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Potentiality and chemical composition of *Bridelia micrantha* (Berth) extracts and its fractions as biofumigant against economically important stored grain insect pests

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ABSTRACT

Purpose: A study was carried out to determine the potentiality of *Bridelia micrantha* (BM) as biofumigant for the control of some economically important insect pests of stored food grains. **Research Method:** BM powder was sequentially extracted with a series of solvents of increasing polarity in a Soxhlet apparatus and concentrated by the rotatory evaporator. The residues were dissolved in 50ml methanol, assayed for insecticidal activity by fumigant toxicity. Effective and active extract which showed maximum activity was selected for analysis using Gas Chromatography-Mass Spectrometry (GC-MS). **Findings:** Ethyl acetate crude extract and its active fraction (50% hexane: 50% ethyl acetate) showed more potent insecticidal activity with increasing concentration and exposure time. Among the insect species *Tribolium castaneum* is more susceptible and *Rhyzopertha dominica* tolerant of the fumigant toxicity treatments. GC-MS analysis revealed that Dibutyl phthalate (96%), 3-Dodecen-1-al (87%), 13-docosen-1-ol (83%) Ethanol-2-(2-butoxyethoxy) (80%), 2-Butenoic acid, 2 propentl (47%), 4-Hydroxyphenylacetic acid (38%) and Phenyl salicylate (30%) were the major constituents out of the eleven bioactive compounds identified. **Research limitations:** There were no limitations to report. **Originality/Value:** The results suggested that *B. micrantha* may be utilized as a good potential herbal fumigant for the management of stored product-insect pests due to its potent insecticidal activity and chemical composition which contains many different chemicals that have different modes of action on target pests and effective in the conservation of the germinative power of the various food grains.

INTRODUCTION

Post-harvest losses and stored food grains quality deterioration caused by insect pests' infestation have become an increasingly important constraint for achieving food security in developing countries as it may cause serious nutritional and economic losses which can be diverse, intense and can lead to upsetting world peace. Heavy infestations of bruchids cause heating of the commodity, leading to quality loss, mold growth which becomes unsuitable for human consumption, impairment of seeds germination rate, weight loss and reduce the commercial value of the produce

Coleoptera is the largest order of insects and the most common and destructive insect pests of stored food grains and processed products; accounted for 10-90% postharvest losses in many tropical and sub-tropical countries, damage in the stored food grains may reach up to 100% if these insects are not adequately and properly managed and controlled (FAO, 1985; Pajni & Tewari, 2002; Gbaye et al., 2011).

Control of stored product insect pests, primarily relied on the use of insecticides and fumigants which resulted in undesirable effects such as pesticide residues in food, toxicity for humans and the environment (Boyer et al., 2012). Owing to attendant shortcomings associated with the use of synthetic insecticides, it has become imperative and unavoidable to find a substitute that is more ecologically friendly, cheap, and easily available with high efficacy. The use of plant materials with insecticidal properties is a traditional method common among resource-poor farmers in rural areas all over the world (Regnault-Roger et al., 2012; Kedia et al., 2015) and may provide potential alternative to currently used synthetic insecticides due to their rich sources of bioactive chemicals for the management of stored grains insect pests (Kim et al., 2002; Park et al., 2003).

Presently, researchers' attention is being geared towards the utilization of plant materials as grain protectants (Adedire et al., 2011) and the tropical regions are well endowed with different plant species, which are used for medicinal purposes in traditional folks (Ojo & Ogunleye, 2013).

Bridelia micrantha (Euphorbiaceae) is a semi-deciduous to deciduous indigenous plant commonly used as a traditional remedy for various ailments (Atindehou et al., 2004; Ngueyem et al., 2008; Green et al., 2011). However, no studies were available regarding the toxicity of *B. micrantha* against stored products insect pests. Although Adesina et al. (2016) reported the insecticidal efficacy of its aqueous extract on *Dysdercus superstitious* under ambient laboratory condition. To overcome the lacunae of information regarding its toxicity, this study was therefore conceived to assess the biofumigant potential of *B. micrantha* extract as an herbal insecticide and its phytochemical constituents as protectants of stored food grains against infestation by economically important storage beetles with a view of establishing a sustainable management strategy.

