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NEMATICIDAL ACTIVITIES OF CHROMATOGRAPHIC FRACTIONS FROM *ALSTONIA BOONEI* AND *BRIDELIA FERRUGINEA* ON *MELOIDOGYNE INCOGNITA*

O.A. FABIYI, G.A. OLATUNJI** AND O. ATOLANI**

Department of Crop Protection, University of Ilorin, Ilorin, Nigeria

*****Corresponding author's email: fabiyitoyinike@hotmail.com

Abstract

The leaves of *Alstonia boonei* (de Wild) and *Bridelia ferruginea* (Benth) were air dried at ambient temperature (27ºC) and subjected to successive cold extraction using n-Hexane, Ethyl acetate and Ethanol. Thirty grams from the resulting crude extracts were further subjected to open column chromatography on silica gel (100-120 mesh grades) using glass column. The chromatographic fractions were tested *in vitro* along with their crudes and carbofuran on *Meloidogyne incognita* juveniles and eggs. The fractions were significantly effective in causing juvenile mortality. 75% concentrations of fraction being more active and showed significant difference from other concentrations. Fractions from *A. boonei* were significantly ($p<0.05$) effective in the first hour of exposure to *M. incognita* juveniles with a percentage mortality of 48.62% which was not significantly different from carbofuran. At 4^{th} and 6^{th} hour of juvenile exposure to treatment, fractions from *A. boonei* were significantly ($p<0.05$) more effective than carbofuran with a percentage mortality of 67.87 and 72.57% against carbofuran with 63.77 and 69.31% mortality, respectively. Mortality increased with increase in exposure time. The fractions were also as effective as carbofuran in inhibiting egg hatch, but there was minimum inhibition among the crude extracts. Spectroscopic analyses revealed that the fractions contain organic compounds that are nematicidal in nature which include phenols, carboxylic acids, aliphatic hydrocarbons, aldehydes, anhydrides, amides, mono and di substituted aromatics and long chain carbon fatty acid esters**.**

Application of toxic chemicals as nematicidal agents has resulted in harmful effects such as environmental pollution as well as making the land infertile. No doubt they are providing hopeful results in the eradication of nematodes, but they are also toxic to important soil organisms which are responsible for soil fertility. Bio-control has been advocated as the best method to cope with losses due to synthetic chemical control. Bio-pesticides are less toxic and also reduce the pollution problems caused by the conventional pesticides. Consequently investigation was made for the leaves of *Alstonia boonei* and *Bridelia ferruginea* for an alternative nematode management strategy that are non persistent, non toxic, biodegradable and environmentally friendly.

**Chemistry Department, University of Ilorin, Ilorin, Nigeria; ***Department of Chemical Sciences, Redeemer's University Mowe, Lagos, Nigeria.

Alstonia boonei De Wild, belongs to the family Apocynaceae. It is used extensively in West Africa for the treatment of malaria, rheumatism and hypertension (Betti, 2004). *Bridelia ferruginea* Benth, belongs to the family Euphorbiaceae, and is commonly found in the savannah regions. The bark extract is used in the formulation of a traditional gargle called "Ogun efu" (Orafidiya *et al*., 1990) in the South Western part of Nigeria. The leaves of *A. boonei* and *B. ferruginea* have been reported to be insecticidal, antifungal and antibacterial (Osawe *et al*., 2007; Adebayo & Ishola, 2009). Our extensive literature review reveals that the nematicidal activity of the two plants has not been reported in literature, thus this present research was carried out to evaluate the nematicidal potential of the chromatographic fractions of the leaves of *A. boonei* and *B. ferruginea*.

Materials and Methods

Preparation of chromatographic fractions: The leaves of *Alstonia boonei* and *Bridelia ferruginea* were collected from their respective habitats and air dried at ambient temperature (27 ºC) for three months, after which they were subjected to successive cold extraction using n-Hexane (Hex), Ethyl acetate (EtOAc) and Ethanol (EtOH). The solvents were all redistilled before use. Each extraction lasted five days, after which it was decanted, filtered and evaporated using rotary evaporator under vacuum. Six plant extracts were obtained coded ALSB/Hex, ALSB/EtOAc, ALSB/EtOH from *A. boone*i and BRDF/Hex, BRDF/EtOAc, BRDF/EtOH from *B. ferruginea*. 30 g of each plant extract was subjected to open column chromatography on silica gel 100-120 mesh grade using a glass column. The initial mobile phase, petroleum ether was allowed to flow at an appropriate steady rate of 1.5 ml per minute and eluted fractions were collected at 50 ml per fraction. As the chromatography progressed, the polarity of the eluting solvent was increased using the mixture of dichloromethane, ethyl acetate and methanol as applicable to each extract from the result of their thin layer chromatography (TLC) test. The collected fractions were evaporated using rotary evaporator under vacuum.

