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Effect of neem leaf extracts (*Azadirachta indica*) and synthetic pesticide (Carbofuran) on the root-knot nematode (*Meloidogyne* spp) of cowpea (*Vigna unguiculata* L. Walp)

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Abstract
The nematicidal effect of aqueous *Azadirachta indica* leaf extract and a standard synthetic nematicide, carbofuran 3G were evaluated for their effect on pathogenicity of *Meloidogyne spp* on cowpea and on the growth and yield of cowpea in a screen house. There were ten treatments and a control replicated 3 times in a Completely Randomized Design to give a total of 33 pots. All the pots received the same number of root knot nematode inoculation which was 1000 eggs/5 mls. The test plant (neem) was applied at five concentrations (100%, 80%, 60%, 40%, 20%) while the carbofuran was applied at five concentrations also (1000 mgL^{-1}, 750 mgL^{-1}, 500 mgL^{-1} 25 0mgL^{-1}, 100 mgL^{-1}), at the rate of 5ml per treatment. Data on the plant height and number of leaves affected were taken 2 weeks and 4 weeks after inoculation with nematodes while data on other parameters were taken at harvest (60 days after planting). Overall performance of neem in controlling the plant was found to be better than that of carbofuran. At harvest, galling was significantly lower in carbofuran treated plants compared to the neem treated plants. The control plants were most galled with poor yield and had stunted growth. The moderate concentrations of both neem (80%, 60%, and 40%) and carbofuran (750 mgL^{-1}, 500 mgL^{-1} and 250 mgL^{-1}) performed significantly ($P < 0.05$) better than control plants in suppressing the effects of root-knot nematode on yield and growth of cowpea. These suggest that the neem leaf extract have nematicidal property and would be effective in the management of root-knot nematodes. Therefore, farmers could adopt it as alternative to carbofuran or other synthetic nematicidal chemicals in controlling root-knot nematode on farmlands.

Keywords: Azadirachta indica, carbofuran, Meloidogyne spp, Vigna unguiculata

Introduction
Cowpea, *Vigna unguiculata* (L.) Walp is a dicotyledonous plant belonging to the family Fabaceae [1]. It is of major importance to the livelihood of millions of people in the tropics [2]. It is an important legume grown by both small and commercial farmers in Nigeria. The importance of cowpea lies in its high protein content and the fact that it fixes atmospheric nitrogen into the soil. It has been estimated that 3.3 million tonnes of dry grains of cowpea are produced worldwide with Nigeria producing 2.1 million tonnes making it the world largest producer [3]. In Nigeria and other West African countries, the most grown and eaten legume is cowpea and it is mainly cultivated in the northern states of Nigeria. It is an essential component of sustainable agriculture in marginal lands and drier regions of the tropics, where rainfall is scanty and soils are sandy with little organic matter [3]. Cowpea was originated in Africa and is widely grown in Africa, Latin America, South-east Asia and in the southern United States.
Cowpea plants are infected by various species of nematodes that are commonly known as thread worms, round worms, or eel worms and nemas [4]. [5] reported that the major problem of cowpea is pests and diseases which do not only cause yield but also discourage most farmers from cultivating the crop. One of the major limiting factors to the profitable cowpea production is the damage caused by plant-parasitic nematodes especially *Meloidogyne* spp. [6]. According to [7], the extent of damage is influenced by the cultivar, nematode species, level of soil infestation and environment.
Nematodes as a whole live under a variety of conditions and are known to occur everywhere. The plant nematodes or phyto-nematodes are of agricultural importance. Plant-parasitic nematodes are microscopic roundworms that are widely distributed and persist as soil plant pest for indefinite period [8]. They are very small in size, about 0.01 mm in length. In agricultural land, plant nematodes are usually confined to the top, 20 to 25 cm of soil though this is variable according to the type of soil, moisture content, host plant and climatic conditions [4]. Most important nematodes are *Meloidogyne*, *Globodera*, *Rotylenchus* and *Tylenchulus* which are enlarged and swollen and may be spherical, oval or lemon shaped. These nematodes cause considerable loss to every part of the plants. Plant parasitic nematodes cause diseases of plants and are responsible for approximately 50% of overall plant damage [9]. Root-knot nematodes (*Meloidogyne spp*.) are found in all temperate and tropical areas, and are among the most damaging plant pathogens worldwide [10]. [11] reported that *Meloidogyne* spp. was among the most prevalent plant parasitic nematode of yam farm in Nigeria. Root-knot nematodes infect roots of cowpea plants resulting in considerable losses. The yield loss is associated with conspicuous galls that disrupt water and nutrient uptake [12]. [13] reported yield losses of more than 90% in high population. The damage they cause to plants usually increase with the

