



Management of Root-knot Disease in Okra with Poultry Manure and Leaf Extracts of *Senna alata*

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Authors' contributions

This work was carried out in collaboration among all authors. Author DOE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IAU and AEU managed the analyses of the study. Author DOE also managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This research was conducted with the aim of evaluating the nematicidal effect of aqueous leaf extracts of *Senna alata*, and poultry manure against *Meloidogyne incognita* on okra (cv. Clemson spineless). The concentration of *S. alata* leaf extract (0.20, 0.40, 0.60, 0.80 g/ml) and a control (0.00 g/ml) were combined with poultry manure (0.00 t/ha) being the control and (15.00, 20.00 t/ha,) applied before planting of okra seeds. Each okra plant was inoculated with 5,000 larvae of *M. incognita*. The plants were grown to fruiting stage. The results indicated that application rate of the poultry manure at 15.00 t/ha and 20.00 t/ha and leaf extract of *S. alata* concentration of 0.60 and 0.80 g/ml significantly ($p < 0.05$) reduced galling relative to their respective controls. The susceptibility status of the okra cultivar was changed from highly susceptible to resistant in all plants treated with the combination of 0.80 g/ml of leaf extract and 20 t /ha poultry manure. Also, application of the leaf extract alone changed the gall index from 5.00 to 2.00. The best interaction was observed at the application rate of poultry manure at 20 t/ha with 0.60 g/ml leaf extract, where the resistant status was reduced from susceptible (GI=5.00) to resistant (GI=2.25). However, the

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amended okra plant and application of leaf extract concentration significantly enhanced plant growth, number of leaves, shoot dry weight and root fresh weight accumulation. When 0.60 g/ml of the plant extract was combined with 20 t/ha of the poultry manure, growth and yield of okra plant were highest. The *S. alata* leaf extract at 0.80 g/ml was phytotoxic as the okra plant showed reduction in growth attributes and pod yield. Therefore, this eco-friendly approach in the management of root –knot disease of okra could be adopted after proper identification of the nematicidal constituents of the leaf extract of *S. alata* and field trials.

Keywords: *Senna alata*; *Meloidogyne incognita*; okra; poultry manure; management and botanicals.

1. INTRODUCTION

Okra, *Abelmoschus esculentus* is an economically important vegetable crop that belongs to the genus *Abelmoschus*, family; *Malvaceae*. It originated probably from East Africa and today is widely distributed in the tropics and sub-tropical regions of the world [1]. Okra is grown for its edible green seed pods that serve as soup thickeners [2]. Commercially, it is cultivated in India, Turkey, Iran, West Africa, Bangladesh, Brazil and southern United State [3] with India as the largest producer covering an area of 179,233 million hectares and production rate of about 345 million tonnes [4]. Okro provides an important source of vitamins, calcium, potassium, carbohydrate and protein in large quantities which are important in disease prevention [5, 6]. The essential amino acids that okra contains are comparable to that of soybean making it a crucial part of the human diet. The plant has immense medical and industrial values [7]. Alcohol extract from the leaves can eliminate oxygen free radicals, alleviate renal tubular-interstitial disease, improve renal function and reduce proteinuria [8]. It has been reported to prevent cancer and heart disease [6] as well as mucilage in mid-wifery [9]. Okro helps in lowering the blood sugar level by blocking the absorption of sugar in the intestinal tract. Okra fruits are eaten to treat involuntary discharge of semen [7].

The cultivation of okra is limited by both biotic and abiotic stress factors. Poor yield of okra in Nigeria has been attributed in part to root knot disease caused by *Meloidogyne spp.* The major limiting factor for its cultivation is the incidence of okra yellow vein mosaic virus [OYVMV] which is transmitted by white fly. Fruit are mostly yellow, small, tough and fibrous once infected by this virus. Also, a number of insect pests like *Earias vittela*, *Helicoverpa armigera*, *Thrips tabaci*, *Alphes gossypie*, *H. armigera* and *Bamisia tabaci* are the notorious and major insect pests of okra [10,11]. In order to improve the performance and yield of okra, there is need to find ways to combat these emerging pests. Although,

chemical control of nematode pest remains the most effective control measure but there some serious constraints. Chemical nematicides are very toxic to mammals and beneficial soil microfauna, pollute groundwater and have residual effect on farm produce [12]. The use of plant extracts and antagonistic microorganisms as a component of integrated nematode management is fast gaining wide acceptance [13].

