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Bioactive chemical composition and insecticidal potential of *Mitracarpus villosus* [Swartz] DC for the management of stored product beetles

Jacobs Mobolade Adesina, Yallappa Rajashekar, Thomas Inomisan Ofuya and Michael Olufemi Ashamo

Abstract
Control of insect pests attacking stored food grain has become a major concern in the developing nations to ensure food security. A study was carried out to explore the biochemical composition and insecticidal potential of *Mitracarpus villosus* as grains protectant for the post-harvest protection of stored grains. Results revealed that the plant possess insecticidal properties as the fumigant and repellent activity was significantly ($p<0.005$) influenced by dosage, insect species and duration of exposure. It was also observed that in all the treatments *Tribolium castaneum* was more tolerant to the plant active fractions than other insects. The GC-MS analysis revealed the presence of Methyl 20-methyl-heneicosanoate, Heptadecanoic acid, Tetracosanoic acid, 2-Bromododecane, 7-Hexadecenioc acid and Nonane, 2, 6-dimethyl as the principal chemical compounds present in the sub active fractions. The bioactive compounds did not elicited any adverse effect on seed viability. In conclusion, the research findings reveal that the plant could be successfully developed as a natural biofumigant and incorporated as a promising grain protectant for the control of storage beetle infestations.

Keywords: active fractions, biofumigant, grain protectant, seed viability, *Tribolium castaneum*

Introduction
Stored food grains suffer heavy loss due to insect pest infestation leading to economic damage and deteriorates the quality of food grains and food products. *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* ranks as the most important pest of stored cereals worldwide. The larvae and adult cause damage to grains by feeding inside them causing maximum seed damage, weight loss and infested seeds are less nutritious and unfit for human consumption (Rawat and Srivastava, 2011) [20].

Management of stored beetles against insect infestation is known currently to depend on the use of various insecticides and fumigants (Park *et al*. 2003) [18]. These chemicals are effective for insect pest control but produce several adverse effects on the consumers and environment due to their indiscriminate and enormous use. These drawbacks have necessitated the search for sustainable alternatives that are readily available, affordable and less detrimental to the environment (Ileke *et al*. 2013) [13].

Recently, researchers have focus considerable attention towards screening plant secondary chemical compounds for developing insecticides for the control of stored product insect pests and various findings has confirmed that plant bioactive compounds are capable of controlling insect pests extending sub lethal effects on their biological processes (Awoyinka *et al*. 2006, Rahman & Talukder 2006, Tarigan *et al*. 2016) [8, 19, 21]. The use of plant extracts and plant products is gaining attention due to proven specificity, low toxicity to non-target organisms and low residual toxicity in the ecosystem and may offer sustainable, biodegradable environmentally friendly and safer alternative to synthetic insecticides by acting as promising grain protectants.

Mitracarpus villosus [Swartz] DC (Family: Rubiaceae) is an annual herb which is widely used in the traditional system of medicine as an antibiotic for the management of several disease conditions (Jegede *et al*. 2005, Abere *et al*. 2007, Adamu *et al*. 2017) [14, 3, 5]. The plant has been reported to contain various classes of secondary metabolite which are responsible for its bioactivity (Ekpendu *et al*. 1994, Jegede *et al*. 2005, Aboh *et al*. 2015) [11, 14, 4]. In spite of various chemical compounds isolated from this plant, most of the reported

bioactivity studies focused only on the antimicrobial activity ignoring completely its insecticidal potentials.

Hence the present study was taken up to investigate the potential fumigant toxicity and repellent action of *M. villosus* bioactive chemical composition as a food grains protectant against stored beetles infestation.

Materials and Methods

Matured *M. villosus* was collected from Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria, washed with tap water, shade dried at room temperature and powdered using electrical blender. Extract from the pulverized plant materials was obtained with the help of a Soxhlet apparatus, sequentially with a series of solvents of increasing polarity viz., petroleum ether, hexane, ethyl acetate, chloroform, acetone and methanol for 7-8 hrs. The extracts were evaporated and concentrated with rotary evaporator under low pressure, below 60 ˚C to make it solvent free.

Adult *S. oryzae*, *R. dominica* and *T. castaneum* were collected from the infested wheat grains obtained from local grocery store at Ima Keithel market, Manipur, Imphal, India. These insects were reared with the clean and uninfected wheat grains in a glass jar at 30 °C ± 1 °C, 70 ± 5% relative humidity (RH) with sufficient aeration. The cultures contained all developmental stages of the respective species and the pooled mixture of individual species served as mixed-age cultures for the plant extract fumigant activity tests.

The active extract (10g) 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 and methanol. Ten fractions of 300 ml each was 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 and methanol. The active sub-fractions showing insecticidal activity was pooled and collected. The purity of the active sub fractions was analyzed by using GC-MS.

