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Effect of Manure and Inorganic Fertilizers on Vegetative, Generative Characteristics, Nutrient, and Secondary Metabolite Contents of Mungbean

✉**Sutrisno, Eriyanto Yusnawan**

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Indonesian Legumes and Tuber Crops Research Institute, Indonesia

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Abstract

A field experiment was conducted in the upland of Muneng Probolinggo during early dry season (April – June 2015) to study the effect of manure and inorganic fertilizers on vegetative, generative growth, nutrient, and secondary metabolite contents of mungbean (*Vigna radiata* L.). The treatments consisted of (1) no fertilizer, (2) 10.4, 18, 60 kg of N, P, K ha^{-1}, (3) 22.5: 22.5: 22.5 kg of NPK ha^{-1}, (4) 5000 kg ha^{-1} manure, and (5) 11.25:11.25:11.25 kg of NPK ha^{-1} + 2500 kg ha^{-1} manure. These treatments were arranged in a randomized completely block design with three replications. Application of manure, inorganic fertilizer, and its combination significantly stimulated several vegetative characters especially number of nodes, number of clusters, fresh weight of biomass, and number of nodules. At the generative characters, application of manure and inorganic fertilizers also significantly increased pod dry weight and grain dry weight. Among all treatments, NPK inorganic fertilizer (T2) gave the highest vegetative and generative growth which was shown on biomass fresh weight, pod dry weight and grain dry weight per plant. NPK fertilizer and manure applications increased total flavonoid and phenolic contents as well as antioxidant activity. This combination treatment therefore, could be suggested in mungbean cultivation to increase seed quality.

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✉ Correspondence Author:
Jln. Raya Kendalpayak. KM 8 PO Box 66 Malang 65101,East Java, Indonesia
E-mail: uthisharun@gmail.com

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INTRODUCTION

Mungbean is nutritionally and functionally important for human health as it contains carbohydrates, proteins, vitamin, and minerals as well as secondary metabolites such as phenolic acids and isoflavones that have antioxidant activities. Mungbean has been used for food products (Shi *et al.*, 2016), beverages, health therapies as well as beauty products (Wongekalak *et al.*, 2011). The increase of quantity and quality demand of mungbean triggers effort in increasing quantity as well as nutritional values of these beans. The improvement in crop cultivation is one of the break through to address this issue.

Fertilization modification has been generally conducted to improve the mungbean cultivation. An application of NPK fertilizers that supply nitrogen, phosphate, and potassium as sources of nutrients is effective to increase the growth and yield of mungbean through increasing the number of nodules, leaves, pods, and seeds per plant (Malik *et al.,* 1988). However, the continuous application of NPK leads to increase the soil compactness, decrease the soil fertility, soil porosity, and C-organic level (Chaudhary *et al.,* 2017) as well as soil microorganism population (Wei *et al.,* 2017). The decrease in soil fertility consequently would result in the decrease in mungbean yield as well as the nutritional quality.

Application of manure increases soil fertility. Macro and micro nutrients become more available and soil microorganism population is more abundance (Qin *et al.,* 2015; Eo & Park, 2016). This application also improves the soil physical properties such as porosity and water holding capacity (Tadesse *et al.,* 2013). However, effect of manure fertilizer does not significantly increase seed yield in a short period. To address this issue, therefore, a combination application of manure and NPK fertilizers can be one of the solutions to maintain and to improve soil fertility and ultimately to increase the yield potential of mungbean (Manna *et al.,* 2007; Sharma *et al.,* 2013). In addition, other studies showed that combination of manure and NPK fertilizers increased crop yield higher than that of NPK fertilizer treatment (Wei *et al.,* 2016).

