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Plant-Based Extracts For The Control Of Root-Knot Nematode *(Meloidogyne Incognita L.)* Infestation On Tomato *(Solanum Lycopersicum L.)*

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Abstract:
Over the years, the search for animal-safe, environmentally friendly and human-safe methods for sustainable control of nematode has increasingly focused on natural alternatives. In tomato production, lowering the population of nematodes minimizes economic loss, enhances vegetative growth and ultimately improves yield. This study considers the control of root knot nematode on tomato using selected plant extracts as organic amendments in Girei Local Government Area, Adamawa State, Nigeria. A pot experiment was conducted at the Plant Science Botanical Garden of Modibbo Adama University, Yola, Girei Local Government Area, Nigeria. The experiment evaluated leaf extracts of bitter leaf (Vernonia amygdalina), false ashoka (Polyalthia longifolia) and jatropha (Jatropha curcas) for their nematicidal effects. The experimental design was a Completely Randomized Design (CRD) with four replications, including a control treatment. Four amendment rates (10g, 20g, 30g and 40g) of ground leaf extracts were applied to 25kg of sterilized soil contained in perforated polythene bags, while control bags received no amendment. Four tomato seeds were sown per bag, and seedlings were inoculated four weeks after planting with 50 juveniles of M. incognita. Data were collected weekly on vegetative and reproductive parameters, including number of leaves, branches and flowers. The data were analyzed using Analysis of Variance (ANOVA), and treatment means were separated using the Least Significant Difference (LSD) test at a 5% probability level. The results showed that all plant extract treatments significantly reduced nematode populations compared to the control. The 20g amendment rate was particularly effective across treatments. The study therefore recommends the use of these plant extracts by farmers, as they are affordable, readily available, environmentally friendly, and require minimal technical expertise.

Keywords: *Nematodes; Tomato; Plant Extract; Pest Infestation.*

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I. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the world's most highly consumed vegetables due to its status as a basic ingredient in a large variety of raw, cooked or processed foods. It belongs to the family *Solanaceae*, which includes several other commercially important species. Tomato can be grown in a variety of geographical zones in open fields or greenhouses, and the fruit can be harvested by manual or mechanical means. Under certain conditions (e.g. rejuvenation pruning, weeding, irrigation, frost protection, etc.), this crop plant can be perennial or semi-perennial, but commercially it is considered as annual, [1].

Tomato grows in most parts of Nigeria, however the best area is the savannah agro-ecological zone where diseases and pests affecting tomatoes are less common. Major producing areas lie between latitudes 7.5°N and 13°N and within a temperature range of 25°C - 34°C. These areas in the northern parts include states like Bauchi, Benue, Borno, Kano, Kaduna, Kwara, Plateau and Jigawa while areas in the southern states include Delta and Oyo. Tomatoes are warm season crop and are sensitive to high humidity and rain. Thus, increase in yield is experienced in well drained, sandy loam and rich in humus soils. The planting season is between August and September. However, where irrigation farming is practiced, the best time for planting is during dry season, [2].

In spite of the development of various protection measures, economic important crop such as tomato unfortunately suffers severely from several diseases caused by fungi, bacteria, nematodes or by adverse environmental conditions, [3]. Control measures such as host plant resistance rotation with non-hosts sanitation

and avoidance, destruction of residual crop root, and judicious use of chemical nematicides have been reported to effectively control root-knot nematodes, [4]. However, the use of resistant varieties remains the most viable option particularly for small-scale farmers with limited resources, [5]. Even with these control strategies, root-knot nematodes are often cited as major limiting factor of crop production. The use of chemicals (chemical nematicides) for nematode control also have some setbacks such as concerns over environmental pollution, financial cost, toxicity and resistance development in nematode population, [6]. Taking into account the world-wide distribution of root-knot nematodes, it is therefore necessary to find effective and feasible control measure. Thus, the search for safer alternatives motivated this study.

