International Journal of Plant & Soil Science

18(3): 1-6, 2017; Article no.IJPSS.34574
ISSN: 2320-7035

Nematicidal Efficacy of Neem (*Azadirachta indica,* A. Juss) Seed Extract against *Meloidogyne incognita*

Kiran Singh[1*] and Sumayya Hali[1]

[1]Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Authors' contributions

This work was carried out in collaboration between two authors. Author KS formulated the concept, designed the study, supervised the research work and corrected the manuscript writing. Author SH managed the experimental work, performed the statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2017/34574
Editor(s):
(1) Omer Kilic, Bingol University, Turkey.
Reviewers:
(1) Montaser Fawzy Abdel-Monaim, Plant Pathology Research Institute, Agricultural Research Center, Egypt.
(2) Mahmoud M. A. Youssef, National Research Centre, Egypt.
(3) Tariq Mukhtar, Pir Mehr Ali Shah Arid Agriculture University, Pakistan.
(4) Tchabi Atti, University of Lomé, Togo.
(5) Paola Del Serrone, Council for Agricultural Research and Economics Crea, Italy.
Complete Peer review History: http://www.sciencedomain.org/review-history/20688

Original Research Article

Received 31st May 2017
Accepted 24th July 2017
Published 28th August 2017

ABSTRACT

Aim: The aim of this study was to evaluate the nematicidal efficacy of aqueous extract of seeds of *Azadirachta indica* on *Meloidogyne incognita,* the most common and harmful gall causing nematodes in this region, with the broader objective of controlling various kind of root knot nematodes using neem and a step towards being organic and saving environment.
Experimental Design: Random sampling.
Duration of Study: One cropping season.
The root-knot nematodes cause damage and loss of quality of products. The control of root knot nematodes require the use of nematicides, which are harmful to humans, it's environment and useful soil bacteria which contribute in nitrogen fixation. This experiment was carried out to evaluate the nematicidal potential of aqueous extract of neem seeds against root knot nematode (*Meloidogyne incognita)*. Ten to forty percent of neem seed extracts (aqueous) were prepared and applied to the potted plants and the result was monitored for two weeks. The result obtained from experiments showed that the plant treated with 10% aqueous extract of neem had the lowest

**Corresponding author: E-mail: ksinghj@gmail.com;*

mortality among *Meloidogyne incognita* (8.33%) and had the lowest weight gain (9.82 gm) as well as the highest number of galls (2.5%), while treatment with 40% aqueous extract of neem seeds showed the mortality of 67.5% and weight gain of 11.33 gm as well as less number of galls (0.8%) after two weeks of treatment. Thus based on these findings it can be concluded that neem seed extract has potentials to control root knot nematodes and local formers can use it in order to avoid bioaccumulation of synthetic nematicides. Use of neem based nematicides will keep the tomato cheap, safe and environmental friendly.

Keywords: Meloidogyne incognita; root knot nematodes; Azadirachta indica; nematicides; tomato etc.

1. INTRODUCTION

Tomato (*Lycopersicum esculentum* Linn) is an edible often red fruit/berry of the nightshade *Solanum lycopersicum*, which is consumed in diverse ways, including raw, as an ingredient in many dishes /sauces, salad, and drinks. [1], it is botanically a fruit; it is consider as a vegetable for culinary purposes. Tomato is grown for its fleshy berry, red or yellow when ripe containing vitamins A and C [2].

The tomato is essential for balanced diet and maintenance of good health as it plays an important role in neutralizing the acids produced during the digestion of meat and other proteins and fatty acids. It promotes gastric secretion, acts as blood purifier and keeps intestines in good condition.

