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Original Article

Bionematicidal Potentials of *Azadirachta indica* (A. juss), *Vernonia amygdalina* (DEL), *Manihot esculenta, Carica papaya. L.* and *Citrus sinensis* on *Meloidogyne incognita* of *Capsicum annuum*, Var. Bell

Godwin P. Angaye[1], Helen .O Imafidor[1], Nzeako O. Sidney[1] and Tariwari C.N Angaye[2*]

[1]Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria
[2]Ecotoxicology Research Unit, Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

***Corresponding Author**
Tariwari C.N Angaye
Ecotoxicity Research Group,
Department of Biological Sciences,
Niger Delta University, Wilberforce Island,
Bayelsa State, Nigeria.
E-mail: maktarry@yahoo.com

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Abstract

Plant parasitic nematodes especially *Meloidogyne* species are linked to global reduction or loss in the yield of agricultural produce. If plant diseases are left unchecked, it could lead to food shortage. Synthetic nematicide could induce toxicity, environmental degradation and are expensive. Root-knot nematodes (RKN) are devastating as it affects many economically important agricultural crops globally. The bionematicidal potential of pepper plant nematode was investigated with five plants (*Azadirachta indica*, *Vernonia amygdalina, Manihot esculenta, Carica papaya.* and *Citrus sinensis*) in a two-phased trial, at concentrations of 20, 30 and 40g within exposure periods of 30, 60 and 90-days. Generally, Compared to the control, all bionematicides similarly showed significant nematotoxocity for both trials with significant difference ($p < 0.05$). Thus the degrees of nematotoxicity are reported as: *A. indica* > *V. amygdalina* > *C. sinensis* > *C. papaya* > *M. esculentus*. Based on the findings of this research, we therefore conclude that the tested plant demonstrated significant levels of nematotoxicity and can be so regarded as Bionematicides.

1. Introduction

The worldwide distribution of root-knot nematodes (*Meloidogyne* species), and their extensive host range, and associations with fungi and bacteria in disease complexes rank RKNs amongst the major pathogens affecting crops [1]. Plant parasitic nematodes are responsible for global agricultural losses amounting to an estimated $157 billion annually [2]. The root-knot nematodes (RKN) seriously affect many economically important agricultural crops worldwide [3]. It has been estimated that 10% of world crop production is lost as a result of plant parasitic nematode damage [4]. This represents one third of the total losses attributed to pests and diseases [5].

Nematode not only suppresses the plant growth but also interferes in the nodulation, nitrogen fixation and adversely affects the overall yield [6]. The disease is manifested by the formation of galls in the root accompanied by stunted growth, chlorosis and loss of vigour of the plant [7]. They play an important role in reducing yield and quality of fruits besides the life span of plants [8]. The effects nematodes have on crop production and yield is made worse by a number of abiotic factors such as temperature, soil moisture and soil pH [9]. This intensifies the challenge facing agricultural scientists today to secure food for a world population that increased by 3 billion (1970-2006) to the current 6.5 billion people, and is predicted to continue growing [10]. This population increase has occurred predominantly in developing, mostly tropical countries, where the majority (820 million) of hungry people live, and a number currently growing at the rate of 4 million a year [9].

Several pests and diseases reduce the nutritive value of crop plants and influence their growth and development [8]. But one of the pests that inhibit the dream of African countries from achieving food sustainability is a group of plant parasitic nematodes commonly referred to as Root Knot nematodes of the Genus *Meloidogyne* [11 - 13]. They are the most abundant and most damaging of plant parasitic nematodes [4]. They are a great threat among all nematode species and one of the most harmful pests in both tropical and subtropical regions, causing extensive economic damage worldwide [14]. These pests affect the quantity and quality of crops [8].

In order to reduce the production losses caused by the various nematode species, several control methods have been suggested. Nematodes may be controlled with chemicals to a certain extent, but these may cause adverse environmental effects and, generally, nematicides are too expensive for subsistence farmers [15]. Control of root-knot nematodes is inevitable so as to make vegetable production profitable, thus developing new and environmentally friendly control methods is required. Currently, soil fumigants and nematicides are used to control nematodes. However, the need to reduce dependence on nematicides, imposed by legislation and consumers, requires the development of new management strategies. Biological control as an alternative is an important tool for plant-parasitic nematode management [16].