In agricultural developing countries like Nigeria, it has been noted that among root-knot nematodes, *Meloidogyne incognita,* causes immense damage to vegetable crops. *Meloidogyne* spp (root-knot nematodes) are important pests of *Solanum lycopersicun* worldwide, [7]. Four major species namely *M. arenaria, M. hapla*, *M. javanica* and *M. incognita* have been reported to infect tomatoes in the tropics, [8]. These species cause gall or root-knot on infected plants. Other symptoms include stunted growth, wilting, and poor fruit yield. Infection by *Meloidogyne incognita* can increase root weight and decrease shoot weight, [9]. Root-knot nematodes *Meloidogyne* spp. cause high levels of economic loss in many agricultural crops worldwide. They are capable of severally damaging wide range of crops causing dramatic yield losses. It is in view of these setbacks that we are motivated to investigate plant extracts-based control of root knot nematodes infestation in tomato since one of the major problems of nematode management and control in Nigeria is that the target farmers are still peasant who cannot afford the necessary synthesis nematicides and who lack the essential technical skills to safely and effectively apply them, [10]. Therefore, the control of nematode by some plants extracts appear to provide one of the best options in Nigerian situation for good reason which include the ease of acceptability of the practice by the target farmers, the cost effectiveness of the strategy, the abundance and availability of the required items and most importantly, the safety of the method of application as reported by the authors in [11]. Other researchers that worked on plant extracts in the management and control of root knot nematodes are [12], [13], [14], [15], to mention a few.

II. Material And Methods

Experimental Site

The experiment was conducted in one season during the dry season between November and December in Plant Sciences Botanical garden of Modibbo Adama University Yola located in Girei Local Government Area of Adamawa State, Nigeria.

Collection and Sowing of Seed

Healthy tomato seeds cultivar (UTC) were obtained from a certified seed market in Jimeta, Adamawa State, Nigeria. The seeds were sown into 25cm diameter perforated polythene bag each containing the three leaves extracts differently while, the control bags were sown with sterilized soil without leaf extract. The seeds were sown 2cm depth and watered weekly using 1 litre container of water per bag and the soil were loosen from time to time to avoid compaction using hand fork.

Experimental Design

The design for the experiment was a Completely Randomized Design (CRD) and was replicated four (4) times including control. The treatments are bitter leaf (Vernonia amygdalina), false ashoka (Polyalthia longifolia) and Jatropha (Jatropha curcas).

Collection and Preparation of Plant Leaves

Leaves of bitter leaf (Vernonia amygdalina), false ashoka (Polyalthia longifolia) and Jatropha (Jatropha curcas) were collected with shears from their respective plant locations within and outside Modibbo Adama University Yola and spread on polythene sheets in a corridor for one week to air dry under shade. The plant leaves were grounded using grinding machine separately into fine particles adopting the method of the authors in [16]. Ten (10) g of the grounded leaves of bitter leaf (Vernonia amygdalina), false ashoka (Polyalthia longifolia) and Jatropha (Jatropha curcas) were each separately mixed in 100ml distilled water in an electric blender for 10 minutes and left for 72 hours. Each were considered as a standard solution of 100 % concentration and then kept in a freezer.

Collection and Sterilization of Soil

Top soil (sandy-loam soil) was collected within Modibbo Adama University Yola and sterilized with oven to a temperature of 100^{o}C and was maintained for 1 hour in Plant Science Laboratory, Modibbo Adama University, Yola, Nigeria.

Amendment Rate

Weight of 10g, 20g, 30g and 40g each of the grounded leaves were mixed separately with 25 kg of sterilized soil in a 25cm diameter perforated polythene bag. The control plant was measured into perforated polythene bag without mixture using method of the authors in [16].

Collection of Root-knot Nematode from Soil and Root

The pathogen was obtained from a gardener at Federal Housing Estate Bajabure, Girei Local Government Area. Sample of root knot nematode (Meloidogyne incognita) was collected from paw-paw plants root showing characteristic symptoms of gall formed and was carefully uprooted at 5 to 10cm depth using shovel in a zig-zag form from rhizosphere of the diseased plants with approximately 1kg of soil. The sample collected was placed in a polythene bag and taken to Plant Science Laboratory, Modibbo Adama University Yola for analysis using the method of the authors in [17].

Extraction of root-knot nematode from soil

The samples were extracted in Plant Sciences Laboratory, Modibbo Adama University Yola using the method described by [18] as described below:

Soil sample (20g) were mixed and measured in 250cm3 beaker; a wet tissue paper was placed into plastic sieve and placed on a plastic tray. The soil was poured into the sieve and water was added to it as needed in the tray and allowed for three days at room temperature of about 26^{o}C. The sample was covered to reduce evaporation and water was added as needed. The water from the soil sample (suspension) was collected into a beaker and 5ml was measured into a petri-dish and observed under a light microscope.

