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Characterization, phytochemical analysis and nematicidal activity of *Daniella oliveri* leaves against *Meloidogyne incognita*

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Abstract

This study was carried out to identify and evaluate the nematicidal potential of *Daniella oliveri* leaves. The leaves of *D. oliveri* were air dried and extracted with n-hexane, there after acetone. The concentrated extracts were subjected to chromatography on a silica gel packed column. The nematicidal activity of the fractions were evaluated in the laboratory using a 4x4x3 factorial experiment, while the crude extracts were tested on *Meloidogyne incognita* infecting *Abelmoschus esculentus* in the field. Three hundred juveniles were used for the assessment. Acetone extract coded DNLO/Me_2CO caused a significant increase in plant height, number of leaves, number of fruits per plant and fruit weight per plant as compared with Carbofuran ($p<0.05$). In the laboratory, chromatographic fractions from acetone extract had the highest percentage juvenile mortality. The infrared analysis of the fractions revealed the presence of anhydride and carbonyl stretching frequencies at 3638, 1702, 1735 and 1771 cm^{-1}. The Nuclear Magnetic Resonance (NMR) of the fractions showed signals that agreed with ozoic and daniellic acids. Preliminary phytochemical screening of the leaves revealed the presence of flavonoids, steroids, terpenoids, reducing sugars, phenols, alkaloids, glycosides and carbohydrates. Nematicidal activity of *D. oliveri* leaves is being reported for the first time. The crude extracts and chromatographic fractions are potent at very low concentrations compared with standard carbofuran. Therefore, the leaves of *D. oliveri* could be employed as a viable source of natural nematicide instead of synthetic toxic nematicides.

A*belmoschus esculentus* (L.) Moench is an important vegetable in South Western Nigeria, where the immature and mature fruits are used to prepare slimy soups and sauces. The young leaves are served as spinach. The medicinal value and application in industry such as plasma replacement, blood volume expander has been highlighted. The mucilage is highly water soluble. The protein content is about 20% comparable to soya bean. The oil content (20%) and fatty acid composition is similar to cotton seed oil (Siemonsma & Kouame, 2004). However, *A. esculentus* is attacked by a large number of pests and diseases. Nematodes of the genus *Meloidogyne* are a major problem. Chemical control is hazardous because crop harvesting is frequent and with pesticide residue provided to the market and inadvertently get to the consumers. In this fact, there is need to search an alternative control for *Meloidogyne* spp., on *A. esculentus*. Such alternative control involves the use of natural nematicides derived from plants. *Daniella oliveri* (Rolfe) belongs to the family Caesalpiniaceae is used as treatment of gastrointestinal parasitism of small ruminants and also against kwashiorkor (Kabore *et al*., 2010; Ige, 2011). The leaves of *D. oliveri* has been proved to effective in circumcision wound dressing and malaria fever treatment by the Igede people of Nigeria (Igoli *et al*., 2005). *Daniella oliveri* has also been used in the treatment of venereal diseases, stings, bites and pulmonary troubles (Tomoda *et al*., 1980).

The intractable problem of environmental pollution of synthetic nematicides has led to the resurgence of interest in bio-pesticides as sources of novel compounds to manage nematode problems, thus investigation was made to evaluate *D. oliveri* leaves for probable solution to nematodes on *A. esculentus*.

Materials and Methods

Sample and sample preparation: The leaves of *D. oliveri* were collected from the University of Ilorin campus farm located at lat: 8°, 29´ N, long: 4°, 40´ E of the Greenwich meridian and authenticated in the herbarium with voucher no UIH964. The leaves were air dried at room temperature (27 °C) for two months after which they were grinded in laboratory mill. Powdered material (1 kg) was extracted with n-hexane and later with acetone for five days each. The extracts were filtered and concentrated using rotary evaporator under vacuum to obtain the crude extracts coded DNLO/Hex and DNLO/Me_2CO from hexane and acetone, respectively. Forty gram each of the crude extracts was subjected to open column chromatography on silica gel using glass column 10 cm dia., and 50 cm long.

