

Investigation of Some Selected Botanicals as Potential Nematicides in the Management of *Meloidogyne incognita*

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ABSTRACT

Botanicals were evaluated in this study for nematicidal properties in the management of *Meloidogyne incognita*. Gas Chromatography Mass Spectrometry (GC-MS) analysis of aqueous extracts of six selected plants (*Chromolaena odorata*, *Ficus mucosa*, *Senna occidentalis*, *Parquetina nigrescens*, *Synedrella nodiflora*, and *Tamarindus indica*) were carried out at the Department of Chemical Engineering, University of Ilorin, to determine their nematicidal activities on *M. incognita*'s egg hatchability and larvae mortality. The treatments were applied at concentrations of 0% (control), 50%, 75%, and 100% at the exposure time of 24 hrs, 48 hrs, and 72 hrs for each aqueous extracts. Data obtained were chemical compounds, egg hatchability and larvae mortality. Results obtained from (GC-MS) analysis revealed the presence of active ingredients in each aqueous extracts such as dimethanesulfonate, Oleic Acid, n-Hexadecanoic acid, Dibenzofuranamine, Methoxy, and Butyric acid. All the tested aqueous plant extracts at different concentrations were capable of causing egg hatch inhibition and juvenile mortality after 24 hrs, 48 hrs, and 72 hrs of exposure. Total juvenile mortality and egg hatched inhibition were observed in 100% concentration of all aqueous extract. Meanwhile, minimum mortality of juveniles and maximum egg-hatch were observed in the control. All screened botanical extracts were effective on *M. incognita* nematodes under in-vitro experiment. However, *Chromolaena odorata* and *Senna occidentalis* had highest values of juvenile mortality of 19.67% and 19.33%, respectively. *Senna occidentalis* recorded the smallest number of egg-hatch with 3.67%. The use of these plant extracts should be encouraged instead of harmful synthetic chemicals to curtailed excess damage caused by root knot, *Meloidogyne incognita* nematodes.

Keywords: Hatchability, Inhibition, Juvenile, *Meloidogyne incognita*, Spectrometry.

INTRODUCTION

The derived products from plants and their corresponding metabolites have garnered huge interest at the agricultural levels. Numerous plant

species have been utilized today as antimicrobial. The antimicrobial activity of several plant-derived compounds has been previously reported by (Newman and Cragg, 2012) and a wide array of active components has been identified. A majority

of plant-derived antimicrobials are secondary metabolites, and are produced in response to chemical stimulus from pathogenic microbes or environmental cues (Upadhyay *et al.*, 2014). Being secondary metabolites, these compounds do not directly modulate plant physiology; however, they play a critical role in enhancing plant health and defense. Plants can be affected by diseases through pathogenic microbes which disturb the metabolism of plant cells through enzymes, toxins, growth regulators and other substances (Westerdahl, 2017). *Meloidogyne* species secrete toxic substances and also absorbing food stuff from the host cells for their own use thereby causing a great loss to agricultural products (Baba *et al.* 2023). Examples of pathogens that cause infectious disease on plants include plant parasitic nematodes, fungi, bacteria and viruses which affect plant health by consumption of plant tissues (Upadhyay *et al.*, 2015).

Substances used to kill or control parasitic nematodes are referred to as nematicides. Nematicides in wide use are mostly synthesized organic compounds, though there are some organic compounds of plant origin referred to as "botanicals" and use of other organisms. In most situations, application of chemical nematicides affect human, animal and plant health directly or indirectly by disrupting ecological systems that exist in rivers, lakes, oceans, streams, wetlands, forests and fields (Andrea *et al.*, 2018). Overenthusiastic use of synthetic chemical insecticides led to problems unforeseen at the time of their introduction (Khater, 2012) Plant extracts are commonly referred to as botanicals and are the secondary plant metabolites synthesized by the plant for protective purposes. Some of these compounds are toxic to insects and pathogens (Tagne *et al.*, 2019). Many of the plant botanicals are used both in commercial and subsistence agriculture by small-scale farmers.

Root-knot nematodes are insidious, reduce yield and are silent enemies of most food, horticultural, vegetable, and fibre crops (Izuogu and Abiri, 2015). Indirect damages from plant parasitic nematodes of crop plants include their role in the plant disease complex by making plant roots more susceptible to secondary infection by fungi and bacteria and their

role as vectors of many viruses (Archana *et al.*, 2014).

