International Journal of Agricultural Technology 2023 Vol. 19(6):2639-2650
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Growth and yield response of green mustard under the combination of nitrogen fertilizer and organic amendment in acid soil

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Setyowati, N., Muktamar, Z. and Sentosa, P. (2023). Growth and yield response of green mustard under the combination of nitrogen fertilizer and organic amendment in acid soil. International Journal of Agricultural Technology 19(6):2639-2650.

Abstract The study indicated that the combination of 25% recommended nitrogen synthetic fertilizer and 75% N from chicken manure provided the highest growth and yield of green mustard represented by the shoot height, leaf area, root length, shoot and root weight, and nitrogen uptake. The substitution of 75% N synthetic fertilizer by chicken manure can improve N uptake by green mustard as much as four times higher, and N absorption efficiency increased by almost 1.5 folds. The yield of the fertilizer combination can increase more than 5-fold compared to the recommended N fertilizer and is approximately 80% higher than chicken manure alone. Also, the combination had the highest nitrogen absorption efficiency. The study is significant for sustainable agriculture implementation by reducing up to 75% of nitrogen synthetic fertilizer.

Keywords: Fertilizer combination, Fertilizer substitution, Organic farming, Sustainable agriculture

Introduction

The acidification of soil is a natural process; however, human activities such as excessive use of synthetic fertilizers bring about the degradation of soil fertility. The acceleration of this process increases the excess of aluminum and iron in the soil, leading to toxicity to plants (Herrera and Perez, 2020). The application of urea in acid soil decreases pH and increases the Al saturation of Ultisols, as Wulandari *et al*. (2017) reported. Long-term use of nitrogen fertilizer also reduces the richness of microorganisms (Coolon *et al*., 2013). The main constraints of acid soil such Ultisols are low pH and high Al saturation in soil.

High Al saturation restricts the development of root cells and thickens root

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cell walls, leading to a significant decrease in root growth and root branches (Muktamar *et al*., 1998).

The application of organic fertilizer improves soil fertility. Water hyacinth compost application on Ultisols and Inceptisols increases soil pH, microbial biomass carbon, and total soil carbon and reduces exchangeable Al in soil (Muktamar *et al*., 2017). Another study reported that vermicompost treatment increases pH, organic-C, total N, available P, and exchangeable K of soil (Al-Mamoori *et al*., 2023). However, the main disadvantage of organic fertilizer is the slow release of plant nutrients, leading to lower early plant growth. Significant improvement in nitrogen and phosphorous availability requires two weeks of vermicompost decomposition, as reported by Muktamar *et al.* (2020) and Muktamar *et al*. (2022). On the other hand, this fertilizer has the advantage of having complete nutrient availability and improving soil structure and high moisture content, while the benefit of synthetic fertilizer is quickly releasing nutrients. Adding more soluble fertilizer to organic fertilizer is necessary to ensure nutrient availability for plants.

Nitrogen availability is an essential nutrient, mainly at an early stage of plant development. The combination of synthetic N fertilizer and organic fertilizer is necessary to complement N from organic fertilizer. The substitution of nitrogen from organic fertilizer to synthetic on plant growth and yield has been reported elsewhere (Li *et al*., 2022; Wang *et al*., 2023; Wei *et al*., 2020; Luan *et al*., 2020). However, combining synthetic nitrogen fertilizer with chicken manure and bokashi has yet to be investigated intensively. The study aimed to determine the appropriate combination of synthetic nitrogen with organic fertilizers for the growth and yield of green mustard.

Materials and methods

Experimental site and design

The experimental site was located in Beringin Raya Village, Muara Bangkahulu SubDistrict, City of Bengkulu, approximately 10 meters above sea level. The soil in the area was classified as Ultisols. The study employed a randomized complete block design (RCBD) with three replicates. The treatment was the following:

T_0: Without fertilization (control)
T_1: 100% recommended synthetic N fertilizer without organic fertilizer
T_2: 100% N from chicken manure without synthetic N fertilizer
T_3: 100% N from bokashi without synthetic N fertilizer
T_4: 75% recommended N fertilizer and 25% N from chicken manure

T_5: 50% recommended N fertilizer and 50% N from chicken manure
T_6: 25% recommended N fertilizer and 75% N from chicken manure
T_7: 75% recommended N fertilizer and 25% N from bokashi
T_8: 50% recommended N fertilizer and 50% N from bokashi
T_9: 25% recommended N fertilizer and 75% N from bokashi

Initial soil and organic fertilizer analysis

Initial soil samples were compositely collected using hoe from 5 different sites at 0-20 cm depth. The samples were air-dried at the greenhouse for two days, crushed, sieved with a 0.5 mm screen, and analyzed for total soil organic carbon (TSOC) using the Walky and Black Method, total soil nitrogen (TSN) using the Kyldahl Method, available P with Bray I Method, exchangeable K with Flame-photometry after extraction with 1N Ammonium acetate, and pH at a 1:1 ratio of distilled water and soil. The soil contained 3.7% TSOC, 0.28% TSN, 5.11 mg kg^{-1} available P, 0.26 cmol kg^{-1} exchangeable K, and a pH of 4.52.