number of nematodes present [14]. The symptoms of nematode infection include formation of root galls which results in stunting, nutrient and water uptake reduction, increased wilting, mineral deficiency, weak and poor yielding plant [15].

Presently, various control measures have been adopted for the control of root-knot nematodes. However, the application of chemical nematicide has been found to be more effective measure for control of nematodes. However, the highly toxic residual effect of chemicals on environment and particularly on non-target organism demands an urgent need to develop alternative strategies for the control of nematodes [16]. The interest in developing pesticides of natural origins has increased during recent years, because of the adverse effects of synthetic chemical pesticides, like impact on environment, toxicity to non-target organisms including humans, and resistance development in insect population. In search for more benign acceptable alternatives, the possibilities are being investigated of exploiting nematode antagonistic botanicals for the management of plant parasitic nematodes [17] and [18].

Botanicals (Plant-based pesticidal chemicals) have been found very effective as alternatives to synthetic pesticides in recent times. Some of these botanicals are already being exploited commercially in pest management. Different plant species are tested to identify the sources of nematicidal substances and many of them have shown promising results in the control of plant parasitic nematodes within and outside Nigeria. Botanicals such as *Azadirachta, Eucalyptus, Chrommelina, Sida acuta* and *Targetis* have been found to be effective in the control of nematodes in cowpea, tomato and eggplant fields [19]. These botanicals not only control nematodes but also improve the soil productivity and crop yield by several folds.

The Neem tree, *Azadirachta indica* originated in India and Myanmar, but is now found throughout the Indian subcontinent, and can be grown in subtropical and tropical areas [20]. Neem components have attracted global attention for their insecticidal, fungicidal, bactericidal and nematicidal properties [21]. Crude neem extracts have been used at a local and small-farm level recently in countries where neem plant grows. In the major countries such as USA, Canada and Europe, the commercial neem insecticides have reached the markets. Azadirachtin, one of the components of the neem plant has been commercialized. The toxicological profile of azadirachtin is generally favorable [22].

Against these backgrounds, field investigation was conducted to determine the efficacy of the crude extracts of plant-derived insecticides, *Azadirachta indica* and synthetic insecticides, carbofuran against root-knot nematode, *Meloidogyne* spp of cowpea.

Materials and Methods

Study area

The study was carried out from the month of May to October, 2015 in a screen house situated in Science Village, Faculty of Biosciences, Nnamdi Azikiwe University (6º14'N,6º14.5'N to 7º8.6'E, 7º9'E) Awka, Anambra State (6º25'N, 7º12'E). Awka lies in a Guinea savanna experiencing an annual rainfall of 1000mm – 1500mm with two seasons – the dry and rainy season [23].

Collection and preparation of cowpea

The cowpea, *Vigna unguiculata (L.) Walp*, variety (IT99K-573-1-1) seeds used for the study were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The seeds were surface sterilized in 0.01% $HgCl_2$ for one minute and rinsed in sterile water as recommended by [24].