Nematode management is a practice of maintaining plant parasitic nematodes below the economic threshold level, using chemical and non-chemical methods. The chemical method has been primarily used as the standard to achieve nematode control, but its hazardous and adverse effects on man and his biotic environment, phytotoxicity in plants, contamination of water, and other forms of pollution have stimulated great interest in the search for alternative management methods (Agbenin, 2012). Bio-nematicides possess a spectrum of properties against pathogens. They are cheaper and more benign. In recent times, the use of bio-nematicides of microbial origin has been fast gaining ground in addressing the challenges posed by plant parasitic nematodes (Izuogu *et al.*, 2019). The potential for nematicidal activity of indigenous plants and their products has been reported by many researchers (Yakubu and Izuogu, 2013; Olabiyi *et al.*, 2013; Baba *et al.*, 2020).

There is increasing need to find more acceptable alternatives, therefore, it is pertinent to investigate the active ingredients present in six plants which are *Chromoleana odorata*, *Ficus mucosa*, *Senna occidentalis*, *Parquetina nigrescens*, *Synedrella nodiflora*, and *Tamarindus indica* and to evaluate their efficacy on root-knot nematode (*Meloidogyne incognita*).

MATERIALS AND METHODS

Experimental Site

The phytochemical screening was carried out at the Department of Chemical Engineering, University of Ilorin, Ilorin, Nigeria, while the *in-vitro* testing was done at International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Sources of Botanicals and Preparation

The six plants that were used and analysed with Gas Chromatography-Mass Spectrometry are presented in Table 1. Fresh leaves of the listed botanicals in Table 1 were collected at mid-day in Oke-odo, Tanke and the University of Ilorin campus. They were

Table 1: Six plants that were studied for nematocidal activities

Species	Family	Local name	Part used
<i>Chromolaena odorata</i>	Asteraceae	Akintola, Siam weed	Leaf
<i>Ficus mucoso</i>	Moraceae	Forest sycomore fig	Leaf
<i>Synedrella nodiflora</i>	Asteraceae	Akara aje, node weed	Leaf
<i>Tamarindus indica</i>	Fabaceae	Indian date, Sweet tamarind	Leaf
<i>Parquetina nigrescens</i>	Asclepiadaceae	Bullock, Ogbo	Leaf
<i>Senna occidentalis</i>	Fabaceae	Coffee senna, Negro coffee	Leaf

confirmed at the herbarium in the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria. Botanical leaves were air-dried for 14 days at room temperature of 25-27 °C. The leaves were pulverized using mortar and pestle and later soaked in methanol and concentrated over water bath for 72 hours before carrying out the GC-MS analysis. Aqueous extract of *Ficus mucosa*, *Senna occidentalis* and *Chromolaena odorata* for *in vitro* testing were prepared by soaking 1 kg of the ground leaves in a plastic bucket containing already 4 litre of hot water at 90 °C. The plastic bucket was covered with the lid and was allowed to soak for 24 hours for extraction following the modified method of Oyedunmade and Izuogu (2011). The supernatant was sieved with a muslin cloth in both cases and the aqueous extracts were collected in sample bottles. This served as the stock solution.

Gas Chromatography Mass Spectrometry (GC-MS) of Plant Extracts Analysis

A gas chromatography from Agilent USA hyperated to a mass spectrophotometer (5975 C) with triple axis detector equipped with an auto injector (10 µl syringe) was used for this analysis. All chromatographic separation was performed on capillary column having specification: length; 30 m, internal diameter 0.2 µm, thickness; 250 µm, treated with phenyl methyl silox. Other GC-MS conditions are ion source temperature (EI), 250 °C, interface temperature; 300 °C, pressure 16.2 psia, out time, 1.8 mm, 1 µl injector in Split mode with split ratio 1:50 with injection temperature of 300 °C. The column temperature started at 35 °C for 5 mins and changed to 150 °C at the rate of 4 °C/min. The temperature was raised to 250 °C at the rate of

20 °C/min and held for 5 mins. The total elution was 47.5 minutes.

In a process to define the real identity of compounds present in crude extracts, extracts were subjected to further analysis (quantitative) using GC-MS. Powdered Plant extracts (0.5 g) each was macerated in methanol (1.5 ml) by Soxhlet extractor. Samples were filtered sterilized using 0.22 µM pore size filter unit and analyzed (GCMSQP2010 ULTRA, Shimadzu). The oven temperature was set up from 50 °C with an increase of 20 °C/min to 100 °C for 2 minutes and 20 °C/min to 250 °C for 5 minutes.

