Original Research Article

THE EFFECT OF AIR DRIED LEAF POWDER AND BURNT LEAF ASH OF DIFFERENT PLANTS ON ROOT-KNOT NEMATODE (*Meloidogyne* spp.) ON OKRA (*Abelmoschus esculentus L . Moench*)

ABSTRACT

The effect of air dried leaf powder and burnt leaf ash of different plants on the control of root-knot nematode (*Meloidogyne* spp.) on Okra was investigated in a pot experiment. The experiment was laid out in a Completely Randomized Design with nine treatments each replicated four times. The treatments included: burnt leaf ashes and air dried leaf powders of *Baphia nitida*, *Pentaclethra macrophylla* and *Delonix regia* respectively, a synthetic nematicide (Carbofuran 3G) and two control which included inoculated but untreated (control 1) and un-inoculated and untreated (control 2) pots. The plants were inoculated with 1000 nematode eggs (*Meloidogyne* spp.) two weeks after germination. Three days after, the treatments were applied at the rate of 30 g each while the synthetic nematicide (Carbofuran 3G) at 3 g/per pot. Parameters recorded were: plant height, number of leaves, fresh and dry shoot weights, fresh root weight, number of galls and nematode eggs in roots, number of larva in soil, number and weight of pods per plant. Results obtained indicated significant differences among the treatments in most parameters recorded. Generally, results from air dried leaf powder of *Baphia nitida* and burnt leaf ash of *Delonix regia* were better and compared favorably with the nematicide treated plants.

Key words: Air dried leaf, Burnt leaf ash, Carbofuran, *Meloidogyne* spp., *Abelmoschus esculentus*

INTRODUCTION

Okra as a vegetable crop belongs to the genus *Abelmoschus*, Family *Malvaceae* and has two main species: *Abelmoschus esculentus (L) Moench and Abelmoschus caillei (A. Chev)* (Siemonsma, 1982). It is an annual crop mainly grown as a fruit and leafy vegetable in the tropics (Tamiyu *et al*., 2012). It is widely distributed in the Tropical and Subtropical regions of Asia, Southern Europe, the Mediterranean countries and the Americas (Andres *et al*., 2005). Nigeria is the second largest producer of Okra in the world after India and largest producer in Africa (5.8 million tonnes), followed by Cote d'Ivoire, Ghana and others (FAOSTAT, 2011). In Nigeria, it is widely distributed and consumed in either fresh or dried forms (Farinde *et al*., 2007).

Okra is 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 (Tamiyu *et al*., 2012). In Nigeria, okra is grown across different ecological zones because it serves as a source of income to framers as well as a cheap source of protein, vitamins A, B complex and C and mineral (Ca, P, Fe and K) to many households (Adebisi *et al*., 2007). It is a nutritious vegetable that contains 86.1% ~~of~~ water, 2.2% ~~of~~ protein, 0.2% ~~of~~ fat, 9.7% ~~of~~ carbohydrates, 1.0% ~~of~~ fiber and 0.8% ~~of~~ ash (Saifullah and Rabbani, 2009), hence it plays vital role in human diet. Okra seeds contain greenish yellow edible oil which is also suitable for use as a bio-fuel (Farroq, *et al*., 2010).

Okra is notorious for its susceptibility to root knot nematodes (Noling, 2012). *Meloidogyne* spp. are responsible for about 70-90% of the yield losses in okra (Saufiuddin *et al*., 2011). Almost all the vegetables in tropical and warm temperate regions are severely attacked by plant-parasitic nematodes particularly root-knot nematodes (*Meloidogyne* spp.) (Sikora and Fernandez, 2005). *Meloidogyne* spp. cause wilting, chlorosis, stunted growth, formation of galls in roots often leads to destruction of roots, poor growth, yield and crop failure when nematode population exceeds economic threshold level (Fourie *et al*., 2001; Sikora and Fernandez, 2005). The symptoms for identifying disease caused by *Meloidogyne* spp. is the presence of galls, wilting, loss of vigor, yellowing of leaves (Kayani *et al*., 2012). They have caused up to 80% yield loses in heavily infested soils (Kaskavalci, 2007). However the extent of damage is influenced by the cultivar, nematode species, level of soil infestation and environment (Ononuju, 1999). It is therefore necessary to control root-knot nematodes in order to avoid or minimize yield loses in okra.

