International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
Received: 17-01-2021, Accepted: 18-02-2021, Published: 24-03-2021
www.entomologyjournals.com
Volume 6; Issue 2; 2021; Page No. 122-126

Anti-termitic activities of *Dodonea viscosa* on subterranean termite, *Odontotermes wallonensis* (Wasmann) (Termitidae: Isoptera)

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Abstract
Different antitermitic properties *viz.,* termiticidal, antifeedant, repellent and IGR activities of methanolic extract of *Dodonea viscosa* were investigated against *Odontotermes wallonensis.* The results indicated that Methanolic extract of *D. viscosa* leaves at 1.00 per cent concentration registered the highest mortality when administered through food (96.78%) and applied topically (88.00%). The highest antifeedant activity was recorded at 1.00 per cent concentration, where minimum food consumption (0.78g) was observed. Methanolic extract of *D. viscosa* leaves at 1.00 per cent concentration, repelled 97.67 per cent termites at 48 HAT. The highest intermediate adult development (37.67%) and deformed larvae (36.22%) were recorded at 1.00 per cent of extract on *O. wallonensis* nymphs. The growth of *Termitomyces* was suppressed to the tune of 56.43 per cent by 1.00 per cent of methanolic extract of *D. viscosa* leaves in treated plates.

Keywords: *Odontotermes wallonensis, Dodonea viscosa,* termiticide, antifeedant, IGR

Introduction
Subterranean termites are an economically important crop and timber pests worldwide. They cause extensive damage to lignocellulosic material in temperate and tropical climates [1]. In the past years, termite control has been totally based on chemical insecticides which are harmful to other organisms in environment. Extracts isolated from some plant species may provide alternatives in termite control. It has been reported that the extractives of some plants exhibit antifeedant, repellency and toxicity effects against some termite species [2, 3]. In addition, plant extracts are biodegradable and best alternatives to resolve problems caused by synthetic pesticides. Adlin *et al* [4]. Investigated some invasive plant extracts at 10 per cent on the subterranean termite, *Odontotermes wallonensis* and found that *Lantana camara* was most effective. Our previous scanning with different plant extracts exhibited the less studied plant *Dodonea viscosa* has potential termiticidal activity against subterranean termites. *D. viscosa* (Hop bush), a member of the family Sapindaceae is a shrub distributed in India, Pakistan, Africa and Australia. Various extracts of the plant have shown insecticidal activity against the cotton leafworm, *Spodoptera littoralis* [5]. Hence, it is felt desirable to pursue a study on its termiticidal, antifeedant, antifungal and Insect Growth Regulator effects on *Odontotermes wallonensis.*

Materials and Methods
Extraction of *D. viscosa*
Shade dried leaves of *D. viscosa* were pulverized using electrical mixer grinder. About 90g of leaf powder was weighed and filled in a thimble made up of filter paper. They were kept in the Soxhlet apparatus and extracted with chloroform, hexane, acetone, ethyl acetate, methanol and ethanol for a period of seventy two hours at 40~60°C. The solvent in the extracts was removed by keeping the extracts in water bath at 40° C for 2 hours. All the crude extractives were stored in glass vials at room temperature.

Preparation of stock solution
Stock solution was prepared by dissolving 2.5mg of solvent extract in 25ml of respective solvents to get a solution of 10 per cent concentration. From this stock solution, various concentrations *viz.,* 0.05, 0.10, 0.25, 0.50 0.75 and 1.00 per cent were prepared by serial dilutions and used for further laboratory experiments.

Collection of termites
The workers and soldiers of the termite species *O. wallonensis* were collected from the paddy straw baits in 9.5 X 8cm sized pots installed at various places around Tamil Nadu Agricultural University (TNAU) campus, Coimbatore.