Helium gas was used as the carrier gas at constant flow rate of 8 ml/min. An aliquot of 2 µl of sample was injected into the column with the injector at 280 °C of the split ratio of 10:1. Mass spectra Solution software provided by supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from National Institute of Standards and Technology (NIST) standard database library software.

Machine model: 7890A GC system, 5675 C Inert MSD with triple –Axis Detector, Packing material: Capillary column, Carrier gas: Helium gas, Flow rate: in the instrumentation parameter Temperature programmed, Column length: 30 m, Dimension: 30 m x 250 µm x 0.25 µm, Column: Agilent 19091-433 HP-5 Ms 5%phenyl methyl silox.

Nematode Egg Extraction

Infested soil and galled roots of *Celosia argentea* were collected from a pure culture in the screen-house of Department of Crop Protection, Faculty of

Agriculture, University of Ilorin, Kwara State. *Meloidogyne incognita* eggs from infected roots were extracted using sodium hypochlorite (NaOCl) method as described by Boyhan *et al.* (2003). A solution of sodium hypochlorite and distilled water were prepared in the ratio 1:9 and mixed thoroughly. The solution was added to chopped *M. incognita* infected roots in a beaker and agitated for five minutes. The resulting solution was passed through 250 µm, 70 µm, 45 µm and 25 µm mesh sieves. Larger roots and other debris were collected by 250 µm, 70 µm and 45 µm sieves, while nematode eggs and juveniles were collected by the 25 µm sieve after thoroughly washing with distilled water. The nematode egg suspension was standardized using stirrer such that 2 ml contained approximately 1,100 eggs. An aliquot of 10 ml therefore contained 5,500 eggs of *M. incognita*.

Extraction of *Meloidogyne incognita* Second-stage Juveniles from Root Samples

Using the method described by Coyne *et al.* (2007), infected roots were rinsed under a tap to remove adhering soil particles. The roots were chopped with scissors or knife and a sub sample of 5 g chopped root was weighed using a digital measuring scale. The weighed roots were transferred to a clean tissue paper inside a sieve and placed inside a plastic tray. Water was added to the extraction plates so as to wet the roots, ensuring that the water is sufficient enough. The set up was labeled accordingly. Water from plastic tray was poured into labeled cup after 48 hours, using a wash bottle to rinse the plate. Suspension was allowed to settle and supernatant from the labeled cup was gently decanted. The infranatant portion was used for nematode enumeration. Used tissue papers and roots were discarded and number of nematode population in 2 ml suspension was counted and identified using a compound microscope.

In vitro Testing of botanical extracts for Nematode Juvenile Mortality and Egg Hatchability

This study was carried out at the Nematology and Striga Unit Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (lat

7°22'39"N; long: 3°54'21"E). *Meloidogyne incognita* eggs and juveniles were from the extractions described above. One (1) milliliter of suspension containing approximately 20 eggs or freshly hatched juveniles of *M. incognita* were dropped into 5 ml Enzyme - Linked Immunosorbent Assay (ELISA) well-plates containing approximately 4 ml of each extracts. Distilled water was used in the case of Control. The stock solutions of aqueous extracts of botanicals were at different concentrations of the percentages of 100, 80, 60, and 50 serial dilutions. Each dilution was replicated four times. These ELISA well-plates were kept in a Biological oxygen demand (BOD) incubator at $25 \pm 1^\circ\text{C}$. Larval mortality after 24 hrs, 48 hrs, 72 hrs, and 96 hrs exposure to different dilutions of different treatments was recorded by counting living and dead second-stage juveniles under a stereoscopic binocular microscope. Juveniles or eggs put in distilled water were treated as a control. Larvae that did not respond to touch by a fine needle were counted as dead. Egg masses were put in distilled water and treated as a control following the technique used by Bernard *et al.* (2017). Serial Dilution of aqueous extract of treatments was carried out to arrive at concentrations of 80% (4 ml of concentration + 1 ml of egg or juvenile suspension); 60% (3 ml of concentration + 1 ml of water + 1 ml of egg or juvenile suspension); 50% (2 ml of concentration + 1 ml of water + 1 ml of egg or juvenile suspension); and 0% [Control] (4 ml distilled water + 1 ml of egg or juvenile suspension)

Statistical Analysis

All numerical data collected were subjected to Analysis of Variance (ANOVA) using Genstat's 17th edition and the means were separated using Duncan Multiple Range Test at 5% probability level.

