NEMATICIDAL ACTIVITY OF *ALSTONIA BOONEI* AND *BRIDELIA FERRUGINEA* LEAVES

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Abstract

Chemical environmental hazards warrant researches on green chemistry. Screen house and field studies were conducted to investigate the natural nematicidal potential of *Alstonia boonei* and *Bridelia ferruginea* leaves. The leaves were extracted successively using n- hexane, ethyl acetate and ethanol; the crude extracts were tested on *Meloidogyne incognita* that infects *Corchorus olitorius* in a 9 x 4 x 3 factorial experiment. In the Screen house and field, *Alstonia boonei* ethanol extracts (ALSB/EtOH) caused a significant ($p<0.05$) increase in plant height and number of leaves which compared significantly with that of reference standard carbofuran, whereas *Alstonia boonei* ethyl acetate extract (ALSB/EtOAc) and *Bridelia ferruginea* ethyl acetate extract (BRDF/EtOAc) influenced the production of higher number of branches which was similar to that of carbofuran. Nematodes were significantly ($p<0.05$) absent in the soil and root of plants that received the highest concentrations (75% and 50%) of ALSB/EtOH, ALSB/EtOAc and *Bridelia ferruginea* ethanol extracts. *Corchorus olitorius* plants that received carbofuran and ALSB/EtOH flowered significantly earlier than plants treated with aqueous extracts of *Alstonia boonei* and *Bridelia ferruginea*, which also produced significantly ($p<0.05$) lower plant height, fewer leaves, fewer branches and had nematodes in their root and soil at harvest. Phytochemical screening of plant extracts revealed the presence of alkaloids, flavonoids, anthraquinones, tannins and saponins. The extracts of the leaves of *A. boonei* and *B. ferruginea* were nematicidal and appear to hold promise as natural, biodegradable and environmentally friendly alternative crop protectants against *Meloidogyne incognita*.

Key words: *Alstonia boonei*, *Bridelia ferruginea*, *Meloidogyne incognita*, *Corchorus olitorius*, nematodes, phytochemical screening.

1. Introduction

Root-knot nematodes (*Meloidogyne spp*) are an important pest of vegetables in the tropics [1]. *Corchorus olitorius*, an important vegetable in the south western Nigeria is highly susceptible to root-knot nematode attack. Many different species are known to cause damage, but the most destructive nematode responsible for enormous yield loss in *C. olitorius* is the root-knot nematode [2]. The damage inflicted by this group of nematodes is characterized by numerous and pronounced swellings or galls on the roots of susceptible host plants. Several control measures have been explored to control root-knot nematodes, including the use of chemical nematicides. Synthetic chemicals constitute the world's most effective nematode control strategy, which has provided nematode control for more than ten decades [3]. A prominent chemical used in nematode control is carbofuran (*2, 2-dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate*) sold under different trade names, which is toxic and not environmentally friendly. Carbofuran has been banned in the United State for use on food crops due to its reported toxicity. However, its legal use is still permitted in many other countries US-EPA [4]. Carbofuran has been reported to be toxic to birds, higher animals and humans, as it is a powerful endocrine disruptor capable of causing transient alterations in the concentration of many hormones in animals and humans, leading to serious reproductive problems even at extremely low concentrations [5, 6]. It has a sub-lethal toxicity to animal testes [7, 8] among other side effects. Because of its high water solubility (351 ppm at 25 °C) and low adsorption coefficient, carbofuran is relatively mobile in soil and in surface runoff. Consequently, carbofuran has the potential to contaminate lakes, streams, and groundwater [9]. In animal studies, carbofuran is presumed to be metabolized via oxidation to 3-hydroxycarbofuran and then to 3-ketocarbofuran (Fig. 1) [10]. However, it undergoes hydrolysis under alkaline conditions. Other metabolites obtained in animal studies include *carbofuranphenol, 3-hydrocarbofuran-7-phenol, N-*

hydroxymethyl carbofuran, 3-ketocarbofuran, and 3-ketocarbofuran phenol [11].These compounds become conjugated and excreted in the urine and bile.

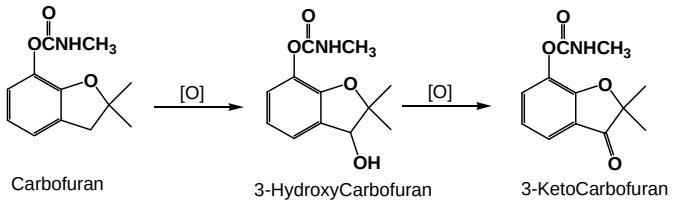

Figure 1: Metabolism of carbofuran

Moreover, nematicides are generally expensive and hazardous because of the residual effect on crop, soil and non-target organisms. Thus, a search for an alternative control for plant parasitic nematodes becomes an imperative. Such an alternative control measure involves the use of bio-pesticides. Efforts have been made to study the nematicidal potential of some plant extracts on plant parasitic nematodes. In an experiment on the integrated management of root-knot nematode *M. javanica* infecting tomato using amendments of oil cakes, bone and horn meals, it was observed that there was increased growth in tomato and reduced nematode multiplication [12]. *Tagetes tennifolia*, *Tagetes patula* and *Tagetes erecta* were found to reduce significantly the population of *Pratylenchus penetrans* on potato [13]. High doses of three organic nematicides were observed to have maximum reduction in *M. incognita* populations [14]. However, there are still several natural materials whose potential remain untested and untapped. *Bridelia ferruginea* is used as a mouthwash for the treatment of furred tongue, while *Alstonia boonei* is mainly employed in the treatment of malaria [15]. Tannins and phenolics have been reported in a preliminary phytochemical investigation of the bark of *B. ferruginea* [16], however, the nematicidal potential of *A. boonei and B. ferruginea* leaves has not been investigated or reported in literature. This research was therefore conducted to determine the phytochemical components present in the leaves of *Alstonia boonei* and *Bridelia ferruginea* and to compare the efficacy of different solvent extracts of the test plants and carbofuran on *Corchorus olitorius* infected with *Meloidogyne incognita* in the screen house and field, thereby identifying a natural nematode control with potential to substitute for the synthetic carbofuran.

