Original Research Article

Journal of Biological Research & Biotechnology

Bio-Research Vol. 22 No.1; pp. 2234-2241 (2024). ISSN (print):1596-7409; eISSN (online):2705-3822

Nematicidal effects of *Azadirachta indica* A. Juss (Neem) seeds on Meloidogyne species (root-knot nematodes)

[§]Danahap Luka Sambo, Ocheme Esther Lucia, [§]Okechalu Ode Brains

Department of Plant Science and Biotechnology, University of Jos, Nigeria

[§]**Joint corresponding author email ids: (DLS)** danahapluka@yahoo.com / (OOB) odebrain@yahoo.com

Abstract

The presence of bioactive substance in plant extracts with nematicidal activity on *Meloidogyne* spp can reduce crop damage in nematode control and to minimized the use of chemical nematicide which may be toxic to man and the environment. An *in vitro* experiment was conducted to investigate the bioactive effects of aqueous and methanolic seed extract of neem on root-knot nematodes. The nematodes were isolated by modified funnel Baerman method and identified by comparative morphology, descriptions and lattice keys. Ten mils of homogenized nematodes suspension (50 juveniles) replicated 5 times and kept at room temperature were exposed to varied concentrations (50, 100, 150, 200, and 300 mg/ml) of the seed extracts of test plants for different time periods; 6, 12, and 24 hours. Distilled water served as a control. Data on dead nematodes was recorded for each period of exposure. The data was analysed using Analysis of Variance (ANOVA). Phytochemical screening of crude seed extracts was carried out using standard methods. The results showed that aqueous seed extract of *A. indica* caused 100 % nematode juvenile mortality at the highest's concentration (300mg/ml) within 24 hours of exposure while the methanolic seed extract of *A. indica* caused 100% mortality within 8 hours of exposure. The results revealed that the higher the concentration of both aqueous and methanolic (50 mg/ml-300 mg/ml) seed extract of A. indica, the higher the mortality of root-knot juveniles. Mortality due to the extracts were significantly higher than those of the control at $P < 0.05$. The plant bioactive chemical screening of the test seed extract revealed the presence of anthraquinones, alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids and terpenes. Both methanolic and aqueous seed extract of *Azadirachta indica* had nematicidal properties with potential for a biological nematicide.

Keywords: Nematicidal, *Azadirachta indica*, Neem seed, *Meloidogyne* species

Received September 18, 2023; **Revised** February 04, 2024; **Accepted** February **6**, 2024; **Published** March 9, 2024.

https://dx.doi.org/10.4314/br.v22i1.2
Journal Homepage: http://www.bioresearch.com.ng.
Publisher*: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria*

INTRODUCTION

The controlling of plant parasitic nematode with extracts from plants is gaining acceptability due to reduce environmental pollution from pesticides. Now, that there are restrictions on the use of non-biotic nematicides because of their toxic residues on the environment (Harish *et al.,* 2008).

The search for plant extracts to replace conventional nematicides would be safer with less negative effects (Ntalli *et al*., 2010a). Plants are known to produce bioactive substances that are used to generate metabolic reactions (Okechalu *et al*., 2020).

Plant derived extracts have long been a subject of research in an effort to develop alternatives to conventional nematicides usually safer and with minimal residual effects (Ntalli *et al*., 2010; Ntalli and Caboni, 2012). These nematicidal substances obtained from plants can be considered to be bio deteriorated by microorganisms. Thereby, resulting in decomposed products. In this case, fewer residues are expected to result from the use of these products (Aoudia *et al*., 2012).

Some medicinal plants are believed to have natural products, in the form of alkaloids, flavonoids, phenols, quinone, tannins, saponins, sterols, and volatile essential oils. Plants bioactive substances, display various anti pathogenic properties (Akhtar *et al.*, 2008). These phytochemicals showed nematicidal properties on plant parasitic nematodes (Khan *et al*., 2017; Singh *et al*., 2015). In addition, plant natural products are easily biodegradable; therefore, they do not tend to pollute the environment (Akhtar *et al.*, 2008).

The phylum nematodes which consist of a large species of nematodes are also called roundworms (Kiontke and Fitch, 2013). These organisms are microscopic widely distributed and exist in the soil as plant pests (Obuezie and Ikpeze, 2012). Nematodes are found in both free-living and parasitic forms in plants (Iqbal *et al.*, 2016). Plant parasitic nematodes are long, slender with smooth surface and young ones has slender bodies but adults are swollen which deviate from the normal structure (Kumar and Yadav, 2020; Goss, 2008). Plant-parasitic nematodes are known to cause damage to many crops in the tropics and subtropics, where they are identified to cause serious yield losses on a wide range crop (Luc *et al*., 2005).

