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Effectiveness of Bio-organic Fertilizer Formulas to Increase Productivity of Upland Rice and Dehydrogenase Activity in Lampung Acid Upland

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ABSTRACT

Development of acid upland in Indonesia still has constraints. To solve the constraint, environmental friendly technology by using organic matter and beneficial microbes is necessary. This study aimed to determine the effectiveness of bio-organic fertilizer formulas for increasing productivity of upland rice plant in acid upland. This study used a randomized block design with 11 treatments using un-treatment as a control and some combination of NPK and bio-organic fertilizer. Each treatment was repeated three times, the size of plot was 5 m x 5 m. Upland rice var. Situ Patenggang was used as a plant indicator, with cultivication spacing of 40 cm x 15 cm. Combination treatment of NPK and bio-organic fertilizer increased population of P-solubilizer and N-fixer bacteria, plant height and tillers. Treatments of NPK-rec, ¾ NPK-rec and combination of ½ NPK-rec, ¾ NPK-rec and NPK-rec with both bio-organic formulas gave the weight of dried unhusked upland rice equal was 1.92 to 2.56 t ha^{-1} significantly higher compared to the control. The highest RAE was generated by treatment of NPK-rec combined with Bio-Organic Formula II about 137%. Bio-Organic Formula II could increase the growth and productivity of upland rice var. Situ Patenggang and could increase the efficiency udeof ½ NPK fertilizer dosage recommendations or equivalent with 125 kg urea, SP-36 100 kg, and 35 kg KCl ha^{-1} and increased the dehydrogenase activity.

***Keywords*:** Acid upland, bio-organic fertilizer, dehydrogenase

INTRODUCTION

Acid upland dominates land in Indonesia, especially in the wet temperate regions such as Sumatra, Kalimantan and Papua island, is about 102,817,113 hectares or 69.4% of the total land (Mulyani 2006). Acid upland in wet tropical areas is dominated by soils with type as Alfisol, Ultisol and Oxisol.Its characteristic causes low productivity (Hakim 2002), thus an obstacle to be developed. Upland rice is widely cultivated by crops in acid upland but until now its production has not been optimal yet. Major problems of acid upland are low pH, low fertility and high Al saturation (Ward *et al*. 2010; Heim *et al*. 2003) so plants cannot grow and produce well.

Indegenous resources in Indonesia have not been used to optimize the productivity of the soil and upland rice in acid upland yet such as organic matter and *Plant Growth Promoting Microbes* (PGPM).Various PGPM are able to stimulate plant growth because of their role in improving the availability of nutrients, nutrient capture, producing phytohormones (Kloepper and Schroth 1978), and producing anti-pathogen substances to defend themselves against microbial diseases. PGPM are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Kloepper, 1994; Glick 1995; Cleyet-Marcel *et al*. 2001).

Based on research conducted by Fitriatin *et al.* (2011) the use of phosphate solubilizing microbes like *Penicillium* sp. and *Pseudomonas* sp. can increase the production of upland rice in acid upland areas about 16%. Phosphatase enzyme produced microbes which are able to release P from Fe sorption that it will be available for plants. Application of bio-organic fertilizer can reduce the environmental damage. Chemical fertilizers generate much more greenhouse gases such as N_2O because of their inefficient utilization by crops but bio-fertilizer may reduce this effect (Kennedy *et al*. 2004). In order to successfully integrate biological and chemical approaches, it is necessary to more clearly define their effects on rhizosphere ecology under agricultural production conditions and develop dose–response data for establishment of populations in the rhizosphere (Kokalis-Burelle *et al*. 2006). This study aimed to determine the effectiveness of bio-organic fertilizer formula to increase productivity of upland rice in acid upland.

MATERIALS AND METHODS

Bio-organic Formula

The materials used in this study are Bio-Organic Fertilizer Formula II, Formula III, urea, SP-36, KCl and upland rice seeds (Situ Patenggang variety). Selected bio-organic fertilizers were achieved from a previous study (Santosa *et al.* 2011).