2. Materials and methods

2.1 Sample and Sample Preparation

The two test plants, viz., *Alstonia boonei* and *Bridelia ferruginea*, were obtained from their natural habitats. *A. boonei* was collected from Fiditi village in Oyo state area of Nigeria, whereas *B. ferruginea* was obtained from Bode Saadu village in Kwara State, Nigeria and authenticated by a taxonomist in the Department of Botany of the University of Ilorin, Ilorin, Nigeria. The leaves were air dried at ambient temperature (27^0C), after which they were pulverized using a laboratory mill (Christy and Norris Ltd type 8). From the resulting powder, 1000g each was packed in a 10-liter aspirator for successive and exhaustive cold extraction using redistilled n-hexane (Hex), ethyl acetate (EtOAc) and ethanol (EtOH). Each extraction lasted 5 days, after which it was decanted, filtered and evaporated using a rotary evaporator under vacuum to get concentrated extracts. Six plant extracts were obtained and coded ALSB/Hex, ALSB/EtOAc, and ALSB/EtOH from *Alstonia boonei* and BRDF/Hex, BRDF/EtOAc, and BRDF/EtOH from *Bridelia ferruginea*.

2.2 Aqueous Extraction

Five-hundred grams of each plant material was soaked in a liter of water for five days, after which it was decanted, filtered and allowed to evaporate to semi-solid substances. These substances were coded ALSB/H_2O and BRDF/H_2O for *Alstonia boonei* and *Bridelia ferruginea*, respectively.

2.3 Phytochemical tests

Phytochemical screening was carried out using the concentrated extracts according to standard methods [17, 18]. Minor modifications were made to such procedures.

2.4 Carbofuran Application

Carbofuran 3G, obtained from a chemical store in Ilorin, Nigeria, was weighed out at rates equivalent to 1.0, 1.5 and 2.0 kg a.i/ha for the screen house and field experiments. The screen house received 0.25 g, 0.37 g and 0.49 g, respectively, whereas the field received 20 g, 30 g and 40 g, respectively. The control did not receive any carbofuran application in the screen house and field trials.

2.5 Screen house Experiment

A screen house measuring 14.6 m by 4.5 m was constructed. One hundred and eight plastic containers of 11 liter capacity each, already filled with 15 kg soil (readily sieved and pasteurized for four hours at 60 ^{0}C) according to standard method were arranged in the screen house in a 9 x 4 x 3 factorial experiment that used a randomized complete-block design (RCBD) [19]. The plastic buckets were placed on bricks to avoid microbial infestation in the course of the experiment. Both the first and the second trials of the experiment, which were conducted between June and August 2010 and 2011, had the same design and arrangement in the screen house. Thirty grams of each plant extract was dissolved in 100 ml of water, (i.e., 0.3 g/ml) to form the stock and thereafter various dilutions were made from it. The dosage of application was 0%, 25%, 50% and 75% for all crude extracts. The aqueous extract had the similar dilution, whereas carbofuran was applied in the solid form.

2.6 Field Experiment

Two trials were conducted at the University of Ilorin Teaching and Research farm in two consecutive years between July and September in both 2010 and 2011. The experimental design was also a 9 x 4 x 3 factorial RCBD, which consisted of nine treatments at four levels, each replicated three times. A portion of land measuring 40 m by 30 m was ploughed and harrowed. This was divided into four plots of 19.25 m by 14.5 m each with an alley way of 0.5 m in between them. Each of the four plots was subdivided into 27 beds of 1.5 m by 4 m (6.0 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, whereas 30 cm was used in the row between each plant stand [20].

2.7 Planting Operations

In both years, seeds were sown at a depth of 0.3 cm in small holes made in the centre of the sterilized soil in the experimental pots in the screen house and covered with soil, whereas the same was done at a specified spacing on each bed in the field. The plants were watered daily in the screen house and kept free of weeds by hand weeding in the field. Germination of seeds started on the fourth day after planting, and the plants were thinned down to one plant per pot in the screen house and one plant per stand in the field on the 10th day after germination.

2.8 Nematode Inoculation and Treatment Application

After the 14th day of planting, 450 juveniles of *Meloidogyne incognita* were inoculated close to the roots of *Corchorus olitorius* plants in each of the 108 plastic pots, and the roots of each plant in the field. Seven days after inoculation, the plants close to the root were treated with extracts of *Alstonia boonei* and *Bridelia ferruginea* at various rates in banded form at 75, 50, 25, and 0 % both in the screen house and field trials. Carbofuran 3G was applied in the solid form at rates equivalent to 1.0, 1.5, and 2.0 kg a.i/ha in the screen house and field experiments. This was done for both the first and second trials.

2.9 Data Collection and Statistical Analysis

Data were collected from the experimental pots and the field on the following parameters: Plant height, number of leaves, and number of branches (all on weekly basis). Days to 50% flowering was recorded; shoot weight, root weight, nematode population in 250g soil sample, nematode population in 5g root sample and root gall rating were assessed in the laboratory after harvest. 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 [21].

