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Efficacy of some aqueous plant extracts against cotton stainers, *Dysdercus superstitious* (Herrich Schaffer) (Hemiptera: Pyrrhocoridae)

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Abstract
The objective of the present study was to investigate the insecticidal efficacy of *Bridelia micrantha, Chasmanthera dependens* and *Vernonia cinerea* under ambient laboratory conditions in the management of *Dysdercus superstitious* (Herrich Schaffer) (Hemiptera: Pyrrhocoridae). The incidence of each aqueous extracts on *D. superstitious* for repellency and mortality was monitored at 24 h interval for 4 days, while nymph emergence was monitored and recorded. The result indicated that *D. superstitious* repellency, mortality and nymph emergence were concentration (dosage) and exposure time dependent. Across treatment, dishes treated with 2.0 ml extract significantly (P<0.05) influenced the bioactivity of the insects on treated surface compared to untreated surface. Adult insects exposed to 2.0 ml extracts for 24 and 48 h exposure time significantly (P<0.05) exerted greater adult repellency (90%), mortality (90%) and suppressed nymph emergence (0%). Among the three tested plants *B. micrantha* showed the best effective values followed by *C. dependens* and *V. cinerea* respectively in significantly repelling the insect from treated surface, caused adult mortality and inhibited nymphs emergence. Hence use of *B. micrantha* to suppress cotton stainers infestation on okra or cotton plants is herewith discussed.

Keywords: Efficacy, *Bridelia micrantha, Chasmanthera dependens, Vernonia cinerea, Dysdercus superstitious,* Cotton stainer

1. Introduction
Cotton stainer, *Dysdercus superstitious* Herrich Schaffer, (Heteroptera: Pyrrhocoridae) is a major pest of cotton. Both nymphs and adults feed on developing flowers and fruits, resulting in bud shedding, abortion of young bolls. Continuous infestation leads to the reduction in yield, infestation by nematospora fungus and lint staining in older bolls [1]. The control measures have largely been dependent on the use of chemical pesticides including organochlorines, organophosphates, carbamates and pyrethroids [2, 3]. In view of the hazardous effects of synthetic insecticides on non-target organisms including man and the environment [4, 5], the need for suitable and effective alternatives for the said purpose are in demand. Botanical insecticides can be one of the best alternatives for these hazardous chemicals. They are plant-derived insecticides, either naturally occurring plant materials or the product simply derived from such plants [6].

Chasmanthera dependens (Hochst), (Menispermaceae) a Nigerian traditional medicine plant commonly called *Chasmanthera*, occurs commonly in forest margins, savanna and secondary forest, often near rocks, but sometimes also in dense and moist evergreen forest, semi-deciduous forest and riverine forest, up to 1500 m altitude. It is widely used in traditional medicine for the treatment of several diseases that include red-eye infections [7], venereal diseases and management of fractures [8].

Bridelia micrantha (Hochst.) Baill. (Euphorbiaceae), also known as Coast gold leaf in English is a semi-deciduous to deciduous tree of up to 20 m tall, with a dense rounded crown and tall, bare stem which is widespread in Africa [9]. In Nigeria, *B. micrantha* stem bark is used in traditional medicine for treating diabetes [10]; in South Western Nigeria a leaf decoction is used traditionally as part of recipe for the management of diabetes mellitus and inflammation of joints [11].

Vernonia cinerea (Asteraceae) is a terrestrial annual erect herb. It grows up to 80 cm high. It can be found in roadside, open waste places, dry grassy sites and in perennial crops during plantation [12]. *V. cinerea* is an important medicinal plant having application in abortion, cancer and various gastrointestinal disorders [13].

In literature, there seem to be dearth of empirical information on the insecticidal efficacy of *B. micrantha*, *C. dependent* and *V. cinerea* although both plants are used for ethnomedicinal purpose in some South-Western states of Nigeria. However, there are no reports on use of these plants against the insect pests. Hence the purpose of this study is to evaluate the insecticidal efficacy of *B. micrantha, C. dependent* and *V. cinerea* on bioactivity of cotton stainers.

