Growth and yield responses of black soybean to bioactivatorenriched goat manures amended with Trichoderma

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Fitriani, D., Podesta, F., Harini, R. and Fahrurrozi, F. (2023). Growth and yield responses of black soybean to bioactivator-enriched goat manures amended with Trichoderma. International Journal of Agricultural Technology 19(1):53-62.

Abstract Soybean growth and yield could be improved by combining the application of natural functional fungal species of *Trichoderma* with goat manure organic fertilizer. Results indicated that the use of bio-activator enriched goat manure significantly influenced plant height, shoot fresh weight, number of mature pods and grain weight per plant, but not weight of 100 grains. The use of *Trichoderma* significantly affected the grain weight per plant and weight of 100 grains. However, it did not significantly influence in plant height, shoot fresh weight, and number of mature pods. The best composition of bioactivator-enriched goat manures was stale rice+cow blood+goat manure, or cow rumen+cow blood+goat manurein which produced higher number of mature pods and grain weight per plant than those of fertilized with synthetic fertilizer. In addition, *Trichoderma* amendment on black soybean increased the number of mature pod and weight of 100 grains. The application 15 g plant⁻¹ was considered the recommended dosage of *Trichoderma* for black soybean production.

Keywords: Bio-activator, Black Soybean, Cow blood, Cow rumen, Goat manure, Trichoderma

Introduction

Black soybean (*Glycine max* L. *Merrill* var. malika) is one the growing Indonesian soybean production to meet the market demands as the national consumption continues to increase over the year. Efforts to improve domestic production continue to be developed and there have been declining trends of Indonesian soybean import billion in 2020 (Jayani, 2021). Successful soybean production requires proper nutrient values lately, i.e., US\$ 1.1 billion in 2018, US\$1.06 billion in 2019, and US\$ 1 production requires proper nutrient management in order to sustain soil fertility and crop productivity, for example, in terms of source of fertilizers, dosages and application techniques.

Recommended fertilization dosages for soybean in ultisol soil of Bengkulu were 350 kg Urea ha⁻¹, 300 kg TSP ha⁻¹, 300 kg KCl ha⁻¹, and 20 tons

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ha⁻¹ organic fertilizer ha⁻¹ (Purba et al., 2018). However, the regular uses of synthetic fertilizer might be harmful to soil environment and eventually decrease crop productivity. The use of organic fertilizer must be introduced to the farmer as a part of integral effort to have sustainable agricultural practices. The use of organic fertilizer must be taken into account to ensure the sustainability of land resources and eventually crop productivity.

The use of solid organic fertilizer (SOF) has been practiced as long as the agriculture itself. Organic fertilizer is generally produced by decomposing the organic materials with the assistance of microorganisms act as decomposers and provides nutrients for crop growth and development. Solid organic fertilizer might be produced from animal manures or in combinations with any other local organic materials in the surrounding production sites. Indeed, organic farming systems provided many advantages to the growers, including reduced pest attacks, energy inputs and greenhouse gas emission, leaching of pesticides into water bodies and developed soil health quality (chemical, physical and biological). Animal manures are among the most common sources of SOF after the selected manure get decomposed for four to five weeks. Goat manures have been also used as the source of organic material in production of SOF. According to (Hartatik and Widowati, 2006) fresh goat manures contained N, P, K and organic C as much as 1.41%, 0.54%, 0.75 %, and 46.51%, respectively. Other source of nutrient available from the slaughtering houses is the cow blood. This waste can be used for organic fertilizer production (Ernawati et al., 2015). Cow blood contained N, P and K as much as 4.94%, 0.16% and 0.93%, respectively (Roy *et al.*, 2013). Roles of microbial decomposers are very important in the production of SOF to meet recommended standard of SOF for crop production and growing media.

The use of local-based microbial decomposers for production of SOF from goat manures is one of the methods to maintain the sustainability of agricultural resources. Locally available microbial decomposer is classified as bio-activator. Bio-activator is generated from solid or liquid natural substances which contained non-toxic and promoting microorganisms to decompose organic matter (Zaman *et al.*, 2020). Among the local organic wastes that can be used as source of bio-activator is cow rumen from the slaughtering houses. Cow rumen could be used as bio-activator for organic matter decomposers. Cow rumen liquid contained xylanolytic bacterium, *i.e.*, *Bacillus* sp., *Celummonassp, Lactobacillus* sp., *Pseudomonas* sp., and *Acinetobacter* sp. Recently, combination of cow blood with stale rice, or yeasts, or cow rumen as bio-activator enriched in production SOF from cow manures were concluded to be as effective as the use commercial bio-activators (Podesta *et al.*, 2021). These findings suggested that the use of stale rice, or yeasts, or cow rumen as

bio-activators, and cow blood from slaughtering house was promising to be expanded in for SOF production from goat manures.