2. Materials and Methods

2.1 Study area: The experiment was conducted in the Parasitology laboratory of the Department of Animal and Environmental Biology and The Green House area of the Department of Plant Science and Biotechnology of University of Port Harcourt, Abuja Campus, Choba, Port Harcourt, Rivers State, Nigeria. The coordinates and exact location of the Green House area was determined using GPS (global positioning system) and found to be N04° 54" 26'. E06° 55" 39'.

2.2 Soil Analysis and Sterilisation: Samples of garden soil were first analysed to determine the physico-chemical properties and found to consist of the following: clay 16.8%, silt 12.8%, sand 70.4%, Cu 13.2 mg/g, Zn 2.76 mg/g, Fe 86.35 mg/g, Mn 35.22mg/g, Al 0.00 Cmol/kg, Na 0.351 Cmol/kg, K 0.093 Cmol/kg, Mg 0.535Cmol/kg, Ca 0.246 Cmol/kg, P 12.22 mg/g, N 0.293%. The soil was heat-sterilized at 60 °C for 45 minutes. This process raised the soil temperature to an average that is lethal to parasitic nematodes and other soil fauna and flora [17]. The sterilized soil was allowed to cool and later packaged and stored in 10cm by 8cm polythene bags, which served as pots for the experiment.

2.3 Procurement and Preparation of Biocontrol Agents – Treatments: Fresh green leaves of Neem (*Azadirachta indica*) and Bitter Leaf (*Venonia amygdalina*) were be purchased at Swahli Market in Yenagoa LGA in Bayelsa State. Fresh leaves of Cassava (*Manihot esculentus*), Pawpaw (*Carica papaya*) and Orange (*Citrus sinensis*) will obtained from different farms in Okoloba Town in Kolokuma/Opokuma Local Government Area of Bayelsa State. After procurement, the leaves were be properly washed and air dried separately in a well-ventilated room at room temperature ranging from 26-30 °C for 6 weeks and were later spread in direct sunlight for one hour before blending them into fine powder [18]. These were later packed in black polythene bags and stored in air and watertight containers away from direct sunlight [19].

2.4 The Experimental Design: The experiment was conducted under field conditions in a completely randomized block design. 180 potted plants were planted in 5 groups. Thirty six (36) plants were in each group, out of which 9 plants served as the Control. These potted plants were inoculated each 1000 eggs/juveniles/adults of *M. incognita* and were treated with 20g, 30g, and 40g of the powder of the bio-nematicides, (with the exception of the control). Group A was inoculated with 1000 eggs/juveniles/adults of *M. incognita* and was treated with 20g, 30g, and 40g of neem (*A. indica*) powder in three replicates.

2.5 Preparing and Planting the Nursery: A good mixture of topsoil, compost and coarse river sand in ratio 3:2:1 [20], was heat-sterilized at 60°C for 45 minutes [17]. The sterilized soil was placed in 4 plastic baskets (to avoid overcrowding) occupying about 80% of the container. Care was taken not to firm the soil too much for easy transplanting [17]. After planting, the nurseries were placed in the Green house to keep the soil moist. The plants were regularly watered and allowed to grow for 3 weeks after germination before they were transplanted to the field [20]. 20g of Hot pepper (Big Sun) the seed of the pepper cultivar (Capsicum annuum var. bell) used for this experiment was purchased from Agritropic Vilmorin Limited-Vegetable Seeds for Nigeria, RC: 338009, Ibadan, Nigeria.

2.6 Transplanting: Using a hand trowel the seedlings were transplanted from the nursery to the pots in the field 3 weeks after germination [20]. The plants were regularly watered and unwanted plants (weeds) were removed. NPK fertilizer (15:15:15) was applied at the rate of 5 g per plant 2 weeks after transplanting [18].