Sterilization of soil

Soil used for the study consists of top loam soil and sandy soil in the ratio of 2:1. The soil mixture was moistened and then put in a cut drum, covered and heated until it reached a temperature of 80°C and maintained at this temperature for 20 minutes. After cooling down, 4kg of the soil is put in a perforated plastic pot, as recommended by [24].

Source and preparation of the aqueous neem leaf extract

Fresh Neem leaves were collected from Nnamdi Azikiwe University, along Faculty of Law building. They were left to air dry at room temperature for seven days and then ground with a clean, dry milling machine to obtain Neem leaf powder, adopted from [25]. A stock solution of the Neem leaf was prepared by soaking 50g of the neem leaf powder in 200ml of distilled water contained in 500ml flask for 12hrs [26]. Crude extract (A1) was obtained by filtering through Whatman No. 1 filter paper. The 5 ml of the crude extract was diluted with 1.25, 3.33, 7.5 and 20 ml distilled water to obtain five concentrations namely (100%, 80%=5ml crude extract+1.25ml of distilled water, 60%=5ml crude extract+3.33ml distilled water, 40%=5ml crude extract+7.5ml distilled water, 20%=5ml crude extract+20ml distilled water).

Source and preparation of carbofuran solution

Wormforce ®, carbofuran 3G was purchased from an agro-chemical shop in Onitsha, Anambra State. Stock solution ($1000 mgL^{-1}$) of the carbofuran was prepared by accurately weighing out 100 mg of the carbofuran granules using a digital balance and then transferred into a volumetric flask, 100ml of Acetonitrile was measured out using a measuring cylinder, it was used as diluent. The Acetonitrile was added to the 100mg of the carbofuran in the volumetric flask to dissolve, and then filtered with Whatman No. 1 filter paper [27]. The filtrate of different volumes (7.5 ml, 5.0 ml, 2.5 ml, and 1.0 ml) of the above solution were pipetted out in 10 mL standard flasks and made up to the mark of 10mL with acetonitrile to get 750 mgL^{-1}, 500 mg L^{-1},250 mg L^{-1}, and 100 mg L^{-1} carbofuran respectively.

Source of the root-knot nematodes, *Meloidogyne* spp

The Root knot nematodes, *Meloiogyne spp* used in the present study were collected from an already created inoculum of Indian Spinach, *Basella alba* at the Plant Protection Unit, National Root Crops Research Institute, Umudike, Abia State, Nigeria. The nematodes eggs were extracted from nematode infected roots of the Indian spinach, *Basella alba*. Galled root pieces of *B. alba* containing egg masses were cut into small pieces and placed in a domestic blender with 500 ml of 0.25% hypo (sodium hypochlorite, NaOCI), covered, and was blended for 15 seconds at low speed [28]. This was done in order to digest the gelatinous matrix encasing the eggs. The solution was then poured through two nested sieves, 200 mesh (75 µm) and 500 mesh (25 µm). Eggs in the 500 mesh sieve were washed free of NaOCI solution with water using a wash bottle into a 1000mL beaker. The cut roots in the original container were washed twice with water to obtain additional eggs.

The number of eggs per 1 mL of egg suspension was estimated by counting 3 samples of 1 mL each using a Doncaster's counting dish under a stereomicroscope and a working mean of eggs/mL was estimated, for example, 213 eggs/mL.

Inoculation of the root knot-nematode and bioassay

Four kilogramme of the sterilized mixture of top loam and sandy soil were weighed into perforated plastic pots of 20 cm diameter and 30 cm depth. The pots were perforated to avoid water logging. Three seeds of the cowpea were planted into each of the plastic pot and later thinned down to one healthy plant per pot. The pots were replicated three times in a Completely Randomized Design (CRD) at mean temperature of 27.3 °C, Relative humidity of 44.5 % obtained using a thermo-hygrometer. After two weeks of planting, each of the cowpea plants was inoculated with 1000 eggs/5mL egg suspension of *Meloidogyne* spp, into 5cm holes in the soil, around the plants as close as possible to the roots as recommended by [25]. Two weeks after inoculation (4 weeks after planting), 5 mls of the various concentrations of the treatments, the aqueous neem leaf extract and the carbofuran solution were injected into the soil using a 10 mL syringe. Control plants, inoculated with nematodes without any of the pesticides were also included.


