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Improvement of local upland rice utilizing mixture of microbes: resistance, yield and reduction of chemical fertilizer usage

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Abstract

Local upland rice is important and contributes to the total production of rice in Indonesia. However, its contribution was stiil low compared to paddy rice. It is essential to improve the resistance of local upland rice crops to pathogens, to increase its yield potential, and to reduce chemical fertilizer use. This can be accomplished by the use of a mixture of microbes i.e, trichoderma, rhizobacteria and michoriza. The objectives of the experiment were to study the resistance of rice plants to disease, rice yield and to reduce the chemical fertilizer use for upland rice. This research used Completely Randomized Design in a split plot pattern consisting of two factors. The first factor was fertilizer dose, with three levels, and the second factor was a mixture of microbes, consisting of four levels. The research results showed that the mixture of microbes siginificantly influenced the rice yield components such as panicle length, 1000 grain weight and yield. The microbes significantly improved rice yield, for up to 5 ton/ha compared to farmer's fertilization method, yielding only 1-2 ton/ha. The microbes could improve resistance of rice plants against the disease infected by *Helminthosporium* sp. on vegetative and generative phases. The microbe use reduced fertilizer usage for up to 25%. The study concludes that the mixture of microbes induces resistance, improves rice yield and reduces chemical fertilizer use. This study needs to be continued at different field locations to get additional data to support the finding results.

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Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population. Asia accounts for 90% of the world's total rice area and production. In India, rice is grown on approximately 45 million ha annually with a production of 104 million tons. India's rice demand is estimated to rise to 122 million tons in 2020, which is equivalent to an overall increase of 22% in the next 10 years (Singh *et al.*, 2013). However, the current evidence shows declining factor productivity and a plateau in rice yields due to fatigued natural resources, declining water table, increasing labour scarcity and energy shortage, escalating fuel prices, and changing climatic conditions. Therefore, yield gains have to be achieved by using less water, labour, land and energy.

Similarly, in Indonesia rice is also a very important staple food source. Various attempts have been made to maintain the rice supply up to the consumer level, but the increase in rice production is still very volatile from season to season. Although self-sufficiency in rice was achieved back in 2008, and according to the BPS (2013) that the production of rice in 2012 almost reached 70 million tons, and a surplus of about 3.29 million tonnes or 5 % compared to the year 2011, these conditions have not yet reached the sovereignty and sustainability national food security. As a result, Indonesia still importes rice anually. Protection measures need to be undertaken to address the growing need for rice due to population growth, limited land capacity, and biotic and abiotic factors. Global Food Security Index 2012, published by the Economist Intelligence Unit, showed that Indonesia ranks only 64th with a value of 46.8 out of the highest value of 100; in comparison, Malaysia at number 33, China ranks 38th, Thailand ranks 45th, Vietnam and the Philippines at the 55th and 63rd, respectively (Basri, 2013).

Efforts to keep the national rice production has been done by the government through the establishment of new fields, repairing and construction of irrigation, the release of improved varieties, and assistance of production inputs to farmers.

However, a variety of potential resources was not fully utilized, such as the availability of dry land to develop upland rice, appropriate technology to increase production and availability of germplasm resources, which are not inferior in taste and nutrient content.

In general, cultivation of upland rice (*Oryza sativa* L.) was conducted on dry land that is not inundated with water throughout the year and source of the water needs only from soil moisture from rainfall. Potential of local upland rice germplasms is quite large, which have been traditionally been bred and cultivated by farmers in various districts. The results of the research showed that some local cultivars like Endokadia, Bakala, and Enggalaru had a somewhat resistant response to blast disease (*Pyricularia oryzae*) with the disease severity of 5 % to 10 %, as compared with the disease severity on the national Situpatenggang variety of more than 50 % (Taufik *et al.*, 2009a).