2.7 Extraction and Sterilization of Nematodes: Capsicum plants infested with root knot nematodes (M. incognita) were collected from a pepper farm at Gokana LGA in Rivers State. Eggs were extracted from the infested roots. The roots (about 10g) was chopped into smaller pieces with scissors and immersed in 100ml of 5% Sodium hypochlorite (NaOCl) solution and was vigorously shaken for about 3 minutes. The chopped pieces of roots was quickly passed through a 200 mesh (75um) over a 500 mesh (26um).The egg masses, juveniles and adult species of nematodes that were trapped on the 500 mesh were washed in a gentle stream of tap water to remove residual Sodium hypochlorite (NaOCl) solution. The remaining roots pieces in the 200 mesh (75um) were rinsed with tap water to recover additional nematodes, egg masses and juveniles. [21, 22].

2.8 Standardization of Innocula: The volume of the suspension was standardised to 50ml. Aliquot of 1ml of each suspension will be taken with a pipette into a counting tray after bubbling air through the suspension for homogenecity and counting will be done with the aid of a microscope and the number of eggs/ juveniles/adults of M. incognita per ml estimated.

2.9 Inoculation: 1000 eggs/juveniles/adults of M. incognita were inoculated to each of the 180 plants 4 weeks after transplanting. Holes were made in a triangular form, 2cm from the pepper plant. The eggs/ juveniles/adults of M. incognita in the suspension were then dispensed into the holes made around the roots of each plant and was be covered with the soil [18].

2.10 Treatment: 48 hours after inoculation, each of the plants (the control excluded) were treated with the 20g, 30g and 40g of the powder of the *Azardirachta indica, Venonia amygdalina, Manihot esculentus, Carica papaya* and *Citrus sinensis*, accordingly.

2.11 Bioassays: Bioassay on the soil and root of pepper plants was carried out every 30 days after inoculation for 3 months (90 days) to determine the nematode population and the rate of infection. Twelve (12) plants from each group were uprooted. Later counting was be done with binocular microscope using glass slides after staining with Lugol's iodine to enhance visibility. The volume of each suspension was standardised to 20ml. Aliquot of 0.1ml of each suspension was taken with a pipette and homogenized by blowing air through it and counting was be done with the aid of a light microscope. Nematode population in the soil was determined using the modified Bearmann's funnel technique according to Whitehead and Hemingway [5].

2.12 Statistical Analysis: SPSS software version 20 was used to carry out the statistical analysis of the. The data were expressed as Mean ± standard deviation. A one-way analysis of variance was carried out at P = 0.05, and Duncan Statistic was used to determine the source of the detected differences.

3. Results

Tables 1 and 2 presents the nematoxicity activities at 30, 60 and 90-days extracted from 5g of roots of Capsicum treated with 20, 30 and 40g of all Nematicides for 1st and 2nd trials respectively. While *A. indica* was the most active treatment indicating total nematocidal action at all concentrations (20, 30 and 40g) and exposure (30, 60 and 90 - days after treatment). Also, it was worthy of note that results of the first and second trial, at 30 days displayed total nematoxicity (0.00) , amongst the various concentrations for all tested bionematicides with no significant difference ($p>0.05$). Compared to the control there was significantly different ($P<0.05$) in both Tables 1 and 2.

Table 1: First trial: Determination of nematode population 30 days, 60 days and 90 days extracted from 5g of roots of *Capsicum* treated with 20g, 30g and 40g of all Nematicides

	Population of Nematodes in the Roots of Treated Crops After Treatment			
Bionematicide	**Concentrations (g)**	**30 days (AT)**	**60 days (AT)**	**90 days (AT)**
control	0	224.67±4.51b	264.33±34.20b	300.66±11.50f
A. indica	20	0.00±0.00a	0.00±0.00a	0.00±0.00a
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
V. amygdalina	20	0.00±0.00a	3.67±0.58a	6.00±1.00b
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
M. esculentus	20	0.00±0.00a	10.66±1.53a	26.00±1.73e
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
C. papaya	20	0.00±0.00a	10.67±0.58a	19.67±1.15d
	30	0.00±0.00a	6.00±1.00a	12.67±1.55c
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
C. sinensis	20	0.00±0.00a	7.33±0.58a	18.00±2.00d
	30	0.00±0.00a	0.00±0.00a	5.67±0.58ab
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a