3. Results and discussion

Carbofuran and *Alstonia boonei* ethanol extract were more effective than the other treatment materials, as they produced plants with significantly ($p<0.05$) higher height from the 6th week after planting (WAP) to the end of the experiment at the 14th week (Figure 2), and this was closely followed by plants treated with *Alstonia boonei* ethyl acetate extract. A similar result was obtained in the field (Tables 1 and 6). Plants that received the aqueous extracts of the two test plants (ALSB/H_2O and BRDF/H_2O) had significantly lower plant heights throughout the period of study (Tables 1 and 6). Height of plants treated with 75 and 50% concentration were significantly ($p<0.05$) better than those treated with 25% concentration of the plant materials. All treatments significantly increased the number of leaves compared with the untreated control plants (0%), but plants treated with ALSB/EtOAc, ALSB/EtOH and CBFN had significantly more leaves compared with other treatments (Tables 2 and 7).

Figure 2: Left - Nematode infected *Corchorus olitorius* plants without treatment in the field. Middle - Nematode infected *Corchorus olitorius* plants treated with plant extracts in the field. Right - Comparative plant heights of *Corchorus olitorius* in the screen house. A: Treated with ALSB/EtOH, B: Treated with ALSB/H2O; C: Treated with BRDF/H2O; D: Treated with BRDF/EtOH

There was an increase in the number of branches among plants treated with CBFN, ALSB/EtOAc and BRDF/EtOAc (Tables 3 and 8), whereas branching was significantly less in plants that received other forms of treatment. Treatment and level of application of plant materials were significant for days to 50% flowering of *Corchorus olitorius* plants, as plants that received CBFN and ALSB/EtOH flowered significantly earlier in the screen house and field trials (Tables 4 and 9). Shoot and root weights were also significantly ($p<0.05$) heavier in plants treated with the highest (75 and 50%) concentrations of CBFN and ALSB/EtOH (Tables 4 and 9); the higher doses of treatments with CBFN, ALSB/EtOH, ALSB/EtOAc and BRDF/EtOH were also effective as there were no nematodes in 250g soil sample and 5g root sample at harvest, and consequently no galling occurred (Tables 5 and 10). The bioactive compounds (alkaloids, anthraquinone, flavonoids, glucosides, tannin and saponin) present in the test plants (Table 11) could be the reason for their efficacy in controlling *Meloidogyne incognita* in *Corchorus olitorius*. The observed nematicidal effect corroborates the traditional uses of the two test plants (Ndukwe *et al.*, 2007). Some chemical compounds of the indole alkaloid group (alstonine, astonidine and porphine) and triterpenoids have been identified in *A. boonei* [22]. Deverall [23] reported the pesticidal astringency and phyto-repellant nature of flavonoids and other bioactive compounds present in plants. Fabiyi and Atolani[24] reported the efficacy of the aqueous and powdered extracts of *Lawsonia inermis* on *Meloidogyne spp.* infecting *Corchorus olitorius*, they found out that there was increase in vegetative growth of *Corchorus olitorius* plants treated with 15% aqueous concentration, and nematode population in root and soil at harvest was significantly reduced. Apart from the nematicidal properties of the plant materials, the plant extracts must have also added to the nutrient status of the soil, thus enhancing crop growth and yield. Gautam and Goswami [25] reported improved yield in cowpea when neem cake was used in the treatment of *Meloidogyne incognita*. The fractionation of the active principles in the two test plants will help identify the compounds responsible for the nematicidal activity observed in this study. Thus, the leaves of *A.boonei* and *B. ferruginea* contain substances that are nematicidal, and can be used as bio-pesticides against *Meloidogyne incognita*.

Table 1**:** The main effect of treatment and level of application of plant extracts and carbofuran on PLANT HEIGHT (cm) of *Corchorus olitorius* infected with *Meloidogyne incognita*. (Screen house)

Treatments	3 WAP		6WAP		10WAP		14WAP	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
ALSB/Hex	9.15^{c}	9.59^{cd}	40.26^{e}	47.00^{e}	87.16^{e}	97.00^{e}	129.31^{e}	144.18^{e}
ALSB/EtOAc	11.53^{ab}	12.09^{b}	51.38^{b}	57.61^{b}	103.11^{b}	111.28^{b}	144.71^{b}	154.11^{b}
ALSB/EtOH	12.14^{a}	14.23^{a}	56.01^{a}	61.30^{a}	108.70^{a}	115.40^{a}	149.22^{a}	158.03^{a}
ALSB/H_2O	7.09^{d}	8.29^{d}	33.15^{g}	43.25^{f}	77.10^{g}	90.07^{g}	121.18^{g}	136.81^{g}
BRDF/Hex	8.01^{cd}	8.73^{d}	37.12^{f}	46.63^{e}	81.35^{f}	93.65^{f}	124.11^{f}	140.16^{f}
BRDF/EtOAc	11.33^{ab}	9.62^{cd}	44.19^{d}	50.02^{d}	93.49^{d}	100.23^{d}	134.02^{d}	147.26^{d}

BRDF/EtOH	11.14[ab]	10.59[c]	47.82[c]	54.19[c]	99.64[c]	106.19[c]	139.25[c]	151.32[c]
BRDF/H_2O	6.31[e]	6.43[e]	28.21[h]	40.18[g]	73.04[h]	86.31[h]	116.46[h]	132.00[h]
CBFN	12.19[a]	14.19[a]	56.75[a]	61.00[a]	109.61[a]	115.69[a]	149.68[a]	158.71[a]
S.E.M	0.27	0.31	1.92	1.79	2.32	2.49	3.22	3.35
LEVEL (%)								
0	1.94[d]	3.15[d]	15.29[d]	22.15[d]	56.71[d]	68.05[d]	91.19[d]	98.21[d]
25	4.23[c]	6.74[c]	27.41[c]	30.53[c]	69.88[c]	75.40[c]	104.69[c]	107.38[c]
50	8.61[b]	10.32[b]	32.12[b]	37.28[b]	74.12[b]	82.11[b]	108.05[b]	113.51[b]
75	11.10[a]	13.21[a]	39.26[a]	42.19[a]	80.55[a]	87.09[a]	115.12[a]	121.67[a]
S.E.M	0.11	0.28	1.19	0.82	2.15	1.93	2.88	2.91

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the new Duncan's multiple range test.