Various upland rice cultivars of Southeast Sulawesi also had different morphological characters and tastes or aroma. For example, Bakala is a brown rice cultivar that contains quite high vitamin B so it is best consumed by toddlers. Loiyo cultivar is black non-glutinous rice that is useful for consumers who do not like sticky rice (Wahab, 2011). Endokadia rice cultivar has a distinctive aroma with a fluffier rice quality. Unfortunately, the community cultivates the cultivars without the introduction of technology, and only cultivates the potential germplasms marginally.

So far, farmers rely heavily on the use of pesticides to address pest or plant disease, or otherwise do not perform control measures at all because of the inability to buy pesticides. It requires the introduction of technology of trichoderm, mycorrhizae, and rhizobakteria as a consortium that can be used to enhance the resilience and crop production. Results of previous studies showed that the application of the three biological agents in cashew, chili, pepper, and corn, proved to increase endurance and crop production (Taufik *et al.*, 2010ab; Halim 2009). Based on the results of the research, consortium of biological agents can be a solution to improve the resistance and upland rice production.

Materials and methods

Research Location and Period

The field research was conducted in an experimental garden; propagation of mycorrhizal and biological agents was performed in the laboratory of Plant Pests and Diseases and screen house of Agrotechnology Department, Faculty of Agriculture, University of Halu Oleo.

Experimental Design

The experiment used a Split Plot pattern using the basic design of randomized block design (RBD). The first factor was fertilization with three levels: 100 % (P1), 75% (P2) and 50% (P3) recommended fertilizer dose, while the second factor was biological agents consortium, with four level: no consortium (K0), trichoderma and mycorrhizal consortium (K1), trichoderma plus Rhizobacteria (K2), and rhizbacteria with mycorrhiza (K3), therefore there were 12 treatment combinations, with three replicates, so that there were 36 experimental units.

Application of Rhizobacteria and Trichoderma sp. Formulation

Rhizobacteria biological agent was in the form of ready-made formulations obtained from the Microbiology Laboratory of UGM. *Trichoderma* sp biological agent was from the collection of the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, University of Halu Oleo, propagated in glutinous rice medium.

Preparation of Mycorrhiza

Mycorrhizae was applied as follows: (1) Soil medium was taken from the field at the rhizosfer area, (2) maize seeds were sterilized with a solution of formalin aceto-alcohol FAA),(3) mycorrhizal propogul was placed in medium in polibag with the depth of ± 5 cm, (4) corn seeds were planted, (5) water was performed,(6) crop was left up to the desired age, (7) the top of the plant was cut, left the soil dry, (8) wasted plant roots were stored in plastic bags with proper label, (9) root stainings were performed, and (10) observation using a microscope (Brundret *et al.*, 1996). Mycorrhiza was mixed with the seeds before planting by 2 g for each planting hole.

Land preparation, planting and harvest

Pot size for each treatment was 4 m × 5 m. Planting was done by making planting holes with spacing of 80 cm × 40 cm; in each planting hole 3-5 seeds were planted. Fertilizers were given in accordance with the appropriate treatments. Harvest was done when the plants were already showing signs of maturity, where 80-90% of the rice ears were physiologically mature, e.i. ear marked in goldish yellow (yellow-ear cultivars) or black (black-ear cultivars), and at the end of the flag leaves coloured brown and dry.

Observation Variables: Evaluation of Resistance against Pathogens

Observations performed were incubation period and the severity of disease. Severity scoring was based on Ou (1985) with a slight modification, i.e. 0 = no symptoms, 1 = brown spot with a diameter less than 0.5 mm; 2 = brown spot with a diameter of about 0.5 - 1 mm; 3 = lesio elliptical with a diameter of 1-3 mm; 4 = lesio or rhombic spots with a diameter of 3 mm; 5 = about more than half of the leaves have necrosis. Scores 0-3 were categorized as resistant and scores 4 and 5 were categorized as susceptible. As for the formula used was:

Formula:

$$KP = \frac{\Sigma (ni \times vi)}{(N \times Z)} \times 100\%$$

Description:

Kp: Severity of disease

vi: Scores on the infected category

ni: number of leaves on infected category

Z: Scores on the highest infected category

N: Number of leaves observed

Observations were performed on the vegetative phase of plant height and number of maximum tillers. Plant height was measured from the base of the stem to the tip of the highest leaf, while the number of tillers was by counting the number of tillers that grew. For the generative phase, variables observed were days to harvest, grain percentage, weight of 1000 grains and grain yield. Days to harvest were calculated from planting until harvest.

