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EVALUATION OF EGG HATCHABILITY AND LARVAL MORTALITY OF METHANOLIC EXTRACTS OF *CHROMOLAENA ODORATA* AND *ANNONA SQUAMOSA* ON *MELOIDOGYNE INCOGNITA*

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Abstract: ***Chromolaena Odorata* and other associated species of *Annona Squamosa* have been reported be nematicidal. This research was intended at providing more information on the nematicidal activity of *Chromolaena odorata* and exposure the nematicidal action of *Annona squamosa*. Ten grams each of the powders were drenched in cold 100 ml of methanol for one week to produce 100,000 mg/kg stock solution, filtered and concentrated. One ml of nematode suspension that contained 60 eggs of *M. incognita* extracted with sodium hypochlorite, one ml of nematode suspension that contained 60 one week old second-stage juveniles dispensed into glass blocks that contained one ml of the extracts. Percentage inhibition and juvenile mortality were estimated. Dry powders were analyzed for Infrared and phytochemicals. Water extracts inhibited egg-hatch of *Meloidogyne incognita* (25-100%) and killed the juveniles (2.3-100%). Water extracts, 20,000 mg/kg, 30,000 mg/kg as from fourth day had 100% mortality of the juveniles. The IR revealed alcohols, amines, unsaturated/aromatic compounds and phenols. The phytochemicals identified were saponins, tannins, phenols, alkaloids, flavonoids, anthraquinones and cardenolides. Saponins were of the highest amounts followed by tannins and phenols. These botanicals were inhibitors of egg-hatch, lethal to juveniles of *Meloidogyne incognita* and might be attributed to the phytochemicals.**

Keywords: **Meloidogyne incognita, Egg hatchability, Larval mortality, Nematicidal activity.**

1. INTRODUCTION

Plant-parasitic nematodes are found wherever plants grow and are known to be among the limitations of producing food for man and his livestock. The predictable annual yield losses due plant-parasitic nematodes in the world's major crops are documented about 12.3% and 14% in the developing countries (Ngangbam and Devi, 2012).

The root-knot nematodes *Meloidogyne* spp. such as *Meloidogyne incognita* is noteworthy pest of crops ranging from vegetables, cereals, legumes to perennial crops (Sikora and Fernandez, 2005). The root-knot nematodes, due to their high reproductive potential and wide host ranges are disreputably difficult to handle. *Meloidogyne* spp. requires 99.9% manage in order to put off the succeeding build up of damaging populations (Chaudhary and Kaul, 2013). The root-knot nematode lays eggs in the protective gelatinous matrix (Abad *et al.*, 2009). The infective stage of the root-knot nematode is the second-stage juvenile (J2) which hatches out from the egg, which invade the roots by active incursion (Curtis, 2008). The

J2 initiates the formation of a permanent feeding site that consists of giant cells which function as specialized sinks (Jones *et al*., 2013), that results in damaging property like gall formation on plants. Any measure that prevents the hatching of the J2 from eggs and or killing of the J2 before their incursion of roots would be sufficient management practice. Thus, the present research was aimed at evaluation of methanolic extracts of *Chromolaena odorata* and *Annona squamosa* for root-knot nematode control, *Chromolaena odorata* has been reported to be nematicidal. This study could be the first report on *Annona squamosa* for nematicidal activity.

2. MATERIALS AND METHODS

Preparation of the Plant Extracts

Leaves and roots of Siam weed (*Chromolaena odorata*) were collected from the Crop Garden of the Sholavandan. Leaves and barks of custard apple (*Annona squamosa*) were collected within Vivekananda College premises. The plant parts were air-dried for three weeks, which were blended to powder and methanol was used to extract the toxic principles from the plant parts. Ten grams each of air-dried, ground Siam weed leaves and roots, custard apple leaves and barks were soaked in 100 ml methanol for one week at room temperature, and then filtered through two layers of muslin cloth (Abbas *et al*., 2009). The filtrates were designated as 100,000 mg/kg stock solution, and dilutions were carried out by adding 10 ml of stock solution to 90 ml of methanol to produce concentrations of 10,000 mg/kg, 20 ml of stock solution added to 80 ml of methanol to produce 20,000 mg/kg and 30 ml of stock solution to 70 ml methanol to produce 30,000 mg/kg solutions for all the various plant parts (Olabiyi *et al*., 1992). The water extracts were concentrated to one-fifth of the volume (Abbas *et al*., 2009) with the Thin Film Evaporator for three hours at 45 ^{o}C at laboratory.

In Vitro Experiments on Hatching and Juvenile Mortality of Meloidogyne Incognita

The hatching and mortality tests of *M. incognita* were carried out in the Research Laboratory of the Department of zoology, Vivekananda College. Temperatures of 24±1.5^{O}C to 28.5 2.5^{O}C and 79 1.15% to 96 2.15% RH were observed throughout the period of the experiments using Brannan Whirling Hygrometer. Hatching and mortality tests of *Meloidogyne incognita* were carried out in the Research Laboratory of the Department of zoology, Vivekananda College.

