ORIGINAL RESEARCH ARTICLE   **OPEN ACCESS**

EFFICACY OF AQUEOUS EXTRACTS OF *RHIZOPHORA MANGLE* L. BARK IN THE MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON OKRA

*Adebowale A. Tanimola, Amina O. Asimiea and Sonia Polycarp

Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, Nigeria

ABSTRACT

Meloidogyne incognita (*MI*) contributes to yield losses in okra worldwide. *Rhizophora mangle* (*RM*) has been used in the management of insects, but not on *MI*. A pot experiment was laid out in completely randomized design to determine the effects of aqueous bark extracts of *RM* and carbofuran on *MI*-infected okra. *Meloidogyne incognita*-infected okra plants were treated with *RM* aqueous extracts of 20%, 10%, 5% weight per volume (w/v) and carbofuran 3 kg. a.i./ha one week after inoculation (WAI). Data were collected on growth parameters fortnightly till 8 WAI. At 8 WAI, data were collected on fruit weight, fresh shoot and root weights, dry shoot weight, Gall index, nematode population and reproductive factor; and were appropriately analyzed. *Rhizophora mangle* at 20% w/v compared favourably with carbofuran in terms of growth and yield of treated okra. Okra treated with carbofuran and *RM* at 20% w/v had significantly higher fresh and dry shoot weight, and fruit yield than infected-untreated okra. *Rhizophora mangle* at varying concentration levels and carbofuran reduced gall index, nematode population and reproductive factor of *MI* in treated okra significantly ($p < 0.05$) compared to infected-untreated okra. Efficacy of *RM* extract was concentration dependent in the order of 20% w/v >10% w/v >5% w/v. Aqueous bark extracts of *RM* especially at 20% w/v promises to be an effective environment-friendly nematicide in the management of *M. incognita*-infected okra.

INTRODUCTION

Okra has high nutritional value containing carbohydrate, protein, fats, minerals, and vitamins (El-Kader *et al.,* 2010) as well as calcium, ascorbic acid and iodine (Uwah *et al.,* 2010). It has medicinal benefits (Gurbuz, 2003; Gosslau and Chen, 2004) with antioxidant properties due to inherent components like carotenoids, phenolic compounds and flavoinoids (Gemede *et al.,* 2014). Okra is also cultivated for industrial use as fibre (Hussain *et al*., 2012). The world okra production was estimated at 4.54 million tons in 2010 and cultivated on 0.43 million hectares with India as the world's largest producer (67.1%) and then Nigeria (15.4%) (Varmudy, 2011).

Corresponding author: **Adebowale A. Tanimola,**
Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, Nigeria.

Several pests such as plant-parasitic nematodes, especially the root-knot nematodes (*Meloidogyne* species) amongst others cause damages and contribute to low yields in okra (Hussain *et al.,* 2012; Mukhtar *et al.,* 2014). Crop loss and yield decrease attributed to *Meloidogyne* species is widely reported (Sikora and Fernandez, 2005; Moens *et al.,* 2009). Mukhtar *et al.* (2014) reported a reduction in yield of okra caused by *M. incognita* on okra with damage greater in younger plants than in older plants. Anwar and McKenry (2012) reported that *M. incognita* was the most abundant plant-parasitic nematode affecting vegetables such as carrots, chilies, cabbage, cucumber, pumpkin, and okra amongst others and were responsible for 35% yield loss in okra. In Nigeria, *M. incognita* has been reported as a bane in okra production (Akinlade and Adesiyan, 1982). The use of biopesticides and botanical pesticides in the management of *M. incognita* had been reported effective compared with inorganic nematicides (Fawole, 2009; Claudius-Cole *et al.*, 2010; Mazid *et al.,* 2011; Tanimola and Akarekor, 2014). The use of conventional