Formula II consists of: Isolate ES60.D7(N-fixer with nitrogenase activity was 0.13×10^{-2} µmol C_2H_4 hr^{-1} and produces IAA 8.57 ppm), ES71.A (N-fixer with nitrogenase activity was 12.68×10^{-2} µmol C_2H_4 hr^{-1} and produced IAA 5.42 ppm), ES54.B4 (produced chitinase and as an antagonist to *Pyriculariagrysea*/Blast disease), and SRPCG1(P-solubilizer), while Formula III consists of : isolate SR2.D2 (N-fixer with nitrogenase activity was 0.50×10^{-2} µmol C_2H_4 hr^{-1} and produced IAA 8.49 ppm), SR1.P1 (N-fixer with nitrogenase activity was 12.29×10^{-2} µmol C_2H_4 hr^{-1} and P-solubilizer) and SR7.6 (produced chitinase and as an antagonist to *Pyriculariagrysea*).

Experimental Designs

The research was conducted at the Experimental Farm of Acid Upland of Indonesian Soil Research Institute in Taman Bogo, Probolinggo, East Lampung, Lampung. Experiments used a randomized block design, the treatments consist of (1) Control (untreated), (2) Inorganic fertilizer (NPK) in dosage recommendations, (3) ¾ NPK-recommendations (4) Bio-Organic Formula II, (5) ½ NPK-recommendation + Bio-Organic Fertilizer Formula II, (6) ¾ NPK-recommendation + Bio-Organic Fertilizer Formula II, (7) NPK-recommendation + Bio-Organic Formula II, (8) Bio-Organic Formula III, (9) ½ NPK-recommendation + Bio-Organic Fertilizer Formula III, (10) ¾ NPK-recommendation + Bio-Organic Fertilizer Formula II, and (11) NPK-recommendation + Bio-Organic Formula III. Each treatment was repeated three times, the size of plot was 5 m×5 m. Plant indicators was upland rice var. Situ Patenggang, with cultivation spacing of 40 cm×15 cm. Treatment that did not use Bio-Organic Formula given straw compost 2 $t.ha^{-1}$ at planting as a basic treatment. While, Bio-Organic Formula II or Formula III Bio-Organic compost was a straw that has been enriched with various constituent microbial isolates Formula II or Formula III, given 2 $t.ha^{-1}$ at planting.

Observations include: soil nutrient C, N, P, K before treatment, soil microbial populations (P-solubilizing bacteria and N-fixing bacteria), plant height, number of tillers, weight of dried harvested rice,weight of dried unhusked rice, and activity of enzyme dehydrogenase. The weight of dried unhusked rice yield from the use of Bio-Organic Formula was determined Relative Value Agronomic Effectiveness (RAE) as follows (Macchay *et al.* 1984):

$$\mathrm{RAE} = \frac{\text{Yield of Bio}-\text{organic Formula}-\text{control}}{\text{Yield of recommendation fertilizer}-\text{control}}\,\mathrm{X}\ 100\%$$

Determination of Enzyme Activity

Dehydrogenase enzyme is generally used to determine the microbial activity in the soil (Casida 1977). Dehydrogenase activity was determined by a colorimetric method. Measurement technique used 2,3,5-Triphenyl Tetrazolium Chloride (TTC) (Casida *et al.*1964). A sample of 5 g soil was put into tube, and then 5 ml of 3% TTC was added. A sampel of 5 g soil was added with 5 ml of Tris-HCl buffer pH 7.8 (for soil with a pH range<5) as a control. The samples were mixed on vortex and incubated at 37 ^{o}C. After 24 hours, a product from enzyme activity, was extracted by adding 20 ml methanol and shaker for 2 hours. The samples were collected in a volumetric flask. Then it was the extract was filtered using Whatman filter paper 5, and rinsed two times with10 mL methanol. The filtrate was then diluted with additional methanol to a final volume 50 ml. Then measured at 485 nm with methanol as a blank, results of enzyme activity is expressed in µg $TPFg^{-1}$ sample.

Data Analysis

The effect of treatments was used analysis of variance (ANOVA), followed by further testing using Duncan multiple range test (DMRT) at 5% level (Gomez and Gomez 1976), using the IRRI Stat program.