Hatching Tests of Meloidogyne Incognita Eggs

The water extracts of Siam weed leaves and roots, and African custard apple leaves and barks, described above were used. One ml that contained 60 *Meloidogyne incognita* eggs extracted with sodium hypochlorite method (Hussey and Barker, 1973) was dispensed in a glass block and one ml of water extracts of the above mentioned plant parts at concentrations of 10,000 mg/kg, 20,000 mg/kg, and 30,000 mg/kg respectively were added. The effective concentrations were 5,000 mg/kg, 10,000 mg/kg and 15,000 mg/kg respectively. The treatments were applied in Completely Randomized Design and replicated four times. The set ups were monitored daily for egg-hatching for seven days. Percentage inhibition was calculated using a modified formulated by Clarke and Shepherd (1964). Percentage inhibition = 100 – (100 – Hs/Hw) where Hs was the number of eggs hatched in substance, HW was the number of eggs hatched in water. The modified formula was percentage inhibition = 100 (Hs/Hw) where Hs was the number of eggs hatched in substance and HW was the number of eggs hatched in water. A repeat experiment on the hatching test was carried out. Drops of 0.5% streptomycin were added to the extracts to prevent microbial growth.

Juvenile Mortality Tests of Meloidogyne Incognita

A 100 ml suspension of *Meloidogyne incognita* eggs extracted with sodium hypochlorite method (Hussey and Barker, 1973) was incubated at room temperature for one week and hatched second-stage juveniles were recovered with the Pie-pan method (Whitehead and Hemming, 1965). One ml that contained 60 second-stage juveniles was dispensed into a glass block that contained one ml of water extracts at concentrations of 10,000 mg/kg, 20,000 mg/kg and 30,000 mg/kg, which brought the effective concentrations to 5,000 mg/kg, 10,000 mg/kg and 15,000 mg/kg. The set-up was a Completely Randomized Design and replicated four times. The treatments at effective concentrations used for the inhibition and juvenile mortality experiments were as follows:

Water extracts of the test plants

1. Water extract of *Chromolaena odorata* leaves at 5,000 mg/kg
2. Water extract of *Chromolaena odorata* leaves at 10,000 mg/kg

3. Water extract of *Chromolaena odorata* leaves at 15,000 mg/kg
4. Water extract of *Chromolaena odorata* roots at 5,000 mg/kg
5. Water extract of *Chromolaena odorata* roots at 10,000 mg/kg
6. Water extract of *Chromolaena odorata* roots at 15,000 mg/kg
7. Water extract of *Annona squamosa* leaves at 5,000 mg/kg
8. Water extract of *Annona squamosa* leaves at 10,000 mg/kg
9. Water extract of *Annona squamosa* leaves at 15,000 mg/kg
10. Water extract of *Annona squamosa* bark at 5,000 mg/kg
11. Water extract of *Annona squamosa* bark at 10,000 mg/kg
12. Water extract of *Annona squamosa* bark at 15,000 mg/kg
13. Control (Methanol)

The set ups were monitored for seven days for dead *Meloidogyne incognita* juveniles. The juveniles were assumed to be dead if they failed to react to touch (Fatoki and Fawole, 1999; Rotimi and Moens, 2005). Percentage juvenile mortality was calculated as follows: Percent juvenile mortality = number of juveniles killed or dead/ total number of juveniles x 100 (Khan, 2009). Drops of 0.5% streptomycin were added to the extracts to prevent microbial growth. A second experiment was carried out.

Infrared (IR) Analysis of Siam Weed Leaf and Root, African Custard Apple Leaf and Bark

Powders of Siam weed leaf and root; and African Custard Apple leaf and bark were used for the Infrared analysis in the Multi-Disciplinary Central Research Laboratory of the University of Ibadan, Ibadan. The identification of the functional groups was carried out in the Phytochemical Research Laboratory, Department of Chemistry, University of Ibadan, Ibadan.

Phytochemical Analysis of Chromolaena Odorata and Annona Squamosa

The phytochemical analysis of the plant samples was carried out in the Pharmacognosy Department of the University of Ibadan, Ibadan using standard procedures to identify the constituents as described by Trease and Evans (1989) and Sofowora (1993) to test for tannins, saponins, flavonoids and alkaloids, anthraquinones (Borntrager test) and cardenolides using the Keller-Killiamis test or Kedde's test (Harborne, 1973; Chhabro *et al*., 1984).

3. RESULTS AND DISCUSSION

Results

Effect of Water Extracts of Chromolaena Odorata and Annona Squamosa on Egghatch of Meloidogyne Incognita

In (Table 1) there were significant differences with the range (25-100%) ($P \leq 0.05$) in percentage inhibition of *Meloidogyne incognita* egghatch among the control (methanol) and concentrations of water extracts of *Chromolaena odorata* and *Annona squamosa* during the period of seven days. There were no significant differences ($P \leq 0.05$) in percentage inhibitions among the water extract concentrations of the test plants.