Evaluation of termiticidal and antifeedant activity of various solvent extracts of *Dodonea viscosa* on *O. wallonensis*
Workers and soldiers of *O. wallonensis* were collected by using bait and used for the laboratory study. Screening was carried out in the Post Graduate laboratory, Department of Entomology, TNAU, Coimbatore. The protocol suggested by Kang *et al.*[6] was used for screening. Nine cm diameter corrugated card board sheets were dipped in plant essential oil solutions and placed in Petri dishes. Fifty numbers of termites (45 workers and 5 soldiers) were randomly selected from stock population and released into Petri dishes containing the treated card board sheets. Standard check in termiticidal activity and antifeedant activity experiments were 0.1 per cent Chlorpyrifos 20 EC and Azadirachtin 0.03% @ 1 per cent respectively. Water served as control. The treatments were replicated three times. Experiment was conducted under laboratory conditions (25±1°C and 70±5% RH). A few drops of water were dripped on card board sheets at 2 hours interval. Mortality was recorded after every 2 hours up to 48hrs. Insects were treated as dead when

they become immobile and have shown no activity to the external stimuli. The weight of the card board sheets were also recorded at 6, 12, 18, 24, 30, 36 and 48 hours after treatment (HAT) and mean food consumption was calculated.

Evaluation of repellent activity of *D. viscosa* on *O. wallonensis*

Filter paper of diameter 9cm divided into two equal halves were kept in Petri plates. The first half was treated with various concentrations (0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 %) of *D. viscosa* extracts. The second half was left untreated. Control was similarly prepared in which one half of filter paper received distilled water and another half was left untreated. Petri plates were left uncovered for 4hrs at ambient condition for solvent evaporation. Then 250µl of distilled water was added to each filter paper. Forty five workers and five soldiers were released into Petri plates and covered with lids. This set up was kept in BOD (25±1º C and 70±5% RH) and covered with an opaque black sheet to eliminate the effect of light. The per cent repellency was recorded in 2, 4, 6, 8, 10, 12, 24, 36 and 48 HAT depending on number of existing insects on the treated half of filter paper.

Evaluation of insect growth regulator activity of *D. viscosa* on *O. wallonensis*

The pseudergates (P_3 or P_4) of *O. wallonensis* were used for the experiment. The different concentrations of *D. viscosa* extracts (0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 %) were prepared and applied with a syringe @ 5ml on the filter papers arranged in the round Petri plates. The control was similarly prepared in which filter papers had received only distilled water and check used was Diaflubenzuron 25% WP @ 1 per cent concentration. Pseudergates numbering fifty per replication were released. Three replications were maintained for each treatment. The experimental set up was kept for three weeks in BOD incubator at 25±1°C. Once in a week the following observations *viz.*, deformed nymphs and intermediate underdeveloped workers were observed.

Statistical analysis

The data on percentage values and number were transformed into arcsine and square root values, respectively before subjecting them to statistical analysis [7]. Analysis of variance was done in AGRESS and AGDATA Packages. Duncan's Multiple Range Test [8] was applied for comparing the treatment means.

Results and Discussion

Termiticidal activity of *D. viscosa* solvent extracts on *O. wallonensis*

All the solvent extracts of *D. viscosa* at higher concentrations showed good to moderate termiticidal activity against *O. wallonensis* (Table1). After 48 hours, the cumulative mean per cent mortality was higher at 1 per cent concentration of all the solvent extracts. Among them, highest mean per cent mortality (100%) was observed in methanol extract. Ethanol, ethyl acetate, hexane, chloroform and acetone extract recorded 93.00, 83.00, 81.00, 69.00 and 56.00 per cent mortality at the same concentration. All the solvent extracts were significantly different ($p<0.05$) from control. There was a corresponding increase in the mortality of *O. wallonensis* with increase concentrations of *D. viscosa* solvent extracts. El-Din and El-Gengaihi also determined the insecticidal property of extracts *Dodonaea* under the laboratory for their effects against the Egyptian cotton leaf worm, *Spodoptera littoralis* and found promising results [9]. Subashini *et al.* conducted the laboratory experiment and has proved that this plant is highly potential against controlling *Helicoverpa armigera* [5].

