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Nitrate reductase, chlorophyll content and antioxidant in okra (*Abelmoschus esculentus* Moench) under organic fertilizer

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Abstract

Okra is often considered useful in traditional medicine because it contains secondary metabolites such as alkaloids, terpenoids, and flavonoids and young fruits and leaves are rich in minerals and dietary fiber. The objective of this study was to assess the growth and quality of okra pods at different plant spacing and litter compost dosages. Results showed that plant height, crop growth rate, yield, pod weight and, fruit circumference increased with the increasing dose of N compost. The highest nitrate reductase activity was at 50 cm row spacing and 150 kg N ha^{-1} interaction. Chlorophyll content at 75 cm row spacing was higher than 50 cm row spacing. Chlorophyll content was higher at 150 kg N than 0 kg N ha^{-1}, 50 and 100 kg N ha^{-1}. Level of antioxidants decreased with the increase of N given both in row spacing of 50 cm and 75 cm.

Key words: Okra, compost, growth, antioxidant, quality, row spacing, nitrate reductase, chlorophyll

Introduction

Okra (*Abelmenthus esculantus*) is a vegetable which successfully grow in tropical and subtropical regions. It is a member of the Malvaceae family, a large and diverse family that includes many important crops. Fruit of okra is used as a traditional medicine because it contains secondary metabolite components such as alkaloids, terpenoids and flavonoids (Adetuyi and Ibrahim, 2014). It can cure irritation of the stomach and colon, sore throat, gonorrhea (Lim, 2012), and can also lower blood sugar (Dubey and Mishra, 2017). The fruit may be consumed as cooked vegetables (Gemede *et al*., 2015). Its fruits grow quickly and can be harvested one week after flowering. Fresh pods are low in calories (20 per 100 g), no fat, high in fiber, and have several valuable nutrients *viz*., 30 % of the recommended levels of vitamin C (16 to 29 mg), 10 to 20 % of folate (46 to 88 g) and about 5 % of vitamin A (Kumar *et al*., 2010). Okra plays an important role in the human diet because it is a good source of carbohydrate, proteins, fiber, minerals and vitamins, including vitamin C (dos Santos *et al*., 2013).

Optimum spacing can reduce the competition for sunlight, water and nutrients. Spacing that is too wide or narrow will produce okra with low quality. Maize grain yield of the 0.51 m row spacing with the 80 kg N ha^{-1} fertilizer rate was 14.6 % higher than the conventional 0.76 m row spacing (Barbieri *et al*., 2013). Corn grown at 76 cm row spacing produced higher yields than that grown in 38 cm rows (10.5 *vs.* 10.3 Mg ha^{-1}) (Farnham, 2001). The reduction of row spacing from 100 to 50 cm increased maize grain yield linearly (Sangoi *et al*., 2001).

Sources of organic materials include compost, green manure, manure (Gulshan *et al*., 2013). Organic fertilizers have beneficial effects on the composition of nutrients, structure, aggregation, infiltration, ratios, microbes and other biological activities of the soil (Sanni, 2014). Compost can provide adequate nutrition, improve the process of plant metabolism and increase production. When nutrients are available in appropriate proportions, the activity of plant photosynthesis will proceed well, in presenc of increased light interception (Subbarao and Ravi, 2001). Researches on organic fertilizer have been made, but effect of fertilization with an organic source and row spacing should be studied further. This research studies compost and row spacing effect on growth, yield, physiology, and quality of okra.

Materials and methods

The study was conducted at an experimental field of Faculty of Animal Husbandry and Agriculture of Diponegoro University (7°44 N, 108°35 E and an altitude of 250 m above the sea level), from May to October 2017. The type of soil was Oxisol. An average temperature ranged from 21.0 °C to 32.4 °C, while the relative humidity ranged from 75.2 to 79.6 %. The average annual rainfall was 2013 mm. The results of soil analysis were as follows: N content (0.17 %) with a C / N ratio of 8.35, P (0.16 %), K (0.26 %) and C (1.42 %). The manure analysis had total N (1.74 %), P (0.66 %), total K (1.08 %) and total C (11.43 %). Litter compost had a total N, P, K, C of 1.32, 0.18, 0.85, and 11.51 %, respectively.

The design used in this study was a complete randomized design of 2 x 4 factorial pattern with 3 replications, where the first factor was row spacing (50 and 75 cm) and the second factor was the dosage N compost (0, 50, 100 and 150 kg N. ha^{-1}). Land was prepared with 3 x 3 m plot size as 24 plot with the distance between plots 0.5 m. Compost was made by dung and applied at a dose of 10 tons/ha (9 kg/plot) and litter compost was used according to treatment (0, 50, 100 and 150 kg N ha^{-1}). Basic fertilizer was given during plot preparation. Okra seeds were sown one week after manuring with row spacing according to treatment (50 cm and 75 cm) with 2 seeds/hole and after growth, 1 plant/hole was allowed to grow.