A combination of organic and inorganic fertilizers also improves seed quality. Phenolic, flavonoid, and vitamin C contents increase in fennel seeds harvested from field treated with organic and NPK fertilizers (Salama *et al.,* 2015). Organic fertilizer treatments on soybean crops increase total phenolic and flavonoid contents as well as protocatechuic acid, p-hydroxybenzoic acid, chlorogenic acid, caffeic acid, quercetein, genistein, and daidzein (Taie *et al.,* 2008). Although studies on the application of fertilizer combinations increase nutrition and secondary metabolite contents in some crops, similar research on mungbean is still limited (Shi *et al.,* 2016). In Shi *et al.* (2016) study, however, no combination of organic and inorganic fertilizers was carried out to examine the nutritional composition and antioxidant activity. This study therefore, aimed to determine the effects of manure and NPK fertilizers as well as the combination of both fertilizers on vegetative, generative growth, nutrition, and secondary metabolites of mungbean.

METHODS

The experiment was conducted in Muneng research station, Probolinggo, East Java, Indonesia from April to June 2015. Muneng research station is located at 10 m above sea level with climate type of E1 (Oldeman), range of temperature between 32°C and 36°C, humidity of 74-80 %, and 2000 mm/year of rainfall. The properties of the soil was alfisol and general chemical and physical characteristics of the soil were presented in Table 1 and 2. Soil chemistry before and after planting, residual fertilizers in the soil and in the crops, and fertilizer intake by the crops have been thoroughly investigated and reported by Kuntyastuti *et al.,* (2015).

This experiment consisted of five treatments, i.e. (0) no fertilizer; (1) 10.4, 18, 60 kg of N, P, K fertilizers ha^{-1}; (2) 22.5: 22.5: 22.5 kg of NPK ha^{-1}; (3) 5000 kg manure ha^{-1}, and (4) combination of 2500 kg manure ha^{-1} + 11.25:11.25:11.25 kg of NPK ha^{-1}. Vima-1 mungbean cultivar with harvesting time of 56 days after planting (dap) was cultivated at spacing of 40 cm x 15 cm. The plot size in each treatment was 4 x 4 m^2. Treatments were arranged in a randomized completely block design with four replications.

Observation on agronomic performances included plant height, number of branches, number of peduncle, stem length from base to the first trifoliate, stem diameter, length of main root, length of lateral root, number of lateral root, root weight, number of nodules, plant biomass, dry pods weight per plant, dry seed weight, number of pod per plant, dry seed coat weight per plant, and dry weight per pod. Seed quality including protein, starch, amylose contents, total flavonoid, total phenolic contents and antioxidant activity was investigated.

Total protein content was estimated using the micro kjeldahl method (AOAC, 2005). Briefly,

Table 1. General chemical characteristics of alfisol soil and cow manure used in Muneng research station, Probolinggo East Java in dry season 2015

Chemical unit of alfisol soil characterization	Value	Chemical unit of cow manure characterization	Value
pH H_2O (1:5)	8.00	pH H_2O (1:5)	8.03
pH KCl (1:5)	6.80	C-organic Kurmis (%)	22.7
C-organic Kurmis (%)	0.77	N-organic (%)	1.24
N-total Kjedahl (%)	0.07	N-NH_4 (%)	0.13
P_2O_5 Bray-1 (ppm)	82.9	N-NO_3 (%)	0.20
K (NH_4OAc pH 7.0, cmol$^+$ kg^{-1})	0.60	N-total (%)	1.56
Na (NH_4OAc pH 7.0, cmol$^+$kg^{-1})	0.87	P-total HNO_3 + $HClO_4$ (%)	0.94
Ca (NH_4OAc pH 7.0, cmol$^+$kg^{-1})	16.0	S-total HNO_3 + $HClO_4$ (%)	0.45
Mg (NH_4OAc pH 7.0, cmol$^+$kg^{-1})	6.53	K-total HNO_3 + $HClO_4$ (%)	1.79
SO_4 (NH_4OAc pH 4.8, ppm)	4.88	Na-total HNO_3 + $HClO_4$ (%)	0.01
CTC (NH_4OAc pH 7.0, cmol$^+$kg^{-1})	49.7	Ca-total HNO_3 + $HClO_4$ (%)	3.14
Al-dd (KCl 1 N, cmol$^+$kg^{-1})	0.00	Mg-total HNO_3 + $HClO_4$ (%)	13.7
H-dd (KCl 1 N, cmol$^+$kg^{-1})	0.10	Fe-total HNO_3 + $HClO_4$ (%)	0.80
Fe (DTPA, ppm)	5.61	Zn-total HNO_3 + $HClO_4$ (%)	0.004
Zn (DTPA, ppm)	0.27	Cu-total HNO_3 + $HClO_4$ (%)	0.002
Cu (DTPA, ppm)	5.96	Mn-total HNO_3 + $HClO_4$ (%)	0.067
Mn (DTPA, ppm)	59.6	CTC (NH_4OAc pH 7.0, cmol$^+$kg^{-1})	57.7

