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In vitro nematicidal activity of some *Aloe* species extracts on eggs and second-stage juveniles of *Meloidogyne incognita*

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Abstract

The nematicidal activity of acetone extract (AE) and water extract (WE) of leaves and roots of *Aloe schweinfurthii* (ASF), *Aloe succrotina* (AST), *Aloe vera* (AVR), *Aloe chinensis* (ACS), *Aloe arborescens* (AAR), *Aloe keayi* (AKY), *Aloe macrocarpa* (AMC) and *Aloe schweinfurthii* x *Aloe vera* (ASV) on egg-hatching and mortality of second-stage juveniles (J_2) of *Meloidogyne incognita* was investigated *in vitro*. Extracts were tested at concentrations of 50,000 mg/kg and 25,000 mg/kg in an experiment laid out in completely randomized design in the laboratory. Data were collected on inhibition of egg-hatching, mortality of juveniles and analyzed using ANOVA ($P \leq 0.05$). Both concentrations of *Aloe* species extracts inhibited egg-hatching and second-stage juveniles mortality was observed significantly when compared with water control. The AE of AKY leaves at 50,000 mg/kg was the most effective in egg-hatching inhibited (95.4±1.7%), followed by AVR (94 ± 0.8%) and AST (88 ± 1.4%). Water extracts of leaves of AKY, AVR and AST inhibited egg-hatching by 85.5±1.2%, 77.8 ± 0.7% and 81±1.3%, respectively. The AE of AKY, AVR, AST and WE of AKY leaves at 50,000 mg/kg were the most effective in J_2 mortality with 100% recorded at 48 hr after exposure to extracts. The AE extracts of AKY, AVR, AST and WE of AKY and AVR roots at 50,000 mg/kg had 100% J_2 mortality at 72 hrs. This study reveals that *Aloe* species have nematicidal activity and used in the management of *M. incognita*.

Keywords: *Meloidogyne incognita*, eggs, second-stage juveniles, *Aloe* species, acetone, mortality.

Root-knot nematodes (*Meloidogyne* species) are one of the major plant-parasitic nematodes causing diseases in plants all over the world (Radwan *et al*., 2007; Osei *et al*., 2010). *Meloidogyne incognita* a notable species of the root-knot nematodes had been identified as a major reason for yield reduction in crops (Sikora *et al*., 2008; Olowe, 2010). Synthetic nematicides were adjudged effective in nematode control (Fawole, 2009; Osei *et al*., 2010). However, the hazards, they pose as environment pollutants, high cost of purchase and the need for skilled manpower in their application necessitate the need for alternative options (Bell, 2000; Adekunle & Fawole, 2003; Osei *et al*., 2010).

The emphasis of pest management strategies as environment-friendly methods and the use of botanicals encouraged as other methods were effective for the control of plant-parasitic nematodes (Chitwood, 2002; Fawole, 2009). In the course of evolution, plants have acquired effective defense mechanisms against arthropods that feed on them and some of these are based on their chemical components. Plant species are known to contain natural pesticidal materials, but many of them have not been extracted profitably (Prakash & Rao, 1997; Chitwood, 2002). Few of the plants already explored and known to produce economically important organic compounds, pharmaceuticals and pesticides; but many species of higher plants not surveyed (Satish *et al*., 2007). It is on the understanding of these natural potentials in plants that development of new pesticides for pest control is based (Chitwood, 2003).

Information on the screening or evaluation of diverse plants for pest management was not sufficient (Satish *et al*., 2007), though a few reports exist (Fatoki & Fawole, 1999; Adekunle & Akinlua, 2007; Fawole, 2009). There is need to discover more plants that have nematicidal potentials or properties, environment-friendly and low costs of production and application (Whitehead, 1998; Adekunle & Akinlua, 2007; Ofuya, 2009). The use of botanicals will be a more reliable method for plant protection and expected to play an increasingly prominent role in the development of future commercial pesticides for crop protection strategies (Gottlieb *et al*., 2002).

Aloe species are of the family Asphodelaceae and commonly found in Africa (Adodo, 2004; Omino, 2010). Aloes have been of ancient use medicinally in treating human ailments such as catarrh in Egypt (Adodo, 2004). Some *Aloe* species such as *Aloe vera* have found so much relevance in medicine and cosmetic industries, but little is known of their pesticidal use in agriculture (Hussein & Mosood, 1975; Mahmood *et al*., 1979; Tucker *et al*., 1989; Shelton, 1991). In Nigeria, there is little information on the nematicidal potentials of the *Aloe* species (besides *A. vera*) found within the country. The study was carried out to determine the nematicidal activity of some *Aloe* species on root-knot nematode (*M. incognita*).

