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The Effects of the Application of *Trichoderma Asperellum* and Biochar on Growth and Productivity of Rice Cultivated by the SRI Method and on Soil Quality

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Abstract. Continuous use of chemical fertilizers to increase productivity often causes disruption to essential soil nutrients and environmental degradation and adversely affects soil rhizosphere microorganisms. This study aimed to determine the efficacy of the joint application of *Trichoderma asperellum* and biochar for increasing rice productivity using the SRI method and for improving the quality of paddy fields. This study consisted of eight treatments: T0BC (standard application of NPK without *Trichoderma asperellum* and biochar), T1BG (*Trichoderma asperellum* + NPK), T2BC (husk biochar + NPK), T3BC (coconut shell biochar + NPK), T4BC (mangrove biochar mangove + NPK), T5BG (*Trichoderma asperellum* + husk biochar + NPK), T6BC (*Trichoderma asperellum* + coconut shell biochar + NPK), and T7BC (*Trichoderma asperellum* + mangrove biochar + NPK). A randomized block research design was applied with three replications. Observations were conducted on plant height, number of tillers, leaf area index, number of panicles, number of pithy grains, number of empty grains, yield per pot, yield per hectare, and soil nutrient. The results showed that the application of a combination of *Trichoderma asperellum* and positive biochar increased the observed growth and yields 55.31% higher than the full dose of inorganic fertilizer. Combined application also increased pH, total N, available P, and Kdd, thereby increasing the nutrient content of paddy fields. Collectively, *Trichoderma asperellum* and biochar increased soil fertility and nutrient absorption, and encouraging the growth of *Trichoderma asperellum* increased the population in the rhizosphere.

Keywords: *Biochar, SRI, Rhizosphere, Trcihoderma asperellum*

1. Introduction

The SRI (System of Rice Intensification) method in rice cultivation can increase soil productivity, save labor and water, lower production costs, and increase rice yields [1]. The SRI works by changing the management of rice cultivation and the use of soil, water, and nutrients in rice production. The SRI with an aerobic system causes beneficial microbes to live in abundant population, increasing rice quality, number of tillers, and rice crop yields [2],[3].

The role of microbes in increasing rice yields in the SRI method has been tested in several research studies. The application of organic fertilizers to lowland soil containing microbes increases the growth of rice plants and soil nutrient quality, which is influenced by the activity and population of soil microbes [4]. According to some studies [5][6], the application of indigenous microbes had increased the yield of rice using the SRI method.

The use of bioorganic compost containing indigenous microbes *Trichoderma* spp., *Pseudomonas fluorescens*, and *Azotobacter* sp., increases the yield of rice cultivated using the SRI method and the

soil nutrient content of paddy fields [7][8]. *Trichoderma* spp. particularly is a multifunctional fungus present in almost all types of soil, including paddy fields. This fungus reproduces rapidly in the root area of plants, including the roots of rice plants. *Trichoderma* sp. requires organic matter to expand its breeding zone.

Organic matter such as biochar can contribute to soil nutrient levels by increasing water retention capacity, nutrient exchange capacity, and soil structure [9]. Biochar is a carbon black compound produced by pyrolysis at very high temperatures with no, or limited, oxygen supply. The higher surface area of biochar facilitates better soil structure with higher water content and nutrient capacity.

Elita et al. [8] in their exploration of the fungus *Trichoderma* sp. in Lima Puluh Kota Regency found *Trichoderma asperellum* in the root rhizosphere of rice of the Kuriak Kuning variety and *Trichoderma harzianum* and *Trichoderma asperellum* in the root rizosphere of rice of the Pandan Wangi and Junjuang varieties. Information about the potential of the application of *Trichoderma asperellum sp*. and biochar to rice cultivation using the SRI method has yet to be available. This research provides useful information that sustainable and environmentally sound production technology can increase the growth and yield of rice using the SRI method.

Therefore, this research was carried out to figure out the effects of the application of *Trichoderma asperellum* sp. and biochar on the growth and productivity of rice cultivated using the SRI method and on the quality of paddy fields. The purpose of this study was to evaluate the role of the application of *Trichoderma asperellum* and biochar of certain types in significantly increasing the yield of rice using the SRI method and the quality of paddy fields.

