Nematicidal Effect of Aqueous Extract of *Ocimum gratissimum* (L.) Leaves on *Meloidogyne incognita* on Okra

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**ABSTRACT**

Aqueous extract of *Ocimum gratissimum* (20% w/v) leaves at two doses of 10 and 20 ml, and carbofuran were evaluated for nematicidal effect on *Meloidogyne incognita*-infected okra in a pot experiment. The experiment was laid out in completely randomized design with five treatments (*O. gratissimum* 20 ml, *O. gratissimum* 10 ml, carbofuran at 3 kg a.i./ha, inoculated-ununtreated and uninoculated control) in four replicates. Okra seedlings at two weeks after sowing were inoculated each with 5,000 eggs of *M. incognita* except uninoculated control. Okra seedlings were treated with carbofuran and aqueous extracts of *O. gratissimum* leaves at one week after inoculation (WAI). Data were collected on gall index at 7 WAI on number of leaves, plant height and number of fruits at 12 WAI. Aqueous leaf extract of *O. gratissimum* at the rate of 20 ml offered nematicidal effect comparable with carbofuran evident in improvement of growth, fruit production and reduction in root damage compared to inoculated-ununtreated okra. *Meloidogyne incognita*-infected okra treated with 10 ml aqueous leaf extract of *O. gratissimum* were not effective in the management of the nematode since infected-treated okra died at eight WAI. Aqueous extract of *Ocimum gratissimum* (20% w/v) leaves at 20 ml/5 kg soil compared effectively with carbofuran in the management of *M. incognita* on okra.

**INTRODUCTION**

Okra, *Abelmoschus esculentus* (L.) Moench is one of the most important vegetables in Nigeria (Christo and Onuh, 2005; Farinde et al., 2007). It originated probably from East Africa and today is widely distributed in the tropics, sub-tropics and warmer portions of the temperate region (ECHO, 2003). Okra is economically important and can be found in almost every market in Africa (Christo and Onuh, 2005). Katung and Kashina (2005) reported that okra is consumed by many throughout Nigeria and its leaves are also fed to livestock. Industrially, okra mucilage is usually used to glace certain papers and also useful in confectionery among other uses (Markose and Peter, 1990).

Plant-parasitic nematodes (PPNs) are one of the major bio-constraints to production of okra with a notable PPN, *Meloidogyne incognita* reported on okra in Nigeria (Akinlade and Adesiyan, 1982; Adesiyan et al., 1990). *Meloidogyne incognita* causes major damages on okra worldwide and is implicated in yield loss of 90.9% in okra at an inoculum density of 3-4 juveniles/g soil under field conditions (Bhatti and Jain, 1977; Mangala and Mauria, 2006). The symptoms of *M. incognita* infection include formation of root galls which results in reduction in growth, nutrient and water uptake, increased wilting, mineral deficiency, weak and poor yielding plants (Abad et al., 2003). Vegetable crops grown in warm climates can experience severe losses from root-knot nematodes, and are often routinely treated with chemical nematicides (Adesiyan et al., 1990).

Chemical control is expensive and is only economically viable for high value crops, and could create a potential hazard to the environment and human health. Moreover, there has been deregistration of some hazardous nematicides and there is increasing pressure on farmers to use non-chemical pest control methods that do not pollute the environment to satisfy the concept of organic agriculture (Lampkin, 1990; Adekunle and Fawole, 2003). For these reasons, there is a shift to the use of other options such as resistant crop varieties, crop rotation, natural products with nematicidal potentials such as plant extracts, root exudates (botanicals), biocontrol, cultural practices amongst others in managing PPNs (Chitwood, 2002). Plant extracts have the advantages of being cheap and readily available over the conventional nematicides. Some plant extracts have been reported effective, cheap and environment-safe for the management of PPNs in Nigeria (Oyedunmade et
al., 1992; Adegbite and Adesiyin, 2001; Adekunle and Fawole, 2003; Fawole, 2009). Botanicals, being environment-safe in an environmentally conscious world also hold promise for their acceptability and use by resource-poor African farmers.