A number of approaches aimed at controlling root knot nematodes through application of nematicides [14,15], organic soil amendments [16-18], cultural management, physical and biological measures [12,19-23] have been advocated. The chemical control of this nematode is very expensive and also not desirable because chemical nematicides may adversely affect the agro-system and also has detrimental effects on numerous beneficial or non-target microbes in the soil. Presently, researchers have diverted their attention to manage plant nematodes through the use of organic amendments [12,16-18,22] and to develop integrated approaches against the pest because often, any single approach may not be effective to manage the plant nematode appropriately. On this note, the present study evaluates the effect of poultry manure and leaf extracts of *Senna alata* on root-knot disease on okra.

2. MATERIALS AND METHODS

2.1 Experimental Site and Source of Material

The experiment was conducted in the Screenhouse of the Faculty of Agriculture, University of Calabar. Okra variety (cv. Clemson spineless) susceptible to root knot nematode was used in this research. The seed was obtained from National Institute for Horticultural Research (NIHORT) Ibadan, Oyo state. Poultry manure was obtained from Animal Science Farm, Calabar Teaching and Research Farm.

Senna alata was sourced from a fallow land at Eburutu Barrack, Ikot Ansa, Calabar.

2.2 Building up of Nematode Population/ Inoculum Preparation

A pure stock culture of *M. incognita* was multiplied on susceptible water leaf plants in the Screenhouse in a steam-sterilized sandy loam soil. The nematode population was considerably built up at full maturity of the water leaf plants, the plants were slightly watered, this was to soften the soil and thus allow for easy uprooting of the plants. The shoot system above the heavily galled root system was cut off and galled root properly washed with water to remove adhering soil particles. The gall root was then placed in moisten polythene bag and taken to the laboratory inoculation preparation. Heavily galled roots of the water leaf plants were uprooted 8 weeks after planting and washed clean with tap water. The galled roots were cut into 1-2 cm segments for larvae extraction. The galled roots were then placed in a blender and distilled water added, blended at 5 seconds interval. The blended material was emptied into 1000 ml beaker and more water added to it and stirred. 30 ml of the suspension was poured into a nematode counting dish and the number of the larvae was counted with the use of a stereomicroscope. An average of such five counts gave approximately 5000 second stage juveniles per 30 ml of solution which was the inoculum density required [24].

2.3 Preparation of *Senna alata* Leaf Extract

Fresh green leaves of *Senna alata* were harvested from its natural habitat and thoroughly washed, chopped into pieces and then ground into a paste. The ground leaf was weighed as 200 g, 400 g, 600 g and 800 g into separate plastic buckets. 1000 ml of distilled water was added to each container and allowed to stand for 24 hours. It was then filtered through a doubled fold muslin cloth. Thus, the filtrate had concentration of 0.20, 0.40, 0.60 and 0.80 g/ml, respectively.

2.4 Soil Analysis for Physiochemical Properties and Pre-Plant Nematode Density

The composite soil sample (0-15cm) depth was subjected to routine soil analysis and also nematode population density was examined in the Soil Science Teaching and Research

Laboratory, Department of Soil Science University of Calabar.

2.5 Preparation of Poultry Manure

Poultry manure was prepared by air drying in the shade for two weeks before application. The poultry manure was applied at the rate of 15 and 20 t/ha, that is 25 g and 34 g per pot, respectively, two weeks before planting. Soil with no amendment of poultry manure served as the control (0 t/ha).

2.6 Application of Treatment

Surface soil (0-15 cm) was collected from a fallow land in the Crop Science Teaching and Research Farm. 200 g of the composite soil sample was analysed for pre-plant nematode density using the methods of Coyne et al. [25]. Sixty plastic pots with diameter 15 cm and depth 25 cm (4 litres capacity) perforated at the bottom were filled each with 3.0 kg of unsterilized top soil and seeds of okra were planted in each pot.