The GC-MS analysis of the active fractions of *M. villosus* bioactive chemical compounds were analyzed using an Agilent Technologies 7890A gas chromatograph equipped with an Agilent Technologies 5975C inert XL EI/CI mass selective detector operated in electron impact mode. The HS-SPME medium polar fiber was conditioned for 15 minutes at 250 °C, prior to the experiment to fully remove any contaminants. Helium was used as the carrier gas at a flow rate of 1ml/min and detector gases were hydrogen and air. Initial column oven temperature was 40 °C for 3 minutes and then it was increased at 10 °C/min up to 280 °C, where it was held for 3 minutes and maintained constant for 16 minutes. The sample was analyzed in the full scan mode with a scan speed of 10,000 amu/s, a mass range of 40–300 *m/z* and a sampling frequency of 25 spectra/s; interface and ion source temperatures were 250 and 200 °C, respectively. Identification of the bioactive components in the sample was based on retention times and Mass spectra were compared with those in the Wiley 275 L mass spectral library.

The insecticidal activity of *M. villosus* extracts dissolved in a known volume of methanol was carried out by fumigant toxicity against *T. castaneum* at varying concentrations (5 - 100 µg/ L) in desiccators (0.85-l) that served as the fumigation chambers. Ten adult *T. castaneum* were released into each desiccator, a Whatman No. 1 filter circle (9 cm size) was placed to serve as an evaporating surface for injecting extracts. For each concentration there were five replicates with equal number of untreated control replicates. Active extract (Petroleum ether), which showed maximum fumigant activity, was selected for the isolation of the insecticide compounds.

In another experiment fumigant activity of the active sub fractions and its bioactive compounds were tested on *R. dominica*, *S. oryzae*, and *T. castaneum*. The adult insects were exposed to a range of doses of active sub fractions and its bioactive compounds (5 - 40 µg/ L) for 24 h in desiccators (0.85-l) that served as the fumigation chambers under ambient laboratory conditions. For each insect species, there were five replicates per dose with an equal number of untreated control replicates with a Whatman No. 1 filter circle (9 cm size) placed to serve as an evaporating surface for injecting extracts. Gas-tight micro syringes were used for injecting the fumigant. The death rate of pest was observed after 24 h of exposure and LC_{50} were determined from dose response data using probit analysis (Finney, 1971) [12].

Repellence effect of the plant bioactive compound was conducted by dividing filter papers (Whatman N∘ 1, diameter 9 cm) into two halves and were treated with 0.5ml 50:50 petroleum ether and ethyl acetate sub fraction applied as uniformly as possible with a pipette to one half of the filter paper. The treated filter papers were air dried, and then placed at the centre of each Petri dish. Ten adult insects were released in the middle of each disk and covered with plastic tape with some holes to prevent insects from escaping. (Ambadkar & Khan, 1994) [11]. The numbers of insects that settled on each half of paper disks were recorded after 2 and 4 h from the beginning of the test. Percentage of repellency (PR) was calculated as follows and the repellency (%) was calculated by following formula:

$$\text{Repellency } (\%) \frac{C-E}{T} = \times 100$$

The study was arranged in a Completely Randomised Design and replicated five times. Percentage motarlity data were transformed using arcsine transformations prior analysis and were later subjected to one way analysis of variance (ANOVA). Significant differences between treatments were determined using Tukey's multiple range test at $P \leq 0.05$ with SPSS statistical analysis software (Version 22.0). Probit analysis was equally done to determine the LD_{50}.

Results

Results from the insecticidal activity tested by fumigation against *T. castaneum* showed that *M. villosus* extracted with petroleum ether was more effective in exerting adult mortality 24 h after infestation than other solvent sources. The insect mortality increased significantly with increase in dosage (Fig 1). Thus, the results obtained suggest good potential for the use of *M. villosus* for insecticidal activity.

Fumigant activity of sub active fraction of *M. villosus* extract clearly demonstrated that the plant bioactive compounds possessed potent fumigant activity against the

stored grains beetles (*S. oryzae*, *R. dominica* and *T. castaneum*) as all the treatments were found significantly superior in mortality of the beetles compared to control (Table 1). Insect mortality was found to be dosage-exposure time dependent as the sub active fraction dosage and exposure period increases mortalities also increased with stored grains beetles exposed to 100 µg/l resulted in 100% mortalities within 48h of treatment. It was also observed that insecticidal activity of the plant extract was significantly (p<0.005) influenced by insect species; in all the treatments *T. castaneum* was more tolerant than other insect pests while *S. oryzae* was more susceptible at 24h in all treatments.