Materials and Methods

***Aloe* species collection:** Eight *Aloe* species were obtained from institutions and gardens in Nigeria i.e., *Aloe chinensis*, *A. vera*, *Aloe succrotina*, *A. keayi*, *A. macrocarpa*; *A. vera* x *A. schweinfurthii*, *A. arborescens* and *A. schweinfurthii*. Aloe plants collected were properly identified by botanists and curators from Departments of Botany, Forest Resources Management of the University of Ibadan; Forestry Research Institute of Nigeria, Ibadan and Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. Suckers of each *Aloe* species were later transferred into five-litre pots (20 cm diam., and depth of 18 cm) in six replicates per species and grown for a minimum of three years till when used.

Source of inoculums: Culture of *M. incognita* was maintained on *Celosia argentea* in the screen house located at the National Horticultural Research Institute (NIHORT), Ibadan. The nematode was properly identified by extracting adult females of *M. incognita* from the *M. incognita* infected *Celosia argentea* roots by teasing with sharp needle in water. The perineal patterns of the adult female were prepared to confirm the identity of *M. incognita* as described by Eisenback *et al*., (1981).

Preparation of extracts: The leaves and roots of the collected *Aloe* species were air-dried for eight weeks in the laboratory at ambient tropical conditions and later milled into fine powder using Kenwood® dry mill. Ten grammes powder of each *Aloe* species (leaf and root separately) were weighed on a Mettler balance (Model P1210) into 200 ml conical flasks and 100 ml of distilled water was added to each of them (Olabiyi *et al*., 1992; Das *et al*., 2010). The set-ups were kept on the laboratory bench at ambient temperature for 72 hrs. The extracts were later filtered through Whatman No.1 filter paper five times to ensure clarity and the filtrates were concentrated in rotary evaporator at the Ajibola Taylor Toxicology Laboratory in the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan (UI). The filtrate obtained for each was considered as the stock extract (100, 000 mg/kg). Similar procedure was followed in extraction with acetone.

Screening for nematicidal potentials of *Aloe* species extracts against *Meloidogyne incognita*: *In vitro* assessment of effects of extracts of leaves and roots of eight *Aloe* species was carried out using two experiments; egg-hatching inhibited and mortality of second stage juveniles (J_2) of *M. incognita*. These experiments were carried out in the Nematology

Research Laboratory of the Department of CPEB, UI, from August to September, 2008 (at a mean temperature 28 ± 0.3 °C and relative humidity (RH) of 84.7 ± 0.6% and from November to December, 2011 at a mean temperature 27 ± 0.2 °C and RH of 79.1 ± 0.8%.

Egg-hatching inhibition: Eggs of *M. incognita* were extracted from the infected roots of *C. argentea* using the method of Hussey & Baker (1973). Nematode egg suspension (1 ml) containing 50 eggs of *M. incognita* were dispensed into each of the transparent glass blocks arranged in a completely randomized design (CRD) in the laboratory with four replications. One ml each of the stock extract (100,000 mg/kg) of acetone and water extracts of leaves and roots of *Aloe* species was dispensed into the glass blocks containing nematode eggs (1 ml) suspension to obtain an effective concentration of 50,000 mg/kg of either water or acetone extract. This also applied to concentration of 25,000 mg/kg in which one ml of prepared extract of 50,000 mg/kg was dispensed into the glass block containing one ml of nematode eggs suspension. The water control was obtained with one ml of distilled water and one ml of nematode egg suspension; also acetone control was prepared with 1ml of *M. incognita* suspension dispensed into 1 ml acetone.

The eggs were incubated at ambient temperature and relative humidity in the Laboratory. Glass covers were appropriately used to cover the glass blocks to prevent evaporation. Hatched juveniles were counted every 24 hrs for seven days under a dissecting microscope. The hatched juveniles were picked out of the extract every day. The second trial was conducted as in the first trial without any modifications for verification. Hatched eggs counted from each extract each day and egg-hatching (%) inhibition determined (Clarke & Shepherd, 1964). The percentage inhibition of egg-hatching of *M. incognita* was used as index of nematicidal ability of the extracts of *Aloe* species.