Hatching was suppressed with increase in the concentrations of extracts of the test plants resulting in reduced percentage hatchability.

The increase in the concentrations of water extracts and increase in duration of exposure of *Meloidogyne incognita* to the various water extracts of *Chromolaena odorata* and *Annona squamosa* therefore, resulted in increases in percentage inhibition as fewer juveniles hatched. At 15,000 mg/kg *Chromolaena odorata* root and *Annona squamosa* leaf from day 5 had 100% inhibition while *Chromolaena odorata* leaf and *Annona squamosa* bark had 100% inhibition as from day six. By the third day percentage inhibitions ranged from 73% (*Chromolaena odorata* leaf) to 87% (*Annona squamosa* leaf) at

15,000 mg/kg. Percentage inhibitions were higher in *Annona squamosa* leaf and *Chromolaena odorata* root than in the other treatments.

Juvenile Mortality of Meloidogyne Incognita

There were significant differences ($P \leq 0.05$) in percent mortality of *Meloidogyne incognita* juveniles among water (control) and water extracts of the test plants as from day two seven (Table 2). An increase in both concentrations of water extracts and in duration of exposure of *Meloidogyne incognita* juveniles resulted in significant increases ($P \leq 0.05$) in percent mortality of the nematodes. An increase in effective concentrations of water extracts of *Chromolaena odorata* leaf from 5,000 mg/kg to 15,000 mg/kg resulted in significant increase in percent mortality from 2.3 to 5.0 and an increase in the concentrations of water extracts of *Chromolaena odorata* root from 5,000 mg/kg to 15,000 mg/kg led to a significant increase in percent mortality from 2.6 to 5.2 on day one.

Corresponding significant increases in percent mortality occurred with the water extracts of the other test plants on day one, by day four, percent mortality of water extracts of *Chromolaena odorata* leaf, *Chromolaena odorata* root, *Annona squamosa* leaf and *Annona squamosa* bark at 5,000 mg/kg, 10,000 and 15,000 mg/kg were increased.

Hundred percent mortality, occurred at varying periods of exposure and concentrations of the water extracts from day five to seven. Water extracts of *Chromolaena odorata* leaf at 15,000 mg/kg had completely killed *Meloidogyne incognita* juveniles (100%) by day five and, at 10,000 mg/kg by day seven and at 5,000 mg/kg by day seven. All of the water extracts at 10,000 mg/kg and15,000 mg/kg by day four had 50% mortality and 100% mortality by day seven. The second trial results followed the trends of the first trial.

Infrared and Phytochemical Tests

The functional groups identified by the IR analysis showed that *Chromolaena odorata* leaf contained alcohols, amides, alkanes, carbonyl, unsaturated/aromatic, double bonds, carboxyl, and phenol groups (Table 3). *Chromolaena odorata* root contained alcohols, alkanes, unsaturated/aromatic, phenol and metallic groups.

Annona squamosa leaf contained alcohols, alkanes, unsaturated/aromatic, double bonds, phenol and metallic groups. *Annona squamosa* bark contained alcohols, alkanes, unsaturated/aromatic and phenol groups. The active chemical ingredients in the various plants from the phytochemical tests, showed that *Chromolaena odorata* root and leaf contained alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins, (Table 4).

Annona squamosa leaf contained alkaloids, phenols, flavonoids, saponins, cardenolides and tannins while *Annona squamosa* bark contained alkaloids, phenols, tannins, flavonoids, saponins, cardenolides and anthraquinones. The concentrations of some of the phytochemicals in the test plants are as shown in Table 5. *Chromolaena odorata* root contained total phenols 14.3 mg/g, tannins 14.5 mg/g, flavonoids 1.5 mg/g, saponins 34.8 mg/g and alkaloids 11.5 mg/g.

Chromolaena odorata leaf total phenols 38.6 mg/g, tannins 41.0 mg/g, flavonoids 7.7 mg/g, saponins 331.7 mg/g, alkaloids 12.2 mg/g.

Annona squamosa leaf total phenols 31.0 mg/g, tannins 31.7 mg/g, flavonoids 11.5 mg/g, saponins 103.3 mg/g, alkaloids 12.0 mg/g and *Annona squamosa* bark total phenols 91.2 mg/g, tannins 97.6 mg/g, flavonoids 0.5 mg/g, saponins 156.2 mg/g, alkaloids 14.7 mg/g. Saponins were of the highest concentrations in all the botanicals, followed by tannins, total phenols, alkaloids and flavonoids with the least concentrations.

Discussion:

Hatching tests are useful in screening for nematicidal activity in plant extracts, because counting hatched juveniles than counting immobile juveniles in a particular J2 population (Oka *et al.*, 2000).