The percentage of filled grain was done by counting the number of filled grain of randomly and diagonally predefined sample clumps. Weight of 1000 grains was measured by weighing 1000 filled grains, with moisture content of 14 %. Grain yield was calculated by creating a clean sample plots with a line separating a clump of plants around the experimental plots, and weighing the harvest of all the clumps that exist on the clean experimental samples.

Observation data of upland rice growth were analyzed using analysis of variance, followed by Duncan's Multiple Range Test (DMRT) at the confidence level of 95 %, with the help of SAS 9.1 program.

Results and discussion

Severity of Brown Spot Disease

Brown spot disease *Helminthosporium oryzae* can be observed two weeks before the maturation phase of rice ears.

The average severity of brown spot disease reached 70% on rice plants with treatment without biological agents. The facts showed that biological agents consortium could reduce the severity of disease on rice plants, as evidenced by the relatively low severity of blast disease and *H. oryzae*, brown spots disease, especially on vegetative phase. However, the spot disease tended to increase when the rice ears had been filled, about two weeks before harvest, so the pathogen infection did not significantly contribute in reducing crop yield. Taufik *et al.* (2011) have reported the application of biological agents in reducing the severity of diseases, where rizobacteria were able to suppress the occurrence of basal stem rot disease and yellow disease in pepper plants for up to 8.75 %.

Table 1. Average number of panicles in each treatment factor.

Interaction PxK	P1 (recommendation)	P2 (75% dose)	P3 (50% dose)	$BNJ_{0,05}$
K0 (tanpa agens hayati)	$15{,}09^{P}$ a	$13{,}38^{Q}$ a	$13{,}05^{P}$ A	3,52
K1 (Tricho+Mikoriza)	$15{,}05^{P}$ a	$14{,}81^{Q}$ a	$14{,}38^{P}$ A	
K2 (Tricho+Rhizo)	$16{,}72^{P}$ a	$19{,}08^{P}$ a	$14{,}00^{P}$ A	
K3 (Rhizo+Mikoriza)	$18{,}28^{P}$ a	$14{,}09^{Q}$ a	$14{,}95^{P}$ A	
$BNJ_{0,05}$		5,61		

Plant Height

Results of field observations indicated that there was no interaction between fertilization factor (P) and disease consortium factor (K), except for the variable of number of panicles. However, there was an interesting fact that the single factor of recommended fertilization dose for upland rice plants, gave the highest plant height. Plant height response showed that there was no interaction between the fertilization and the biological agents factor. Independently, the treatment of biological agents did not significantly different among treatment levels, but the fertilization factor showed significantly different among fertilization levels.

Average height of plants treated with recommended dose of fertilizer had a margin reaching 8 cm higher than the plants given 50% recommended dose of fertilizer. Biological agents treatment was still unable to enhance height of upland rice plants at 11 weeks after planting (WAP).

It seemed that fertilization, particularly N using recommended dose, enhanced upland rice plant height. This was possibly because adequate nitrogen causes better plant vegetative growth. Pringadi *et al.* (2007) have reported that upland rice Situ

Patenggang, Batutegi, and line BP760F-2-2-1-1-PN had an average of 69.9 cm plant height at the N fertilizer dose of 135 kg per ha. The higher dose of N will enhance plant height, and significantly different from other treatments.