Second-stage juveniles mortality test: Water and acetone extracts of the leaves and roots of the eight *Aloe* species were prepared as previously described in egg-hatching inhibition experiment. The experiments were carried out under similar environmental conditions as in egg-hatching studies. Eggs of *M. incognita* extracted from infected *Celosia* roots in using the method of Hussey & Barker (1973) were incubated in the laboratory at ambient temperature for seven days. Freshly hatched second-stage juveniles (J_2) were later extracted using the pie-pan method (Whitehead & Hemming, 1965). The population of J_2 was estimated by counting in a Doncaster counting dish (Doncaster, 1962) under the stereomicroscope.

One ml of suspension containing 50 second-stage juveniles (J_2) was dispensed into each of the transparent glass blocks arranged in CRD in the laboratory. The extracts were prepared and treatments administered to J_2 in similar manner as in egg-hatching experiment. Glass covers were used to cover the blocks so as to prevent evaporation and each treatment was replicated four times. Dead juveniles, on the assumption of straight-line shape position and failure to react to stimuli when touched with a picking needle and counted every 24 hrs for seven days. Such counted dead juveniles were picked and transferred into distilled water for confirmation. The percentage mortality of second-stage juveniles of *M. incognita* was used as index of nematicidal ability of the extracts of *Aloe* species.

Data analysis: The data were arcsine transformed prior to analysis so as to conform to normal distribution. Data were statistically analyzed using analysis of variance with SAS (2009) statistical package and means separated using Fisher's Least Significant Differences (LSD) at 5% level of significance. After analysis, the back-transformed data were used in the presentation of results.

Results

Effects of acetone and aqueous extracts of *Aloe* leaves and roots on egg-hatching of *M. incognita*: At day 7 of exposure of *M. incognita* eggs to extracts, acetone extract of *A. keayi* leaves at 50,000 mg/kg inhibited egg-hatching of *M. incognita* by 95.4%, but this was not significantly higher than egg-hatching inhibited in *A. vera* at 50,000 mg/kg (94%). Acetone extracts of *A. succrotina*, *A. schweinfurthii* at 50,000 mg/kg and *A. keayi* at 25,000 mg/kg inhibited egg-hatching of *M. incognita* by 88.8, 86.8 and 86.5%, respectively. The lowest egg-hatching inhibition of 61.1% was recorded in *A. chinensis* at 25,000 mg/kg. No egg-hatching inhibited was observed in water control at the end of the experiment, whereas acetone control egg-hatching inhibited by 100%. On the seventh day of exposure of eggs of *M. incognita* to aqueous extracts of eight *Aloe* leaves, *A. keayi* (50,000 mg/kg) had 85.5% egg-hatching inhibition which was higher ($P \leq 0.05$) than egg-hatching inhibition of 77.8% in *A. vera* (50,000 mg/kg). The least egg-hatching inhibition of 51.2% was recorded in water extract of *A. chinensis* leaf (25,000 mg/kg). At day 7, all the eggs of *M. incognita* in the water control had no affected egg-hatching. All the aqueous extracts of *Aloe* leaves screened gave above 50% egg-hatching inhibited at the end of the experiment (7 days). Also at day 7, acetone extract of *A. keayi* root (50,000 mg/kg) significantly inhibited egg-hatching of *M. incognita* by 87.7% than *A. vera* (80.2%) and *A. chinensis* (73.7%). The least inhibition of 45.1% was recorded in *A. arborescens* (25,000) mg/kg. On the seventh day, *A. keayi* water root extract (50,000 mg/kg) observed highest egg-hatching inhibition 81.5% that was significantly higher than *A. vera* (75.7%), *A. succrotina* (66.9%) and *A. chinensis* (60.4%) roots at 50,000 mg/kg. The least egg-hatching inhibited of 38.2% was recorded in *A. schweinfurthii* x *A. vera* roots (25,000 mg/kg) (Table 1).

Table 1. *In vitro* effects of acetone and water extracts of *Aloe* species on egg-hatching (%) inhibition of *M. incognita*.