2. Methodology

Isolation of Indigenous *Trichoderma asperellum* was carried out at the Microbiology Laboratory of the Payakumbuh State Agricultural Polytechnic. Indigenous *Trichoderma* spp. was isolated from the soil in the rhizosphere area of rice of the Kuning Kurik variety in Nagari Taram, Harau District, Lima Puluh Kota Regency at coordinates 0^{o} 12' 25" S, 100^{o} 41' 33" E. Soil from the rice rhizosphere area was taken into a volumetric flask that already contained 100 ml of sterile distilled water at 1 gram and then stirred. The aqueous solution in isolation was taken at 1 ml into a Petri dish for culturing on Potato Dextrose Agar (PDA) medium. Observation of characteristics was based on Watanabe (2010). The selected *Trichoderma* isolates were sub-cultured for purification. Observations were performed on selected single spores, which were then re-cultured on PDA medium. The resulting 5-day-old cultures were subjected to molecular tests at the Balitbu Solok Laboratory for *Trichoderma asperellum* identification.

Indigenous *Trichoderma asperellum* propagation

Indigenous *Trichoderma asperellum* was obtained from the rhizosphere of rice of the Kuniang Kuriak variety. From the exploration, isolation, and characterization conducted *Trichoderma* spp. isolates were obtained. Molecular tests were later carried out to determine the fungal isolates found. The result of the molecular tests on the *Trichoderma asperellum* isolates is presented in Figure 1.

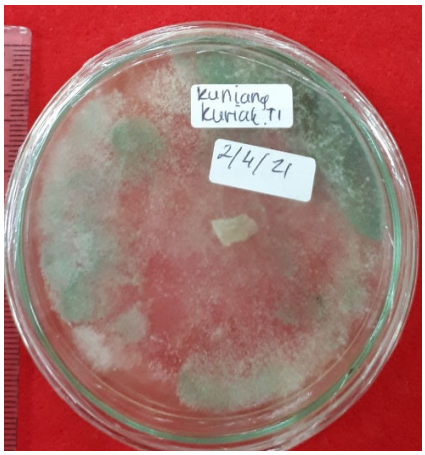

Figure 1. Indigenous *Trichoderma asperellum* from the rhizosphere of rice of the Kuniang Kurik variety

The *Trichoderma asperellum* was propagated using husk and bran media. Husks were soaked overnight and then drained. They were mixed with brans evenly at a 1:2 ratio and put into plastic bags at 250 grams. The plastic bags were pierced with a needle to let the air to enter. They were then steamed for one hour and left to cool down for 30 minutes. The *Trichoderma asperellum* culture from the Petri dish was diluted with 100 ml of distilled water, taken with a syringe, and isolated into the plastic. It was incubated for 7 days. The *Trichoderma asperellum* was then ready for propagation.

Experimental design and treatment

This study used a completely randomized design with eight treatments, namely

1. T0BC = (NPK standard application without *Trichoderma asperellum* and biochar),
2. T1BG (*Trichoderma asperellum* + NPK),
3. T2BC (husk biochar + NPK),
4. T3BC (coconut shell biochar + NPK),
5. T4BC (mangrove biochar + NPK),
6. T5BG (*Trichoderma asperellum* + husk biochar + NPK),
7. T6BC (*Trichoderma asperellum* + coconut shell biochar + NPK), and
8. T7BC (*Trichoderma asperellum* + mangrove biochar + NPK).

All the treatments were repeated three times. N was administered at 135 kg/ha, P_2O_5 was at 45 kg/ha, and K_2O was at 75 kg/ha. P and K fertilizers were given two days before planting, and N fertilizers were given three times at one-third of the dose one week, 25 days, and 50 days after planting. Biochar and *Trichoderma asperellum* were given a week before planting at a dose of 100 grams/pot each.

Microbial population in the rhizosphere soil

A calculation of the microbial population in the rhizosphere soil was carried out 8 weeks after planting on destructive samples. *Trichoderma harzianum* population observations were carried out by taking soil samples in each treatment using the modified serial dilution plate method [10]. Soil samples were taken and weighed at 1 g and then added with 9 ml of sterile distilled water in 12 ml test tubes and homogenized. After a homogeneous state was reached, 1 ml of the sample was taken and then added with another 9 ml of sterile distilled water in test tubes until reaching 10^{-3}. The samples were then poured on PDA medium in Petri dishes and inoculated for 3 days. The results of the inoculation were dissolved in sterile distilled water and dropped on a haemocytometer to count the number of spores with a density of 10^6 under a light microscope. The formula to determine the number of *Trichoderma harzianum* spores is as follows: S = t . dx 106 (n x 0.25).