Source: Kuntyastuti *et al.,* 2015.

Tabel 2. Physical characteristics of alfisol soil of Muneng research station, Probolinggo East Java in dry season 2015

Physical unit of alfisol soil characterization	Value
Kjeldahl (cm hour^{-1})	3.21
Density (g cm^{-3})	1.20
Specific gravity (g cm^{-3})	2.40
Porosity (%)	50.00
Water content of pF 2.5 (cm^{-3} cm^{-3})	0.33
Water content pF 4.2 (cm^{-3} cm^{-3})	0.16
Water holding capacity (%)	17.0
Sand (%)	19.0
Dust (%)	62.0
Clay (%)	19.0
Texture class	Dusty clay

Source: Kuntyastuti *et al.,* 2015.

destructive and distillated sample were conducted prior titration. Titration was conducted by adding 0.02 N HCl to the sample. Total protein content was calculated by multiplying the N total with the correction factor.

Starch content was analyzed with acid hydrolysis following the Nelson-Somogy method (Sudarmadji *et al.,* 1997). The Nelson and arsenolmolybdat solution were used to estimate the starch content of the sample. Absorbance values were measured at 540 nm using a spectrophotometer. A standard of reduction sugar curve was used as a reference to estimate the starch content.

Amylose content was analysed based on the method developed by Juliano (1979). Acetic acid solution (1N) and iodine were used to measure amylose content in the sample. The absorbance values at 620 nm were measured using a spectrophotometer. An amylose standard was used as a reference.

Determination of total flavonoid content. Mungbean flour extract was diluted in distilled water (1:5 v/v) in a glass tube and mixed thoroughly. A solution of 5% $NaNO_2$ (150 μL) was added into the tube, vortexed, and incubated for 6 min at room temperature. Aluminum chloride (300 μL) was added and the mixed solution was incubated for 5 min. Sodium hydroxide (1M, 1000 μL) was reacted in the mixed solution and distilled water was added to the final volume of 5000 μL. After thoroughly mixed, absorbance values of the sample were read using a spectrophotometer at 510 nm. Catechin equivalents per gram of sample (mg CE g^{-1} sample) were used to express total flavonoid contents in the sample (Heimler *et al.,* 2005; Xu & Chang, 2007).

Determination of total phenolic content. Folin Ciocalteu's reagent was used to estimate total phenolic content in mungbean extract (Singleton *et al.,* 1999; Xu & Chang, 2007). Sample in distilled water (1:60 v/v) was reacted with Folin-Ciocalteu's reagent (250 μL), then sodium carbonate (750 μL) was added. The mixed solution was vortexed and incubated for 8 min at T room. Additional distilled water (950 μL) was pipetted and the mixture was incubated in the dark room for 2 h. Absorbance values of samples were recorded using a spectrophotometer at 765 nm. Total phenolic contents in samples were expressed as gallic acid equivalents per gram of sample (mg GAE g^{-1} sample).

DPPH free radical scavenging activity. Antioxidant activity of mungbean flour extract was estimated using 1 mM ethanolic DPPH solution (Xu & Chang, 2008a; Xu & Chang, 2008b). In DPPH solution, mungbean extract (1:19 v/v) was reacted, vortexed, and incubated for 30 min in the dark at room temperature. The absorbance values of sample (A_{sample}) and blank ($A_{control}$) were recorded using a spectrophotometer at 515 nm. Percent discoloration which reflects DPPH scavenging activity was calculated as follows: percent discoloration = $[1-(A_{sample}/A_{control})] \times 100$. The DPPH free radical scavenging activity of each sample was expressed as micromoles of Trolox equivalent per gram of sample (μmol TE g^{-1} sample).