nematicides like carbofuran is expensive, required skill and also environment-unfriendly especially when applied indiscriminately. Management of pests with botanicals is being encouraged due to the high degradability of these products when used. However, many plants are yet to be screened for pesticidal potentials against specific pests such as plant-parasitic nematodes (Ofuya, 2009). Mangrove forests are considered to be of great ecological, economic and social significance (Gopal and Chauhan, 2006). Thathoi *et al*. (2013) posited that approximately 90% of mangroves are distributed in South-East Asia, America, and Africa and that they are adapted to high salinity, tidal flooding and abundance of nutrients. Mangroves are used for medicinal purposes (Revathi *et al.,* 2013; Meenakshi and Jayaprakash, 2014), coastal reclamation (Kitaya *et al.,* 2002) as well as biopesticides (Meenakshi and Jayaprakash, 2014). The red mangroves, *Rhizophora mangle* L. contains phytochemicals such as saponins, glycosides, flavonoids, and tannins, which have been used as an effective larvicide against some 4th instar larvae of *Anopheles gambiae* (Angaye *et al.,* 2014). Other mangroves have steroids, terpenes and alkaloids in them (Meenakshi and Jayaprakash, 2014). Also, some biological compounds isolated from these mangrove plants have antibacterial, antifungal, antiviral and anthelminthic properties (Piyusha *et al*., 2012). Mangrove plants contain phytochemicals which are reported to be effective in the management of plant-parasitic nematodes (Chitwood, 2002). It is important that new botanicals for nematode management should be identified and appropriate methods of their usage be determined to preserve the environment and increase okra production. This study seeks to proffer a safer and more environment-friendly nematicide for the control of *M. incognita* on okra. The mangrove plants grow abundantly in the Niger-Delta, inexpensive, readily available and technically manageable by local farmers. This study provides information on the efficacy of aqueous extract of *Rhizophora mangle* bark on *Meloidogyne incognita* infecting okra.

MATERIALS AND METHODS

Study site

The research was conducted at the Teaching and Research Farm of the University of Port-Harcourt, Rivers State, Nigeria. The University is located at latitude 4.42°N and longitude 4.51°E with 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 *Rhizophora mangle* bark the seeds of okra (Choba local) were procured from Choba market in Rivers State, Nigeria. *Rhizophora mangle* bark was collected locally from a timber market at Illoabuchi within Rivers State.

Preparation of *Rhizophora mangle* aqueous extracts and carbofuran

Rhizophora mangle bark was air-dried for seven weeks and ground into powder using a commercial milling machine. Aqueous extracts of different concentrations of *Rhizophora mangle* were obtained by varying the weight of the powder in a constant volume of water. For instance, 25 g, 50 g and 100 g of red mangrove bark were soaked in 500 ml of water for three days to obtain 5%, 10%, and 20% weight per volume (w/v) concentrations of aqueous extracts of *R. mangle*, respectively.

The resulting suspension was filtered using a 200 mesh to obtain the extract. Also, carbofuran 3G was applied as treatment at the rate of 3 kilogramme active ingredient per hectare (a. i./ ha).

Experimental design

The pot experiment was laid out in a completely randomized design. 30 plastic pots (12 liter) of 28 cm diameter and 26 cm depth were each filled with 10 kg of steam-sterilized sandy-loam top soil. Two okra seeds were sown per pot and eventually thinned to one seedling per pot at one week after sowing. The experiment had six treatments with five replicates each. The treatments were; *M. incognita*-inoculated okra treated with *R. mangle* 5 % w/v, inoculated okra treated with *R. mangle* 10% w/v, inoculated okra treated with *R. mangle* 20% w/v, inoculated okra treated with Carbofuran 3 kg.a.i./ha, *M. incognita*-inoculated okra with neither *R. mangle* nor Carbofuran (Control 1 or C1), and uninoculated okra with neither *R. mangle* nor carbofuran (Control 2 or C2). *Meloidogyne incognita* eggs were extracted from infected roots of okra using the method of Hussey and Barker (1973). The eggs were appropriately quantified using standard procedures. At two weeks after sowing (WAS), each okra seedling was inoculated with 10,000 eggs of *M. incognita* except the uninoculated control okra plants. The inoculation was carried out by introducing nematode egg suspension of 2 ml containing 10,000 eggs of *M. incognita* using hypodermic syringe into four holes at a depth of 4 cm made at the base of the roots of each okra seedling. The holes were later covered with soil. Distilled water was introduced for uninoculated control okra. 200 ml of varying concentrations of aqueous extracts of *R. mangle* and carbofuran were applied as drench near to the root system of okra per pot at three weeks after sowing, except plants assigned uninoculated and inoculated controls treatments.