On a worldwide bases distribution of nematode species differ greatly. Some are found in every country such as certain *Meloidogyne* species while some are restricted in certain places e.g., *Nacobbus* species or host specific, such as *Heterodera carotae* which infect only carrots. Some crops may be susceptible to certain nematodes pests while others may have a wide range of genera and species associated with them. These crops such as sugar cane and rice, create difficulties in nematode control principles. Although plant parasitic nematodes are among the most widespread pests, they are the most salient and costly to control (Webster, 2004), This research is aimed at searching for nematicidal effect of seed extracts of neem on *Meloidogyne* species (root-knot nematodes) obtained from infected plants.

MATERIALS AND METHODS

Study area

The study was carried out in the nematology laboratory University of Jos, Plateau State, Nigeria

Sampling and authentication of plant material

The seeds of *Azadirachta indica* (Neem) were obtained from Quanpan local government area, plateau state, Nigeria and taken to taxonomy unit where it was subjected to proper identification and authentication.

Preparation of plant materials

The seed were air dried under shade at room temperature (25 ^{o} C) for two weeks, then pulverized into powder with mortar and pestle. The powders were sieved and stored in air-tight bottles until needed.

Extraction of neem seed powder

The extraction of the bioactive substances from neem seed powder was carried out using cold maceration method as described by Pagi and Patel (2017). Fifty-grammes (50 g) each of the powder was weighed with a top loader balance

and it was then transferred into a large flask (bottles) the content was soaked with 500 ml of water, and same applied to methanol and allowed to stand for three (3) days at room temperature. The content was shaken and stirred with a sterile glass rod at intervals. The suspension was then filtered with a sterile muslin cloth and Whatman number one (No.1) filter paper inserted in a funnel. The plant residue was subjected to several parts of rinse and filtration to attained an exhaustive level of extraction.

The extracts were prepared at different concentrations by dissolving variable weights of powdered residue in distilled water, this was done by taken 0.5, 1.0, 1.5, 2.0, and 3.0g and dissolved in 10 ml to achieve 50, 100, 150, 200 and 300mg/ml respectively.

Source of nematode inoculum

Tomato and carrot roots with galls, a symptom typical of root-knot nematode infection was randomly collected from various infested farms. The roots were brought to the laboratory for the isolation of nematodes.

Isolation and identification of root-knot nematodes

The modified Baermann funnel method (Okechalu *et al*., 2020) was adopted for the isolation of nematodes (Okechalu *et al.,* 2020; Okechalu and Wonang, 2015). Infected roots with galls were placed in Petri dishes containing 5 mills of distilled water to moisten the nematodes and then teased apart. The teased roots and distilled water were inverted into funnels tied to a short piece of rubber tube attached to a test tube. The entire set up was filled with water and were made air tight with masking tape. The funnels were covered up with thin layer of cotton wool. The whole set up was supported in an upright position on a table.

Water was added to the brim of the funnels; thus, allowed for free movement of nematodes, the set up was allowed to stand for 24hours. Nematodes juveniles that hatched and swam through the cotton wool down ward to the bottom of the test tube (Hooper *et al*., 2005).

Root-knot nematodes were identified by comparative morphology, descriptions, lattice keys and infection often identify by swelling in roots that look like ‘knots or galls that are large and easy to see with naked eye. Female shapes are oval while male characters are stylet length and shape, stylet cone length, head shape, and distance of esophageal gland outlet from stylet base (Jepson, 2024).

Estimation of nematode population

The population of the nematodes was estimated by counting the number of active juveniles in 1ml of homogenized suspension of inoculums under a binocular microscope at the magnification of x10. Each 1ml of homogenized suspension of inoculum consists of an average of 50 juvenile nematodes.

***In-vitro* nematicidal test**

The *in-vitro* nematicidal assay of the prepared seed extracts on juveniles of nematodes was undertaken in nematology Laboratory. A varied concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g of seed extract were added to 10 m1 of homogenized nematodes (50 juveniles) to achieve 50, 100, 150, 200, and 300 mg/ml. Each was replicated 5 times and kept at room temperature. The mixtures were examined at intervals of 6, 12, and 24 hours for life and dead nematodes. The life and dead nematodes were assessed by touching the juveniles with a fine needle to trigger movement thus, observing whether immobilize or alive (Abbasi *et al*., 2008). These were counted and recorded. Juvenile nematodes suspension in distilled water serves as control.