Aloe species	Concentration (mg/kg)	Egg-hatching (%) inhibited at day 7 after exposure to extracts			
		AEL	AER	WEL	WER
A. schweinfurthii	50,000	86.8	62.5	64.5	52.7
	25,000	73.9	55.5	53.4	40.2
A. succrotina	50,000	88.8	71.4	81	66.9
	25,000	80.3	58.3	71	50.2
A. vera	50,000	94	80.2	77.8	75.7
	25,000	84.1	67.8	67.4	59.8
A. arborescens	50,000	76.3	68.9	59.9	46.2
	25,000	66.9	45.1	59.9	39.9
A. keayi	50,000	95.4	87.7	85.5	81.5
	25,000	86.5	72.3	76.8	66.5
A. macrocarpa	50,000	76.9	63	64.6	49
	25,000	69.1	55	52.5	41.9
A. schweinfurthii x *A. vera*	50,000	74.6	69.4	64.6	41.8
	25,000	61.9	51.9	58.5	38.2
A. chinensis	50,000	73.8	73.7	65.4	60.4
	25,000	61.1	60.1	51.2	49.8
Acetone		100	100	0	0
Water	0	0	0	0	0
LSD ($P \leq 0.05$)		4.6	2.8	5.4	4.3

AEL = Acetone extracts of leaves of *Aloe* species; AER = Acetone extracts of roots of *Aloe* species; WEL = Water extracts of leaves of *Aloe* species; WER= Water extracts of roots of *Aloe* species.

Effects of acetone extracts of *Aloe* leaves on mortality of second-stage juveniles (J_2) of *M. incognita*: The results of *M. incognita* juveniles mortality test presented in Table 2 and revealed that 100% mortality of second-stage juveniles recorded at day 2 with the application of acetone extracts of *Aloe* leaves of *A. succrotina*, *A. vera* and *A. keayi* at 50,000 mg/kg. This was significantly higher ($P \leq 0.05$) than the mortality observed in *A. keayi* (25,000 mg/kg) with 86.7%, *A. chinensis* at 50,000 mg/kg (86.6%) and the least recorded in *A. schweinfurthii* x *A. vera* at 25,000 mg/kg (49.7%). No mortality was observed in water control only at day 2. At day 3, 100% mortality was observed in *Aloe* leaves of *A. vera* (25,000 mg/kg), *A. keayi* (25,000 mg/kg), *A macrocarpa* (50,000 mg/kg), *A. schweinfurthii* x *A. vera* (50,000 mg/kg) and *A. chinensis* (50,000 mg/kg). On the fourth day of the exposure of J_2 of *M. incognita* to acetone extracts of *Aloe* leaves, *A. schweinfurthii* (50,000 mg/kg), *A. macrocarpa* (25,000 mg/kg) and *A. schweinfurthii* x *A. vera* (25,000 mg/kg) all had 100% mortality of J_2. No mortality was recorded in water control. On the day 7, all the acetone extracts of *Aloe* leaves screened at both concentrations provided 100% mortality of *M. incognita* J_2 followed by *A. chinensis* (25,000 mg/kg) with 95.9%, *A. arborescens* (25,000 mg/kg) with 93.1% and water control with 6.5%.

Table 2. Effects of acetone extracts of leaves of *Aloe* species on cumulative mortality (%) of second-stage juveniles of *M. incognita*.

Aloe species	Concentration (mg/kg)	Cumulative mortality (%) of J_2 days after application of extracts						
		1	2	3	4	5	6	7
A. schweinfurthii	50,000	55	70.7	99.4	100	-	-	-
	25,000	40.1	54.7	82.3	92.9	100	-	-
A. succrotina	50,000	67.2	100	-	-	-	-	-
	25,000	42.9	55.2	65.8	100	-	-	-
A. vera	50,000	76.2	100	-	-	-	-	
	25,000	42.2	64.2	100	-	-	-	-
A. arborescens	50,000	37.4	52.2	87.7	100	-	-	-
	25,000	28.9	49.8	69.1	78.1	86.1	90.6	93.1
A. keayi	50,000	86.3	100	-	-	-	-	-
	25,000	59.8	86.7	100	-	-	-	-
A. macrocarpa	50,000	59.5	86	100	-	-	-	-
	25,000	39.3	71.2	94.5	100	-	-	-
A. schweinfurthii x *A. vera*	50,000	50.3	66.6	100	-	-	-	-
	25,000	29.8	49.7	87.5	100	-	-	-
A. chinensis	50,000	68.3	86.6	100	-	-	-	-
	25,000	48.5	76	83.7	85.7	89.8	93.4	95.9
Acetone		100	-	-	-	-	-	-
Water	0	0	0	0	0	0	3.9	6.5
LSD ($P \leq 0.05$)		6.1	5	4.3	2.5	1.9	1.6	1

