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Integrated effect of different N-fertilizer rates and bioslurry application on growth and N-use efficiency of okra (*Hibiscus esculentus* L.)

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Abstract: Bioslurry obtained from biogas plants has the potential to reduce the use of expensive chemical fertilizers and increase yields. A field experiment was conducted to investigate the growth response and yield production of okra fertilized with various combinations of bioslurry and nitrogen fertilizer. The experiment was planned according to randomized complete block design with 3 replications. Bioslurry was analyzed for its composition and was applied at the rate of 600 kg ha⁻¹ along with 50%, 75%, and 100% of the recommended dose of N fertilizer for the production of okra. Phosphorus and potassium fertilizers were added according to recommended rates per hectare. Compared to inorganic N alone, the application of bioslurry alongside NPK fertilizers applied at reduced rates significantly increased the okra fruit yield as a consequence of 14%–31% enhanced plant height, 12%–14% additional branches per plant, and 25%–36% more fruits per plant. Moreover, bioslurry improved root length by 13%–45%, which resulted in an increased N uptake by plants and improved N use efficiency. Our results suggest that the application of bioslurry alongside reduced rates of N fertilizer is a viable strategy for the sustainable production of okra, especially under semiarid climatic conditions.

Key words: Biogas waste, effective efficiency, lady finger, organic farming

1. Introduction

Balanced fertilization is one of the most important factors in maximizing the yield potential of various crops. High output from limited resources and intensive farming results in the accelerated use of chemical fertilizers, which pose certain threats to the environment and to humans (Zhu and Chen, 2002). Limitations in the food supply for an ever-increasing population is a major challenge for agricultural researchers (Sáez et al., 2012). In the recent past, intensive use of chemical fertilizer was one of the most suitable tools for getting a higher yield for food security. To get a high yield by limiting the use of chemical fertilizers and supplementing them with organic-based fertilizers is a new concept for sustainable agriculture. Integrated use of fertilizers for high-yielding crops can decrease dependency on chemical fertilizers (Zia et al., 1992).

Application of nitrogen not only increases the growth and fruit yield of crops, but also improves soil characteristics by affecting soil microflora and fauna. The lack of N in soil may lead to poor plant growth due to a decline in soil productive potential and fertility status. Therefore, N is the most essential element of plant nutrition; plants take it up in significant amounts. Sufficient N supply improves cell

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division, foliage production, and photosynthetic activity of the plant, thus producing higher numbers of flowers and fruits (Sharma and Yadav, 1996). Optimal use of N improves dry matter, especially the economic parts of the plant (i.e. flowers and fruits). However, N availability to plants depends on the source, soil type, and environmental conditions, which may affect crop performance. Therefore, a crop's N supply should be synchronized to its demand. N losses are observed in every type of soil and management of such losses should always be a top priority when considering the study of N supply to crops (Salazar et al., 2011).

Knowledge about the integrated use of organic material with chemical fertilizers could enable the development of new agricultural approaches for improving N management and contribute to developing models of sustainable agriculture (Sáez et al., 2012). This approach would provide new information for increasing the N use efficiency (NUE) in plants. It is an excellent factor for estimating the behavior of plants in particular growth environments. NUE provides insights regarding the proper use of fertilizers, which is essential to maintaining productivity, timing, and source of N application. Deficiency of N has a clear effect on crop growth and the production of economic products. Soil organic amendments such as bioslurry and composts are valuable sources of plant nutrients, particularly N (Takahashi et al., 2010). Organic amendments such as bioslurry provide essential nutrition to crops through their decomposition and may also act as soil conditioners (Cameronet al., 2004). Most developing countries are trying to get rid of expensive chemical fertilizers, especially N, by supplementing them with some organic-based sources.

To supplement chemical fertilizers, use of bioslurry as a soil amendment is being considered a high priority in the world because of its positive effect on soil productivity and its safe disposal (Weyman-Kaczmarkowa et al., 2000). Bioslurry is an aerobically digested organic material that is obtained from biogas plants. It is an inexpensive, environmentally friendly, and renewable source of nutrients for plants (Islam et al., 2010). Application of bioslurry improves soil structure and aeration, increases water-holding capacity, and diversifies nutrients for sustainable crop productivity (Zhu and Chen, 2002; Yu et al., 2010). N is the main nutrient in bioslurry. Factors influencing N availability from bioslurry are its inorganic N content, digestion process (aerobic or anaerobic), C:N ratio, pH, the method and timing of application, and physicochemical properties of the soil (Warman and Termeer, 2005). Bioslurry has shown positive effects on restoring soil fertility and on the mobilization and recycling of soil nutrients, which are at alarmingly low levels in Pakistan (Sarwar et al., 2008).