Chicken manure and bokashi were gathered from the Agriculture Commercial Store. The organic fertilizer was gently ground, screened with a 0.5 mm sieve, and analyzed for the concentration of C, N, P, and K using wet extraction. The pH was measured at a 1:5 ratio of distilled water and sample. Chicken manure had 27.1% total carbon, 3.50% total N, 0.80% total P, 0.64 % total K, and the pH of 8.04, while bokashi contained 18,17% total carbon, 2.41% total N, 0.38% total P, 0.57% total K and the pH of 7.92.

Experimental procedure

The experimental site was prepared by clearing the land and plowing using a hoe to a depth of 20 cm. Thirty practical bed of 90 cm x 90 cm was established with a spacing of 0.5 m among plots and 1 m among block. Each plot was limed 2 weeks before planting using dolomite at a dose of 239 g $plot^{-1}$, equivalent to 2960 kg ha^{-1}. The lime was homogeneously mixed at 0-10 cm soil depth.

Green mustard seeds were sowed in a plastic seed tray with a media of soil incorporated with compost at a ratio of 1:1 (v/v). A seed was implanted in every hole and gently concealed with media. The seed tray was covered with a black plastic sheet and placed in a wooden rack in the greenhouse. The seed was watered twice a day to maintain the moisture of the media. The tray was uncovered when the seed germinated and placed in direct sunlight. The nursery ended for two weeks after germination. The seedling was transplanted to an experimental plot at a spacing of 20x20 cm. Four samples per experimental unit were allocated randomly at the middle row of the plant a week after transplanting.

Applying organic fertilizer as treatment was carried out during the soil tilt a week before transplanting. Basal fertilization was applied at transplanting using KCl at a dose of 100 kg ha^{-1} and phosphorous fertilizer (SP36) at 150 kg ha^{-1}. The recommended N requirement was calculated from nitrogen uptake by green mustard (224 kg N ha^{-1}). Nitrogen fertilizer was applied twice, 1/3 of the recommended N fertilizer seven days after transplanting (DAP) and the rest at 21 DAT. Nitrogen fertilizer was applied in a row between plants at a 5 cm depth and covered directly with soil to avoid evaporation. Fertilization occurred at approximately 09:00 a.m. During the experiment, the plant was frequently watered in the morning and late afternoon when necessary. Weed was controlled manually, while pest control was performed using a pesticide with an active ingredient of Deltamethrin every two weeks. After 35 DAT, green mustard was harvested by cutting the stem base while the root was excavated using a shovel after wetting the soil. The root was gently cleansed from the dirt.

Experimental variables included shoot height, number of leaves, leaf area, shoot fresh and dry weight, root length, root fresh and dry weight, shoot/root ratio, nitrogen uptake, N absorption Efficiency, and soil pH. Nitrogen uptake was calculated by the multiplication of leaf nitrogen concentration by dry weight (Putra *et al*., 2015), while the N absorption Efficiency was calculated as follows:

$$\text{Eh} = \frac{Sp - Sk}{Hp} \times 100\%$$

Eh = N absorption Efficiency (%), Sp = N uptake by fertilized plant , Sk = N uptake by unfertilized plant and Hp = N fertilization rate (Bhaskoro *et al.,* 2015).

Data analysis

Data were analyzed using ANOVA at a probability level of 5%, and treatment means were compared using LSD 5%.

Results

The result showed that N fertilizer combined with chicken manure of bokashi significantly affected shoot height, number of leaves, leaf area, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, shoot/root ratio, N uptake, and N absorption efficiency as indicated in Table 1.