RESULTS AND DISCUSSION

Characteristics of Soil Sample Before Treatment

The data analysis showed that the soil sample from Taman Bogo has characteristics as very acid soil (pH.H_2O=4.3), low soil organic C (1.51%), very low N total (0.15%), low available P (7 ppm), and low K (10 mg 100 g^{-1}). Besides that, the cation exchange capacity (CEC) is very low (3.39 Cmol kg^{-1}), the base saturation (35%) is low and the Al saturation (47%) is very high.

Dosage of NPK-recommendation

Based on the analysis of the soil characteristics above, we can be stated briefly that to obtain adequate nutrient for rice plant growth required the addition of organic matter and fertilizer, especially N, P, and K with a dose of 250 kg urea ha^{-1}, 200 kg SP-36 ha^{-1} and 75 kg KCl ha^{-1}.

Characteristics of Compost

Compost used in this study was compost from rice straw composted in the location of study. The analysis result shows that the compost has characteristics as very high organic C content (24,18%), low total N content (0,15%), low available P (0,09%), and medium K (2,14%). The compost used in this study about 2 Mg ha^{-1}.

Soil Microbial Populations of Upland during Primordial Age

The population of P-solubilizer bacteria and N-fixer bacteria when upland rice at primordial age are shown in Table 1. This study indicated that the population of P-solubilizer bacteria after treatment increased compared to before treatment at the time of primordial. Treatment of ½ NPK-recommendation combined with F II, ¾ NPK-recommendation combined with F II, and ¾ NPK-recommendation combined with F III significantly increased population of P-solubilizer bacteria compared to control treatment, ranged in 6.860-7.267 log10 cfu g^{-1}. The solubilization of phosphorus in the rhizosphere is the most common mode of action implicated in plant growth promoting rhizobacteria that increase the nutrient availability to host plants (Richardson 2001). P deficiency has been recognized as one of the main limiting factors in upland rice production in many parts of the world (Sahrawat*et al*. 2001). Deficiency in N and P in upland rice is quite common (Fageria and Breseghello 2001). Apart from P fertilization, microbial P mobilization would be the only possible way to increase available phosphate for upland crops (Goldstein 1986). N-fixer bacterial populations also increased after treatment compared to before treatment. There are differences in the effect of treatment on on the population of N-fixer bacteria. NPK treatment, and various NPK combinations with bio-organic formula significantly increased population of N-fixer bacteria on upland rice, but the use of bio-organic formula which was not accompanied by NPK fertilization can increase the population of N-fixer, bacteria although it was lower than the control. N2-fixer bacteria can affect plant growth directly by the synthesis of phytohormones and vitamins, inhibition of plant ethylene synthesis, improve nutrient uptake, and enhance stress resistance (Deb Roy *et al.* 2009). Since plants are the main supplier of organic materials in terrestrial ecosystem, they indirectly affect soil microbial communities, as well as diverse ecosystem processes especially at the rhizosphere (Kowalchuk*et al.* 2002). Resource availability for the soil microflora is limited to organic compounds of decayed leaves and root exudates which they use to generate cellular energy (Smith

Table1. Effect of NPK fertilizer, Bio-organic formula, and combination of NPK with bio-organic formula on the population of P-solubilizer and N-fixer bacteria at primordial stage of upland rice plant.

No.	Treatments	Bacterial population (log10 cfu g^{-1})	
		P-solubilizer	N-fixer
A	Before	5.301	5.397
B	After		
1	Control (Untreated)	6.213 c	10.930 ab
2	NPK-recommendation	6.100 c	10.607 bc
3	¾ NPK-rec	6.493 bc	9.797 def
4	Bio-Organic Formula II (F II)	6.537 bc	10.023 c-f
5	½ NPK-rec + F II	7.267 a	10.480 bcd
6	¾ NPK-rec + F II	6.880 ab	10.203 b-e
7	NPK-rec + F II	6.363 bc	9.723 ef
8	Bio-Organic Formula III (F III)	6.237 c	9.453 ef
9	½ NPK-rec + F III	6.213 c	9.473 ef
10	¾ NPK-rec + F III	6.860 ab	9.270 f
11	NPK-rec + F III	6.60 bc	11.350 a