It contains lycopene; a powerful antioxidant that helps prevent prostate cancer. Its consumption has been associated with decreased risk of breast cancer, neurodegenerative disorders and protection of skin against harmful UV rays [3]. Being rich in Vitamin A, it is important for bone growth, cell division and differentiation as well as in the regulation of immune system and maintaining surface linings of eyes, respiratory, urinary and intestinal tracts while its high percentage of Vitamin C is important in forming collagen, a protein that gives structures to bones, cartilage, muscle and blood vessel. It also helps in maintaining capillaries, bones and teeth and aid in absorption of iron. The folic acid present in tomato helps in depression by preventing excess of homocysteine from forming in the body which can prevent blood and other nutrient from reaching the brain [2].

Tomatoes are affected by a large number of pests from the time of first emerging to harvest. Flea beetles, aphids, leaf miners, stink bugs and fruit worms cause foliage damage in the field, but their fruit damage and disease spreading problems can be very serious.

Root-knot nematodes are plant-parasitic nematodes, exist in soil in areas with hot climates or short winters and cause approximately 5% of global crop loss, [4]. Root-knot nematode larvae infect plant roots, causing the development of root- galls that drain the plant's photosynthates and nutrients. Infection among young plants may be lethal, while infection to mature plants causes decreased yield and quality [4]. Root-knot nematode (*Meloidogyne* spp) is one of the three most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. Root-knot nematodes are distributed worldwide and are obligate parasites of the roots of thousands of plant species, [4].

The elimination of nematodes from crops is essential for export quality, particularly of high-value horticultural products. Several general purpose fumigants and nematicides give excellent control on nematodes in soil and their efficacy is related to their high volatility at ambient temperatures.

All nematicides remain in the topsoil where there is greatest microbial activity. Once nematicides or their degradation products are flushed through the upper soil layers their persistence may be extended and cause great damage to the useful microbial flora and contribute in toxicity of surface and ground water. Chemical nematicides are highly toxic compound even at high LD_{50} values and their use should therefore be restricted to skilled operators who take adequate safety precautions, [5].

The use of botanical nematicides is one of the alternative methods suggested by Nematologist for nematode control. Botanicals such as *Azadirachta*, *Eucalyptus*, *Chrommelina*, *Sida acuta* and *Targetis* have been found to be effective in the control of nematodes of cowpea plants in field conditions [6]. These botanicals not only controls nematodes but also improve the soil fertility and crop yield by several folds,

therefore, this study was carried out to evaluate nematicidal efficacy of aqueous extract of seeds of *Azadirachta indica* against *Meloidogyne incognita* the most common nematode species in this area.

2. MATERIALS AND METHODS

2.1 Study Area

Sokoto State is located in the extreme North-West part of Nigeria, it is located in the Sudan Savannah zone between longitude 4°8' E and 6°5'E and latitude 12°0'N and 13°54'N (11), with an annual average temperature of 28.3°C (82.9°F), however, maximum daytime temperature most of the year is generally under 40°C (104.0°F) and the dryness makes the heat bearable. The warmest months are February to April when daytime temperature can exceed 45°C (113.0°F). The raining season lost from June to October. The Kwalkwalawa area from where the tomato sample and the nematodes were collected is located in Sokoto, Usmanu Danfodiyo University Permanent site area.

2.2 Raising the Tomato Nursery

Thirty plastic pots of 20 cm in diameter and 30 cm in height, were obtained and divided in groups each of five pots. Each pot was filled with 1 kg of autoclaved soil which was sterilized at 121°C for 15 minute. One week old seedlings of tomato were transplanted and raised separately.

2.3 Neem Seed Collection

Azadirachta indica seeds were obtained from neem tree from the Biological garden of Usmanu Danfodiyo University, Sokoto and transported to the herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto (Nigeria) together with other parts of plants such as leaves and stem, for identification and authentication.

2.4 Extraction of *Meloidogyne incognita*

Soil and root sample were collected from an infested tomato farm in Kwalkwalawa area along the Usmanu Danfodiyo University main campus, by digging around the rhizosphere of the tomato plant to a depth of 30 cm below surface using hand shovel. The collected soil was transported to lab and kept under room temperature for extraction of nematode species.