Effects of water extracts of *Aloe* leaves on mortality of second-stage juveniles of *M. incognita*: At day 2, 100% mortality of J_2 was recorded in *A. keayi* (50,000 mg/kg) significantly higher than those of *A. vera* (50,000 mg/kg) with 90.2%, *A. keayi* (25,000 mg/kg) with 81.3%, *A succrotina* (50,000 mg/kg) with 80.5% and the least mortality of 33.7% from *A. arborescens* (25,000 mg/kg). On the third day, *A. vera* (50,000 mg/kg) and *A. keayi* (25,000 mg/kg) recorded 100% mortality of J_2 of *M. incognita*. No mortality was recorded in water control. At day 4, *A. succrotina* (50,000 mg/kg) had 100% J_2 mortality and least mortality of 49.1% in *A. arborescens* (25,000 mg/kg). At day 7, water extract of *A. arborescens* (50,000 mg/kg) leaf recorded 100% J_2 mortality and the lowest mortality of 62.4% was recorded in *A. macrocarpa* (25,000 mg/kg). Water control had 6.9% mortality of J_2 of *M. incognita* (Table 3).

Table 3. Effect of water extracts of *Aloe* leaves on cumulative mortality (%) of second-stage juveniles (J_2) of *M. incognita*.

Aloe species	Concentration (mg/kg)	Cumulative mortality (%) of J_2 days after application of extracts						
		1	2	3	4	5	6	7
A. schweinfurthii	50,000	49	65.2	89.4	95.9	100	-	-
	25,000	30.4	55.5	70.1	83	93.5	98.1	98.1
A. succrotina	50,000	59.3	80.5	93	100	-	-	-
	25,000	38.2	45.7	65.5	69.9	87.2	89.6	92.1
A. vera	50,000	73	90.2	100	-	-	-	-
	25,000	52.3	78.2	88.9	94.9	100	-	-
A. arborescens	50,000	36.7	42.3	49.8	55.7	61.7	84.1	100
	25,000	26.7	33.7	44.6	49.1	56.9	69.9	90.7
A. keayi	50,000	78.2	100	-	-	-	-	-
	25,000	61.6	81.3	100	-	-	-	-
A. macrocarpa	50,000	41.6	49.5	53	54.5	60.5	77.3	82.3
	25,000	36.1	41.5	46.4	49.4	51.3	56.4	62.4
A. schweinfurthii x *A. vera*	50,000	47.2	71.7	80.7	86.6	96.6	100	-
	25,000	36.6	61.5	67.1	73.7	79.3	84.8	92.4
A. chinensis	50,000	38	47	60.2	72.9	86.9	100	-
	25,000	32.5	37.5	45.8	51.7	54.7	73.9	88.7
Water	0	0	0	0	0	3.9	5.4	6.9
LSD ($P \leq 0.05$)		4.8	5.5	5.4	4.5	4	3.4	2.8

Effects of acetone extracts of eight *Aloe* species roots on mortality of second-stage juveniles of *M. incognita*: At the second day of exposure of J_2 of *M. incognita* to acetone root extracts, *A. keayi* (50,000 mg/kg) showed the highest mortality of 93.2% among the *Aloe* roots compared with the mortalities recorded in *A. vera* (78.9%), *A. succrotina* (75.8%) at 50,000 mg/kg and the least mortality of 40.7% recorded in *A. arborescens* (25,000 mg/kg). There was no mortality observed in water (control). At day 3, mortality of *M. incognita* J_2 was observed in acetone extracts of *A. succrotina*, *A. vera* and *A. keayi* roots at 50,000 mg/kg (100%). The lowest J_2 mortality of 57.1% was recorded in *A. macrocarpa* (25,000 mg/kg). At day 4, *A. schweinfurthii* roots (50,000 mg/kg), *A. vera* (25,000 mg/kg) and *A. keayi* (25,000 mg/kg) achieved 100% mortality. On the seventh day, all acetone extracts of *Aloe* roots provided mortality *M. incognita* J_2 in *A. chinensis* (25,000 mg/kg) with 94.6% followed by *A. macrocarpa* (25,000 mg/kg) with 91.9% and whereas, water control 2.9% (Table 4).