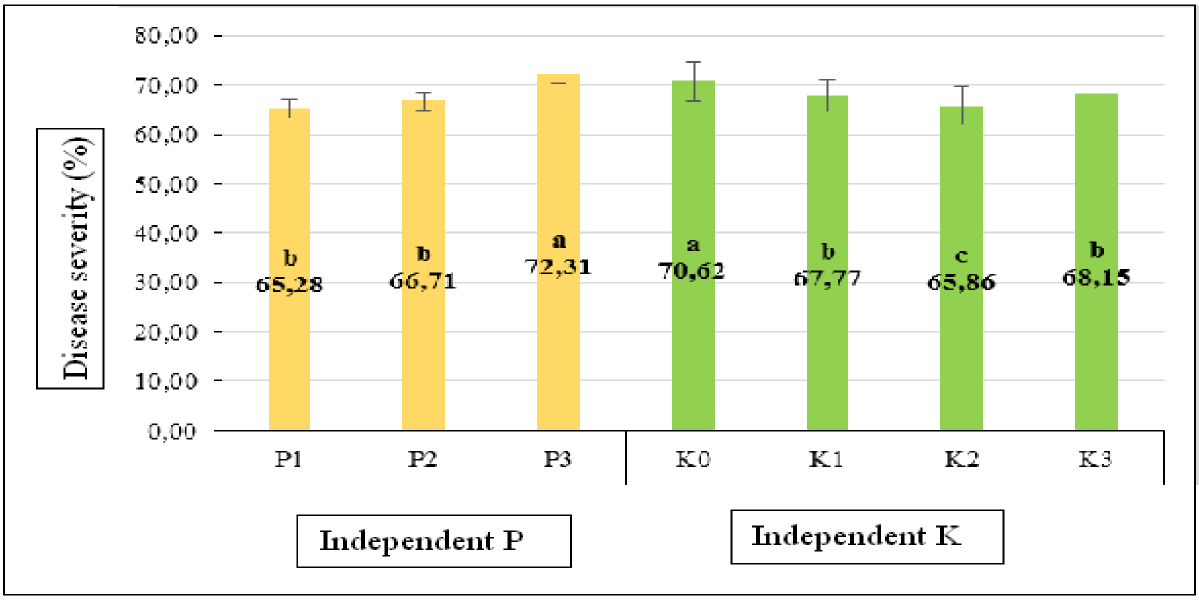

Fig. 1. Average severity of brown spot disease (*H. oryzae*), two weeks before maturation phase.

Number of Tillers

Only the independent fertilizer treatment gave a significant effect to observed variable of number of tillers at the end of the observation period (11 weeks after planting, WAP), and was still consistent with the observation at 7 WAP. Fertilization treatment of P1 produced the highest number of tillers and was significantly different with fertilization treatment of 50 % recommended dose.