The studied variables were plant height, crop growth rate (CGR), okra yield, pod weight, pod girth, nitrate reductase activity (NR) and chlorophyll content, crude protein and antioxidant activity of okra fruit. Okra was harvested at 60 to 70 days when the size of the okra was 10 to 12 cm.

The nitrate reductase (NR) activity was measured using Krywult and Bielec (2013) method with slight modification. The chlorophyll a and chlorophyll b were determined spectrophotometrically according to the Arnon method (Rajalaksmi and Banu, 2015). The total phenolic compounds were measured according to Orak (2006). All samples were prepared and measured in triplicate. The total phenolic contents were expressed as milligrams of gallic acid equivalents (mg GAE/g extract).

The data obtained were analysed using ANOVA to see treatment effect on the observed variables and if there was a significant effect, Duncan Multiple Range Test was used to see the difference between treatment means (Steel and Torrie, 1980)

Results

Plant height and crop growth rate: Okra plant height at different row spacing and N compost doses are presented in Table 1. The row spacing and dosage of nitrogen did not have a significant interaction effect. Plant spacing influenced the plant height and N compost also had direct efffect. Duncan multiple range test results showed that the N dosage of compost of 100 and 150 kg N ha^{-1} gave the plant height of 40.9 and 43.7 cm which differed significantly from those without fertilization. The increase in N compost dose increased 4.6 to 34.05 % height compared with that without N appliaction.

The interaction between row spacing and N compost increased CGR. At the row spacing of 50 cm, the increase of CGR 167 reached up to 300 percent while the row spacing of 75 cm produced CGR of 66.6 % higher than row spacing of 50 cm. Fruit yield at row spacing of 75 cm was 36.8 % higher than those with the row spacing of 50 cm. Dose N of 100 kg ha^{-1} significantly increased the yield by 67.1 % compared to that without N, whereas the dosage of 150 kg N was able to increase the yield by 94.3 % compared to that without N.

Okra yield, pod weight and pod girth: The interaction between row spacing and N compost increased fruit weight. At the row spacing of 50 cm, it increased the weight 25.7 to 41.7 % with increased dosage from 50 to 150 kg N ha^{-1}, compared to that without N compost. The highest pod girth occurred at the row spacing of 50 cm, which was 2.06 cm at a compost dose of 150 kg N ha^{-1}. Increased N compost increased pod girth by 2.4 to 8.9 % at row spacing of 50 cm, while at row spacing of 75 cm the increase in pod girth was 8.3 to 21.9 % compared to that without compost.

Nitrate reductase activity, chlorophyll content, protein and antioxidant of okra: The highest NR value in row spacing of 50 cm and the compost dose of 150 kg N ha^{-1} was 1.879 µmol $NO_2.g^{-1}$ which was significantly different from all treatments (Table 2). An increased N compost dosage from 50 to 150 kg N ha^{-1} increased NR 30.5 to 179.6 % compared to that without N with the row spacing of 50 cm (Table 2). An increased N compost dosage from 50 to 150 kg N ha^{-1} increased NR 120.9 to 134.9 %.

Table 1. Plant height, CGR, okra yield, pod weight, pod circle under row spacing and N Compost doses

Treatment	Plant height (cm)	CGR (g/day)	Okra yield (ton/ha)	Pod weight (g)	Pod girth (cm)
Row spacing 50 cm					
N Compost					
0	33.6	0.03 c	6.6	13.2 d	1.68c
50	35.3	0.08 bc	8.1	16.6 cd	1.72 c
100	43.9	0.12 ab	9.8	17.8 d	1.79b
150	49.0	0.12 ab	10.3	18.7 cd	1.83b
Row spacing 75 cm					
N Compost					
0	31.7	0.14 a	7.4	20.3 bcd	1.69c
50	32.9	0.14 a	10.0	22.2 abc	1.83b
100	37.9	0.16 a	13.5	27.6 ab	1.85b
150	38.3	0.15 a	16.8	30.2 a	2.06a
Row spacing 50 cm	40.9 a	0.09a	8.72 b	16.58 b	1.76b
Row spacing 75 cm	35.2 b	0.15 a	11.93 a	25.07 a	1.86a
N Compost					
0	32.6 b	0.085	7.0 b	19.05	1.69c
50	34.1 b	0.11	9.0 b	20.45	1.78b
100	40.9 a	0.14	11.7 a	22.10	1.82b
150	43.7 a	0.14	13.6 a	22.70	1.95a
ANOVA					
Row spacing	*	*	*	*	*
N Compost	*	ns	*	ns	*
Row spacing * N Compost	ns	*	ns	*	*