Okra (Hibiscus esculentus L.) is an annual vegetable crop grown throughout the tropical and subtropical parts of the world, either as the sole crop or intercropped (Emuh et al., 2006). It is a chief vegetable crop grown for its immature pod that can be used as a boiled or fried vegetable, or it may be added to salad or soup (Kashif et al., 2010). Okra plays an immense role in the human diet, providing carbohydrates, fats, proteins, vitamins, and minerals (Abd El-Kader et al., 2010). Okra is rich in unsaturated fatty acids, like linoleic acid, that are generally deficient in the human diet (Savello et al., 1980). The edible portion of the pod contains approximately 88% water, 2.1% protein, 0.2% fat, 7.2% carbohydrates, 1.7% fiber, and 0.2% ash (Aykroud, 1963). Despite the high nutritive value of okra, optimum yields and quality have not been attained because of a continued decline in soil fertility and a decreased use of organic amendments (Akanbi et al., 2010).

Much information is available about the use of organic matter for vegetables; however, our main emphasis here is to determine the impact of bioslurry on the NUE, growth, and yield of local okra cultivars. We hypothesized that application of bioslurry to the soil, along with inorganic N fertilizers, would improve NUE in okra and ultimately its growth and yield.

2. Materials and methods

2.1. Bioslurry and soil characteristics

Two-week-old bioslurry from cattle dung in dry form was obtained from already-installed village biogas plants in the vicinity of Faisalabad and was analyzed for physicochemical properties before application (Table 1). At the time of seed bed preparation, a sun-dried and ground form of bioslurry was incorporated into the soil to prevent N loss from a surface application of the bioslurry.

Soil samples were collected at depths of 0-15 cm before sowing and were analyzed for soil physicochemical properties (Table 1). Organic matter in the soil was calculated using the Walkley and Black (1934) method, while organic matter in the bioslurry was determined using the loss of weight on ignition method. By using the procedure of Moodie et al. (1959), a sandy clay loam soil textural class was established with the help of a triangle used in the international textural classification system. Total N was estimated following Gunning and Hibbard's method (Jackson, 1962) of sulfuric acid digestion and distillation of ammonia into 4% boric acid by a micro-Kjeldahl apparatus. P was determined following Olsen's method (Jackson, 1962) and available K was determined using a flame photometer (US Salinity Lab, 1954). Soil pH was determined in a saturated paste (250 g of soil saturated with distilled water) using a pH meter (JENCO Model-671 P). Clear extract of the aforementioned paste was obtained using a vacuum pump. Electrical conductivity was measured from the saturated soil extract using an EC meter (Digital Jenway Conductivity Meter Model 4070). After harvesting, the residual effect of bioslurry on the soil was also estimated, and N, P, K, and organic matter of the soil was determined by following proper procedures as described above.

2.2. Field experiment and layout

A field experiment was conducted at an experimental area of the Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. The experiment was laid down according to a randomized complete block design (RCBD), keeping row-to-row distances at 45 cm and plant-to-plant distances at 20 cm. The dimension of each plot was $6 \times 2 \text{ m}^2$. The treatment plan included the control (without application of fertilizer and bioslurry) (T₁), bioslurry at 600 kg ha⁻¹ (T₂), 100% of the recommended dose of an inorganic N fertilizer (T₃), bioslurry at 600 kg ha⁻¹ plus 50% of the recommended dose of an inorganic N fertilizer (T₅), and

Analysis of bioslurry							
C:N	pН	OM (%)	N (%)	$P_{2}O_{5}(\%)$	K ₂ O (%)		
39.5	7.3	49.9	1.59	1.75	1.32		
Soil analysis	Soil analysis						
Treatments	pН	ОМ	Total N	Ext. P	Ext. K		
		(%)	(%)	(ppm)	(ppm)		
Presowing							
	7.9	0.72	0.06	6.01	312		
Postharvest							
T ₁	7.8	0.69	0.05	6.51	319		
T ₂	7.8	0.91	0.65	6.70	312		
T ₃	7.9	0.70	0.07	6.80	335		
T_4	7.8	0.98	0.08	6.92	325		
T ₅	7.9	0.96	0.09	7.01	390		
T ₆	7.8	0.98	0.11	6.85	345		

Table 1. Characteristics of experimental bioslurry and soil (presowing and postharvesting).