The extracts were eluted with n-hexane after that with a mixture of equal volume of n-hexane/dichloromethane and finally another equal volume of dichloromethane and diethyl ether. The collected fractions were later combined based on the TLC (pre-coated silica gel 0-25 mesh F_{254}) profile. Spots were detected by UV light (254, 366 nm).

Aqueous extraction: Plant material (500 g) was extracted for a period of five days with a litre of water after that decanted, filtered and allowed to evaporate to a semi-solid substance coded DNLO/H_2O.

Phytochemical tests: Phytochemical screening was carried out using the concentrated extracts according to standard methods (Sofowora, 1993).

Nematode extraction: Root-knot nematode *Meloidogyne incognita* was cultured on *Celosia argentea* and identified on the basis of perineal pattern (Eisenback *et al*., 1981). The second stage juveniles were extracted from the root using the standard tray method (Whitehead & Hemming, 1965).

Egg extraction: Egg-masses from *Celosia argentea* roots were shaked thoroughly with 500 ml of 0.6% sodium hypochlorite (NaOCl) in stopper flasks lasting 2 minutes (Hussey & Baker, 1973) for the purpose of digesting the gelatinous matrix encasing the eggs. The eggs were washed by rinsing with tap water through a 75 µm sieve, placed on a 25 µm sieve and transferred into distilled water forming egg suspension.

Spectroscopic analysis: Infrared spectra of chromatographic fractions were recorded on SHIMADZU 8400s FTIR spectrophotometer at the Department of Chemical Sciences Redeemer's University Mowe, Lagos, Nigeria, while the Proton and Carbon, Nuclear Magnetic Resonance (^{1}H NMR) of fractions were recorded on mercury model 200BB with TMS as internal standard and $CDCl_3$ as solvent, available at the Central Research Laboratory Obafemi Awolowo University Ile-Ife, Nigeria.

Juvenile mortality and egg hatchability assessment: A sample of 20 mg was taken from the chromatographic fractions of *D. oliveri* n-hexane and acetone extract (DNLO/Hex and DNLO/Me_2CO). Similarly, same quantity was also taken from the aqueous and crude extracts of the plant material (DNLO/H_2O, DNLO/Hex/CRD and DNLO/Me_2CO/CRD) + standard carbofuran (CBFN) thus making a total of six treatments for each assessment. The experimental design for each assessment was a 6x4x3 factorial experiment conducted in a randomized complete design (RCD), involving six treatments at four levels and each replicated three times; 20 mg of each chromatographic fraction was dissolved in 50 ml distilled water. From this solution serial dilutions were made for 25, 50 and 75% concentrations without distilled water. The crude extracts and carbofuran were diluted in the same way. For the mortality test 300 freshly hatched juveniles of *M. incognita* were used in each Petri dish, and also 300 eggs were used in the hatchability test at room temperature. Counting was done uder stereo microscope. The dead juveniles did not respond to the touch of fine needles.

Field experiment: Two trials were conducted at the University of Ilorin, Teaching and Research farm in two consecutive years between July and September, 2009 and 2010. A 4x4x3 factorial experiment fitted into randomized complete block design (RCBD) which consisted of four treatments at four levels and replicated three times. A portion of land measuring 30 x 20 m was prepared. This was divided into four plots of 14.25 x 9.5 m with an alleyway of 0.5 m in between the plots. Each of the four plots was subdivided into twelve beds of 1.5 x 4 m (6 m^2) in size, with an alley way of 0.7 m in between the beds in a row and 1.25 m in a column. On each bed, a spacing of 50 cm was used in the line, and 30 cm was used in the row between each plant stand (Denton, 1997).

Planting operations: Seeds were sown in both years at a depth of 0.3 cm using specified spacing in the field. The seeds started germination on the fourth day after planting. Ten days after germination the plants were thinned down to a plant per stand.