Plant bioactive chemical screening

The seed extract was screened for plant bioactive chemical constituents to determine presence of anthraquinones, alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids using standard phytochemical screening procedures as described by the Association of Official Analytic Chemists (A.O.A.C, 2007).

Statistical analysis

Data collected was analyzed using Analysis of Variance (ANOVA) at 5 % level of significance. Means were separated using Least Significant Difference (LSD).

RESULTS

Quantitative yield of extract

The quantitative yields in grams and percentage of different extracts employed in the study are shown in Table 1. The highest percentage (12.96 %) yield was obtained from aqueous extract of *Azadirachta indica* seeds while lowest percentage yield (7.08 %) was obtained from methanolic extract of same seed.

Table 1: Percentage yield of *A. indica* seed extracts

Parameter	Aqueous	Methanolic
Weight of crude powder	50 g	50 g
Volume of solvent used	500 ml	500 ml
Weight of extract	15.06 g	18.61g
Percentage yield	12.96 %	7.08 %

Plant bioactive chemical screening

Screening for bioactive chemical substance obtained from aqueous and methanolic seed extracts of *Azadirachta indica* A. Juss showed the presence of anthraquinones, alkaloids, carbohydrates, cardiac glucoside, flavonoids. saponins, steroids, tannins and terpenes (Table 2). Alkaloids was found to be highly present in both the aqueous and methanolic extract. Saponins found slightly present in the aqueous extract and moderately present in methanolic extract. Tannins was absent in both the aqueous and methanolic extract. Carbohydrates was slightly present in the aqueous seed extract and absent in methanolic seed extract. Phenols was absent in both aqueous and methanolic seed extract. Steroids was moderately present in aqueous seed extract and highly present in the methanolic seed extract. Anthraquinones found absent in the aqueous seed extract and moderately present in methanolic seed extract. Cardiac glycoside was absent in the aqueous seed extract and highly present in methanolic seed extract. Terpenes was found to be absent in the aqueous seed extract of neem and highly present in the methanolic seed extract.

Table 2: Plant bioactive chemical screening results of neem seed extract

Constituents	Aqueous	Methanolic
Alkaloids	+++	+++
Saponins	+	++
Tannins	-	-
Flavonoids	+++	+++
Carbohydrates	+	-
Phenols	-	-
Steroids	++	+++
Anthraquinones	-	++
Cardiac glycoside	-	+++
Terpenes	-	+++
Percentage yield of plants	15.09	18.61

Key: - = absent; + = slightly present; + = moderately present; +++ = highly present

***In-vitro* nematicidal test**

The results of this test indicated that the aqueous and methanolic seed extract had nematicidal properties on nematode juveniles. It indicates nematode mortality with increase time of exposure and concentration of the extracts (Tables 3 and 4).

Death and immobilisation of nematode juveniles recorded were highest when exposed at higher concentration of 300 mg/ml of the aqueous and methanolic extracts. This was followed by 200 mg/ml, 150 mg/ml, 100 mg/ml, and 50 mg/ml respectively, for both extracts. Thus, mortality of root knot juveniles increases at higher concentrations (Tables 3,4, 5 and 6).

The, mortality of nematodes increases with concentration and time of exposure to aqueous seed extracts of *Azadirachta indica* at 300 mg/ml and 24 hours respectively. The least mortality (46 %) occurred at a concentration of 50mg/ml at 24 hours. The least percentage mortality (57.60 %) was recorded for 50 mg/ml at 24 hours. Generally, the different concentrations of methanolic seed extract of *Azadirachta indica* had higher percentage mortality at shorter exposure time than those of aqueous seed extract (Table 4 and 5).

Results revealed that juvenile death occurred in the distilled water that was used as control but at a very slow rate (Tables 3, 4, 5, and 6). The percentage mortality in the control after 24 hours was 27.20 %.

Statistical analysis indicates that mean mortality of various concentrations of the seed extracts were significant compare to control at 5 % level of significant. This revealed significant mortality at ($P < 0.05$).

