

Evaluation of Nematicidal Activity of Aloe Vera Against *Meloidogyne Incognita* Associated with Tomatoes in Jos North L.G.A.

John Walter Chinaka*, Adentan Olusolape Esther*, Ihum, Temitope A**, Olorundare Olufunke Olufunke*.

*Federal College of Forestry Jos, PMB 2019, Jos, Plateau State, Nigeria

**Nigeria Stored Products Research Institute, Ilorin, Kwara State, Nigeria

Abstract- Nematicidal activity of *Aloe vera* plant at different concentration treatments were evaluated to determine its effect on root-knot nematode. The study was conducted at the Federal College of Forestry Jos, Plateau State of Nigeria between March and May, 2017. Nematicidal activity of *Aloe vera* was tested on *M. incognita* associated with tomatoes using 80mg/ml, 70mg/ml, 60mg/ml, 50mg/ml and 40mg/ml of the extract. Modified Baermann Funnel Method was used for nematode extractions and 70% ethanol was used for *Aloe vera* extraction analysis. Seedlings of the tomato plant grown in a sandy-loam soil were inoculated with 400 eggs of root-knot nematodes, *Meloidogyne incognita* on nursery bed. The nematicidal activity of the *Aloe vera* ethanolic extract was evaluated based on tomato plant height, root length, shoot weight, and fruit yield. A complete randomized design (CRD) was used and data collected were analyzed using analysis of variances (ANOVA) to determine the significant differences. The results showed that there was a significant difference at $p \leq 0.05$ level. The progressive growth and yield of the tomato plant was significantly increased (compared to the control), indicating nematicidal activity of *Aloe vera* extract. The highest result was obtained using 80mg/ml treatments which showed tomato plant height in week one at 18.10cm, which was higher in week three at 36.30cm. There was a marked difference when compared with control treatments. The results obtained indicated *Aloe vera* extract contain active component that exerted inhibitory effect against nematodes, *Meloidogyne incognita* and can be used as a bio-control agent.

Index Terms- *Lycopersicon esculentum*, Nematicidal, *Aloe vera*, *Meloidogyne incognita*

I. INTRODUCTION

Tomato (*Lycopersicon esculentum*) is an edible red fruit of *Solanum lycopersicum*, belongs to the nightshade family Solanaceae Jibia, et al., 2014 [1]. Tomato is one of the most important tropical vegetable crop widely used throughout the world. In recent years, root-knot nematodes *Meloidogyne* spp. problem has become a threat to tomato cultivations. Yield loss due to nematode cause diseases to nearly all plant crops of Economic importance with estimated losses of US \$125 billion per year World-Wide Chitwood, 2003 [2]. They can cause significant plant damage ranging from negligible injury to total destruction of plant materials. Nematodes had long been known to attack crops but had been studied less than the insects, this is

because of their minute nature. Control of root-knot nematodes has been primarily accomplished through chemical nematicides. However, indiscriminate use of chemical pesticides causes great threat to human being, animals, vegetation and to the environment as a whole due to their non-target effect, hazardous nature and besides they are expensive. So with the increasing awareness of possible deleterious effects of the chemicals, biological controls of plant pathogen have received considerable attention Gaima, et al., 2005 [3]. Leaf of *Aloe vera* extracts apply directly to the soil will tend to offer a more nematode control, environmentally friendly and chemical-free possibilities as there is an urgent need to replace pesticides with alternative means of control that are less toxic and more environmentally friendly. Many investigators had managed root-knot nematodes by using some plant dried powder of certain ornamental plants Akhtar and Mohmood, 1993 [4]. In this research, activity of the leaf extracts of *Aloe vera* is study as nematicides for the control of root-knot nematodes, *Meloidogyne incognita* attacking tomato.

II. MATERIALS AND METHODS

A. Sample Collection

Infected tomato plants for extraction of nematode eggs and seedlings of tomatoes used for tomatoes cultivation were collected from farms around Fari-gada area of Jos, Plateau State. The seedlings were transplanted in the nursery of Federal College of Forestry, Jos. 100g of leaves of *Aloe vera* plant were collected periodically from the nursery and taxonomically at the Herbarium of Federal College of Forestry, Jos.