Data collection

Data were collected on growth parameters such as plant height (cm) using measuring tape, number of leaves that was visually counted, and stem diameter (mm) using electronic digital Vernier caliper immediately after inoculation and fortnightly till termination of experiment at eight weeks after inoculation (WAI). At 8 WAI, data were collected on fresh fruit weight (g), fresh shoot and root weights (g) per okra plant using an electronic weighing balance. Each root system was carefully dug out and rated for galls to determine the gall index 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. Dry shoot weight (g) was also determined after the fresh shoot was oven-dried at 70° C for 48 hours and weighed using electronic balance. Eggs of *M. incognita* were extracted from infected roots of okra using the method of Hussey and Barker (1973). The infected roots were properly rinsed with water to remove dirt and chopped into 1-2 cm. The chopped roots were put into a conical flask. Sodium hypochlorite solution (0.5%) was poured into conical flask and shaken for four minutes. The content was poured into sieves of 200 mesh placed on 500 mesh. The 200 mesh sieve was used to retain the roots and debris, while the 500 mesh sieve was used to retain eggs. The eggs retained were later rinsed into a beaker using wash bottle and the content was allowed to settle down and later decanted.

The egg population was determined by dispensing 1 ml of the nematode egg suspension into a counting dish and counting was carried out under a compound microscope using tally counter. Second-stage juveniles of *M. incognita* were also extracted from the soil (Whitehead and Hemming, 1965). 200 ml soil was collected from each thoroughly mixed pot and then taken to the laboratory where it was set up in an extraction tray. Water was poured between the extraction tray and the sieve. The set-up was left for 48 hours after which the nematode suspension was poured into sample bottles and stored for counting in a refrigerator at 10° C The final nematode population (Pf) was calculated by the addition of eggs and second-stage juveniles' population. Also, reproductive factor (RF) was calculated using the formula Pf/Pi in which Pi= initial nematode population of 10,000 eggs. The experiment was repeated with no modification.

Data analysis

Data collected from the two trials were combined for analysis and means presented. Nematode population counts were transformed using Log_{10} (x+1) prior to analysis to ensure conformity to normal distribution and the back transformed means were presented. Data were analyzed using analysis of variance and means were separated with least significant difference (LSD) at 5% probability level using Statistical Analysis System (SAS, 2009).

RESULTS

Effects of aqueous extracts of *Rhizophora mangle* and carbofuran on growth of *M. incognita*-infected okra

Effects of *Rhizophora mangle* aqueous bark extract and Carbofuran on growth of *Meloidogyne incognita*-infected okra plants were assessed using plant height, number of leaves and stem diameter; and the results are presented on Tables 1, 2 and 3, respectively. Table 1 showed variation in mean plant height between 2 WAI and 8 WAI across treatments.

Table 1. Effects of different concentrations of aqueous extract of *R. mangle* bark and Carbofuran on mean plant height (cm) of *M. incognita*-infected okra

	Weeks after inoculation			
	2	4	6	8
C2	22.14	41.54	49.86	55.20
C1	14.14	21.68	25.94	22.54
R. mangle 5% w/v	18.46	34.40	42.72	48.22
R. mangle 10% w/v	17.32	24.60	29.76	34.02
R. mangle 20% w/v	22.12	40.92	46.32	57.38
Carbofuran 3 kg.a.i/ha	18.10	31.96	37.26	41.42
LSD (P≤0.05)	5.97	12.6	14.1	15.2

C1=inoculated-untreated; C2= uninoculated.

Table 2. Effects of different concentrations of aqueous extract of *R. mangle* bark and Carbofuran on number of leaves of *Meloidogyne incognita*-infected okra

	Weeks after inoculation			
	2	4	6	8
C2	41.0	58.0	91.0	102.0
C1	29.0	43.0	60.0	69.0
R. mangle 5% w/v	38.0	68.0	83.0	96.0
R. mangle 10% w/v	30.0	58.0	73.0	84.0
R. mangle 20% w/v	41.0	73.0	98.0	119.0
Carbofuran 3 kg.a.i/ha	38.0	75.0	92.0	104.0
LSD (P≤0.05)	10.2	22.9	21.9	28.5

C1=Inoculated-untreated; C2= Uninoculated.

All the treated plants had averagely the same mean plant height after inoculation. Eight weeks after inoculation, the tallest okra plants were those treated with *R. mangle* 20% w/v, but were not significantly (P≤0.05) taller than uninoculated control okra. However, at 8 weeks after inoculation (WAI), the plants treated with *R. mangle* at all concentrations and Carbofuran were significantly (P≤0.05) taller than the inoculated control plants. Two weeks after inoculation, there was no significant (P≤0.05) difference in the number of leaves amongst treatments (Table 2). At 8 WAI, *R. mangle* 20% w/v had the highest mean number of leaves which was not significantly (P≤0.05) more than number of leaves of plants treated with Carbofuran and uninoculated okra plants (Table 2). Effects of different concentrations of aqueous mangrove bark extract and carbofuran on stem diameter of *M. incognita*–infected okra showed that plants treated with *R. mangle* 20% w/v had the highest mean stem diameter which was not significantly (P≤0.05) higher than stem diameter of okra plants treated with Carbofuran and uninoculated okra at 8 WAI (Table 3).