Table 2: The main effect of treatments and level of application of plant extracts and Carbofuran on PLANT HEIGHT (cm) of *Corchorus olitorius* infected with *Meloidogyne incognita* (Field Experiment).

Treatments	3WAP		6WAP		10 WAP		14WAP	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
ALSB/Hex	10.38 [c]	13.21 [d]	25.83 [e]	34.72 [e]	60.71 [e]	63.11 [e]	90.05 [e]	93.03 [e]
ALSB/EtOAc	12.51 [ab]	17.04 [b]	37.39 [b]	50.16 [b]	72.50 [b]	81.19 [b]	107.11 [b]	119.63 [b]
ALSB/EtOH	13.48 [a]	18.39 [a]	40.77 [a]	53.82 [a]	77.23 [a]	86.46 [a]	112.61 [a]	126.81 [a]
ALSB/H_2O	8.19 [d]	9.40 [f]	23.17 [f]	26.00 [g]	50.00 [g]	54.00 [g]	79.40 [g]	80.18 [g]
BRDF/Hex	9.26 [c]	11.08 [e]	23.46 [f]	29.65[f]	56.80 [f]	59.35 [f]	84.00 [f]	87.72 [f]
BRDF/EtOAc	12.00 [b]	13.27 [d]	28.07 [d]	39.28 [d]	64.29 [d]	70.41 [d]	95.07 [d]	102.07 [d]
BRDF/EtOH	12.40 [b]	15.33 [c]	30.12 [c]	46.23 [c]	69.38 [c]	77.00 [c]	101.26 [c]	111.61 [c]
BRDF/H_2O	7.08 [d]	8.51 [f]	21.31 [g]	23.45 [h]	48.61 [h]	50.08 [h]	71.89 [h]	74.05 [h]
CBFN	13.69 [a]	18.14 [a]	41.36 [a]	54.26 [a]	78.21 [a]	86.74 [a]	113.40 [a]	127.00 [a]
S.E.M	0.39	0.26	1.27	1.16	1.81	2.13	1.49	1.83
Level (%)								
0	2.30 [d]	2.00 [d]	10.73 [d]	14.45 [d]	34.29 [d]	41.11 [d]	65.18 [d]	70.00 [d]
25	5.61 [c]	5.80 [c]	17.10 [c]	24.05 [c]	49.31 [c]	62.46 [c]	83.77 [c]	87.53 [c]
50	9.22 [b]	10.16 [b]	28.36 [b]	28.53 [b]	60.00 [b]	68.49 [b]	91.23 [b]	95.13 [b]
75	12.03 [a]	14.68 [a]	36.41 [a]	36.10 [a]	68.19 [a]	78.15 [a]	99.15 [a]	106.16 [a]
S.E.M	0.46	0.13	1.55	0.51	2.22	1.45	1.78	2.18

Means in a segment of a given column followed by the same letter are not significantly different at $P<0.05$ using the new Duncan's multiple range test.

Table: 3 The main effect of treatment and level of application of plant extracts and carbofuran on NUMBER OF LEAVES of *Corchorus olitorius* infected with *Meloidogyne incognita*. (Screen house)

Treatments	3 WAP		6WAP		10WAP		14WAP	
	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial
ALSB/Hex	10.15[d]	10.08[d]	51.19[d]	56.82[d]	120.23[d]	130.00[d]	163.37[d]	167.23[d]
ALSB/EtOAc	17.00[a]	18.73[a]	60.72[a]	69.28[a]	134.60[a]	139.75[a]	175.13[a]	179.36[a]
ALSB/EtOH	17.23[a]	18.69[a]	61.38[a]	69.33[a]	134.96[a]	140.19[a]	175.00[a]	179.41[a]
ALSB/H_2O	9.26[de]	9.03[de]	42.65[f]	47.35[f]	112.08[f]	122.11[f]	156.25[f]	161.26[f]
BRDF/Hex	10.06[d]	10.16[d]	47.22[e]	52.11[e]	116.13[e]	126.51[e]	160.24[e]	164.11[e]
BRDF/EtOAc	12.31[c]	13.32[c]	54.15[c]	61.29[c]	126.11[c]	133.16[c]	167.31[c]	171.40[c]
BRDF/EtOH	14.21[b]	15.11[b]	57.21[b]	65.09[b]	130.04[b]	137.09[b]	171.29[b]	175.21[b]
BRDF/H_2O	9.19[de]	9.31[de]	36.00[g]	43.28[g]	108.59[g]	117.15[g]	152.10[g]	158.22[g]
CBFN	17.14[a]	19.00[a]	61.44[a]	69.15[a]	134.78[a]	140.30[a]	175.38[a]	179.00[a]
S.E.M	0.36	0.28	0.94	1.09	2.10	2.09	3.22	3.11
LEVEL (%)								
0	4.18[d]	6.49[d]	21.33[d]	24.19[d]	50.39[d]	59.18[d]	82.33[d]	107.42[d]
25	10.11[c]	11.33[c]	30.00[c]	32.00[c]	62.14[c]	67.17[c]	90.71[c]	115.00[c]
50	15.37[b]	16.01[b]	41.76[b]	43.17[b]	78.45[b]	83.03[b]	110.43[b]	131.07[b]
75	18.00[a]	19.25[a]	53.29[a]	56.29[a]	90.21[a]	95.11[a]	122.23[a]	143.10[a]
S.E.M	0.23	0.41	0.82	1.36	1.93	2.39	2.27	3.26

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the new Duncan's multiple range test.