Table 1. Result of ANOVA for variables of growth and yield of green mustard

Variable	Calculated F	Table F at 5%	Significance
Shoot height	66.71	2.39	*
Number of leaves	21.53	2.39	*
Leaf area	30.84	2.39	*
Shoot fresh weight	4639.66	2.39	*
Shoot dry weight	380.46	2.39	*
Root length	16.23	2.39	*
Root fresh weight	38.65	2.39	*
Root dry weight	414.14	2.39	*
Shoot/Root ratio	17.63	2.39	*
Nitrogen uptake	490.24	2.39	*
Nitrogen absorption efficiency	211.72	2.51	*

Shoot height

Plant height is an indicator of the change of agronomic character. The study showed that the combination of N fertilizer and chicken manure or bokashi significantly influenced the height of green mustard. The treatment of T_6 provided the highest green mustard compared to others. The shoot height of treatment T_8 was not significantly different from T_5, T_9, T_7, and T_2. Treatment T_6 had 55.39% and 66.97% higher mustard than T_1 and T_0, respectively (Table 2).

Root length

Investigation of root length aims to provide information on the capability of the root to absorb water and plant nutrients. It is observed that there was a prominent effect of combined N and organic fertilizer on root length. Treatment T_6 exhibited the longest root and significantly differed from other treatments, while the shortest was treatment T_0. Root length for treatment T_5 did not vary from treatments T_2, T_3, T_4, T_8 and T_9. The root for treatment T_6 was 42.87% and 62.4% longer than treatments T_1 and T_2, respectively (Table 2).

Root weight

The result of the data analysis showed the combination of N fertilizer and organic fertilizer prominently increased root fresh weight (Table 2). The most significant root fresh weight was attained by treatment T_6, while the lowest was by treatment T_0 (control). The root fresh weight of treatment T_1 did not differ from control (T_0), and that of treatment T_2 was similar to T_7, T_8, and T_9. Root fresh weight for T_6 increased by 80.36% and 87.44% compared to T_1 (recommended dose) and T_0 (control).

In root dry weight, treatment T_6 provided the highest dry weight while the lowest was control (T_0). Root dry weight for treatment T_8 was essentially similar to that of T_9. The same pattern was observed in treatments T_3 and T_4. Root dry weight in treatment T_0 (control) and recommended dose (T_1) was 81.34% and 72% lower than treatment T_6, respectively (Table 2)

Table 2. The effect of N fertilizer combined with chicken manure or bokashi on selected growth variables of mustard

Treatment	Selected growth variables			
	Shoot height (cm)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
T_0	15.73 g	7.72 e	0.71 f	0.14 h
T_1	22.62 f	11.73 d	1.11 of	0.21 g
T_2	31.79 d	14.37 bcd	2.7 c	0.39 d
T_3	22.54 f	12.84 cd	1.65 de	0.23 fg
T_4	25.95 e	11.79 d	1.45 e	0.25 f
T_5	34.83 bc	16.04 b	3.47 b	0.58 b
T_6	42.18 a	20.53 a	5.65 a	0.76 a
T_7	31.74 cd	16.00 bc	2.54 c	0.51 c
T_8	35.20 b	12.40 d	2.3 cd	0.35 e
T_9	33.78 bc	15.07 bcd	2.44 c	0.36 e

Note: Numbers followed by the same letter in the same column are not significantly different using LSD 5%., T_0: control (without N), T_1: 100% N synthetic fertilizer (recommended dose), T_2: 100% N chicken manure, T_3: 100% N bokashi, T_4: 75% N + 25% N chicken manure, T_5: 50% N + 50% N chicken manure, T_6: 25% N + 75% N chicken manure, T_7: 75% N + 25% N bokashi, T_8: 50% N + 50% N bokashi and T_9: 25% N + 75% N bokashi.

Leaf number and area

The leaf is vital for photosynthesis and determines light energy absorption and conversion for developing vegetative and generative organs. The study results showed that 25% N fertilizer and 75% N from chicken manure (T_6) had the most significant number of leaves; the fewest number was the control (T_0). However, the number of leaves for treatment T_6 did not differ from T_2, T_5, and T_8. Treatment T_6 increased the number of leaves by 37.76% and 35.59% compared to treatment T_1 and T_0 (Table 3).

In general, an increase in the number of leaves was followed by the expansion of leaf area, leading to the rise in the photosynthesis process, which will improve the development of plant biomass. The study indicated that combining N fertilizer with chicken manure or bokashi substantially increased the leaf area of green mustard. The highest leaf area was obtained in the treatment of T_6, while the least was control. Treatment T_6 had a similar leaf area with T_2, T_7, T_8, and T_9. The leaf area in T_6 was 69.72% and 82.18% higher than in T_1 and T_0 (Table 3).