Nematode inoculation and treatment application: Fourteen days after planting, 300 freshly hatched juveniles were inoculated around the root of each *A. esculentus*. Seven days after inoculation the plants close to the root were treated with extracts of *D. oliveri* in banded form at 75, 50, 25 and 0%. The serial dilution for plant extracts was prepared as 30 g of each plant extract was dissolved in 100 ml of water, which is equivalent to 0.3 g/ml, and 5 ml plant extract + 15 ml water = 25%; 10 ml plant extract + 10 ml water = 50%; 15 ml plant extract + 5 ml water = 75%. Carbofuran 3G was applied in the solid form at rates equivalent to 1, 1.5 and 2 kg ai/ha. This was done for both the first and second trials.

Data collection and statistical analysis: From the field, data on parameters such as plant heights, number of leaves, number of fruits per plant and fruit weight per plant were collected. Days to 50% flowering was recorded as observed, while, nematode population in 250 g soil sample, nematode population in 5 g root sample and root gall rating were assessed in the laboratory after harvest using the rating scale (0-5) described by Das & Sukul (1986). All data were subjected to analysis of variance. Treatment means were separated using the Duncan's new multiple range test at 5% level of probability (Gomez & Gomez, 1984).

Results

Spectroscopic analysis: The result of the infrared analysis of the chromatographic fractions (Table 1) revealed the presence of various functional groups which is a pointer to the various types of compound present in the chromatographic fractions of *D. oliveri* leaves. A few of the diagnostic bands establishes the presence of hydrocarbons in the isolate, while some gave an indication of mono substituted aromatic rings. The NMR data also supports the presence of lactone and daniellic acids.

Table 1. Infrared analysis.

Wave number (cm^{-1})	Functional group
3638	O-H stretch (Phenol)
2932	C-H stretch aliphatic
2870	C-H stretch aliphatic
1771	C=O of anhydride
1735	C-O stretch lactone
1702	C=O in cyclopentanone
1570	C=O of aromatic ester
1022	C-O of phenol

Nuclear Magnetic Resonance (NMR) analysis: ^1H NMR: d 0.8; d1.04 (Me_2-CH-); m 1.0 (-CH-Me_2); m 1.6 (-CH_2-); d 4.4 (CH_2-O-)

The spectroscopic data above agrees with the data established for lactone and daniellic acid (Mills, 1973; Olatunji, 1998).

Phytochemical screening: Result of the phytochemical screening of the three extracts from *Daniella oliveri* (Table 2) depicted that all the class of phytochemicals are present in *D. oliveri* acetone extract (DNLO/Me_2CO). Anthraquinone and reducing sugars are absent in the n-hexane extract (DNLO/Hex), while in the

aqueous extract (DNLO/H_2O) steroids, reducing sugars and anthraquinone were absent.

Table 2. Phytochemical screening.

Phytochemicals	DNLO/ Hex	DNLO/ Me_2CO	DNLO/ H_2O
Alkaloids	+	+ +	+
Anthraquinones	-	+ +	-
Flavonoids	+	+ +	+
Glycosides	+	+ +	+
Reducing sugars	-	+	-
Saponins	+	+ +	+
Steroids	+	+ +	-
Tannin/Polyphenols	+	+ +	+
Terpenoids	+	+ +	+

DNLO/Hex= *Daniella oliveri* hexane extract, NLO/Me_2CO= *Daniella oliveri* acetone extract, DNLO/H_2O= *Daniella liveri* aqueous extract, - = Absent, + = Traceable amount, + + = Appreciable amount.

Juvenile mortality: The chromatographic fraction from the *D. oliveri* acetone extract (Me_2CO) was significantly more effective than all the other treatments including the standard check carbofuran, with a percentage mortality of 51.38% as opposed to carbofuran (CBFN) which had 46.02% at the fourth hr of exposure of juveniles treatments ($p<0.05$). Similar observation was seen in the activity of the chromatographic fractions at 12 hrs (Table 3).

On day two, all the treatments had a hundred percent mortality except the aqueous extract which had 93.68%. The level of application of the treatments was also significant with the hundred percent concentrations recording the highest percentage mortality throughout the period of the experiment.

Table 3. Comparative effects of chromatographic isolates, crude extracts on mortality (%) of *Meloidogyne incognita* juveniles in the laboratory.