Table 4. *In vitro* effect of acetone extracts of *Aloe* roots on cumulative mortality (%) of second-stage juveniles (J_2) of *M. incognita*.

Aloe species	Conc. (mg/kg)	Cumulative mortality (%) of J_2 days after application of extracts						
		1	2	3	4	5	6	7
A. schweinfurthii	50,000	43.5	57	67.1	100	-	-	-
	25,000	26.9	48.9	58.6	77.7	100	-	-
A. succrotina	50,000	48.1	75.8	100	-	-	-	-
	25,000	35.2	52.9	70.9	87.5	100	-	-
A. vera	50,000	61.1	78.9	100	-	-	-	-
	25,000	47.7	59.6	80.3	100	-	-	-
A. arborescens	50,000	41.6	56.4	65.1	91.3	100	-	-
	25,000	29	40.7	58.1	76.5	84.6	100	-
A. keayi	50,000	68.2	93.2	100	-	-	-	
	25,000	52.8	73.2	89.6	100	-	-	-
A. macrocarpa	50,000	36.4	51	69.3	86.5	100	-	-
	25,000	31.8	48.5	57.1	64.7	74.8	84.4	91.9
A. schweinfurthii x *A. vera*	50,000	38.2	51.1	60.2	72.9	100	-	-
	25,000	32.3	49.8	59.8	74.7	88.1	100	-
A. chinensis	50,000	34.5	66.4	74.3	83.3	100	-	-
	25,000	31.5	44.8	53.7	66.9	77.8	86.7	94.6
Acetone		100	-	-	-	-	-	-
Water	0	0	0	0	0	0	0	2.9
LSD ($P \leq 0.05$)		6.4	5.9	5.6	4.7	2.6	1.5	1.3

Conc= concentration

Effects of water extracts of Aloe roots on mortality of second-stage juveniles of *M. incognita*: On the third day, aqueous extracts of *A. keayi* and *A. vera* roots at 50,000 mg/kg recorded 100% mortality of J_2 and this was significantly higher than J_2 mortality obtained in *A. succrotina* (87.3%), *A. schweinfurthii* (85.5%) 50,000 mg/kg and the least mortality of 43.1% in *A. chinensis* (25,000 mg/kg). Water control had no mortality at day 3. On the fourth day, only *A. succrotina* (50,000 mg/kg) significantly mortality of *M. incognita* J_2 (100%) and least J_2 mortality of 52.9% was recorded in *A. chinensis* (25,000 mg/kg). At day 5, *A. schweinfurthii* (50,000 mg/kg), *A. vera* (25,000 mg/kg), *A. arborescens* (50,000 mg/kg) and *A. keayi* (25,000 mg/kg) recorded 100% mortality of J_2 of *M. incognita*. No mortality was recorded in water control. At day 7, water extracts of *A. chinensis* (50,000 mg/kg) and *A. succrotina*, *A. schweinfurthii* x *A. vera*, *A. arborescens*, *A. macrocarpa*, *A. chinensis* roots at 25,000 mg/kg recorded J_2 mortality of 96.1, 97.9, 95.5, 95.1, 92.9 and 90.6 % respectively. Water control had 2.01% mortality of J_2 of *M. incognita* (Table 5).

Table 5. Effect of water extracts of *Aloe* roots on cumulative mortality (%) of second-stage juveniles (J_2) of *M. incognita*.

Aloe species	Concentration (mg/kg)	Cumulative mortality (%) of J_2 days after application of extracts						
		1	2	3	4	5	6	7
A. schweinfurthii	50,000	23.9	60.9	85.5	92.5	100	-	-
	25,000	15.3	33	50.8	72.6	88.4	100	-
A. succrotina	50,000	45.8	69.2	87.3	100	-	-	-
	25,000	30.3	39.9	60.1	57.2	80.8	92.9	97.9
A. vera	50,000	68.3	86.6	100	-	-	-	-
	25,000	40.2	50.3	76.9	91.5	100	-	-
A. arborescens	50,000	30.5	53.3	70.1	90.4	100	-	-
	25,000	27.9	36.6	49.5	59.5	72.9	89	95.1
A. keayi	50,000	70.5	88	100	-	-	-	-
	25,000	46.3	57.3	64.67	88.6	100	-	-
A. macrocarpa	50,000	34.4	46.1	61.6	82.9	97	100	-
	25,000	24.7	38.4	51	62.7	71.7	85.9	92.9
A. schweinfurthii x *A.* vera	50,000	29.1	41.9	50	62	89.2	100	-
	25,000	21.8	34	44.2	52.9	59.9	80.2	95.5
A. chinensis	50,000	31.5	39.5	51.8	67.4	74.4	84.2	96.1
	25,000	20.3	33.7	43.1	52	70.3	79.7	90.6
Water	0	0	0	0	0	0	0	2
LSD ($P \leq 0.05$)		4.7	5.9	4.7	5.4	4.5	3.2	2.9