 T_1 = Control (without application of fertilizer and bioslurry), T_2 = bioslurry at 600 kg ha⁻¹, T_3 = 100% of the recommended N dose from an inorganic N fertilizer, T_4 = bioslurry at 600 kg ha⁻¹ plus 50% of the recommended N dose from an inorganic N fertilizer, T_5 = bioslurry at 600 kg ha⁻¹ plus 75% of the recommended N dose from an inorganic N fertilizer, T_6 = bioslurry at 600 kg ha⁻¹ plus 100% of the recommended N dose from an inorganic N fertilizer. All treatments except T_1 also had recommended P and K fertilizers.

bioslurry at 600 kg ha⁻¹ plus 100% of the recommended dose of an inorganic N fertilizer (T_6). All treatments except T_1 (control) contained full recommended doses of P and K. For okra, recommended rates of N, P, and K are 180, 90, and 40 kg ha⁻¹, respectively. Inorganic fertilizers sources applied for N, P, and K were urea, diammonium phosphate, and potassium sulfate, respectively.

Okra seeds were sown in soil plots on wedges amended with the above-described treatments, and canal water (pH 7.86, EC = 0.45 dS m^{-1}) was used for irrigation.

2.3. Plant morphological growth characteristics

Morphological growth performance of the okra plants was assessed at different growth stages based on plant height, number of leaves per plant, tap root length, and leaf area. From each plot, 10 plants were randomly tagged. Data regarding plant height were recorded after 30 and 90 days after sowing (DAS), while shoot girth, number of branches per plant, number of leaves plant, leaf area, fruit fresh weight, and root length from all the plots were recorded at the stage of plant maturity. The fully expanded (third, fourth, and fifth from the shoot) leaves were used for measuring leaf area. Total leaf area was measured with a Delta-T Device Ltd. (UK, 2000), using the Delta-T Digital Image Analysis protocol. Readings were taken as an average of 3 readings for each leaf.

Plant roots, shoots, and fruits were first oven-dried at 105 °C until they achieved a constant weight, then ground to determine N concentration. After grinding, the fruit, shoot, and root material (0.1 g) was digested in a mixture of sulfuric acid and hydrogen peroxide. N concentrations in roots, shoots, and fruit were determined by distillation of ammonia into 4% boric acid by a micro-Kjeldahl apparatus. N uptake by root, stem plus leaves, and fruit were calculated using the following formula:

N uptake= N concentration × respective dry weight.

Different forms of N use efficiencies were calculated by using the following formulae:

DNE $(\alpha/\alpha) =$	Yield in treated plot-Yield in control plot
r ive (g/g) –	Nutrient uptake in treated plot-Nutrient uptake in control plot
ANE $(g/g) =$	Yield in treated plot-Yield in control plot
	Amount of N applied

where PNE is physiological N use efficiency, ANE is agronomic N use efficiency, and ANR is apparent N recovery.

For harvest index, the following formula was applied:

$$HI(\%) = \frac{Fruit yield}{Biological yield} \times 100$$

Benefit cost ratio (Rs) = $\frac{\text{Income generated}}{\text{Total cost occurred}}$

For HI, PNE, and ANE, calculations were made on dry weight basis.

2.4. Statistical procedure

Statistical procedures were applied to analyze the data (Steel et al., 1997) using a RCBD; means were compared using the least significant difference test at a 5% probability level.

