

Effects of Five Application Rates of Powdered *Vernonia amygdalina* Leaf in the Management of *Meloidogyne incognita* on Okra (*Abelmoschus esculentus* (L.) Moench)

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Abstract: *Vernonia amygdalina* (VA) leaf has been used in the management of *Meloidogyne incognita* (MI) on crops at diverse formulations and application rates. Determination of optimum application rate of VA for the management of MI on okra will ensure sustainability of VA and reduce cost of management. Effects of five rates of powdered VA leaf and carbofuran in managing *M. incognita* on okra were determined in screenhouse and field experiments laid out in completely randomized design and randomized complete block design, respectively. Two-week old okra seedlings were each inoculated with 5,000 eggs of MI except the uninfected control. Powdered VA leaf was applied at 50, 100, 150, 200, 250 kg/ha and carbofuran at 3 kg a. i./ha to MI-infected okra at one week after inoculation (WAI). At 8 WAI, data were collected on vegetative growth, fruit weight, gall index (root damage), nematode population and reproduction. Quantification of phytochemicals in VA leaf was also carried out. Data were analysed using analysis of variance and means partitioned with least significant difference ($P \leq 0.05$). Activity of VA leaf on MI-infected okra was rate dependent (higher rate > lower rate). *Vernonia amygdalina* leaf at 50, 100, 150, 200, 250 kg/ha and carbofuran at 3 kg ai/ha significantly improved vegetative growth by 51.2, 85.2, 111.7, 131.8, 159.5 and 147.8% in pot; and 55.1, 114.1, 187.4, 266.7, 317.8 and 232.8% in field, respectively than infected-untreated okra. Rates of 50, 100, 150, 200, 250 kg/ha and carbofuran significantly reduced root damage by 44, 64, 80, 80, 80 and 84% in pot; and 31.5, 47.4, 47.4, 47.4, 47.4, and 60.5% in field, respectively than infected-untreated okra. Nematode population and reproduction rate of MI were reduced in similar trend observed in root damage. There was no significant differences among VA leaf-treated okra at 150, 200 and 250 kg/ha and carbofuran in improvement of growth, yield, reduction of root damage, nematode population and reproduction. *Vernonia amygdalina* leaf had saponins (48.2 mg/g), Alkaloids (19.1 mg/g), flavonoids (5.5 mg/g), tannins (4.5 mg/g) and phenol (4.2 mg/g) that confer nematocidal properties. Powdered VA leaf should be applied at 150 kg/ha in the management of *M. incognita* on okra since it compared effectively with carbofuran and other VA higher rates.

[Tanimola, A. A. And Asimiea, A. O. **Effects of Five Application Rates of Powdered *Vernonia amygdalina* Leaf in the Management of *Meloidogyne incognita* on Okra (*Abelmoschus esculentus* (L.) Moench).** *N Y Sci J* 2018;11(4):1-10]. ISSN 1554-0200 (print); ISSN 2375-723X (online). <http://www.sciencepub.net/newyork>. 1. doi:[10.7537/marsnys110418.01](https://doi.org/10.7537/marsnys110418.01).

Keywords: *Vernonia amygdalina*, *Meloidogyne incognita*, carbofuran, application rate, management, phytochemicals, okra.

Introduction

Plant products have been found effective for crop protection purposes worldwide because of the advantages of cheapness, easy availability and environment-friendliness over the conventional synthetic nematicides (Adegbite and Agbaje, 2007; Lale, 2007; Ofuya, 2009; Claudius-Cole *et al.*, 2010). However, issues of formulation, standardization and commercialization to meet the ever growing needs for crop protection in small, medium, and large scale farms remain largely unresolved (Lale, 2007; Ofuya, 2009). Commonly used formulations for botanicals in Nigeria and most developing countries are powders, crude water extracts, oils, ash, mixed formulations, amongst others (Ofuya, 2009).

Okra, *Abelmoschus esculentus* (L.) is a very valuable economic vegetable crop in the diets of most individuals in Nigeria and the world (Agwu and

Ezigbo, 2005; Udoh *et al.*, 2005; Adenipekun *et al.*, 2009). Plant-parasitic nematodes contribute significantly to decline in okra yield amongst other constraints (Udoh *et al.*, 2005; Sikora and Fernandez, 2005). Root-knot nematodes, *Meloidogyne* species are one of the major plant-parasitic nematodes on okra and the crop is highly susceptible to these nematodes (Akinlade and Adesiyun, 1982). *Meloidogyne incognita*, a major species of root-knot nematodes found on okra in Nigeria and worldwide is responsible for decline in yield thereby reducing the economic value of okra (Adekunle and Akinlolu, 2007).