Plate 1: Pods of plant treated with *A. indica*, Plate 2: Pods of control plant

Watering, weeding and fertilizer application

Following the germination of the cowpea seeds, the plants were watered daily and were kept weed free by hand weeding as recommended by [25]. The pots were treated with 0.9g of urea fertilizer, recommended by [26].

Data Collection

Growth/yield parameters

Visual observation and measurement were taken from plant in each pot and data recorded. The parameters studied included; plant height, number of leaves, pod length, pod weight.

Root knot index

Rating of galls was obtained by observing the number of galls on the roots of cowpea after harvest. The assessment was done on 1-5 scale [29].

Score	Rating	Reaction
0	0% of root galled	Immune
1	1-5% of root galled	Resistant
2	6-25% of root galled	Fairly resistant
3	26-50% of root galled	Fairly susceptible
4	51-75% of root galled	Susceptible
5	76-100% of root galled	Very susceptible

Rating of Root Galls by [29]

Statistical Analysis

All numerical data collected were subjected to Analysis of Variance (ANOVA) and Duncan New Multiple Range Test was used to separate means at 5 % (*P*=0.05) level of significance. The statistical package used was SPSS version 21. 0

Results

The results show the effect of different concentrations of the aqueous *A. indica* leaf extract on mean height of cowpea plant at 2 weeks after inoculation of the root knot nematodes. It was observed that there was significant (*P*< 0.05) increase in the height of plants and number of leaf treated with the crude neem extract at 100% concentration compared to plants subjected to other concentrations of the extract. At this high concentration of 100%, the plant height was 83.33± 3.4 and the number of leaf was 25.00± 2.0 after 2 weeks of inoculation. As the week of inoculation was increased to 4 weeks, the height and number of leaf of cowpea plant increased significantly to 97.77± 1.8 and 29.67± 1.5 respectively (Table 1). A similar trend was observed at the lower concentrations of the plant crude extract. Similarly, cowpea plants treated with synthetic insecticide, carbofuran at highest concentration recorded plant height and number of leaf of 69.00± 4.6 and 16.00± 2.0 respectively after 2 weeks of inoculation (Table 2). There was also significant (P< 0.05) increase as the week of inoculation progressed to 4 weeks. The plants in the treated groups performed better than the plants in the control group (Table 1 and 2). The control plant experienced stunted growth when compared with treated plants. Immediately after harvest, the fresh root weight, number of galls and weight of pods were taken and the results showed that in *A. indica* treated plants, the fresh root weight, number of galls, number of pods and weight of pods (Plate 1) were respectively 1.27± 0.02, 4.67±0.3, 4.00±0.0 and 2.05±0.2 at highest concentration (Table 3). Similarly, cowpea plants treated with carbofuran at the highest concentration recorded fresh root weight, number of galls, number of pods and weight of pods of 1.24±0.02, 3.33±0.3, 3.67±0.3 and 1.63±0.2 respectively (Table 4). The plants treated with the two insecticides at the lowest concentration performed poorly compared to the highest concentration. The number of galls produced in the control treatment was 13.00±0.6 while the control recorded significantly lower number of pods and the weight of pods (Plate 2) compared to treated plants (Table 4).