Another natural functional microorganism in rhizosphere is the presence of fungus from species of *Trichoderma*. The roles of this species have been reported to positively affect growth of soybean roots. The association of this fungus with soybean roots has been reported to increase soybean growth (Bononi *et al.*, 2020). Nevertheless, there has been very limited information on the uses cow blood from slaughtering as source of nutrients in combinations with goat manure in the production of SOF from goat manures and its interactions with *Trichoderma* sp for black soybean production. The experiment aimed to determine the interaction effects of bioactivator-enriched goat manures and *Trichoderma* sp. and the effects of *Trichoderma* sp. on growth and yield of black soybean.

Materials and methods

This polybag experiment was conducted from September to December 2020, at Experimental Station of Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Bengkulu, in Bengkulu Tengah, Indonesia at elevation of 50 m above sea level (3°, 44', 2" South Latitude and 102°, 20', 31.9'' East Longitude).

The experiment was arranged as in factorial experiment in Complete Randomized Design with three replications. The first factor was bioactivatorenriched goat manures; B1) 1 kg of yeast + 20 L of cow blood + 20 kg goat manure + 1 L diluted white sugar (0.24%, 47.62%, 47.62% and 0.24% of total mixtures), (B2) 1 kg stale rice + 20 L of cow blood + 20 kg goat manure + 1 L diluted white sugar (0.24%, 47.62%, 47.62% and 0.24% of total mixtures), (B3) 20 L cow rumen + 20 L of cow blood + 20 kg goat manure + 1 L diluted white sugar (32.86%, 32.86%, and 1.72 % of total mixtures), and (B4) control, recommended synthetic fertilizer (350 kg ha⁻¹ Urea, 300 kg ha⁻¹ TSP and 300 kg ha⁻¹ KCl). The second factor was *Trichoderma* dosages; (T1) 0 g plant⁻¹, (T2)15 g plant⁻¹, (T3) 30 g plant⁻¹, and (T4) 45 g plant⁻¹.

Each treatment of bioactivator-enriched goat manure was applied to plant with dosages of 200 g polybag⁻¹. Meanwhile, synthetic fertilizer Urea, TSP and KCl was applied at the dosages of 7.5, 5.25, and 4.5 g polybag⁻¹, respectively. The application of Trichoderma treatments into the growing media in the polybag was conducted at a week before planting. Each treatment unit consisted of three plants grown in each polybag to make a total of 144 polybags.

Production of bioactivator-enriched goat manures was conducted by method proposed by (Safitri *et al.*, 2021). Each of all treatment formula mixture and then fermented in a wrapped-up tarpaulin and for two weeks. Every for day the mixtures were opened and stirred and rewrapped up for further fermentation.

Growing media of this experiment was top soil of ultisol, which was previously treated with 4 tons ha⁻¹ of dolomite for a week to reach soil pH of 6, and each planting bag (black polyethylene bags of 40 x 40 cm in size) was filled with 10 kg limed soil. Two seeds of black soybean (*var.* Anjasmoro) were sown in each polybag and the worse soybean growth was removed at 14 days after sowing.

Manual irrigation was applied to all experimental plants, when there was no precipitation, until the growing medium reached field capacity, indicated by dripping water from the bottom of polybag. Manual weeding and soil upraising were conducted at 14, 28 and 42, 56, 70 days after planting. All pests were physically controlled by removing their presence in the polybags.

Crops were harvested at 85 days after planting. The effects of treatment were determined in term of (1) plant height at 42 days after planting (cm), (2) shoot fresh weight (g), (3) number of branches, (4) number of mature pods (g), (5) weight of grains plant⁻¹ (g), and (6) weight of 100 grains (g). Data were subjected to homogenous test before analysis of variance by using Statistical Analysis System at $P \le 0.05$. Means of treatment effects were compared using Least Significantly Difference at $P \le 0.05$.

Results

The use of bio-activator in the production of goat manure composts significantly influenced plant height, shoot fresh weight, number of mature pods and grain weight plant⁻¹, but not weight of 100 grains plant⁻¹. The application of *Trichoderma* significantly affected the grain weight plant⁻¹ and weight of 100 grains. However, it did not significantly influence plant height, shoot fresh weight, and number of mature pods. There were no interaction effects of bio-activators and *Trichoderma* on all observed variables. Summary of treatment effects on growth and yields of black soybean are presented in Table 1.