Vernonia amygdalina holds promise as an effective botanical pesticide for the management of root-knot nematodes (Iwalokun *et al.*, 2004; Adeniyi *et al.*, 2010). Organic extract from *V. amygdalina* was reported toxic to *M. incognita* and it contains compound with nematocidal, antimicrobial and

insecticidal activities (Iwalokun *et al.*, 2004). Tanimola *et al.* (2015) posited that powdered *V. amygdalina* leaf was effective in managing *M. incognita* on okra. Many workers linked the nematicidal activity of *V. amygdalina* to the active ingredients (phytochemicals) in the plant (Iwalokun *et al.*, 2004; Adeniyi *et al.*, 2010). Tanimola *et al.* (2015) reported the presence of alkaloids, tannins, saponins, flavonoids and cardenolides in a preliminary investigation into the phytochemicals in *V. amygdalina* leaf. Also, infrared analyses revealed some functional groups such as phenols, carboxylic acid and amines in *V. amygdalina* leaf (Tanimola *et al.*, 2015). There is need to quantify these phytochemicals in *V. amygdalina* leaves so as to achieve standardization and effectiveness in its usage.

Most botanicals are being applied indiscriminately in large quantities which sometimes increase the cost of production of crops especially when appropriate rate that will achieve effective pest management are not determined. This study was carried out to quantify the phytochemicals in *Vernonia amygdalina* leaf and determine the optimum application rate of powdered *V. amygdalina* leaf that will effectively improve growth, yield, and manage *M. incognita* on okra with a view to reducing the cost of management.

Materials and Methods

Experimental Location

The screenhouse and field experiments were carried out from March to May 2014 and June to September 2017 at the Crop and Soil Science Research Farm, Faculty of Agriculture, University of Port Harcourt (latitude 04°53'38.3" N and longitude 0.06°54'38.0"E), Choba, Rivers State, Nigeria. The research location is at an elevation of 18 m above sea level, temperature of 28-33° C and with rainfall ranging from 2000-2680 mm per annum (GEM, 2012).

Sources of Okra Seeds and Carbofuran .

Okra seeds (V35) were purchased from Rivers State Agricultural Development Programme (ADP), Rumuokoro, Port Harcourt, Rivers State. Carbofuran (3G) was purchased from Amens Agricultural Services, Ibadan, Oyo State, Nigeria.

Source and Preparation of Botanical

Vernonia amygdalina leaves were collected from Victoria Garden, Port Harcourt, Rivers State. The leaves were air-dried on the laboratory bench for six weeks and milled using Kenwood® blender into powder. Milled leaves were packed into 1 litre-bottle, appropriately labelled and kept in a dark cupboard in the laboratory till when needed.

Soil Sterilization

Top loamy-sand soil was collected from the Crop and Soil Science Research Farm and sterilized for four

hours at a temperature of 80° C using a 25 litre-metal drum. Soil was allowed to rest for three weeks in bags kept in the Laboratory prior to use so that the soil can regain stability.

Quantification of Phytochemicals in *Vernonia amygdalina* Leaf

The test for presence and quantification of phytochemicals in *V. amygdalina* leaf were carried out in the Pharmacognosy Research Laboratory and Multidisciplinary Central Research Laboratory respectively, University of Ibadan, Oyo State, Nigeria.

Preparation of Extracts

Methanolic extracts of the samples were prepared following the method of Chan *et al.* (2006). 25 ml of methanol was added to 0.5 g of sample contained in a covered 50 ml centrifuge tube, and shaken continuously for one hour at room temperature. The mixture was centrifuged at 3,000 rpm for 10 minutes, and then the supernatant was collected and stored at -20° C until analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content of each extract was determined according to the Folin–Ciocalteu method used by Chan *et al.* (2006). Some 300 µl of extract was dispensed into test tube (in triplicates). To this was added 1.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 ml of Na₂CO₃ solution (7.5 w/v). The reaction mixture was mixed, allowed to stand for 30 minutes at room temperature before the absorbance was measured at 765 nm (using a spectrophotometer) against a blank prepared by dispensing 300 µL of distilled water instead of sample extract. TPC was expressed as gallic acid equivalent (GAE) in mg/g material. The calibration equation for gallic acid was $Y = 0.0645x - 0.0034$ ($R^2 = 0.9997$).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using aluminium chloride method as reported by Kale *et al.* (2010). 0.5 ml of extract was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was mixed, allowed to stand at room temperature for 30 minutes, before absorbance was read at 514 nm using a spectrophotometer. TFC was expressed as quercetin equivalent (QE) in mg/g material. The calibration equation for quercetin was $Y = 0.0395x - 0.0055$ ($R^2 = 0.9988$).

