trichoderma* and entomopathogenic fungi, referred to as the control, was used as a basis for comparison in calculating the percentage differences in tiller numbers following maximum growth. (Δx) was used to represent the comparative values. Measurements were taken of the highest number of panicles, the grain yield weight at harvest, and the weight of 1,000 grains in a cluster. Additionally, the percentage of Δx for each treatment was calculated. The severity of sundep pest attack symptoms has been assessed on two occasions: (i) at 24 DAP, which represents the pest attack from the nursery stage to one week after planting, and (ii) at 56 DAP, which measures the aftereffects of the initial attack during the nursery and planting stages until the end of the vegetative growth phase. All technical abbreviations are explained when first used. The attack symptom index is calculated using formula (1).

$$IS = [\sum_{k=0}^{nk}(\mathrm{n}i)/(\mathrm{n}.k)]\,100\% \tag{1}$$

provided that IS = intensity of pest attack symptoms (%) , i = numerical value (score) of plants with the appropriate symptoms (Table 1), ni = number of clumps with a score of i, N = number of clumps observed per experimental unit, and k = the highest score of symptoms.

Table 1. Symptom score scale of rice plant damage due to stem borer attack on paddy rice plants score

Score	Damage symptom
0	No damage occurred
1	As many as 1-25% of damaged leaves turn yellow or turn white and die
2	As much as 25-50% of damaged leaves turn yellow/white or experience death, or panicles turn white
3	As much as 50-75% of damaged leaves turn yellow or die and turn white, or white panicles
4	More than 75% of damaged leaves turn yellow and turn white and the plants die

Observational data were processed using a Variety Analysis at the 5% and 1% level, followed by the HSD test at the 5% and 1% level to determine the difference in effect between treatments.

3 Result and Discussion

3.1 The development of the number of tiller

Table 2. The average effect of Trichodema and entomopathogenic fungi on the growth of the number of tillers per clump of lowland rice 14-56 DAP

Treatments	Number of tillers per clump				Δx
	14 DAP	28 DAP	42 DAP	56 DAP	(%)
T0E0	4.60±0.10 e	11.67±0.05 e	21.82±0.05	33.82±0.68 d	-
T0EB	4.97±0.13 c	14.76±0.17 d	23.02±0.05	35.83±0.72 c	5.93
T0EM	4.83±0.06 d	11.67±0.15 e	22.41±0.20	35.48±0.66 c	4.92
T1E0	4.74±0.06 d	15.69±0.06 c	23.02±0.84	38.66±0.83 b	13.43
T1EB	5.84±0.19 a	18.66±0.05 a	25.14±0.44	39.15±0.90 a	15.76
T1EM	5.74±0.19 b	17.93±0.30 b	24.67±0.37	38.33±0.94 b	13.34
HSD 5%	0.07	0.09	0.34	0.48	-

Note: T0= without Trichoderma, T1= Trichoderma, E0= without entompathogens, EB = *B. bassiana*, EM = *M. anisopliae*; letters accompanying the mean value of different treatments in one column indicate different effects; Δx: percentage increase in the number of tillers to treatment without biological agents at 56 DAP

The use of Trichoderma and entomopathogenic fungi produced a statistically significant effect ($p<0.01$) on the number of tillers in rice plants throughout the observation period. Table 2 outlines the average impact that the treatment had on the number of tillers. During the growth phase, the treatment using the biological agent achieved a maximum percentage increase of 5.93-15.76% in the number of tillers.

The proliferation of rice plant tillers indicates the impact of abiotic factors, particularly nutrient availability [30] and growth-promoting agents [31]. Additional to nutrition, there exists significant speculation of the effect of entomopathogenic and *Trichoderma* fungi on plant tiller growth. Similar findings were demonstrated through experimentation with *M. anisopliae* [32] and *B. bassiana* [33], which can greatly impact the increase in rice plant tillers. Additionally, research has indicated the participation of *Trichoderma* [34], which can raise the number of tillers per rice clump by as much as 20%.

3.2 Intensity of symptoms of pest attack

There was a noticeable distinction in the severity of symptoms of sundep insect pests across all treatments utilizing biological agents, as contrasted with the treatment that lacked *Trichoderma* and entomopathogenic fungi. Furthermore, the application of *Trichoderma* in combination with entomopathogenic fungi had a noteworthy impact on decreasing the pest's attack index. Table 3 displays the mean score of each attack symptom index, with each entry indicating the proportion of symptom index reduction observed in treatments where biological agents were used.

Table 3. The mean effect of *Trichoderma* and entomopathogenic fungi on the symptom index of stem borer attack on lowland rice plants 28 and 56 DAP

Treatments	Stem borer symptom intensity			
	28 DAP	Δx (%)	56 DAP	Δx (%)
T0E0	27.34±1.56 a	-	33.59±1.56 a	-
T0EB	15.63±2.55 c	42.86	23.44±1.80 c	30.23
T0EM	16.41±1.56 c	40.00	25.78±2.99 b	23.26
T1E0	21.09±1.56 b	22.86	26.56±1.80 b	20.93
T1EB	12.50±2.55 a	54.29	20.31±1.80 d	39.53
T1EM	14.06±1.80 d	48.57	17.97±1.56 3	46.51
HSD 5%	1.36	-	1.42	-

Note: T0= without Trichoderma, T1= Trichoderma, E0= without entompathogens, EB = *B. bassiana*, EM = *M. anisopliae*; letters accompanying the mean value of different treatments in one column indicate different effects; Δx: Percentage of reduction in the intensity of stem borer attack symptoms