Each seedling was inoculated with 5,000 larvae of *M. incognita* by pouring 30 ml of the prepared inoculum into the three holes made around each stand. Inoculation took place 2 weeks after plant emergence. The application of plant extract took place simultaneously with inoculation of plant with *M. incognita*. *Senna alata* leaf extract was applied at the rate of 30 ml per pot for each concentration. Pots where *S. alata* leaf extract was not applied served as control. A total of 60 pots were used for this study. The pots were perforated at the bottom to allow for drainage and were labelled according to the poultry manure and *Senna alata* treatment combination. The nematode inoculation was done at two weeks after emergence of crop in the early hours of the day. A planting hole was made at the centre of each pot, 30 ml of the inoculum was poured into the hole. All the 60 pots were inoculated with nematode inoculum.

2.7 Experimental Design

The experimental design was laid out as a 3x5 factorial in a completely randomized design (CRD) with four replications. The three rates of poultry manure application (0, 15, 20 t/ha) were combined in a factorial fashion with the five concentrations of *Senna alata* leaf extract (0.00, 0.20, 0.40, 0.60 and 0.80 g/ml) to give 15 treatment combination and 60 experimental units.

2.8 Data Collection

Data on plant height and number of leaves were collected every two weeks after planting for 8 weeks. At maturity, the following data were collected: number of galls per root system, fresh weight of the root, fresh weight of shoot, dry weight of root, dry weight of shoot. Fresh weight of the root was obtained by uprooting the plants after slightly watering the pot to soften the soil thus aiding uprooting. The adhering soil particles were thoroughly washed with water and properly separated based on treatment. A sharp knife was used to separate the shoot from the root and their respective weight taken with a weighing balance. The number of galls per root system was determined with the aid of a hand lens. Gall index was obtained according to [26], using the following scale;

0= zero gall,
 1=1 or 2 galls
 2=3-10 galls.
 3= 11-30 galls
 4=30-100 galls
 5= more than 100 galls per root system.

The dry weight of the shoot and root were obtained using the weighing balance after oven drying in a hot air oven at a temperature of 70°C for 48 hours.

2.9 Statistical Analysis

A one way analysis of variance (ANOVA) was used to test the significance of the treatments. Treatment means were compared using Fisher's Least Significant Differences (F-LSD) at 5% level of probability. All statistical analysis were performed with GenStat software, 8th edition.

3. RESULTS

3.1 Effects of Poultry Manure and *Senna alata* Extracts on Number of Galls/Root System and Gall Index

Table 1 show the effects of different concentrations of *S. alata* leaf extract and soil amendment with poultry manure (PM) on number of galls per root system and gall index (GI) of okra plants inoculated with *M. incognita*. Successive increase in the concentration of *S. alata* leaf extract significantly ($P < 0.05$) inhibited root galling of okra. Similarly, successive

increase in the rate of amendment of soil with poultry manure led to a significant ($P < 0.05$) decrease in root galling. The interaction between *S. alata* leaf extract and poultry manure amendment was significant. The least number of galls was obtained when soil was amended with 20 t/ha PM and 0.80 g/ml of *S. alata* leaf extract. The result on gall index showed that the Okra variety used in this trial was highly susceptible with a GI of 5.00 with no treatment applied (Table 1. and Fig. 1a). There was a significant ($P < 0.05$) decrease in gall index with increase in the rate of poultry manure amendment. Plants amended with 20t/ha PM were rated moderately susceptible (G.I= 3.25) compared with the unamended control with GI = 4.30, rated susceptible. The interaction between poultry manure and plant extract was significant. The lowest gall rating (GI = 2.25), with plants rated resistant was obtained when soil was amended with 20 t/ha PM and with application of 0.80 g/ml *S.alata* leaf extract (Table 1. and Fig. 1b).



Fig. 1a. Heavily galled roots of okra not treated (P0S0)

P0 - unamended with poultry manure
 S0 - 0.00 g/ml (control) leaf extract of *S. Alata*

3.2 Effects of Poultry Manure and Plant Extract of *Senna alata* on Plants Height (cm) at 4 and 6 WAP

Table 2 shows the effects of different concentrations of *S. alata* leaf extract and poultry manure amendment on plant height of okra at 4 and 6 weeks after planting (WAP). Successive increase in the concentration of *S. alata* leaf extract up to 0.60 g/ml significantly increased the growth of okra plant at both period of growth. However, there was no significant difference ($p > 0.05$) between plants treated with 0.20 g/ml and 0.80 g/ml of *S. alata* extract at 4 and 6 WAP. Similarly, successive increase in the level of poultry manure amendment significantly ($P < 0.05$) enhanced the growth of okra plants. For both period of growth evaluated, the interaction between *S. alata* leaf extract and

poultry manure amendment was significant. The combination of 0.60 g/ml of *S. alata* leaf extract and 20 t/ha PM produced significantly ($P < 0.05$) the tallest plants.