The active fraction of *M. villosus* extract had LC50 values of 34.49, 24.96, 29.15 µL/L and LC99 61.10, 54.18 and 60.73 µL/L against *T. castaneum S. oryzae* and *R. dominica* respectively. In all the treatments *S. oryzae* was more susceptible than other insect pests (Table 2).

The bioactive chemical composition of the active fractions (petroleum ether 50: ethyl acetate 50) of *M. villosus* was summarized in Table 3. A total of 13 bioactive chemical composition were identified and the principal chemical compounds in the active sub fractions were Methyl 20-methyl-heneicosanoate (99%), Heptadecanoic acid (95%), Tetracosanoic acid (93%), 2-Bromododecane (86%), 7-Hexadecenioc acid (78%), Nonane, 2, 6-dimethyl (64%), 5-Butyl nonane (60%), Dodecane, 2,6-methyl (43%), Tetradecane, 4-Methyl (43%) and Octane, 3-5-dimethyl decane (41%). The toxicological effects of the plant extract can be attributed to the presence of the various chemical components identified as the main bioactive volatile components and act as insecticides to the stored grains insect pest.

The repellency rates of the insect pests that visited the stored food grains impregnated with *M. villosus* sub active fraction were influenced by the concentrations of extracts as shown in Table 4. Repellency rate increased proportionally with doses which translated that the highest concentration of 1.587 mg/cm^2 significantly (P<0.005) repelled more of the insect compared the lowest concentration of 0.080 mg/cm^2 (Table 4). Also the results indicates that repellent activity of the *M. villosus* sub active fraction varied depending on the insect species with intervals of time.

Discussion

M. villosus is a promising food grains protectant with regards to the use of botanicals as both the crude plant extracts and its chromatographic sub active fractions produced toxic effects by fumigant activity which is strategic for the management of food grains insect pests as fumigants are able to penetrate deeper into storage packaging and structures. It is evident that, duration of exposure, insect species and the dosage of plant extract played an important role in exerting the lethal effects and this support the findings of Abdullah *et al*. (2017) [1].

Repellency is an important property to be considered in controlling insect pests of stored food grains, because the higher the repellency, the lower the infestation and percentage grains damage and weight loss. The sub active fraction of *M. villosus* extract exhibited repellent effect at the different doses used in relation to insect species because there is positive correlation between repellency and doses in which repellency increased with increase of doses. This is an indication of the presence of bioactive chemical substances present in the plants that makes the insect move away from the source of the stimulus. This is consistent with the findings of Ukeh and Umoetok (2011) [22], Ogbonna *et al*. (2014) [17] in which bioactive compound isolated from *Zingiber officinale* is found to be good repellents to *T. castneum, R. dominica* and *Prostephanus truncatus.* Jemaa and Khouja (2011) [15] also substantiate the fact that some plants secrete volatile substances that lure insect away from them.

The observed insecticidal and repellent properties might be attributed to the synergistic effects of its wide array of major and minor bioactive components and this study authenticates the earlier findings by Abdullahi *et al*. (2011) [2], Degri and Mailafiya (2013) [10], Adesina and Ileke (2014) [6], Adesina (2014) [7] and Mamadou *et al*. (2014) [16] who all reported reported the insecticidal activity of *M. villosus* against *Culex quinquefasciatus* larvae, *Helicoverpa armigera*, *Podagrica* spp, *Acanthomia tomentosicoliss* and stored grains insect infestation respectively. The reduction in the boring, sucking and feeding activities of these insect pests might be partly due to their repulsion by *M. villosus* extract (Babarinde *et al*. 2008) [9]. While, Yusuf and Muhammed (2009) [24] attributed the reduction in *Callosobruchus maculatus* infestation in stored cowpea to the strong pungent odour and taste of *M. villosus* leaf powder.