Determination of Tannin Content

Tannin content of samples was determined according to the method of Padmaja (1989). Sample (0.1g) was extracted with 5 ml of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes. The mixture was centrifuged at 3,000 rpm for 20 minutes. 0.1 ml of the supernatant was added with

7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorbance was measured at 760 nm using a spectrophotometer. Blank was prepared with distilled water instead of the sample. Tannin content was expressed as tannic acid equivalent (TAE) in mg/g material. The calibration equation for tannic acid was $Y = 0.0695x + 0.0175$ ($R^2 = 0.9978$).

Determination of Total Saponins (TSP)

Total saponins (TSP) were determined by the method of Hiai *et al.* (1976) as described by Makkar *et al.* (2007). 0.5 g of sample was extracted with 25 ml of 80% aqueous methanol by shaking on a mechanical shaker for 2 hours, after which contents of the tubes were centrifuged for 10 min at 3,000 rpm. In a test tube, an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 were added. The reaction mixtures in the tubes were heated in a water bath at $60^\circ C$ for 10 minutes. Then tubes were cooled in ice for four minutes and then allowed to acclimatize to room temperature. Subsequently, the absorbance was measured in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

Total Alkaloid Determination

The total alkaloid content in the samples was measured using 1, 10-phenanthroline method described by Singh *et al.* (2004). 100 mg sample powder was extracted in 10 ml 80% ethanol. This was centrifuged at 5000 rpm for 10 min. Supernatant obtained was used for the further estimation of total alkaloids. The reaction mixture contained 1ml plant extract, 1 ml of 0.025M $FeCl_3$ in 0.5M HCl and 1 ml of 0.05M of 1, 10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with the temperature maintained at $70 \pm 2^\circ C$. The absorbance of red coloured complex was measured at 510 nm against reagent blank using a spectrophotometer. Alkaloid contents were estimated and it was calculated with the help of standard curve of quinine (0.1 mg/ml, 10 mg dissolved in 10 ml ethanol and diluted to 100 ml with distilled water).

Pot Experiment

Experimental design, sowing and thinning of okra

Forty (7 litre) pots of diameter 21 cm and depth 20 cm were filled with steam-sterilised loamy-sand soil (5 kg/pot) and arranged using completely randomised design (CRD) with eight treatments and five replications per treatment. The treatments were; *M. incognita* infected-untreated, uninfected, carbofuran at 3 kg ai/ha, milled *V. amygdalina* leaf at 50 kg/ha (5 g/pot), 100 kg/ha (10 g/pot), 150 kg/ha (15

g/pot), 200 kg/ha (20 g/pot) and 250 kg/ha (25 g/pot). Two seeds of okra (V35) were sown per pot and later thinned to one plant per pot at a week after sowing (WAS).

Extraction of *Meloidogyne incognita* Eggs

Meloidogyne incognita eggs were extracted from infected roots of okra plants grown as culture for *M. incognita* using the hypochlorite method (Hussey and Barker, 1973). The infected roots were properly rinsed with water to dislodge dirt and soil particles and chopped into 1-2 cm. The chopped roots were put into conical flask. Then, 0.5% hypochlorite solution was poured into the conical flask and shaken vigorously for four minutes. The content was poured into 200 mesh sieve nested over 500 mesh sieve. The 200 mesh sieve retained debris and roots, while the 500 mesh sieve retained the eggs. The eggs retained in 500 mesh sieve were later rinsed into a beaker using a wash bottle. The content was allowed to settle and later decanted. One millilitre aliquot of suspension containing eggs of *M. incognita* was later dispensed into Doncaster counting dish (Doncaster, 1962) and counted under stereo microscope. The egg suspension was concentrated such that one millilitre had 5,000 eggs.