Fig. 1b. Very few galls on okra roots treated with P₂S₄

P₂ - 15 t/ha application rate of poultry manure
S₄ - 0.80 g/ml leaf extract of *S. alata*

3.3 Effects of Poultry Manure and Plant Extract of *S. alata* on Number of Leaves Per Plant at 4 and 6 WAP

Table 3 shows the effects of poultry manure and plant extract of *S. alata* on okra plant inoculated with *M. incognita*. Successive increase in the concentration of *S. alata* up to 0.60 g/ml significantly increased ($P < 0.05$) the number of leaves produced by okra plants. The highest number of leaves were obtained when 0.60 g/ml of *S. alata* was applied. However, at 0.80 g/ml, there was decreased in the number of leaves produced by the okra plant. Similarly, for both period of sampling, successive increase in the level of poultry manure amendment significantly ($P < 0.05$) increased the production of leaves by okra plant. The interaction between plant extract and poultry manure amendment was significantly ($P < 0.05$).

3.4 Effect of Poultry Manure and Plant Extract of *Senna alata* on Fresh Root Weight and Dry Weight of Shoot of Okra

Table 4 presents the result of the leaf extract of *S. alata* and poultry manure on fresh root weight and dry shoot weight of okra infected with *M. incognita*. Increase in plant extract concentration significantly ($p < 0.05$) increased the weight of fresh root and dry shoot of okra. Plant drenched with 0.60 g/ml of the plant extract concentration had the highest weight. However, above 0.60 g/ml of the leaf extract, there was a significant

decrease in the weight of fresh root and dry weight content of the dry shoot. Increased in the level of poultry manure amendment significantly ($p < 0.05$) enhanced the fresh root and the dry weight content of the okra shoot. The interaction between plant extract and poultry manure amendment was significant ($p < 0.05$) for both fresh root and dry shoot weight.

3.5 Effect of Poultry Manure and Plant Extract of *S. alata* on Number of Pod and Weight of Fresh Pod of Okra

Table 5 shows the effect of poultry manure and plant extract of *S. alata* on number of pod and weight of fresh pod of okra inoculated with *M. incognita*. Increase in the concentration of *S. alata* to 0.60 g/ml significantly ($p < 0.05$) increased the number of pods produced. At 0.80 g/ml of the leaf extract, there was significant inhibition in the number of pods produced by okra plant. Successive increase in the level of poultry manure led to a significant increase ($p < 0.05$) in pod yield of okra and number of pods produced. The interaction between the plant extract concentration and poultry manure was significant ($p < 0.05$). Amendment of soil with 20 t/ha poultry manure and application of 0.60 g/ml of *S. alata* leaf extract significantly ($p < 0.05$) produced the highest yield of okra fruit.

4. DISCUSSION

The result obtained showed that the application rate of the poultry manure and leaf extract of *S. alata* differed significantly in their efficacy on gall suppression as compared to the control with no treatment application. There was improvement in the yield of okra following the poultry manure application. These results agreed with the report of some earlier investigators [12,27,28]. The organic amendment could have helped in the stimulation of biological activities that suppressed the nematodes. Significant difference in the time of application and the rate of application of poultry manure in the suppression of root galling and egg mass production by root-knot nematode was earlier reported [13]. Khan et al. [23] reported that phytochemicals possess strong nematicidal effects and can be used effectively in an integrated disease management program against root knot nematodes. Therefore, the phytochemicals in extracts of *S. alata* may have contributed in suppressing the nematodes and improving okra yield in the present study. Okra plants planted in soil amended with 20 t/ha poultry manure had fewer galls and the

susceptibility rating was reduced from highly susceptible to resistant. The poultry manure could have decomposed in the soil and released some nematicidal compounds like ammonia, nitric acid, organic acids, that may have adversely affected the development of nematodes and improved plant growth and tolerance to nematodes [27,29,30]. In another way, the poultry manure may have released basic nutrients needed to support a faster growth of the okra plants at a rate that the nematodes strength could not match. Faruk [12] reported a reduced gall index in onion plants following application of poultry manure which corroborates

the present findings. Notwithstanding, multiple mechanisms may operate simultaneously to retard the strength of the nematodes while improving okra yield [30].