Leaf extracts of certain plants are known to have nematicidal or nematostatic properties against several plant-parasitic nematodes (Siddiqui and Alam, 1989; Hussain et al., 1996). Scent leaf, Ocimum gratissimum L. is of the family Fabaceae and widely distributed in the tropical and warm temperate regions where it is grown for the essential oils in its leaves and stems (Sulistiarini, 1999). Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties (Dubey et al., 2000). The plant extract is used against gastrointestinal helminths of animals and man (Fakae, 2000; Pessoa et al., 2002; Holets et al., 2003). This justifies the trial of aqueous extract of O. gratissimum as an alternative in the management of Meloidogyne incognita. The study was carried out to determine the efficacy of aqueous extract of O. gratissimum leaves (20% w/v) at rates of 10 and 20 ml as a botanical nematicide in comparison with a synthetic nematicide, carbofuran in the management of M. incognita on okra.

MATERIALS AND METHODS

Study location:
The study was carried out at the Research Farm and Laboratory of the Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, Rivers State, Nigeria

Sources of okra seeds, Ocimum gratissimum and carbofuran:
OKra seeds (V35) were collected from the Rivers State Agricultural Development Project (ADP), Port Harcourt, Rivers State, Nigeria. Fresh green leaves of O. gratissimum were collected from the Faculty of Agriculture Farm within University of Port Harcourt; whereas, carbofuran was purchased at Amens Agricultural Services, Ibadan, Oyo State, Nigeria.

Soil collection and sterilization:
Top sandy-loam soil was collected within a depth of 0-15 cm at the Crop and Soil Science Research Farm, University of Port Harcourt, and was steam-sterilized using standard procedures. The soil was then allowed to cool and later stored in bags for four weeks to facilitate rest and to regain its stability.

Experimental design:
Five-litre pots (22 cm diameter and 21 cm depth) were filled with steam-sterilized soil (5 kg/pot) and arranged using completely randomized design. The five treatments with four replicates in the study were; aqueous extracts of O. gratissimum leaves at 10 ml/5 kg of soil, O. gratissimum at 20 ml/5 kg of soil, carbofuran at 3 kg a.i./ha, inoculated-untreated plant and uninoculated control (No infection by M. incognita).

Sowing of okra seeds, extraction and inoculation of M. incognita:
Two seeds of okra were sown per pot and later thinned to one plant per pot at one week after sowing (WAS). The extraction of eggs of M. incognita from M. incognita-infected okra roots was achieved using the hypochlorite method (Hussey and Baker, 1973). The infected roots were properly rinsed with water to dislodge dirt and chopped into 1-2 cm. The chopped roots were placed in a conical flask and 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 on 500 mesh sieve. The 200 mesh sieve was used to retain the roots and the debris, while the 500 mesh sieve was used to retain the eggs which were later rinsed into a beaker using a wash bottle. The content was allowed to settle down and later decanted. Eggs were later counted in a Doncaster dish (Doncaster, 1962) under stereomicroscope at X40 magnification. Each plant was inoculated with 5,000 eggs of M. incognita at 2 WAS except uninoculated okra.

Preparation, storage and application of aqueous extract of Ocimum gratissimum leaves:
Four hundred gram of thoroughly washed and chopped fresh green leaves of O. gratissimum was crushed in a wooden mortar. Two litre of hot water (70°C) was added to the plant paste, kept for one hour and filtered through muslin cloth to obtain the aqueous extract concentration of 20% w/v. The extract obtained was considered as the stock solution and it was stored in a sterile plastic container and kept in a refrigerator at 4°C.