Extraction of root-knot nematode from root

The juveniles were extracted using the Baermanns method in [19] as follows:

Root collected was carefully washed to remove soil particles. Galled roots of the plants was cut into small pieces and poured into a blender, water was added. The roots were macerated into the blender. Double ply serviette paper was laid in each sieve and then placed in a tray. The content in the blender was poured into each sieve and water was gently poured into each tray and left on a flat slab for 48 hours to allow the nematode migrate through the serviette into the water in the tray and water was added as needed. The nematode suspension in the tray was collected and standardized such that 5ml contained approximately 50 juveniles.

Identification of root knot nematode species

The identification of the nematode species was carried using a perineal pattern method. Mature females were dissected out from large galls on the roots of tomato plants using a sharp dissecting needle (D201/01). Perineal pattern slides were prepared and examined under a stereo dissecting microscope (S6 Basic Stereo Zoom LED 7x-45x) to study their characteristics. The species were identified on the basis of their characteristic morphology involving the head morphology, stylet, vagina, anus, plasmids, tail terminal, lateral lines and annulation as reported by [20].

Counting of nematode

The counting of nematode was carried out using method described by [21] as follows:

A 100x and 400x eyepiece compound microscope was used for the counting. A 5ml from sample was taken and poured into a counting disk (60 x 15mm). Plain slide was used to support the counting dish. The nematode sample was allowed to settle down and counting was at 25-50 x magnification. Focusing knob was adjusted close to the specimen to give sharp image of nematode while tally form method was used for the counting. The sample was counted at least twice for accurate result.

Inoculation of Nematode

The tomato plants were inoculated with nematode four (4) weeks after planting for it to be active. The population of about 50 juvenile nematodes per plant was used. Holes of about 2cm deep and 1cm wide each were made in the soil around each seedling to expose the roots. The second-stage juvenile nematode suspension was applied three times in a week into each hole using syringe. The treatment was replicated three times for the period of one week.

Application of Leaves Extracts

Seed of tomato cultivar (UTC) was grown in a sandy-loam soil for four (4) weeks with 50 juveniles of root-knot nematode in perforated polythene bags were inoculated and then allowed to be active. Plant extracts from three (3) plant species of bitter leaf (Vernonia amygdaline), false ashoka (Polyalthia longifolia) and Jatropha (Jatropha curcas) were applied four (4) weeks after inoculation at four different concentration levels (10g, 20g, 30g and 40g). The control plant was grown without mixture by modifying the method of [17].

III. Data Collection

Four weeks after germination, data were collected weekly on root knot nematode population, number of leaves, branches, flowers produced and leaves shaded per plant were counted. About 50 juveniles nematode was inoculated into each hole per plant after four (4) weeks of planting. The total number of leaves and leaves shaded, branches and flowers produced were counted at weekly interval per plant.

IV. Data Analysis

The data collected at the end of the research were subjected to analysis of variance (ANOVA) and means were compared using Least Significant Difference (LSD) at 5 % probability level. While the LSD was employed in identifying significant difference between treatment means, the determination of statistical significance of treatment effects on yield and growth parameters were obtained using ANOVA.

V. Results

Effect of Leaf Extracts on the Performance of Tomato Plant Infected with *Meloidogyne incognita*

Studies have shown that there is a general reduction in nematode (*Meloidogyne incognita*) infestation of tomato upon application of leave extracts. The result showed that, there was significant difference ($p<0.05$) between the treated and control plants. The treated plant had the highest number of branches produced (4.67) compared to the control plant (2.00). It was observed that there was significant difference among plant treated with *Polyalthia longifolia*. The result showed that, there was significant ($p<0.05$) difference among plant growth with *Vernonia amygdalina* having the highest number of branches (5.33) followed by *Polyalthia longifolia* with (4.67) and *Jatropha curcas* having the lowest (4.00). It was observed that there was significant difference among plant treated with *Vernonia amygdalina*. Plant extracts of *Vernonia amygdalina* had the highest number of flower produced (3.33) followed by *Polyalthia longifolia* (3.33) and *Jatropha curcas* had the lowest number (2.33). It was also observed that there was significant difference among plant treated with *Jatropha curcas*. Plant treated with *Vernonia amygdalina* leaves extract had the highest number of leaves produced (18.00) followed by *Polyalthia longifolia* had (17.00) and *Jatropha curcas* had the lowest number of leaves produced (12.00) as shown in Table 1.