Soil Chemical Analysis

Initial soil samples were taken from the initial soil before being entered into pots. Nutrient soil analysis was carried out after treatments by taking 250 g of soil sample from each experimental pot

based on [11] eight WAP because during this week rice plants enter the generative phase. Flooding was carried out to achieve a soil anaerobic condition. The results of the initial soil analysis are presented in Table 1.

Table 1. Analysis of Soil Chemical Properties

No	Soil Chemical Properties	Unit	Nominal/ Criteria
1	pH in H_2O (1:2.5)		5.90 low acidity
2	Organic ingredients	%	2.62 low
	C-organic	%	1.52 low
	N-total	%	0.13 low
	C/N		11.69 low
3	P-available (Bray-2)	Ppm	5.28 very low
4	K-can be exchanged	me.100 g^{-1}	0.14 low
5	Cation Exchange Capacity	me.100 g^{-1}	11.78 low

Preparation of Planting Medium

The soil medium was taken from the rice fields of Taram, Harau District, Lima Puluh Kota Regency. Soil was taken compositely at a layer depth of 0–20 cm. The soil was air-dried and then sieved through a 2 mm sieve at 11 kg pot^{-1}. In this study, no organic matter or compost was given to prevent the influence of other microbes apart from those used in the treatments (indigenous Trichoderma spp.). Analysis of soil nutrient content was carried out at the beginning of the study and after treatments eight weeks after planting (MST).

Plant maintenance included irrigation, weed control, and plant pest/disease control. Irrigation was carried out by watering until a state of stagnation occurred at 2 cm above the soil surface at most and allowing to dry, with a watering interval of three days. Weed control was conducted by pulling weeds, while mechanical pest control was conducted for grasshoppers. Harvest was performed 110 days after planting (DAT) when 90%–95% of the grains turned yellow.

Observation

The growth parameters measured were plant height, number of tillers, number of productive tillers per clump, number of pithy grains per panicle, number of empty grains per panicle, weight of 1,000 grains, percentage of pithy grains, percentage of empty grains, weight of dry grains per clump, root length, and root dry weight. Total number of grains per panicle and number of empty grains per panicle were calculated from three panicles per pot. All data were analyzed statistically using a test at 5% probability.

Data analysis

The data obtained were analyzed statistically using Statistical Analysis System (SAS) 9.1 for Windows. If there were significantly different data results, Duncan's Multiple Range Test (DMRT) was then carried out to see the difference in treatment at the 5% level.

3. Results and Discussion

3.1 Soil Chemical Analysis Results

The pre-study analysis of the paddy field soil aimed to determine the nutrient status of the soil before treatments. In Table 1, it can be seen that the pH of the soil was in a slightly acidic status. The organic

matter was at low conditions, with 1.52% C-organic soil and 0.13% N-total. The results of the soil analysis after treatments are presented in Table 2.

Table 2. Analysis of soil nutrients eight weeks after planting

Soil Chemical Properties	T0		T1		T2		T3		T4		T5		T6		T7	
pH	6.10	D	6.40	BC	6.37	BC	6.27	CD	6.30	C	6.60	A	6.53	AB	6.27	CD
C-organic (%)	1.52	C	2.43	B	2.39	B	2.47	B	1.63	C	2.84	A	2.39	B	2.63	AB
N-total (%)	0.143	D	0.21	BC	0.21	BC	0.197	C	0.153	D	0.223	A	0.207	BC	0.217	AB
C/N	10.64	C	12.15	AB	11.75	B	11.56	AB	10.64	C	12.73	A	12.18	AB	11.93	AB
P- avail. (ppm)	6.73	D	11.36	AB	10.05	C	10.01	C	9.83	C	11.59	A	11.52	A	10.41	AB
K- c b e (me.100 g^{-1})	0.150	E	0.2	BC	0.197	C	0.193	C	0.173	D	0.220	A	0.213	AB	0.200	BC
CEC (me.100 g^{-1})	11.65	D	13.48	ABC	12.91	BG	12.17	C	11.87	D	13.96	A	13.71	AB	13.03	BC

The numbers in the column followed by the same capital letter are not significantly different according to SD0.05

The results of the analysis of the average pH, C-organic, and N-total of the soil indicated the best soil quality. T5 was able to allow for a neutral soil acidity level most suitable for the SRI method on the rice root medium. This is supported by the best C/N ratio of 12.73 in T5. The initial growth of rice plants cultivated with the SRI method could be optimal in this treatment. The role of available P- and K-dd in suitable soil CEC could increase the plant metabolic ability.