Extraction of juveniles: Root-knot nematode *Meloidogyne incognita* which was identified on the basis of perineal pattern (Eisenback *et al*., 1981) was maintained on *Celosia argentea* and the second stage juveniles (J_2) were extracted from the root using the Whitehead & Hemming (1965) tray method.

Extraction of eggs-masses: Egg-masses collected from *Celosia argentea* roots were vigorously shaken with 500 ml of 0.8% Sodium hypochlorite in stopper flasks for 2 min (Hussey & Barker, 1973), so as to digest 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 measurements: The Ultra violet visible (UV) of the resulting chromatographic fractions were taken on Aquamate UV-visible spectrophotometer V_4. 60 at the Chemistry Department, University of Ilorin. The infra-red spectra were recorded on SHIMADZU 8400_S FTIR spectrophotometer at the Department of Chemical Sciences Redeemer's University Mowe, Lagos, Nigeria, while Gas Chromatography Mass Spectroscopy (GC/MS) of the fractions was done at NACRIT Zaria, Nigeria.

Juvenile mortality and egg hatchability assessment: Three groups of chromatographic fractions from each extract were taken according to polarity and weight, they were designated fraction A, B and C, thus making (18) eighteen chromatographic isolates, plus their crudes and reference standard, carbofuran. These gave a total of twenty five (25) treatments for each assessment. The experimental design for each assessment was a 25 x 4 x 3 factorial experiment fitted into Completely Randomised Design (RCD), involving twenty five treatments at four levels and each replicated three times. 10 mg of each group of chromatographic fractions was dissolved in 40 ml distilled water, this forms 100% concentration from which serial dilutions were made into 25, 50 and 75% concentration, while distilled water served as 0%. The same dilutions were used for the crude extracts and carbofuran. 450 freshly hatched juveniles of *M. incognita* were used in each Petri dish, and also 450 eggs were used for the hatchability test. The Petri dishes were incubated at room temperature. Counting was done under the stereo microscope. The juveniles which did not respond to the touch of fine needle were considered dead.

Statistical Analysis: All data collected were subjected to analysis of variance (ANOVA) and significant means separated with the Duncan's multiple range test (Gomez & Gomez, 1984).

Results

Juvenile mortality: Table 1 shows the effect of chromatographic fractions and carbofuran on the mortality of *Meloidogyne incognita* juveniles over time. The chromatographic fractions were significantly ($p<0.05$) effective in causing juvenile mortality, in 1st and 2nd hour of juvenile exposure to treatment. There was no significant difference between carbofuran and ALSB/EtOHc, they both recorded the highest percentage mortality among all the treatments, while no mortality was recorded for any of the crude extracts. It was observed that ALSB/EtOHc was significantly ($p<0.05$) better than carbofuran after 4th and 6th hour of juveniles exposure to treatment, with a percentage mortality of 67.87 and 72.57% as against carbofuran with 63.77 and 69.31% mortality, respectively. At day one carbofuran and ALSB/EtOH fractions had 100% mortality, while ALSB/EtOAcC had 92.17% mortality. The highest percentage mortality recorded among *B. ferruginea* fractions was 83.7% after a day. On the 4th day all fractions had 100 % mortality except three extracts in which Hexane (Hex) was used as solvent. Among the crude extracts ALSB/EtOH/CRD was significantly

Table 1. The effect of different concentrations of chromatographic isolates and carbofuran on percentage mortality of *Meloidogyne incognita* juveniles.