Table 1: Mean effects of *A. indica* on height of cowpea plant and number of leaves at 2 and 4 weeks after inoculation respectively

Treatment(%)	**2 Weeks Height(cm)**	**Number**	**4 Weeks Height(cm)**	**Number**
100	83.33± 3.4	25.00± 2.0	97.77± 1.8	29.67± 1.5
80	65.33± 6.4	14.00± 1.0	81.98± 6.8	19.33± 1.3
60	55.67±1.5	13.00± 2.7	69.67± 2.4	17.67± 1.5
40	53.00±2.8	12.00± 1.7	67.40± 2.5	15.00± 1.7
20	34.67±3.2	9.00± 0.0	52.67± 4.3	11.00± 1.0
Control	34.33±1.2	9.00± 0.0	44.00± 3.6	10.00± 1.0

Table 2: Mean effects of carbofuran on height of cowpea plant and number of leaves of cowpea plant at 2 and 4 weeks after inoculation respectively

Treatment (mgL^{-1})	**2 Weeks Height**(cm)	**Number of leaf**	**4 Weeks Height**(cm)	**Number of leaf**
1000	69.00±4.6	16.00± 2.0	91.33± 6.4	20.00± 1.2
750	47.23±0.9	12.67± 0.7	64.20± 2.9	17.67± 1.5
500	41.00±1.0	11.00± 0.6	62.67± 3.1	16.00± 1.0
250	38.33±1.8	10.00± 1.0	58.17± 2.3	15.00± 1.7
100	32.33±0.9	10.00± 0.6	52.17± 1.0	12.67± 0.7
Control	34.33±1.2	9.00± 0.0	44.00± 3.6	10.00± 1.0

Table 3: Mean Fresh root weight (g), Number of galls on plant roots, Number of pods per plant and weight of pods per plant of cowpea plant treated with *A. indica* after harvest.

Treatment (%)	**Fresh root weight (g)**	**Number of galls**	**Number of pods**	**Weight of pods (g)**
100	1.27± 0.02	4.67± 0.3	4.00± 0.0	2.05± 0.2
80	1.34± 0.02	6.67± 0.3	2.67± 0.3	1.64± 0.2
60	1.45± 0.02	7.67± 0.3	2.33± 0.3	1.34±0.4
40	1.62± 0.03	9.00± 0.0	2.00± 0.6	1.27±0.3
20	1.94± 0.04	10.67± 0.3	1.67± 0.3	1.00±0.2
Control	2.39± 0.04	13.00± 0.6	1.33± 0.3	0.54±0.1

Table 4: Mean Fresh root weight (g), Number of galls on plant roots, Number of pods per plant and weight of pods per plant of cowpea plant treated with Carbofuran after harvest

Treatment (mgL^{-1})	**Fresh root weight (g)**	**Number galls**	**Number pods**	**Weight pods (g)**
1000	1.24± 0.02	3.33± 0.3	3.67± 0.3	1.63±0.2
750	1.32± 0.02	6.00± 0.0	3.00± 0.0	1.00±0.4
500	1.42± 0.02	7.33± 0.3	2.33± 0.3	1.07±0.4
250	1.57± 0.03	8.67± 0.3	2.00± 0.0	0.83±1.4
100	1.89± 0.04	10.33± 0.3	1.67± 0.3	0.72±0.1
Control	2.39± 0.04	13.00± 0.6	1.33± 0.3	0.54±0.1

Discussions

Field study was conducted to evaluate the nematicidal potential of aqueous crude neem leaf extract and a standard synthetic nematicide, carbofuran 3G at different concentrations. Various workers have emphasized the advantages and potential use of synthetic insecticides in controlling plant diseases but very little work has been done to investigate natural plant products as nematicides, particularly against nematode disease of cowpea [24].

The results of the present study on the effect of the two insecticides on the yield and growth parameters (plant height, number of pods, number of leaves and weight of pods) of cowpea plant show that there was generally increased plant growth and yield on plants treated with aqueous *A. indica* leaf extract compared to those treated with carbofuran. This is in accordance with the work done by [30] who reported that extract of certain weeds are able to cause substantial mortality of plant parasitic nematode thereby improving plant growth and yield. Similarly, [31] also found that neem leaf extracts compared with carbofuran reduced nematode population in infected cowpea plant with an accompanied increase in yield. Therefore, the results of the present study have shown that *A. indica* has some nematicidal ability. Also, the general increase in plant height and number of leaves could be attributed to the fact that incorporation of soil with botanicals provide organic matter to the plant and therefore additional nutrient that improved the growth and yield of the cowpea plant [32].