3. Results

3.1. Plant growth parameters

Statistically significant ($P \le 0.05$) higher growth performance of okra plants was recorded in plots where bioslurry was applied alongside different rates of N fertilizer as compared to the control and recommended

inorganic N without bioslurry. Stem height and root length of okra differed according to the different treatments. The differences among treatments in terms of stem height were significant in the whole growth period of okra (Table 2). Application of bioslurry without inorganic N fertilizer also showed a significant positive effect on stem height when compared with the control. Stem height, measured at 30 and 90 DAS, was the highest when plants were supplied with bioslurry along with 100% of the recommended dose of an inorganic N fertilizer (T2), followed by the plots receiving bioslurry supplemented with 75% and 50% of the recommended dose of N from inorganic N fertilizer (Table 2). Similarly, maximum root length was consistently observed in the plants treated with 100% of the recommended inorganic N dose alongside bioslurry application (T_6) , as shown in Table 2. Plants in the control (T_1) , bioslurry (T_2) , and 100% recommended inorganic N dose (T₃) treatments showed consistently lower growth compared to other treatments in terms of the whole growth parameters that we have observed.

Similarly significant results were obtained in terms of number of fruits per plant, along with maximum calculated fresh fruit weight per hectare. The highest values were obtained in the treatments receiving higher doses of N alongside bioslurry (Table 3). The maximum number of fruits was found in T_6 , followed by T_5 and T_4 . The actual effect of the application of bioslurry combined with different rates of inorganic N on total dry mass of different

Table 2. Effect of bioslurry on the physical growth parameters of okra in the presence of recommended and reduced inorganic N fertilization under field conditions. Each value is the mean of 4 replicates.

Treatments	Shoot length (cm)		Root length	Stem girth	Branches	Leaves	Leaf area
	30 DAS	90 DAS	(cm)	(cm)	plant ⁻¹	plant ⁻¹	(cm)
T ₁	31 d	52 e	21 d	1.30 c	3.8 c	7.3 c	685.4 e
T ₂	42 c	78 d	27 с	1.39 c	4.0 c	12.7 b	733.3 d
T ₃	51 b	116 c	29 bc	1.72 b	5.0 b	13.5 b	1117.1 c
T_4	68 a	132 b	33 b	2.52 a	7.3 a	14.2 ab	1288.5 a
T ₅	69 a	135 ab	31 b	2.42 a	7.3 a	15.1 a	1206.2 b
T ₆	71 a	140 a	38 a	1.78 b	4.7 b	15.5 a	1202.1 b

Note: Mean values with different letters in the same column differ significantly ($P \le 0.05$).

 T_1 = Control (without application of fertilizer and bioslurry), T_2 = bioslurry at 600 kg ha⁻¹, T_3 = 100% of the recommended N dose from an inorganic N fertilizer, T_4 = bioslurry at 600 kg ha⁻¹ plus 50% of the recommended N dose from an inorganic N fertilizer, T_5 = bioslurry at 600 kg ha⁻¹ plus 75% of the recommended N dose from an inorganic N fertilizer, T_6 = bioslurry at 600 kg ha⁻¹ plus 100% of the recommended N dose from an inorganic N fertilizer. All treatments except T_1 also had recommended P and K fertilizers.

yield components of okra was estimated. Maximum physiological growth in terms of total dry mass, shoot dry mass, and root dry mass was observed in the treatment where maximum N was applied along with bioslurry (T_6). All treatments significantly differed from one another for dry matter in different yield components (Figure 1). Although compared to the control growth performance of okra was improved by either recommended inorganic N (T_3) or bioslurry (T_2) alone, their performances were not as good as those of the rest of the treatments.

3.2. N uptake and N use efficiency

Application of inorganic N alongside bioslurry significantly improved N uptake by plants, roots, and fruits and stems in conjunction with leaves (Figure 2). N content in roots was almost the same in all treatments; however, fruits showed a significant increase in N uptake on application of bioslurry along with inorganic N, as compared to the application of either inorganic N or bioslurry on their own (Figure 2). Compared to N- and bioslurry-only treatments, shoot and total plant N uptake was almost doubled when

Table 3. The effect of bioslurry on fruit yield and economic feasibility of organic farming for okra production in the presence of recommended and reduced inorganic N fertilization under field conditions. Each value is the mean of 4 replicates.