In the *in vitro* tests, water extracts of *Chromolaena odorata* leaf and root, *Annona squamosa* leaf and bark at effective concentrations of 5,000 mg/kg, 10,000 mg/kg, 15,000 mg/kg inhibited *Meloidogyne incognita* egghatch. An increase in concentrations and exposure time, percentage egghatch inhibitions increased, which was similar to the findings of Fatoki and Fawole (1999) and Nath and Mukherjee (2000).

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Table 1: Percentage Inhibition of *Meloidogyne Incognita* Egghatch at Various Water Extract Concentrations *of Chromolaena Odorata and Annona Squamosa* for a Period of Seven Days*

		Percentage Inhibition at Daily Intervals						
Treatment	Concentration (mg/kg)	1	2	3	4	5	6	7
Water (Control)	0	23.0(24.2)	14.4(21.7)	11.0(19.3)	9.8(18.0)	8.4(16.2)	5.1(13.0)	4.2(11.8)
C. Odorata Leaf	5,000	60.0(50.3)	58.0(49.4)	50.0(44.4)	40.0(39.0)	38.0(37.5)	23.0(28.0)	18.0(24.8)
C. Odorata Leaf	10,000	50.0(44.4)	48.0(43.6)	40.0(39.0)	36.0(36.8)	33.0(34.7)	15.0(22.3)	11.0(19.3)
C. Odorata Leaf	15,000	40.0(39.0)	25.0(30.0)	23.0(28.0)	20.0(26.1)	18.0(24.8)	0(0)	0(0)
C. Odorata Root	5,000	73.0(58.2)	68.0(55.0)	60.0(50.3)	58.0(49.4)	20.0(26.1)	15.0(22.3)	13.0(21.1)
C. Odorata Root	10,000	68.0(55.0)	63.0(52.1)	55.0(47.7)	45.0(42.0)	23.0(28.0)	11.0(19.3)	0(0)
C. Odorata Root	15,000	60.0(50.3)	50.0(44.4)	30.0(32.6)	18.0(24.8)	0(0)	0(0)	0(0)
A. Squamosa Leaf	5,000	63.0(52.1)	48.0(43.6)	45.0(42.1)	38.0(37.6)	20.0(26.1)	15.0(22.3)	11.0(19.3)
A. Squamosa Leaf	10,000	50.0(44.4)	30.0(32.6)	20.0(26.1)	18.0(24.8)	16.0(23.5)	11.0(19.3)	0(0)
A. Squamosa Leaf	15,000	30.0(32.6)	15.0(22.3)	13.0(21.1)	11.0(19.3)	0(0)	0(0)	0(0)
A. Squamosa Bark	5,000	75.0(59.3)	68.0(55.0)	55.0(47.3)	48.0(43.6)	30.0(32.6)	15.0(22.3)	11.0(19.3)
A. Squamosa Bark	10,000	68.0(55.0)	60.0(50.3)	50(44.4)	47.0(42.8)	16.0(23.5)	11.0(19.3)	0(0)
A. Squamosa Bark	15,000	43.0(40.5)	38.0(37.5)	30.0(32.6)	28.0(31.3)	13.0(21.1)	0(0)	0(0)
LSD 0.05		24.5(29.3)	15.3(22.9)	12.1(17.4)	10.4(18.6)	10.1(18.0)	2.4(8.6)	3.6(10.3)

* Data are means of four replicates, *C. odorata* = *Chromolaena odorata*, *A. squamosa* = *Annona* squamosa Transformed values in parentheses

Table 2: **Cumulative Percentage Mortality of *Meloidogyne Incognita* Juveniles at Various Concentrations of Water Extracts of *Chromolaena Odorata* and *Annona Squamosa* for a Period of Seven Days***