RESULTS AND DISCUSSION

Plant growth characteristics

Treatments of NPK fertilizer, manure, and its combination were effective to increase some agronomic characters such as number. Number of fertile nodes, number of peduncles, the stem length from base to the first trifoliate, and stem diameter. The best response of those agronomic characters was achieved by the treatment of NPK fertilizer followed by the combination of NPK and manure fertilizers. However, other agronomic characters like plant height and number of branch were not significantly affected by all fertilizer treatments (Table 3).

The application of T1 (10.4, 18, and 60 kg of N, P, K fertilizers ha^{-1}) was the most effective treatment to stimulate the length of stem to trifoliate, number of fertile nodes, and stem diameter. The increase of these parameters was 20 to 28 % higher than those of no fertilizer treatment. The difference of NPK dosage also influenced the difference in number of peduncles. Number of peduncles of T1 was less than that of T2 (22.5:22.5:22.5 kg of NPK fertilizers ha^{-1}) even though number of fertile nodes and the length of stem were higher in T1. This increase of vegetative growth may be related to the level of N and P intake. The treatment of T1 provided the highest N intake compared to the other treatment, whereas T2 treatment showed the highest of P intake as reported by Kuntyastuti *et al.* (2015).

NPK application enriched the availability of macro nutrients, nitrogen, phosphate, and potassium in the soil. These chemicals therefore, were readily absorbed by the crops. In crop metabolism, these nutrients are utilized in carbohydrate synthesis, cellulose, proteins, hormones, and enzymes. All these processes triggered the growth of plant organs such as stem length, stem diameter, and number of fertile nodes as investigated in this present study. This result was in line with the previous studies conducted by Mandal *et al.,* (2009) and Bandyopadhyay *et al.,* (2010). In their studies, an application of NPK also triggered the growth of vegetative crops.

The different composition of nutrients in NPK fertilizers affected plant metabolisms and plant growth. The small amount of specific nutrient in a mixed fertilizer could be a limiting factor in normal plant growth. Plant growth obtained from crops treated with T2 (22.5:22.5:22.5 kg of NPK fertilizers ha^{-1}) was slower than the crops treated with T1 (10.4, 18, 60 kg of N, P, K fertilizers ha^{-1}). This could be caused by less amount of potassium in T2 than that of T1 treatments. Increased dose of potassium also increased soybean plant growth according to (Parvej *et al.,* 2015; Parvej *et al.,* 2016).

Application of manure in a single treatment was unable to trigger the highest plant growth. However, the combination of manure with a half dose of T2 (T4 treatment) showed significant influence to the plant growth. Number of fertile nodes, stem length from base to the first trifoliate, and the number of peduncles were equal to T1 treatment. In addition, the combination treatment was slightly higher than the growth plant in T2 (22.5:22.5:22.5 kg of NPK fertilizers ha^{-1}) treatment (Table 3). This research was supported by the previous studies in which combination of manure and NPK fertilizers were able to stimulate the vegetative growth similar or faster than the growth from NPK fertilizer (Manna *et al.,* 2007; Wei *et al.,* 2016). In addition, the combination of manure and a half dosage of NPK fertilizer ha^{-1} increased vegetative growth of crops similar to the growth from a full dose of NPK fertilizer ha^{-1} (Bandyopadhyay *et al.,* 2010). The addition of manure to inorganic fertilizers gave advantages since manure slow released nutrients, improve physical, biological, and chemical properties of soil continuously.