Treatments	Exposure time								
	1hr	2hrs	4hrs	6hrs	8hrs	Day 1	Day 2	Day 3	Day 4
ALSB/Hex $_A$	31.35^{f}	36.59^{g}	48.06^{g}	51.53^{g}	60.16^{f}	80.08^{fg}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/Hex $_B$	31.27^{f}	36.73^{g}	48.33^{g}	51.41^{g}	60.38^{f}	81.36^{f}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/Hex $_C$	31.39^{f}	37.00^{g}	50.27^{f}	53.22^{f}	63.49^{e}	83.13^{e}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/Hex/CRD	0.00^{j}	0.00^{k}	0.00^{p}	8.03^{lm}	9.39^{m}	19.69^{o}	27.40^{j}	38.56^{g}	55.50^{f}
ALSB/EtOAc $_A$	37.14^{e}	44.07^{e}	49.00^{fg}	56.01^{e}	68.52^{d}	85.00^{d}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/EtOAc $_B$	39.12^{d}	47.16^{d}	52.18^{e}	59.21^{d}	68.21^{d}	88.12^{c}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/EtOAc $_C$	42.26^{c}	49.28^{c}	56.36^{c}	62.51^{c}	71.01^{c}	92.17^{b}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/EtOAc/CRD	0.00^{j}	0.00^{k}	8.97^{o}	9.89^{l}	10 .90^{l}	28.41^{m}	36.76^{h}	48.20^{e}	59.35^{e}
ALSB/EtOH $_A$	45.27^{b}	47.40^{d}	54.49^{d}	72.22^{a}	77.00^{b}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/ EtOH $_B$	48.10^{a}	56.07^{b}	64.29^{b}	72.10^{a}	79.35^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/ EtOH $_C$	48.62^{a}	58.76^{a}	67.87^{a}	72.57^{a}	79.51^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
ALSB/ EtOH/CRD	0.00^{j}	0.00^{k}	19.53^{n}	25.27^{k}	32.67^{k}	45.51^{l}	53.31^{f}	64.37^{c}	72.10^{c}
BRDF/Hex $_A$	21.15^{i}	26.74^{j}	36.53^{m}	43.47^{j}	51.24^{ij}	68.77^{k}	76.06^{e}	85.71^{b}	91.51^{b}
BRDF/Hex $_B$	21.02^{i}	27.38^{j}	36.29^{m}	43.15^{j}	51.51^{ij}	69.11^{k}	76.10^{e}	85.89^{b}	91.63^{b}
BRDF/Hex $_C$	21.04^{i}	27.41^{j}	39.23^{kl}	45.63^{i}	54.30^{h}	69.24^{k}	79.21^{d}	86.00^{b}	92.00^{b}
BRDF/Hex/CRD	0.00^{j}	0.00^{k}	0.00^{p}	0.00^{n}	8.10^{mn}	19.29^{o}	24.78^{k}	32.74^{h}	49.24^{h}
BRDF/EtOAc $_A$	23.79^{h}	30.13^{i}	40.05^{k}	46.02^{i}	52.20^{i}	71.12^{j}	79.34^{d}	100.00^{a}	100.00^{a}
BRDF/EtOAc $_B$	23.54^{h}	30.20^{i}	40.16^{k}	46.10^{i}	53.02^{hi}	71.09^{j}	83.24^{c}	100.00^{a}	100.00^{a}
BRDF/EtOAc $_C$	24.00^{h}	33.00^{h}	42.12^{j}	51.68^{g}	56.18^{g}	73.69^{i}	85.52^{b}	100.00^{a}	100.00^{a}

BRDF/EtOAc/CRD	0.00^{j}	0.00^{k}	0.00^{p}	8.65^{lm}	10.48^{l}	25.09^{n}	33.01^{i}	43.51^{f}	53.98^{g}
BRDF/EtOH $_{A}$	27.65^{g}	35.09^{g}	44.91^{i}	49.29^{h}	56.29^{g}	78.17^{h}	100.00^{a}	100.00^{a}	100.00^{a}
BRDF/EtOH $_{B}$	27.58^{g}	35.36^{g}	45.09^{i}	51.13^{g}	56.00^{g}	78.20^{h}	100.00^{a}	100.00^{a}	100.00^{a}
BRDF/EtOH $_{C}$	28.00^{g}	39.12^{f}	47.26^{gh}	56.29^{e}	63.63^{e}	83.70^{e}	100.00^{a}	100.00^{a}	100.00^{a}
BRDF/EtOH/CRD	0.00^{j}	0.00^{k}	0.00^{p}	9.62^{l}	11.44^{l}	27.80^{m}	40.26^{g}	51.93^{d}	63.20^{d}
CBFN	48.89^{a}	58.95^{a}	63.77^{b}	69.31^{b}	78.79^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
S.E.M	2.21	2.54	2.76	3.12	3.43	3.65	3.92	4.16	4.01