Table 3. Effects of different concentrations of aqueous extract of *R. mangle* bark and Carbofuran on mean stem diameter (mm) of *M. incognita*-infected okra

	Weeks after inoculation			
	2	4	6	8
C2	4.27	5.85	7.10	7.87
C1	2.45	4.34	5.23	6.19
R. mangle 5% w/v	4.27	6.37	7.42	7.94
R. mangle 10% w/v	3.97	6.08	7.22	7.85
R. mangle 20% w/v	4.77	6.97	8.01	8.66
Carbofuran 3 kg.a.i/ha	4.62	6.54	7.60	8.11
LSD (P≤0.05)	1.41	1.15	1.16	1.27

C1=Inoculated-untreated; C2= Uninoculated.

Effects of aqueous extracts of *Rhizophora mangle* and carbofuran treatments on fresh shoot and root weights, dry shoot weight and fruit weight of *M. incognita*-infected okra

Okra plants treated with Carbofuran recorded the highest mean fresh shoot weight which was not significantly (P≤0.05) higher from plants treated with *R. mangle* mangrove at 20% w/v and uninoculated okra (Table 4). The inoculated-untreated okra plants (C1) recorded the highest mean fresh root weight which was significantly (P≤0.05) higher than all other mangrove treatments, Carbofuran and uninoculated plants. The highest mean dry shoot weight was recorded in the uninoculated okra plants and this was significantly (P≤0.05) higher than mean dry shoot weights of other okra plants. However, there was no significant difference in the mean dry shoot weights of okra treated with carbofuran and aqueous bark extracts of *R. mangle* 20%, 10% and 5% w/v treated okra plants. Inoculated-untreated plants had the significantly lowest mean dry shoot weight among treatments. Carbofuran-treated plants had the highest mean fresh fruit weight that was not significantly higher than fruit weight recorded in uninoculated and *R. mangle* 20% w/v treated okra plants (Table 4)

Effects of aqueous extracts of *Rhizophora mangle* and carbofuran on gall index, egg population, second-stage juveniles, final nematode population and reproductive factor of *M. incognita* on okra

There were very few galls on the roots of the plants treated with carbofuran plants and the highest level of galling was observed in inoculated-untreated okra plant which was

Table 4: Effects of different concentrations of aqueous mangrove bark extract and Carbofuran on fresh shoot weight (g), fresh root weight (g), fruit weight (g) and dry shoot weight (g) of *M. incognita*-infected Okra

Treatments	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Fruit weight (g)
C2	17.80	10.00	7.60	11.60
C1	9.60	17.00	1.80	0.00
R. mangle 5% w/v	12.00	8.20	2.20	3.60
R. mangle 10% w/v	12.00	6.40	3.32	8.00
R. mangle 20% w/v	20.60	8.20	3.80	10.80
Carbofuran 3 kg.a.i/ha	22.40	8.20	4.00	16.80
LSD (P≤0.05)	9.57	4.18	2.95	6.61

C1=Inoculated-untreated; C2= Uninoculated.

Table 5. Effects of different concentrations of aqueous mangrove bark extract and Carbofuran on egg population, J2 population, final population and reproductive factor of *M. incognita*-infected okra

Treatments	Gall index	Egg population	J2 population	Final nematode population	Reproductive factor
C2	0	0	0	0	0
C1	4.4	96000	6000	102000	10.2
R. mangle 5% w/v	3.0	49200	3600	52800	5.2
R. mangle 10% w/v	1.6	8000	2000	10000	1.0
R. mangle 20% w/v	1.4	3600	2800	6400	0.6
Carbofuran 3 kg.a.i/ha	1.2	3100	2200	5300	0.5
LSD (P≤0.05)	0.4	11030	1055	11356	1.1

C1=Inoculated-untreated; C2= Uninoculated. J2= Second-stage juveniles.