Table 1: Effect of Leaf Extracts on the Performance of Tomato Plant Infected with *Meloidogyne incognita*

Treatments (g)	Branches	Flower Produced	Number of Leaves
V. amygdalina	5.33 ± 1.20	3.33 ± 0.88[a]	18.00 ± 5.29[a]
P. longifolia	4.67 ± 0.66	3.33 ± 0.66[a]	17.00 ± 3.05[a]
J. curcas	4.00 ± 0.57	2.33 ± 0.33[a]	12.00 ± 1.15[ab]
Control	2.00	1.00	4.00
LSD	2.301	1.694	8.799
F - Value	3.082	7.505	4.074
P - Value	0.062	0.004	0.031
Remark	NS	S	S

Means followed by the same letter (s) within the same treatment column are not significantly different at 5% level of probability according to Least Significant Differences (LSD)

* NS - Not Significant S - Significant

Different Concentration of Leaf Extracts of *Vernonia amygdalina* on *Meloidogyne incognita* infected Tomato Plant

The result on different concentration of leave extracts of *V. amygdalina* shows that there is significant difference ($p<0.05$) among the doses. The concentration at 10g of *V. amygdalina* extract produced higher number of branches with (5.33) while concentration at 30g produced the lowest (4.00). It was observed that there was no significant difference in the number of branches produced. The result of the study showed that, concentration at 40g of *V. amygdalina* extract produced much flower with (3.67) while concentration at 30g produced fewer flowers (2.33). There was no significant difference ($p<0.05$) observed among the doses in the number of flower produced. The dose at 10g of *V. amygdalina* extract produced higher number of leaves with (18.00) than the dose of leaf extract at 30g with the lowest number of leaves (12.00). The study showed that there was significant difference ($P<0.05$) only among doses at 10g, 20g and 30g as shown in Table 2.

Table 2: Different Concentration of Leaves Extracts of *Vernonia amygdalina* on *Meloidogyne incognita* Infected Tomato Plant

Leaf Extract (g)	Branches	Flower Produced	Number of Leaves
10	5.33 ± 1.20	3.33 ± 0.88^{a}	18.00 ± 5.29^{a}
20	4.67 ± 0.66	3.33 ± 0.66^{a}	17.00 ± 3.05^{a}
30	4.00 ± 0.57	2.33 ± 0.33^{a}	12.00 ± 1.15^{ab}
40	4.67 ± 0.66	3.67 ± 0.33^{a}	15.00 ± 0.00^{a}
LSD	2.301	1.694	8.799
F - Value	3.083	7.808	4.064
P - Value	0.068	0.004	0.033
Remark	NS	S	S

Means followed by the same letter (s) within the same treatment column are not significantly different at 5% level of probability according to Least Significant Difference (LSD)

* NS - Not Significant S - Significant

Different Concentration of Leaf Extracts of *Polyalthia longifolia* on *Meloidogyne incognita* infected Tomato Plant

The result of the study showed that, dose at 40g had the highest number of branches (8.33) compared to the dose at 20g with the lowest number of branches (3.67). It was observed that, there was no significant difference ($p<0.05$) among the doses. The result on the number of flowers produced as the lowest flower produced was (2.33) at 10g and the highest number of flowers produced was (3.33) at 40g. There was significant difference ($p<0.05$) observed only between doses at 40g and 30g. The result obtained on the number of leaves produced among the doses showed that dose at 30 g had the highest number of leaves (27.33) compared to the extract dose at 10g with the lowest number of leaves (3.67). There was significant difference ($p<0.05$) difference among the doses but none was observed between dose 30g and 40g as shown in Table 3.

Table 3: Effect of Different Concentrations of Leaf Extracts of *Polyalthia longifolia* on *Meloidogyne incognita* Infected Tomato Plant

Leaves Extract (g)	Branches	Flower Produced	Number of Leaves
10	3.67 ± 0.66^{bc}	2.33 ± 0.88	3.67 ± 3.28
20	3.67 ± 0.66^{bc}	3.00 ± 0.00	13.00 ± 3.60
30	7.33 ± 1.76^{ab}	2.67 ± 0.66	27.33 ± 9.82
40	8.33 ± 1.66^{a}	3.33 ± 1.20	25.67 ± 5.04
LSD	3.787	8.623	15.415
F - Value	5.661	0.855	3.096
P - Value	0.012	0.523	0.067
Remark	S	NS	NS

Means followed by the same letter (s) within the same treatment column are not significantly different at 5% level of probability according to Least Significant Difference (LSD)

* NS - Not Significant S - Significant

Different Concentration of Leaf Extracts of *Jatropha curcas* on *Meloidogyne incognita* infected Tomato Plant