Inoculation of *M. incognita* and Application of Treatments

At two weeks after sowing (WAS), each okra seedling was inoculated with 5,000 eggs of *M. incognita*. Six holes were made around roots of each seedling to a depth of 5 cm and one ml egg suspension containing 5,000 eggs of *M. incognita* was dispensed into the holes around the rhizosphere of each okra seedling and the holes were later covered with soil. At one week after inoculation (WAI), a total of eight treatments were applied; *M. incognita* infected-untreated, uninfected, carbofuran at 3 kg ai/ha, milled leaves of *V. amygdalina* at 50 kg/ha, 100 kg/ha, 150 kg/ha, 200 kg/ha and 250 kg/ha. Necessary cultural practices such as weeding, watering amongst others were carried out till when experiment was terminated.

Data Collection

At inoculation and fortnightly till eight weeks after inoculation (WAI), data were collected on plant height (cm) and number of leaves per plant. Okra roots were carefully removed per pot, rinsed with water to dislodge soil and were rated for galls using the scale of Taylor and Sasser (1978); 0=No galls or egg masses; 1=1-2 galls or egg masses; 2=3-10 galls or egg masses; 3=11-30 galls or egg masses, 4=31-100 galls or egg masses and 5= more than 100 galls or egg masses. Fresh shoot and root weights (g) were determined using a Mettler® balance. The fresh shoot was oven-dried at a temperature of $70^\circ C$ for 48 hours and later weighed on Mettler® balance to determine dry shoot weight (g). Soil of each pot was thoroughly

mixed and 200 ml soil was taken using a graduated beaker for extraction. The second-stage juveniles (J2) of *M. incognita* were extracted from each sample using the pie-pan method (Whitehead and Hemming, 1965). The population of J2 was determined in similar manner to that of egg population.

Field Experiment

The field experiment was laid out using randomised complete block design on a land area of 159 m² (9.4 m x 17 m) with eight treatments replicated nine times. The research plot was divided into three blocks of 1.8 m x 17 m each. There was an alley of 1 m between each block. Each block was later subdivided into eight sub-plots of 1.8 m x 1 m each with an alley of 1 m between two subplots to accommodate the eight treatments. The treatments were assigned randomly within each block. The pre-planting soil samples were taken to determine the initial population of *M. incognita* present in the research plot. The treatments on the field were similar to that in the pot experiment. Plots assigned *M. incognita* uninfested per block was denematized with carbofuran 3 kg. a. i./ha at three weeks before sowing of okra seeds.

Two okra seeds were sown per hole at a spacing 60 cm x 30 cm and later thinned to one seedling per hole at one week after sowing (WAS). Each treatment within a block has three rows with 3 okra plants per row to produce a plant population of nine okra plants. Since the nematode population was low, each okra seedling was inoculated with 5,000 eggs in the manner in the pot experiment at two WAS. Rhizosphere soil around each okra were carefully removed to a band of 3 cm and milled leaves of *Vernonia amygdalina* at rates of 50 kg/ha, 100 kg/ha, 150 kg/ha, 200 kg/ha and 250 kg/ha and carbofuran 3 kg. a.i./ha were applied at a week after inoculation (WAI). The roots were carefully covered with the soil. Four okra plants were randomly tagged for data collection per treatment within a block. Data were collected in similar manner like in pot experiment.

Data Analysis

Nematode counts were transformed using logarithm transformation [$\text{Log}(x + 1)$] prior to analysis. Data obtained from two trials per pot and field experiments were combined prior to analysis due to similarity in values. Data were analyzed using analysis of variance with SAS (2009) statistical package for all the treatments tested and means separated using Fisher's Least Significant Difference (LSD) at 5% level of probability. Back-transformed data were presented in results.

Results

Concentration of Phytochemicals in *Vernonia amygdalina* Leaf

Concentrations of phytochemicals in milled *Vernonia amygdalina* leaf are presented in Table 1. Saponins had the highest concentration among phytochemicals identified and quantified in *V. amygdalina* leaf, followed by alkaloids, and the lowest concentration of 4.3 mg/g was recorded for phenol.

Effects of Five Application Rates of Powdered *Vernonia amygdalina* Leaf and Carbofuran on the Growth of *Meloidogyne incognita*-infected Okra

The effects of varying rates of air-dried *V. amygdalina* leaf and carbofuran on growth of *M. incognita*-infected okra were assessed using plant height (cm) and number of leaves (Table 2). *Meloidogyne incognita*-infected-okra treated with carbofuran at eight weeks after inoculation (WAI) had the tallest okra plants but this was not significantly taller than okra treated with *V. amygdalina* leaf at 200 and 250 kg/ha. On the field, all treated plants with either carbofuran or *V. amygdalina* leaf had significantly taller plants than *M. incognita* infected-untreated (Table 2). There was no significant difference in plant height of uninfected okra plants and treated okra with *V. amygdalina* leaf at 100,150, 200, 250 kg/ha, and carbofuran at 3 kg.a.i./ha.

