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Management of root-knot nematode (*Meloidogyne* spp.) on okra (*Abelmoschus esculuntus* (L.) Moench) with aqueous sesame seed extract

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Abstract

Okra (*Abelmoschus esculuntus* (L.) Moench) is a popular vegetable in Ghana but its production is limited by nematode infestation of soils. Pot experiments were carried out in a plant house at the Nyankpala campus of the University for Development Studies (UDS), Tamale to assess the effect of aqueous sesame seed extract on root-knot nematodes (*Meloidogyne* spp.) of okra. The experiment was laid out in a completely randomized design with each of the four treatments replicated four times. Treatments consisted of three levels of sesame seed extract (10, 20 and 30 g/50ml) per pot and a control (0 g/50ml). All pots were inoculated with 1000 root-knot nematode eggs a week before the application of the treatments. Data collected included plant height, number of leaves, stem girth, fruit weight, fresh root weight, number of fruits, nematode eggs per 50 g of fresh soil and root galling index. The results showed that aqueous extract of *S. indicum* at 30 g/50 ml (w/v) suppressed root-knot nematodes better than the control. Similarly, okra plants treated with *S. indicum* had the lowest infection index (root gall). There were significant differences ($P<0.05$) among the treatment means for number of fruits and fruit weight of okra between aqueous sesame seed extract at 30 g/50 ml and the control. Yield of okra can be enhanced and nematode population reduced through the application of sesame seed extract preferably at 30 g/50ml

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Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is an annual crop grown mainly as a fruit and leafy vegetable in the tropics (Tiamiyu *et al*., 2012). It is widely distributed in the tropical and subtropical regions from Africa to Asia, Southern Europe, the Mediterranean countries, and the Americas (Andras *et al.,* 2005). In Ghana, okra is being produced by both small scale farmers and commercial concerns on large commercial farms to support the export drive (Norman *et al*., 2011). Okra is an important constituent of most local dishes in West Africa. It is used as a soup thickener and may also be served with rice and other food types (Tiamiyu *et al*., 2012). Okra seeds contain greenish-yellow edible oil which is suitable for use as a biofuel (Farroq *et al.,* 2010).

Among the numerous constraints facing okra farmers in Ghana is nematode infestation. Root-knot nematodes (*Meloidogyne* spp.) are serious pests of okra which can cause root galling (Stirling and Pattison, 2008) early wilting, leaf browning, suppression in plant growth and reduction of photosynthetic pigments (Khan and Khan, 1994) resulting in yield reduction, poor fruit quality, and reduced shelf-life (Sasser, 1989). There is public outcry against synthetic pesticides, because of their harmful effects on man, animals, plants and the environment as a whole as well as the high cost involved in their development and registration (Chitwood, 2002), high toxicity and persistence and hazards posed to non-target species and agricultural workmen (Oka *et al*., 2014). As a result, there is a growing preference for plant products which are less harmful, effective, easily degraded, pollution-free, leave no harmful residues, are cheaper and not toxic to host plants and humans (Amadioha, 2003; Siddiqui and Allam, 1989).

Extracts of various plants including sesame (*Sesamum indicum* L.) castor bean,chrysanthemum, velvet bean, crotalaria, indigo, neem and tephrosia (Ameen, 1996, Chitwood, 2002) have been reported to be effective against nematodes (Alashalaby and Noweer, 2003; Araya and Caswell-Chen, 1994). According to Youssef and El-Nagdi (2004), sesame seed cake significantly reduced nematode galls, egg-masses and prevented nematode build-up on squash plants. Since sesame is abundant in the study area, there is the need to explore its potential for the management of root-knot nematode under local conditions. This study therefore sought to evaluate the efficacy of aqueous sesame seed extract against root-knot nematodes and determine its effect on growth characteristics and yield of okra plants.

Materials and methods

Study area

The experiment was carried out from November, 2011 to February, 2012 in a plant house of the Faculty of Agriculture, University for Development Studies, Nyankpala in the Tolon Kumbungu district of the Northern Region. The area lies within the Guinea savanna zone of Ghana and experiences a unimodal annual rainfall of about 1000 – 1200 mm unevenly distributed from April to November. The temperature distribution is uniform with mean monthly minimum and maximum values of 21^{o}C and 32^{o}C respectively (SARI Annual report, 2012). The minimum and maximum relative humidities are 52% and 85% respectively. The soil is moderately drained sandy loam free from concretion developed from voltaian sand stone and classified as Nyankpala series (SARI Annual report, 2012).