Management of root-knot nematodes with synthetic nematodes can be very effective (Sikora and Fernandez, 2005; Adegbite and Agbaje, 2007). However, there is public outcry against synthetic nematicides due to their toxicity, persistence and hazards posed to non-target species and agriculturists (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, cheaper and not toxic to host plants and humans (Amadioha, 2003). These plants if explored for nematicidal activity might be alternatives to synthetic nematicides (Siji *et al*., 2010). (Have these products been safety tested? Plants also make toxic products and burning can create more. This should be addressed, either way!)

The objective of this study is to determine the effect of air dried leaf powder and burnt leaf ash of *Baphia nitida, Pentaclethra macrophylla and Delonix regia* on the population of root-knot nematode (*Meloidogyne* spp.) on the growth and yield of okra, gall formation and also to compare the effect of these plant materials with the synthetic nematicide (Carbofuran 3G).

MATERIALS AND METHODS

LOCATION OF EXPERIMENTAL SITE

The experiment was carried out in the Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria located on latitude $5^0 2^I$N and longitude $7^0 33^I$E. It lies in the humid tropical rain forest zone with annual rainfall of 1916 mm per annum, altitude of 112m above sea level and relative humidity of 76% with temperature range of 19-35^0C (N.R.C.R.I, 2010).

EXPERIMENTAL DESIGN

The experiment was laid out in a Completely Randomized Design (CRD) on a platform in an open field using plastic pots. Okra variety *Pusa suwaan* was planted. There were nine treatments replicated four times including the control, giving a total of 36 pots.

EXPERIMENTAL MATERIALS

Source of seed: Seeds of okra variety *Pusa suwaan* were obtained from National Root Crop Research Institute Umudike, Abia State, Nigeria.

Treatments: The materials used were: burnt leaf ash and air dried leaf powder of *Baphia nitida*, *Pentaclethra macrophylla* and *Delonix regia* and a synthetic nematicide (Carbofuran 3G).

Treatments' sources and preparation: The plant materials? Not plants?, leaves? or roots ? were obtained within the school premises while the synthetic nematicide (Carbofuran 3G) was obtained from a chemical store. The plant materials were collected and washed under running tap to remove soil particles and reduce contamination by microorganisms. They were then spread on a clean platform for air drying. This was done to reduce the moisture content after which each material was divided into two parts: one part was ground into powder using an electric grinder while the other part was burnt to ash in a controlled environment and each part were put in a clean plastic container ready for application.

Soil preparation and sterilization: Top soil was collected and sifted to remove large soil particles, stones and plant~~s~~ debris. The soil was poured into a cut metal drum. It was moistened and a source of heat applied underneath. It was sterilized at 80^{0}C for 20 minutes after which it was left to cool (Ononuju *et al.*, 2014).

Extraction of nematode eggs from inoculum: Eggs of root-knot nematodes were extracted from the heavily galled roots of *Basella alba* (Ceylon spinach). The galled roots were washed in tap water and cut into pieces of 2cm long and placed in a beaker containing 200 mls of 0.5% NaOCl (Sodium hypochloride) solution and ~~was~~ shaken vigorously for about 5minutes (Hussey and Barker, 1973). This was done to prevent egg damage while the gelatinous matrix is being removed. The solution was poured over two nested sieves of 75µm mesh 25µm mesh to collect the eggs. Eggs in the 25µm mesh sieve were rinsed with cold water and washed into a beaker. The cut roots in the original beaker were washed twice with water to obtain additional eggs. The number of eggs in 1ml of water was estimated by counting four samples of a milliliter each using Doncaster's counting dish under a stereomicroscope and the average taken (Hussey and Barker, 1973).

Sowing of seeds: Okra seeds were sown at two seeds per hole in plastic pots with diameter 22.5cm containing 6kg sandy loam soil mixture that has been sterilized. Two weeks after, the seedlings were thinned down to a healthy plant per pot.