3.2 Vegetative growth of rice plants cultivated using the SRI method

The application of *Trichoderma* spp. and biochar resulted in rice plant height, number of leaves per clump, and leaf area index that were significantly different from those resulted without *Trichoderma* spp. (see Table 3).

Table 3. Average Plant Height, Number of Leaves per Clump, and Leaf Area Index (ILD)49-56.

Treatment	Plant Height (cm)		Number of Leaves (Leaves/Clump)		LAI (49-56 DAP)	
T0	91.38	D	204.44	B	3.12	E
T1	105.43	C	273.29	A	4.59	A
T2	100.50	C	262.40	AB	3.71	C
T3	93.54	C	228.82	B	3.18	D
T4	96.33	C	225.67	B	3.23	D
T5	125.53	A	284.40	A	4.64	A
T6	112.71	B	273.84	A	4.36	A
T7	112.68	B	255.72	A	4.21	AB

The numbers in the column followed by the same capital letter are not significantly different according to LSD0.05

Table 3 shows that the application of *Trichoderma* spp. and biochar (T5, T6, and T7) resulted in the greatest plant heights among all the treatments. The application of *Trichoderma* spp. alone (T1) and a

combination of *Trichoderma* spp. and biochar (T5, T6, and T7) also had the greatest results in terms of number of leaves and ILD. *Trichoderma* spp. can act as plant growth-promoting fungus (PGPF) to promote plant growth, improve overall plant health, create a favorable environment, and produce large amounts of secondary metabolites [12].

3.3 Rice plant generative growth

The results of the application of *Trichoderma* spp. and biochar in average generative growth in terms of number of panicles per clump, number of seeds per panicle, and weight of 1,000 seeds are presented in Table 4.

Table 4. Average Number of Panicles per Clump, Number of Seeds per Panicle, and Weight of 1,000 Seeds at 12% Moisture Content

Treatment	Number of Panicles (Panicles/Clump)		Number of Pithy Seeds (Grains/Panicle)		Weight of 1,000 Seeds (g)	
T0	33.00	D	147.67	C	17.41	D
T1	46.00	B	232.33	B	19.92	B
T2	38.00	C	190.67	C	19.72	B
T3	36.00	C	165,67	C	19.11	C
T4	37.00	C	187.67	C	19.43	C
T5	52.00	A	248.00	A	21.02	A
T6	49.33	B	215.97	A	20.50	AB
T7	48.00	B	230,67	C	20.45	AB

The numbers in the column followed by the same capital letter are not significantly different according to LSD0.05

Average number of panicles, number of pithy grains, and weight of 1,000 grains are 3 variables derived from yield components. Number of panicles often plays a major role in supporting grain yields because the addition of just 1 panicle can add multiple grains, which can result in a significant difference in the treatments with *Trichoderma* spp. The four treatments with *Trichoderma* spp., T1, T5, T6, and T7, had a strong influence on the number of panicles. Two of the *Trichoderma* spp. treatments had high numbers of coniadia, namely 49.375 x 10^8 and 47.500 x 10^8. These conidia's ability to improve the root zone was strong, allowing the roots' ability to absorb nutrients to be improved (data on phosphorus content in leaves) (Table 6). As a result, the number of panicles was also higher than the other treatments.

The use of beneficial microorganisms (e.g., *Trichoderma* spp.) can increase the metabolism of primary or secondary plants and crop yields [13]

Table 5. Average Seed Yield, Grain Yield, and Leaf Phosphorus Levels

Treatment	Seed Yield (g/Clump)		Grain Yield (t/Ha)		Leaf Phosphorus Content (%)	
T0	91.38	F	9.60	D	0.15	E
T1	105.43	C	14.11	BC	0.19	B
T2	100.50	D	11.43	C	0.19	B
T3	93.54	E	10.30	C	0.18	C
T4	96.03	E	10.33	C	0.18	C
T5	125.53	A	14.91	A	0.21	A
T6	112.71	B	14.19	B	0.19	B
T7	112.68	B	14.12	B	0.17	D