Treatments	Time (hrs) of exposure for juveniles to extract				
	4	8	12	24	48
DNLO/Hex	40.16^{c}	54.21^{c}	67.25^{c}	92.35^{b}	100^{a}
DNLO/Me_2CO	51.38^{a}	65.14^{a}	79.07^{a}	100.00^{a}	100^{a}
DNLO/H_2O	26.75^{f}	40.08^{f}	51.11^{f}	76.12	93.68^{b}
DNLO/Hex/CRD	30.62^{e}	47.39^{e}	56.63^{e}	80.00^{d}	100^{a}
DNLO/Me_2CO/CRD	35.39^{d}	51.82^{d}	62.44^{d}	85.61^{c}	100^{a}
CBFN	46.02^{b}	61.18^{b}	72.71^{b}	100.00^{a}	100^{a}
S.E.M.	1.14	1.37	1.54	2.01	2.38
Treatment level (%)					
0	0.00^{d}	0.00^{d}	0.00^{d}	3.06^{d}	5.68^{d}
50	27.19^{c}	41.80^{c}	59.30^{c}	84.21^{c}	100^{a}
75	32.76^{b}	47.30^{b}	65.00^{b}	90.11^{b}	100^{a}
100	39.24^{a}	53.29^{a}	71.66^{a}	96.00^{a}	100^{a}
S.E.M.	0.11	0.18	0.26	0.39	1.12

Values with different alphabets along the same column are statistically different at $p< 0.05$.

DNLO/Hex = *Daniella oliveri* hexane extract chromatographic fraction, DNLO/Me_2CO = *Daniella oliveri* acetone extract chromatographic fraction, DNLO/H_2O = *Daniella oliveri* aqueous extract, DNLO/Hex/CRD = *Daniella oliveri* hexane extract crude, DNLO/Me_2CO/CRD = *Daniella oliveri* acetone extract crude, CBFN = Carbofuran, S.E.M. = Standard error of mean.

Egg hatchability: Effect of the chromatographic fractions, crude extracts and carbofuran on percentage egg hatch of *M. incognita* juveniles (Table 4) was significantly inhibited by the treatments at day one ($p<0.05$). A few egg hatches were recorded in the crude and aqueous extracts on day four of the experiment, with the aqueous extract recording the highest percentage egg hatch of 2.19%.

There was no significant difference between the levels of treatment application, all levels inhibited egg hatch except the 0% control recorded egg hatch throughout the period of observation.

Table 4. Comparative effects of chromatographic isolates, crude extracts on *Meloidogyne incognita* eggs hatching (%) in the laboratory.

Treatments	Time (hrs) of exposure for eggs to extract			
	24	48	72	96
DNLO/Hex	0.00[a]	0.00[a]	0.00[a]	0.00[a]
DNLO/Me_2CO	0.00[a]	0.00[a]	0.00[a]	0.00[a]
DNLO/H_2O	0.00[a]	0.41[b]	0.86[c]	2.19[d]
DNLO/Hex/CRD	0.00[a]	0.09[a]	0.25[b]	0.68[c]
DNLO/Me_2CO/CRD	0.00[a]	0.00[a]	0.00[a]	0.13[b]
CBFN	0.00[a]	0.00[a]	0.00[a]	0.00[a]
	NS			
S.E.M.	0.00	0.12	0.53	0.71
Treatment level (%)				
0	18.26[b]	27.77[b]	38.91[b]	46.69[c]
50	0.00[a]	0.03[a]	0.08[a]	0.15[b]
75	0.00[a]	0.00[a]	0.00[a]	0.00[a]
100	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M.	0.02	0.06	0.13	0.19

Means in column followed by the same letters are not significantly different at $p< 0.05$.

DNLO/Hex = *Daniella oliveri* hexane extract chromatographic fraction, DNLO/Me_2CO = *Daniella oliveri* acetone extract chromatographic fraction, DNLO/H_2O = *Daniella oliveri* aqueous extract, DNLO/Hex/CRD = *Daniella oliveri* hexane extract crude, DNLO/Me_2CO/CRD = *Daniella oliveri* acetone extract crude, CBFN = Carbofuran, NS = Not significant.