Table 4: The main effect of treatment and level of application of plant extracts and carbofuran on NUMBER OF LEAVES of *Corchorus olitorius* infected with *Meloidogyne incognita.* (Field Experiment).

Treatments	3WAP		6WAP		10 WAP		14WAP	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
ALSB/Hex	12.00^{c}	11.21^{d}	62.68^{d}	59.86^{d}	258.91^{d}	331.28^{d}	411.39^{d}	450.03^{d}
ALSB/EtOAc	16.32^{a}	18.01^{a}	84.33^{a}	79.11^{a}	316.71^{a}	368.62^{a}	456.68^{a}	482.45^{a}
ALSB/EtOH	14.21^{b}	16.00^{b}	83.70^{a}	78.64^{a}	315.54^{a}	368.35^{a}	455.79^{a}	481.71^{a}
ALSB/H_2O	10.04^{d}	10.07^{e}	51.63^{f}	43.25^{f}	230.89^{f}	311.31^{f}	395.22^{f}	432.81^{f}
BRDF/Hex	11.12^{cd}	11.74^{d}	56.15^{e}	50.31^{e}	242.14^{e}	345.31^{e}	407.30^{e}	443.31^{e}
BRDF/EtOAc	13.11^{b}	13.07^{c}	67.32^{c}	63.21^{c}	264.12^{c}	343.61^{c}	424.08^{c}	461.06^{c}
BRDF/EtOH	15.06^{a}	16.32^{b}	72.14^{b}	70.46^{b}	273.81^{b}	351.24^{b}	431.35^{b}	472.23^{b}
BRDF/H_2O	9.22^{de}	10.61^{de}	43.18^{g}	37.44^{g}	219.75^{g}	302.74^{g}	381.64^{g}	421.25^{g}
CBFN	16.10^{a}	18.10^{a}	84.15^{a}	79.08^{a}	317.08^{a}	368.11^{a}	456.28^{a}	482.19^{a}
S.E.M	0.47	0.37	1.89	1.03	1.58	1.82	1.11	1.85
Level (%)								
0	6.30^{d}	7.22^{d}	26.72^{d}	22.81^{d}	78.29^{d}	74.00^{d}	123.12^{d}	130.14^{d}
25	13.01^{c}	12.15^{c}	66.51^{c}	54.03^{c}	194.17^{c}	207.22^{c}	338.55^{c}	373.91^{c}
50	17.29^{b}	16.49^{b}	74.68^{b}	63.14^{b}	210.86^{b}	216.53^{b}	354.30^{b}	386.00^{b}
75	20.07^{a}	22.00^{a}	82.19^{a}	70.96^{a}	226.41^{a}	229.13^{a}	370.01^{a}	402.63^{a}
S.E.M	0.57	0.28	2.32	1.32	1.93	2.41	1.43	1.30

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new Duncan's multiple range test.

Table 5: The main effect of treatment and level of application of plant extracts and carbofuran on NUMBER OF BRANCHES of *Corchorus olitorius* infected with *Meloidogyne incognita*. (Screen house).

Treatments	3 WAP		6WAP		10WAP		14WAP	
	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial
ALSB/Hex	0.00^{a}	0.00^{a}	0.00^{c}	0.00^{c}	8.00^{d}	9.34^{d}	13.27^{d}	14.12^{d}
ALSB/EtOAc	0.00^{a}	0.00^{a}	4.39^{a}	5.09^{a}	17.41^{a}	18.00^{a}	25.16^{a}	27.10^{a}
ALSB/EtOH	0.00^{a}	0.00^{a}	2.00^{b}	3.24^{b}	12.22^{b}	13.25^{b}	18.23^{b}	19.26^{b}
ALSB/H_2O	0.00^{a}	0.00^{a}	0.00^{c}	0.00^{c}	5.31^{ef}	6.29^{ef}	10.31^{ef}	11.32^{e}
BRDF/Hex	0.00^{a}	0.00^{a}	0.00^{c}	0.00^{c}	6.12^{e}	7.15^{e}	11.08^{e}	13.62^{d}
BRDF/EtOAc	0.00^{a}	0.00^{a}	4.28^{a}	5.11^{a}	17.63^{a}	18.28^{a}	25.09^{a}	27.39^{a}
BRDF/EtOH	0.00^{a}	0.00^{a}	2.00^{b}	3.54^{b}	10.83^{c}	11.12^{c}	15.44^{c}	16.35^{c}
BRDF/H_2O	0.00^{a}	0.00^{a}	0.00^{c}	0.00^{c}	5.06^{ef}	6.18^{ef}	9.02^{f}	11.26^{e}
CBFN	0.00^{a}	0.00^{a}	4.06^{a}	5.42^{a}	17.95^{a}	18.00^{a}	25.33^{a}	27.14^{a}
S.E.M	0.00	0.00	0.13	0.24	0.48	0.52	0.74	0.73
LEVEL (%)								
0	0.00^{a}	0.00^{a}	0.00^{c}	0.00^{c}	8.09^{d}	9.47^{d}	12.04^{d}	13.07^{d}
25	0.00^{a}	0.00^{a}	1.15^{b}	1.73^{b}	12.11^{c}	13.06^{c}	17.01^{c}	18.44^{c}
50	0.00^{a}	0.00^{a}	2.03^{ab}	2.82^{ab}	14.22^{b}	15.28^{b}	20.46^{b}	21.39^{b}
75	0.00^{a}	0.00^{a}	3.21^{a}	3.46^{a}	16.32^{a}	17.75^{a}	23.51^{a}	24.79^{a}
S.E.M	0.00	0.00	0.07	0.10	0.47	0.48	0.88	0.69

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the new Duncan's multiple range test.