2. Materials and methods

2.1. Collection and Preparation of Plant Materials

Stem barks of *B. micrantha, C. dependens* and *V. cinerea* leaves were collected from rain forest region around Basola village (6° 47´ N, 4° 52´ E) in Odigbo Local Government area of Ondo State, Nigeria. The parts collected (500g) from each plant were washed with running water to remove dirt, and were allowed to drain and thereafter, pounded using wooden mortar and pestle. The extracts were obtained by soaking the pounded materials separately in 2 liters of cold water each for 48 h. Then the crude extracts were separated from the plant materials using muslin cloth and stored at room temperature until further use

2.2. Collection of insects

Adult cotton stainers were collected from infested okra plants in the horticultural garden of Rufus Giwa Polytechnic Teaching, Research and Commercial Farms, Owo, Ondo State, Nigeria (7° 11´ N and 5° 35´ E). The insects were collected and kept in a cylindrical plastic jar covered with wire mesh to permit air flow and then taken to the laboratory for use.

2.3. Repellency bioassays of the extracts against adult cotton stainers

Bioassays were conducted under ambient laboratory conditions of 28 ± 2 ˚C temperature and 80±2% relative humidity in the Crop Protection Laboratory, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria (7° 11´ N and 5° 35´ E) during the months of April – August, 2015. The repellent action of the extracts against the insects was evaluated by area preference method as reported by Obeng-Ofiori *et al.* [14]. In this study, test areas consisted of 10cm Whatman No.1 filter papers cut in half. Different test extracts and concentrations i.e. 0.5, 1.0, 1.5 and 2.0 ml were applied to half filter paper disc as uniformly as possible with syringe. The other halves of each filter paper were not treated. The treated half discs were air-dried and full disc remade by placing in the Petri dish. Each filter paper was placed in a Petri dish and five insects placed at the centre of the paper and covered with the dish lid and bounded with rubber bands. Each treatment was replicated three times and laid in a completely randomized design (CRD). The number of insects presents on the control (non-treated) and the treatment (extract treated) strips were recorded after 24, 48, 72, 96 and 120 h respectively.

2.4. Contact Toxicity of Extracts

All treated Petri dishes were maintained in the laboratory and mortality was determined at 24 h interval after infestation. The insects were considered dead if appendages did not respond after being viewed properly or touched. Dead insects were removed immediately, and bioassay was carried out daily till day 5 (120 h).

2.5. Effect of Plant Extracts on Oviposition

On day 5 (120 h) after infestation (DAI), the total number of eggs laid by female cotton stainers during infestation period were counted and recorded; and 13 DAI, nymphs' emergence was observed. This was monitored and recorded for three consecutive days.

2.6. Data Collection and Analysis

Completely Randomized Designs (CRD) was adopted for the experiment and each treatment was replicated three times. Values on percentage repellency (PR), percentage mortality and percentage of eggs hatched (nymphs emergence) were computed from the data using the following formulas.

(i) Percent repellency (PR) =

$$\frac{\text{number of insects present on control strip} - \text{no of insects present in treated strip}}{\text{number of insects present on control strip} + \text{no of insects present in treated strip}} \times \frac{100}{1}$$

(ii) Percentage Adult Mortality =

$$\frac{\text{Total number of dead insects after treatment}}{\text{total number of insect before treatment}} \times \frac{100}{1}$$

(iii) Percentage of eggs hatched =

$$\frac{\text{Total number of emerged nymphs}}{\text{total number of eggs laid}} \times \frac{100}{1}$$

Data collected were subjected to Analysis of Variance (ANOVA) after arcsine transformation and treatment means separated by using Least Significant Differences (LSD) at $P \leq 0.05$ [15].

3. Results

3.1. Repellant effects of plant extracts on Cotton Stainers

The results for the laboratory study on repellency reveal that percentage repellency values vary over exposure time. As exposure time increases, the percent repellency value reduces. Maximum repellency values were observed at 24 and 48 h after infestation (HAI). The dish treated with 2.0ml and 1.5ml has the highest repellency values followed by those treated with 1.0ml and 0.5ml respectively, which means that the repellant effect are directly proportional to the concentrations applied and exposure time (Table 1).

Table 1: Percentage adult repellency of cotton stainers treated with different plant extracts.