At 8 WAI, *M. incognita*-infected okra treated with *V. amygdalina* leaf at 150 kg/ha, 200 kg/ha, 250 kg/ha and carbofuran had the same number of leaves which was significantly higher than number of leaves in *M. incognita* infected-untreated okra. However, there was no significant difference in the number of leaves of carbofuran and *V. amygdalina* leaf- treated okra at the rates of 100-250 kg/ha. The trend observed on number of leaves on the field was similar to that in pot.

Effects of Five Application Rates of Powdered *Vernonia amygdalina* Leaf and Carbofuran on the Fruit Weight of *Meloidogyne incognita*-infected Okra

The highest fresh fruit weight was obtained in the uninfected okra plants in the pot experiment and this was significantly higher than fruit weights of other treated plants (Table 3). There was no significant difference in the fruit weights of *M. incognita* infected okra treated with *V. amygdalina* leaf at the rates of 100-250 kg/ha and carbofuran at 3 kg. a.i./ha. *Meloidogyne incognita*-infected-untreated and *V. amygdalina* leaf-treated okra at 50 kg/ha did not produce fruit when the experiment was terminated (Table 3). In the field, okra treated with *V. amygdalina* leaves at 250 kg/ha produced the highest fruit weight that was significantly higher than fruit weights of other okra plants. All treated plants with either *V. amygdalina* leaves or carbofuran produced more fruits and had higher fruit weights than infected-untreated okra (Table 3).

Effects of Five Application Rates of Powdered *Vernonia amygdalina* Leaf and Carbofuran on Fresh Shoot and Root Weights (g), and Dry Shoot Weight (g) of *Meloidogyne incognita*-infected Okra

Meloidogyne incognita-infected okra treated with *V. amygdalina* leaf at 250 kg/ha had significantly higher fresh shoot weight than other treated okra in the greenhouse (Table 4). The lowest fresh shoot weight was recorded in infected-untreated okra. *Meloidogyne incognita*-infected okra treated with *V. amygdalina* leaf at 50, 100, 150, 200, 250 kg/ha and carbofuran at 3 kg. a.i./ha had their shoot growth improved by 55.1, 114.1, 187.4, 266.7, 317.8 and 232.8%, respectively. All treated okra had significantly higher shoot weights than *M. incognita* infected-untreated okra (Table 4).

The highest fresh root weight was obtained in *M. incognita* infected-untreated okra and this was significantly higher than fresh root weights of other okra plants (Table 4). Okra treated with powdered *Vernonia amygdalina* leaf at 50 kg/ha recorded the lowest fresh root weight amongst the treated okra (Table 4). On the field, there was no significant difference in the fresh root weights of uninfected and *V. amygdalina* treated okra at rates of 200, 250 kg/ha and carbofuran 3 kg. a.i./ha (Table 4).

In the pot experiment, the uninfected okra had the highest dry shoot weight which was not significantly higher ($P \leq 0.05$) than dry shoot weights of carbofuran and *V. amygdalina* leaf treated okra at 250 kg/ha (Table 4). However, all *Meloidogyne incognita*-infected okra treated with powdered *V. amygdalina* leaf and carbofuran had significantly higher fresh shoot weight than infected-untreated okra. The lowest dry shoot weight was obtained in infected-untreated okra. In the field trial, uninfected okra plants had the highest dry shoot weight, but this was not significantly higher than dry shoot weights obtained in *V. amygdalina* treated okra at rates of 100-250 kg/ha and carbofuran. Infected-untreated okra had the lowest dry shoot weight on the field (Table 4).

Effects of Five Application Rates of Powdered *Vernonia amygdalina* Leaf and Carbofuran on Gall Index (Root Damage) of *Meloidogyne incognita*-infected Okra

The highest significant level of root damage (gall index) was obtained in the infected-untreated okra compared to the treated okra plants in the greenhouse (Table 5). Application rates of 50, 100, 150, 200, 250 kg/ha and carbofuran significantly reduced root damage by 44, 64, 80, 80, 80 and 84% in pot; and 31.5, 47.4, 47.4, 47.4, 47.4, and 60.5% in field, respectively than infected-untreated okra. However, there was no significant difference in reduction of root damages among *M. incognita*-infected okra treated with *V. amygdalina* leaf at 150, 200, 250 kg/ha and carbofuran 3 kg. a. i./ha. Similar trend in root damage

and reduction assessment was obtained on the field, but carbofuran significantly reduced root damage in *M. incognita*-infected okra than the *V. amygdalina*-treated okra plants (Table 5). There was no significant difference in the root damages by *M. incognita* in okra treated with *V. amygdalina* leaf at 100, 200 and 250 kg/ha.