Sterilization of soil and planting

Topsoil was collected and sifted to remove large particles, stones, plastic materials and plant debris. The soil was mixed with river sand in the ratio 3:1 (v/v) respectively and sterilized using a metal tray for 30 minutes at 103^{o}C with fire wood as the source of heat and maintained at that temperature for 1 hour. The sterilized soil was spread on a large metal sheet after heating and left over night to cool off before it was used. Five kilograms (5 kg) of the sterilized soil was placed in each of 32 plastic pots measuring 7 litres. Two seeds of okra var. Legon Fingers were planted at stake in each pot. Watering was done immediately and then continued on a daily basis.

Extraction of root-knot nematode eggs

The inoculum was obtained from infested cabbage roots which were collected from a vegetable farm on the University campus. The roots were washed thoroughly with clean water, dried and then chopped into pieces of about 3 cm with a sharp knife. The eggs of the nematodes were extracted from the infested roots as described by Hussey and Barker (1973). One hundred grams (100 grams) of chopped roots were placed in a bottle before adding 0.5% sodium hypochlorite solution and then shaken vigorously for four minutes. The sodium hypochlorite solution containing the nematode eggs and the root debris was quickly poured through a 200 μm mesh sieve nested over a 500 μm mesh sieve. The residual sodium hypochlorite in the two sieves was rinsed several times by placing them under slowly running tap water. The eggs were collected from the 500 μm sieve into a clean beaker and covered.

Counting of root-knot nematode eggs

The nematode eggs were counted using a Doncaster counting tray. The egg suspension was agitated with a pipette. An aliquot suspension of 1 ml was pipetted into the counting tray. A hand tally counter and a stereo microscope were used to count the eggs. The count was repeated two more times to ensure accuracy. The total number of eggs in 1 ml was multiplied by the total egg suspension to obtain the total number of eggs in the suspension.

Preparation of sesame seeds aqueous extracts

Dried sesame seeds were collected, washed thoroughly and ground in sterile mortar with a pestle. Fifty milliliters (50 ml) of water was added to each of 10, 20 and 30 grams of sesame seed powder. The mixture was passed through a 2-ply silktex tissue in a funnel to give various aqueous filtrates. The filtrates were labelled as 10 g/50 ml, 20 g/50 ml and 30 g/50 ml concentrations.

Inoculation of okra seedlings

Inoculation was done three weeks after planting. Three holes about 3 cm were created around each of the plants. One millilitre (1 ml) of egg suspension was placed in each of the three holes around each plant using a syringe. The holes were then covered lightly with soil. Two days after inoculation, the extracts were applied to each of the plant according to the treatment. A single hole was created near each plant and the extract applied in each hole. A final application was carried out at four weeks after the first application.

Experimental design

The pots were arranged in a completely randomized design (CRD). There were three treatments and a control each replicated four times. Treatments consisted of three levels of sesame seed extract (10, 20 and 30 g/50ml) and a control (0 g/50ml).

Data collection and analysis

Data were collected on the following parameters: plant height, number of leaves, stem girth, and root-knot nematode population in the soil, fruit weight, fresh root weight and number of fruits. Galling was determined by uprooting each plant gently, washing in a bucket and then dipping in water contained in a beaker and then observing through the beaker. Rating was based on Bridge and Page (1980) gall rating chart.

The data were subjected to Analysis of variance Genstat (Version 11), and treatment means separated using the least significant difference (LSD) at $P<0.05$.

Results

Effects of sesame seed extract on growth characteristics of okra

Plant height

Generally plants treated with aqueous seed extract of *S. indicum* were significantly taller ($P<0.05$) than those of the control from the second to the eighth week after planting (Table 1). Plants treated with 30 g/50 ml of the aqueous sesame seed extract produced taller plants than those treated with 10 g/50 ml and 20 g/50 ml.