Application of aqueous extract of Ocimum gratissimum leaves and carbofuran:
At one week after inoculation (WAI), soil around roots of okra were carefully excavated, granules of carbofuran at 3 kg a.i./ha (0.3 g/pot), 10 ml and 20 ml of the stock solution of aqueous extract of O. gratissimum were applied around roots of okra seedlings they were assigned to. The roots were appropriately covered after application of aqueous extract of O. gratissimum and granules of carbofuran.
Data collection:

Growth parameters such as plant height (cm) and number of leaves were collected at 12 WAI. At maturity (12 WAI), yield parameters, such as numbers of fruits per plant was visually counted, and fresh fruit weight (g) was determined using Mettler ® balance. The okra roots after each harvest were carefully dug out and rated for galls using the scale of Taylor and Sassar (1978);

\[0=\text{No} \\text{galls or egg} \text{masses, } 1=1-2 \text{galls or egg masses, } 2=3-10 \text{galls or egg masses, } 3=11-30 \text{galls or egg masses, } 4=31-100 \text{galls or egg masses, and } 5=\text{more than} \text{100 galls or egg masses}\]

Data analysis:

Data were analysed using analysis of variance (ANOVA) with SAS (2009) and means were partitioned using Fisher’s least significant difference (LSD) at 5% level of probability.

Results:

Effects of aqueous extracts of Ocimum gratissimum and carbofuran on growth of M. incognita-infected okra

At 12 weeks after inoculation (WAI), carbofuran-treated okra showed the highest mean plant height (45.5 cm), but this was not significantly higher than those of okra treated with aqueous extract of O. gratissimum at 20 ml and uninoculated plants. Infected-untreated okra and those treated with aqueous extracts of O. gratissimum at 10 ml had died at eight WAI before the experiment was terminated at 12 WAI.

At 12 WAI, okra treated with aqueous extracts of O. gratissimum at 20 ml/5 kg had the highest mean number of leaves (13.3) and this was significantly more (P≤0.05) than from other treatments. However, inoculated-untreated and infected okra treated with 10 ml of aqueous extract of O. gratissimum died before 12 WAI.

Table 1: Effects of Ocimum gratissimum and carbofuran on growth of M. incognita-infected okra at 12 WAI.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated-untreated</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>O. gratissimum 20 ml</td>
<td>44.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Carbofuran 3 kg a.i/ha</td>
<td>45.5</td>
<td>11.3</td>
</tr>
<tr>
<td>O. gratissimum 10 ml</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>43.3</td>
<td>11.0</td>
</tr>
<tr>
<td>LSD (P&lt;0.05)</td>
<td>6.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>

WAI= weeks after inoculation. a.i./ha=active ingredient/hectare

Effects of Ocimum gratissimum and carbofuran on number of fruits and fresh fruit weight of M. incognita-infected okra.

The results presented in Table 2 shows that M. incognita-infected okra treated with carbofuran at 3 kg a.i./ha as well as the plants treated with aqueous extract of O. gratissimum at 20 ml produced two fruits at the time the experiment was terminated, but this was not significantly higher than number of fruits produced in uninoculated okra. The highest fresh fruit weight of 10.4 g was recorded in okra treated with carbofuran and this was significantly higher than fruit weights in O. gratissimum (20 ml) treated and uninoculated okra.

Table 2: Effects of Ocimum gratissimum and carbofuran on number of fruits and fruit weight (g) of M. incognita-infected okra.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of fruits</th>
<th>Fruit weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated-untreated</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>O. gratissimum at 20 ml</td>
<td>2.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>2.0</td>
<td>10.4</td>
</tr>
<tr>
<td>O. gratissimum at 10 ml</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>1.3</td>
<td>8.3</td>
</tr>
<tr>
<td>LSD (P&lt;0.05)</td>
<td>1.0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Effects of aqueous extracts of Ocimum gratissimum and carbofuran on gall Index (root damage) of M. incognita-infected okra:

The highest level of galling of 4.5 was recorded in untreated M. incognita-infected okra and this was significantly higher than the level of galling recorded in other inoculated and treated okra (Table 3). The lowest gall index was observed in carbofuran-treated okra (1.3), but this was significantly lower than that of aqueous extract of O. gratissimum (20 ml) treated okra (2.0). However, carbofuran and aqueous extract of O. gratissimum (20 ml) treated okra significantly had lower gall indices than inoculated-untreated okra (Table 3).
Table 3: Effects of aqueous extracts of Ocimum gratissimum and carbofuran on gall index of okra infected with M. incognita

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Gall index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated-untreated</td>
<td>4.5</td>
</tr>
<tr>
<td>Ocimum gratissimum at 20 ml</td>
<td>2.0</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>1.3</td>
</tr>
<tr>
<td>Ocimum gratissimum at 10 ml</td>
<td>4.0</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>0.0</td>
</tr>
<tr>
<td>LSD(d&lt;0.05)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Discussion:

Effects of Ocimum gratissimum on growth and yield of M. incognita-infected okra:

Aqueous extracts of Ocimum gratissimum showed nematicidal activity on M. incognita, but the activity is dependent on the volume of extracts applied as evident in the 20 ml of the extracts that showed more potency than the 10 ml in terms of improvement in growth, yield and reduction in root damage. The nematicidal effectiveness of O. gratissimum has been reported by some workers (Holets et al., 2003; Olabiyi and Oyedunmade, 2003) which corroborated the finding in the study. Olabiyi and Oyedunmade (2003) reported that marigold (Tagetes erecta), African basil (Ocimum gratissimum), nitta (Hyptis suaveolens) and rattle weed (C. retusa) have nematicidal properties that prevented egg-hatching and killed juveniles of root-knot nematodes in vitro. It might be that 20 ml aqueous extracts of O. gratissimum leaves applied on okra might either have reduced or prevented hatching of M. incognita eggs and killed second-stage juveniles thereby mitigated adverse effects of M. incognita on growth and yield of okra. It thus means that M. incognita-infected okra plants treated with 20 ml of aqueous extracts of O. gratissimum leaves were more able to perform basic physiological functions that ensured improvement in growth and yield compared with inoculated-untreated okra plants.

The death of M. incognita-infected okra treated with 10 ml of aqueous extract of O. gratissimum leaves before 12 WAI showed that the menace of M. incognita could not be managed at this dosage. This might be that the quantities of active principles in the 10 ml rate were not enough to exert nematicidal control. The view that nematicidal activity of botanicals is application rate dependent has been corroborated by reports of other workers (Radwan et al., 2007; Tiwari et al., 2011). At such, M. incognita might have damaged the root system in inoculated-untreated and 10 ml Ocimum aqueous extract-treated okra which thus made it difficult for translocation of water and nutrients that are necessary for growth and yield in plants. This might have led to early death of inoculated-untreated okra seedlings and those treated with 10 ml of the aqueous extract of O. gratissimum (20% w/v). However, the application of 20 ml aqueous extract of O. gratissimum leaves in managing M. incognita-infected okra promoted better growth and yield than 10 ml dose. Also, the 20 ml extract also compared effectively with carbofuran in improving growth of treated okra.

The death of the okra inoculated with M. incognita but untreated at 8 WAI is attributed to the damaging effect of M. incognita infection on plants as no treatment was applied. Infection of roots by nematodes alter uptake of water and nutrients and interferes with the translocation of minerals and photosynthates leading most times to wilting and eventually death (Williamson and Hussey, 1996). Such alterations change the shoot:root ratio (Anwar and Van Gundy, 1989) and expose the plants to other pathogens. In fact, root-knot nematode infections on plants cause galls on the roots and galls are known to serve as nutrient sinks in infected plants since nutrients are redirected from shoots to roots (Melakeberhan et al., 1990). Also it could be due to impairment of the roots in absorbing nutrients from the soil for good growth and yield caused by the action of Meloidogyne incognita (Adegbite and Agbaje, 2007).