Table 1: Termiticidal activity of various solvent extracts of Dodonea viscosa on O. wallonensis

Dviscosa leaf extract	Cumulative mean per cent mortality* at HAT**													
	0.05%		0.1%		0.25%		0.50%		0.75%		1%		Control	
	24	48	24	48	24	48	24	48	24	48	24	48	24	48
Hexane	0.00 (0.67) ^{b}	0.00 (0.67) ^{c}	1.00 (5.71) ^{b}	10.50 (18.63) ^{c}	12.50 (20.71) ^{a}	23.33 (28.93) ^{b}	20.33 (27.19) ^{b}	35.33 (36.41) ^{b}	45.00 (42.35) ^{b}	56.33 (48.62) ^{b}	63.30 (52.59) ^{c}	81.00 (64.41) ^{c}	0.00	3.33
Acetone	0.00 (0.67) ^{b}	0.00 (0.67) ^{c}	1.33 (6.62) ^{a}	8.00 (16.52) ^{d}	7.33 (15.66) ^{c}	15.00 (22.90) ^{e}	8.00 (16.62) ^{f}	17.68 (25.59) ^{d}	20.00 (26.59) ^{e}	33.66 (35.66) ^{d}	39.30 (38.78) ^{e}	56.00 (48.67) ^{e}	1.33	1.33
Chloroform	0.00 (0.67) ^{b}	0.00 (0.67) ^{c}	0.00 (0.67) ^{d}	0.00 (0.67) ^{e}	0.00 (0.67) ^{f}	16.00 (23.73) ^{d}	10.50 (19.00) ^{e}	33.33 (35.03) ^{c}	18.56 (25.42) ^{e}	45.66 (42.40) ^{c}	35.30 (36.26) ^{f}	69.00 (55.84) ^{d}	3.00	8.70
Ethyl acetate	0.00 (0.67) ^{b}	0.00 (0.67) ^{c}	0.00 (0.67) ^{d}	0.00 (0.67) ^{e}	4.99 (12.98) ^{d}	20.00 (26.69) ^{c}	18.33 (25.45) ^{c}	35.66 (36.59) ^{b}	22.33 (28.35) ^{d}	54.00 (47.20) ^{b}	44.70 (41.68) ^{d}	83.00 (65.85) ^{c}	1.00	1.00
Methanol	0.33 (3.29) ^{a}	21.09 (27.28) ^{a}	0.33 (3.29) ^{c}	25.52 (30.45) ^{a}	8.66 (17.07) ^{b}	27.33 (31.57) ^{a}	15.68 (23.26) ^{d}	41.00 (39.70) ^{a}	25.33 (30.48) ^{c}	69.02 (55.68) ^{a}	82.33 (65.53) ^{a}	100.00 (87.13) ^{a}	0.00	2.70
Ethanol	0.00 (0.67) ^{b}	12.33 (20.67) ^{b}	0.00 (0.67) ^{d}	19.00 (25.83) ^{b}	4.00 (11.47) ^{e}	23.33 (28.84) ^{b}	25.00 (29.90) ^{a}	33.68 (35.46) ^{c}	50.99 (45.29) ^{a}	67.00 (54.24) ^{a}	76.00 (60.91) ^{b}	93.00 (75.25) ^{b}	1.33	2.33
SEd	0.016	0.161	0.030	0.202	0.144	0.301	0.258	0.345	0.488	0.725	0.617	1.172	-	-
CD(P=0.05)	0.036	0.352	0.066	0.442	0.314	0.657	0.563	0.753	1.063	1.580	1.344	2.555	-	-

*Mean of 3 replications

**HAT-Hours after Treatment

Figures in parentheses are arc sin transformed values

Means followed by same letter(s) are not significantly different at 5% level by LSD

Termiticidal activities of methanolic extract of *D. viscosa* leaves on *O. wallonensis*