Table 6: The main effect of treatment and level of application of plant extracts and carbofuran on NUMBER of BRANCHES of *Corchorus olitorius* infected with *Meloidogyne incognita*. (Field Experiment).

Treatments	3WAP		6WAP		10 WAP		14WAP	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
ALSB/Hex	0.00^{a}	0.00^{a}	3.10^{c}	3.08^{c}	15.22^{d}	17.81^{d}	19.31^{d}	21.00^{d}
ALSB/EtOAc	0.00^{a}	0.00^{a}	6.38^{a}	6.19^{a}	27.60^{a}	29.10^{a}	35.78^{a}	37.59^{a}
ALSB/EtOH	0.00^{a}	0.00^{a}	4.11^{b}	4.01^{b}	22.08^{b}	24.21^{b}	29.06^{b}	31.02^{b}
ALSB/H_2O	0.00^{a}	0.00^{a}	1.32^{d}	1.41^{d}	11.53^{f}	13.21^{e}	15.64^{f}	16.19^{ef}
BRDF/Hex	0.00^{a}	0.00^{a}	1.19^{d}	1.26^{d}	12.04^{e}	14.00^{e}	16.33^{e}	17.16^{e}
BRDF/EtOAc	0.00^{a}	0.00^{a}	6.25^{a}	6.33^{a}	27.55^{a}	28.32^{a}	35.11^{a}	36.58^{a}
BRDF/EtOH	0.00^{a}	0.00^{a}	4.81^{b}	4.00^{b}	20.13^{c}	22.33^{c}	27.22^{c}	28.19^{c}
BRDF/H_2O	0.00^{a}	0.00^{a}	1.26^{d}	1.18^{d}	10.18^{g}	12.09^{f}	14.20^{g}	15.21^{f}

CBFN	0.00[a]	0.00[a]	6.07[a]	6.41[a]	28.07[a]	29.17[a]	36.41[a]	37.83[a]
S.E.M	0.00[a]	0.00[a]	0.20	0.08	0.42	0.38	0.62	0.51
Level (%)								
0	0.00[a]	0.00[a]	0.00[d]	0.00[d]	11.09[d]	11.72[d]	17.01[d]	18.15[d]
25	0.00[a]	0.00[a]	3.00[c]	2.71[c]	17.34[c]	16.61[c]	22.23[c]	23.19[c]
50	0.00[a]	0.00[a]	4.33[b]	4.10[b]	21.16[b]	20.19[b]	26.19[b]	27.24[b]
75	0.00[a]	0.00[a]	5.14[a]	6.31[a]	25.00[a]	25.00[a]	31.68[a]	33.58[a]
S.E.M	0.00	0.00	0.25	0.20	0.44	0.42	0.59	0.63

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the new Duncan's multiple range test.

Table 7: The main effect of treatment and level of application of plant extracts and carbofuran on YIELD ATTRIBUTES of *Corchorus olitorius* infected with *Meloidogyne incognita* (Screen house)

Treatments	Days to 50% flowering		Fresh shoot weight (g)		Fresh root weight (g)	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
ALSB/Hex	49.21[e]	47.39[e]	111.00[e]	114.61[e]	38.02[e]	40.22[e]
ALSB/EtOAc	32.15[b]	31.53[b]	125.11[b]	128.39[b]	81.00[b]	84.30[b]
ALSB/EtOH	27.92[a]	26.74[a]	130.86[a]	132.58[a]	87.13[a]	92.41[a]
ALSB/H_2O	63.00[g]	61.31[g]	101.20[g]	104.41[g]	29.63[g]	31.08[g]
BRDF/Hex	56.13[f]	55.74[f]	107.42[f]	110.33[f]	34.46[f]	36.12[f]
BRDF/EtOAc	44.03[d]	43.58[d]	116.27[d]	119.70[d]	69.14[d]	71.48[d]
BRDF/EtOH	38.43[c]	37.49[c]	121.09[c]	124.51[c]	75.02[c]	78.19[c]
BRDF/H_2O	69.19[h]	68.61[h]	95.74[h]	98.27[h]	23.77[h]	25.14[h]
CBFN	28.26[a]	27.00[a]	131.26[a]	133.07[a]	87.28[a]	92.27[a]
S.E.M	0.53	0.57	0.60	0.69	1.07	1.20
LEVEL (%)						
0	53.05[d]	52.71[d]	43.26[d]	48.09[d]	18.33[d]	22.82[d]
25	36.18[c]	34.41[c]	79.17[c]	84.53[c]	33.76[c]	37.92[c]
50	30.06[b]	28.25[b]	103.42[b]	108.40[b]	59.61[b]	63.37[b]
75	24.49[a]	23.11[a]	119.51[a]	124.38[a]	73.86[a]	77.60[a]
S.E.M	1.07	1.20	1.39	1.46	1.51	1.62

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new Duncan's multiple range test.