Plant extracts	Dosage (ml)	% of repellency h after infestation				
		24 h	48 h	72 h	96 h	120 h
B. micrantha	0.5	34.6 ± 2.5	34.6 ± 2	26.5 ± 2.2	26.5 ± 1.8	26.5 ± 2.6
	1.0	44.6 ± 2.5	34.5 ± 2.6	34.63 ± 3	36.6 ± 3.2	26.56 ± 2.6
	1.5	63.8 ± 3.2	50.7 ± 4.8	42.7 ± 2.6	50.7 ± 2.8	34.6 ± 3.2
	2.0	76.7 ± 3.4	90.0 ± 4.2	50.7 ± 2.2	50.7 ± 2.2	42.7 ± 4.8
LSD (5%)		35.4	18.5	18.6	5.4	NS
C. dependens	0.5	34.6 ± 2.5	26.5 ± 2	26.5 ± 2.4	26.5 ± 1.3	26.5 ± 2.5
	1.0	34.6 ± 2.5	34.63 ± 2.6	26.5 ± 2.5	26.6 ± 3.2	26.56 ± 2.6
	1.5	34.8 ± 3.2	34.6 ± 4.8	26.5 ± 2.5	26.5 ± 2.8	26.5 ± 3.2
	2.0	76.9 ± 2.3	42.7 ± 2.6	42.7 ± 2.6	34.6 ± 2.2	34.6 ± 4.8

LSD (5%)		31.2	3.14	18.6	NS	NS
V. cinerea	0.5	26.6 ± 2.5	26.5 ± 2	26.5 ± 2.2	26.5 ± 1.8	26.5 ± 2.6
	1.0	34.6 ± 2.5	26.5 ± 2.6	34.63 ± 3	26.6 ± 3.6	26.56 ± 1.6
	1.5	34.6 ± 3.2	42.7 ± 2.4	34.6 ± 2.6	26.7 ± 2.4	26.5 ± 1.2
	2.0	42.7 ± 3.4	34.63 ± 4.2	34.6 ± 2.2	42.7 ± 2.2	26.5 ± 1.8
LSD (5%)		NS	NS	NS	13.1	NS

The result for *B. micrantha* extract (Table 1) showed that at 24, 48, 72 and 96 h post treatments, the dishes treated with 2.0ml recorded significant repellent (P<0.05) activity compared to other dosage rates, while at 120 HAI repellency did not differ significantly (P>0.05). The repellency of surfaces treated with 2.0ml concentration of *C. dependens* extract was significantly high at 24, 48, and 72 and 120 h after infestation; while it showed non-significantly different (P>0.05) at 96 and 120 h after infestation (HAI) (Table 1). Significant repellency was recorded only at 96 h for *V. cinerea* extract (Table 1).

3.2. Effect of plant extracts on adult mortality of cotton stainers

The data on percentage adult mortality of the cotton stainers were presented in Tables 2-4. The result shows that mortality increases after 48 HAI and the results (values) were at par to each other. Mortality was very low at 0.5 ml and 1.0 ml concentrations, compared to mortality response at 1.5 ml and 2.0 ml respectively.

Table 2: Percentage adult mortality of cotton stainers treated with *B. micrantha* extract.

Treatments	Percentage adult mortality after infestation				
Dosage	24h	48h	72h	96h	120h
0.5ml	0.0±0.0	0.00±0.0	30.78±1.7	43.07±2.8	43.07±2.4
1.0ml	13.07±1.5	21.93±2.6	51.14±2.5	35.00±2.2	46.92±2.7
1.5ml	30.78±2.9	30.45±1.6	35.00±2.3	46.92±2.2	81.14±4.8
2.0ml	35.00±2.3	39.23±2.6	51.14±2.5	59.21±3.2	90.00±3.2
LSD (5%)	7.42	NS	18.85	13.19	16.97

The treatment with *B. micrantha* (Table 2) gives best result as it showed quick knock down effect, followed by *C. dependens* (Table 3). While lowest mortality was observed in the treatment with *V. cinerea* at 24 and 48 HAI (Table 4).