		Percentage Mortality Days after Inoculation													
	Concen-	1		2		3		4		5		6		7	
Treatment	tration (mg/kg)	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Water (Control)	0	1.4 (6.7)	0(0)	4.11(11.6)	1.8 (7.7)	7.8(1 6.2)	5.5(1 3.5)	13.7(21.7)	11.7 (20)	19.9(26.4)	19.9(26.4)	28.2(32.0)	30.1(33.2)	37.5 (37.7)	44(4 1.5)
C. odorata Leaf	5000	2.3 (8.6)	0.9 (5.4)	7.5(1 5.8)	4.1(11 .6)	19.4(26.1)	10.3(26.1)	33.6(35.3)	20.0(26.5)	50.2(45)	32.2(34.5)	70.1(56.8)	54.7(47.6)	91.9(7 3.3)	86.4(59.7)
C. odorata Leaf	10000	3.6(10.8)	2.2 (8.5)	10.5(18.9)	7.4(15 .7)	28.9(32.4)	15.3(23.0)	52.4(46.3)	26.0(30.6)	78.2(62.1)	43.7(41.3)	97.3(80.4)	69.7(56.6)	100 (90)	100 (90)
C. odorata Leaf	15000	5(12 .8)	3.2(1 0.5)	14.2 (2.0)	8.8(17 .2)	41.4 (40)	19.8(26.4)	71.4(57.5)	35.0(36.2)	100 (90)	99.0(84.2)	100 (90)	100 (90)	100 (90)	100 (90)
C. odorata Root	5000	2.6 (9.2)	1.8 (7.7)	7.2(1 5.5)	5(12.9)	20.3(26.7)	10.3(18.7)	35.7(36.6)	20.0(26.5)	54.4(47.4)	33.7(35.4)	100 (90)	78.7(62.5)	100 (90)	100 (90)
C. odorata Root	10000	3.9(11.3)	3.6(1 0.9)	12.2(20.4)	8.8(17 .2)	29.0(32.5)	18.3(25.3)	47.4(43.4)	33.2(34.5)	69.4(56.4)	55.3(48.0)	93.5 (75)	87.3(69.1)	100 (90)	100 (90)
C. odorata Root	15000	5.2(13.1)	4.5(1 2.2)	17.2(24.4)	10.3(1 8.7)	41.5 (40)	20.0(26.5)	67.4 (55)	35.9(36.8)	96.9(79.7)	61.6(51.7)	100 (90)	96.7(79.5)	100 (90)	100 (90)
A. senegalensis Leaf	5000	2.2 (8.5)	0.9 (5.4)	7.4(1 5.7)	4.1(11 .6)	26(3 0.5)	10.3(18.7)	47(4 3.2)	19.5(26.2)	71.6(57.7)	33.6(35.4)	97.2 (80)	53.3(46.8)	100 (90)	81.2(64.3)
A. senegalensis Leaf	10000	3.4(10.6)	1.8 (7.7)	11.4(19.6)	6.9(15 .2)	32.8(34.8)	14.9(22.7)	57.2(49.1)	26.8(31.1)	79.7(63.1)	44.7(41.9)	97.5 (80)	70.3(56.9)	100 (90)	100 (90)
A. senegalensis Leaf	15000	5.9(14.0)	3.6(1 0.9)	15.9(23.4)	9.3(17 .7)	43.5(41.2)	17.9(25.0)	72.4(58.2)	31.8(34.3)	99.5(85.5)	51.8(46.0)	100 (90)	83.5(66.0)	100 (90)	100 (90)
A. senegalensis Bark	5000	1.8 (7.7)	0.9 (5.4)	5(12. 8)	3.6(10 .9)	11.1(19.4)	9.3(1 7.7)	28.3 (32)	18.0(25.1)	48.3(43.9)	32.6(38.8)	72.3(58.2)	51.7(45.9)	96.8(7 9.4)	78.5(62.3)
A. senegalensis Bark	10000	3.9(11.3)	1.8 (7.7)	12.8(20.9)	5.9(14 .0)	31.4 (34)	12.8(20.9)	53.6 (47)	24.4(29.6)	75.3(60.1)	40.5(39.5)	100 (90)	94.7(76.7)	100 (90)	100 (90)
A. senegalensis Bark	15000	6.4(14.5)	4.5 (12.2)	19.8(26.3)	12.2(2 0.4)	44.3(41.4)	23.1(28.7)	72.1(58.1)	63.6(52.8)	100 (90)	99.8(87.4)	100 (90)	100 (90)	100 (90)	100 (90)
LSD 0.05		1.4 (6.7)	1.0 (5.7)	2.7 (9.4)	2.0 (8.1)	4.1(1 1.6)	2.6 (9.2)	5.7(1 3.8)	4.0(1 1.5)	7.2(1 5.5)	5.9(1 4.0)	13.9(21.8)	8.5(1 6.9)	3.1(10. 1)	35.9(36.8)

* Data are means of four Replicates, *C. Odorata* = *Chromolaena Odorata*, *A. Squamosa* = *Annona Squamosa*, T1 = First Trial, T2 = Second Trial, concentration of water extracts in mg/kg. Transformed values in parentheses

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Table 3: Functional Chemical Groups of *Chromolaena Odorata* Leaf and Root, and *Annona Squamosa* Leaf and Bark Identified by Infrared Analysis

Functional Groups									
Plant and part	Alcohol	Amine	Alkane	Carbonyl	Unsaturated/aromatic	Alkene	COOH	Phenol	Metal (Fe)
C.o Leaf	+	+	+	+	+	+	+	+	-
C.o Root	+	+	+	-	+	-	-	+	+
A.s Leaf	+	+	+	-	+	+	-	+	+
A.s Bark	+	+	+	-	+	-	-	+	-

Key

\+ = indicates presence, - indicates absence, *C. o = Chromolaena odorata, A. s = Annona squamosa*

Table 4: Active Chemical Ingredients in *Chromolaena Odorata* Leaf and Root, *Annona Squamosa* Leaf and Bark