RESULTS

The GC-MS Analysis of Methanolic Extract of *Chromolaena odorata* Leaves

The methanolic extract of *Chromolaena odorata* leaves revealed several peaks which represents different compounds present in the chromatogram

Table 2: GC-MS analysis of methanol extract of *Chromolaena odorata* leaves

Peak No.	Compound Name	Molecular Formula	Molecular Weight.(g/mol)	Retention Time (min)	Peak Area (%)
1	1,9-Nonanediol, dimethanesulfonate	C ₁₁ H ₂₄ O ₆ S ₂	316.00	36.27	16.92
2	1,2-Dimethyl-4-oxocyclohex-2-enecarboxaldehyde	C ₉ H ₁₂ O ₂	152.19	36.58	3.17
3	1,4-Eicosadiene	C ₂₀ H ₃₈	278.50	36.77	6.13
4	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.40	37.77	38.59
5	Phytol	C ₂₀ H ₄₀ O	128.17	38.59	12.19
6	cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	282.50	38.86	23.01

by Gas Chromatography –Mass spectrometry (GC-MS) analysis. The peaks in the chromatogram were integrated and were compared with the database of spectra of known components stored in the Gas Chromatography-Mass Spectrometry library. The identification of phytochemical compounds was based on the peak area, retention time and molecular formula and hereby presented in Table 2. The results revealed the presence of 1, 9-Nonanediol, dimethanesulfonate, Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha, Bicyclo [3.1.1] heptane, 2,6,6-trimethyl- (16.92%); 1,2-Dimethyl-4-oxocyclohex-2-enecarboxaldehyde, 4a(2H)-Naphthalenemethanol, octahydro-, Oleyl alcohol, acetate (3.17%); 1,4-Eicosadiene, 9-Octadecyne,

1-Methoxy-3-(2-hydroxyethyl) nonane (6.13%); n-Hexadecanoic acid, Tetradecanoic acid (38.59%); Phytol, Isophytol, Butyric acid, 3-pentadecyl ester (12.19%); cis-Vaccenic acid, 9,12-Octadecadienoic acid (Z,Z)-, (Z)6,(Z)9 Pentadecadien-1-ol (23.01%).

The GC-MS Analysis of Methanolic Extract of *Ficus mucosa* Leaves

Compounds identified from the GC-MS analysis of methanolic extract of *Ficus mucosa* leaves at different retention time and peak area is presented in Table 3 and these were: Bicyclo[3.1.1]heptane 2,6,6-trimethyl-; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; n-Hexadecanoic acid; Phytol and Oleic Acid.

Table 3: Bioactive compounds identified in methanolic extract of *Ficus mucosa* leaves

Peak No.	Compound Name	Molecular Formula	Molecular Weight.(g/mol)	Retention Time (min)	Peak Area (%)
1	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	C ₁₀ H ₁₈	138.25	36.27	5.73
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.50	36.77	2.00
3	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.40	37.76	34.12
4	Phytol	C ₂₀ H ₄₀ O	296.50	38.58	12.75
5	Oleic Acid	C ₁₈ H ₃₄ O ₂	282.50	38.87	45.40

The GC-MS Analysis of Methanolic Extract of *Senna occidentalis* Leave

The first set of compounds identified with less retention time (36.27 min) were four (4); Bicyclo [3.1.1]heptane, 2,6,6-trimethyl-(1.alpha.,

2.beta.,5.alpha.) while Bicyclo[3.1.1]heptane, 2,6,6-trimethyl- while 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- took the longest retention time (38.878 min) to identify.

Table 4: Bioactive compounds identified in methanol extract of *Senna occidentalis* leaves

Peak No.	Compound Name	Molecular Formula	Molecular Weight.(g/mol)	Retention Time (min)	Peak Area (%)
1	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl(1.alpha.,2.beta.,5.alpha.)	C ₁₀ H ₁₈	138.25	36.27	18.60
2	1-Pentadecyne	C ₁₅ H ₂₈	208.38	36.58	2.73
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.5	36.77	5.44
4	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.40	37.783	21.69
5	Phytol	C ₂₀ H ₄₀ O	128.17	38.590	25.34
6	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280.40	38.878	26.19

The GC-MS Analysis of Methanolic Extract of *Parquetina nigrescens* Leaves

The major five (5) compounds in *Parquetina nigrescens* were n-Hexadecanoic acid, 4-Bicyclo [3.1.1] heptane, 2, 6, 6-trimethyl, Phytol and 3-Dibenzofuranamine (Table 5).