When the methanolic extract of *D. viscosa* administered through topical application, highest mortality (96.78%) was recorded at 1 per cent concentration after 48 hours. It was followed by 0.75 (69.02%), 0.50 (41.09%) and (27.20%). Control recorded 6.87 per cent mortality (Table2). Chlorpyrifos recorded cent per cent mortality at 12 HAT. When the methanolic extract of *D. viscosa* administered through topical application, highest per cent mortality (88.00%) was recorded at 1 per cent concentration of methanolic extract of *D. viscosa* leaves after 48 hours of exposure (Table 3). Results of the present study coincided with Sohail [10] where methanolic extracts of *D. viscosa* at 40

per cent concentration exhibited good termiticidal activity (LT_{50} 27.5h) against *Macrotermes obesi.* Nisar *et al.* evaluated the potential termiticidal activity of *D. viscosa* leaves extracts and maximum LT_{50} (167.83 h) was observed with chloroform extract at 1 per cent concentration whereas minimum LT_{50} (66.68 h) was observed with n- Hexane extract at 10% concentration [11]. LT_{50} in leaf extracts/solvents were significant lower than their respective controls. The phenolics (flavonoids) have been isolated from leaves of *D. viscosa* and three to four types of kaempferol were identified [12]. Teffo *et al.* demonstrated the isolation of kaempferol from *D. viscosa* with antibacterial and antioxidant properties, yet no reference so far has been found to explain the effect of kaempferol on the biology of termites [13].

Table 2: Termiticidal activity of methanol extract of *D. viscosa* leaves on *O. wallonensis* *Mean of 3 replications

Methanol extract of D. viscosa leaves	Cumulative mortality per cent at HAT**								
	2	4	6	8	10	12	24	36	48
0.05%	0.00 (0.58) b	0.00 (0.58) d	0.98 (5.73) d	1.67 (5.68) g	3.00 (10.00) e	4.78 (20.86) c	5.33 (21.36) f	10.78 (30.65) e	21.09 (27.49) f
0.10%	0.00 (0.58) b	0.00 (0.58) d	0.00 (0.58) f	1.85 (7.42) f	2.33 (8.76) e	4.66 (20.66) c	8.00 (23.92) e	12.58 (31.42) e	25.52 (30.18) e
0.25%	0.00 (0.58) b	0.00 (0.58) d	1.00 (5.76) d	2.33 (7.75) e	4.00 (11.46) d	5.70 (21.74) c	8.66 (24.65) e	11.99 (31.29) e	27.20 (31.55) e
0.50%	0.00 (0.58) b	0.00 (0.58) d	0.78 (5.13) e	1.98 (8.83) c	4.66 (12.42) d	7.33 (22.31) c	15.68 (28.86) d	33.00 (36.96) d	41.09 (39.83) d
0.75%	0.00 (0.58) b	1.98 (8.10) c	3.66 (11.07) c	7.00 (8.04) d	11.97 (20.27) c	15.00 (28.48) c	25.33 (33.34) c	50.09 (40.49) c	69.02 (56.54) c
1.0%	0.00 (0.58) b	2.66 (9.36) b	5.00 (12.92) b	12.99 (15.29) b	20.66 (26.88) b	48.07 (41.42) b	59.00 (45.06) b	88.33 (48.58) b	96.78 (78.43) b
Chlorpyrifos 20 EC @0.1%	3.00 (9.80) a	18.00 (25.23) a	45.33 (42.44) a	70.69 (21.15) a	83.00 (66.32) a	100.00 (89.41) a	100.00 (89.41) a	100.00 (89.41) a	100.00 (89.41) a
Control	0.00 (0.58) b	0.00 (0.58) d	0.00 (0.58) f	0.00 (0.58) h	0.00 (0.58) f	0.12 (1.98) d	0.99 (5.68) g	3.66 (11.07) f	6.87 (15.11) g
SEd	0.060	0.056	0.147	0.092	0.707	3.903	0.388	1.133	1.069
CD (P=0.05)	0.127	0.119	0.311	0.196	1.499	8.333	0.823	2.403	2.266