Notwithstanding, at 60 days after treatment, the population of nematodes was 3.67 for *V. amygdalina*, 10.66 for *M. esculentus*, 10.67 for *C. papaya* and 7.33 for *C. sinensis* at 20g, being significantly different (P<0.05) from the control (Table 1). Summarily results of the first trial, of nematode population for 30, 60 and 90 days extracted from 5g of roots of *Capsicum* treated with 20g, 30g and 40g of all Nematicides showed the pattern of activities as: *A. indica* > *V. amygdalina* > *C. sinensis* > *C. papaya* > *M. esculentus*. The highest bionematicidal activity was achieved at 20g for *A. indica* at 0.00, 30 days after treatment (AT); followed by *V. amygdalina* (6.00) 90 days AT, *C. sinensis* (20.67) 90 days AT, *C. papaya* (21.00) and *M. esculentus* (30.00) 90 days AT. There was significant difference (P>0.05) in nematoxicity of the treatment compared control (Table 2).

Table 2: Second Trial: Determination of nematode population 30 days, 60 days and 90 days extracted from 5g of roots of *Capsicum* treated with 20g, 30g and 40g of all Nematicides

	Population of Nematodes in the Roots of Treated Crops After Treatment			
Bionematicide	**Concentrations (g)**	**30 days (AT)**	**60 days (AT)**	**90 days (AT)**
control	0	272.00±27.22b	291.67±30.99c	277.33±52.56c
A. indica	20	0.00±0.00a	0.00±0.00a	1.67±0.58a
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
V. amygdalina	20	0.00±0.00a	0.00±0.00a	6.00±0.00ab
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
M. esculentus	20	0.00±0.00a	12.00±2.00ab	30.00±3.00b
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
C. papaya	20	0.00±0.00a	16.00±0.00b	21.00±1.00ab
	30	0.00±0.00a	8.0000ab	19.00±2.00ab
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
C. sinensis	20	0.00±0.00a	6.00±2.00ab	20.67±1.53ab
	30	0.00±0.00a	0.00±0.00a	14.00±2.00ab
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a

Table 3 and 4 presents the nematoxicity activities at 30 days, 60 days and 90 days extracted from 10g of roots of Capsicum treated with 20, 30 and 40g of all Nematicides for 1st and 2nd trials For 30 days the highest population was achieved at 20g of the plant materials. However, in the first trial the highest population was observed for *M. esculentus* (47.00). Basically there was significance variation (P<0.05) between the different concentration of individual plant apart from *A. indica* and across the various plant materials (Table 3). Whereas in the 2nd trial the highest population was observed in *C. papaya* (62.67). There was significance variation (P<0.05) between the different concentration of individual plant apart from *A. indica* and across the various plant materials that has at least population (Table 4). For 60 days, the highest population was achieved for *M. esculentus* (75.00) and *C. papaya* (79.67). Significant variation (P<0.05) exist between the variation plant material for each of the concentration set (Table 3).

Table 3: First Trial: Determination of nematodes population from 10g of soil around root of *Capsicum* treated with 20g, 30g and 40g of all bio-nematicides

	Population of Nematodes in the Roots of Treated Crops After Treatment			
Bionematicide	**Concentrations (g)**	**30 days (AT)**	**60 days (AT)**	**90 days (AT)**
Control	0	315.67±34.59e	358.67±21.13	385.33±14.47h
A. indica	20	0.00±0.00a	0.00±0.00a	2.33±0.58a
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
V. amygdalina	20	0.00±0.00a	17.67±1.53b	16.00±1.00b
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
M. esculentus	20	36.00±2.65c	75.00±2.00e	104.00±3.00g
	30	21.00±1.73b	44.00±3.46d	60.67±2.08f
	40	19.67±0.58d	46.00±0.00d	62.67±0.58f
C. papaya	20	62.67±1.53d	79.67±0.58e	103.33±2.52g
	30	39.33±1.15c	41.00±4.00d	62.67±2.52f
	40	17.00±2.00b	28.66±1.53c	37.00±2.00d
C. sinensis	20	37.33±0.58c	44.00±3.00d	50.67±1.55e
	30	20.00±1.00b	41.00±1.73d	56.67±1.55ef
	40	2.00±0.00a	20.33±1.54bc	27.00±1.00c

Similar trend was observed in 2nd trial, with highest population achieved for *M. esculentus* (80.00) and *C. papaya* (77.67). There were significant variation (P<0.05) exist between the different plant material for each of the concentration set (Table 4). While at 90 days, the highest nematode population was achieved for *esculentus* (104.00) and *C. papaya* (103.33) (Table 3) furthermore at 2nd trail for 90 days, the highest population of nematode were achieved for *M. esculentus* (98.33). There were significant difference (P<0.05) exist between the various plant material for each of the concentration set (Table 4).