Extraction of nematodes was carried out according to the methods of cob [7], using decantation and sieving techniques. The soil sample was poured into a bucket half-filled with water. The mixture of soil and water was then stirred gently to remove any lump and allowed to settle for about 30 seconds. The supernatant which contained most of the nematodes was decanted and poured through a series of sieves of varying mesh sizes (2.0 mm, 1.00 mm and 0.5 mm). Some of the suspension that was collected in basin, poured again for the second time over the sieves for maximum collection of nematodes. The residue from each sieve was collected in Petri-dishes.

The isolated nematodes were washed with 0.9% normal saline buffer and were picked by forceps and placed in 10(%) formalin solutions for preservation. The nematodes were transferred in petri-dishes and kept for an hour; after which they were fully extended with the head free. They were washed thoroughly under running tap water and stored for identification.

2.5 Identification of Nematodes

The nematodes were placed on slide, mounted in lactophenol, and examined under light microscope for the shape of the head, body and other structures. The Identification was done using a pictorial guide and confirmed by a senior nematologist.

2.6 Inoculation of *M. incognita* in Test Pots

Forty juveniles were inoculated into each of 5 groups (4 treatments and one positive control/without treatment) and were mixed gently [8], while the remaining last group was inoculated but treated with cypermethrin (standerd nematicide) and served as negative control.

2.7 Screening of Seeds of *Azadirachta indica* for Nematicidal Activity

This was carried out in accordance with the methods described by Akhtar and Mahmood [9]. Neem fruit obtained and identified were de-pulped and air dried. The dried seeds were pounded using pestle and mortar Then 100 g of dried powder was weighed and dissolved in 100 ml of distilled water. This solution was then sieved using a Muslim cloth, and was labeled as the stock solution (100%). This stock solution

was used in order to obtain varying concentrations ie.10%., 20%., 30%., and 40% by serial dilution. To compare the efficacy of the tested plant with standard nematicides, cypermethrin was used.

Four concentrations of the neem seeds extract were applied to the test groups with one group without treatment which serve as control and one group treated with cypermethrin to serve as standard nematicide. The plants were watered regularly. After two weeks of treatment the tomato plant was removed from each pot and growth, weight and galling status of plant was observed. For the nematicidal efficacy, the plants from each pot were uprooted and the soil of the pots was sieved (as mentioned in extraction technique). The nematodes were extracted from the soil and mortality was recorded. No nematode body movement (whether dry or wet) and no response to a needle probe was taken as evidence of death.

2.8 Data Analysis

The data were analyzed using simple analysis to calculate percent death of nematodes and result were presented in percentage.

3. RESULTS

The result of this study showed that neem seed extract had potency to kill nematodes of tomato plant.

Table 1 showed the mortality of nematode parasites of tomato treated with aqueous extract of neem seeds. The highest mortality (100%) was observed in the case of cypermethrin treated plants, while the tomatoes plants treated with neem seed extract showed less mortality. Mortality recorded was dose dependent. Lowest concentration ie 10% of seed extract showed 8.33% mortality among nematodes, while the mortality observed in the pots treated with neem extract at 40% concentration was 67.5%.

Table 2 showed that the highest weight gain was observed in case of cypermethrin treated plant (19.16 gm) while the tomatoes treated with neem seed extract showed the lowest weight gain (i.e 10% having 9.82 gm weight, while plants treated with 40% of neem seed solution had 11.33 gm weight. Untreated control showed a plant weight of 9.16 gm.

Table 3 showed the number of galls produced in tomatoes plants treated with varying concentrations of neem seed extract. The result showed that the 10% treatment with aqueous extract of neem seed had the highest number of galls with 2.5 while 20% and 30% had 1.6 galls and plants treated with 40% having the lowest number of galls 0.8. The untreated control had the highest number of galls 7.5 while no gall formation was observed in the plants treated with cypermethrin.