Number of nodules of Vima 1 varied among fertilizer treatments. Nodules obtained from T1 treatment were the highest, with 10.6% increase compared to the control (Table 4). This result was in line with the previous study conducted by Mandal *et al.,* (2009). Surprisingly, the combination of manure and NPK fertilizers was unable to increase the number of nodules. In fact there was 33% decrease of nodules in T4 treatment compared to the control (Table 4). This decrease might be caused by the high nitrogen content in the soil as reported by Kuntyastuti *et al.* (2015). The high availability of nitrogen in soil would inhibit the growth of rhizobial organisms (Supanjani, *et al.*, 2006). Fujikake *et al.,* (2002) also reported that the increase in nitrogen concentration significantly inhibited soybean nodules. However this result was not in agreement with other reports (Mandal *et al.,* 2009; Singh *et al.,* 2017). Mandal *et al.* (2009) and Singh *et al.* (2017) observed that combination of manure and inorganic fertilizers increased the number of nodules.

Applications of fertilizers did not significantly influence the root growth. The main root length, lateral root length, number of lateral roots and fresh weight of lateral roots were not affected by all fertilizer applications. No effect on the root growth could be caused by the application of fertilizers did not immediately change physical properties of the soil. According to Lin *et al.* (2016), the difference of root growth was influenced by physical properties of soil such as soil density.

The weight of plant biomass increased significantly in all fertilizer treatments except for manure application (Table 5). The increase of 11 and 23% of biomass weight was observed in the crops treated with inorganic fertilizers (T1 and T2 treatments). The combination of manure and inorganic fertilizer treatment gave the same fresh biomass as T1. Unlike as observed in fresh biomass, dry pod harvested from crops treated with combination of manure and inorganic fertilizers was not significantly influenced. The same result was found in dry seed weight/plant.

Pod and seed dry weights obtained from T2 fertilizer treatment were the highest among other treatments. The increases were 26 and 42 % compared to the control, respectively. The in-

Table 3. Effect of inorganic fertilizers and manure on plant vegetative growth of mungbean

Treatment	Plant height (cm)	Number of branchs **	Number of fertile nodes	Number of Peduncles	Trifoliate stem length (cm)	stem diameter (mm)
T0	51.51^{a}	0.93^{a}	5.60^{bc}	4.20^{a}	8.53^{c}	5.24^{b}
T1	55.78^{a}	1.10^{a}	6.87^{a}	3.31^{b}	10.98^{a}	6.33^{a}
T2	52.76^{a}	0.93^{a}	5.19^{c}	3.85^{a}	8.79^{bc}	6.32^{a}
T3	48.70^{a}	0.93^{a}	5.77^{bc}	3.43^{b}	9.87^{abc}	4.98^{b}
T4	54.97^{a}	1.07^{a}	6.42^{ab}	4.00^{a}	10.08^{ab}	5.56^{ab}
Average	52.74	0.99	5.97	3.76	9.65	5.69
CV	5.32	13.35	7.40	4.99	8.48	8.78

Note: values in the same column followed by the same letter were not significantly different based on LSD test at α 0.05. **): 2x transformation with square root

T0	No fertilizer	T3	5000 kg manure ha^{-1}
T1	NPK 10,4:18:60 kg ha^{-1}	T4	2500 kg manure ha^{-1}+ 11.25:11.25:11.25 kg NPK ha^{-1}.
T2	22.5:22.5:22.5 kg of NPK ha^{-1}		

crease of pod and seed dry weight was not observed in combination treatment both manure and inorganic fertilizers. However, other studies suggested that combination treatment of manure and inorganic fertilizers was able to increase the yield equal to the additional of manure as many as 4 ton ha^{-1} (Bandyopadhyay *et al.,* 2010) or even higher up to 5 ton ha^{-1} (Singh *et al.,* 2017). The addition of 2.5 ton ha^{-1} of manure such as conducted in this current study was not enough to increase the yield.

Protein contents were significantly influenced by the application of high potassium fertilizer as observed in T1 (Table 6). The increase of total protein content up to 14.5% was recorded in T1. Similar results was also reported by Haq & Mallarino (2005) who investigated the significant increase of protein contents caused by the increase dose of potassium fertilizer.