Means in a column followed by the same letter are not signifantly different at 5% level by DMRT and Paul 1990; Panjeshahi and Ataei 2008). The rhizosphere is surrounding the plant root surface and being affected by plant root activities. Plant roots secrete mucigel (Jenny and Grossen-bacher 1963) to supply carbohydrate sources to soil microorganisms (Jimenez *et al.* 2003). Rice roots also provide polysaccharides, amino acids and organic acids (Kimura*et al.* 1977; Kimura *et al.* 1983), as do upland plants (Rovira 1969). The ecological impact of microbial inoculants in soil has often been characterized in terms of size and composition of specific microbial groups. However, these approaches do not provide a comprehensive view of the impact of an inoculant on the functioning of the soil ecosystem (Doyle and Stotzky 1993). Enzyme activities have been proposed as a tool to monitor changes in soil ecology resulting from the interactions between inoculants and indigenous microbial populations of soil (Doyle and Stotzky 1993; Vazquez *et al.* 2000).

Upland Rice Plant Height

The results of upland rice plant height are presented in Figure 1. There are differences in the effect of treatment on both plant heights at 30, 60,

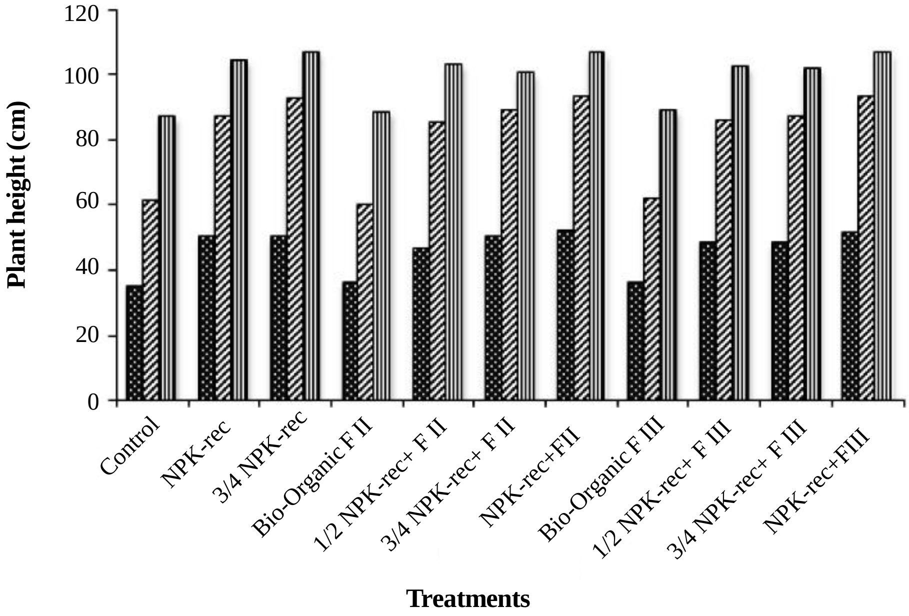

Figure 1. Effect of NPK fertilizer, Bio-organic formula, and combination of NPK with Bio-organic formula on plant height of upland rice at ▣: 30 DAT, ▨: 60 DAT and ▥: 90 DAT.