B. Laboratory Extraction of Nematodes

The infested young plant which showed sign of stunted growth and scanty leaves were used for the nematode extraction. Large and small roots showing swelling varying from round shaped sphere-like galls to elongated spindle from large numbers of individual galls were carefully up-rooted and washed gently to remove the soil directly attaching to the tomato. Suspected galls on the root were carefully transported to the laboratory where they were excised and teased with the use of forceps and teasing pins in order to expose and release the nematode from the root tissues. The extraction of the root-knot Nematodes from the host plant roots was done using the Modified Baermann Funnel Method Southey, 1972 [5]. A regular funnel with a piece of rubber tube about 25-30cm long attached to its stem and in turn connected to the test-tube tightly held together with the aid of

masking tape was constructed. The setup was kept in an upright position using a table stand with small regular holes and filled with distilled water to the brim of the funnel's stem. Cotton wools was placed in the funnel to assume the shape of the funnel so that the water slightly covered the wool before the teased root samples for the extraction to be place on the wool and then cover with water to prevent the samples from drying. The set-up was then allowed to stand for 24 hours. The juvenile Nematodes being very active readily passed through the cotton wool down the funnel stem and were collected at the bottom of the test-tube. The test-tubes were then carefully removed and their contents were centrifuged at 2000 RPM (Revolution per minute) for five (5) minutes in order to concentrate the nematode juveniles in the test-tubes which were then taken for examinations, four hundred (400) total number of nematode were estimated in the each test-tubes used.

C. Identification of Nematodes

Suspension of nematode extract was centrifuged at 200rpm, the residue in the test-tube of extracted nematode was dropped onto a grease-free microscopic glass slide and covered with a cover slip, then viewed under a compound microscope at low power magnification (x10) and high power magnifying lens (x40) and identify using identification aid.

D. Preparation of the Plant Material

The leaf extracts was prepare from fresh and healthy *Aloe veraplant*, they were cut vertically. The plants then dried at room temperature and pulverized using mortar, along with pestle and sieved into powder; using 60 mesh screens Vats, *et al.*, 1995 [6]. The powdered leaves were soaked in 70% ethanol and was left to stand for 72 hours (3days) and then filtered using Whatman's filter paper No. 30 Gundare, 2007 [7]. Water was allowed to evaporate from the filtrate using hot plates at a temperature of 50°C suitable for even evaporation while still maintaining the active components within the filtrates especially the heat labile ones. The resultant powdered extracts were collected and then used for the nematocidal tests. Different concentrations of the leave Extracts were prepared by dissolving variable concentration of the extracts in distilled water as follows:

1. 0.8g of *Aloe verato* 10ml of distilled water
 $0.8\text{g in }10\text{ml} = 0.08\text{g/ml}$
 $= 80\text{mg/ml}$
2. 0.7g of *Aloe verato* 10ml of distilled water
 $0.7\text{g in }10\text{ml} = 0.07\text{g/ml}$
 $= 70\text{mg/ml}$
3. 0.6g of *Aloe verato* 10ml of distilled water
 $0.6\text{g in }10\text{ml} = 0.06\text{g/ml}$
 $= 60\text{mg/ml}$
4. 0.5g of *Aloe verato* 10ml of distilled water
 $0.5\text{g in }10\text{ml} = 0.50\text{mg/ml}$
 $= 50\text{mg/ml}$
5. 0.4g of *Aloe verato* 10ml of distilled water
 $0.4\text{g in }10\text{ml} = 0.04\text{g/ml}$
 $= 40\text{mg/ml}$

E. Planting of Tomato and Determination of Nematicidal Activity

Two weeks old seedlings of tomato planted obtained from Faringada was transplanted in nursery of Federal College of Forestry in six different pots containing sterilized loamy soil. Three blocks in area of about 300m² partitioned into five (5) plots with 1m alley each in-between plots and blocks, was prepared, this was done to avoid treatments interaction. Each plot was 50m² for one treatment between the tested nematicidal extract. Four hundred (400) total numbers of the estimated nematode were thoroughly mixed with the plots soil to which the transplanted tomatoes was planted. Various concentrations (80mg/ml, 70mg/ml, 60mg/ml, 50mg/ml, 40mg/ml,) of extract were applied by bare surface placement at 3cm into the soil and 1cm away from the tomato plant after 21 days of nematode inoculation. 5 milliliters (5ml) each of the concentrations was applied per plant and the plants were watered regularly Jibia, *et al.*, 2014 [1]. Tomato plants that were treated with water only served as the control. Each treatment was replicated four (4) times and laid out in a randomized complete design. Plant height, fruit yield, shoot weight and root length at final harvest were recorded. The growing plant in each plots were examined at intervals of three weeks for three mouth.

III. PARAMETERS

A. Plant Height (cm)

The plant height were obtained by taking measurement (cm) of plant height in each plot in the nursery using a measuring tape. The data were recorded at interval of two (3) weeks in three (3) replicates.