Table 3: Mean mortality of nematodes juvenile at varied concentrations of aqueous seed extract of *Azadirachta indica* at different exposure time

Treatment	Conc. (mg/ml)	Time of Exposure (Hours)			LSD
		6	**12**	**24**	
Neem Aqueous Extract	50	9.00 ± 3.81^{e}	17.20 ± 1.92^{e}	23.00 ± 2.74^{e}	4.88
	100	12.60 ± 6.66^{d}	20.40 ± 6.77^{d}	28.00 2.92^{d}	
	150	17.80 ± 3.19^{c}	26.20 ± 3.19^{c}	31.20 ± 3.03^{c}	
	200	20.00 ± 4.58^{b}	30.00 ± 3.61^{b}	42.80 ± 3.96^{b}	
	300	31.20 ± 6.34^{a}	39.80 ± 3.27^{a}	50.00 ± 0.00^{a}	
Control	Water	2.60 ± 0.89	10.00 ± 2.92	13.60 ± 2.07	
L.S. D	4.88				

*Pairs of means that differ by more than their LSD are significantly different at 0.05 level of significant.

Table 4: Mean mortality of nematodes juvenile treated with varying concentrations of methanolic seed extract of *Azadirachta indica* at different exposure time

Treatment	Conc. (mg/ml)	Time of Exposure (Hours)			LSD
		6	**12**	**24**	
Methanolic Neem Extract	50	16.80 ± 4.03^{e}	23.00 ± 5.34^{e}	28.80 ± 4.09^{d}	3.96
	100	22.00 ± 2.55^{d}	28.60 ± 3.21^{d}	31.20 ± 4.03^{c}	
	150	24.20 ± 3.77^{c}	30.20 ± 3.70^{c}	34.80 ± 3.35^{b}	
	200	38.40 ± 2.41^{b}	42.20 ± 4.87^{b}	50.00 ± 0.00^{a}	
	300	50.00 ± 0.00^{a}	50.00 ± 0.00^{a}	50.00 ± 0.00^{a}	
Control	Water	2.60 ± 0.89	10.00 ± 2.92	13.60 ± 2.07	
L.S. D	3.96				

*****Pairs of means that differ by more than their LSD are significantly different at 0.05 level of significant.

Table 5: Percentage Mean mortality of nematodes juveniles treated varied concentrations of aqueous seed extracts of *Azadirachta indica* at different exposure time

Treatment	Conc. (mg/ml)	Time of Exposure (Hours)			LSD
		6	**12**	**24**	
Aqueous Neem Extract	50	18.00 ± 4.03^{e}	34.40 ± 5.34^{e}	46.00 ± 4.09 ^{d}	4.88
	100	25.20 ± 2.55^{d}	40.80 ± 3.21^{d}	56.00 ± 4.03^{c}	
	150	35.60 ± 3.77^{c}	52.40 ± 3.70^{c}	62.40 ± 3.35^{b}	
	200	40.00 ± 2.41^{b}	60.00 ± 4.87^{b}	85.60 ± 0.00^{a}	
	300	62.40 ± 0.00^{a}	79.60 ± 0.00^{a}	100.00± 0.00^{a}	
Control	Water	5.2 ± 0.89	20.00 ± 2.92	27.20 ± 2.07	
L.S. D	4.88				

*****Pairs of means that differ by more than their LSD are significantly different at 0.05 level of significant.

Table 6: Percentage Mean mortality of nematodes treated with varying concentrations of methanolic seed extracts of *Azadirachta indica* at different exposure time

Treatment	Conc. (mg/ml)	Time of Exposure (Hours).			LSD
		6	**12**	**24**	
Methanolic Neem Extract	50	33.60 ± 4.03^{e}	46.00 ± 5.34^{e}	57.60 ± 4.09 ^{d}	4.88
	100	44.00 ± 2.55^{d}	57.20 ± 3.21^{d}	62.40 ± 4.03^{c}	
	150	48.40 ± 3.77^{c}	60.40 ± 3.70^{c}	69.60 ± 3.35^{b}	
	200	76.80 ± 2.41^{b}	84.40 ± 4.87^{b}	100.00± 0.00^{a}	
	300	100.00± 0.00^{a}	100.00± 0.00^{a}	100.00± 0.00^{a}	
Control	Water	5.2 ± 0.89	20.00 ± 2.92	27.20 ± 2.07	
L.S. D	3.97				