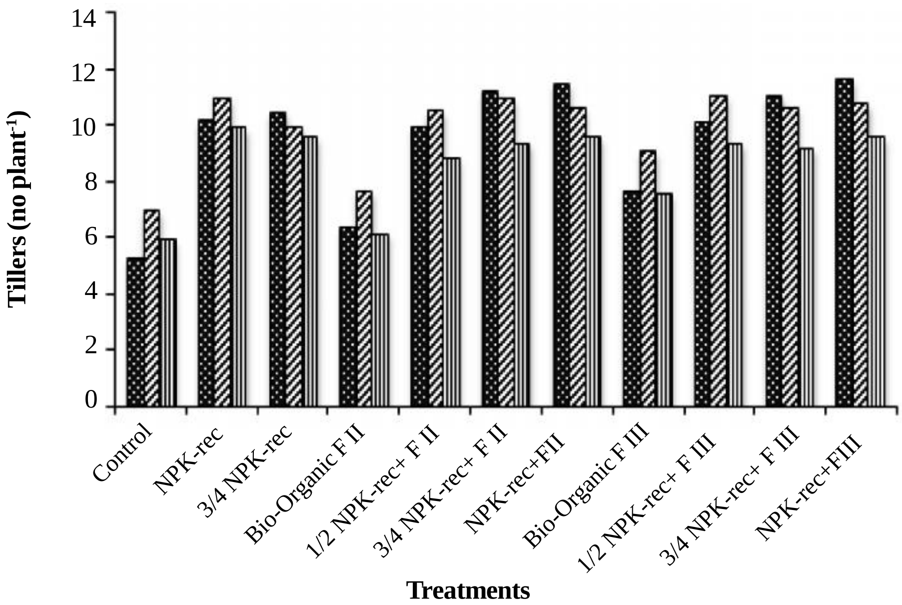

Figure 2. Effect of NPK fertilizer, Bio-organic formula, and combination of NPK with Bio-organic formula on plant tillers of upland rice at ▣: 30 DAT, ▨: 60 DAT and ▥: 90 DAT.

and 90 days after transplanting (DAT). These results show that treatments of NPK-recommendation, ¾ NPK-rec and combination of ½ NPK-rec, ¾ NPK-rec and NPK-rec with the both of bio-organic formula gave an equal and plant height was significantly higher than the control treatment and Bio-Organic of Formula II or Formula III. This means that the use of Bio-Organic of Formula II or Formula III was most effective for improving upland rice plant height if followed by ½ NPK - recommendation dosage.

Number of Tillers

The number of upland rice tillers are presented in Figure 2. There are differences in the effect of treatment on the number of tillers in both ages of 30, 60, and 90 days after transplanting (DAT). These results indicate that the use of Bio-Organic Formula II is most effective for increasing the number of upland rice tillers if followed by a dose of ½ NPK recommendation but the use of Bio-Organic Formula III without combination with NPK recommendation was less effective in increasing the number of tiller. Inoculation with N-fixer bacteria like *A. lipoferum* also can increase plant height and tiller number of rice plants (Nayak *et al.* 1986). In the field, Balandreau (2002) found that the increasing yield was around 1.8 Mg ha^{-1} (22% increase).

The Weight of Dried Harvested Rice

Results of weight of dried harvested rice are presented in Table 2. There are differences in the effect of treatment on weight of dried harvested rice. The highest yield achieved by treatment of ½ NPK-rec combined with Bio-Organic Formula III at 4.86 Mg.ha^{-1}, was significantly higher than control, Bio-Organic Formula II, Bio-Organic Formula III, and ¾ NPK-rec combined with Bio-Organic Formula III, but it is not different from the six other treatments. While the control treatment, Bio-Organic Formula II, and Bio-Organic Formula III produced equivalent weight of dried harvested rice *i.e.* from 1.43 to 1.89 Mg.ha^{-1}. While the ¾ NPK-rec combined Bio-Organic Formula III produced weight of dried harvested rice at 3.19 t.ha^{-1} which was no different than the Bio-Organic Formula III. Thus, the use of Bio-Organic Formula II and Formula III were most effective in increasing the weight of dried harvested rice if followed by a dose of ½ NPK fertilizer recommendations. Kennedy *et al.*(2004) reported that bio-fertilizer application increased grain yield and N uptake by rice grain significantly, although urea application was reduced by 50% to 55 kg ha^{-1}. In farmers' fields, the application of this bio-fertilizer also increased rice yield up to 1.1 Mg ha^{-1}. Bacteria in the rhizosphere influence plant growth and interact with plant roots. The results suggest that the bacteria in the rice rhizosphere can be changed depending on aerobic and anaerobic conditions of fields (Doi*et al.* 2007).

The Weight of Dried Unhusked Upland Rice

The net weight of dried unhusked upland rice var. Situ Patenggang is presented in Table 2. There are differences in the effect of treatment on the net weight of dried unhusked upland rice. NPK treatment, and various NPK combined with Bio-

Table 2. Effect of NPK fertilizer, bio-organic formula, and combination of NPK with bio-organic formula on the weight of dried harvested rice and dried unhusked rice of upland rice (Situ Patenggang variety).