MATERIALS AND METHODS

Experimental location and condition

The study was conducted in the Insect Chemical Ecology Laboratory, Institute of Bioresources and Sustainable Development, Takyelpat, Imphal, Manipur, India under ambient conditions of 27±2 ºC and 75±5% RH and a photoperiod of 10:14 (L:D).

Insect culture

The initial stocks of *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) used for the study were obtained from naturally infested wheat and rice

grains from Food Corporation of India warehouse Imphal, Manipur, North East, India (24.81° N, 93.93° E). About 150-200 adults of *T. casteneum, S. oryzae* and *R. dominica* from natural infested food grains were sub-cultured in a glass jar sealed with muslin and reared by maintaining insects on wheat flour with 2% yeast powder and whole wheat grains respectively in the laboratory until the emergence of adults (Mishra et al., 2012).

Collection and preparation of the plant material

Leaves of *Bridelia micrantha* were collected from Owo, metropolis, Ondo State, Nigeria. The identity of plant was confirmed and authenticated at the Department of Forestry and Wood Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria. The leaves were washed with distilled water and air-dried for seven days and milled to powder using a domestic blender. The powdered material was subjected to extraction sequentially with a series of solvents of increasing polarity viz., petroleum ether, hexane, ethyl acetate, chloroform, acetone and methanol for 7-8 hrs in a Soxhlory evaporator under low pressure. Chlorophyll removal from the dark-green residue was done by using activated charcoal. The chlorophyll free filtrate was then concentrated, and the solvent evaporated. The dried residue was a dissolved in respective solvents on weight by volume (w/v) basis, making it 100% stock solution and was stored in sealed glass vials and maintained in a refrigerator (4 ºC) until further use (Murasing et al., 2017). Extract, which showed potent insecticidal activity was selected for the isolation of the bioactive chemical compounds.

Insecticidal activity of *B. micrantha* crude extracts and sub active fractions

The insecticidal activity of *B. micrantha* (Table 1) was carried out by residual film technique against the *S. oryzae*. The residual film was done in a desiccator (0.85-L) that served as the fumigation chamber without any food media. Ten adults *S. oryzae* were placed in each desiccator and a Whatman No. 1 filter paper (9 cm size) was placed to serve as an evaporating surface for injecting extracts. Crude extracts of *B. micrantha* were applied at 5-100 µg L^{-1} which was prepared in dissolved known volume of methanol using a gas-tight microsyringe. Three replicates were set up for each concentration and control. Mortalities were recorded after 24 hrs. Insects were considered dead if they do not respond to probing using a blunt probe. The concentration that caused higher mortality after 24 hrs, was considered potent and selected for the isolation of the insecticide compounds. Insecticidal activity of the active fractions was tested by fumigation on adult *R. dominica, S. oryzae* and *T. castaneum* separately. Ten adult insects of all the species were exposed to a range of doses of active subfractions (5- 0 µg L^{-1}) and its bioactive compounds without food media for 48 hrs in a desiccator (0.85-L) and a Whatman No. 1 filter paper (9 cm size) was placed to serve as an evaporating surface for injecting the extracts. For each species, there were five replicates per dose with an equal number of untreated control replicates. LC_{50} were determined from dose-response data using probit analysis (Finney, 1971).

Insecticidal activity of *B. micrantha* sub active fraction extracts on mixed-age insect cultures

In another experiment, rearing media containing mixed-age cultures of individual species were weighed separately in 50 g aliquots and transferred into cloth bags of size 20 cm × 14 cm. These cloth bags were placed individually in 0.85 L desiccators that served as the fumigation chambers. The desiccators were provided with holes sealed by rubber septa for injecting active subfractions (dissolved in a known volume of solvent) of *B. micrantha*. At the end of the exposure, the test insect bags were taken out of the

desiccators. The contents of the bags were transferred to individual bottles (12×5 cm size) and kept at the rearing temperature and humidity conditions for eight weeks. The insects, which emerged from wheat (*R. dominica* and *S. oryzae*) or survived as adults (*T. castaneum*) in their respective media were checked at weekly intervals for eight weeks. Similarly, counts were made in untreated control batches every week. Percentage kill was determined by taking the survival/emergence in the controls as 100%. In each, bioassay mortality was recorded and those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead.