Similarly during the second trial as presented in table 2, the least nematicidal activity was achieved at 20g for *M. esculentus* (12.00), *C. papaya* (16.00) and *C. sinensis* (6.00), being not significantly different (P>0.05) from the bionematicides with nematode population control (Table 2). At 90 days, only *A. indica* demonstrated total nematotoxicity against the nematodes. All other bionematicides at 20g had had moderate activities, with *M. esculentus* indicating the least activity with nematode population of 26.00. Basically, there was significant variation (p<0.05) between the different concentrations of individual treatment, apart from *A. indica* and across the various plant materials (Table 1). Similarly during the second trial, the activities indicated similar trend (*A. indica* > *V. amygdalina* > *C. sinensis* > *C. papaya* > *M. esculentus* >). The highest population was achieved at 20g for *V. amygdalina* (6.00) 90 days, *M. esculentus* (30.00), *C. papaya* (21.00) and *C. sinensis* (20.67), being not significantly different (P>0.05) from the plant materials with nematode population control (Table 2).

Table 4: Second trial determination of nematodes population from 10g of soil around root of *Capsicum* treated with 20g, 30g and 40g of all bio-nematicides

	Population of Nematodes in the Roots of Treated Crops After Treatment			
Bionematicide	**Concentrations (g)**	**30 days (AT)**	**60 days (AT)**	**90 days (AT)**
control	0	331.67±10.50h	368.67±23.86g	443.67±12.01j
A. indica	20	0.00±0.00a	0.00±0.00aa	4.00±1.00a
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
V. amygdalina	20	0.00±0.00a	14.00±2.00bc	21.00±2.00b
	30	0.00±0.00a	0.00±0.00a	0.00±0.00a
	40	0.00±0.00a	0.00±0.00a	0.00±0.00a
M. esculentus	20	47.00±2.00g	80.00±4.00e	98.33±1.15i
	30	23.00±2.00d	46.00±2.00d	63.00±2.00f
	40	14.00±2.00c	25.00±2.00c	40.00±2.00d
C. papaya	20	41.00±0.2.00f	77.67±3.51e	88.00±2.00h
	30	21.00±0.2.00d	46.67±2.31d	70.33±2.52g
	40	2.00±0.00ab	12.00±2.00b	20.33±3.51b
C. sinensis	20	33.67±2.31e	40.33±3.51d	64.00±3.00f
	30	22.33±2.52d	36.67±2.31d	52.00±2.00e
	40	7.00±2.00b	24.00±2.00c	28.33±3.06c

4. Discussion

The results obtained in Tables 1 and 2 for the root bioassay of *Capsicum* treated with the leaf powder of *A. indica* indicated a 100% nematicidal effect in all the different concentrations. When compared with the results obtained in the control group, it shows that *A. indica* can effectively control plant parasitic nematodes in the field. This result agrees with earlier findings by many authors and researchers, such as Egunjobi and Afolami [23], Khan, [24], Adegbite and Adesiyan [25], Kuman and Khana [26]. Their results showed that *A. indica* had strong nematicidal properties in the laboratory and field work. The presence of active ingredients in the leaves contributed to an overall reduction in the nematode load and in some cases complete elimination of plant parasitic nematodes in the roots and soil leading to better growth in plants.

The results obtained in the 2nd Trial (Tables 2 and 4) followed the same trend. It showed a complete eradication of nematode in all treatments, except for the group treated with 20g which showed the presence of nematodes in 90 days. This could be as a result of various environmental factors, considering the fact that it was carried out in a different year. The effects of the extract could be gradually depleting because of its lower quantity. In this case it agrees with earlier reports that the efficacy of plants extracts is influenced by the concentration and duration of exposure [27]. Also the concentration of active ingredients in neem seed and leaf extract may differ depending on the environmental condition, the years of collection and the geographical area of the neem tree [28].