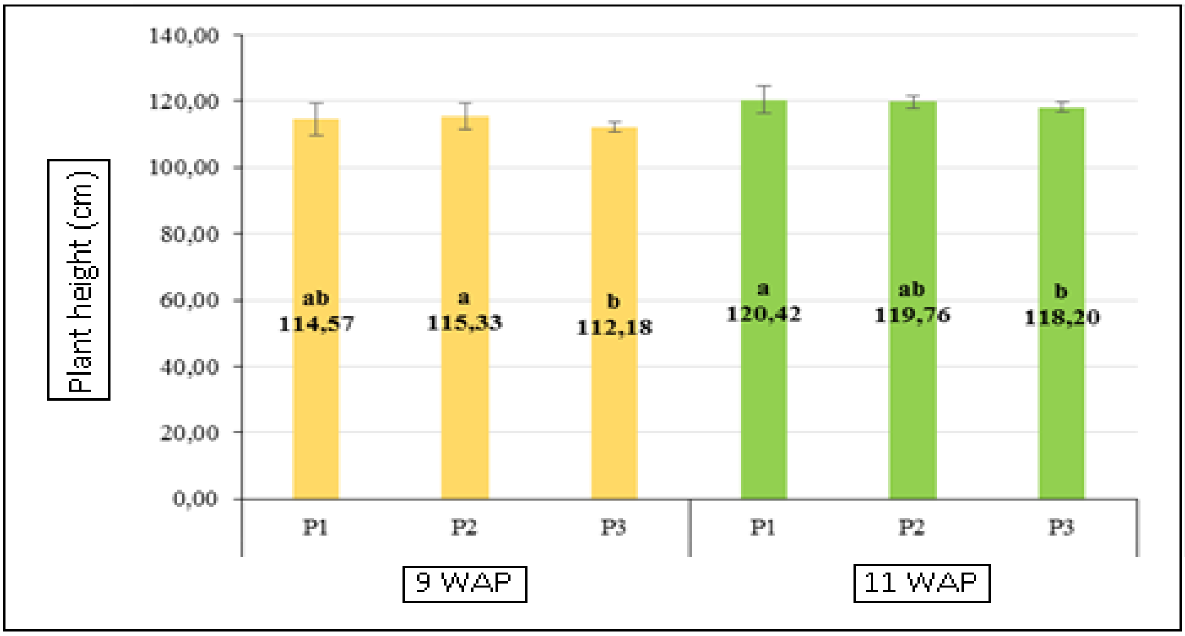

Fig. 2. Average plant height of Bakala upland rice cultivar at 9 and 11 weeks after planting.

The difference in number of tillers in both treatments reached four tillers (Table 1).

As for plant height data, the fertilization treatment gave a significant independent effect on variable of number of tillers. Fertilization according to the recommended dose produced the highest number of tillers. The high number of tillers implicated the sufficient amount of nutrients availability for rice plants, so that increasing tiller number in line with increasing age of the plants.

Pringadi (2007) noted that medium-altitute upland rice, that was given N fertilizer, produced the highest number of tillers in Situ Bagendit variety, line BP720C-Si-5-1-1-2 and BP0760F-2-2-1-1-PN, which was significantly different with plants supplemented with a low dose of N fertilizer. Adequacy of nutrients of N, P and K on upland rice crops treated with the recommended dose of fertilizer or 75% of the recommended dose resulted in an increase in the ability of leaves to perform photosynthesis to increase the accumulation of fotosintate, distributed to all parts of the plant.