Repellency bioassays

Bioassays for repellency were conducted following the area preference method (Obeng-Ofiori et al., 1997). In this study, test areas consisted of 10cm Whatman No.1 filter papers cut in half. Different 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 a 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 ten adults *R. dominica, S. oryzae* and *T. castaneum* were placed at the centre of the paper and covered with the dish lid and bounded with paraffin wax (Adesina et al., 2016). Each treatment was replicated three times and laid in a completely randomized design (CRD). The number of insects on the two half of the Petri dish was recorded after four hrs from the beginning of the test. The percent repellence (PR) was calculated by the formula (1):

$$PR = \frac{Nc-Nt}{Nc+Nt} \times \frac{100}{1} \quad (1)$$

where Nc is the number of beetles present in the control half paper, and Nt the number of beetles present in the treated half paper with extract (Inyang & Emosairue, 2005)

Isolation of active subfractions

The active extract was subjected to column chromatography using a glass column (length, 50 cm; diameter, 3 cm) packed with silica gel (60-120 mesh) and eluted with Hexane followed by a stepwise gradient of ethyl acetate, chloroform, acetone and methanol. Three fractions of 300 ml each were collected, concentrated under reduced pressure, and assayed for insecticide activity. Fractions showing insecticide activity were pooled into active fractions and further concentrated. Active fraction was once again loaded on to a silica gel column (length, 50 cm; diameter, 3 cm) eluted with a stepwise gradient mixture of hexane, ethyl acetate, chloroform, acetone awning insecticidal activity were pooled and collected. The purity of the active subfractions was analyzed by using GC-MS.

Gas Chromatography Mass Spectrometry (GC-MS) analysis and identification of bioactive components

The GC-MS analysis of the active subfractions from plant extracts was carried out using Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP-6890 gas chromatograph (Agilent Technologies UK Ltd., South Queensferry, West Lothian EH30 9TG) with an HP5 column (30×0.25 mm id, 0.25 µm film thickness) and an MS detector (Agilent Technologies). The oven temperature was programmed from 70°C (after 2 minutes) to 325°C at 4°C/min. while the final temperature was held for 10 minutes at 240°C. The ion source was set at 240°C and electron ionization at 70 Ev. Helium was used as the carrier gas (1 mi/min). The split ratio was 1:25 with the scan range of 35 to 425 amu. Ethyl acetate methanol fraction (1.0 µL), was manually injected into the GC/MS. The components of the extract were identified

based on the comparison of their retention indices and mass spectra with the standards, the Wiley 275 Library of Mass Spectra database (Wiley, New York) of the GC/MS system and published data.

Seed viability test

Wheat and green gram samples were selected from wholesome seed lot and treated with 50, 100, 200 and 400µg/l doses of the active fractions of *B. micrantha* extracts. The treated seeds were then air-dried and assayed for seed viability test at 24 and 72 h after treatment exposure time. Twenty grains of each seed were randomly selected from each treatment and soaked in distilled water for about 30 min, and kept on filter paper (Whatman No. 1) in a petri dish, moistened daily with distilled water and allowed to germinate at room temperature (25 ± 2ºC). After 48 and 72 h, germinated seeds were counted and percentage germination was calculated as follows (2):

$$\% \text{ Seed viability} = \frac{\text{Number of seed that germinated}}{\text{Total number of seed placed in Petri dish}} \times \frac{100}{1} \quad (2)$$

Five replications each were made for the treatment and the control.

Statistical analysis

All the experiments were laid in Completely Randomised Design with five replications containing ten unsexed insects in each replication and subjected to statistical analysis. Percentage mortality was corrected using the formula suggested by Abbot (1925). The experimental data in percentage were subjected to angular transformation before analysis. Statistical analysis of the angular transform data was done using Fisher's method of Analysis of Variance (ANOVA). Significant treatment means were separated by Tukey Test using a computer programme, SPSS (Version 20) and alphabetic notation was used to denote significant or non-significant differences among the treatment means. Mean lethal concentration (LC_{50}) was computed by following the probit analysis using a computer programme, SPSS (version 20.0).