No.	Treatments	Weight of dried harvested rice (Mg ha^{-1})	Weight of dried unhusked rice (Mg ha^{-1})
1	Control (Untreated)	1.43 d	0.82 c
2	NPK-recommendation	3.72 ab	2.09 ab
3	¾ NPK-rec	3.40 ab	2.01 ab
4	Bio-Organic Formula II (F II)	1.62 d	0.90 c
5	½ NPK-rec + F II	3.60 ab	2.28 ab
6	¾ NPK-rec + F II	3.51 ab	2.24 ab
7	NPK-rec + F II	4.07 ab	2.56 a
8	Bio-Organic Formula III (F III)	1.89 cd	1.09 c
9	½ NPK-rec + F III	4.86 a	2.18 ab
10	¾ NPK-rec + F III	3.19 bc	1.92 b
11	NPK-rec + F III	3.68 ab	2.42 ab

Means in a column followed by the same letter are not significantly different at 5% level by DMRT.

Organic Formula significantly increased the net weight of dried unhusked upland rice, but the use of Bio-Organic Formula which was not accompanied by NPK fertilization was not able to increase the net weight of dried unhusked upland rice. Treatments of NPK-rec, ¾ NPK-rec and combination of ½ NPK-rec, ¾ NPK-rec and NPK-rec with both bio-organic formula gave the net weight of dried unhusked upland rice equal to 1.92 to 2.56 $t.ha^{-1}$ significantly more higher compared to the control treatment and both formula of Bio-Organic which were 0.82 to 1.09 $t.ha^{-1}$, respectively. However, of the various treatment combinations between the use of Bio-Organic with various doses of NPK, ¾ NPK-rec combined with Bio-Organic Formula III was a treatment that provided the lowest net weight of dried unhusked upland rice at 1.92 $t.ha^{-1}$, lower than NPK-rec combined with Bio-Organic Formula II. This suggests that the use of Bio-Organic fertilizer without NPK was not able to increase the net weight of dried unhusked upland rice, but the use of Bio-Organic Formula II or Formula III if followed by a dose of ½ NPK fertilizer increased the net weight of dry unhusked upland rice equivalent to the use of ¾ NPK-rec and NPK-rec.

Relative Agronomic Effectiveness (RAE) Value

The results of the RAE of various treatments are shown in Table 3. It appears that the highest RAE generated by treatment of NPK-rec combined with Bio-Organic Formula II was about 137,0% and the lowest RAE generated by treatment of Bio-Organic Formula II was about 6,3%. Treatment ¾ NPK-rec had RAE about 93.7% and other treatments that gave RAE <100% respectively were Bio-Organic Formula III and ¾ NPK-rec combined with Bio-Organic Formula III (Table 3). While other treatments that provided RAE> 100% from the lowest to the highest respectively were ½-NPK-rec + Bio-Organic Formula III,¾ NPK-rec + Bio-Organic Formula II, ½ NPK-rec + Bio-Organic Formula II, and NPK-rec + Bio-Organic Formula III. Based on the value of RAE that the Bio-Organic Formula II can be selected as the formula that can be used to increase the productivity of upland rice var. Situ Patenggang in acid upland. The use of this formula should be combined with NPK fertilizer at a dose of at least ½ NPK recommendation.

Dehydrogenase Activity

The activity of dehydrogenase enzyme of various treatments is presented in Figure 3. Dehydrogenase activity among treatments either early planting, primordial or harvested was not significantly different, but it was significant different related to rice growth stage and fertilizer application (Figure 3). Dehydrogenase activities in all treatments either in primordial or harvested time were significant different compare to early planting, while dehydrogenase activity on highest condition in harvested time. Treatment ½ NPK recommendation + F II has the highest dehydrogenase activity on primordial and harvested time, this treatment promoted activities of other microbes within soil and rhizosphere of rice, therefore, the rice grew well than other treatments. Control treatment was likely to have higher dehydrogenase activity than treatment of NPK-recommendation, indicated that microbial activity within this treatment was not disrupted because there were not external inputs such as inorganic fertilizer. The chemical alters the number and activity of microorganism and affects

Table 3. Effect of NPK fertilizer, Bio-organic formula, and combination of NPK with bio-organic formula to *Relative Agronomic Effectiveness* (RAE) of upland rice, Situ Patenggan variety.