Number of leaves

Sesame seed extract treated plants recorded a higher number of leaves than those of the control but the differences were not significant (Table 2) except at 6WAP when plants treated with 20 g/50ml and 30 g/50ml recorded a significantly higher ($P<0.05$) number of leaves.

Stem girth

Plants treated with 20 g/50ml of sesame seed extract recorded the highest stem girth, followed by those treated with 30g/50ml and 10g/50ml but the differences were not significant ($P>0.05$). However there were significant differences ($P<0.05$) between the treatments and the control (Table 3).

Table 1. Effect of aqueous sesame seed extract on height.

Treatments	Plant height (cm)			
	2WAP	4WAP	6WAP	8WAP
10g/50ml	11.90	18.60	28.80	34.50
20g/50ml	13.42	21.07	27.90	38.50
30g/50ml	13.68	17.02	33.80	40.30
Control	11.67	14.65	22.10	30.00
LSD (0.05)	1.10	1.51	1.69	2.13
CV (%)	9.80	5.30	3.70	3.70

WAP= Weeks after planting.

Treatments	Number of leaves			
	2WAP	4WAP	6WAP	8WAP
10g/50ml	6.25	8.00	9.50	10.00
20g/50ml	6.75	8.25	10.00	11.25
30g/50ml	6.50	8.50	10.00	11.25
Control	6.50	7.75	8.50	10.00
LSD (0.05)	1.31	2.13	1.10	2.13
CV (%)	12.60	16.40	13.10	12.50

WAP= Weeks after planting

Fruit weight, fresh root weight and number of fruits

Plants treated with 30 g/50ml produced the heaviest fruits which were comparable to those produced by plants treated with 10 g/50ml but significantly heavier ($P<0.05$) than fruits produced by plants treated with 20 g/50ml and the control (Table 4). Application of sesame seed extract did not affect fresh root weight significantly ($P<0.05$). However, plants treated with 30 g/50 ml recorded the least mean root weight of 1.75 while those of the control recorded the heaviest weight of 2.10 g. Plants treated with 30 g/50ml produced the highest number of fruits (4.25) followed by those treated with 20 g/50ml (3.0) and 10 g/50ml (2.5) and control (1.75).

Influence of sesame seed extract on root galling and nematode population

Generally, plants treated with sesame seed extract recorded significantly lower ($P<0.05$) severity of galling and mean population of root-knot nematode (Table 5). Plants of the control treatments had severe galls on the main roots while those grown in sesame seed extract amended soils had fewer galls, which were limited to the feeder roots. Roots of plants treated with 30 g/50 ml recorded the least number of galls (0.25) which was significantly lower ($P<0.05$) than those of plants treated with 10 g/50 ml (4.0), 20 g/50 ml (1.0) and the control (6.5). The population of second stage juveniles (J2) of *Meloidogyne* spp. recorded at harvest indicated that, okra plants treated with aqueous sesame seed extract at the different rates of 10 g/50 ml, 20 g/ 50 ml and 30 g/50 ml had the lowest population densities. For instance, plants treated with 30 g/50ml had a mean population of 1.06 which was significantly lower ($P<0.05$) than those of the other treatments and the control (Table 5).

be attributed to the feeding habits of nematode, which has been confirmed by Noling (1999) report that nematode-infested plants become stunted due to root dysfunction, reduction of rooting volume and efficient utilization of water and nutrients. Similarly, Radwan *et al.* (2009) reported a significant increase in height and a corresponding reduction in *Meloidogyne incognita* infestation of tomato plants treated with sesame cake at the rate of 5 g/kg soil.

Discussion

Effect of sesame seed extract on growth and yield of okra

Plants treated with 30 g/50 ml of aqueous sesame seed extract produced significantly taller ($P<0.05$) plants than those treated with 10 g/50 ml, 20 g/50 ml and the control. Lower height of control plants could

Table 3. Effect of aqueous sesame seed extract on stem girth.