Field experiments: *Daniella oliveri* acetone extracts crude (DNLO//Me_2CO) and carbofuran (CBFN) were significantly more effective than the other treatments as they produced plants with significantly ($p<0.05$) higher heights from the 3rd week after sowing (WAS) to the end of the experiment at the 8th week after planting (Table 5). This was closely followed by plants treated with *D. oliveri* hexane extracts crude (DNLO/Hex). Plants treated with aqueous extract had significantly lower heights throughout the period under study. All treatments significantly increased the number of leaves, compared with the untreated control plants (0%), but plants treated with DNLO/Me_2CO/CRD and CBFN had significantly more number of leaves compared with the other treatments (Table 6). There was an increase in number of fruits per plant among plants treated with CBFN and DNLO/Me_2CO/CRD, whereas number of fruits per plant was significantly ($p<0.05$) fewer in plants that received other forms of treatments. Fruit weight per plant was also significantly heavier in plants treated with carbofuran, acetone and hexane extract (crude) of *Daniella oliveri* as compared to the aqueous extract and the untreated control plants (Table 7 and 8).

Nematodes were also significantly absent in the 250 g soil sample and 5 g root sample of plants treated with *D. oliveri* acetone extract and carbofuran (DNLO/Me_2CO/CRD and CBFN). The higher doses of treatments with CBFN and DNLO/Me_2CO/CRD were also effective in reducing nematode population in 250 g soil sample and 5 g root sample at harvest.

Consequently no galling occurred (Table 9). Treatments and level of application of plant extracts was significant on days to 50% flowering as plants treated with 100 and 75% concentration of CBFN, DNLO/Me_2CO and DNLO/Hex flowered significantly earlier than plants treated with aqueous and the untreated control plants (Table 10).

Table 5. Effects of plant extracts on plant height of *Abelmoschus esculentus* infected with *Meloidogyne incognita* in the field.

Treatments	3rd WAS		5th WAS		8th WAS	
	1st Trial	2nd trial	1st Trial	2nd Trial	1st Trial	2nd Trial
DNLO/Hex/CRD	8.08^{b}	7.72^{b}	22.11^{b}	25.00^{b}	38.28^{b}	37.00^{b}
DNLO/Me_2CO/CRD	10.53^{a}	11.64^{a}	27.74^{a}	29.68^{a}	45.83^{a}	46.77^{a}
DNLO/H_2O	7.31^{bc}	7.22^{b}	15.09^{c}	18.43^{c}	30.11^{c}	29.91^{c}
CBFN	11.15^{a}	12.01^{a}	28.19^{a}	30.26^{a}	46.10^{a}	47.13^{a}
S.E.M	1.21	1.23	1.45	1.49	1.62	1.65
Treatment level (%)						
0	5.02^{c}	5.13^{d}	12.54^{d}	13.02^{d}	26.29^{d}	27.86^{d}
50	6.25^{c}	7.00^{c}	17.19^{c}	18.23^{c}	31.06^{c}	30.95^{c}
75	8.14^{b}	9.21^{b}	20.33^{b}	21.00^{b}	36.45^{b}	35.80^{b}
100	10.31^{a}	11.57^{a}	25.16^{a}	26.30^{a}	41.28^{a}	45.18^{a}
S.E.M.	1.33	1.37	1.42	1.40	1.71	1.89

Means in column followed by the same letters are not significantly different at $p< 0.05$.
WAS = Weeks after sowing.

Table 6. Effects of plant extracts on number of leaves of *Abelmoschus esculentus* infected with *Meloidogyne incognita* in the field.