Treatment level (%)	**1 hr**	**2hrs**	**4hrs**	**6hrs**	**8hrs**	**Day 1**	**Day 2**	**Day 3**	**Day 4**
0	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{b}	3.17^{b}
25	20.15^{c}	31.80^{c}	40.79^{c}	48.68^{c}	61.13^{c}	84.28^{c}	93.12^{c}	100.00^{a}	100.00^{a}
50	22.27^{b}	34.40^{b}	44.59^{b}	53.85^{b}	66.96^{b}	88.49^{b}	97.05^{b}	100.00^{a}	100.00^{a}
75	28.57^{a}	37.24^{a}	47.89^{a}	57.31^{a}	72.55^{a}	95.87^{a}	100.00^{a}	100.00^{a}	100.00^{a}
S.E.M	0.17	0.19	0.54	0.63	1.47	1.62	1.95	1.61	0.62

Each value is a mean of three replicates. Values with different alphabets along the same column are statistically different at $p < 0.05$

($p<0.05$) better with a percentage mortality of 72.1%, while BRDF/Hex/CRD was significantly low with a percentage mortality of 49.24%. Generally mortality increased with increase in exposure time of fractions to juveniles, but *A. boonei* fractions were significantly more effective than *B. ferruginea* fractions. The effect of level of concentration of fractions was also significant throughout the time of exposure, there was increase in percentage mortality with increase in concentration of fractions.

Egg hatchability: Table 2 depicts the effect of chromatographic fractions and carbofuran on percentage of egg hatching of *Meloidogyne incognita*. The fractions were significantly ($p<0.05$) effective in inhibiting egg hatch, as there was no egg hatch recorded in the fractions throughout the period of observation. Egg hatch was significantly ($p<0.05$) low in all the crude extracts than in the control. ALSB/EtOH/CRD (*Alstonia boonei* ethanol crude) was significantly more effective than other crude extracts with a cumulative egg hatch of 0.64% at the end of the experiment, while BRDF/Hex/CRD (*Bridelia ferruginea* hexane crude) had the highest cumulative egg hatch of 4.29%. Egg hatch was significantly low with increase in concentration of fractions. The 0% (control) had 83.21% hatches on day 8, while 75% concentration had 0.27%.

Spectroscopic results: The UV of fractions from *A. boonei* in dichloromethane (DCM) reflects the presence of indole and methoxy-indoles, while that of *B. ferruginea* also in DCM shows the presence of flavonoids, esters and ketones. The infra-red analysis depicts that the fractions from *A. boonei* and *B. ferruginea* contains aliphatic and aldehyde C-H stretching functional groups at 2956-2850 cm^{-1} which is associated with long chain hydrocarbons, alicyclics and aromatics, and carbonyl stretching frequencies of lactones, ketones and esters at 1735, 1730, 1725 and 1717 cm^{-1}. The presence of indole alkaloids in the chromatographic fractions of *A. boonei* was complemented by the N-H stretching frequencies in some of the fractions from *A. boonei*, this is associated with primary and secondary amines as well as amides. The GC/MS spectra gave an insight into the organic compounds present in the chromatographic fractions. The fractions from *A. boonei* confirmed specific compounds such as friedlan-3-one, hexadecanoic acid, methyl ester, 11-octadecanoic acid, methyl ester, heptadecyl ester, gamma sitosterol, pentadecanal, ethyl tetracosanoate, tricosanoic acid, methyl ester, 2-pentadecanone-6,10,14,-trimethyl, octadecane. The results of fractions from *B. ferruginea* revealed the presence of alpha-amyrin acetate, lupeol, lup-20(29)-en-3-one, beta-amyrin, 1-cyclohexene-1-butanol, 2,6,6-trimethyl, 14b-octadecahydro-2-H-picen-3-one, delta (sup^{9})-cis-oleic acid

Table 2. The effect of different concentrations of chromatographic isolates and carbofuran on percentage egg hatch of *Meloidogyne incognita* eggs.