RESULTS

Insecticidal activity of crude *B. micrantha* extracts

The mean mortality percentage of adult *S. oryzae* to different concentration and exposure periods of *B. micrantha* crude extracts is shown in Figure 1. The extracts killed the adults *S. oryzae* by fumigant action. Fumigant toxicity of *B. micrantha* crude extracts against adults *S. oryzae* gradually increased with increasing exposure time and concentration level; the duration-concentration dependent increased percent mortality was observed in all solvent sources after an interval of different exposure time. Complete mortalities (100%) of adult insects were recorded after 24 hours of exposure time from desiccators treated with 1.0mg/l concentration of crude ethyl acetate extract thus proved to be the most potent fumigant, was selected for further activities.

Fumigant toxicity of *B. micrantha* ethyl acetate extract active fraction

Ethyl acetate extract of *B. micrantha* and its 50% hexane and 50% ethyl acetate fraction showed fumigant toxicity in mild to moderate range against *S. oryzae*, *T. castaneum* and *R. dominica* infestation on stored food grains. Adult mortality varied with concentration, exposure time and insect species. The fumigant properties exhibited by *B. micrantha* may be

due to the volatile chemical compounds identified from the plant (Table 1). Complete mortality (100%) was recorded from all the insect species exposed to the highest concentration (100 µg/l), irrespective of the exposure time. *T. castaneum* exposed to different concentrations of the plant extract showed to be more susceptible and *R. dominica* tolerant to the fumigant toxicity (Table 2).

Repellence activity of *B. micrantha* ethyl acetate extract active subfraction

B. micrantha ethyl acetate extract active fraction (50% hexane: 50% ethyl acetate) had a repellent effect against adults of *T. confusum, S. oryzae* and *R. dominica*. This is due to the odours emitted by plants, which inconvenienced the insect pests; whereas the insect species significantly oriented away from the treated surface in the direction of the non-treated surface. In general, the repellent activity showed a clear dose-dependent relationship, repellency of the subfraction increased with increasing concentrations. The higher concentration induced the maximum percentage repellency of 64.66, 73.46 and 77.36% against *T. confusum, S. oryzae* and *R. dominica* respectively Whereas, the minimum percentage of repellency were recorded at the lower concentration (Table 3). All the tested concentrations variously repelled the tested insects and ultimately conferred protection on stored food grains against infestation and damage.

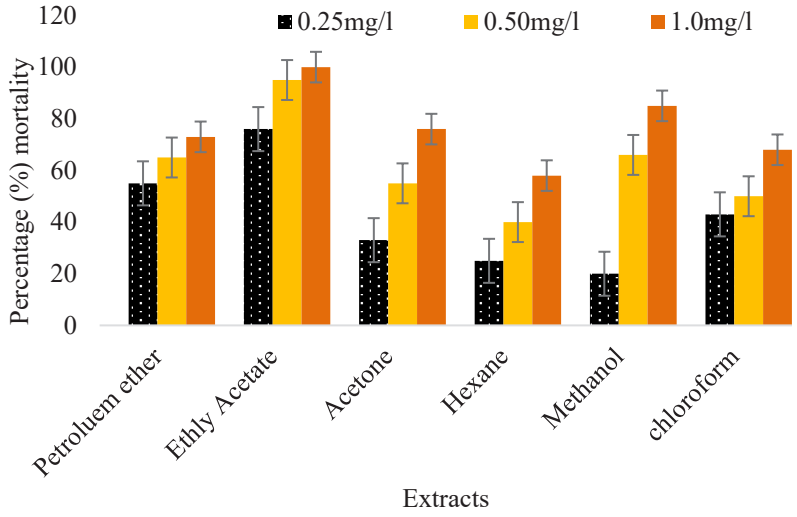

Fig. 1. Insecticidal activity of crude *B. micrantha* extracts

Table 1. Mean Adult mortality of *B. michranta* Ethyl acetate extract active subfractions against some economically important stored grain insect pests