*****Pairs of means that differ by more than their LSD are significantly different at 0.05 level of significant.

DISCUSSION

The results obtained from this research revealed that the aqueous and methanolic extract of *Azadirachta indica* contains biologically active components such as alkaloids, carbohydrates, flavonoids, cardiac glycosides, anthraquinones, saponins, steroids and serpenes. This finding is similar to that of Okechalu *et al* (2020) who reported that the leaf extracts of *Commenlina benghalensis* and *Bidens pilosa* contain some biological active ingredient which include alkaloid, flavonoid, tannin, saponin, steroid, cardiac glycoside and Phlobattam. They attributed their finding to the presence of the bioactive components in the extracts that suppressed the nematode activities. These bioactive compounds are said to be nematicidal (Ilker *et al.*, 2016; Bawa *et al*., 2014). These bioactive substances may be responsible for nematicidal activities of seed extracts in this study. Khan *et al.* (2017) also reported the presence of alkaloids, flavonoids, tannins and saponins in weeds from India and further said they could be responsible for the observed mortality of *Meloidogyne* population *in vitro*. *In-vitro* experiment showed that the nematicidal activities of the aqueous and methanolic seed extracts of neem increased with increase in concentration and time of exposure. This agreed with the report of Okechalu *et al.,* (2020), who reported similar findings in the leaf extracts of *Ricinus communis* L. and *Azadirachta indica.* Hossain *et al.,* (2003), revealed that *Azadirachta indica* seeds contains nematicidal properties.

The results of this study showed that the longer the time of exposure and the higher the concentration of neem seed extract, mortality of root-knot nematode juveniles also increased. Generally, methanolic extract gave a higher mortality within a shorter time of exposure. This may be as a result of higher yield of the extract by methanol compared to water. Also, the methanol extract had more bioactive constituents than the water extract. These may be accounted for the higher mortality as compared to the water extract. The mortality recorded in the control treatment could be attributed to starvation.

CONCLUSION

This study has shown that, both the aqueous and methanolic seed extract of neem had nematicidal properties. How ever, it can be concluded that the methanolic seed extract *was* capable of reducing the population of root-knot nematode faster than aqueous seed extract of *Azadirachta indica.* The seed extract of neem that have nematicidal properties can be formulated into a biological nematicide. It is suggested that further studies be carried out in a field experiment to fully ascertain nematicidal potential of the seed extracts.

Conflict of interest

Authors have no conflict of interest to declare.

Author contribution

OEL wrote the first draft and helped in the collection and preparation of materials. DLS was involved in conceptualization, design and editing of the draft. OOB also helped in conceptualization/design and edited the draft.

Acknowledgement

The authors wish to appreciate the laboratory staff in Botany Lab II and Professor P.A Wuyep Research Laboratory. They assisted in the course of the bench work. We appreciate Mr Satkat, a Masters student in our laboratory who helped in the extraction of the processed neem seed.

REFERENCES

Abbasi, W., Ahmed, N., Zaki, N., and Shaukat S. (2008). Effect of *Barleria acanthoides* Vahl. Nematode infection and growth of infected okra and brinjal plants. *Pakistan Journal of Botany*, **40:** 2193-2198.

Akhtar, Y., Yeoung, R., Isman, M.B. (2008). Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta. Phytochemicals Review* **7:**77– 88.

Association of Official Analytical Chemists, A. O. A. C. (2007). Official Methods of Analysis, 18th Edition, Published by Inc.; 2200 Wilson Boulevard: Arlington, Virginia, USA.

Aoudia, H., Ntalli, N., Aissani, N., Yahiaoui-Zaidi, R., Caboni, P. (2012). Nematotoxic phenolic compounds from *Melia azedarach* against *Meloidogyne incognita*. *Journal of Agriculture and Food Chemistry* **60:** 11675–11680.

Bawa, J.A., Mohammed, I., and Liodi, S. (2014). Nematicidal effect of some plant extracts on root-knot nematodes (*Meloidogne incognita*) on Tomato (*Lycopersicon esculentum*). *World Journal of Life Science and Medical Research*: **3 (3)**: 81 – 87.

Caboni. P., Ntalli, N.G., Aissani, N., Cavoski, I. and Angioni, A. (2012). Nematicidal activity of *Ailanthus altissima* against *Meloidogyne javanica*. *Journal of Agriculture and Food Chemistry* **60:** 1146–1151.

Goss, C. R. (2008). Plant parasite nematode of coffee; (1st edition). Springer Netherlands. DOI: 10.1007/978-1-4020-8720-2.

Harish, S., Saravanakumar, D., Radjacommare, R., Ebenezar, E.G. and Seetharaman, K. (2008). Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. *Biological Control* **53:** 555–567.

Hooper, D.J., Hallmann, J. and Subbotin, S. (2005). Methods for extraction, processing and detection of plant and soil nematodes. Wallingford, Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, **2** 53-86).