Effects of Five Application Rates of Powdered *Vernonia amygdalina* Leaf and Carbofuran on *Meloidogyne incognita* Population on Okra

In the pot experiment, infected-untreated okra obtained the highest significant egg population of *M. incognita* than in other okra plants (Table 6). Other treated okra significantly reduced egg population when compared with infected-untreated. However, the highest reduction was obtained in carbofuran-treated okra and this was not significantly more reduced than *V. amygdalina* leaf-treated okra at 250 kg/ha. Similar trend to that of pot experiment was obtained in the egg population on the field (Table 6).

The highest second-stage juvenile population (J2) of *M. incognita* was obtained in infected-untreated okra in the greenhouse trial. The highest level of reduction in J2 population was obtained in okra plants treated with carbofuran, but this was not significantly higher than reduction in J2 population obtained in *V. amygdalina* leaf-treated okra at 100, 200 and 250 kg/ha. The trend in the field trial was similar to that in the pot experiment. The trend in the final nematode population both in the pot and field trials were similar to trends earlier reported for both eggs and second-stage juvenile populations (Table 6).

Effects of Five Application Rates of Powdered *Vernonia amygdalina* Leaf and Carbofuran on Reproduction of *Meloidogyne incognita* on Okra

There was no significant difference in the rates of reproduction of *M. incognita* on okra in all treated plants with either *V. amygdalina* leaf and carbofuran in both the pot and field trials (Table 7). However, the rate of reproduction of *M. incognita* was significantly more reduced in all okra treated with *V. amygdalina* leaf and carbofuran than infected-untreated okra. The activity of *V. amygdalina* leaf at 150, 200 and 250 kg/ha was as reported in the nematode population data.

Discussion

Meloidogyne incognita-infected okra treated with five rates of *V. amygdalina* leaf and carbofuran performed better in growth when compared to infected-untreated okra due to the fact that the botanical could have enhanced the soil as a nutrient source. This view was supported by Pessoa *et al.* (2002) when they reported increase in plant growth parameters of okra such as plant height and number of leaves when *Occimum gratissimum* powder was applied at 50, 100 and 250 kg per plot on *M.*

incognita-infected okra. It could also mean that *Vernonia amygdalina* possessed some nematicidal ingredients that might have killed the nematodes such as that their population were reduced below economic injury level. At such reduced population, the nematode will not be able to cause economic damage to the plant in terms of its growth and yield (Onifade and Fawole, 1996; Harve and Kamath, 2004; Orech *et al.*, 2005; Tanimola and Adesiyun, 2006). All *M. incognita* infected okra treated with *V. amygdalina* leaf at 150 kg/ha, 200 kg/ha, 250 kg/ha and carbofuran increased growth when compared with infected-untreated okra. These observations showed that the treatments applied had controlling effect on *M. incognita* by reducing the adverse effects on growth of okra. The differences observed in the performance of varying rates of *V. amygdalina* could be as a result of the varying quantities of active nematotoxic ingredients (phytochemicals) per rate in which higher application rate performed better than lower rate.

The nematicidal activity of *V. amygdalina* could also be attributed to the presence of phytochemicals in them such as saponins, alkaloids, tannins, phenols and flavonoids which have also been confirmed present and quantified in this botanical in this study (Chitwood, 2002, Adedire *et al.*, 2003, Musyimi *et al.*, 2008). Alkaloids may be toxic to nematodes by inhibiting the movement of *M. incognita* juvenile or hatching of eggs and by inducing death (Jenkins *et al.*, 1998). Flavonoids, such as quercetin have been reported to inhibit reproduction of *M. javanica* as a soil drench at 400 g/ml (Osman and Viglierchio, 1988; Chitwood, 2002). Tannins have exhibited toxicity towards *M. incognita* juvenile and provided some degree of control of galling when applied (Chitwood, 2002). Saponins have been reported to possess cell membrane-breaking property in which they bind with the lipid membrane of cells, making the cells more permeable and at the same time more fragile, enabling a loss of cell contents through leakage (Bassetti and Sala, 2005). Bassetti and Sala (2005) posited that inhibition of egg-hatch and mortality of the second-stage juveniles of *M. incognita* due to application of botanical extracts with saponins might be due to disruption of membranes that facilitated penetration of other lethal ingredients. Iwalokun *et al.* (2004) reported that *Vernonia amygdalina* possesses antibacterial, antifungal, anti plasmodial and nematicidal properties which also gave credence to its nematicidal activity on *M. incognita* in this study.