The result showed significant difference ($p<0.05$) among the doses. The dose of *J. curcas* leaf extract at 30g produced the highest branches (10.33) while the dose at 10g produced the lowest branches (6.67). There was no significant difference ($p<0.05$) observed among the doses. The dose of *J. curcas* leaf extract at 20g produced the lowest flower (1.67) while the dose at 30g produced the highest flower (2.67). There was no significant difference ($p<0.05$) observed on flower produced among the doses. The dose at 30g of *J. curcas* extract produced the highest number of leaves (39.33) while the dose at 40g dose of *J. curcas* extract produced the lowest leaves (22.33). There was significant difference ($p<0.05$) observed between dose 30g and other doses on the number of leaves produced as shown in Table 4.

Table 4: Effect of Different Concentrations of Leaf Extracts of *Jatropha curcas* on *Meloidogyne incognita* Infected Tomato Plant

Leaves Extract (g)	Branches	Flower Produced	Number of Leaves
10	6.67 ± 0.88^{b}	1.67 ± 0.33	24.00 ± 3.46^{b}
20	8.33 ± 0.88^{ab}	1.67 ± 0.66	27.67 ± 4.66^{ab}
30	10.33 ± 0.88^{c}	2.67 ± 0.33	39.33 ± 2.72^{a}
40	7.33 ± 1.45^{ab}	2.00 ± 0.57	22.33 ± 5.48^{b}
LSD	3.007	1.409	12.011
F - Value	8.744	1.833	9.895
P - Value	0.003	0.199	0.002
Remark	S	NS	S

Means followed by the same letter (s) within the same treatment column are not significantly different at 5% level of probability according to Least Significant Difference (LSD)
* NS - Not Significant S - Significant

VI. Discussion Of Findings

This study focused on plant extract-based control of root knot nematode infestation on tomato. The result of this study showed that the control of root-knot nematode using three plant extracts namely: leaf extract of Jatropha curcas (Jatropha), Vernonia amygdaline (Bitter leaf) and Polyalthia longifolia (False ashoka) managed the population of Meloidogyne incognita in tomato plant. Based on the findings of the studies, the effect of leaf powders of Vernonia amygdalina, Polyalthia longifolia and Jatropha curcas used as plant extracts to control Meloidogyne incognita in the soil shows that all the treatments significantly ($p<0.05$) suppressed the development of Meloidogyne incognita in the soil as compared to the control.

Studies have shown that there is a general reduction in nematode (Meloidogyne incognita) infestation of tomato upon application of leave extracts between the treated and control plants. The treated plant had the highest number of branches produced compared to the control plant. Plant extracts of V. amygdalina had the highest number of flower produced while plant treated with J. curcas leaves extract had the highest number of leaves produced. This is in line with the work of [22] on the number of branches, flowers and leaves produced.

With reference to different concentrations of leave extracts among the doses, the concentration at 10g of V. amygdalina extract produced higher number of branches. The concentration at 40g of V. amygdalina extract produced much flower. The dose at 10g of V. amygdalina extract produced higher number of leaves. This is in line with the work of [23] on the concentration of leave extracts among doses. The result obtained on the number of leaves produced among the doses showed that dose at 30g had the highest number of leaves compared to the extract dose at 10g had the lowest number of leaves. The result showed significant difference ($p<0.05$) among the doses. The dose of J. curcas leaf extract at 30g produced the highest branches. The dose at 30g of J. curcas extract produced the highest number of leaves while the dose at 40g dose of J. curcas extract produced the lowest leaves. This study agrees with the work of [18] on the use of leaf extracts at different concentrations.

VII. Conclusion

Conclusively, while the findings of this study showed that the three extracts were effective, it was observed that the leaf extracts of Jatropha curcas demonstrated significant nematicidal potential in controlling root-knot nematodes and gives the best growth and yield of tomato in comparison to the other two. This is because the Jatropha curcas extract significantly reduce the nematode population by inhibiting their reproduction, egg hatching and reducing root galling. This indicates that controlling root knot nematode (Meloidogyne incognita) with leaf extract is a potential viable venture. The result obtained from this study is in consonance with existing literature advocating for continued use of plant extracts in controlling root knot nematode. Further research in the field is recommended especially under different geographical locations to ascertain these findings and to explore possibility of processing those leaf extracts in large quantity and the use of other plant extracts for the control and management of other nematodes.

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