Dosage (µg L^{-1})	% Adult mortality (Mean ± SE)					
	R. dominica		*S. oryzae*		*T. castaneum*	
	24 h	48 h	24 h	48 h	24 h	48 h
0	0.0± 0.0^{f}	0.0 ±0.0 ^{f}	0.0±0.0 ^{f}	0.0±0.0 ^{f}	0.0±0.0 ^{f}	0.0±0.0 ^{f}
10	5.00±2.39^{a}	6.53±0.97^{a}	4.62±2.81^{a}	5.46±2.21^{a}	7.94±1.33^{a}	9.92±2.06^{a}
20	18.49±0.52^{b}	26.56±0.47^{b}	18.11±1.44^{b}	25.81±0.43^{b}	19.98±0.7^{b}	33.22±0.38^{b}
40	23.56±0.45^{c}	47.8±1.31^{c}	30.26±0.38^{c}	53.8±0.16^{c}	26.74±2.18^{c}	49.7±2.4 ^{c}
60	45.21±2.16^{d}	72.5±4.79^{d}	42.15±2.5^{d}	75.03±2.89^{d}	55.57±2.46^{d}	80.0±2.84^{d}
80	75.0±2.89^{e}	92.0±2.93^{e}	77.31±0.86^{e}	95.83±2.89^{e}	84.9±3.4 ^{e}	97.55±2.28^{e}
100	100.0±0.0^{f}	100±0.0 ^{f}	100.0±0.0^{f}	100±0.0^{f}	100.0±0.0^{f}	100 ±0.0^{f}

Each value is a mean ± SE of five replicates. Mean within the column followed by different supper script(s) alphabet are significantly different at ($P \leq 0.05$) using Tukey's test.

Chemical composition of sub active fraction of *B. micrantha* extract

Table 4 shows the chemical composition of the active subfraction (50% hexane: 50% ethyl acetate) of *B. micrantha*. Result revealed that Dibutyl phthalate (96%), 3-Dodecen-1-al (87%), 13-docosen-1-ol (83%) Ethanol-2-(2-butoxyethoxy) (80%), 2-Butenoic acid, 2 propentl (47%), 4-Hydroxyphenylacetic acid (38%) and Phenyl salicylate (30%) were the major constituents out of the eleven bioactive compounds identified from *B. micrantha* active fraction.

Effect of grain protectants on seed viability

The bioactive compounds derived from the active fraction of *B. micrantha* extract (50% hexane: 50% ethyl acetate) were observed to be effective in the preservation of the germinative power on the treated grains (Table 5). All the treated grains germinated successfully within the range of 90-97.67% and it can be deduced that the active fractions of the plant extract used for control of storage beetles in this trial did not have any negative impact on the germinative ability of the grains.

Table 2. Fumigant activities of B. micrantha ethyl acetate extract active subfractions against some economically important stored grain insect pests

Insects	LC_{50}	LC_{90}	Slope ± SE	Chi-square
T. castaneum	68.39 (55.33 – 77.42)	212.63 (235.08 -381.75)	0.93±0.26	34.94
S. oryzae	74.53 (60.36 – 84.64)	384.28 (118.09 – 405.22)	0.73±0.65	40.05
R. dominica	96.50 (83.29 – 110.54)	266.93 (238.44 – 316.45)	0.52±0.16	62.51

Values in parenthesis represent confidence limits.

Table 3. Repellence effect of *B. micrantha* ethyl acetate extract sub active fraction against economically important stored product insect pests

Percentage Repellence (%) Concentration (mg/cm^2)	*T. castaneum*	*S. oryzae*	*R. dominica*
0	0 ± 0.0^f	0 ± 0.0^d	0 ± 0.0^e
0.080	9.87 ± 1.72^a	10.33 ± 2.08^a	9.68 ±1.6^a
0.158	14.93 ±1.76^b	16.33 ±2.03^{ab}	18.33±2.01^{ab}
0.396	38.68 ±2.41^c	40.48±1.45^b	31.00 ±2.73^b
0.793	46.33 ±1.20^d	42.59±2.7^b	44.33 ±1.73^c
1.587	64.66 ±2.48^e	73.46 ±2.95^c	77.36 ±2.9^d

Each value is a mean ± SE of five replicates. Mean within the column followed by different supper script(s) alphabet are significantly different at (P≤0.05) using Tukey's test.