**HAT-Hours after Treatment
Figures in parentheses are arc sin transformed values
Means followed by same letter(s) are not significantly different at 5% level by LSD

Table 3: Termiticidal activity of methanol extract of *D. viscosa* leaves on *O. wallonensis* –Topical application method

Methanol extract of *D. viscosa* leaves	Cumulative mortality per cent at HAT**								
	2	4	6	8	10	12	24	36	48
0.05%	0.00 (0.58) b	0.00 (0.58) c	0.50 (4.01) f	1.00 (5.73) e	2.66 (9.42) f	3.98 (11.52) e	6.00 (14.25) e	9.33 (24.95) e	20.00 (26.41) f
0.10%	0.00 (0.58) b	0.00 (0.58) c	0.66 (4.69) e	1.00 (5.73) e	1.67 (7.46) g	4.00 (11.61) e	7.17 (15.62) e	10.03 (25.39) e	23.33 (28.91) ef
0.25%	0.00 (0.58) b	0.00 (0.58) c	0.89 (5.38) d	1.06 (5.88) e	3.00 (9.95) e	4.66 (12.45) e	7.33 (15.69) e	10.98 (26.05) e	25.00 (29.91) e
0.50%	0.00 (0.58) b	0.00 (0.58) c	0.99 (5.68) d	2.30 (8.76) d	5.08 (13.00) d	10.66 (19.14) d	16.00 (24.19) d	28.78 (34.76) d	33.00 (35.26) d
0.75%	0.00 (0.58) b	0.00 (0.58) c	1.99 (8.13) c	3.00 (9.99) c	6.33 (14.51) c	12.99 (21.21) c	20.98 (27.11) c	45.99 (40.71) c	50.00 (45.21) c
1.0%	0.00 (0.58) b	0.66 (4.66) b	3.08 (10.10) b	7.00 (15.30) b	13.99 (22.23) b	32.05 (35.43) b	48.36 (43.83) b	63.33 (46.42) b	88.00 (70.23) b
Chlorpyrifos 20 EC @0.1%	2.98 (9.99) a	12.33 (20.55) a	35.00 (36.48) a	55.79 (48.29) a	72.03 (58.08) a	100.00 (65.14) a	100.00 (89.41) a	100.00 (89.41) a	100.00 (89.41) a
Control	0.00 (0.58) b	0.00 (0.58) c	0.00 (0.58) g	0.00 (0.58) f	0.00 (0.58) g	0.12 (1.98) f	0.99 (5.68) f	3.66 (11.07) f	6.87 (15.11) g
SEd	0.002	0.010	0.231	0.084	0.130	0.624	1.179	1.115	1.213
CD (P=0.05)	0.006	0.021	0.490	0.178	0.277	1.323	2.499	2.365	2.572

*Mean of 3 replications
**HAT-Hours after Treatment
Figures in parentheses are arc sin transformed values
Means followed by same letter(s) are not significantly different at 5% level by LSD

Antifeedant activity of methanolic extract of *D. viscosa* leaves on *O. wallonensis*

Different concentrations of methanolic extract of *D. viscosa* leaves were evaluated for their antifeedant action on *O. wallonensis.* Food consumption at 1.00 per cent concentration was minimum when compared to lower concentrations of the extract (Fig.1). Ohmoura *et al.* proved the antifeedant activity of some flavonoids such as kaempferol and their related compounds against *C. formosanus* [14]. Praveena *et al.* found that *D. viscosa* extract has antifeedant activity against spotted bollworm [15] and their achievements were in conformity with the results of this study. Flavonoids (kaempferol) present in *D. viscosa* leaves extract are responsible for antifeedant activity.