The numbers in the column followed by the same capital letter are not significantly different according to LSD0.05

Average seed yield per pot is a set of yield components as with average number of panicles per pot, number of pithy seeds per panicle, and weight of one thousand seeds. T5 was able to consistently support the grain yield per pot. Even the nutrient content of phosphorus (P) in the leaves (Table 4) also demonstrated the same thing. It is known that physiologically P plays a major role in plant metabolism that takes place in the leaves and in distributing energy to each pithy grain. However, it is number of panicles that must be considered as the biggest factor in encouraging grain production (yield per pot and/or yield per ha). *Trichoderma* spp. is able to produce growth-promoting substances that can stimulate plant growth and increase photosynthesis and biomass production [14].

Table 6. Average *Trichoderma* spp. Conidial Population under a Microscope Calculated Using a Haemocytometer

Treatment	***Trichoderma*** spp. Conidial Population
T0	18.125×10^8
T1	38.750×10^8
T2	35.625×10^8
T3	30.625×10^8
T4	27.500×10^8
T5	49.375×10^8
T6	47.500×10^8
T7	40.000×10^8

Trichoderma spp. conidia are the forerunner to the subsequent increase in conidial populations in carrier materials such as biochar. The more suitable the biochar, the higher the number of conidia in the condition in the root zone. Several treatments that stood out in terms of number of conidia were T5, T6, and T7 (Table 5). The number of conidia that were able to encourage root activity of rice plants as shown by ILD, yield, and production components can be clarified by the following observation variables (Tables 6). Microbes, including fungi and beneficial bacteria from the rhizosphere soil, play an important role in providing nutrients by dissolving organic nutrients. These rhizosphere microorganisms also increase the availability of nutrients for plants and thereby increase plant growth and productivity by increasing soil fertility.

The combination application of *Trichoderma* spp. and biochar coupled with N-P-K fertilizer had a positive effect on soil properties (Table 2). Soil properties such as pH, organic C, total N, available P, and available K were significantly affected by the application of *Trichoderma asperellum* and biochar. The maximum fungal population was recorded at T5, followed by T6, T7, and T1. According to [15], soil properties such as organic matter, total N, available P, and available K were significantly affected by *Trichoderma* spp.

The results of this study showed that the application of *Trichoderma* spp. and biochar had a very significant positive effect on the growth of rice under the SRI method. The joint application of

Trichoderma spp. and biochar showed an increase in the performance of both. The synergistic effect of *Trichoderma* spp. and biochar played a more significant role in rice growth than did control. The results of previous research [8] showed that *Trichoderma* spp. increased plant height, number of tillers, number of panicles, number of pithy grains, and yield. The results of this study as shown in Tables 2, 3, and 4 showed that the application of *Trichoderma* spp. and the joint application of *Trichoderma* spp. and biochar increased plant height, number of leaves, leaf area index, number of panicles, number of pithy grains, and rice yield using the SRI method.

The application of *Trichoderma* spp. with biochar provided new changes to the soil, increasing soil fertility, improving soil structure, and increasing soil microbiota interactions. Biochar serves as a medium for expanding the colonization zone of beneficial fungal roots through the growth and elongation of hyphae. Cell proliferation of *Trichoderma* spp. hyphae increases soil fertility and contributes to more efficient nutrient absorption for plants, thereby increasing plant growth [16]. According to [17], biochar has a pore space structure that facilitates root proliferation and better absorption of nutrients, increasing root biomass.

The effect of the application of *Trichoderma* spp. with biochar was significantly higher than the application of NPK fertilizer alone. According to [18], there was an increase of 114% in wheat chlorophyll content thanks to the combined application of *Bacillus amyloliquefaciens* and biochar, which was higher than the increase made by control. [19] reported that the application of biochar increased the production of photosynthetic pigments. *Trichoderma* spp. and biochar interacted synergistically to increase chlorophyll and number of leaves, which was directly related to nitrogen absorption and photosynthesis rate.

4. Conclusion

Collectively, *Trichoderma asperellum* and biochar increased soil fertility and nutrient absorption, and encouraging the growth of *Trichoderma asperellum* increased the population in the rhizosphere. The application of a combination of *Trichoderma asperellum* and positive biochar increased the observed growth and yields 55.31% higher than the full dose of inorganic fertilizer.

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