Besides these, variation is a natural phenomenon in life, so changes are bound to occur. However, looking at the overall result and comparing it with the control, the nematicidal effects of the *A. indica* powder still stands out. Roots of the control and lower concentration treatments were found to be more favourable to root knot nematode activities. More eggs were, therefore, deposited on their roots than roots of plant treated with the higher extract concentrations, agreeing with Alashalaby and Noweer, [29], that a reduction in the population and absence of root knot nematode in the root of *Capsicum* treated with *A. indica* could be attributed to poor penetration and a retardation in nutritive activities and fecundity of nematodes.

The group treated with *V. amygdalina* also indicated a high level of nematotoxicity, especially in the groups treated with higher concentration. This further establishes *V. amygdalina* as bio-nematicidal, thus agreeing with findings of previous researchers such as Onyenobi and Aghale [30] and Imafidor and Angaye [31] that bitter leaf extract was toxic to root knot nematodes under both laboratory and field conditions. Like was observed in *A. indica*, in the group treated with lower concentration, there was presence of nematodes in this group in 30 days and 60 days. This could be attributed to the lower concentration of the extract that was losing its efficacy with time of exposure. This agrees with previous findings that concentration of extracts and length of time of exposure of plant extracts do influence their nematicidal potential and that the lower the concentration, the shorter the effective time would be [27]. The results obtained in the 2nd Trial (Tables 3 and 4) also followed similar trend. When compared with the control group, the nematicidal potentials of *V. amygdalina* is evident. More nematodes were extracted from the control compared to those treated with the extract. There was a 100% nematotoxicity in the group treated with higher concentrations all through the period of growth.

In the group treated with *M. esculentus* the lower concentration showed the presence of nematode in the later part of the research. This could be attributed to the fact that the length of exposure reduced the efficacy of the extract because of the lower concentration used. This result corresponds with the findings of Joymatti *et al*., [32] and Madhusudanan *et al*., [33] who reported that juvenile mortality was concentration dependent. Roots of lower concentration of extract and the control were found to be more favourable to the activities of root knot nematode. More eggs were found to be deposited on their roots compared with the higher concentrations. In the present study the powdered leaves of *M. esculentus* was found to have a nematotoxic effect on root knot nematodes. This agrees with works of earlier researchers who worked with other parts of *M. esculentus* such as the root extracts and the dry peel. Ramakrishnan and Mohandas [34] and Balagopalan and Rajalakshmi [35], found out that water extract of cassava roots and dry peel, contains moderate amounts of cyanogenic compounds.

Mohandas and Nambisan [36] suggested that these nematicidal properties should be exploited and developed into a biopesticide to control *M. incognita*. Though they worked on the root and dry peel (rind), from the results obtained from this study it seems the same active ingredient is present in the dry leaves of *M. esculentus*. This can also be exploited and used in controlling plant parasitic nematodes. The results obtained in the 2nd Trial (Table 4) also reflected this nematicidal effect. When higher doses are used, better results would be obtained as was observed when the treated plants are compared with the control group.

C. papaya also exerted a significant level of nematotoxicity, following the trend of other extracts. The group treated with highest dose used in the research exhibited 100% nematicidal effect. This confirms and establishes earlier findings that concentration of extract and the time of exposure play a significant role in the nematotoxity of substances as opined by Joymatti *et al*., [32] and Madhusudanan *et al*., [33]. The lower doses registered the presence of nematode in 60 days and 90 days, but the group treated with 40g of *C. papaya* exhibited complete nematicidal action. This agrees with findings of Olabiyi *et al*., [18] who reported that application of high concentrations of leaf extracts of pawpaw caused a significant decline in the population of nematode and subsequently improved growth and yield.