Shoot weight and shoot/root ratio

The combination of 25% N synthetic fertilizer and 75% N from chicken manure (T_6) was superior in shoot fresh weight to other treatments, as seen in Table 3. Treatment 8 had a similar shoot fresh weight to treatment 2. Treatment T_6 had an 89.7% increase in shoot fresh weight compared to the control, while 85.33% recommended N fertilizer. A similar fashion was observed in the shoot dry weight of green mustard, where T_6 was the greatest compared to other treatments. Shoot dry weight in the treatment T_5 did not significantly differ from T_2. So did T_7 and T_9, T_1 and T_3. The treatment T_6 increased this variable by approximately 81% to T_1 and more than 92% to T_0 (control).

The combination of N fertilizer with chicken manure or bokashi significantly affected the shoot-root ratio of green mustard. The highest shoot-root ratio was provided by treatment T_8, while the lowest was the treatment T_0 (control) (Table 3). The study also resulted in treatment T_1 did not differ from treatments T_3, T_4, T_5, and T_7. The shoot-root ratio in treatment T_1 was 42.36% lower than in treatment T_6.

Table 3. Combining N fertilizer with chicken manure or bokashi affects selected growth and yield components

Treatment	Selected growth and yield components						
	No. of leaf	**Leaf area (cm^2)**	**N uptake (g/plant)**	**Shoot fresh weight (g)**	**Shoot dry weight (g)**	**Shoot-root ratio**	**N absorption efficiency (%)**
T_0	6.33 e	39.35 e	0.012 g	14.72 i	0.56 g	3.91 f	
T_1	6.83 de	66.86 de	0.038 f	20.96 h	1.34 f	6.68 e	2.55 of
T_2	9.66 ab	128.26 bc	0.094 d	80.72 c	3.99 c	10.49 b	2.19 g
T_3	7.33 cde	71.43 d	0.041 f	23.48 g	1.54 f	7.37 e	0.73 h
T_4	7.75 cd	104.65 c	0.051 e	28.30 f	1.87 e	8.05 cde	2.31 fg
T_5	10.25 ab	147.81 b	0.143 b	100.52 b	4.07 c	8.03 cde	5.49 b
T_6	10.83 a	220.77 a	0.206 a	142.87 a	7.03 a	9.51 bcd	6.37 a
T_7	8.16 c	136.01 b	0.072 d	68.63 e	2.98 d	7.66 de	4.92 c
T_8	10.33 ab	144.94 b	0.112 c	82.27 c	4.68 b	14.32 a	4.10 d
T_9	9.91 b	148.93 b	0.098 d	73.08 d	3.26 d	9.98 b	2.75 e

Note: Numbers followed by the same letter in the same column are not significantly different using LSD 5%., T_0: control (without N), T_1: 100% N synthetic fertilizer (recommended dose), T_2: 100% N chicken manure, T_3: 100% N bokashi, T_4: 75% N + 25% N chicken manure, T_5: 50% N + 50% N chicken manure, T_6: 25% N + 75% N chicken manure, T_7: 75% N + 25% N bokashi, T_8: 50% N + 50% N bokashi and T_9: 25% N + 75% N bokashi.

Nitrogen uptake and N absorption efficiency

The combination of N fertilizer and chicken manure or bokashi significantly increased N uptake by green mustard. Table 3 indicated that treatment T_6 had the highest N uptake while the lowest was control. Similar N uptake was observed in T_2, T_7, and T_9; also, in T_1 and T_3. Treatment T_6 enlarged N uptake by 81.29% and 94.09% compared to T_1 and control, respectively. Table 3 also shows that the nitrogen absorption efficiency of green mustard fertilized by 25% N fertilizer combined with 75% N (T_6) from chicken manure was the highest compared to other treatments. The fertilizer combination was approximately 1.50 folds more efficient than the recommended N fertilizer (T_1).

Discussion

The study revealed that the combination of 25% N fertilizer with 75% N from chicken manure (T_6) exhibited the greatest growth and yield of green mustard. Also, green mustard without N fertilization had lower growth and yield than that of N synthetic fertilizer or organic fertilizer. Nitrogen availability is essential for plant growth, mainly the development of chlorophyll. Sufficient N will accelerate plant growth and increase plant protein content (Kogoya *et al.*, 2018).