The treatment of plant with the leaf extract of *S. alata* significantly reduced root galling by the nematode species. The highest concentration of extract inhibited galling most. The plant *S. alata* contains some antimicrobial compounds with wide application in ethnomedcines as documented by Newwinger [31]. The antimicrobial properties of the leaf extract played a very crucial role in reducing nematode effect on

Table 1. Effects of poultry manure and plant extract of *Senna alata* on number of galls/root system and gall index of okra inoculated with *M. incognita*

Poultry manure t/ha	Number of galls/root system <i>S. alata</i> concentration (g/ml)					Mean
	0.00	0.20	0.40	0.60	0.80	
0.00	138.50	109.25	85.50	58.00	34.00	85.00
15.00	92.50	74.75	50.75	38.75	20.25	55.40
20.00	61.50	46.75	24.50	16.50	9.50	31.75
Mean	97.50	76.92	53.58	37.75	21.22	
Gall index*						
0.00	5.00	5.00	4.00	4.00	3.50	4.30
15.00	4.00	4.00	4.00	4.00	3.00	3.80
20.00	4.00	4.00	3.00	3.00	2.25	3.25
Mean	4.33	4.33	3.67	3.67	2.92	
					Number of galls	G.I
F-LSD (0.05) for comparing <i>S. alata</i> (s) mean:					4.20	0.16
F-LSD (0.05) for comparing poultry manure (PM) mean:					3.25	0.13
F-LSD (0.05) for comparing (SXPM) interaction mean:					7.27	0.28

*0 = Immune 1 = highly resistance 2 = Resistance 3 = moderately susceptible
4 = susceptible 5 = highly susceptible

Table 2. Effects of poultry manure and plant extract of *Senna alata* on plant height (cm) at 4WAP and on 6WAP on okra infected with *M. incognita*

Poultry manure t/ha	4WAP (cm) <i>S. alata</i> concentration (g/ml)					Mean
	0.00	0.20	0.40	0.60	0.80	
0.00	18.38	19.75	22.00	23.25	20.88	20.85
15.00	25.88	29.00	32.38	33.75	30.27	30.20
20.00	30.03	33.75	34.62	37.12	30.27	30.25
Mean	24.76	27.50	29.67	31.37	27.80	
6WAP						
0.00	22.00	23.50	26.50	28.25	23.00	24.65
15.00	27.50	30.90	34.13	36.00	32.75	32.26
20.00	32.20	36.25	37.25	40.00	34.25	35.99
Mean	27.23	30.22	32.62	34.75	30.00	
					4WAP	6WAP
F-LSD (0.05) for comparing <i>S. alata</i> (s) mean:					0.90	1.07
F-LSD (0.05) for comparing poultry manure mean:					0.69	0.83
F-LSD (0.05) for comparing (SXPM) interaction mean:					1.55	1.8

the okra plants. Higher plants as source of bioactive compounds continue to play a dominant role in the maintenance of human health and also management of disease in plant. Reports available on green plant represent a reservoir of effective chemotherapeutants, they are non –phytotoxic, more systemic and biodegradable [13,23,32,33]. The secondary metabolite is involved in the defence of the plant. It contains chrysophanic acid and anthroquinin, tanins, steroids, alkaloids which are all describe as phytochemicals [34]. The above bioactive compounds earlier submitted by authors are suggestive of the potential of *S. alata* leaf extract to reduce the activities of nematodes and support okra yield as observed in this study.