C sinensis also exhibited some level of nematotoxicity on *M. incognita* with increase in concentration though at lower frequency, but when compared with the control, it stands out clear as having nematotoxic effects. This is quite interesting because Tsai [37] reported that the extracts of fresh peels of lemon, orange, and grapefruit showed

significant nematostatic effect against *M. incognita* second stage juveniles after 48 h treatment and that while different parts of *Citrus* plants have been examined and there are reports on the nematicidal activity of orange plant. He also reported that the existence of nematicidal principles in the leaves of orange plant cannot be ruled out without experiments [37]. There is a dearth of material on the nematicidal potentials of orange leaves. This is not unrelated to the fact that it is not economical or productive to go about defoliating orange plants. Even though it contain some nematicidal principle, from the results obtained in this work, it is hard to get the leaves in bulk. *C. sinensis* is a fruit rich in vitamin C and it is an expensive fruit. The leaves play an important role in photosynthesis, so going about defoliating them will greatly reduce yield, income and nutrition. So other parts such as the seed, pulp and peel should be looked at, since these can be obtained as wastes without any economic disadvantage.

All the different plant extracts used exhibited some level of nematicidal action both in the 1st and 2nd Trials when compared with the control groups. This agrees with the assertion of Sukul [38], who reported that many plants belonging to 57 families possess nematicidal properties and it is possible to use these plants to control root knot nematodes. Nematicidal principles exist in so many plants and in various parts of the plant [37]. *A. indica*, however, still emerged as the plant with the highest nematicidal potentials, followed by *V. amygdalina*. The difference between *M. esculentus* and *C. sinensis* did not stand out. *C. papaya* exhibit the lowest bio-nematicidal potentials in the root bioassay. Better results can be obtained in all extracts with increase in concentration. These can easily be obtained except for *V. amygdalina* and *C. sinensis*.

V. amygdalina is used as a leafy vegetable for various soups in the south-south region of Nigeria and a good source of income. The economic advantage should be weighed and compared to ascertain which is more beneficial, either to use it to control plant parasitic nematodes or to use it for food directly or sold in the market for nutritionally purposes and economic/financial benefits. This points came up because obtaining *V. amygdalina* is becoming more difficult and very expensive. The farmers benefit more when they process, package and sell the vegetable to housewives and bachelors as food than selling it in bulk to researchers at a lower price to control nematodes. Focus should be on the other three and further experiments carried with newer plants.

The soil bioassay of *Capsicum* treated with *A. indica* (Table 4) showed a complete absence of nematodes in soils treated with higher concentrations. However in 90 days both in the 1st and 2nd Trial (Table 4) the presence of nematode was recorded in *Capsicum* treated with 20g. These could be attributed to lower concentration of extract exposed over a long period of time, which led to a decline in the active ingredients in the neem powder. This agrees with findings of Akhtar and Mahmood [39], Joymatti *et al*., [32] and Madhusudanan *et al*., [33] that the mortality of root-knot nematode juveniles was found to depend on the concentration of the extract and the time of exposure. This is also in line with Alashalaby and Noweer [29], who reported that neem extract significantly reduced the total number of root knot nematode juveniles and inhibited egg hatch in roots and soil. The number of nematodes recovered was low in the highest concentration compared to the control.

V. amygdalina powder (Table 4) was found to reduce the nematode load in the soil. The higher concentrations both in the 1st Trial and 2nd Trial (Table 4) exhibited complete nematicidal action. These further establishes *V. amygdalina* as having nematicidal properties thus agreeing with works of early researchers such as Onyenobi and Aghale [30], and Imafidor and Angaye [31], on its use to control plant parasitic nematodes. Even at lower concentration, when compared with the control group, its nematicidal properties stands out. To get the best results higher concentrations should be used to control plant parasitic nematodes, both in the field and laboratory settings. These will completely inhibit the growth, reproduction and proliferation of the parasites and improve crop yield since they can also serve as organic amendments.

M. esculentus was also indicate moderate nematicidal activity compared with that of the control group. Better results could be achieved if treatment concentration is increase. The result showed that the lower the concentration, the higher the proliferation of nematodes. The lower concentration group allowed more reproduction and growth of nematodes. This is evident even in the 1st months both in the 1st Trial and 2nd Trial, unlike other bio-nematicides discussed so far, which indicated the presence of nematodes only in the lowest concentration and mostly in the 3rd months. These showed that *M. esculentus* nematicidal ability is lower and better results would only be achieved with higher concentration over time. This agrees with Madhusudanan et al., [33] who asserted that toxicity to nematodes by compounds in *M. esculentus* was related to the concentration of these compounds and the exposure time.