B. Root Length (cm)

The root lengths were obtained by taking measurement (cm) of root length in each plot in the nursery using a measuring tape. The data were recorded at interval of two (3) weeks in three (3) replicates.

C. Shoot Weight (g)

The shoot weights was obtained using the weighing balance in which the shoot was un-plucked and weighed. The data obtained were recorded at interval of two (3) weeks in three (3) replicates.

D. Fruit Yield of Plant

The plant yields were obtained by counted with hand fruit yield within each plots in the field. Data obtained were recorded at interval of two (3) weeks in three (3) replicates.

IV. DATA ANALYSIS

Data collected were analyzed using analysis of variance, ANOVA to determine the significant difference between the concentrations of the *Aloe vera* plant extract and mean separation were made using Duncan multiple ratio test, DMRT.

V. RESULTS AND DISCUSSION

A. Results

Table 1: Identification of nematodes

Nematode	Microscopic Features
Juvenile	<ul style="list-style-type: none"> - Head not offset with truncated cone shape when viewed laterally. - Stylet knob is prominent and rounded.
Adult male nematode	<ul style="list-style-type: none"> - The head is not offset with a high truncate cone shape. - The head cap is clearly annulated. - The head cap is with stepped outline in lateral view. - Annule number behind head cap very variable usually 1-3 on sub-lateral head sector. - Conus of stylet longer than shaft. - Stylet knob is prominent usually of greater width than length with flat concave or toothed anterior.
female Adult nematode	<ul style="list-style-type: none"> - The body is spherical with projecting neck. - Head with 2 or 3 annule behind the head cap. - The cuticle thickening at base of relaxed stylet. - Stylet knobs are drawn out laterally. - Dorsal arch is high and rounded.

Table 2: Evaluation of Nematicidal effect of Aloe vera extract base on tomato length

Treatment (mg/ml)	Plant height (cm)		
	Week 3	Week 6	Week 9
80	18.10 ^a	21.00 ^a	36.30 ^a
70	13.00 ^b	20.10 ^a	34.10 ^a
60	12.20 ^b	17.00 ^b	29.00 ^b
50	10.00 ^{bc}	15.33 ^b	27.00 ^{bc}
40	8.00 ^c	11.00 ^c	23.40 ^{cd}
Control	7.00 ^c	10.00 ^c	22.00 ^e
SE±	1.00	0.79	1.41

Mean followed by the same superscript in a column are not significantly different from each other.

Table 3: Evaluation of Nematicidal effect of Aloe vera extract base on tomato root length

Treatment (mg/ml)	Root length (cm)		
	Week 3	Week 6	Week 9
80	9.00 ^a	11.00 ^a	22.20 ^a
70	5.10 ^b	8.00 ^b	20.00 ^{ab}
60	4.10 ^{bc}	5.40 ^c	18.00 ^{bc}
50	2.00 ^{cd}	4.30 ^{cd}	17.00 ^{bcd}
40	2.00 ^{cd}	3.00 ^{cd}	15.30 ^{cd}
Control	1.27 ^d	2.00 ^d	14.10 ^d
SE±	0.67	0.82	0.94

Mean followed by the same superscript in a column are not significantly different from each other.

Table 4: Evaluation of Nematicidal effect of Aloe vera extract base on tomato shoot weight

Treatment (mg/ml)	Shoot weight (g)		
	Week 3	Week 6	Week 9
80	12.40 ^a	18.70 ^a	29.20 ^a
70	9.21 ^b	15.50 ^b	24.60 ^b
60	6.11 ^c	11.22 ^c	20.40 ^c
50	6.12 ^c	11.21 ^c	20.30 ^c
40	6.11 ^c	11.11 ^c	20.30 ^c
Control	2.17 ^d	5.13 ^d	11.30 ^d
SE±	0.02	0.34	0.06

Mean followed by the same superscript in a column are not significantly different from each other.

Table 5: Evaluation of Nematicidal effect of Aloe vera extract base on tomato yield

Treatment (mg/ml)	Fruit Yield		
	Week 3	Week 6	Week 9
80	6.00 ^a	16.00 ^a	27.00 ^a
70	4.00 ^{ab}	14.00 ^{ab}	24.00 ^{ab}
60	4.00 ^{ab}	14.00 ^{ab}	24.00 ^{ab}
50	3.00 ^{ab}	13.00 ^{ab}	23.00 ^{ab}
40	2.00 ^b	12.00 ^b	22.00 ^b
Control	6.00 ^a	16.00 ^a	26.00 ^a
SE±	1.00	1.00	1.00

Mean followed by the same superscript in a column are not significantly different from each other.