Table 3: Percentage adult mortality of cotton stainers treated with C. *dependens extract.*

Treatments	Percentage adult mortality after infestation				
Dosage	24h	48h	72h	96h	120h
0.5ml	0.0±0.0	0.0 ±0.0	17.70±0.23	30.78±1.7	35.00±2.3
1.0ml	0.0±0.0	21.93±2.6	38.78±2.4	39.23±2.2	46.92±2.2
1.5ml	0.0±0.0	17.70±0.3	38.85±2.2	47.30±3.4	54.99±3.8
2.0ml	8.85±0.1	30.78±1.7	40.03±2.1	51.18±2.9	81.15±2.6
LSD (5%)	NS	NS	20.65	18.73	18.55

B. micrantha, 2.0ml treated dish recorded significantly high (P<0.05) adult mortality compared to other dosage rate at 24, 72, 96 and 120 HAI and at 48 HAI *B. micrantha* did not exhibited significant (P>0.05) adult mortality effect (Table 2). Adult mortality of insect exposed to 2.0ml *C. dependens* extract recorded non-significantly difference (P>0.05) at 24 and 48 hours but differ significantly (P<.0.5) at 72, 96 and 120 HAI (Table 3). In dishes treated with *V. cinerea* extract at 2.0ml concentration, adult mortality differ significantly (P<0.05) compared to other dosage rate at 48 72, 96 and 120 HAI, while it was not different significantly (P>0.05) at 24 HAI (Table 4). Among the three plant extracts tested, *B. micrantha* was found to be superior (most toxic) to all other extracts evaluated under laboratory conditions.

Table 4: percentage adult mortality of cotton stainers treated with *V. cinerea* extract.

Treatments	Percentage adult mortality after infestation				
Dosage	24h	48h	72h	96h	120h
0.5ml	0.0 ± 0.0	0.0 ± 0.0	26.56 ± 0.35	30.78 ± 1.9	46.92 ± 2.1
1.0ml	0.0±0.0	17.71±0.2	35.0 ± 2.3	35.0 ± 2.3	46.92 ± 2.2
1.5ml	0.0±0.0	17.71±0.2	54.99 ± 3.8	54.99 ± 3.7	54.99 ± 3.7
2.0ml	17.7±1.4	35.1±2.2	54.99 ± 2.8	54.99 ± 2.2	72.29 ± 4.8
LSD (5%)	NS	21.58	13.79	13.79	18.32

3.3. Effect on oviposition

Table 5: Percentage of eggs hatched after infestation when treated with the three plants.

Treatments	Percentage eggs hatched when treated with different aqueous extracts		
Dosage	*B. micrantha*	*C. dependens*	*V. cinerea*
0.5ml	55.75 ±2.2	64.86 ±3.4	55.55±2.4
1.0ml	58.54±3.8	63.99 ±2.3	53.19 ±3.4
1.5ml	13.06 ±0.5	40.50 ±2.4	28.64 ±1.8
2.0ml	0.0±0.0	11.95±1.2	11.2 ±0.4
LSD (5%)	36.52	49.83	32.47

The results presented in Table 5 show the percentage of eggs hatched. The results revealed that in all the plant extracts evaluated, the lowest dosage rate (0.5 ml and 1.0 ml) did not significantly (P>0.05) reduce percentage egg hatched. While at 2.0ml it greatly suppressed the percentage of egg hatched in dishes treated with *C. dependens* and *V. cinerea* and no egg hatched was recorded at 2.0ml dosage rate in dish treated with *B. micrantha*. From the results, 1.5ml and 2.0ml treated dishes with aqueous extract of *B. micrantha* showed great impact (effect) in reducing cotton stainers oviposition compared to all other extracts at the same dose. Thus, preventing the insects from laying large number of eggs or hatch the eggs laid.

4. Discussion

Cotton stainer, *D. superstitious* (Herrich Schaffer), (Heteroptera: Pyrrhocoridae) is a serious pest in many parts of Tropics and Subtropics attacking cotton and okra plants. In light of recent interest in plant based secondary chemistry into products suitable for integrated pest management, necessitated this study.