Table 5: Bio-active compounds in methanol extracts of *Parquetina nigrescens* of leaves

Peak No.	Compound Name	Molecular Formula	Molecular Weight.(g/mol)	Retention Time (min)	Peak Area (%)
1	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	C ₁₀ H ₁₈	138.25	36.26	2.00
2	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.4	37.77	39.38
3	Phytol	C ₂₀ H ₄₀ O	128.17	38.59	22.01
4	3-Dibenzofuranamine	C ₁₂ H ₉ NO	183.21	38.85	36.61

The GC-MS Analysis of Methanolic Extract of *Synedrella nodiflora* Leaves

The list of constituents in the methanolic extract of *Synedrella nodiflora* is given in Table 6. The major components are 2-Methoxy-4-vinylphenol, n-Hexadecanoic acid and 9-Octadecenoic acid, (E)-

Table 6: Bio-active ingredients in methanol extract of *Synedrella nodiflora* leaves

Peak No.	Compound Name	Molecular Formula	Molecular Weight.(g/mol)	Retention Time (min)	Peak Area (%)
1	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150.17	21.75	3.68
2	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.4	37.74	49.31
3	9-Octadecenoic acid, (E)-	C ₁₈ H ₃₄ O ₂	282.5	38.85	47.01

The GC-MS Analysis of Methanolic Extract of *Tarmarindus indica* Leaves

The leaves of *T. indica* GC-MS analysis exhibited the presence of five Bicyclo[3.1.1]heptane, 2,6,6-trimethyl(1.alpha.,2.beta.,5.alpha.), n-Hexadecanoic acid, Butyric acid, 3-tetradecyl ester 9 and 12-Octadecadienoic acid (Z,Z)- (Table 7)

Table 7: Bio-active constituents identified in methanol extract of *Tarmarindus indica* leaves

Peak No.	Compound Name	Molecular Formula	Molecular Weight.(g/mol)	Retention Time (min)	Peak Area (%)
1	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl(1.alpha.,2.beta.,5.alpha.)	C ₁₀ H ₁₈	138.25	36.26	3.01
2	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.40	37.77	38.70
3	Butyric acid, 3-tetradecyl ester	C ₁₈ H ₃₆ O ₂	284..50	38.59	1.77
4	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280.40	38.88	56.52

Effect of Aqueous plant extracts on egg-hatch inhibition of *Meloidogyne incognita*

The results of the effect of aqueous extract of *Ficus mucosa*, *Senna occidentalis* and *Chromolaena odorata* on *Meloidogyne incognita* egg-hatch inhibition for 24, 48 and 72 hours consecutively were presented in Table 8. Twenty (20) eggs were dropped in aqueous extract of the leaves against three concentrations 50%, 60%, 80% and Control (Distilled water). The result revealed that the egg-hatch inhibition with plant extracts increased with increase in exposure time from 24 to 72 hours. Control (distilled water) recorded highest number of hatched eggs (19.00 i.e.

95% percentage egg hatch) and conversely, had the lowest egg-hatch inhibition (5%). This was followed by 80% aqueous extract of *Ficus mucosa* (4.67 i.e. 23.2% egg-hatched and 76.8% egg-hatch inhibition) and 80% aqueous extract of *Chromolaena odorata* (5.67 i.e. 28.4% hatched and 71.6% egg-hatch inhibition) after 72 hours of time exposure. *Senna occidentalis* extract observed the lowest egg hatched amongst the botanicals (3.67 i.e. 18.35% egg hatched and 81.65% egg-hatch inhibition) at 80% concentration of the aqueous plants extract and at 72 hours of time exposure.