Starch content in seeds significantly increased in all fertilizer treatments, however, the increase was not different among all fertilizer applications (Table 6). The increase was in the range of 13.8 to 19.1 % higher than that in control. The increase in starch content due to fertilizer applications was also reported by other study (Manna *et al.,* 2007). Although fertilizer application increased starch contents, the amylose contents were not influenced by the fertilizer treatments. The increase of total starch contents as investigated in this study may be due to the increase of other starch compounds such as amylopectin. The increase of amylopectin was influenced by the application of organic and inorganic fertilizers (Oktavia *et al.,* 2008).

Total flavonoid content in seeds increased significantly with the application of manure in combination with NPK fertilizers (Table 7). The increase in total flavonoid content of this treatment was the highest among other treatments. The increase of total flavonoid content in the combination treatment reached up to 28% higher than the control. The application of cow manure increased this content of 15% compared to the control, while treatment of NPK fertilizers (T2), the increase was 8.5%. The application of manure increased total flavonoid content 5.7 % higher than the application of NPK (T2).

High flavonoid content in seeds harvested from the combination treatment as well as manure only suggested that manure played an important role in increasing the flavonoid contents. Manure application indirectly influenced the increase of flavonoid contents. The increase of these compounds is directly caused by the interaction among rhizobium bacteria, arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria, and nematodes. Manure application supplies organic materials which enrich soil microbe population such as bacteria, fungi, and nematodes which then interact with plant roots (Qin *et al.,* 2015; Zhong *et al.,* 2010). The interaction between soil microbes and plant roots increase the synthesis of flavonoids (Sugiyama & Yazaki, 2014).

Similar as observed in flavonoid contents, total phenolic contents also increased significantly in the combination treatment of manure and NPK fertilizers (Table 7). The increase of 37% higher of total phenolic contents was recorded, whereas the increase of these compounds in NPK fertilizer treatment (T1) was 26% higher than the no fertilizer. The increase of total phenolic contents from crops treated with the combination

Table 4. Effect of inorganic fertilizers and manure on root growth and nodule of mungbean

Treatment	Length of main root	Length of lateral root	Number of lateral roots*	Root fresh weight**	Number of nodules*
T0	16.04[a]	10.32[a]	3.27[a]	1.24[a]	3.19[ab]
T1	16.62[a]	11.00[a]	3.58[a]	1.54[a]	3.53[a]
T2	14.32[a]	11.00[a]	3.04[a]	1.29[a]	2.42[bc]
T3	13.75[a]	10.00[a]	2.99[a]	1.19[a]	2.91[abc]
T4	14.56[a]	10.39[a]	3.01[a]	1.27[a]	2.13[c]
Average	15.06	10.54	3.18	1.30	2.83
CV	12.66	12.51	10.37	9.62	15.22

Note: value in the same column followed by the same letter were not significantly different based on LSD test at α 0.05. *): Transformation with square root **): 2x transformation using square root

T0 No fertilizer
T1 10.4, 18, 60 kg of N, P, K ha^{-1}
T2 22.5:22.5:22.5 kg of NPK ha^{-1}
T3 5000 kg manure ha^{-1}
T4 2500 kg manure ha-1+ 11.25:11.25:11.25 kg NPK ha^{-1}.

Table 5. Effect of inorganic fertilizers and manure on plant generative growth of mungbean

Treat-ment	Biomass fresh weight	Pod dry weight/ plant	Seed dry weight/ plant	Number of pods/ plant	Seed coat dry weight/ plant	Weight/ pods
T0	56.25[bc]	11.10[bc]	6.91[b]	12.20[a]	4.19[a]	0.91[ab]
T1	62.36[ab]	12.39[ab]	8.13[b]	13.18[a]	4.26[a]	0.94[a]
T2	69.49[a]	14.04[a]	9.87[a]	14.44[a]	4.17[a]	0.98[a]
T3	49.56[c]	10.09[c]	7.19[b]	12.18[a]	2.90[b]	0.83[bc]
T4	61.70[ab]	11.88[bc]	8.14[b]	14.97[a]	3.74[a]	0.79[c]
Average	59.87	11.90	8.05	13.39	3.85	0.89
CV	10.54	9.38	11.04	10.36	10.47	4.87

Note: values in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

T0 No fertilizer
T1 10.4, 18, 60 kg of N, P, K ha^{-1}
T2 22.5:22.5:22.5 kg of NPK ha^{-1}
T3 5000 kg manure ha^{-1}
T4 2500 kg manure ha^{-1}+ 11.25:11.25:11.25 kg NPK ha^{-1}.