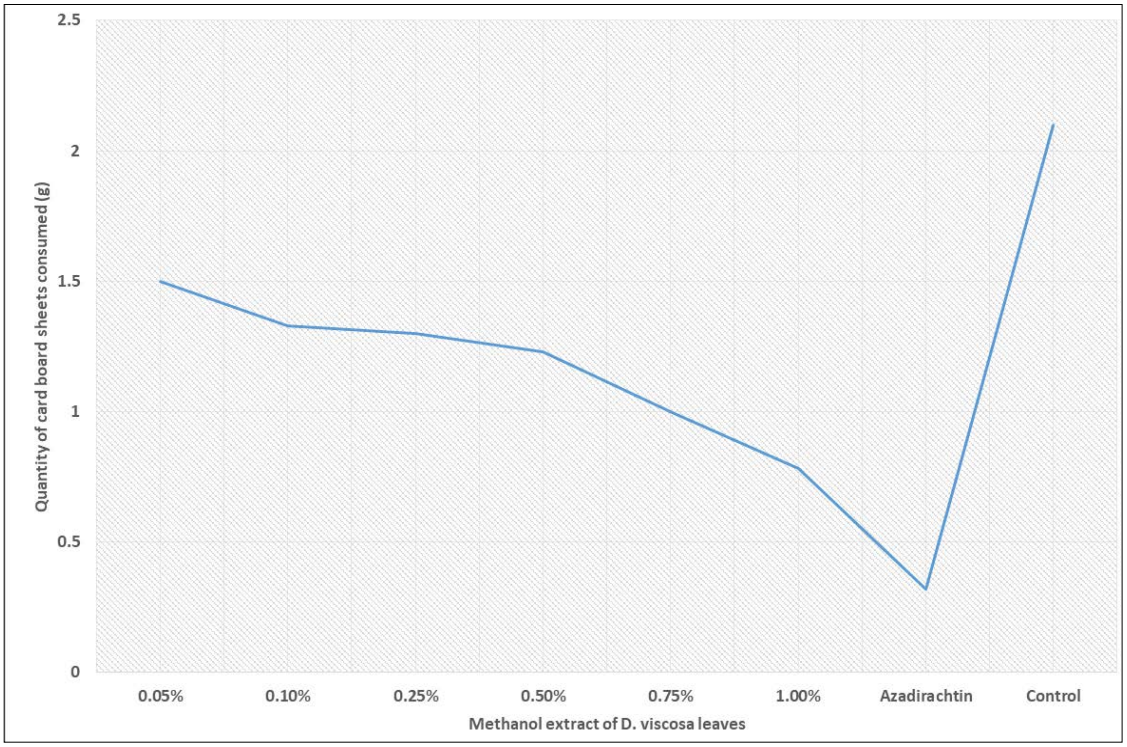

Fig 1: Antifeedant activity of methanol extract of *D. viscosa* leaves on *O. wallonensis*

Repellent activity of methanolic extract of D. viscosa leaves on O. wallonensis

Methanolic extract *D. viscosa* leaves at 1.00 per cent concentration effectively repelled the termites (97.67%) and the per cent repellency was decreased with the lower concentrations (Fig. 2). Nisar confirmed the repellent activity of n-Hexane extract of *D. viscosa* leaves at 10 per cent concentration on *O. obesus* [16]. Minimum tunnel length (22.67mm) was observed in n-Hexane extract at 10 per cent concentration.