The reduction in attack symptom intensity through biological agent treatment hints at a mechanism that does not have a direct impact on symptom intensity. This is due to the fact that there is no possibility of contact between insect pests while they are in their egg stage or as caterpillars. Stem borers, which lay their eggs on the exterior of plant tissues coated in saliva [35], provide an example of this. Newly hatched larvae penetrate the stem to feed on internal tissues during the vegetative and reproductive phases of plant growth, leading to the manifestation of symptoms [36]. It is noteworthy that symptoms of this stem borer attack may arise not only from *S. innotata*, but also from other insects such as *Gryllotalpidae* sp., which infests the base of the stem and root. The level of tolerance can be measured by assessing the severity of damage caused by borer attacks in the field [37-38]. White panicles (whiteheads) are an informative marker of plant resistance, susceptibility, and tolerance to attack by stem borer larvae. Additionally, it is a parameter used by farmers to determine the optimal timing of insecticide spraying [39-40] in order to prevent yield loss.

The experimental treatment did not involve direct contact between the host and the spores. Nevertheless, the entomopathogenic fungi effectively stimulated growth in rice plants (Table 2), which may significantly boost resistance against larval attack (Table 3). Various research results have demonstrated the effectiveness of *B. bassiana* in mitigating a range of pest attacks, such as stem borers [41-42]. Furthermore, *M. anisopliae* has proven successful in protecting rice plants from borer pests, leading to optimal vegetative growth [43]. Alternatively, the application of *Trichoderma*, while not pathogenic, has been found to lessen the severity of attack symptoms and promote increased rice crop yields [44-45].

3.3 Production

The use of *Trichoderma* and entomopathogenic fungi, as well as their interaction, significantly impacted the maximum number of panicles produced by plants and the grain weight per clump. However, there was no significant effect observed on the weight of 1000 grains. The average interaction effect on the maximum number of panicles, grain weight, and 1000 grain weight is displayed in Table 4.

Table 4. Mean effect of *Trichodema* and entomopathogenic fungi on maximum number of panicles, grain weight. and the weight of 1000 grains per clump of lowland rice

Treatment	Maximum number of panicles		grain weight per clump		weight of 1000 grains	
		Δx (%)	(g)	Δx (%)	(g)	Δx (%)
T0E0	33.82±0.16 d	-	105.51±4.27 e	-	31.03±1.04	-
T0EB	35.83±0.11 c	5.93	116.33±3.18 d	10.26	32.44±0.52	4.58
T0EM	35.48±0.28 c	4.92	114.50±3.02 d	8.53	32.20±0.29	3.78
T1E0	38.11±0.49 b	12.69	120.66±4.20 c	14.36	31.73±0.32	2.25
T1EB	39.15±0.10 a	15.76	128.36±4.94 a	21.66	32.88±0.05	5.95
T1EM	38.33±0.18 b	13.34	123.80±3.46 b	17.33	32.30±0.32	4.10
HSD 5%	0.21	-	2.00	-	Ns	-

Note: T0= without Trichoderma, T1= Trichoderma, E0= without entompathogens, EB = *B. bassiana*, EM = *M. anisopliae*; letters accompanying the mean value of different treatments in one column indicate different effects; Δx: maximum number of panicles, grain weight, and the weight of 1000 grains of grain per clump of lowland rice, ns = not significant

There is a significant difference in the percentage of panicles and grain weight per clump across all biological treatments, as shown in Table 4. Additionally, biological agents play a direct role in providing nutrients and plant growth regulators, as well as an indirect role in inducing plant responses that increase tolerance to borer attacks. The plant has a tolerance mechanism that compensates for damage despite the increase in attack symptoms [46]. In contrast, the control treatment exhibits susceptibility as the growth period progresses without the aid of biological agents. This may be due to larval disturbance and physical pressures like temperature and humidity that affect them [47]. During stem borer attacks, young rice plants divert nutrients to undamaged areas of the plant, stimulating tiller formation and growth, ultimately resulting in increased productivity [48-49]. The production parameters in treatments without biological agents show the effectiveness of this tolerance mechanism, although results are significantly lower than treatments using biological agents (refer to Table 4). The infection intensity did not sharply increase from 28 to 56 watersheds, particularly when using biological agents (see Table 3). This suggests an enhancement in plant tolerance to borer attacks, likely due to the contribution of nutrients, especially N [50]. The activities of all biological agent's population in the soil encourage an increase in soil N, H, and C [51-52]. These are the outcomes of the fungal biomass decomposition process within the soil [53].

4 Conclusion

The use of biological agents, such as the fungus *Trichoderma esperellum* and entomopathogenic fungi, had a significant impact on the growth and yield of M70D rice cultivar, grown on land known to be endemic to rice stem borer (*Scirpophaga sp*.) pests. The combination of these two factors significantly aided in the growth of tiller numbers and reducing pest attacks, resulting in an increase in the maximum number of panicles and grain weight per clump. However, it did not have a significant effect on the weight of 1000 grains per clump. The combination application of *Trichoderma asperellum* with *Beauveria bassiana* and *Metarhizium anisopliae* resulted in an increase in the maximum number of tillers by 39.53% and 46.51%, respectively. Meanwhile, the reduction in the severity of rice stem borer attack symptoms at 28 and 56 days after planting (DAP) was 48.57-54.29% and 39.53-46.51%, respectively. The maximum number of panicles increased by 15.76% and 13.34%, respectively, and the grain weight per hill increased by 21.66% and 17.33%.

Trichoderma asperellum and these two isolates of entomopathogenic fungi have the potential to be used to protect the health and production of lowland rice plants on land endemic to rice stem borer attacks.

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