The good performance of carbofuran-treated plants in terms of improved growth and yield obtained in this study may be due to the efficacy of the nematicide in controlling *M. incognita* and other parasitic nematodes on okra (Tanimola and Godwin-Geigin, 2009). This view was supported in the report of

Akinlade and Adesiyun (1982) in which they found carbofuran effective in controlling *M. incognita* on okra. Adesiyun *et al.* (1990) also asserted to the effectiveness of carbofuran when they evaluated the toxicity of three systemic nematicides (carbofuran, temik and miral) against root-knot nematodes attacking tomato plant and opined that carbofuran is an effective nematicide. Adegbite and Agbaje (2007) reported that application of carbofuran resulted in increased growth and yield of *M. incognita* infected yam varieties. All these workers attested to the efficacy of this nematicide in controlling root-knot nematodes. The poor growth recorded for the infected-untreated plants were probably due to the stunting action in infected okra facilitated by *M. incognita* and impairment of the efficiency of the roots in absorbing nutrients and water from the soil for good growth and yield (Tanimola and Adesiyun, 2006; Adegbite and Agbaje, 2007).

The highest fresh root weight recorded in infected-untreated okra was due to the presence of galls. The presence of galls on root is an indication of the root damage level and nematode population. The observations on root damage and nematode population showed that all treated plants with either powdered *V. amygdalina* or carbofuran had lesser root damage and nematode population than infected-untreated plants. However, the effective rates start at 100 kg/ha to 250 kg/ha to manage *M. incognita* on okra. The reduction in galling might be due to the fact that the nematode activity, development and reproduction were affected by nematicidal ingredients in *Vernonia amygdalina*.

Powdered *Vernonia amygdalina* leaf at rates of 100-250 kg/ha were effective in improving growth, yield and suppressing *M. incognita* population and damage on okra compared with carbofuran-treated okra. However, rates of 150, 200 and 250 kg/ha showed similar effectiveness in the management of *M. incognita* on okra comparable to carbofuran. The rate of 150 kg/ha is considered optimum in the improvement of growth, yield and management of *M. incognita* on okra since it will also facilitate sustainable use of this botanical resource and reduction in the cost of managing *M. incognita* on okra with *V. amygdalina* leaf.

Table 1: Concentration of phytochemicals in *Vernonia amygdalina* leaf

Phytochemical	Concentration (mg/g)
Saponins	48.2
Alkaloids	19.1
Flavonoids	5.5
Tannin	4.5
Phenol	4.3

Table 2: Effects of five application rates of powdered *Vernonia amygdalina* leaf and carbofuran on growth of *M. incognita*-infected okra at eight weeks after inoculation

Treatments	Plant height (cm)		Number of leaves	
	Pot	Field	Pot	Field
Uninfected	25.9	29.73	17.0	13.16
Infected-untreated	13.8	5.55	10.0	8
<i>V. amygdalina</i> 50 kg/ha	20.9	20.8	15	11.16
<i>V. amygdalina</i> 100 kg/ha	24.7	23.88	16.0	12
<i>V. amygdalina</i> 150 kg/ha	26.2	24.11	18.0	12.3
<i>V. amygdalina</i> 200 kg/ha	27.1	25.11	18.0	12.83
<i>V. amygdalina</i> 250 kg/ha	27.5	28.01	18.0	12.66
Carbofuran 3 kg. a.i./ha	27.9	27.85	18.0	13
LSD (P≤0.05)	0.7	5.4	2.5	1.96

Table 3: Effects of five application rates of powdered *Vernonia amygdalina* leaf and carbofuran on fruit weight of *M. incognita*-infected okra at eight weeks after inoculation