Hossain, M.A, Ahmed, M.U., Yasmin, L., Rashid, M.H. and Nasim, M.U. (2003). Use of neem seed extract in

controlling root knot nemetode (*Meloidogyne javanica*) of sweet gourd. *Pakistan Journal of Pathology* **2(3)** 161-168.

Ilker, K., Dolunay E. and Pervin E. (2016). Effects of some plant extracts on root-knot nematodes *In-vitro* and *In-vivo* Conditions *Turkish Journal of Entomology* **40 (1).**

Iqbal, S.; Jones, M.G.K. Thomas, B., Murray, B.G., Murphy, D.J., Eds. (2016). Academic Press: Oxford, UK, Nematodes in *Encyclopedia of Applied Plant Sciences*; ISBN 9780123948083.

Jepson, S. B. (2024). Identification of root-knot nematodes *Meloidogyne* species. Department of Nematol, rothamsted express station Harpenden, Hearts, abi (ABI) @ copyright from 1987, AL5 2JQ, U pp. 265.

Khan, A., Asif, M., Tariq, M and Rehman, B (2017). Phytochemical investigation of nematostatic and nematicidal potential of weed extracts against *Meloidogyne incognita in vitro*. *Asian Journal of Biological Sciences.* **10 (2)**:38-46.

Kiontke, K.; Fitch, D. H.A. (2013) Nematodes. *Current. Biology. **23*:** 862–864.

Kumar, Y., and Yadav, B.C (2020). Plant-Parasitic Nematodes: Nature's Most Successful Plant Parasite. *International journal of research and review* **7(3):** 379-386.

Luc, M., Sikora R. A. and Bridge, J. (2005). Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford, UK, CABI Publishing, pp. 871.

Ntalli, N.G., Ferrari, F., Giannakou, I., Menkissoglu-Spiroudi, U. (2010a). Phytochemistry and nematicidal activity of the essential oils from 8 Greek Lamiaceae aromatic plants and 13 terpene components. *Journal of Agriculture Food Chemistry* 58: 7856–7863.

Ntalli, N.G., Caboni, P. (2012). Botanical nematicides: A review. *Journal of Agriculture and Food Chemistry* **60:** 9929– 9940.

Ntalli, N.G., Ferrari, F., Giannakou, I., Menkissoglu-Spiroudi, U. (2010b). Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece. *Pest Management Science* **67 :** 341–351.

Ntalli, N.G., Menkissoglu-Spiroudi, U., Giannakou, I. (2010c). Nematicidal activity of powder and extracts of Melia azedarach fruits against *Meloidogyne incognita*. *Applied Biology* **156:** 309−317.

Obuezie, C.B. and Ikpeze, O.O. (2012). Parasitic nematodes of maize in farms at Oba, Idemili South Local Government Area of Anambra state, Nigeria. J*ournal of Occupational Safety and Environmental Health.* **1(1):**73-78.

Okechalu, O.B. and Wonang, D.L. (2015). The response of eleven sweet potatoes (*Ipomoea batatas* (L) Lam). Cultivas to infection by *Meloidogyne* species in Jos, Nigeria. *Journal of Pharmacy and Biological Sciences.* **10:** 42- 49.

Okechalu, O.B., Gutol, N., Danahap, L.S. and Agaba, O.A. (2020). Nematicidal activities of methanolic leaf extracts of *Azadirachta indica* A. Juss and *Ricinus communis* L. on the root-knot nematode (*Meloidogyne Species*) of Potato (*Solanum tuberosum* L.*) Nigerian Journal of Botany,* **33(1):** 81 -96.

Okechalu, O. B. Oyeyinka, J., Danahap, L. S (2020). *In-vitro* evaluation of the nematicidal potential of leaf extracts of *Commenlina benghalensis* and *Bidens pilosa* on root-knot nematode (*Meloidogyne* Spp) *Journal of Natural sciences Research* 10 (4): 40.

Pagi, N and Patel, P. (2017). Antimicrobial activity and phytochemical screening of Aloe vera (*Aloe barbadensis* Miller). *International Journal of current Microbiological Applied Science* **(1):** 1-10.

Singh, S., Singh, B. and Singh, A.P. (2015). Nematodes, a threat to sustainability of agriculture. *Procedia Environmental Sciences*, **29:** 215-216.

Webster, J. M. (2004)). Principles and practice of nematode control in crops. Academic Press, Melbourne, pp 1–12.