Discussion

Aqueous and acetone extracts of the leaves and roots of the eight *Aloe species* tested were effectively egg-hatching inhibited and causing mortality of the second-stage juveniles of *M. incognita*. This showed that *Aloe* species have nematicidal properties and this view corroborated findings of pesticidal properties in some *Aloe* species (Hussein & Mosood, 1975; Pandey & Hasseb, 1988; Ukoimah & Okah, 2006; Arunkumar & Muthuselvam, 2009; Mbagwu *et al*., 2010). However, previous reports have been limited to the commonly found *Aloe* species such as *Aloe vera* and *Aloe ferox*, amongst others. There were variations in the nematicidal activity exhibited in terms of egg-hatching inhibition and mortality of second-stage juveniles of *M. incognita* among the *Aloe* species screened. Acetone and water extracts of *A. keayi*, *A. succrotina* and *A. vera* exhibited more nematicidal effects than the other *Aloe* species tested. The three *Aloe* species recorded higher egg-hatching inhibition at the end of the *in vitro* study and equally mortality of *M. incognita* second-stage juveniles faster in two to three days after exposure of juveniles to extracts with *A. keayi* being outstanding than other *Aloe* species tested.

The nematicidal activity observed in these *Aloe* species might be due to the presence of active principles (phytochemicals) in them such as alkaloids, saponins, tannins, amongst others. Phytochemicals had been reported to confer pesticidal abilities in plants (Chitwood, 2002; Adekunle & Fawole, 2003; Adeniyi *et al*., 2010). Some of these phytochemicals such as tannins, saponins have nematicidal properties and linked to disruption of membranes in organisms thereby facilitating penetration of toxic principles to the detriment of such organisms (Agrios, 2005; Bassetti & Sala, 2005; D'Addabbo *et al*., 2010). Saponins also called saponocydes reported to possessed cell membrane-breaking property. In particular, saponins bind with the lipid membrane of cells, making the cells more permeable and at the same time more fragile, enabling a loss of cell contents through leakage (Bassetti & Sala,

2005). The contents enclosed by such membranes, thus altered as the eggs were hindered from undergoing embryogenesis and subsequently destroyed. Also, alkaloids in plants have been reported to exhibit nematicidal activity on root-knot nematodes (Chitwood, 2003). Phenolics have been reported to show toxicity to insects, fungi, bacteria, nematodes and weeds (Koul, 2008). Tannins inhibit pathogenic fungi and also have anthelmintic, antimicrobial properties; whereas alkaloids have antimicrobial, anthelmintic and antidiarrhoeal abilities (Gills, 1992; Chitwood, 2002; Tiwari *et al*., 2011).

Phytochemicals reported as the basis for antimicrobial activity of some *Aloe* species (Arunkumar & Muthuselvam, 2009; Mbagwu *et al*., 2010). Arunkumar & Muthuselvam (2009) and Mbagwu *et al*., (2010) reported bioactivity of some *Aloe* species might be related to the presence, types and quantities of phytochemicals in them. In *Aloe* species, the saponins have a purifying, antiseptic and antimicrobial action (Basseti & Sala, 2005). The nematotoxic effect of the extracts may also be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve cytoplasmic membrane of nematode cells and interfering with enzyme protein structure (Knobloch *et al*., 1989; Claudius-Cole *et al*., 2010). `The mechanisms of many plant extracts may include denaturing and degrading proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Konstantopoulou *et al*., 1994; Claudius-Cole *et al*., 2010).