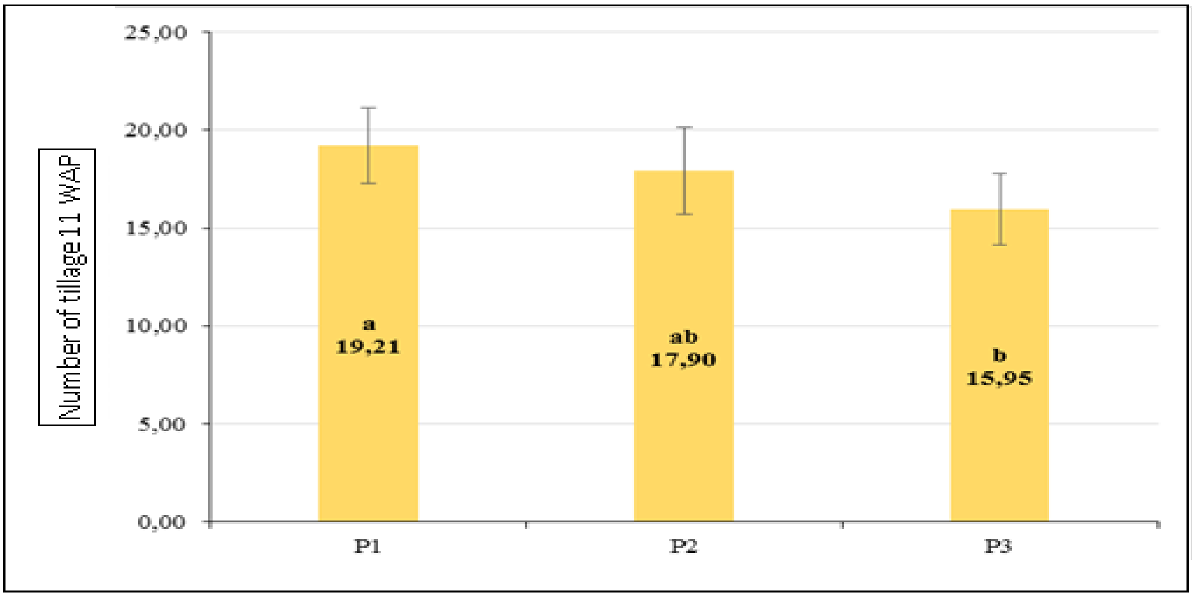

Fig. 3. Average number of tillers of Bakala upland rice cultivar at 11 WAP.

A group of microbial that gained a lot of attention and was often used in cultivation systems both in a single inoculation and in a double inoculation was rhizobacteria and Mycorrhiza. *Mycorrhiza arbuskula* that is symbiosis with crop roots, can increase the absorption of nutrients N, P and K, and improve the efficiency of water use, increase the osmotic tension of plant cells in soil with lo water content so that the plants perform their life and are able to increase the vegetative growth rate and crop production (Scheublin *et al.*, 2004 *in* Thangadurai *et al.*,2010).

Various studies have reported that VA *Mycorrhizae arbuskula* can increase the growth rate of crops including cocoa seedlings, improve the efficiency of water use and improve crop resistance to drought (Sasli, 2004).

Chakrabarti *et al.* (2010) reported that the application of mycorrhizal fungi and Rhizobium bacteria produced a double effect of healtier root growth of *Vigna mungo*, because it contained higher indole acetic acid (IAA) compared to plant root that was not inoculated with both microorganisms. It was reported that tryptophan compound was not only found in root nodules but also in young leaves.

Number of panicles

Research results (Tabel 1) showed that there was an interaction effect between the combination of fertilization (with 75 % recommended dose) and a consortium of trichoderma and rhizobacteria (P2K2) on the number of panicle, which was observed after 50 % of plant samples had produced panicles. The treatment combination resulted in an average of 5 panicles more than other combinations.

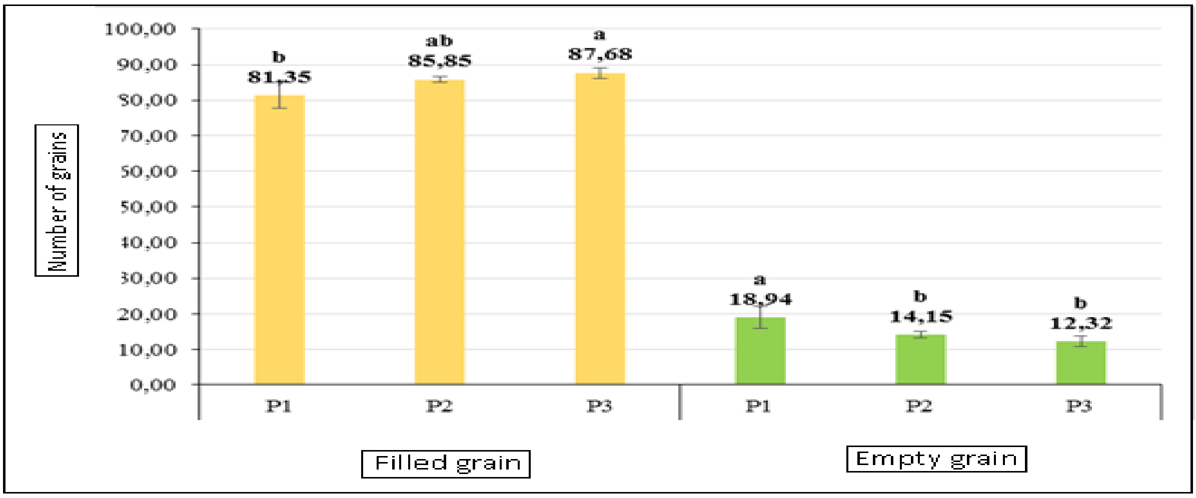

Fig. 4. Average percentage (%) of filled grain of Bakala upland rice cultivar.