4. DISCUSSION

The result showed a significant reduction in number of nematodes obtained from the surrounding root of tomato treated with varying concentration of the neem seed extracts. The inoculated but untreated seedlings had the highest number of nematodes with an average of 52.3 which was observed significantly higher than those seedlings treated with extract concentrations at all levels. Those seedling treated with the highest concentration (40%) of neem seed extract recorded a higher mortality among nematodes with 67.5% than those of the lower concentrations of 30% (27.5%); 20% has (16.66%), 10% (8.33%) mortality respectively.

Table 1. Mortality among nematodes treated with varying concentrations of neem seed extracts after two weeks

Treatments	No of nematodes inoculated	No. of dead nematodes after 2 weeks of treatment				% mortality
		1st Set	2nd Set	3rd Set	Average	
10% Neem seed extract	40	2	3	5	3.33	08.33
20% Neem seed extract	40	7	8	5	6.66	16.66
30% Neem seed extract	40	11	10	12	11.00	27.5
40% Neem seed extract	40	29	27	25	27.00	67.5
Cypermethrin 10EC	40	00	00	00	00.0	100
Untreated	40	47	52	58	52.3	0.00

Table 2. Weight of tomatoes plant inoculated with nematodes and treated with varying concentrations of neem seed extract

Treatments	Average initial weight of tomato plants	Weight of tomato plant after two week of inoculated, treated				Weight gain
		1st Set	2nd Set	3rd Set	Average	
10% Neem seed extract	6.50	16.25	16.30	16.40	16.32	09.82 gm
20% Neem seed extract	6.52	16.30	16.47	16.44	16.40	09.88 gm
30% Neem seed extract	6.54	16.52	16.57	16.51	16.53	09.99 gm
40% Neem seed extract	6.51	17.83	17.84	17.85	17.84	11.33 gm
Cypermethrin 10EC	6.53	19.49	19.57	19.67	19.57	13.04 gm
Untreated	6.52	15.69	15.76	15.59	15.68	09.16 gm

Table 3. Average number of galls recorded in tomato plants after treatment with varying concentrations of neem seed extract

Treatments	No of nematodes inoculated	No of galls produced				No of galls %
		1st Set	2nd Set	3rd Set	Average	
10% neem seed extract	40	1	1	1	1	2.5
20% neem seed extract	40	2	0	0	0.6	1.6
30% neem seed extract	40	0	2	0	0.6	1.6
40% neem seed extract	40	1	0	0	0.3	0.8
Cypermethrin 10EC	40	0	0	0	0.0	0.0
Untreated (control)	40	2	3	4	3.0	7.5

The result, showed significant variation in the formation of galling in tomato treated with different levels of concentrations of the extracts. All the levels of treatments displayed lower number of galls over the inoculated untreated; which had the highest number of galls (7.5%) it was observed among the treated seedlings, as the concentration of the extract decreased, the extent of galling increased ie 40%(0.8), 30%(1.6), 20%(1.6) 10% (1.0). while the cypermethrin having (0.00%) of gall formation. The reduction in the number of nematodes may be due to presence of some bioactive substances in the test extract which may had deleterious or harmful effect on the nematodes. The active compound present in neem is *azadiractin* [10] which has harmful effect on nematodes. Azadirachtin has two profound effects on insects, at the physiological level. Azadirachtin blocks the synthesis and release of molting hormones (ecdysteroids) from the prothoracic gland, leading to incomplete ecdysis in immature insects and also cause sterility in adult female. In addition, azadirachtin is a potent antifeedant to many insects [11]. Inability to consume food or metabolism may contribute to death among treated nematodes. Neem oil is effective against soft-bodied insects and mites and disulfides in the neem oil likely contribute to the bioactivity [12,13].