Table 8: Effects of plant extracts on egg hatch of *Meloidogyne incognita*

Plant extracts	Conc.	Mean number of egg hatched and time of exposure					
		24 hours		48 hours		72 hours	
<i>Ficus mucosa</i>	80%	1.33ab	at 24 hours	3.33a	at 48 hours	4.67ab	at 72 hours
	60%	1.67ab	at 24 hours	5.67c	at 48 hours	6.67c	at 72 hours
	50%	2.67b	at 24 hours	6.33d	at 48 hours	6.67c	at 72 hours
Control	0%	3.67c	at 24 hours	8.67e	at 48 hours	18.67h	at 72 hours
<i>Senna occidentalis</i>	80%	0.00a	at 24 hours	3.33a	at 48 hours	3.67a	at 72 hours
	60%	0.67a	at 24 hours	4.67b	at 48 hours	5.67b	at 72 hours
	50%	1.67ab	at 24 hours	5.33c	at 48 hours	6.00c	at 72 hours
Control	0%	5.67e	at 24 hours	10.67f	at 48 hours	17.33f	at 72 hours
<i>Chromolaena odorata</i>	80%	3.33c	at 24 hours	4.33b	at 48 hours	5.67b	at 72 hours
	60%	5.67e	at 24 hours	6.67d	at 48 hours	9.33d	at 72 hours
	50%	5.67e	at 24 hours	8.67e	at 48 hours	10.67e	at 72 hours
Control	0%	4.67d	at 24 hours	11.67g	at 48 hours	18.33h	at 72 hours

Means followed by the same letters within columns are not significantly different according to Duncan Multiple Range Test at P<0.05

Key – % = Percentage; Conc. = Concentration

Effect of plant extracts on second-stage *Meloidogyne incognita* juvenile's mortality

Aqueous botanical extracts were found to cause juvenile mortality of *M. incognita*. The mortality of the nematode was directly proportional to the concentration of extract used. The highest juvenile mortality was observed at 80% concentration of botanical extracts, while the control (distilled water) recorded the least number of juvenile mortality (Table 9). Number of nematode mortality was in the range of 0 to 20, the result revealed mortality of second-stage juveniles of *M. incognita* with plant leaf extracts was increased with the increase of the exposure time from 24 to 72 hours. Extract of *Chromolaena odorata* recorded highest

(19.67) mortality among tested extracts against second-stage juveniles of *M. incognita* at 80% concentration, followed by *Senna occidentalis* with mortality of 19.33 and *Ficus mucosa* recorded mortality of 17.33 after 72 hours of exposure. At 60% concentration of the aqueous plant extract, *Chromolaena odorata* observed the highest mortality of juveniles as 15.67, followed by *Senna occidentalis* and *Ficus mucosa* with mortality of 15.33 and 13.33, respectively. At 50% concentration, the extract of *Chromolaena odorata* and *Senna occidentalis* recorded mortality with 13.33, while *Ficus mucosa* recorded lowest mortality of 9.33 (Table 10).

Table 9: Effects of plant extracts on Second-stage juvenile mortality of *Meloidogyne incognita* at hours of exposure

Plant extracts	Conc.	Mean mortality of second-stage juveniles of <i>M. incognita</i> and time of exposure							
<i>Ficus mucosa</i>	80%	8.33de	at 24 hrs	11.33d	at 48 hrs	17.33d	at 72 hrs		
	60%	5.33cd	at 24 hrs	8.33c	at 48 hrs	13.33c	at 72 hrs		
	50%	4.67c	at 24 hrs	5.67b	at 48 hrs	9.33b	at 72 hrs		
Control	0%	1.33ab	at 24 hrs	3.33a	at 48 hrs	6.33ab	at 72 hrs		
<i>Senna occidentalis</i>	80%	9.67e	at 24 hrs	13.67a	at 48 hrs	19.33e	at 72 hrs		
	60%	7.67d	at 24 hrs	9.67cd	at 48 hrs	15.33cd	at 72 hrs		
	50%	4.67c	at 24 hrs	6.67bc	at 48 hrs	13.33c	at 72 hrs		
Control	0%	0.67a	at 24 hrs	3.33a	at 48 hrs	5.33a	at 72 hrs		
<i>Chromolaena odorata</i>	80%	5.67cd	at 24 hrs	11.67de	at 48 hrs	19.67d	at 72 hrs		
	60%	4.33bc	at 24 hrs	11.33d	at 48 hrs	15.67cd	at 72 hrs		
	50%	3.33b	at 24 hrs	8.67c	at 48 hrs	13.33c	at 72 hrs		
Control	0%	1.33ab	at 24 hrs	4.33ab	at 48 hrs	6.33ab	at 72 hrs		

Means followed by the same letters within columns are not significantly different according to Duncan Multiple Range Test at $P < 0.05$