Meanwhile, at the observation on post vegetative phase, the consortium of biological agents seemed to have a positive influence on the plant generative growth. It seemed that the fertilization effect was faster on vegetative phase, while a consortium of biological agents affected the generative phase. This indicated that fertilizing with synthetic chemicals affected faster than with natural ingredients, including microbial biological agents. This research showed that the use of biological agents as an alternative microbial control was promising to be used as a non-conventional alternative solution. The combination of fertilizer using 75 % recommendation dose and the consortium of trichoderm and rhizobacteri produced the highest number of tillers. It seemed that a dose of 75% fertilizer gave a good influence for microbial biological agents to stimulate higher generative growth (number of panicles) compared to the other treatment combinations. Some reports indicated that it requires the right balance of nitrogen content in the soil, as it can lead to competition between plants and microbes in getting nitrogen. Magill and Aber (1998) reported that soil microbial growth was limited by nitrogen, and thus fertilization may reduce competition between plants and microbes in obtaining N, and simultaneously will increase the growth of plants and microbes themselves. The results of this study along with the recent study reported by Naseri *et al.* (2013) showed that biological or microbial fertilizers could substitute the N fertilizer. They suggested to use 150 kg N/ha for rapeseed plant, instead of 200 kg N/ha. The reduction of N fertilizer use caused a reduction of pollutants in the soil and provided sustainable agriculture.

Percentage of filled grain

Independent fertilization treatment resulted in significantly different averages of percentage of filled grain of Bakala cultivar between treatment P1 and P3. Treatment P3 resulted in average of 6% higher filled grain yield than in P1 treatment.

Weight of 1000 grains

The observation on the weight of 1000 grains showed that the K3 treatment (rhizobacteri plus mycorrhizae) was significantly different from K0 treatment (without biological agents). There was a tendency that the application of the biological agents consortium could improve weight of 1000 grains of rice cultivar bakala, compared with treatment without biological agent; weight of 1000 grains of rice on K3 treatment was 2 g heavier than the grain on treatment without biological agents (Figure 5).

The use of the consortium of biological agents with fertilization of less recommended dose can increase rice production variables such as, percentage of filled grains, weight of 1000 grains and upland rice production. The percentage of filled grains increased with decreasing doses of synthetic chemical fertilizer, while the variable of weight of 1000 grains increased with the use of biological agent consortium.

Similarly, the use of the consortium of biological agents was significantly capable of increasing the production of upland rice compared with no consortium. The treatment of Rhizobacteria biological agents plus mycorrhizae produced the highest upland rice production. Rhizobacteria mixed with mycorrhizae was sinergically able to trigger the growth and production of upland rice. This was presumably caused by rhizobacteria ability to assist plants in accessing nutrients from the soil or as a bio - fertilizer. Naseri *et al.* (2013) outlined that the use of bio - fertilizers Azotobacter and Pseudomonas, otherwise known as rhizobacteria, increased crop yields of Rapeseed by 15.8 % and 13.7 %, respectively. Both microbes provided a dual effect on the production of upland rice. Woysessa and Assefa (2011) reported that *Pseudomonas fluorescens* significantly increased the average of root dry weight by 39 %, the ratio of roots and shoots by 42 % and yield of tef (*Eragrostis tef*) variety DZ-Cr-37 by 45 %. Nasaruddin (2012) who noted that the application of Azotobacter and mycorrhizae simultaneously gave a dual effect on seedling growth variables, dry weight and better root growth of cocoa seedlings.