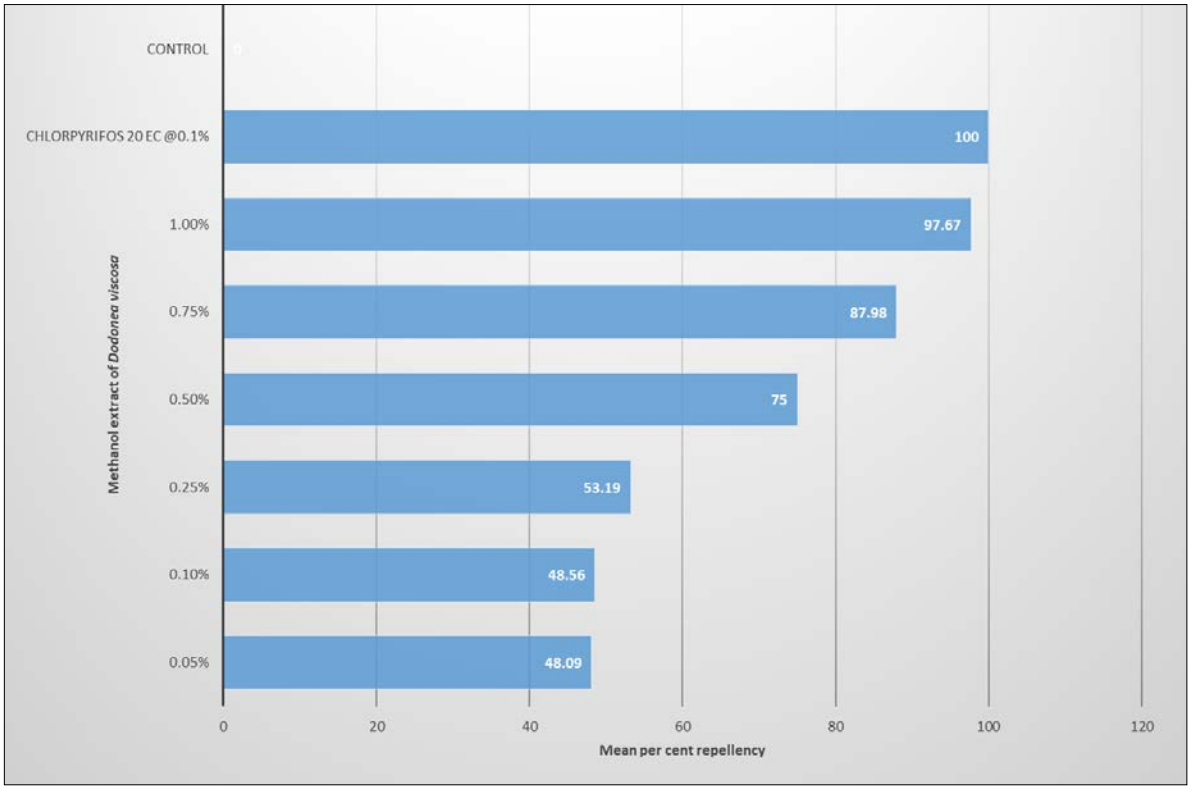

Fig 2: Repellent activity of methanol extract of D. viscosa leaves on O. wallonensis

IGR activity of methanolic extract of *D. viscosa* leaves extract on *O. wallonensis*

Methanolic extracts of *D. viscosa* at 1.00 per cent concentration showed significant IGR activity on the termite nymphs (Table 4). The overall mean per cent intermediate development was highest (37.67%) at 1.00 per cent of

methanolic extract of *D. viscosa* leaves. It also recorded maximum per cent deformed larvae development (41.67%) and pre-soldiers development (36.22%). In another study, *D. viscosa* plant extract at different concentrations showed significant IGR activity against *A. aegypti* mosquitoes and there was no successful pupation and adult emergence at 500 µg/mL [17]. Abdelaziz and Omer also recorded the hexane extract of *D. viscosa* tested against *Spodoptera littoralis* was the most effective, which gave the highest larval mortality and reduction in hatchability, fecundity and pupation [18].

Conclusion

Scientists from different parts of the world in their studies are using plants and their by-products to control pests, including termites, because plants are eco-friendly and can play a larger role in the control of termites. Due to increasing interest in the development of plant materials as an alternative to chemical pesticides, this study examined the effect of *D. viscosa* leaf extract against *O.wallonensis.* This study has revealed the anti-termitic properties *viz.*, termiticidal, antifeedant, repellent and IGR activities of *D. viscosa* where the methanolic extract of leaves @ 1 per cent showed the best results against *O. wallonensis.* Consequently, by further investigation in future and by separating the dominant components of *Dodonea*, an economical and eco-friendly pesticide can be developed for controlling termites.

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