Treatments	No. of fruits per plant	Fresh fruit weight (t ha ⁻¹)	Harvest index (%)	Cost-to-benefit ratio
Control (T ₁)	3.2 d	3.77 d	11.9 с	0.58 e
BS* (T ₂)	3.4 d	4.63 d	12.4 c	1.18 d
N** (100%) (T ₃)	5.9 c	8.39 c	16.0 ab	3.64 c
$BS + N (50\%) (T_4)$	8.6 b	11.69 b	16.3 a	6.38 a
BS + N (75%) (T_5)	9.0 b	11.09 b	15.6 b	5.86 b
$BS + N (100\%) (T_6)$	10.9 a	14.39 a	15.5 b	6.30 a

Note: Mean values with different superscript letters in the same column differ significantly ($P \le 0.05$). *: Bioslurry, **: inorganic N.

Note: The control lacked the application of fertilizers and bioslurry. All treatments except the control had recommended P and K fertilizers.



Figure 1. The effect of bioslurry on the dry matter content of various parts of okra in the presence of recommended and reduced inorganic N fertilization under field conditions. Note: all treatments contained N and P fertilizers, except the control.

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Figure 2. The effect of bioslurry on N uptake by various parts of okra in the presence of recommended and reduced inorganic N fertilization under field conditions. Note: all treatments contain N and P fertilizers, except the control.

bioslurry was applied to the soil, even with lesser amounts of inorganic N.

Three types of NUE were calculated (Table 4) for exploring the N use effectiveness of the combined application of organic and inorganic sources of N. Maximum ANE was observed where only bioslurry was applied. Minimum ANE (5.66 g g⁻¹) was observed where inorganic N fertilizers was used; however, it improved from 12.19 to 16.43 g g⁻¹ when different rates of inorganic N was applied alongside bioslurry (Table 1). The best ANR (393.4%) was observed with the application of bioslurry without inorganic N (Table 4). However, application of inorganic N on its own failed to recover N added into the soil; as a result, the application of 100% of the recommended dose of inorganic N on its own resulted in minimum ANR (30.2%). However, it improved from 30.2% to 158.5% with combined applications of bioslurry and inorganic N.

Table 4. The effect of bioslurry on different forms of okra N use efficiency in the presence of rec	ommended
and reduced inorganic N fertilization under field conditions. Each value is the mean of 4 re	plicates.

Treatments	Agronomic use efficiency (g g ⁻¹)	Apparent N recovery efficiency (%)	Physiological N use efficiency (g g ⁻¹)
Control	-	-	-
BS*	22.61 a	393.4 a	5.75 d
N** (100%)	5.66 d	30.2 e	18.74 a
BS + N (50%)	16.43 b	158.5 b	10.37 c
BS + N (75%)	12.19 c	112.4 c	10.84 c
BS + N (100%)	12.45 c	90.9 d	13.69 b

Note: Mean values with different superscript letters in the same column differ significantly (P \leq 0.05). *: Bioslurry, **: inorganic N.

Note: The control had neither fertilizers nor bioslurry. All treatments except the control had recommended P and K fertilizers.

3.3. Residual effect of bioslurry

Although a clear picture of the residual effects of bioslurry application cannot emerge after a single season of study, increased nutrient and organic matter concentrations in the soil showed a positive tendency regarding the residual effects of bioslurry application (Table 1). Application of a mineral fertilizer along with bioslurry has demonstrated a positive contribution on the availability of N, P, and K content in soil. Although an increasing trend in the content of organic matter and N concentration in the soil after harvesting okra crops has been observed, long-term observation is required to determine any noticeable change. This was too short a period to notice the changes in the soil properties brought about by the application of bioslurry. Still, a postharvest increase in the concentration of extractable P and K is quite encouraging.

4. Discussion

Our results showed that the application of bioslurry alongside different percentages of recommended doses of inorganic N sources, as compared to 100% of the recommended dose of inorganic N alone (T₂), significantly improved the physical parameters of plant growth (i.e. stem height, stem girth, number of branches and leaves per plant, and leaf area) (Table 2). Total dry matter yield (root, shoot, and fruit) subsequently increased with the increment of higher doses of inorganic N when combined with bioslurry. However, this increment was due to increased shoot growth, whereas the fruit yield showed insignificant behavior in treatments when bioslurry was used in combination with chemical N fertilizer (Figure 1). More fruits per plant and a higher fruit weight increased the HI. Use of bioslurry alongside the N fertilizer significantly improved HI as compared to the control and the plots without bioslurry treatment; however, the HI was highest in the plot fertilized with 50% of the recommended N along with bioslurry (Table 3). Improved HI might be due to higher N utilization in the root environment because of the modification of soil characteristics and decreased N losses that resulted in better fruit sets and translocation of assimilates to developing pods. These results were found to be in agreement with those of Akanbi et al. (2010). Moreover, the benefit-to-cost ratio was also the highest when bioslurry was applied with half of the recommended N dosage, due to economical use of available N under the presented conditions (Table 3).