Plant Species and Part	Alkaloids	Cardenolides	Anthraquinones	Saponins	Tannins	Flavonoids
C.o Leaf	+	+	+	+	+	+
C.o Root	+	+	+	+	+	+
A.s Leaf	+	+	-	+	+	+
A.s Bark	+	+	+	+	+	+

Key

\+ = indicates presence, - indicates absence, *C. o = Chromolaena odorata, A. s = Annona Squamosa*

Table 5: Concentrations of some Phytochemicals in *Chromolaena Odorata* Leaf and Root, *Annona Squamosa* Leaf and Bark

Plant & Part	Total Phenols (mg/g)	Tannins (mg/g)	Flavonoids (mg/g)	Saponins (mg/g)	Alkaloids (mg/g)
C. Odorata Root	14.3	14.5	1.5	34.8	11.5
C. Odorata Leaf	38.6	41.0	7.7	331.7	12.2
A. Squamosa Leaf	31.0	31.7	11.5	101.3	12.0
A. Squamosa Bark	91.2	93.6	0.5	156.2	14.7

In all concentrations of *Chromolaena odorata* and *Annona squamosa*, the juvenile hatching was suppressed, which was similar to the findings of Wiratno *et al*., (2009). The plant extracts of *Chromolaena odorata* and *Annona squamosa* were toxic and lethal to the second-stage juveniles of *M. incognita* at all concentrations which led to their mortality. An increase in concentrations of the extracts and exposure time resulted in increased percentage mortality rates of the juveniles. Water extracts could be directly nematostatic (Onifade and Egunjobi, 1994). Therefore, *Chromolaena odorata* and *Annona squamosa* extracts possessed and showed strong ovicidal and larvicidal activities against *Meloidogyne incognita* which were similar to findings of Adegbite and Adesiyan (2005). The nematicidal activity of the extracts was concentration and exposure time dependent (Rotimi and Moens, 2005). The inhibitory potential of the extracts does not necessarily correspond with their ability to induce juvenile mortality. Some extracts causing high juvenile mortalities did not always inhibit egg hatch effectively. In general, the extracts were less effective against egg hatch as compared to mortality of emerged juveniles (Khurma and Singh, 1997). The nematicidal activities could be attributed to the active chemical constituents called phytochemicals contained in the two test plants. Phytochemicals in plant products may control nematodes by direct killing, preventing penetration by causing paralyzation, causing the loss of host-finding ability, repulsion or by some unknown mechanisms (Tsai, 2000). *Chromolaena odorata* and *Annona squamosa* plant parts contained high amounts of saponins, followed by tannins and total phenols.

Saponins are plant secondary metabolites which cause heamolysis, with inhibitory effects on DNA, RNA and proteins in mammals (Fatoki and Fawole, 2000). These compounds are reported to cause reduction in membrane integrity of cells by the formation of transmembrane pores (Bernards *et al*., 2006) and increases cell membrane permeability to macromolecules (Francis *et al*., 2002). This influences eggshell permeability, and when eggshells are permeable, the unhatched juveniles are susceptible to toxic compounds from plant extracts (Curtis *et al*., 2009). Saponins work by interacting with the cuticle membrane of the larvae, ultimately disarranging the membrane, which may be the most probable reason for larval death (Ghayal *et al*., 2010). The nematicidal activity of saponins could be attributed to their ability to inhibit cholesterol accumulation in egg and/or larva (Ibrahim and Srour, 2013).

Tannins act as a defense mechanism in plants against pathogens and herbivores (Kumbasli *et al*., 2011). Tannins induce changes in the morphology of pathogens through action on cell membranes by destabilization of cytoplasmic and plasma membranes, inhibition of extracellular microbial enzymes and metabolism and substrate deprivation required for microbial growth (Ciocan and Bara, 2007; Min *et al*., 2008). Phenols alter root attractiveness to nematodes and induce resistance of the plant to nematode development and infestation was correlated to the phenols level in the roots results in delaying the formation of giant cells and poor nutrition of larvae (Stirling, 1991). Phenolic compounds are reported to be involved in host resistance (Pegard *et al*., 2005). Phenols are involved in causing tolerance of cells against the invasion and development of nematodes (Siji *et al*., 2010). Phenolic compounds act as constitutive protection agents against invading organisms, function as signal and plant defense molecules, is involved in resistance to biotic and abiotic stress (Joachim *et al*., 2007). Alkaloids are complex compounds found occurring naturally in plants, insoluble in aqueous hydroxide but soluble in aqueous

hydrochloric acid, toxic to insects (Fatoki and Fawole, 2000) and toxic to plant-parasitic nematodes. The alkaloids present in *Chromolaena odorata* have shown nematostatic and nematicidal effects on plant-parasitic nematodes (Thoden *et al*., 2009). The mode of action of alkaloids is suggested to the inhibition of protease activity (Wen *et al*., 2013). Flavonoids are a class of phenolic compounds that have anti feeding and attracting deterrent properties, thus are toxic to insects, fungi, bacteria, nematodes and weeds (Koul, 2008). They are synthesized by plants in response to microbial infection, and their activity is probably due to their ability to form complex with extracellular and soluble proteins, and to complex with microbial cell walls, also disrupting microbial membranes (Ciocan and Bara, 2007).