Treatments	Plant girth (cm)			
	2WAP	4WAP	6WAP	8WAP
10g/50ml	0.43	0.47	0.58	0.68
20g/50ml	0.60	0.64	0.71	0.80
30g/50ml	0.475	0.55	0.66	0.76
Control	0.35	0.40	0.50	0.55
LSD (0.05)	0.21	0.18	0.20	0.17
CV (%)	28.80	21.80	20.40	15.10

WAP= Weeks after planting

Although the differences were not significant ($P<0.05$), plants treated with sesame seed extract produced a higher number of leaves and wider stems than the control plants with 30 g/50 ml being the best. The inhibition of growth in the control plants could have resulted from reduced productivity (Khan and Saxena, 1992) due to infestation of the roots of seedlings soon after germination (Ploeg, 2001). Abid (1996) report that sesame, neem, cotton, mustard and castor oil cakes significantly inhibited the development of root galls on okra, tomato, brinjal and mungbean with subsequent increase in plant growth is a further confirmation of the inhibition of growth in control plants of this study.

Table 4. Effect of aqueous sesame seed extracts on mean fruit weight, root weight and number of fruits at harvest.

Treatments	Fruit weight (g)	Fresh root weight (g)	Number of fruits
Control	5.20	2.10	1.75
10 g/50 ml	12.40	2.06	2.50
20 g/50 ml	11.50	1.96	3.00
30 g/50 ml	16.40	1.75	4.25
LSD (0.05)	6.63	0.43	0.96
CV (%)	40.90	13.60	20.90

Plants treated with 30 g/50ml produced the highest number of fruits which were also heaviest. The production of a higher number and heavier fruits in plants treated with sesame seed extract could be due to a better translocation of water and nutrients to the shoots (Sikora and Fernandez, 2005) than the control plants whose roots were highly infested or galled, leading to a reduction in uptake and transportation of nutrients as reported by Hussey (1985) and Caveness and Ogunforowa (1985).

Roots of the control plants were heavier than those of plants treated with the sesame seed extract. This could be due to the higher number of galls formed on their roots (Caveness and Ogunforowa, 1985).

Effect of sesame seed extract on infestation of okra plants by root-knot nematode

Generally, plants treated with sesame seed extract recorded significantly lower ($P<0.05$) galls and mean population of root-knot nematode. Treatment with 30 g/50 ml was the most effective, a confirmation of Abid (1996) report that development of root galls decreased with an increase in concentration of the extracts and the duration of dip treatment. The 30 g/50ml treatment which was the highest concentration may have released more chemicals into the soil which inhibited the entry of root knot-nematodes into the roots of plants as reported by Gommers *et al.* (1982). The lower mean population of root-knot nematodes in plants treated with the sesame seed extract confirms Alashalaby and Noweer (2003) report that aqueous neem and sesame extract significantly reduced the total number of root knot nematode juveniles and inhibited egg hatch in peanut roots and soil. Mean number of juveniles recovered was low in the highest concentration compared to the control treatment. This confirms the report by Joymatti *et al.* (1998) that juvenile mortality was dependent on the concentration of extract of ginger extract. Sesame extract has been found to contain a mixture of unsaponifiable materials such as sesamin, sesamolin, and sesamol which are nematicidal (Radwan *et al.*, 2009). Sikora and Fernandez (2005) also found that application of sesame seed extract reduced the incidence of root knot nematodes and the severity of galling on okra roots. According to Akhtar and Mahmood (1993), sesame seed extracts have a systemic activity against nematodes which may have accounted for the lower number of galls and mean population in treated plants.

Table 5. Effect of aqueous sesame seed extract on root galling index and population densities of *Meloidogyne* spp. recovered from rhizosphere of okra.

Treatments	Root Galling (Scale:0-10)	Population Untransformed mean	Transformed mean
Control	6.50	870.00	2.94
10 g/50 ml	4.00	50.00	1.70
20 g/50 ml	1.00	20.75	1.32
30 g/50 ml	0.25	10.50	1.06
LSD (0.05)	0.77	65.10	0.12
CV (%)	16.30	17.10	4.30

Log (x+1) transformed, where x= mean count

0=No gall, 10= severely galled and plant usually died.

Conclusion

Sesame seed extract produced better results than the control for all parameters with 30 g/50ml as the best. It is therefore recommended farmers should apply 30 g/50ml of sesame seed extract for the control of nematodes, since it is safer than synthetic chemicals.

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