Overcoming this may not be a problem, especially in the south-south region, where the harvest and processing of cassava go on almost on a daily basis for the production of cassava products. There is virtually unlimited access to cassava leaves, especially in the rural areas. Farmers and researchers with interest can easily access these leaves almost at no cost for the control of plant parasitic nematodes to improve plant growth and yield. Higher doses, say at 60g to 70g or even more should be used, since cassava leaves is the most abundant of all the extracts used in the research. Focus should also be on the peel and the effluents produced during the conversion of cassava to garri and starch. The peel should be collected and dried and the effluent channelled to a source instead of wasting it. These should be used to treat farm land to reduce nematode load. *M. esculentus* seem to be the cheapest and most available for the control of nematodes. Though a higher concentration is needed, the availability of the product compensates for that, since it is available all through the year and almost everywhere, especially in the south-south region.

C. papaya (Table 4) also exhibited a level of nematicidal action when we look at the results obtained from the different concentration and comparing it with the control group both in the 1st Trial and 2nd Trial (Table 4). This agrees with the findings of Olabiyi *et al*., [18] who reported that application of leaf extracts of pawpaw caused a significant decline in the soil population of nematode pests and subsequently improved growth and yield. Like was observed in *M. esculentus*, the presence of nematode was noticed right from the 1st months in all treatments, though a lower population was observed in the higher concentrations. To get better results higher concentration should be introduced, but like *C. sinensis*, it might be challenging. *C. papaya* is an expensive fruit rich in vitamin A and C. The trees are also difficult to come by and the few ones around are zealously protected by their owners. Being a plant with fewer leaves, except for research purposes, it will be difficult to get a large quantity of leaves to control nematodes in a big farm, because defoliating them will reduce the process of photosynthesis. This will in turn reduce fruit yield.

At the research level this may not be a problem. Though not all variety produce fruits. If one can identify the non-producing ones, the leaves could be channelled to nematicidal uses. If these are obtained higher concentrations of 70g to 80g or more should be applied for better results. The dry peel and seeds should also be looked at since they are wastes, possibilities are they could also contain the nematode controlling principle and active ingredients.

C. sinensis also showed some level of bio-nematicidal potentials when the results obtained from the treatment is compared with the control group. This brings to light the suggestion by Tsai, [37] that the existence of nematicidal principles in the leaves of orange plant cannot be ruled out without experiments. The results showed that the leaves of *C. sinensis* does contain some nematotoxic ingredients. The higher the concentration the lower the nematode population. This agrees with earlier findings that concentration of extracts influences the number of nematodes, growth and crop yield [32, 33, 39]. The number of nematodes in the soil of the control group is higher. Another observation is the presence of nematodes in the 1st months in all treatments, except for 3 plants in the group treated with 40g in the 1st Trial and the 2nd Trial. This suggests that a higher concentration should be applied for better results.

5. Conclusion

The experiment revealed that the treatments used improved the plant health status and exerted a significant control of the nematodes resulting in reduced root galling in *Capsicum*. Fruit production was best

in plants treated with a higher concentration which showed the most effective control of the nematodes. As registered chemical nematicides continue to become more limited and unavailable, it is necessary to develop other management strategies for the control of plant-parasitic nematodes. Most synthetic nematicides are quite expensive, and because of their toxicity they have adverse effects on non-target organisms, such as beneficial soil microorganisms. There is also negative aspects of soil fumigants and nematicides leading to the increasing demand for Biocontrol agents that would lead to less environmentally harmful agricultural practices. This make plants material a potentially valuable alternative to chemical nematicides for nematode management. It is clear that these plant materials can be used as a substitute for synthetic nematicides. They can even reduce nematode populations at greater soil depths than most soil fumigants. In addition, they are also more environmentally friendly than most chemical nematicides because they do not repress other soil microorganisms. However, to successfully incorporate these plant Biocontrol agents into an integrated nematode management program, it is important to select a plant that is effective against the locally occurring nematode populations.

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