It has been reported that extracts of plants that contained saponins and tannins tend to inhibit nematodes egg hatch because of their ovicidal property (Umar and Mamman, 2014). Alkaloids, flavonoids and the other phytochemials have been reported to be nematotoxic in activity (Pavaraj *et al*., 2012).

Chromolaena odorata and *A. squamosa* are inhibitors of *Meloidogyne incognita* egg hatch and toxic and lethal to the juveniles. The ovicidal and larvicidal activities of the two plants can be attributed to the phytochemicals they contained, such as saponins, tannins, phenols, alkaloids, flavonoids, anthraquinones and cardenolides.

REFERENCES

[1] **Abbas S, Dawar S, Tariq M and Zaki MJ (2009).** Nematicidal activity of spices against *Meloidogyne javanica* (Treub) Chitwood. *Pakistan Journal of Biology* **41**(5) 2625-2631.

[2] **Abad P, Castagnone-Sereno P, Rosso M, Engler JA and Favery B (2009).** Invasion, feeding and development. In *Root-Knot Nematodes*, edited by Perry RN, Moens M and Starr JL. (CAB International, Wallingford UK), 163-176.

[3] **Bernards MA, Yousef LF and Nicol RW (2006).** The allelopathic potential of ginsenoides. In *Allelochemicals: Biologicals Control of Plant Pathogens and Diseases*, edited by Inderjit and Mukerjit (KG Springer. The Netherlands), 157-175.

[4] **Chhabro SC, Usio FC and Mshin EN (1984).** The phytochemical screening of Tanzanian medicinal plants. *Journal of Ethnopharma*, **11** 157-159.

[5] **Chaudhary KK and Kaul RK (2013).** Efficacy of *Pasteuria penetrans* and various oil seed cakes in the management of *Meloidogyne incognita* in Chilli pepper (*Capsicum annuum* L.). *Journal of Agricultural Science Technology* **15** 617-626.

[6] **Ciocan ID and Bara II (2007).** Plant products as anti microbial agents, Analele Stiintifice ale Universitatii Alexandru Ioan Cuza *SectiueaGenetica si Biologie Moleculara*, TOM **V111** 151-156. **Clarke AJ and Shepherd AM (1964).** Synthetic hatch agents for Heterodera schachtii Schum, and their modes of action. *Nematologia* **10** 431-453.

[7] **Curtis RHC (2008).** Plant-nematode interactions: environmental signals detected by the nematode's chemosensory organs control changes in the surface cuticle and behaviour. *Parasite* **15** 310-316.

[8] **Curtis RHC, Robinson AF and Perry RN (2009).** Hatch and host location. In *Root-Knot Nematodes,* edited by Perry RN, Moens M and Starr JL. (CAB International, Wallingford UK), 139-155.

[9] **Fatoki OK and Fawole B (1999).** *In Vitro* toxicity of some selected plant extracts on eggs and second-stage juveniles of *Meloidogyne incognita*. *African Journal of Plant Protection* **9** 81-82.

[10] **Fatoki OK and Fawole B (2000).** Identification of nematicidal ingredients from neem leaves, siam weed leaves and roots. *African Journal of Plant Protection* **10** 33-38.

[11] **Francis G, Kerme Z, Makkar HP and Becker K (2002).** The biological action of saponins in animal systems: a review. *British Journal of Nutrition* **88** 587-605.

[12] **Ghayal N, Padhye A and Dhumal K (2010).** Larvicidal activity of invasive weeds *Cassia uniflora* and *Synedrella nodiflora*. *International Journal of Pharma and BioSciences*, **1** 1-10.

[13] **Harborne JB (1973).** *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. (Chapman and Hall, London) 48-228.

[14] **Hussey RS and Barker KR (1973).** A comparison of methods of collecting inocula of *Meloidogyne* spp.*Plant Disease Reporter* **57** 1025-1028.

[15] **Ibrahim MAR and Srour HAM (2013).** Saponins suppress nematode cholesterol biosynthesis and inhibit root-knot nematode development in tomato seedlings. *Natural Products Chemistry and Research* **2** 123. Available: http://dx.doi.org/10.4172/2329-6836.1000123. [Accessed 16 May 2014]

[16] **Joachim H, Makoi JR and Ndakidemi PA (2007).** Biological, ecological and agronomic significance of plant phenolic compounds in the rhizosphere of the symbiotic legumes- Review. *African Journal of Biotechnology* **16**(12) 1358-1368.