From the result, it was observed that the *A. indica* (neem) leaf extract at 100% concentration and carbofuran at 1000mgL^{-1} reduced substantially the nematode pathogenicity thus reduced number of galls and low root weight. Observed symptoms such as reduction in yield of untreated plants, galled roots and stunted growth in some of the plants could be as a result of higher populations of nematode on the untreated plants and plants treated with lower concentrations of the insecticides. The control recorded the lowest growth rates, high galling due to nematode activity at root zone resulting in giant cell formation and high population of nematodes because the nematodes larvae were able to penetrate roots freely and reproduce without any inhibition. The poor performance recorded in the control treatment (nematodes only) in the yield and growth parameters when compared with the treated plants (plant extract and synthetic nematicide) could be attributed to heavy nematode infestation and availability of nutrients [33]. The population build up in the control treatment may have resulted in increased penetration of juvenile stages into the root system to induce parasitic attack and formation of galls. These findings are in line with those of [15]. Galls ranging from slight to severe were observed in some plants in the study indicating the proliferation of nematodes and their active penetration due to the absence or lower rate of nematicidal materials. Reduction in root-knot index may also be attributed to the toxicity or high concentrations of the neem insecticide. [34] found that many farmers in Nigeria use neem for various crop protection practices with a lot of success. Seed dressing of cowpea with neem reduced seedling infestation of disease as much as common seed dressing using synthetic pesticides [34].

Furthermore, plants treated with the crude extract of *A. indica* gave the best result in all growth parameters (plant height, number of leaves, number of pods and pod weight) compared to those treated with carbofuran. This is in accordance with the work done by [31] who compared carbofuran and some selected plant extracts on the biology and pathogenicity of *M. incognita* on cowpea and tomatoes. However, the reason for the poor performance of carbofuran, a synthetic insecticide could be as a result of resistance. Carbofuran, a plant systemic insecticide has been widely used as a soil insecticide for the suppression of nematodes and other soil pests. It is possible that this insecticide had become ineffective as a result of continuous use by the farmers. The case of resistance may be caused by increase in microorganisms capable of quickly degrading the compound after application [35]. Therefore, when used for soil insects, carbofuran should be rotated with a soil insecticide of another class.

Although the active principles in the plant extract were not investigated, the nematicidal effect of the tested plant extract may possibly be 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 [36]. The mechanisms of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation [37].

In conclusion, the present study has shown that the leaves of *Azadirachta indica* (Neem) have nematicidal effect on root-knot nematode *Meloidogyne* species in cowpea plant. High concentrations of the plant crude extract were more effective in controlling the nematodes and as such caused increased plant growth and yield improvement. From the findings of this study, it could be concluded that the incorporation of the *A. indica* (neem) plant leaves into the soil as nematicide treatment to control root knot nematode, *Meloidogyne spp*. could provide a suitable and cheaper alternative for management of *Meloidogyne spp*. and reduce dependence on the use of synthetic nematicide. Although chemical nematicides may be effective in nematode control, their high costs, non-availability at the time of need and the hazards they pose as environmental pollutants may discourage many farmers. Therefore it is recommended that *A. indica* should be adopted as a component of integrated pest management of nematodes.