B. Discussion

Poor yield of tomato has been attributed to nematode diseases Stone, *et al.*, 2000 [8] and tomato plants are highly susceptible to *Meloidogyne incognita* Agbenin, *et al.*, 2004 [9]. The result on Table 1 showed that *Meloidogyne incognita* identify has distinctive features such as head not offset with truncated cone shape, stylet knob prominent and round, conus of stylet longer than shaft. Similar report been made by Orton Williams, 1973 [10] showed *M. incognita* body is spherical with projecting neck, in lateral view, stylet knob are drawn out, the head is clearly annulated, conus of stylet is longer than shaft. The increase in plants parameters such as the plant height, root length, fruit yield and shoot weight shown in Table 2, 3, 4 and 5 suggests that the ethanolic extracts of *Aloe vera* used in this work have potential nematicidal effect on the root knot nematodes (*Meloidogyne incognita*). Jibia, *et al.* 2014 [1] reported that the test plant extracts of *A. indicaleaf*, *C. annuumfruit*, *Z. officinalerhizome*, *P. biglobosaseed* on the micro plots suppressed the population root knot nematodes in tomato. The extent of nematode population reduction was dependent on the rate of application of different concentration of the extracts and time of exposure, inhibition of *M. incognita* reproduction resulted in significant improvement in the tomato growth parameters and yield, a similar report by (Alam, 1991 [11]; Ramesh, *et al.*, 2008 [12] that plant powder treated plant showed increased plant growth parameters and yield and may be due to an additive effect of nutrients produced. It was also earlier reported by Adegbite, 2003 [13], Adegbite and Adesiyun, 2005 [14], Ranjitsingh, *et al.*, 2009 [15] and Umar, 2013 [16] that botanical extract that contained alkaloids, saponins and flavonoids either singly or in combination inhibited of

Meloidogyne spp. In a related work Ferreira, *et al.*, 2013 [17] reported that aqueous extracts of *zinnia peruviana* and *Wedelia* species inhibited *Meloidogyne incognita* when compare to the control by 92.72% and 97.48% respectively. Khan, *et al.*, 2008 [18] reported that extracts of some plants such as onion, garlic, tobacco, cloves and chill were effective against *M. incognita* larvae and caused mortality of juveniles between 82-100%. The ready availability of the organic materials used in this study, its effects on nematode population, plant growth and yield suggest the need for additional studies in the field to evaluate the efficacy and economics for its use in nematode management. It was established in this work that soil treated with the ethanolic extracts had higher reduction in the population of *M. incognita*, which is opposed to that of control experiment in which untreated plants shows significance difference ($p > 0.005$) with an increased poor fruit set up and swollen root galls. Abulusoro, 2004 [19] reported that the susceptibility in tomato plants infected with root-knot nematodes (*Meloidogyne* spp.) show stunted growth, yield loss and conspicuous root galls, but a number of plants are thought to contain biologically active ingredients which when applied in the soil reduced the incidence of plant parasitic nematodes. The responses to pesticide treatment were not compared directly with the results obtained from extract of *Aloe vera* plant tested, because this material showed promising outcome.

VI. CONCLUSION

The results obtained showed the nematicidal content of the extracts inhibited nematodes. The effect of the different extracts of the botanical on the performance of tomato was

significantly different at 5% level of probability. Tomato crop treated with different concentration recorded taller plant, longer root, higher shoot weight and high yield. The treated plant recorded better growth parameters and fewer nematodes due to the nematicidal or nemostatic effect of the different concentrations of extract. It was reported that extracts of plant containing tannins, alkaloids and flavonoids were effective against root-knot nematodes both *in-vivo* and *in-vitro* (Adegbite and Adesiyani, 2005, Anuja and Satyawati, 2007 and Umar, 2013). The result of the study indicated that 80mg/ml was more effective against *M. incognita* and hence improved tomato growth and yield. Although, others treatment were also able to reduced nematode population and were not as effective as the 80mg/ml. Further work should be conducted to determine the actual active ingredient and purify

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AUTHORS

- First Author** – John Walter Chinaka, Msc Microbiology, Federal College Of Forestry, Jos, Nigeria
Second Author – Adentan Olusolape Esther, Msc Entomology And Parasitology, Federal College Of Forestry, Jos, Nigeria
Third Author – Ihum, Temitope A. Msc Environmental Microbiology, Nigeria Stored Products Research Institute, Ilorin, Nigeria
Fouth Author – Olorundare Olufunke Olfunke, Bsc Microbiology, Federal College Of Forestry, Jos, Nigeria
Correspondence Author – John Walter Chinaka, wchinaka@yahoo.com, 08036137846