Table 4. Bioactive chemical composition of active fraction of *Bridelia micrantha*

Compounds	RT	Molecular weight	% match	Abundance
2-Butenoic acid, 2 propentl	3.355	126	47	>70k
Phosphoric acid	6.222	152	11	30k
Decanal	7.514	156	7	40k
Ethanol-2-(2-butoxyethoxy)	8.102	162	80	50k
3-Dodecen-1-al	10.475	182	87	>60k
Fumaric acid, butyl ester	11.591	200	22	>70k
Phenyl salicylate	12.590	214	30	>60k
7-Hexadecanone	15.293	240	20	62k
Dibutyl phthalate	17.337	278	96	>60k
4-Hydroxyphenylacetic acid	18.054	294	38	70k
13-docosen-1-ol	21.461	324	83	60k

Table 5. Seed viability percentage of wheat and green gram treated with different concentrations (mg L^{-1}) *B. micrantha* active fractions

Dosage (µg L^{-1})	% Germination (Mean ± SE)			
	Wheat		Green gram	
	24 h	72 h	24 h	72 h
50	90.00±2.71[a]	93.33±1.23 [a]	91.67±2.86 [a]	95.89±1.62[a]
100	92.50±1.64 [a]	97.53±2.09 [a]	94.33±1.36 [a]	96.67±3.16[a]
200	93.58±2.72 [a]	96.89±0.84 [a]	95.83±2.64 [a]	97.33±3.38[a]
400	91.67±2.68 [a]	94.38±1.67 [a]	90.20±1.82 [a]	94.55±2.36[a]
Control	96.84 ± 2.13[a]	94.37 ± 2.58[a]	95.84±2.39[a]	97.67±2.72[a]

Each value is a mean ± SE of five replicates. Mean within the column followed by the same supper script(s) alphabet are not significantly different at ($P \leq 0.05$) using Tukey's test.

DISCUSSION

Plant products or their constituents have considerable potential as stored grains protectants due to their broad spectrum of activities against insect pests. The insecticidal activity of the plant extract and its active subfractions is dependent on its active chemical constituents and the gross sensitivity of the target stored products insect pest to the active chemical principles (Obeng-Ofiori et al., 1997). This present result of mortality is similar to the work of Khalequzzaman and Khanom (2000) who reported that ethyl acetate extract of Neem (*Azadirachta indica*) leaf showed lowest LD50 value on 5 instar larvae, indicating more toxic action.

The variation experienced for the insecticidal activity of the crude plant extracts could be explained by the fact that a non-polar solvent was incapable to extract some polar compounds and also the structure of some polar molecules were able to bind to the non-polar molecules, thus extracting the plant volatiles differently (Abdoulaye et al., 2018). Mahmoudi et al. (2013) stated that polarity of solvent used and their solubility determines the nature of the compounds present in each plant extract.

The toxicity of plant materials to stored product insects is influenced by the chemical composition of the products and plant part to be used (Lee et al., 2001). This can vary dramatically, even within the same species sources. Higher concentration might be containing more active volatile constituents that were easily received by insect olfaction and caused rapid suffocation to them in comparison with what was obtainable in lower concentrations. In the current study, the chemical composition of the plant active fraction was different from what was obtained from the study conducted by Green et al. (2011) on the n-Hexane subfraction of the same species collected from South Africa. These differences might arise from environmental (climatic, seasonal and geographical), genetic and chemotype differences and nutritional status of plants (Perry et al., 1999). The phytochemical compounds in these plant extracts might have acted like anti-nutrients hindering assimilation of the nutrients (Zijp et al., 2000), in the insect nervous system by disorganizing ion exchange of sodium and potassium, causing insect death (Rajashaker et al., 2012; Zimudzi et al., 2014). Methanolic extracts of *Securidaca longepedonculata* roots contain methyl salicylate acid, which is a volatile compound at 90%, which would be a real natural pesticide against *C. maculatus* (Ojewole, 2008).