Treatments	3rd WAS		5th WAS		8th WAS	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
DNLO/Hex/CRD	6.29^{ab}	6.00^{b}	15.32^{b}	17.67^{b}	30.07^{b}	32.69^{b}
DNLO/Me_2CO/CRD	7.22^{a}	8.35^{a}	19.79^{a}	22.08^{a}	34.85^{a}	36.72^{a}
DNLO/H_2O	5.18^{bc}	6.11^{b}	12.00^{c}	13.59^{c}	24.33^{c}	25.18^{c}
CBFN	7.01^{a}	8.44^{a}	20.25^{a}	22.55^{a}	35.40^{a}	37.00^{a}
S.E.M	1.35	1.41	2.01	2.36	2.49	2.77
Treatment level (%)						
0	2.04^{d}	3.38^{d}	8.00^{d}	10.02^{d}	18.00^{d}	20.19^{d}
50	4.36^{bc}	5.23^{bc}	12.32^{c}	13.59^{c}	24.11^{c}	26.02^{c}
75	5.10^{ab}	6.15^{ab}	15.41^{b}	17.18^{b}	27.21^{b}	29.41^{b}
100	6.28^{a}	7.40^{a}	18.22^{a}	20.38^{a}	31.66^{a}	33.18^{a}
S.E.M.	1.09	1.16	1.23	1.29	1.56	1.61

Means in column followed by the same letters are not significantly different at $p< 0.05$.

Table 7. Effects of of plant extracts on number of fruits per plant of *Abelmoschus esculentus* infected with *Meloidogyne incognita* in the field.

Treatments	3rd WAS		5th WAS		8th WAS	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
DNLO/Hex/CRD	0.00[a]	0.00[a]	6.24[b]	8.20[b]	11.10[b]	10.26[b]
DNLO/Me_2CO/CRD	0.00[a]	0.00[a]	9.85[a]	11.49[a]	16.32[a]	15.09[a]
DNLO/H_2O	0.00[a]	0.00[a]	3.40[c]	5.06[c]	7.15[c]	7.21[c]
CBFN	0.00[a]	0.00[a]	9.61[a]	11.21[a]	16.21[a]	15.53[a]
S.E.M.	0.00	0.00	0.35	0.46	0.61	0.69
Treatment level (%)						
0	0.00[a]	0.00[a]	1.32[d]	2.09[d]	6.25[d]	5.82[d]
50	0.00[a]	0.00[a]	4.11[c]	6.22[c]	9.14[c]	9.26[c]
75	0.00[a]	0.00[a]	6.21[b]	8.21[b]	12.09[b]	11.33[b]
100	0.00[a]	0.00[a]	8.73[a]	10.03[a]	15.31[a]	14.00[a]
S.E.M.	0.00	0.00	0.41	0.44	0.53	0.51

Means in column followed by the same letters are not significantly different at p< 0.05%.

Table 8. Effects of plant extracts on fruit weight (kg) per plant of *Abelmoschus esculentus* infected with *Meloidogyne incognita* in the field.

Treatments	3rd WAS		5th WAS		8th WAS	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
DNLO/Hex/CRD	0.00[a]	0.00[a]	0.76[b]	0.73[b]	0.85[b]	0.87[b]
DNLO/Me_2CO/CRD	0.00[a]	0.00[a]	0.86[a]	0.87[a]	0.96[a]	0.97[a]
DNLO/H_2O	0.00[a]	0.00[a]	0.51[c]	0.54[c]	0.71[c]	0.73[c]
CBFN	0.00[a]	0.00[a]	0.85[a]	0.86[a]	0.97[a]	0.98[a]
S.E.M	0.00	0.00	0.01	0.03	0.06	0.07
Treatment level (%)						
0	0.00[a]	0.00[a]	0.45[d]	0.48[d]	0.61[d]	0.63[d]
50	0.00[a]	0.00[a]	0.60[c]	0.62[c]	0.80[c]	0.82[c]
75	0.00[a]	0.00[a]	0.69[b]	0.70[b]	0.86[b]	0.87[b]
100	0.00[a]	0.00[a]	0.75[a]	0.76[a]	0.89[a]	0.91[a]
S.E.M.	0.00	0.00	0.03	0.05	0.07	0.06

Means in column followed by the same letters are not significantly different at p< 0.05.

Table 9. Effects of plant extracts on nematode populations and root gall index of *Abelmoschus esculentus* infected with *Meloidogyne incognita* in the field.