Table 8: The main effect of treatment and level of application of plant extracts and carbofuran on the YIELD ATTRIBUTE of *Corchorus olitorius* infected with *Meloidogyne incognita*. (Field Experiment)

Treatments	Days to 50% flowering		Fresh shoot weight (g)		Fresh root weight (g)	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
ALSB/Hex	44.11[e]	42.02[e]	130.27[e]	133.0[e]	41.69[e]	44.81[e]
ALSB/EtOAc	27.38[b]	26.17[b]	143.81[b]	146.32[b]	92.31[b]	96.12[b]
ALSB/EtOH	22.12[a]	21.08[a]	149.86[a]	153.88[a]	97.65[a]	102.71[a]
ALSB/H_2O	56.00[g]	55.32[g]	120.00[g]	121.51[g]	32.68[g]	34.60[g]
BRDF/Hex	52.64[f]	50.71[f]	125.32[f]	128.10[f]	37.00[f]	39.16[f]
BRDF/EtOAc	39.48[d]	37.23[d]	134.71[d]	136.00[d]	81.54[d]	83.22[d]
BRDF/EtOH	35.42[c]	33.15[c]	139.57[c]	141.21[c]	87.91[c]	90.56[c]
BRDF/H_2O	62.51[h]	60.10[h]	114.29[h]	117.00[h]	27.00[h]	29.84[h]
CBFN	21.77[a]	20.65[a]	150.28[a]	155.19[a]	98.26[a]	103.00[a]
S.E.M	0.43	0.49	2.97	3.08	0.51	0.92
Level (%)	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
0	58.14[d]	57.01[d]	37.51[d]	42.49[d]	15.24[d]	19.35[d]
25	37.72[c]	35.30[c]	98.58[c]	103.10[c]	46.62[c]	50.26[c]
50	25.28[b]	23.63[b]	122.72[b]	127.40[b]	70.23[b]	74.05[b]
70	19.77[a]	18.18[a]	138.30[a]	141.32[a]	84.34[a]	88.14[a]
S.E.M	0.40	0.47	2.65	2.61	0.23	0.14

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the new Duncan multiple range test.

Table 9: The main effect of treatment and level of application of plant extracts and carbofuran on NEMATODE POPULATIONS and ROOT GALL RATING of *Corchorus olitorius* infected with *Meloidogyne incognita.* (Screen house)

Treatments	Nematode population in 250g soil		Nematode population in 5g root sample		Root gall rating	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
ALSB/Hex	27.31[c]	32.07[c]	16.52[c]	21.19[c]	1.50[b]	1.50[b]
ALSB/EtOAc	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
ALSB/EtOH	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
ALSB/H_2O	40.82[e]	46.24[e]	25.11[e]	30.07[e]	2.10[c]	2.10[c]
BRDF/Hex	35.48[d]	40.30[d]	19.22[d]	24.51[d]	2.00[c]	2.00[c]
BRDF/EtOAc	18.66[b]	23.15[b]	9.06[b]	14.02[b]	0.45[a]	0.45[a]
BRDF/EtOH	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
BRDF/H_2O	53.69[f]	58.41[f]	43.61[f]	48.78[f]	2.60[d]	2.65[d]
CBFN	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M	1.36	1.41	1.22	1.38	0.00	0.00
LEVEL (%)						
0	871.42[c]	962.31[c]	618.28[c]	633.07[c]	5.00[c]	5.00[c]
25	213.68[b]	236.29[b]	83.19[b]	94.50[b]	3.00[b]	3.00[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]
S.E.M	1.03	2.07	1.12	2.18	0.00	0.00

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new Duncan's multiple range test.

Table 10: The main effect of treatment and level of application of plant extracts and carbofuran on the NEMATODE POPULATIONS and ROOT GALL RATING of *Corchorus olitorius* infected with *Meloidogyne incognita.* (Field Experiment)

Treatments	Nematode population in 250g soil		Nematode population in 5g root sample		Root gall rating	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
ALSB/Hex	20.85[c]	28.24[c]	10.80[c]	16.51[c]	1.50[b]	1.45[b]
ALSB/EtOAc	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
ALSB/EtOH	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
ALSB/H_2O	33.29[e]	38.71[e]	19.81[e]	24.42[e]	2.10[c]	2.10[c]
BRDF/Hex	28.02[d]	35.11[d]	13..06[d]	18.54[d]	2.00[c]	2.00[c]
BRDF/EtOAc	11.05[b]	17.68[b]	4.13[b]	7.78[b]	0.45[a]	0.45[a]
BRDF/EtOH	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
BRDF/H_2O	46.59[f]	52.91[f]	37.40[f]	46.36[f]	2.70[d]	2.70[d]
CBFN	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M	0.65	1.59	0.37	1.66	0.00	0.00
Level %	1st Trial	2ndTrial	1st Trial	2nd Trial	1st Trial	2nd Trial
0	863.50[c]	941.10[c]	530.62[c]	617.68[c]	5.00[c]	5.00[c]
25	143.71[b]	169.85[b]	74.02[b]	86.00[b]	3.50[b]	3.50[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]
S.E.M	2.05	3.11	2.36	3.07	0.00	0.00

Means in a segment of a given column followed by the same letter are not significantly different at $p < 0.05$ using the new Duncan's multiple range test.

Table 11: Phytochemical analysis of concentrated plant extracts

Components	ALSB/Hex	ALSB/EtOAc	ALSB/EtOH	BRDF/Hex	BRDF/EtOAc	BRDF/EtOH
Alkaloids	+	+	+	+	+	+
Anthraquinone	+	+	+	-	+	+
Carbohydrate	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Reducing Sugar	+	+	+	-	+	+
Saponin	+	+	+	+	+	+
Steroids	+	+	+	-	+	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+

+ = Positive; - = Negative

4. Conclusion

The extracts from the leaves of *Alstonia boonei* and *Bridelia ferruginea* are nematicidal, with activities similar to carbofuran. The nematicidal activity observed was as a result of the synergistic activities of phytochemicals. In addition to the nematicidal activity, the phytochemicals in the leaves of the plant must also have enhanced the growth of *Corchorus olitorius,* being a degradable organic matter. The degradable nature of the extracts as a result of environmental factors, such as sunlight, heat and microorganism activities, would have undoubtedly enhanced the growth of the *Corchorus olitorius* plants. It is also possible that the decrease in number of the nematodes might have been responsible for the increase in growth of *Corchorus olitorius.* The effectiveness of the plant extracts was observable even at very low concentrations, and they compared well with the control, carbofuran. Future effort would be directed to the identification, quantification, isolation and structural elucidation of the active principles responsible for the observed results. This work has demonstrated that natural organic substances can replace the expensive, toxic and non-environmentally friendly synthetic nematicides in the control of nematodes.