In general, the growth and yield of green mustard fertilized with N synthetic fertilizer had lower growth and yield than fully N from chicken manure but similar to fully N from bokashi. Treatment T_1 has a lower pH (5.5) than treatment T_2. Synthetic N fertilizer apparently increases soil acidity, mainly exchangeable Al, as Liu *et al*. (2023) reported, causing decreased soil pH (Wang *et al*., 2018). An increase in Al saturation in the soil might cause plant toxicity (Ofoe *et al*., 2023). Lower growth and yield of green mustard fertilized with N synthetic fertilizer might also related to the more soluble of this fertilizer. After solubilization of the N fertilizer, the nitrification process forms nitrate, which is more easily leached out of the soil profile. According to Wang *et al*. (2020), synthetic fertilizer application increased nitrate in the subsoil, indicating nitrate leaching. The denitrification process might also lead to the lower performance of green mustard in 100% N synthetic fertilizer. A study by Mulvaney *et al*. (1997) and Perron *et al*. (2019) confirmed that application of N fertilizer increased denitrification. Likewise, the lower performance of green mustard in treatment T_3 compared to T_2 might be due to the lower N, P, and K content in bokashi than in chicken manure. Greater availability of these nutrients supports the performance of green mustard in T_2.

Overall, 25% N synthetic fertilizer and 75% N from chicken manure (T_6) provided the most significant growth and yield of green mustard compared to control, N synthetic at the recommended dose, organic fertilizer alone, and other combinations. This result is associated with several factors. Firstly, organic fertilizer has a complete nutrient even though the concentration is lower than synthetic fertilizer. Organic fertilizer may contain macro and micro plant nutrients, enzymes, hormones, and humic substances (Muktamar *et al*., 2017; Adhikary, 2012; Ghosh *et al*., 2018). Secondly, the highest performance of green mustard in treatment T_6 is attributed to the content of nutrients in chicken manure. Our result showed that the amendment has a higher content of N, P, and K with a similar C/N ratio to bokashi. A low C/N ratio (approximately 7.5) indicates that organic fertilizers are easily decomposed, releasing plant nutrients. This result is in line with that suggested by Maryam *et al*. (2015), where chicken manure has higher N than other manures.

Thirdly, adding 25% N from synthetic to chicken manure will provide N early in green mustard growth. Nitrogen synthetic fertilizer is easily dissolved in soil solution, and N is instantly available for plants. This available N will compensate for the slowly released N from chicken manure, mainly at an early stage of plant growth. According to Eghball *et al*. (2002), the availability of N is a crucial nutrient for crop productivity, mainly at the immediate stage of plant growth. The N compensation will reduce the weakness of chicken manure, which releases N slowly. A study by Muktamar *et al*. (2022) indicated a significant nitrate increase occurred after four weeks of vermicompost incubation in Inceptisols. Another study also confirmed that available N from compost was significant after two weeks of compost application (Ebid *et al*., 2007). Another possible reason is associated with the texture of chicken manure. This organic fertilizer has a fine texture, whereas bokashi is solid with a high fiber content. The finer texture of chicken manure will accelerate its decomposition, discharging faster nutrients than bokashi. A study by Barokah *et al*. (2017) indicated that chicken manure is more effective in increasing the yield of green mustard.

Nitrogen fertilization, either synthetic or organic fertilizer, significantly increased the shoot-root ratio of green mustard, as indicated in Table 3. This result might be attributable to the limited availability of N in the rhizosphere. Shortage of N will prioritize the plant root growth. According to Ericsson (1995), under sufficient N, the shoot retains a higher proportion of assimilates than the root. Consequently, the shoot-root ratio is higher; however, the opposite is true when nutrient (N) is reduced. Likewise, Marschner *et al*. (1996) suggested that root growth is superior when N and P are deficient.

Nitrogen uptake by green mustard was the highest in treatment T_6, as was its absorption efficiency. This result might be attributed to N behavior in soil. Slow-released N from chicken manure will prevent faster N leaching and denitrification from the soil, so its availability to plants will last longer. Ning *et al*. (2022) confirmed that organic fertilizer slowly released plant nutrients and could substitute for synthetic fertilizer. On the other hand, N from easily soluble N fertilizer will immediately be lost from the rhizosphere due to leaching and denitrification. Nitrogen from urea is immediately leached out after rainfall (Mo *et al*., 2022). In the case of bokashi, this solid organic fertilizer contains lower N than chicken manure and might decompose slower due to the high lignin content.

In summary, the combination of 25% N from synthetic fertilizer and 75% N from chicken manure had the greatest growth and yield of green mustard. This fertilizer combination also provided the highest N uptake and the most efficient N absorption. The result implies that chicken manure can substitute 75% of the N requirement for green mustard production. This study contributes to the implementation of sustainable agriculture.

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(Received: 5 September 2023, Revised: 3 November 2023, Accepted: 14 November 2023)