significantly (P≤0.05) higher than the values recorded in other treated plants (Table 5). However, all red mangrove-treated and carbofuran-treated okra plants had significantly lower gall indices compared with those of inoculated-untreated okra. The highest egg population was obtained from roots of inoculated-untreated okra plants and this was significantly higher than those of okra plants treated with *R. mangle* at 20% and 10% w/v. Plants treated with carbofuran the lowest mean number of *M. incognita* eggs in their roots (Table 5). The soil of inoculated-untreated okra had the highest second-stage juveniles and this was significantly higher than other treated soils. Carbofuran-treated soil had the lowest mean number of second-stage juveniles. The soil of all the plants treated with botanicals had lower numbers of second-stage juveniles of *M. incognita* compared to inoculated-untreated okra plants. However, the highest final nematode population was recorded in inoculated-untreated okra plants which were significantly higher than other treatments. Reproductive factor of *M. incognita* was highest in inoculated-untreated okra plants and this was significantly higher (P≤0.05) than plants treated with carbofuran, and all other levels of treatments. carbofuran had the lowest reproductive factor which was not significantly lower than *R. mangle* at 20% w/v.

DISCUSSION

All *M. incognita*-infected okra treated with either the varying concentrations of aqueous extracts of *R. mangle* and carbofuran had better growth than inoculated-untreated plants. The plants treated with botanicals and carbofuran had taller okra plants, mean number of leaves and stem diameter due to the nematicidal effects of the red mangrove plant extract and carbofuran (Chitwood, 2002) which suppressed the adverse effects of *Meloidogyne incognita* on okra growth. Most plants including *R. mangle* have been reported to contain phytochemicals which are pesticidal (Chitwood, 2002; Adeniyi *et al.*, 2010; Piyusha *et al.*, 2012; Angaye *et al.,* 2014). These phytochemicals with nematicidal effects might have facilitated improved physiological processes that resulted in the observed better growth in *R. mangle*-treated plants than in inoculated-untreated okra.

It is suggested that the poor growth reported in the inoculated-untreated okra may be as a result of the stunting action of *M. incognita* and the impairment of the efficiency of the roots in absorbing nutrients from the soil for good growth and yield (Tanimola and Adesiyan, 2006; Adebgite and Agbaje, 2007). Okra plants administered 20% w/v aqueous bark extract of *R. mangle* recorded the best improvement in growth compared with *M. incognita*-infected-untreated due to likely higher concentration of phytochemicals at this treatment level which exerted more nematicidal effects than at other concentration levels. Tiwari *et al.* (2011) opined that efficacy of botanicals on plants are concentration dependent in which the higher concentration is more effective than the lower. Plants treated with carbofuran and *R. mangle* 20% w/v were not significantly different in fresh shoot weight.

Uninfected plants recorded highest dry shoot weight since the plants were able to perform all physiological process and assimilates produced were built into the plant structures, instead of where they were channelled into the feeding zones for *M. incognita* in infected-untreated and okra treated with *R. mangle*. *Meloidogyne incognita* infected-untreated okra plants recorded highest fresh root weight due to the additional weight of the gall that was formed on the roots. The production of fruits in all the *M. incognita* infected okra treated with aqueous bark extract of *R. mangle* and none in infected-untreated okra might be due to reduction in the damaging effects of *M. incognita* in the treated okra plants. This might have facilitated better efficiency of various physiological processes such as photosynthesis that led to better growth and yield compared with infected-untreated okra. Photosynthetates produced through photosynthesis might have been properly partitioned into fruit development in treated okra plants than in managing nematode infection in inoculated-untreated okra. However, the production of fruits in all infected, but treated okra and none in uninfected showed that the infection by *M. incognita* might have hastened the growth and likewise the production of fruits as an escape mechanism to compensate for damages due to infection. The no difference in the fruit weight of carbofuran and aqueous bark extract of *R. mangle* 20% w/v treated okra showed that the botanical at this concentration was effective

and mitigated the destructive effects of *M. incognita* on yield of treated okra The *M. incognita* infected-untreated okra had the highest number of galls, egg population on roots and juvenile population in the soil which implies that *M. incognita* produced freely since there was no check. Carbofuran is effective for the management of *M. incognita* on infected okra evident by significant reduction in root damage, nematode reproduction and population. *Rhizophora mangle* 20% w/v compared effectively in this wise with carbofuran and this might be due to the antihelminthic properties of the plant (Piyusha *et al.*, 2012). From the foregoing, aqueous bark extracts of *R. mangle* promises to be an effective, cheap farmer and environment-friendly botanical nematicide in the management of *M. incognita*-infected okra. Further field trials are suggested to determine effective concentrations and formulations for field application, profitability and to validate the findings in this report.

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