The effects of bioactivator-enriched goat manures composts significantly influenced plant height and shoot fresh weight (Figure 1). Results indicated that soybean fertilized with bioactivator-enriched goat manures, B1 (yeast+cow blood+goat manure), had the lowest plant height compared to other treatments, although it was similar to control (B4, recomended synthetic fertilizers). Plant

height of soybean grown with B2 (stale rice+cow blood+goat manure) and B3 (cow rumen+cow blood+goat manure) are amongst the highest and it was not different to B4.

Variables	$F_{calculated}$		
	Bio-activator	Trichoderma	Interaction
Plant height	3.42*	0.72 ns	1.94 ns
Shoot fresh weight	12.88**	1.46 ns	2.13 ns
Number of mature pods	11.52 **	1.00 ns	2.09 ns
Grain weight per plant	5.47 **	3.72 *	2.14 ns
Weight of 100 grains	0.85 ns	4.27 *	1.78 ns

Tabel 1. Analysis of variances of effects of Bio-activator and Trichoderma on growth and yields of black soybean

Note: *, **, and ns are significantly different at 95%, significantly different at 99%, and insignificantly different at 95% level of confident.

Effects of bioactivator-enriched goat manures on yield of black soybean as indicated by the number of mature pods and grain weight per plant are presented in Figure 2. Black soybean fertilized with B3 formula had the highest number of mature pods, followed by those of fertilized with B2, B1, and B4 formulas (Figure 2A). Meanwhile, crops fertilized with B22 and B3 formulas produced the highest grain weight per plant followed by those fertilized with B4 and B1 formulas (Figure 2B). However, both B1 and B4 formulas provided similar effects to grain number of black soybean.

The effects of Trichoderma on mature pod number and weight of 100 grains of black soybean are presented in Figure 3. Results indicated that the application of *Trichoderma* significantly increased the grain weight plant⁻¹ and weight of 100 grains. Soybean amended with 30 g plant⁻¹ had the highest grain weight plant⁻¹ and weight of 100 grains, although it was not significantly different with those of fertilized with 15 g plant⁻¹. The application of *Trichoderma* with 40 g plant⁻¹ produced similar effects with untreated plant (0 g plant⁻¹) to grain weight per plant and weight of 100 grains. This experiment suggested that the application of 15 g plant⁻¹ is suitable for black soybean production.



Figure 1. Effect of bioactivator-enriched goat manures on plant height (A) and shoot fresh weight (B) of black soybean. [(B1=yeast+cow blood+goat manure, B2=stale rice+cow blood+goat manure, B3=cow rumen+cow blood+goat manure, and (B4=control/recomended synthetic fertilizer; Urea, TSP and KCl). *Treatment means followed by the same letter in each figure are not significantly difference at Least Significantly Difference P* \leq 0.05)



Figure 2. Effect of bioactivator-enriched goat manures on mature pod number (A) and grain weight (B) of black soybean. (B1 = yeast+cow blood+goat manure, B2=stale rice+cow blood+goat manure, B3=cow rumen+cow blood+goat manure, and (B4=control/recomended synthetic fertilizer; Urea, TSP and KCl). *Treatment means followed by the same letter in each figure are not significantly difference at Least Significantly Difference* $P \le 0.05$]



Figure 3. Effect of Trichoderma amendment in goat manure decomposition number of filled pod (A) and weight of 100 grains (B) of black soybean. [(T1= 0 g plant⁻¹, T2=15g plant⁻¹, T3=30 g plant⁻¹, and T4=45g plant⁻¹). Treatment means followed by the same letter in each figure is not significantly difference at Least Significantly Difference $P \le 0.05$]

Discussion

Effects of bio-activator amendment

It appeared that soybean fertilized with bioactivator-enriched goat manures, B1 (yeast+cow blood+goat manure), had the lowest plant height compared to other treatments, although it was similar to control (B4, recomended synthetic fertilizers). Plant height of soybean grown with B2 (stale rice+cow blood+goat manure) and B3 (cow rumen+cow blood+goat manure) are amongst the highest and it was not different to B4. This research suggested that the use of bioactivator-enriched goat manures could replace the effectiveness of synthetic fertilizers for black soybean production. However, bioactivator-enriched goat manures of formula B3 and B3 are better than B1.Trend of increased plant heights was likely in accordance with increased Soybean fertilized with bioactivator-enriched goat shoot fresh weight. manuresB3 had the highest shoot fresh weight, yet it was not different to B4, followed by those of grown with B3. Black soybean grown with B1 formula had the lowest shoot fresh weight. According to (Harmankaya et al., 2008), plant height is an important character since it positively correlates to beans yield.