References

1. Cronquist A. The Evolution and Classification of Flowering plants. (2nd Edition). The New York Botanical Garden, New York, 1988, 555.
2. Quin FM. Importance of Cowpea. In: Advances in Cowpea Research. Printed by Colcocraft, Hong Kong, 1997, 375.
3. IITA. Annual Report, International Institute of Tropical Agriculture Ibadan Nigeria. Pp: 1-3.
   IITA. International Institute of Tropical Agriculture, 2002, 17.
4. Manisegaran S, Soundararajan RP. Pest management in field crops: Principles and Practices. Agrobios (INDIA), Agro house, behind Nasrami Cinema Chopasani road, Jodhpur 342 002, 2012, 213-216.
5. Emechebe AU. Cowpea pathology. In: Grain Legume improvement programme. Annual Report 1981. IITA, Ibadan, Nigeria, 1985, 69-10.
6. Rachie KO, Lawal RD. Grain legumes of lowland tropics. In: Advances in Agronomy. John Wiley and Sons, Chichester, U.K, 1975, 47-48.
7. Ononuju CC. The Use of Natural Nematicides: The best sustainable plant protection option for plant-parasitic nematodes among small scale farmers in Nigeria. Paper presented at the 21st Annual Conf. of Nigerian Society for plant protection. Nigeria institute for oil palm Research (NIFOR), Benin City, Edo State, Nigeria, 1999.
8. Obuezie CB, Ikpeze OO. Parasitic Nematodes of maize in farms at Oba, Idemili south Local Government Area of Anambra state Nigeria. Journal of Occupational Safety and Environmental Health. 2012; 1(1):73-78.
9. Abbasi WMN, Ahmed JM Zaki, Shakau SS. Effect of Barleria acanthoides Vahl. on Root-knot Nematode Infection and Growth of Infected Okra and Brinjal Plants. Pakistan Journal of Botany, 2008; 40(5):2193-2198.
10. Trudgil DL, Blok VC. Apomictic Polyphagous Root-knot Nematode: Exceptionally Successful and Damaging Biotrophic Root Pathogen. Annual Revised Phytopathology, 2001; 39:53-77.
11. Duru VC, Nwankwo EN, Ogbonna Confidence U, Onyido AE, Adewuyi OAA. Survey of plant-parasitic nematode of yam farms in Awka-North Local Government Area, Anambra State, Nigeria. Journal of Applied Bioscience, 2015; 95:8950-8957.
12. Ogbuji RO. The status of root-knot nematodes in the Eastern States of Nigeria. In: Proceedings of the Research Planning Conference on Root-knot nematodes, Meloidogyne spp. IITA, Ibadan, June 7-11, 1981, 44-47.
13. Rose JP, Nusbaum C, Hircumann H. Soybean reduction by lesion, stunt and spiral nematodes. Phytopathology, 1989; 3:189-207.
14. Ploeg A. When Nematode Attack is Important. California Growers, 2001, 12-13.
15. Abad P, Favery B, Rosso M, Castagnone-sereno P. Root-knot Nematode Parasitism and Host Response: Molecular