There were differences observed in the bioactivity of the *Aloe* species tested in this study and these variations might be due to varying quantities and types of phytochemicals present in them. The differences could also be attributed to the type of solvents used for extraction and method of extraction (Qasem, 1996). Similar to the report from some other plants by Radwan *et al*., (2007) reported differences in the nematicidal activity exhibited by the different plants screened for phytochemicals varied in qualitatively and also quantitatively.

The period of exposure of *M. incognita* eggs and J_2 to extracts might have influenced the nematicidal activity of the *Aloe* species in terms of egg-hatching inhibition and mortality of second-stage juveniles. In the egg-hatching studies, the inhibitions to egg-hatching by the various extracts decreased as the period of exposure of eggs of *M. incognita* increased. There exists a direct relationship between time of exposure to extracts and mortality of J_2. Similar observation was made by Fatoki & Fawole (1999) when the trend in egg-hatching inhibition and mortality of J_2 of *M. incognita* were studied *in vitro*. The first two or three days showed either higher egg-hatching inhibition or mortality of J_2 of *M. incognita*.

The earlier explanation for egg-hatching inhibition holds for the high mortality of J_2 of *M. incognita* in days 1 and 2 of exposure to extracts, but the mortality increased with time of exposure to extracts with the highest mortality recorded in all the treatments at the seventh day. It might be that as the period of exposure of the nematode to extracts increases, the juveniles were weakened both in activity and structurally. This condition might have paved way for easy penetration of the active principles in the extracts into the body of the nematodes.

Time of exposure of pathogens to active principles in plant extracts does affected bioactivity (Fabiyi *et al*., 2012). Sharma and Trivedi (2002) from the screening of leaf extracts of 15 plants for their nematicidal properties against *M. incognita* posited that exposure time played an important role in the mortality of the nematode, such that the longer the exposure time to extract, the more the mortality effect on the nematode.

The nematicidal activity observed in solvent-dependent for the *Aloe* species (either leaves or roots). The results showed that acetone extracts

of either *Aloe* species leaves or roots showed better nematicidal activity than water extracts using egg-hatching inhibition and juvenile mortality. Acetone extract has more active principles (phytochemicals) than water (Ncube *et al*., 2008). There was greater penetration of the active principles aided by acetone into egg-shells and cuticles of second stage juveniles of *M. incognita* which in no doubt hastened and increased the toxic effect of these extracts. This view explaining the importance of acetone as a better extraction solvent than water was supported by Tiwari *et al*., (2011). They reported that acetone was a useful extraction solvent, specially for antimicrobial studies when more phenolic compounds were extracted. Eloff (1998) and Das *et al*., (2010) reported that extraction of tannins and phenols was better in acetone than in aqueous methanol. Acetone was good solvent for extraction of phytochemicals (Lale & Ajayi, 1996; Arunkumar & Muthuselvam, 2009; Adedire *et al*., 2011). Acetone control showed nematicidal potentials than water in this experiment regarding the egg-hatching and mortality of second-stage juveniles.

The nematicidal effects recorded in the experiment across the *Aloe* species was also concentration-dependent, irrespective of the solvents of extraction used. The higher concentration in either acetone or water extracts of either leaves or roots of *Aloe* species. This might be because higher concentration of extracts was made from higher amount of plant samples (in terms of weight) which invariably will contain more quantities of phytochemicals than the lower concentration. It might be true then that the more the active principles per concentration determined by amounts of plant samples, the more might be the nematicidal effects of the *Aloe* species extracts on *M. incognita* (Upadhyay *et al*., 2003; Mondale *et al*., 2009; Insunza *et al*., 2001).

Aloe species provided credence to the fact that plants showed nematicidal activity and many plants confirmed effective in the management of plant-parasitic nematodes (Fatoki & Fawole, 1999; Nath & Mukherjee, 2000; Chitwood, 2002; Sharma & Trivedi, 2002; Upadhyay *et al*., 2003; Claudius-Cole *et al*., 2010; Tiwari *et al*., 2011) and phytochemicals (Arunkumar & Muthuselvam, 2009; Mbagwu *et al*., 2010) in some *Aloe* species were posited medicinal or pesticidal in action (Chitwood, 2002; Adedire *et al*., 2003; Thoden *et al*., 2009). The study showed that all the eight *Aloe* species screened showed nematicidal activity, but *Aloe keayi*, *A. succrotrina* and *A. vera* showed greater nematicidal potentials for the management of *M. incognita*.

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