Within the same grow-out period, M. incognita-infected okra treated with O. gratissimum at 20 ml and carbofuran at 3 kg a.i/ha had fruits. Also the non-production of fruit in plants administer with low concentration of O. gratissimum is attributed to the adverse effects of the nematode that might have affected growth, development and subsequently yield. The development of galled roots led to modification in absorption of water and nutrients from soil and their translocation to foliage resulting in foliage chlorosis and stunting of vegetative growth (Bala, 1984). The destroyed root system would not be able to fully explore the soil for water and nutrients thereby leading to poor growth and yield (Clark et al., 2003).

Ocimum gratissimum aqueous extract as botanical nematicide:

Aqueous extract of O. gratissimum at 20 ml dosage compared effectively with carbofuran in the reduction of gall index (root damage). This is an indication that at 20 ml dosage of the extract, nematode activity, development and reproduction were reduced to the extent that M. incognita did not cause significant damage. Hasabo and Noweer (2005) reported that water extract of O. gratissimum was effective in reducing populations of root-knot nematodes in eggplant at 5% w/v concentration. Their finding also indicated that Ocimum-treated plants had lower yields when used at 2.5% w/v concentration, suggesting variation in efficacy related to concentration of the extract and nematode population on the plant. The variation in the effectiveness based on concentration reported by Hasabo and Noweer (2005) and this study might be due to differences in the age of the plant used and formulation (Tiwari et al., 2011). Plants of different ages are reported to possess varying concentration of active principles (Tiwari et al., 2011).
The okra treated with *O. gratissimum* at 10 ml/5kg of soil showed significant galling at seven WAI and this suggests the ineffectiveness of the dosage of 10 ml *O. gratissimum* in managing *Meloidogyne incognita* on okra. At the lower rate, the aqueous extract failed to suppress the damaging action of the nematode thus leading to death of treated okra at 8 WAI. Vegetable crops, including okra are among the most susceptible and worst affected by root-knot nematodes (*Meloidogyne* species) (Sharma et al., 2006; Anwar et al., 2007; Singh and Khurma, 2007).

The nematicidal effect of the *Ocimum gratissimum* extracts is attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock et al., 1989). This suggests that a water soluble component of the extracted oil in adequate concentration may provide better control of the nematode.

**Effectiveness of carbofuran as a synthetic nematicide:**

Carbofuran showed more nematicidal efficacy on *M. incognita* than the two rates of aqueous extracts of *O. gratissimum* in improving growth, yield and management of *M. incognita* on okra evident in significant reduction in galling on okra. The efficiency of carbofuran has been reported by various authors; its action is by causing dysfunction of the nematode by inhibiting development of female organ and egg production (Disanzo, 1977; Steele, 1977). Akinlade and Adesiyan (1982) observed that yields of carbofuran-treated okra were superior to the untreated ones infected with *M. incognita*. The therapeutic effects of carbofuran on *M. incognita* leading to improvement of agronomic parameters in crops have been reported by Kinloch (1982) and Adegbite and Adesiyan (2001). Carbofuran application has been observed to improve plant height in crops infected with *M. incognita* than infected-untreated plants (Adegunle and Fawole, 2003). Once growth is improved in *M. incognita*-infected plants treated with carbofuran, it will surely translate to improvement in yield since the plant will be able to perform basic physiological processes. This agrees with the findings of other workers who recorded increased growth parameters and yield with application of carbofuran (Adegbite and Adesiyan, 2001; Adegunle and Fawole, 2003; Adegbite and Agbaje, 2007).

The uninoculated okra had no galls because of the absence of *M. incognita* infection since the soil used was steam-sterilized to eradicate nematodes and *M. incognita* was also not introduced. This facilitated good growth and yield comparable to treated plants with carbofuran and 20 ml aqueous extracts of *O. gratissimum*.

**Conclusion:**

Aqueous extract of *O. gratissimum* leaves at 20 ml/5kg of soil compared favourably with carbofuran in the management of *M. incognita* okra. Thus, the plant extract can be used in management of root-knot nematodes. Its application is expected to be cheap, easily available and environment-friendly.

**REFERENCES**