*Different superscripts on the same columns and rows indicate a significant difference

Row spacing of 50 cm produced 6.8 % higher total chlorophyll than that of 75 cm row spacing. The total chlorophyll of the okra increased by 55 to 138.3 % with the increment of compost N from 50 to 150 kg N ha^{-1}. N dosage had the significant effect on the crude protein content of okra fruit. Doses of N compost (50 to 150 kg N ha^{-1}) increased the levels of crude protein of okra by 6.8 to 20.7 % compared to those without N. The highest antioxidant content of okra pod in row spacing of 50 cm was 167.57 mg. g^{-1} DW at compost dose of 150 kg N ha^{-1}. The increase of N compost decreased the antioxidant concentration of okra fruit by 13.41 to 75.5 % in row spacing of 50 cm while the row spacing of 75 cm decreased the antioxidant level by 4.1 to 56.8 % due to the increased N dose (50 to150 kg N ha^{-1}) compared to that without compost.

Discussion

The height of the okra plant in 50 cm row spacing was higher than that of the row spacing of 75 cm. The overcrowding due to less row spacing causes shading of plants by each other thus resulting more height due to competition for getting light and also inhibits photosynthesis resulting suboptimal crop production (Stephenson *et al.*, 2011). Taller plants with denser population results in lower pod yields. The pod yield was higher in the row spacing of 75 cm. This differs from Kokare *et al.* (2006) reported

Table 2. Nitrate reductase activity, chlorophyll content, protein and antioxidant of okra at row spacing and N Compost doses

Treatment	NR μmol NO_2 g^{-1}	Chlorophyll (mg/g)	Crude Protein (%)	Antioxidant activity (mg/g DW)
Row spacing 50 cm				
N Compost				
0	0.672 c	0.66 a	15.49a	78.73c
50	0.877 c	0.97 a	16.60a	75.53c
100	1.354b	1.10 a	17.97a	44.95d
150	1.879a	1.40 a	19.18a	34.00e
Row spacing 75 cm				
N Compost				
0	0.573c	0.53 a	15.80a	167.57 a
50	1.266b	0.90 a	16.82a	145.10b
100	1.288b	1.10 a	17.51a	92.53 c
150	1.346b	1.47 a	18.56a	41.10 de
Row spacing 50 cm	1.195a	1.03 b	17.30a	105.61 a
Row spacing 75 cm	1.118b	1.10 a	17.13a	61.51 b
N Compost				
0	0.623d	0,60d	15.64d	123.15 a
50	1.072c	0.93c	16.70c	110.32b
100	1.321b	1.10b	17.74b	70.17c
150	1.613a	1.43a	18.87a	37.95d
ANOVA				
Row spacing	*	*	ns	*
N compost	*	*	*	*
Row spacing *N compost	*	ns	ns	*

*Different superscripts in the same column and row indicate significant difference

that the plant height was 88.33 to 117.33 cm, weight per pod was 9.2 to 10.4 g, yield pod ha^{-1} was 125.29 to 138.50 kg. The plant height in the study was 32.6 to 43.7 cm, lower than Adekiya *et al*. (2019) research result that plant height was 40 cm to 61 m with green manure, and the yield was 13.6 tons ha^{-1} that was higher than Adekiya *et al*. (2019) where pod yields were 10.1 to 11.7 tons ha^{-1}. The more doses of N given from the litter compost, the more degradable organic matter will increase the soil N. Nitrogen fertilization helped in increasing vegetative growth of plants such as plant height. At higher dose of N, higher cell division rate results into increased vegetative growth of plants (Firoz, 2009). Nitrogen application of 50-150 kg N ha^{-1} increased the growth and yield of okra linearly (Firoz, 2009). The crop growth rate was affected by organic matter and N dosage (Purbajanti *et al*., 2016). Litter compost can provide sufficient nutrients to improve the metabolism of plants and increase the antioxidant activity in okra. Compost also has the ability to enrich nutrients in the soil and increase water holding capacity so as to improve the water balance in the soil. Mohammadi *et al*. (2012) described that due to close planting, shading of fellow plants over each other, resulted in elongated plant growth because of competition for getting light and inhibited photosynthesis resulting less crop yield.