This also confirms the works of various workers who investigated the activities of these extracts by showing a reduction in the population level of nematodes. [14]; and Akhtar and Mahmood [9], reported that these substances reduce the population of nematodes species by over 50%. Bunt [15], explained that the extracts rendered the roots of a susceptible plant highly un-favorable to the root-knot nematodes, as a result of which there is poor penetration and later reduction in the biological activities of nematodes, such as feeding and/or reproduction or both.

The fewer number of galls may be indicative of an acquired resistivity conferred on the plant by the extract, or it showed that given dose was not enough to kill nematodes. The result therefore establishes the nematicidal potential of the extract from neem seed. It has been shown that the potential for treatment by the tested plant extract is observed as directly proportional to their concentrations and that at lower concentration, the active ingredient with the nematicidal effect takes longer period to accumulate to the point of potency.

5. CONCLUSION

Based on this research work, it can be concluded that neem seed extract can effectively reduce population of root knot nematodes of tomatoes and farmers can use 40% neem seed extract to control heavy infestation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anonymous Renato Vicario retrieved 1 January. Anti-cancer vitamins and Minerals, Mackowsky MS, RD, CDN, CSCS. Jason, Nutrition. 2014;411. (Accessed 13 February 2014)
2. Villarreal N. Tomato in tropic ADS development oriented literature series Steven B. Breech Seria Edition. 1980;15-25.
3. Parray BA, Ganai AM, Fazili KM. Physiochemical parameters and growth yield of tomato (Lycopersicum esculentum): Role of farm yard manure and neemcake. American-Eurasia J. Agric. Environ. Sci. 2007;2(3):303-307.
4. Sasser NJ, Cater CC. Overview of the international meloidogyne project. In an advanced treatise on meloidogyne. Edited by: Saser JN, Cater CC Raleigh: North Carolina State University Graphs. 1975; 19-24.
5. Bromilow RH. Behavior of nematicides in soil and plants, in factor affecting the application and use of nematicides in Western Europe. Workshop. Nematology Group Association of Applied Biologists. 1980;87-107.
6. Umar I, Muhammad Z, Okusanya BAO. Effect of organic amendments on the control of *Meloidogyne javanica* (Kofoid and White, 1999) Chitwood, 1949) on tomato (Lycopersicon lycopersicum, Mill). Agric., Business Tech. 2010;8:63-77.
7. Cob NA. Notes on mononchus and tylenchulus. Washington J. Aca. Sci. 1913;3(10):287-288.
8. Abubakar U. Studies on the nematodes of cowpea (*Vigna unguiculata* L.) of the savanna regions of northern Nigeria and control of *Meloidogyne incognita* using selected plant extracts and animal manures. Unpublished Ph.D. thesis, Biological Science Dept. Usmanu Danfodiyo University Sokoto; 1999.
9. Akhtar M, Mahmood I. Control of root-knot nematodes by bare-root tip undecomposed and decomposed extracts of neem cake and leaf. Nematol. Medit. 1994;22:55-57.
10. Schmutter H. Which insect pests can be controlled by application of neem seed kernel extract under field conditions. *Z* Angew. Entomol. 1985;100:468-475.
11. Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu Rev Entomol. 1990;35:271–297.
12. Dimetry NZ, Abd El-Salam AME, El-Hawary FMA. Impor- tance of plant extract formulations in managing different pests at- tacking beans in new reclaimed area and under storage conditions. Arch. Phytopathol. Plant Prot. 2010;43: 700–711.
13. Dimetry NZ. Prospects of botanical pesticides for the future in integrated pest management programme (IPM) with special reference to neem uses in Egypt. Arch Phytopathol Plant Prot. 2012;45: 1138–1161.
14. Sangwin NK, Verma KK, Verma BJ. Nematicidal activity of essential oil of cybopongon grass. Nematol. 1985;31:93-99.
15. Bunt JA. Effect and mode of action of some systemic nematicides. Meded Lanbd. Hogesch. Wageningen. 1975;73:1-128.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20688