The results from this study suggest that plant extracts possess insecticidal bioactivity (i.e., spatial repellency, contact toxicity and nymphs emergence suppression as a result of reduced percentage egg hatched) against cotton stainer. The magnitude of these effects differs among plant extracts and concentrations. Repellency was observed to be dependent upon duration of exposure time and concentration of the extracts. The toxic secondary metabolites present in the extracts were responsible for the repellent as well as toxicological action. The overall results indicated that the insect bioactivity increased proportionally with the increase of concentration of the extract. This supports the findings of Jahromi *et al*. [16] and Sagheer *et al*. [17] in which serial concentrations were made and maximum (82.22%) percent repellency was shown at highest concentrations. This study equally supports that the potential of plant extracts to cause repellency increases with concentration and as a result of the bioactive molecules present in the extracts. But, the results are different for the time factor because in their experiment there is no significant effect of time. They utilized the olfactometer method while in our case; the area preference method was used, which showed a progressive decrease in repellency over the time factor. Obeng-Ofiori [14] reported that filter papers have polar surfaces to which some toxicants when applied may be bound to reduce volatilization and therefore become less effective than non-polar surfaces. This assertion corroborates the findings obtained from this study.

Dekker *et al*. [18] showed that several repellent compounds elicit consistent electrophysiological responses in antennae of *Aedes aegypti*. The irritant effect of a product might be due to its action through tarsi on the nervous system [19]. Some individual compounds of essential oils are clearly detected and avoided by mosquitoes through their antennae. Still, the physiological influence of essential oils leading to repellency remains largely unknown [19, 20]. Deciphering the mechanisms underlying repellency might be challenging since this effect may be due to a synergistic effect of several compounds contained in plant extracts.

Results reveal that the treatments with the aqueous extract from *B. micrantha* gave best result as it shows quick knock down effect, whereas the lower mortality was observed in treatments with *C. dependens* and *V. cinerea*. The aqueous extracts from the plants resulted in statistically equal mean mortality over lowest dosage rate against the cotton stainers. Hernandez and Vendramin [21] reported larval mortality above 80 percent with the aqueous extract of *Melia azedarach* and *Azadirachta indica* against S*podoptera frugiperda*. Statistically equal adult mortality was observed with *C. dependens* and *V. cinerea* in this present study. Verma and Srivastava [22] reported that alcoholic extract of *Solanum indicum* reduced the population of *Macrosiphum rosae* L. Similarly, Oudhia [23] obtained mortality of 23-35 percent against orange-banded blister beetle, *Zonabris postulate* Thunb with the aqueous extract of *L. camara*. *B. macrantha* caused more mortality than *C. dependens* and *V. cinerea*. Atwal and Pajni [24] reported 33.3 and 40 percent mortality against *Pieris brassicae* with alcohol extract (10 and 5%, respectively) of *M. azedarach*. Larval mortality of 22.8 percent with methanol extract of *M. azedarach* against *Plutella xylostella* has been reported by Dilawari *et al*. [25].

The three plants extracts significantly lower the percentage of egg hatched which ultimately result in reduced nymph emergence. This suggests that extracts having comparatively more amount of the chemicals with higher molecular weight have shown more oviposition inhibition. Oviposition deterrency however, appeared to be directly related with polarity of the test plant extracts [26]. Annie Bright [27] and Raja *et al*. [28] stated that when eggs were laid on treated seeds, the toxic substance present in the extract may enter in to the egg through chorion and suppressed their embryonic development. It is in agreement with the present study that adult emergence was greatly reduced in treated seeds than control seeds. Keita *et al*. [29] and Sathyaseelan *et al*. [30] reported that *Ocimum basilicum*, *O. gratissimum*, *Prosophis* spp., *Nerium* spp., *Acalypha* Spp., *Catharanthus* spp. and *Vitex* spp. were effective in reducing oviposition in stored product insects.

Several plants used in traditional medicine such as the plants in this research exhibit some degree of effectiveness towards the activities of pests. These plants could serve as useful sources for new pest control agents for effective use in evolving an ecologically sound, economically viable and socially acceptable pest management system owing to the increase in resistance to conventional synthetic insecticides by many insect pests.

5. Conclusion

Based on findings from this study, the effectiveness of aqueous extracts of the tested plants could be arranged in the following order: *B. micrantha* > *C. dependens* > *V. cinerea*. The study conceptualizes that application of the plant extracts on cotton stainers might reduce infestation and damage caused by the insect to okra and cotton crops. From the study it is recommended that further study needs to be conducted to determine the phytochemical properties of the tested plant species so as to determine their modes of action and also the types of compounds responsible for the activities. There is also the need to test the plants extracts on other crops with arrays of pest complex to determine their effectiveness on other insects.

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