4. References

1. Caveness F. E: **End of tour progress report on Nematology**. *Ministry of Agriculture and Natural Resources, Research Division*, Ibadan, Nigeria. Report. 1967, 119.

2. Quin J.C: **Agronomic problems of vegetable production. Proceedings of 1st National Seminar on fruits and vegetables. NIHORT**, Ibadan, Nigeria. 1975.

3. Johnson A.W: **Nematicides, a historical Review: in Veech and Dickson, Vistas on Nematology**. 1987.

4. United State Environmental Protection Agency (US EPA): **Bans Carbofuran Pesticide Residues on Food"**.*Environmental News Service*. 2009.

5. Goad R.T, Goad J.T, Atieh B.H and Gupta RC: **Carbofuran-induced endocrine disruption in adult male rats**. *Toxicology mechanisms and methods*. 2004, 14 (4): 233–9.

6. Lau T.K, Chu W and Graham N: **Degradation of the endocrine disruptor carbofuran by UV, O3 and O3/UV**". *Water science and technology : Journal of the International Association on Water Pollution Research* 2007, 55 (12): 275–80.

7. Baron R.L: **Carbamate insecticides. In Handbook of Pesticide Toxicology**. Hayes, W. J., Jr. and Laws, E. R., Jr., Eds. *Academic Press, New York*, NY, 1991, 3-6

8. Pant N, Shankar R and Srivastava SP: **In utero and lactational exposure of carbofuran to rats: effect on testes and sperm**. *Human & experimental toxicology* 1997, 16 (5): 267–72.

9. Nicosia S, Carr N, Gonzales D.A and Orr M.K. **Off-Field Movement and Dissipation of Soil-Incorporated Carbofuran from Three Commercial Rice Fields**. *J Environ Qual*. 1991, 20: 532-539.

10. United State Environmental Protection Agency (US EPA): **Drinking water criteria document on carbofuran**. Prepared under Program No. 1524 for contract 68-C8-0033 by ICAIR, Life Systems, Inc. Cleveland, Ohio for the U.S. Environmental Protection Agency. 1990.

11. Eisler R.: **Carbofuran Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review**. *U.S. Fish Wildl. Serv. Biol. Rep*. 1985, 85: 36.

12. Khan T.M and Saxena S.K**. Integrated Management of root-knot nematode, *Meloidogyne javanica* infecting tomato using organic materials and *Paecilomyce lilacinus***. *Bioresources Technology*. 1997, 61: 247-250.

13. Kimpinski J, Arenault W.J, Gallant C.E and Sanderson J.B: **The effect of Marigold (Tagetes spp) and other cover crops on *Pratylenchus penetrans* on potato crops**. *Supplement to Journal of Nematology*. 2000.

14. Pandey R: **Studies on the phytonematoxic properties in the extract of some medicinal and aromatic plants**. *International nematology network newsletter*. 2000, 12:31-33

15. Kayode J and Omotoyinbo M.A: **Conservation of Botanicals Used for Dental and Oral Healthcare in Ekiti State, Nigeria.** *Ethnobotanical Leaflets*.200812:7-18.

16. Irobi ON, Moo-Young M, Anderson WA and Daramola SO: **Antimicrobial activity of bark extracts of Bridelia ferruginea (Euphorbiaceae)**. *Journal of Ethnopharmacology*. 1994, 43(3): 185-190.

17. Sofowora A: **Medicinal plants and Traditional Medicine in Africa**. *Spectrum Books, Ibadan*. 1993, 150.

18. Atolani O, Adeyemi SO, Akpan E, Adeosun CB and Olatunji GA:**Chemical Composition and Antioxidant Potentials of *Kigelia Pinnata* Root Oil and Extracts.***EXCLI Journal.* 2011,10:264-273.

19. Gautam C. and Goswami BK: **Different combinations of neem cake and carbofuran against *Meloidogyne incognita* on *Vigna radiate***. *International Journal of Nematology*. 2002, 12: (1): 106-110.

20. Denton L: **A review of *Corchorus olitorius* (L) in Nigeria. In Shippers, R.R and Budd,L (Editors) Proceedings of a Workshop on African Indigenous vegetables, Limbe, Cameroon**. NRI/PGRI, Chatham, UK. 1997, 25-30

21. Gomez KA and Gomez AA. **Statistical Procedures for Agricultural Research**. 2nd Edition John-Wiley and Sons, New York, 1984.

22. Phillipson JD, O'Neill MJ, Wright CW, Bray DH, Warhurst DC (1987). **Plants as Sources of antimalarial and amoebicidal compounds; Medicinal and Poisonous plants of the tropics; proceedings of symposium5-35of the 14th International Botanical Congress**; Berling. 70-78.

23. Deverall BJ: **In Phytochemical EcologyEd**. *Harbone J.B. Academic Press. London.* 1972, 217-234.

24. Fabiyi OA and Atolani O: ***Lawsonia inermis* in the Control of *Meloidogyne* spp. on *Corchorus olitorius***. *EJEAFChe.* 2011, 10: 2000-2006.

25. Ndukwe G, Amupitan JO, Isah Y and Adegoke KS (2007). **Phytochemical and antimicrobial screening of the crude extracts from the root, stem, bark and leaves of *Vitellaria paradoxa***. *African Journal of Biotechnology*. 2007, 6: 1905-1909.