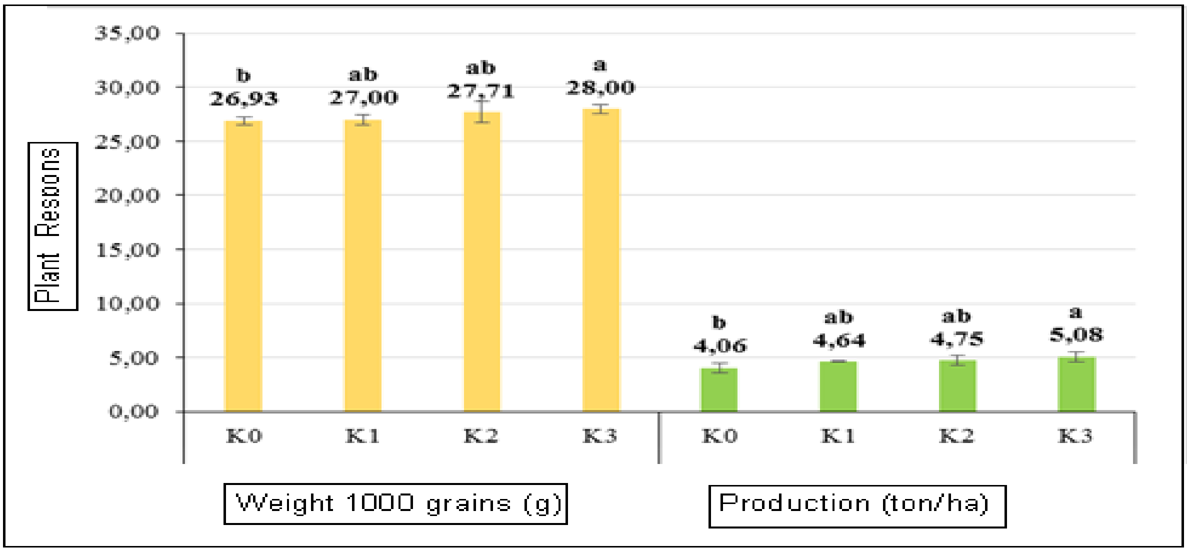

Fig. 5. Average of weight of 1000 grains (g) and average production of Bakala upland rice cultivar.

Bakala Upland Rice Production

The production of local upland rice was fairly good, with an average production of 5 tons/ha on K3 treatment, and was significantly different from the treatment without biological agents (K0). However, there was no significant difference between treatments of biological agents (Figure 5).

The research results showed that the production of upland rice using recommended dose of fertilizer did not significantly different with the production of using 75% and 50% fertilizer dossages. The results also suggested that the reduction on fertilizer dose did not negatively affect the growth of plant height. Even high doses of N could affect the growth of plant height, as reported by Adil *et al.* (2006) that the application of 5g urea per pot caused the plants did not grow, as a result of N content was too high, due to the presence of residual N before planting. According to Birch and Eagle (1969), high doses of urea released high N to the soil so that the resulting N content in the soil is too high for plants that cause poisoning. Chaturvedi (2005) stated that high N fertilization produced optimum plant height.

Accumulation of secondary metabolites produced by microbes produced plants with high enough production. The results of this study proved that the combination of the consortium of biological agents with a lower dose of fertilizer increased rice production to about 5 tons per ha. The results of this study differed from the results obtained by Pirngadi *et al.* (2007) who reported that Situbagendit, Situpatenggang and Batutegi varieties produced only 3-4 tons per ha of dry milled grain, while Toha and Drajat (2006) noted that the yield potential of the upland rice could reach more than 4 tons per ha. The difference could be partially attributed to use of different cultivars and treatments. Both researchers did not use biological agents to help improve plant growth and only relied on synthetic chemicals. However, these results still need to be further tested to yield more consistent data.

Conclusion

Consortium of biological agents is able to replace the role of synthetic chemical fertilizers to enhance the growth and production of local upland rice Bakala. The use of synthetic chemical fertilizer is recommended to be reduced to only 75 % or 50 % of the recommended dose. The consortium of biological agents, in particular rhizobacteria combined with mycorrhizae, effectively enhance the growth and production of rice to more than 5 tons per ha.

This consortium significantly reduced the infection of blast and brown spot diseases, during the vegetative and generative phases of upland rice crop.

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