Inoculation of plants with nematode eggs: Three weeks after emergence, the plants were inoculated with a calculated volume of 1000 eggs of *Meloidogyne* spp.~~ extracted by Hussey and Barker (1973) method~~. ~~This was~~Eggs were applied near the base of plant by making holes around the Okra plants.

Application of treatments: Thirty (30g) each of the air dried leaf powder and burnt leaf ash of *B. nitida, P. macrophylla and D. regia* were applied evenly on the surface of the soil in each bucket according to the treatments and their replications three days after inoculation. A synthetic nematicide (Carbofuran 3G) at 3.0g ai per plant was applied. The controls include plants whose soils were inoculated but untreated (control 1) and un-inoculated and untreated (control 2) respectively.

The treatment combinations were as follows:

1. T1 Nematode + Burnt leaf ash of *Baphia nitida*
2. T2 Nematode + Air dried leaf ash of *Baphia nitida*
3. T3 Nematode + Burnt leaf ash of *Pentaclethra macrophylla*
4. T4 Nematode + Air dried leaf ash of *Pentaclethra macrophylla*
5. T5 Nematode +Burnt leaf ash of *Delonix regia*
6. T6 Nematode + Air dried leaf ash of *Delonix regia*
7. T7 Nematode + synthetic nematicide (Carbofuran 3G).

8. T8 Inoculated but untreated (Control 1)
9. T9 Un-inoculated and untreated (Control 2)

(NPK 15:15:15) was applied at the rate of 100kg/ha to give a rate of 0.4g per bucket. Water was applied to the plant as ~~at when~~ required.

DATA COLLECTION

Data collected at the end of the experiment after twelve weeks included:

- Plant height- measured using a meter rule in centimeter.
- Number of leaves- ~~counted with hand (cm)~~.
- Fresh and dry shoot and fresh root weights- measured using a digital laboratory weighing balance in grams (g).
- Number of eggs in root and larva in soil- extracted using the modified Baermann technique (Hooper, 1969), and counted using Domncaster's counting dish under an electronic stereomicroscope.
- Number of galls in roots- ~~counted with hand~~.
- Number of pods- ~~counted with hand~~.
- Weight of pods- measured in grams (g) using a digital laboratory weighing balance ~~in grams (g)~~.

STATISTICAL ANALYSIS

The data collected were subjected to Analysis of Variance (ANOVA) and means were separated using Least Significant Difference (LSD) at 5% probability level (P<0.05) by using computer software "Genstat Discovery Edition 4".

RESULTS AND DISCUSSION

RESULTS

The effect of treatments on Plant height and Number of leaves are shown in Table 1. ~~On~~ For plant height, significant difference (P<0.05) was observed between control 2 (un-inoculated and untreated) (27.17cm) and all the treatments. There was also a significant difference between the plants treated with air dried leaf powder of *B. nitida* (18.77cm) and control 1 (6.65cm). ~~On~~ For Number of leaves, there was no significant difference recorded among the treatments including the controls. Although, the highest number of leaves (15.00) was recorded for the plants treated with burnt leaf ash of *B. nitida* while the lowest (6.00cm) was recorded for control 1(inoculated but untreated).

Table 1: Effect of treatments on the plant height and number of leaves of okra infested with root-knot nematode (*Meloidogyne* spp.).

Treatments	Plant height(cm)	No. of leaves per plant
Burnt leaf ash of *Baphia nitida*	11.20	15.00
Air dried leaf powder of *Baphia nitida*	18.77	13.00
Burnt leaf ash of *Pentaclethra macrophylla*	12.62	8.00

Air dried leaf powder of *Pentaclethra macrophylla*	12.00	10.00
Burnt leaf ash of *Delonix regia*	11.07	13.00
Air dried leaf powder of *Delonix regia*	7.25	10.00
Synthetic nematicide (Carbofuran 3G)	10.25	12.00
Inoculated but untreated (control 1)	6.65	6.00
Un-inoculated and untreated (control 2)	27.17	11.00
LSD $_{(0.05)}$	6.25	NS

Where, NS = No Significant Difference show +/- values for each measure; provides idea of variability between samples

Table 2 shows the effect of treatments on number and weight of pods. There was no significant difference (P<0.05) between the treatments and the controls on the number of pods. Although the highest number of pods was recorded by control 2 (11) and plants treated with synthetic nematicide (11) respectively, with the least number of pods (5.0) recorded for plants that were inoculated but untreated (control 1). Significant differences (P<0.05) were recorded on the weight of pods. All the treatments differed significantly from control 2 (34.9g), except the plants treated with synthetic nematicide (28.3g). Nevertheless plants treated with air dried leaf powder of *B. nitida* (23.4g) compared favorably with the synthetic nematicide.