Treatments	Exposure time							
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
ALSB/Hex $_A$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/Hex $_B$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/Hex $_C$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/Hex/CRD	0.00 ^{a}	0.00 ^{a}	0.08 ^{a}	0.23 ^{a}	0.78 ^{b}	1.02 ^{c}	1.78 ^{d}	2.03
ALSB/EtOAc $_A$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/EtOAc $_B$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/EtOAc $_C$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/EtOAc/CRD	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.06 ^{a}	0.54 ^{b}	0.98 ^{b}	1.31 ^{c}
ALSB/EtOH $_A$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/ EtOH $_B$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/ EtOH $_C$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
ALSB/ EtOH/CRD	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.07 ^{a}	0.64 ^{b}
BRDF/Hex $_A$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
BRDF/Hex $_B$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
BRDF/Hex $_C$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
BRDF/Hex/CRD	0.47 ^{a}	0.89 ^{b}	1.13 ^{b}	1.62 ^{c}	2.07 ^{d}	3.02 ^{d}	3.86 ^{e}	4.29 ^{e}
BRDF/EtOAc $_A$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
BRDF/EtOAc $_B$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
BRDF/EtOAc $_C$	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
BRDF/EtOAc/CRD	0.00 ^{a}	0.00 ^{a}	0.19 ^{a}	0.73 ^{b}	1.01 ^{c}	1.27 ^{c}	1.53 ^{c}	1.79 ^{c}

BRDF/EtOH $_A$	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
BRDF/EtOH $_B$	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
BRDF/EtOH $_C$	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
BRDF/EtOH/CRD	000 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.11 [a]	0.49 [a]	1.01 [b]	1.22 [c]
CBFN	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
S.E.M	0.08	0.37	0.64	0.87	1.22	1.34	1.92	1.64

Treatment level (%)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
0	11.90 [b]	17.05 [c]	37.90 [c]	41.77 [d]	48.67 [d]	62.69 [d]	74.94 [d]	83.21 [d]
25	0.03 [a]	0.08 [b]	0.22 [b]	0.28 [c]	0.34 [c]	0.39 [c]	0.52 [c]	0.63 [c]
50	0.03 [a]	0.04 [a]	0.08 [a]	0.18 [b]	0.22 [b]	0.26 [b]	0.36 [b]	0.45 [b]
75	0.01 [a]	0.02 [a]	0.05 [a]	0.06 [a]	0.09 [a]	0.12 [a]	0.18 [a]	0.27 [a]
S.E.M	0.14	0.18	0.27	0.36	0.52	0.99	1.13	0.25

Each value is a mean of three replicates. Values with different alphabets along the same column are statistically different at $p < 0.05$

Discussion

The presence of triterpenes, pentacyclic triterpenes and steroids in the extract of *A. boonei* was reported by Faparusi & Bassir (1982) and validated by Kweifo-Okai & Carroll (1992), who confirmed the presence of lupeol palmitate, lupeol linoleate, palmitic and linoleic acid esters in the petroleum ether extract of the leaves of *A. boonei*. However, in this research, friedlan-3-one a triterpene and some fatty acid esters were confirmed in the hexane extract of *A. boonei* leaves through GC/MS. Boonyaratavej *et al.*, (1990), isolated lupeol, beta amyrin and beta sitosterol from the petroleum spirit extract of *B. ferruginea*, while in this study, lupeol, lup-20 (29)-en-3-one, beta-amyrine and alpha-amyrine acetate were identified in the hexane extract. A variety of fatty acid esters have been used to control nematodes *in vitro* and *in vivo*. A mixture of sodium lauryl sulphate and citric acid immobilised some nematodes, this mixture also reduced nematode growth significantly when applied at planting. Some fatty acids that can be in the epoxide, cyclopropane, methylated or hydroxylated forms have also been confirmed to be toxic to nematodes (Pinkerton & Kitner, 2006). Vrain (1980) confirmed that the toxicity of fatty acids on second stage juveniles of *Meloidogyne hapla* increased with increase in carbon number C_3 to C_{11}.The presence of fatty acids and their esters in the chromatographic fractions of *A. boonei* and *B. ferruginea* explain the toxicity of the fractions to *M. incognita*, and why they compared favourably well with carbofuran. The fatty acid esters observed in this study contain long chain carbon numbers, and it has been established that toxicity increase with carbon chain length.

Conclusion

Many researchers have investigated *A. boonei* and *B. ferruginea* root, bark, and leaf extracts, and a number of organic compounds have been isolated and characterised with various positive results as being antibacterial, antifungal, insecticidal, acaricidal, mollucidal and anti-HIV. However, this is the first time the nematicidal activity of the two test plants is being reported. Therefore, they are also nematicidal materials with potential for commercial application in view of the environmental pollution caused by synthetic nematicides. The future looks bright for developing bio-pesticides through fractionation.

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