The interaction between leaf extract of *S. alata* and organic soil amendment with poultry manure in suppressing gall formation was significant. It could be said that, the efficacy of the two control agents used in combination increased the growth and production of the okra plants compared with individual application. *S. alata* concentration at 0.80 g/ml consistently showed significant decreased in growth and the yield of okra suggesting possible phytotoxicity. Generally, it has been established that root galling in plants renders the plant inefficient of water and nutrient absorption and subsequent translocation, which inversely reduced leaf production thereby inhibiting the plants photosynthetic activities and impairs dry weight accumulation [35]. The

Table 3. Effects of poultry manure and plat extract of *Senna alata* on number of leaves per plant at 4WAP and 6WAP of okra infected with *M. incognita*

4WAP						
<i>S. alata</i> concentration (g/ml)						
Poultry manure (t/ha)	0.00	0.20	0.40	0.60	0.80	Mean
0.00	2.25	3.25	3.75	3.75	3.25	3.25
15.00	5.25	5.75	5.75	8.75	6.75	6.45
20.00	7.50	9.50	10.50	11.00	8.75	9.45
Mean	5.00	6.17	6.67	7.83	6.25	
6WAP						
0.00	2.00	2.75	3.25	3.50	2.75	2.85
15.00	4.50	5.00	5.25	7.75	6.75	5.85
20.00	7.25	8.75	9.75	10.00	7.25	8.60
Mean	4.58	5.50	6.08	7.08	5.58	
				4WAP	6WAP	
F-LSD (0.05) for comparing <i>Senna alata</i> (s) mean:				0.45	0.57	
F-LSD (0.05) for comparing poultry manure mean:				0.35	0.44	
F-LSD (0.05) for comparing (SXPM) interaction mean:				0.77	0.98	

Table 4. Effect of poultry manure and plant extract of *Senna alata* on eight of fresh root and dry weight of shoot of okra infected with *M. incognita*

Weight of fresh root (g)						
<i>Senna alata</i> concentration (g/ml)						
Poultry manure (t/ha)	0.00	0.20	0.40	0.60	0.80	Mean
0.00	0.56	0.89	1.68	2.79	1.94	1.57
15.00	2.77	4.94	6.85	7.94	5.79	5.65
20.00	5.12	7.21	9.00	12.00	8.27	8.31
Mean	2.81	4.34	5.83	7.56	5.33	
Dry weight of shoot(g)						
0.00	0.65	1.60	2.25	3.60	2.55	2.13
15.00	2.01	2.91	3.66	5.19	4.01	3.55
20.00	3.44	4.51	6.00	7.07	5.62	5.33
Mean	2.03	3.00	4.00	5.28	4.06	
				Dry shoot weight	fresh root weight	
F-LSD (0.05) for comparing <i>Senna alata</i> (s) mean:				0.22	0.27	
F-LSD (0.05) for comparing poultry manure mean:				0.17	0.21	
F-LSD (0.05) for comparing (SXPM) interaction mean:				0.38	0.46	

Table 5. Effects of poultry manure and plant extract of *Senna alata* on number of pods/plant and weight of fresh pod (g) of okra infected with *M. incognita*

Poultry manure t/ha	Number of pods/plant					Mean
	<i>S. alata</i> concentration (g/ml)					
0.00	0.00	0.20	0.40	0.60	0.80	1.30
15.00	1.00	1.00	1.25	2.00	1.25	2.30
20.00	1.50	2.00	2.50	3.00	3.00	3.00
Mean	2.25	2.75	3.50	4.00	2.50	3.00
	1.58	1.92	2.41	2.83	2.25	
Weight of fresh pod(g)						
0.00	5.76	6.08	6.77	7.43	6.02	6.41
15.00	17.57	21.10	26.62	32.32	29.34	25.39
20.00	26.60	34.59	42.95	45.96	39.47	37.91
Mean	16.65	20.59	25.44	28.57	24.94	
				Pod/plant	Weight of fresh pod	
F-LSD (0.05) for comparing <i>S. alata</i> (s) mean:				0.35	1.40	
F-LSD (0.05) for comparing poultry manure mean:				0.27	1.08	
F-LSD (0.05) for comparing (SXPM) interaction mean:				0.60	2.42	

reduction in root galling could possibly account for improved growth and dry weight yield in plants amended with poultry manure and treated with leaf extract of *S. alata*.

5. CONCLUSION

Poultry manure significantly inhibited root galling in okra plants especially in combination with leaf extract of *S. alata*. The growth and yield of okra plants were also significantly improved following the application of poultry manure and leaf extract of *S. alata*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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