Table 2: Effect of treatments on the number and weight of Okra pods.

Treatments	No. of pods per plant	Weight of pods (g)
Burnt leaf ash of *Baphia nitida*	7.0	21.3
Air dried leaf powder of *Baphia nitida*	10.0	23.4
Burnt leaf ash of *Pentaclethra macrophylla*	7.0	15.6
Air dried leaf powder of *Pentaclethra macrophylla*	8.0	21.4
Burnt leaf ash of *Delonix regia*	10.0	22.0
Air dried leaf powder of *Delonix regia*	6.0	16.1
Synthetic nematicide (Carbofuran 3G)	11.0	28.3
Inoculated but untreated (control 1)	5.0	6.1
Un-inoculated and untreated (control 2)	11.0	34.9
LSD $_{(0.05)}$	NS	9.75

Where, NS = No Significant Difference Would Duncan's multiple range test be better?

The effect of treatments on fresh shoot, dry shoot and fresh root weights are shown in (Table 3). On fresh shoot weight, air dried leaf powder of *B. nitida* (480g) compared favourably with control 1 (205g) but did not with control 2 and other treatments. On dry shoot weight, there was no significant difference recorded among the treatments including the controls. Although the highest dry shoot weight (85.2g) was recorded for the plants treated with burnt leaf ash of *P. macrophylla* while the lowest (30g) was recorded for the inoculated but untreated plants(control 1). On fresh root weight all the treatments significantly differed from control 2 (371g). However, the other treatments did not differ significantly from each other.

Table 3: Effect of treatments on fresh shoot, dry shoot and fresh root weights of Okra infested with root-knot nematode (*Meloidogyne* spp.)

Treatments	Fresh shoot	Dry shoot	Fresh root

	weight (g)	weight (g)	weight (g)
Burnt leaf ash of *Baphia nitida*	133.0	32.8	66
Air dried leaf powder of *Baphia nitida*	480.0	76.8	187
Burnt leaf ash of *Pentaclethra macrophylla*	335.0	85.2	118
Air dried leaf powder of *Pentaclethra macrophylla*	326.0	46.2	57
Burnt leaf ash of *Delonix regia*	308.0	64.0	136
Air dried leaf powder of *Delonix regia*	389.0	63.5	165
Synthetic nematicide (Carbofuran 3G)	362.0	70.0	132
Inoculated but untreated (control 1)	205.0	30.0	122
Un-inoculated and untreated (control 2)	629.0	68.2	371
LSD $_{(0.05)}$	267.1	NS	133.2

Where, NS = No Significant Difference

Effect of treatments on the number of nematode eggs in roots, galls in roots and larva in soil are shown in Table 4. On number of galls in roots, all the treatments compared favourably with control 1, but the treatments did not differ significantly from each other. Similar observations were made on the number of eggs in root. On number of larva in soil, the treatments and the controls did not differ significantly from each other. However apart from control 2 (000), plants treated with nematicide had the least number of larva (126) while the highest number (475) was recorded for plants treated with burnt leaf ash of *P. macrophylla*.

Table 4: Effect of treatments on the population of *Meloidogyne* spp. in root and soil.