Changes in soil nutrient reserves and alterations in root systems under different sources of N supply, including organic sources, might have a direct bearing on the nutrient availability and uptake by crops. Use of bioslurry and chemical fertilizer together are known to spark changes in soil organic matter, nutrient concentrations, bulk density, water-holding capacity, and soil temperature, among others, and ultimately increase NUE (Akanbi et al., 2010). Better N use might be due to a reduction in nitrification and denitrification losses, coupled with favorable growth in the rhizosphere for roots to uptake more N available in the rhizosphere, culminating in more dry matter production and ultimately amplified fruit yield per plant and better root growth. According to Baligar et al. (2007), improved root morphological factors such as length, thickness, surface area, and volume have profound effects on a plant's ability to acquire and absorb nutrients in soil. Mamman et al. (2007) also proved that these parameters influence the ability of roots to penetrate high-density soil layers, to withstand temperature and moisture extremes, and to tolerate toxicities and deficiencies of elements.

For the most part, the conversion of applied N into nitrate (NO₃) and ammonia (NH₃) involves 2 enzymecatalyzed reactions occurring in roots and leaves. Both reactions occur in series so that toxic nitrite (NO₂) may not accumulate in the cell (Yokoyama and Ohama, 2005; Yu et al., 2010). The NH, produced in this reaction is assimilated into amino acids that are subsequently combined into proteins and nucleic acids (Okumoto and Pilot, 2011). Proteins provide a framework for chloroplast, mitochondria, and other structures in which most biochemical reactions occur. An adequate supply of N is associated with high photosynthetic activity, vigorous vegetative growth, and a dark green color (Yu et al., 2010), whereas an excessive amount of N in relation to other nutrients, such as P, K, and S, can delay crop maturity (Gaio-Oliveira, 2005) and render the plant susceptible to many diseases that may reduce HI. If N is used properly in conjugation with needed inputs, it greatly improves crop productivity. Application of N to soil for optimal and economical plant growth is very tricky. N is needed in higher amounts for maximum plant growth; however, the application of full doses of N fertilizer to the soil is not economical because of its volatilization as NH₃ and leaching as nitrate NO₃ (Yokoyama and Ohama, 2005). Therefore, it is always recommended to apply N fertilizer in small installments along with an organic recommendation because it provides conditions conducive to improving the efficiency of N fertilization. Tuyishime et al. (2011) demonstrated that the addition of bioslurry along with different levels of N improves soil nutrient status, physical conditions, and soil microbial community, which enhances N mineralization and reduces N losses. In previous studies it was found that application of organic matter along with different rates of fertilizers significantly increased the NUE of plants (Masarirambi et al., 2010). The results obtained in this study show a great potential in managing N utilization in plants through the use of bioslurry as a soil conditioner, along with reduced levels of recommended N. The influence will, however, depend on the soil type, form,

amount of bioslurry added, and environmental conditions. Increased organic matter, P_2O_5 , N, and K_2O concentrations in the soil (Table 1) will have a positive impact on plant growth for future crops; these results are also found to be in alignment with those of Caravaca et al. (1999).

In conclusion, the application of bioslurry alongside a chemical N fertilizer resulted in an increased N content in different plant parts and ultimately in total N uptake as compared to control plants and plants applied with 100% of the recommended dose of a chemical N fertilizer. The increased N uptake ultimately boosted NUE, showing more assimilation of N in various portions of plants treated with bioslurry in the presence of 50% of recommended chemical N fertilizer. Application of bioslurry alongside different rates of inorganic N improved the plant growth

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and fruit yield of okra. Thus, the application of bioslurry alongside N fertilization at 50% of the recommended inorganic N dose is suggested for a maximized and economical okra fruit yield. Further studies are required to understand the effects of bioslurry application on soil structure, soil organisms, and the biochemical changes in plants. The development of methods for the application of bioslurry to fields is worth investigating.

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