Treatments	Nematode population				Root gall index	
	250 g soil		in 5 g root			
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
DNLO/Hex/CRD	13.39[b]	17.28[b]	8.63[b]	10.61[b]	1.50[b]	1.50[b]
DNLO/Me_2CO/CRD	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
DNLO/H_2O	41.05[c]	43.25[c]	19.51[c]	22.11[c]	2.10[c]	2.10[c]
CBFN	0.00[a]	0.00[a]	0.85[a]	0.86[a]	0.97[a]	0.98[a]
S.E.M	0.15	0.19	0.13	0.14	0.00	0.00
Treatment level (%)						
0	734.29[b]	813.41[b]	463.08[b]	482.51[b]	5.00[b]	5.00[b]
50	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
75	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
100	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M.	1.28	1.46	1.04	1.07	0.00	0.00

Means in column followed by the same letters are not significantly different at $p< 0.05$.

Table 10. Effects of plant extracts on days to 50% flowering of *Abelmoschus esculentus* infected with *Meloidogyne incognita* in the field.

Treatments	Days to 50% flowering	
	1st Trial	2nd Trial
DNLO/Hex	26.14[b]	28.09[b]
DNLO/Me_2CO	21.59[a]	22.76[a]
DNLO/H_2O	32.18[c]	34.62[c]
CBFN	22.02[a]	23.11[a]
S.E.M	1.18	1.21
Treatment level (%)		
0	33.41[d]	34.29[d]
50	28.33[c]	30.08[c]
75	24.09[b]	26.39[b]
100	20.56[a]	21.72[a]
S.E.M.	1.13	1.27

Means in column followed by the same letters are not significantly different at $p< 0.05$.

Discussion

The bioactive compounds like terpenoids, flavonoids, polyphenols, saponins and glycosides present in the plant extracts are responsible for the observed nematotoxic action of the plant materials. This corroborates the reports in literature on the presence of the phytochemicals identified above in *Daniella oliveri* (Ahmadu *et al*., 2007; Adaku & Okwesili, 2008; Bylka *et al*., 2004). Many phytochemicals have reported in the management of plant parasitic nematodes (Fabiyi & Atolani, 2011; Fabiyi *et al*., 2012). The action of the chromatographic fractions indicated the presence of some organic compounds as revealed by the NMR and infrared spectroscopic result of the fractions. This however, underlies the basis for the comparatively higher toxicity of the chromatographic fractions. The infrared analysis revealed that it contains some functional groups such as O-H stretch of phenols at 3638 cm^1, C-H stretching aliphatic at 2932-2870 cm^1, C=O of anhydride at 1771 cm^1 and C=O of aromatic ester at 1570 cm^1. C-H stretching of aliphatic and C=O of aromatic ester is associated with long chain hydrocarbons and aromatics, while the O-H of phenols is an indication of the presence of

phenolic aldehydes in the chromatographic fractions. A number of phenolic compounds and aromatic acids have been stated to be nematicidal. Chlorogenic acid, caffeic acid and Trans cinnamic acid are effective in suppressing egg hatch of *M. incognita* (Mahajan *et al*., 1985). Flavonoid compounds like naringenin and rutin have also been indicated to be effective in suppressing egg hatch (Bajaj, 1999). The NMR of the chromatographic fractions showed signals for lactone that agreed (Olatunji, 1998). Lactone a cyclic ester is a white amorphous solid often employed in the treatment of microbial infections. It has been stated to be effective on *M. incognita* affecting banana in the field (Waele & Romulo, 1998). Some chemical compounds of the terpenoids family like δ-cadinene and α-copaene have been isolated from *D. oliveri* (Schwab *et al*., 2008). The nematicidal activity present in the leaves of *D. oliveri* could be attributed to the synergistic effect of all the phytohemicals and the organic compounds present in the plant.

Conclusion

The nematicidal activity of the leaves of *Daniella oliveri* is being reported for the first time in literature, the observed nematicidal activity was concentration dependent, and this is a good index for dosage application. The toxicity demonstrated by the leaves of *D. oliveri* on *Meloidogyne incognita* suggests that the plant is a promising source of bio-pesticide as an alternative to the environmentally unfriendly synthetic nematicides. *D. oliveri* has been partially characterized to contain lactone, further studies will aim at identifying other components that were not isolated in sufficient quantities.

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