The result of fumigant activity has shown that all insect species were significantly susceptible to the varying concentrations of the extracts' active fractions compared with control and proved to be promising as a control against stored-product insects. It is observed that there are variations in the activities of the plant extract active fractions as considerable differences in mortality of insects' due to extract treatment, were observed in different concentrations and exposure times. The mortality of the insects seems to be due to the

asphyxiation and inhibition of different biosynthetic processes of insect metabolism (Obeng-Ofori et al., 1997). Mishra et al. (2016) concluded that the mode of toxicity of plant products used as phyto-insecticides is through competitive inhibition of acetylcholinesterase and their rapid action against insect pest is suggestive to their neurotoxic mode of action interfering with neuromodulator octopamine (Pooja et al., 2012).

The insect species mortality increased with rising concentrations and exposure times. The observed variability among insect species susceptibilities to the plant extract and its active fractions is a common phyto-insecticides phenomenon. *T. casteaneum* and *R. dominica* more susceptible and tolerant to the toxic effect of the plant extract at all doses and exposure times, respectively. The insect species, differential susceptibility to the active fraction of the extract might be as a result of the difficulties in airflow into respiratory structures of the insects. This upholds El-Nahal et al. (1989) findings on the insecticidal activity of *Acorus calamus* essential oil on adults of five stored-product insect species. In this study, the exposure period seemed to be the utmost important factor affecting the toxic effects of the fumigants rather than the dosage. The toxicity effect of the plant derivative increased with ascending exposure period for all the three insect species. The increase in adult mortality according to ascending exposure period and dosage was due to the increase in the quantity of active ingredients contained in the plant extract.

The repellency effects of the active fractions at the varying concentrations showed the significant difference and were concentration dependent. Results were in accordance with the findings of (Hill & Schoonhoven, 1981; Desmarchelier, 1994) reported that plant products are known to possess repellent activities against stored-product insects. It also affirms the findings of Ko et al. (2009); Cosimi et al. (2009) who reported that repellent activity of plant materials was concentration-dependent, with the highest concentration showing more repellent activity. The evaluated plant extract had a repellent effect against adults of *T. confusum, S. oryzae* and *R. dominica* due to the odour emitted by the plants, which inconvenienced the insect pests; whereas the insect species significantly oriented away from the treated surface in the direction of the non-treated surface. This supports Cox (2004), who opined that repellents could be used to provide protective bands around grain bulks or incorporated into packaging materials to inhibit invasion by pests to prevent insect feeding and/or oviposition.

The germinability of treated seeds and control observed through the standard germination test indicated that the seed germination was above 90% and the treatments showed no significant adverse effect on the seed viability. The results revealed that, all the seeds treatments were found to be effective in maintaining the quality of seed and in controlling the stored beetle infestation, as no abnormality was observed. This indicates that, the plant extracts do not have any adverse effect on seed viability. The findings conclude that these active fractions of the plant extracts used as grain protectants are relatively safe for the grains stored for seed purposes. Findings concur with those of, other researchers who used different plant products as grain protectant and observed no adverse effect of these products on the viability of the treated grains (Trematerra & Hanzotli. 1999; Rajendra et al., 2014; Khinchi et al., 2017). The observed no significant seed impairment recorded in this study may be due to the insecticidal properties and bioactive molecules present in the plant (Schmidt et al., 1991). Renugadevi et al. (2006) opined that active principle β asarone derived from Vasambu rhizome powder prevented bruchid infestation and maintained the viability of seeds.

CONCLUSION

The insecticidal activity of the plant extract and its active fraction might be ascribed to the high volatility exhibited through fumigant activity that might be of great significance for controlling stored product insects. Results of this study indicate that *B. micrantha* extracts might be useful for managing coleopterous insects in enclosed spaces such as storage bins, glasshouses, or buildings because of their fumigant action. The results obtained suggest that crude ethyl acetate extract and its active fractions are promising biofumigants and the plant may be explored as good potential natural fumigant and alternative to chemical insecticides in storehouses to minimize the infestation and damage caused by storage beetles due to their insecticidal activity and chemical composition which contains many different chemicals that have different modes of action on target pests and effective in the conservation of the germinative power of the various stored food grains; likewise ensuring steady grain supply during off-season thus guarantee food security.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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