Treatments	No. of galls in roots	No. of eggs in roots	No. of larva in soil
Burnt leaf ash of *Baphia nitida*	0.75	126.00	350
Air dried leaf ash of *Baphia nitida*	0.25	102.00	250
Burnt leaf ash of *Pentaclethra macrophylla*	0.50	102.00	475
Air dried leaf ash of *Pentaclethra macrophylla*	1.75	202.00	351
Burnt leaf ash of *Delonix regia*	1.00	158.00	425
Air dried leaf ash of *Delonix regia*	0.75	126.00	300
Synthetic nematicide (Carbofuran 3G)	0.00	78.00	126
Inoculated but untreated (control 1)	5.00	575.00	425
Un-inoculated and untreated (control 2)	0.00	000.00	000
LSD $_{(0.05)}$	2.23	272.5	NS

Where, NS = No Significant Difference

DISCUSSION

The results on the effect of the treatments on plant height agreed with the findings of Bawa *et al.*, (2014) in which the plant extracts used had a significant effect on the height of tomato. They are still in agreement with the findings of Kayani *et al.*, (2012) who confirmed that the symptoms for identifying diseases caused by *Meloidogyne* spp. is the presence of galls, wilting, loss of vigor, yellowing of leaves. Also Perry *et al.*, (2009) confirmed that the symptoms also include, yellowing of leaves and stunting, loss of vigor, wilting due to lack of moisture, decay of tissue due to secondary infection and yield loss. The growth reduction in crops such as Okra due to nematode investation varies depending on population density of nematode, level of cultivar susceptibility and environmental condition to which the host is subjected to (Ononuju and Fawole, 2000).

The reduction in the fresh shoot and root weight could be due to slow rate of action of the active ingredients in the plant materials (Barman and Das, 1996), or due to poor nutrients and water flow as a result of ~~formed~~ giant galls formed on the plant roots (Ploeg, 2001) which inhibit the normal growth~~s~~ and development of shoots and roots of plants affected.

The reduced number of nematode population and number of galls compared to control 2 agrees with the observations of Adegbite and Adesiyan (2001) who indicated that the higher the nematode population the higher the number of galls and the higher the ~~number~~ amount of damage done to plants which hinders the transportation of water and mineral nutrients from roots to aerial part of the plant. The results obtained confirmed the findings of Ozores-Hampton (2002) that the use of organic amendments suppressed soil phyto-parasitic nematode populations. Abulusoro *et al*., (2004) reported that ~~the susceptibility~~ susceptibile~~of~~ tomato plants infected with root-knot nematodes (*Meloidogyne* spp.) show stunted growth, yield loss and conspicuous root galls, but that a number of plants are thought to contain biologically active ingredients which when applied in the soil reduce~~d~~ the incidence of plant-parasitic nematodes. 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 lower mean population in treated plants. Also Vats *et al*., (1996) reported reduction of galls and egg masses when some *M. javanica* infected tomato plants were treated with leaf extracts of *Azadirachta indica* and *Eucalyptus tereticornis*.

The reduced number and weight of pods agrees with Bolles *et al.,(*2012) who reported that nematode (*Meloidogyne* spp.) ~~are found to be serious pest of Okra, damaging stands and~~ greatly ~~decreasing~~ decreased and ~~delaying~~ delayed the production of pods. Also, Adesiyan, (1990) reported that some crop varieties could be effective hosts of root-knot nematode ~~that~~ but suffer no statistically significant damage. However the extent of damage is generally influenced by the cultivar, nematode species, level of soil infestation and environment (Ononuju, 1999). According to Gommers (1981) active compounds with nematicide activity which have been found to limit the activities of plant-parasitic nematodes in plants includes alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls. These compounds were extracted from different plants part such as roots, leaves and so on which could be responsible for the positive effects of air dried leaf ash and burnt leaf ash of these plants on the population of nematode in the soil and root as well as in pod formation.

CONCLUSION AND RECOMMENDATION

The different treatments significantly decreased the population of root-knot nematode (*Meloidogyne* spp.). There were significant differences between the controls and the treatments in some of the parameters measured, while there were no significant differences in others. Synthetic nematicide (Carbofuran 3G) appeared to be more effective in the control of root-knot nematode. However, the performance of the synthetic nematicide was not significantly different from the results obtained from air dried powder of *Baphia nitida* and burnt leaf ash of *Delonix regia* at the rate applied. From the findings of this study, air dried powder of *B. nitida* and burnt leaf ash of *D. regia* appeared to have significantly performed better than other treatments and is being recommended as an alternative for the synthetic nematicide (Carbofuran 3G) in the control of root-knot nematode. Notwithstanding, further research work both in green house and field trials at different rates are needed in order to authenticate this findings. Is there any practical way this could be developed for use in growers plots?

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