Competition among plants will not occur when the density of plant population has not reached the threshold as the needs of the plants are met (Maurya *et al*., 2013). Row spacing and N compost affected NR in okra. The highest NR in row spacing of 50 cm and 150 kg N. ha^{-1} was 1.879 μmol $NO_2.g^{-1}$. The higher the N compost dosages, the higher the N will be added to the soil. NR depends on the supply of photosynthates (Wyse, 2014) and water regime (Purbajanti *et al*., 2017). Wtih the higher dosage of litter compost the higher NR was recorded. The row spacing of 50 cm with N litter compost increased the number of okra leaves and better light interception resulting better photosynthesis rate. Nitrate reductase depends on the supply of photosynthates in the form of carbohydrates utilized in the respiration process. According to Chow (2012), the assimilation of nitrate depends on the substrate of organic carbon, reductant, and ATP supplied for photosynthesis and respiratory processes. When nitrogen supply is limited, the photosynthesis and respiration are affected. This dependence is multi-factor in nature which activates compensation or regulatory mechanism. NR catalytic flux is controlled by the substrate availability and the level and activity of functional NR. Nitrate reduction capacity is regulated in relation to the overall plant metabolic level by metabolic sensors and signal transduction pathways. Nitrate reductase at the junction of two energy-consuming pathways, nitrate assimilation, and carbon fixation, results in a controlled response to environmental changes that affect photosynthesis (Baroniya *et al*., 2014). Nitrogen is an important component of plant organs that make up nucleic acids, amino acids, and proteins. Nitrogen is absorbed by the roots and transported into plants in the form of nitrate (NO_3^-), ammonium (NH_4^+) and amino acids. Chlorophyll content at the row spacing of 75 cm (1.10 mg. g^{-1} FW) was higher than that of the row spacing of 50 cm (1.03 mg g^{-1} FW). The highest chlorophyll content was 1.43 mg g^{-1} FW at a dose of 150 kg N ha^{-1}. This value was higher than that obtained by Kokare *et al*. (2006), where leaf chlorophyll of okra was 1.053 to 1.178 mg g^{-1} FW, but it was low as compared to value reported by Nana *et al*. (2014) and William and Qureshi (2015).

Row spacing affected the okra pod girth. It was higher at 75 cm row spacing than at 50 cm. It indicates that the wider row spacing provided better nutrition during reproductive phase. Wider row spacing provides a wider space for plants to grow (Brar and Singh, 2016), and absorb more nutrients so that the resulting okra pod has higher girth.

The weight per pod and the pod girth was influenced by the interaction between row spacing and N compost. This was indicated from the increased weight and girth of pod. Best results were achieved by administering 150 kg of N compost weight (30.2 g weight and a 2.06 cm pod girth). The row spacing of 75 cm could produce fruits with better quality because plants recieved nutrients, moisture and enough sunlight than that with the row spacing of 50 cm. A research by Maurya *et al*. (2013) showed that plants at lower population utilized more photosynthates for fruit development and better quality. Nutritional quality is represented by protein levels and antioxidant levels of okra. The addition of litter compost affected the protein content because of the additional N through litter compost. N applications of 50 to 150 kg N ha^{-1} increased the level of crude okra protein up to 20.6 % compared to those without compost. Levels of okra protein were 16.70 to 18.87 %, which was higher than that reported by Rahman *et al*. (2011), Gemede *et al*. (2015), Adewole and Ilesanmi (2011) (4.47 to 6.7, 2.10 and 7.35 %, respectively). The higher litter compost dosages indued the higher antioxidant content in okra fruit. Row spacing of 75 cm had higher antioxidant content than the row spacing of 50 cm.

Pods and seeds are rich in phenolic compounds with important biological properties such as catechin, oligomers and hydroxycinnamic derivatives (Gemede *et al*., 2015). Antioxidant activity is the ability to prevent auto-oxidation of free radicals mediated by substrate oxidation when it is present in low concentrations. Increased N concentrations also reduce the content of bioactive compounds excluding carotenoids, which interferes antioxidant activity (Núñez-Ramírez *et al*., 2011). Nitrogen fertilization (N) affects pH, soluble solids concentration, titratable acidity, antioxidant activity, vitamin C content and nitrate content (Porto *et al*., 2016). There is an inverse relationship between the availability of N and the concentration of phenolate in plant tissue (Yañez-Mansilla *et al*., 2015). They reported that the plant height, crop growth rate, yield, pod weight and fruit girth increased with increasing doses of compost N.

Present research revealed that the plant height, CGR, yield, pod weight and pod girth increased with increasing doses of compost N. NR increased with increasing dose of N at row spacing of 50 and 75 cm, while antioxidant activity decreased with increasing N dose both at 50 and 75 cm row spacing. Chlorophyll and CP levels increased with the dose of N.

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