Treatments	Fresh fruit weight (g)	
	Pot	Field
Uninfected	18.56	52.43
Infected-untreated	0	5.85
<i>V. amygdalina</i> 50 kg/ha	0	28.63
<i>V. amygdalina</i> 100 kg/ha	11.13	43.31
<i>V. amygdalina</i> 150 kg/ha	10.76	56.96
<i>V. amygdalina</i> 200 kg/ha	12.86	52.41
<i>V. amygdalina</i> 250 kg/ha	12.5	74.01
Carbofuran 3 kg. a.i./ha	11.7	56.31
LSD (P≤0.05)	2.18	7.41

Table 4: Effects of five application rates of *Vernonia amygdalina* leaf and carbofuran on fresh shoot and root weights (g), and dry shoot weight (g) of *Meloidogyne incognita*-infected okra

Treatments	Fresh shoot weight (g)		Fresh root weight (g)		Dry shoot weight (g)	
	Pot	Field	Pot	Field	Pot	Field
Uninfected	25.9	13.46	9.9	3.53	9.3	5.12
Infected-untreated	10.1	5.48	16.9	15.45	2.6	2.35
<i>V. amygdalina</i> 50 kg/ha	15.3	8.50	5.5	9.72	4.4	3.42
<i>V. amygdalina</i> 100 kg/ha	18.7	11.73	6.6	7.05	4.9	4.14
<i>V. amygdalina</i> 150 kg/ha	21.4	15.75	7.9	5.85	7.7	4.55
<i>V. amygdalina</i> 200 kg/ha	23.5	20.1	8.4	4.48	8.0	4.59
<i>V. amygdalina</i> 250 kg/ha	26.3	22.9	8.7	3.56	8.8	4.72
Carbofuran 3 kg. a.i./ha	25.1	18.24	9.6	4.64	8.7	4.28
LSD (P≤0.05)	0.6	4.1	0.4	1.15	0.6	1.01

Table 5: Effects of five application rates of powdered *Vernonia amygdalina* leaf and carbofuran on gall index (root damage) of *Meloidogyne incognita*-infected okra at eight weeks after inoculation

Treatments	Gall index	
	Pot	Field
Uninfected	0.0	0.0
Infected-untreated	5.0	3.8
<i>V. amygdalina</i> 50 kg/ha	2.8	2.6
<i>V. amygdalina</i> 100 kg/ha	1.8	2
<i>V. amygdalina</i> 150 kg/ha	1.0	2
<i>V. amygdalina</i> 200 kg/ha	1.0	2
<i>V. amygdalina</i> 250 kg/ha	1.0	2
Carbofuran 3 kg. a.i./ha	0.8	1.5
LSD (P≤0.05)	0.4	0.4

Table 6: Effects of five application rates of powdered *Vernonia amygdalina* leaf and carbofuran on *Meloidogyne incognita* population on okra

Treatments	Egg population		J2 population		Final nematode population	
	Pot	Field	Pot	Field	Pot	Field
Uninfected	0g	0	0	0g	0f	0
Infected-untreated	489000	250000	26333.3	21300	515333.3	271300
<i>V. amygdalina</i> 50 kg/ha	37333.3	8000	11666.6	5950	49000	13950
<i>V. amygdalina</i> 100 kg/ha	17166.66	4400	6500	2800	23666.6	7200
<i>V. amygdalina</i> 150 kg/ha	10800	3125	4833.3	1666.66	15633.3	4791.66
<i>V. amygdalina</i> 200 kg/ha	9500	4650	4883.3	700	14383	5350
<i>V. amygdalina</i> 250 kg/ha	8000	1850	4166.6	616.66	12166.6	2466.66
Carbofuran 3 kg. a.i./ha	6333.3	1350	4933.3	300	11266.6	1650
LSD (P≤0.05)	76873.0	427.0	5720.5	1184.48	82019.2	13873.77

J2= second-stage juvenile population

Table 7: Effects of five application rates of powdered *Vernonia amygdalina* leaf and carbofuran on reproductive factor of *M. incognita* on okra at eight weeks after inoculation

Treatments	Reproductive factor	
	Pot	Field
Uninfected	0	0
Infected-untreated	103.1	27.13
<i>V. amygdalina</i> 50 kg/ha	9.8	1.39
<i>V. amygdalina</i> 100 kg/ha	4.7	0.72
<i>V. amygdalina</i> 150 kg/ha	3.1	0.47
<i>V. amygdalina</i> 200 kg/ha	2.9	0.53
<i>V. amygdalina</i> 250 kg/ha	2.4	0.24
Carbofuran 3 kg. a.i./ha	2.3	0.16
LSD (P≤0.05)	16.4	1.38

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3/29/2018