No.	Treatments	*RAE* Value(%)
1	Control (Untreated)	-
2	NPK-recommendation	100.0
3	¾ NPK-rec	93.7
4	Bio-Organic Formula II (F II)	6.3
5	½ NPK-rec + F II	115.1
6	¾ NPK-rec + F II	111.8
7	NPK-rec + F II	137.0
8	Bio-Organic Formula III (F III)	21.2
9	½ NPK-rec + F III	107.1
10	¾ NPK-rec + F III	86.8
11	NPK-rec + F III	125.9

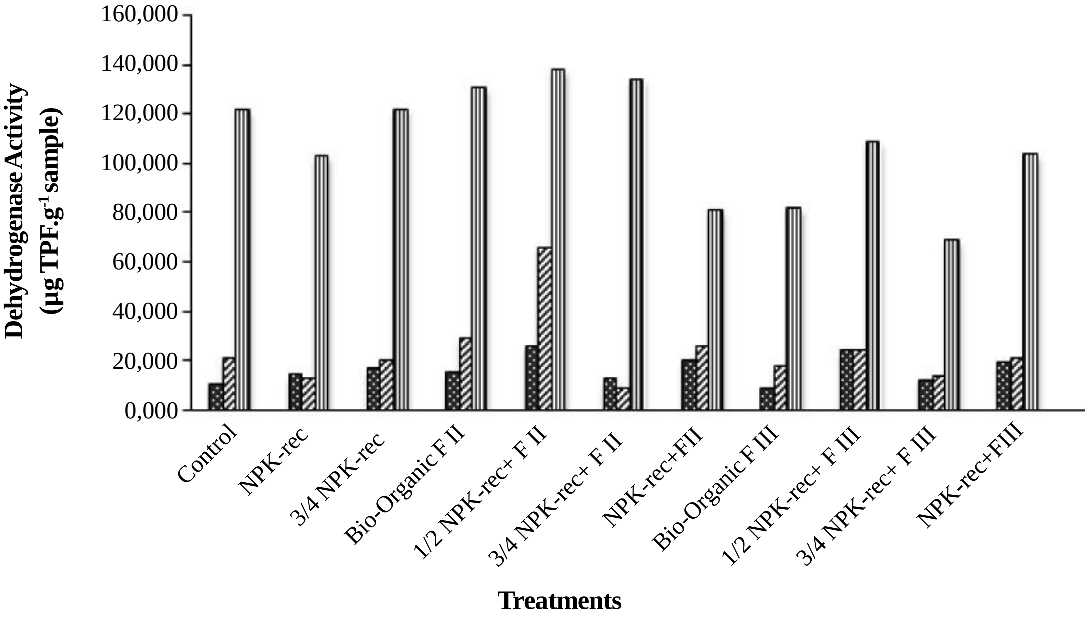

Figure 3. Effect of NPK fertilizer, Bio-organic formula, and combination ofNPKwith bio-organic formula to dehydrogenase activity of upland rice at ■: 30 DAT, ▨: 60 DAT and ▥: 90 DAT.

biochemical processes and fertility of the soil (Wainwright 1978; Shukia 2000). Prasanna *et al.* (2011) reported that organic carbon was significantly increased in all microbe-inoculated treatments, which could be correlated with activities of all the enzymes tested in their study. Mader *et al.* (2011) observed that the application of PGPR improved soil quality as a result of increased soil enzyme activities of alkaline and acid phosphatase, urease and dehydrogenase.

CONCLUSIONS

Bio-organic Formula II which is a composted rice straw enriched with bacterial isolates ES60.D7, ES71.A, ES54.B4, and SRPCG1 can be used to increase the growth and productivity and dehydrogenase activity of upland rice var. Situ Patenggang in acid upland. It can increase the productivity of upland rice when combined with NPK fertilizer at a dose of at least ½ recommendation. It can increase the efficiency use of ½ NPK fertilizer dosage recommendations or equivalent to 125 kg urea, 100 kg SP-36, and 35 kg KCl ha^{-1}.

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