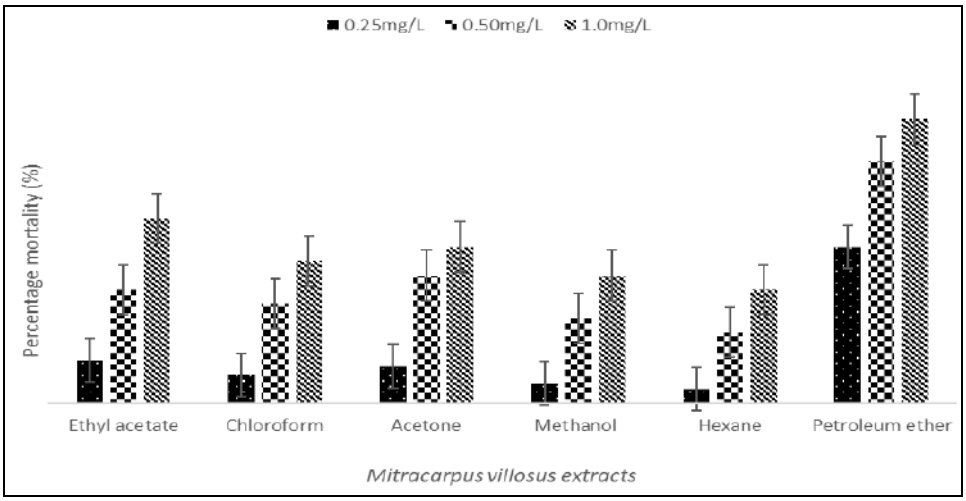

Fig 1: Insecticidal activity of crude *M. villosus* extracts

Table 1: Percentage mortality of stored-product beetles exposed to active sub fractions of *M. villosus* petroleum ether extract

Dosage (µg/l)	% 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	3.7±1.3^{a}	4.3±1.1^{a}	5.2±1.8^{a}	7.6±0.8^{a}	3.4±0.3^{a}	4.9±0.6^{a}
20	15.9±2.2^{b}	25.6±2.8^{b}	17.1±1.4^{b}	30.8±2.1^{b}	16.8±1.2^{b}	29.8±2.8^{b}
40	28.2±2.9^{c}	45.8±2.3^{c}	34.6±3.6^{c}	54.8±3.6^{c}	26.4±3.8^{c}	49.7±2.4^{c}
60	58.1±3.1^{d}	74.7±3.9^{d}	55.1±2.6^{d}	78.9±3.9^{d}	54.7±1.6^{d}	82.1±2.4^{d}
80	72.8±2.8^{e}	85.1±2.3^{e}	76.1±2.6^{e}	93.3±3.8^{e}	68.9±3.4^{e}	89.7±2.2^{e}
100	82.6±3.3^{f}	100±0.0^{f}	91.2±2.8^{f}	100±0.0^{f}	80.8±3.1^{f}	100 ±0.0^{f}

Values represent Mean of five replicates ± SE and value followed by different alphabets in columns are significantly different using Tukey's test ($P \leq 0.05$).

Table 2: Insecticidal activity of active fraction from *M. villosus* against three stored- product insects by fumigant toxicity

Insects	LC_{50}	LC_{90}	Slope ± SE	Chi-square
T. castaneum	34.14	61.10	3.13±0.57	24.59
S. oryzae	24.96	54.18	2.73±0.65	10.15
R. dominica	29.15	60.73	3.02±0.82	18.32

Table 3: Bioactive chemical composition of active fraction of *M. villousus*

Compounds	RT	Molecular weight	% match
Octane, 3-5-dimethyl decane	5.329	142	41
Nonane, 2, 6-dimethyl	6.703	156	64
1-Dodecene	8.254	168	15
5-Butyl nonane	9.594	184	60
Dodecane, 2,6-methyl	12.484	212	43
Tetradecane, 4-Methyl	12.649	212	43
1-Hexadecene	13.565	224	42
2-Bromododecane	15.128	248	86
Pentadecanoic acid	15.88	256	49
7-Hexadecenioc acid	16.691	268	78
Heptadecanoic acid	17.877	284	95
Methyl 20-methyl-heneicosanoate	22.389	354	99
Tetracosanoic acid	24.892	382	93

Table 4: Repellence effect of sub active fraction of *M. villosus* petroleum ether extracts against stored product bettles

Percentage Repellence (%)			
Concentration (mg/cm^2)	*T. castaneum*	*S. oryzae*	*R. dominica*
0	0 ± 0.0	0 ± 0.0	0 ± 0.0
0.080	12.8 ± 2.2^{c}	14.3 ± 2.8^{e}	26.8 ± 3.4^{e}
0.158	51.3 ± 3.4^{bc}	47.6 ± 3.8^{d}	49.4 ± 3.1^{d}
0.396	63.8 ± 2.3^{b}	55.8 ± 2.7^{c}	69.0 ± 3.3^{c}
0.793	88.2 ± 3.1^{ab}	76.1 ± 2.9^{b}	84.8 ± 3.7^{b}
1.587	94.6 ± 2.8^{a}	93.4 ± 3.5^{a}	95.6 ± 3.9^{a}

Values represent mean of five replicates ± SE and value followed by different alphabets in columns are significantly different using Tukey's test ($P \leq 0.05$).

Conclusion

1. Bioactive compounds identified from the plant is responsible for the strong fumigant and repellent effects exhibited against the storage beetles.
2. The plant could be successfully developed as a natural biofumigant and incorporated as a promising grain protectant for the control of storage beetle infestations.
3. For the plant extracts and their bioactive compound to be adopted as novel food grains protectant, further research is required to determine its safety for human health.

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