[17] **Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, Kikuchi T, Manzanilla-Lopez R, Palomares-Rius JE, Wesemael WML and Perry RN (2013).** *Top 10 Plant-parasitic nematodes*, *Molecular Plant Pathology Review*. BSPP and John Wiley and Sons Ltd **14**(9) 946-961. Available: http://dx. doi:10.1111/mpp.12057. [Accessed 20 May 2014]

[18] **Khan SA (2009).** Screening of tomato cultivars against root-knot nematodes and their biological management. Ph.D Thesis, Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan. **Khurma UR and Singh A (1997).** Nematicidal potential of seed extracts: *In vitro* effects on juvenile mortality and egg hatch of *Meloidogyne incognita. Nematologia Mediterranea* **25**(1) 49-54.

[19] **Koul O (2008).** Phytochemicals and insect control: an antifeedant approach. *Critical Review of Plant Science* **27**(1) 1-24.

[20] **Kumbasli M, Bauce E, Rochefort S and Crepin M (2011).** Effects of tree age and stand thinning related variations in balsam fir secondary compounds on spruce budworm *Choristoneura fumiferana* development, growth and food utilization. *Agricultural and Forestry Entomology*, **13**(2) 131-141.

[21] **Min BR, Pinchak WE, Merkel R, Walker S, Tomita G and Anderson RC (2008).** Comparative antimicrobial activity of tannins extract from perennial plants on mastitis pathogens. *Scientific Research and Essay* **3**(2) 66-73.

[22] **Nath RC and Mukherjee B (2000).** *Dioscorea floribunda*, a potential source of nematicides of plant origin. *Nematologia Mediterranea* **28**(2) 145-149.

[23] **Ngangbam AK and Devi NB (2012).** An approach to the parasitism genes of root knot nematodes. *E Science Journal of Plant Pathology* **1** 81-87.

[24] **Olabiyi TL, Babatola JO and Oyedunmade EEA (1992).** *In Vitro* assessment of some plant extracts for their nematicidal properties. In *The Biology and Control of Nematode Pests of Food Crops in Africa*, edited by Fawole B, O. A. Egunjobi OA, Adesiyan SO, Babatola JO and Idowu AA. African Society of Nematologists. (His Will Info Resources Management Limited, Ibadan) 311-322.

[25] **Pavaraj M, Bakavathiappan G and Baskaran S (2012).** Evaluation of some plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita*. *Journal of Biopesticides.* **5**(Supplementary) 106-110.

[26] **Pegard A, Brizzard G, Fazari A, Soucaze O, Abad P and Djian-Caporalino C (2005).** Histological characterization of resistance to different root-knot nematode species related to phenolic accumulation in *Capsicum annuum*. *Phytopathology* **95** 158-165.

[27] **Rotimi O and Moens M (2005).** Effect of leaf extracts of some herbs on the juveniles of *Meloidogyne incognita* (Kofoid and White) Chitwood. *Nigerian Journal of Plant Protection* **22** 95-104.

[28] **Siji JV, Jayaprakas CA, Sheela MS and Mohandas C (2010).** Efficacy of *Cleome viscosa* L. against *Meloidogyne incognita* infestation in okra (*Abelmoschus esculentus* L.). *Thai Journal of Agricultural Science* **43**(3) 151-156.

[29] **Sikora RA and Fernandez E (2005).** Nematode parasites of vegetables. In *Plant-Parasitic Nematodes in Subtropical and Tropical Agriculture* edited by Luc M, Sikora RA and Bridge J, 2nd edition. (CAB International, Wallingford, UK) 319-392.

[30] **Sofowora A (1993).** *Medicinal Plants and Traditional Medicine in Africa*. second edition, Sunshine House (Spectrum Books Ltd, Ibadan, Nigeria) 26- 138.

[31] **Stirling GR (1991).** *Biological Control of Plant-Parasitic Nematodes: Progress, Problems and Prospects*. (CAB International, Wallingford, UK) 20- 265.

[32] **Thoden TC, Hallmann J and Boppre M (2009).** Effects of plants containing pyrrolizidine alkaloids on the northern root-knot nematode, *Meloidogyne hapla*. *European Journal of Plant Pathology* **123** 27-36. **Trease BE and Evans WA (1989).** *Preliminary Screening of Plants for their Chemical Constituents*. (CAB International, Wallingford, UK) 24- 94.

[33] **Tsai BY (2000).** A root penetration bioassay for the screening of nematode control principles. *Plant Pathology Bulletin* **9**(4) 131-136.

[34] **Umar I and Mamman A (2014).** Nematicidal potential of *Faidherbia albida* fruit against *Meloidogyne javanica* on cowpea. *Pakistan Journal of Nematology* **32**(1) 77-83.

[35] **Whitehead AC and Hemming JR (1965).** A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* **55** 25-28.

[36] **Wiratno D, Taniwiryono H, der Berg V, Riksen JAG, Riejens IMCM, Djiwanti SR, Kammenga JE and Murk AJ (2009).** Nematicidal activity of plant extracts against the root-knot nematode, *Meloidogyne incognita*. *The Open Natural Products Journal* **2** 77-85.