Basis of a Sophisticated Interaction. Molecular Plant Pathology, 2003; 4(4):217-224.
16. Anastasiadis IA, Giannakou IO, Prophetou-Athanasiadou DA, Gowen DA. The Combined Effect of the Application of a Biocontrol Agent Paecyliomyces lilacinus, with Various Practices for the Control of Root-knot Nematodes. Crop Protection, 2008; 27:352-362.
17. Chitwood DJ. Phytochemical Based Strategies for Nematode Control. Annual Review of Phytopathology, 2002; 87:250-258.
18. Akhtar M. Current Options in Integrated Management of Plantparasitic Nematodes. Integrated Pest Management Review, 2004; 2:187-197.
19. Umar I, Muhammad Z, Okusanya BAO. Effect of organic amendments on the control of Meloidogyne javanica (Kofoid and White, 1919) Chitwood, 1949) on Tomato (Lycopersicon lycopersicum, Mill). Agriculture, Business and Technology Journal, 2010; 8:63-77.
20. Khalil MS, Abamectin, Azadirachtin. as Eco-friendly Promising Biorational Tools in Integrated Nematodes Management Programs. Journal of Plant Pathology and Microbiology. 2013; 4:174.
21. Gajalakshmi S, Abbasi SA. Neem leaves as a source of fertilizer-cum-pesticide vermicompost. Bioresources Technology, 2004; 92:291-296.
22. Stark JD. Ecotoxicology of Neem. ACS Symposium Series, 2007; 947:275-286.
23. Mogbo TC, Akunne CE. Ecological Survey of Earwigs (Hemimerus talpoides) as Ectoparasites of Wild African Giant Rat (Cricetomys gambianus) in Awka, Nigeria. American Journal of Zoological Research, 2014; 2(1):22-24.
24. Ononuju CC, Nzenwa PO. Nematicidal effects of some plant extracts on egg hatchability and control of Meloidogyne spp. in cowpea (Vigna unguiculata (L.) Walp). African Journal of Plant Science. 2011; 5(3):176-182.
25. Claudius-Cole AO, Aminu AE, Fawole B. Evaluation of plant extracts in the management of root-knot nematode Meloidogyne incognita on cowpea [Vigna unguiculata (L) Walp]. Mycopath, 2010; 8(2)**:**53-60.
26. Umar I, Adamu MY. Nematicidal potential of some botanicals on Meloidogyne javanica in vivo and *in vitro*. Journal of Biopesticides, 2014; 7:137-142.
27. Kumar KV, Naidu NVS. Development and validation of RP-HPLC method for the analysis of carbofuran and in its formulations. Der Pharma Chemica, 2013; 5(1):313-319.
28. Hussey RS, Barker KR. A comparison of methods of collecting inocula for Meloidogyne spp. including a new technique. Plant Disease Reporter, 1973; 57:1025-1028.
29. Taylor AL, Sasser JN. Biology, Identification and Control of root knot nematodes (Meloidogyne spp). Cooperative publication of Department of Plant Pathology, North Carolina State University and US Agency International Development Raleigh, North Carolina, 1978, 111.
30. Shaukat SS, Siddiqui IA, Zarina B. Effects of Some Common Weeds from Pakistan on Plant-parasitic Nematodes *in vitro* and Population Densities and Survival of Meloidogyne incognita in Okra and Brinjal. *Nematologia Mediterranea*, 2004; 32:111-115.
31. Fatoki OK. Comparative effects of Carbofuran and some selected plant extracts on the biology and pathogenicity of M incognita on Cowpea and Tomato. PhD thesis, Faculty of Agriculture, University of Ibadan, Nigeria, 2001, 2013, 135.
32. Yakubu LB, Izuogu NB. Comparative evaluation of some botanicals and carbofuran in the control of root-knot nematode, Meloidogyne spp on cowpea, Vigna unguiculata. Journal of Agriculture and Veterinary Sciences. 2013; 5(2):105-115.
33. Netcher C, Sikora RA. Nematodes parasites of vegetables, in: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, edited by Luc, M., Sikora., R.A. and Bridge, J. Chapter 7, C.A.B. International. 1990.
34. Salako EA, Anjorin ST, Garba CD, Omolohunnu EBA. review of neem biopesticide utilization and challenges in Central Northern Nigeria**.** African Journal of Biotechnology, 2008; 7(25):4758-4764.
35. Pedigo LP, Rice ME. Entomology and Pest Management (5th edition). Pearson and Prentice-Hall Inc, Upper Saddle River, New Jersey, 2006, 733.
36. Knobloch K, Pauli A, Iberl N, Weigand N, Weis HM. Antibacterial and antifungal properties of essential oil components. Journal of Essential Oil Research, 1989; 1:119-128.
37. Konstantopoulou I, Vassilopoulou L, Mawogantisi PP, Scouras G. Insecticidal effect of essential oils: A study of essential oils extracted from eleven Greek aromatic plants on Drosophila auroria. Experientia, 1994; 48:616-619.