Key – % = Percentage; Conc. = Concentration

DISCUSSION

The results of the GC-MS analysis from this study showed that the six botanical extracts screened possessed basic chemical compounds such as Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-(1.alpha.,2.beta.,5.alpha.), 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, Butyric acid, 3-tetradecyl ester (Z,Z,Z), n-Hexadecanoic acid, 2-Methoxy-4-vinylphenol, Phytol and 3-Dibenzofuranamine. The present findings corroborate the outcomes from earlier studies by some researchers such as Bello *et al.* (2013) and Mogaka *et al.* (2023) who reported the inhibitory effect of *Tamarindus indica* and *Carica papaya* leaves on root-knot nematode, *Meloidogyne incognita* egg hatch and larvae mortality and the use of *Senna occidentalis* extract for the inhibition of the growth of plasmodium *in vivo*.

Some of these botanicals such as *Parquetina nigrescens*, *Senna occidentalis*, *Tamarindus indica* and *Ficus mucosa* possess chemical compounds that belong to the terpenoid group which are Bicyclo[3.1.1]heptane, and 2,6,6-trimethyl-(1.alpha.,2.beta.,5.alpha.). Terpenes are group of terpenoids that possessed strong properties of molluscicidal and fungitoxic activities (Westerdahl *et al.*, 2017). One of the major n-Hexadecanoic acid is one of the major phytochemicals isolated from plants in this study and Sadiq *et al.* (2010) reported that this n-hexadecanoic acid shows antiviral activities.

The *in vitro* study revealed that the three aqueous extracts of phytochemicals isolated from plants in this study *Chromolaena odorata*, *Ficus mucosa* and *Senna occidentalis* inhibited egg-hatch and caused juvenile mortality of *M. incognita*. From the result, aqueous extract of *Chromolaena odorata* was the most effective in the mortality of the second-stage juveniles of *M. incognita*, while *Senna occidentalis* had the highest percentage inhibition of egg-hatch. However, control which was distilled water recorded lowest juvenile mortality and egg-hatch inhibition. This work was similar to that of Agbenin *et al.* (2005) that reported

that the extracts of neem leaf and garlic bulb completely inhibited hatching of egg masses of *M. incognita* and were lethal to larvae. The nematocidal activity of aqueous extract of the botanicals against the nematodes might be due to the presence of various bio-active compounds present in the leaves and exposure period. Yakubu *et al.* (2017) reported that Gas Chromatography–Mass spectrometry (GC-MS) analysis of the methanol extract of *Chromolaena odorata* leaves shows the presence of anti-bacterial activity and Qamar *et al.* (2005) report shows that methanolic extract of the leaves of *Lantana camara* exhibited significant mortality rate of root-knot nematode *M. incognita*.

Soil infestation and infection of crops by root-knot nematodes could be lowered by using botanical extract as soil amendment, which is eco-friendly, available, cheaper and cost effective. Their utilisation has advantages over expensive and hazardous chemical nematicides which have toxic effect on flora, fauna and the environment, though effective in the management of nematode pests.

CONCLUSION

The study revealed that chemical compounds such as Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-(1.alpha.,2.beta.,5.alpha.), 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, Butyric acid, 3-tetradecyl ester (Z,Z,Z), n-Hexadecanoic acid, 2-Methoxy-4-vinylphenol, Phytol and 3-Dibenzofuranamine are present in the leaves of botanicals like *Parquetina nigrescens*, *Senna occidentalis*, *Tamarindus indica*, *Chromolaena odorata* and *Ficus mucosa*. All the aqueous leaf extract of botanicals used in this *in vitro* study showed nematocidal activity against *M. incognita*. Aqueous extract of *Chromolaena odorata* leaf was the most effective in mortality against second-stage juveniles of *M. incognita*, while *Senna occidentalis* had the highest inhibition of egg-hatch. Therefore, aqueous extract of botanicals may provide reliable alternative nematicides for the management of root-knot nematodes.

RECOMMENDATIONS

Use of aqueous leaf extract of botanicals to manage problems from root-knot nematodes can replace chemical nematicides which are expensive and unfriendly to the environment especially when not judiciously used. Farmers should be encouraged to adopt the use of leaf extracts like *Senna occidentalis*, *Tamarindus indica*, and *Chromolaena odorata* as nematicides and a further investigation of the botanicals on the field is recommended.

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