The ability of bioactivator-enriched goat manures to have comparable growth with black soybean fertilized with recommended synthetic fertilizer might have related to the presence of microorganisms form the stale rice, yeasts, or

cow rumen in decomposition process of goat manures which eventually produce more nutrients in the goat manures. High nutrient contents in bioactivator-enriched goat manures will not only improve growth, but also yields of black soybean. Results from this experiment suggested that the use of B2 (stale rice+cow blood+goat manure) and B3 (cow rumen+cow blood+goat manure) formulas are comparable and promising to promote black soybean yields. These organic fertilizers are generally superior to that of synthetic fertilizer (B4). Sources of nutrients in B2 and B3 formula are most presumably derived from the goat manures (Hartatik and Widowati, 2006) and cow blood (Ernawati et al., 2015). The addition of microbial decomposers from stale rice and cow rumen might have accelerated the decomposition of goat manures. Both stale rice and yeasts are able to serve as organic material decomposers due to the presence of microorganism decomposers. In addition, cow rumen contained numerous microbial decomposers such as bacteria, protozoa, yeast, and fungi that play significant roles in decomposing organic materials (Basri, 2016). Previously, (Lamid et al., 2006) concluded that rumens contained xylanolytic bacterium, including Bacillus sp., Cellumonas sp., Lactobacillus sp., Pseudomonas sp. and Acinetobacter sp.

The availability of well-composted goat manures around the rooting zone subsequently contributed to the nutrients for black soybean. Animal manures, including goat manure, are considered as good nutrient provider, P, Ca, Mg and K, for plants (Almeida *et al.*, 2019). The use of vermicompost improved the quality of growing medium in terms of soil pH, aggregates, bulk density, water-holding capacity, organic matter, micro and macro-nutrients as well as soil biological properties (Piya *et al.*, 2018). In addition, the use of manure application also elevated enzyme activities in provoking nutrient availability for plant (Šimon and Czak ó, 2014). Such better growing environment due to application of B2 (stale rice+cow blood+goat manure) and B3 (cow rumen+cow blood+goat manure) will eventually increase soybean growth and yields. Increased plant height and leaf number of soybean due to goat manure application has been reported to increase of shoot fresh weight (Rahayu *et al.*, 2021).

Effects of Trichoderma

The uses of *Trichoderma* sp. in agricultural practices have widely applied since it benefits crops through improvement of plant biological control mechanisms, plant diseases resistances, plant growth, decomposition process and bioremediation as well as its application on environmentally friendly agriculture practices (Zin and Badaluddin, 2020). Similar results had been also concluded that the use of *Trichoderma* in soybean increased weight per 100 seeds due to the ability of *Trichoderma* to suppress diseases intensity in soybean crops (Martanto *et al.*, 2020). The use of *Trichoderma*, especially *T. harzianumis*, might improve phosphate availability for the soybean, since this fungus also a phosphate dissolving microorganism that able to work together with phosphate dissolving bacteria (Li *et al.*, 2015). Under organic production systems, the use of organic fertilizer potassium uptakes by sweet corn fertilized with liquid organic fertilizer significantly increased shoot dry weight, weight of husked ear and yield of sweet corn (Fahrurrozi *et al.*, 2018). The use presence of *Trichoderma* was reported to increase soybean growth and yield (Saputra *et al.*, 2021).

In conclusion, black soybean fertilized with bioactivator-enriched goat manures composed of stale rice+cow blood+goat manure and cow rumen+cow blood+goat manure had higher number of mature pods and grain weight per plant than those of fertilized with synthetic fertilizer. The application of *Trichoderma* on black soybean increased the number of mature pod and weight of 100 grains. Recommended dosage of *Trichoderma* for black soybean production was 15 g plant⁻¹. Scaling up this experiment in an open field should be further evaluated in the near future.

Acknowledgements

Sincerely thank Head of Agrotechnology Study Program, Dean of the Faculty of Agriculture and Animal Husbandry, Chancellor of the University of Muhammadiyah Bengkulu for financing this project and funding for this research program. This research was funded by 2021 Applied Research Grant University of Muhammadiyah Bengkulu (Grant Number: 220/LPPM-UMB/2021).

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(Received: 14 April 2022, accepted: 30 December 2022)