Table 6. Effect of inorganic fertilizers and manure on total protein, starch, and amylose contents of mungbean

Treatment	Protein content (dry weight)	Starch content (dry weight)	Amylose content (dry weight)
T0	24.47[b]	41.61[b]	25.06[a]
T1	28.01[a]	47.37[a]	25.53[a]
T2	25.91[b]	49.57[a]	25.46[a]
T3	25.34[b]	49.41[a]	25.58[a]
T4	24.49[b]	47.98[a]	26.27[a]
Average	25.64	47.19	23.03
CV	2.74	1.96	1.57

Note: values in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

T0 No fertilizer
T1 10.4, 18, 60 kg of N, P, K ha^{-1}
T2 22.5:22.5:22.5 kg of NPK ha^{-1}
T3 5000 kg manure ha^{-1}
T4 2500 kg manure ha^{-1}+ 11.25:11.25:11.25 kg NPK ha^{-1}.

of manure and NPK fertilizers and manure only was also reported by Ibrahim, *et al.* (2013) and Bavec *et al.* (2010).

The treatment of manure in combination with NPK fertilizers increased 24% antioxidant activity than control. Manure application only or NPK fertilizers also increased antioxidant activity 8.5% and 3.3% respectively than no fertilizer. This finding supported a previous study in which a combination treatment of organic and inorganic fertilizers as well as manure application also increased antioxidant activity (Ibrahim *et al.,* 2013). This increase was not surprising because flavonoids are one of the largest groups of phenolic compounds which have antioxidant activity (Jeng *et al.,* 2010). In this study, the highest flavonoid contents were observed in seeds harvested from the treatment of manure and NPK fertilizers. Manure applications either single or in combination with inorganic fertilizers trigger the number of soil microbe population, leading to the higher chance of interaction between crops and the microbes (Mandal *et al.*, 2007). This interaction leads to the synthesis of chemical compounds including flavonoids which have high antioxidant activity (Mandal *et al.,* 2007; Jannoura *et al.,* 2014; Montalba *et al.,* 2010).

Table 7. Effect of inorganic fertilizers and manure on total flavonoid, phenolic contents, and antioxidant activity of mungbean

Treatment	Total flavonoid content (mg CE g^{-1})	Total phenolic content (mg GAE g^{-1})	Antioxidant activity (μmol TE g^{-1})
T0	0.764^{cd}	2123^{c}	9.172^{cd}
T1	0.754^{d}	2.308^{bc}	8.841^{d}
T2	0.829^{bc}	2.230^{bc}	9.475^{c}
T3	0.876^{b}	2.458^{b}	9.955^{b}
T4	0.978^{a}	2.911^{a}	11354^{a}
Average	0.840	2.406	9.759
CV	4.09	6.08	2.29

Note: values in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

T0	No fertilizer	T3	5000 kg manure ha^{-1}
T1	NPK 10,4:18:60 kg ha^{-1}	T4	2500 kg manure ha^{-1}+ 11.25:11.25:11.25 kg NPK ha^{-1}.
T2	22.5:22.5:22.5 kg of NPK ha^{-1}		

CONCLUSIONS

Combinations of organic and inorganic fertilizers did not significantly improve the yield, however, the increase of plant secondary metabolites especially total flavonoid and phenolic contents as well as antioxidant activity was observed. The increase of 28 %, 37 %, 28 % was found in total flavonoid, phenolic contents and antioxidant activity in seeds harvested from the crops treated with manure and NPK fertilizers, compared to no fertilizer treatment. The treatment of combination of organic and inorganic fertilizers, therefore, could be suggested to increase the seed quality in mungbean.

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