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Original Contribution

BIO-ACTIVITY OF THREE ESSENTIAL OILS EXTRACTED FROM EDIBLE SEEDS ON THE RUST-RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST.) INFESTING STORED PEARL MILLET.

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ABSTRACT
The ovicidal, repellent and toxic activities of three essential oils extracted from clove, *Syzgium aromaticum* (L.) Merril and Percy; West African black pepper (WABP), *Piper guineense* Schum and Thonn. and Ethiopian pepper, *Xylopia aethiopica* (Dunn) A. Rich were evaluated against the rust-red flour beetle, *Tribolium castaneum* (Herbst.) under laboratory conditions (20 – 39^{O}C; 41 – 58% RH). Five pairs of 2- to 3-day old adult *T. castaneum* were allowed to oviposit for 20 days on 20 g of 20% broken pearl millet grain contained in 50 ml glass jar with a lid. After 20 days, all dead and live insects were removed from the jars. Thereafter, four rates (0.75, 1.50, 2.25 and 3.00 mg) of each essential oil carried in 2 ml analytical grade acetone were used to treat the seeds and progeny emergence was recorded for three generations. The same rates of essential oil as described above were assayed against thirty larvae and adults *T. castaneum* as repellant on a 30 cm^3 Whatman No. 1 filter paper. The number of insects repelled on both treated and untreated half discs of filter paper was recorded after 30 min. Both the ovicidal and repellent treatment were set up in a randomized complete block design with a split plot arrangement and replicated three times. In the toxicity study 30 unsexed adults and larvae of *T. castaneum* were treated with the same rates of essential oils carried in 2 ml analytical grade acetone and admixed with 20 g of 20% broken grain in a 50 ml glass jar with a lid. Adult and larval mortality was taken 24 h after and results were subjected to probit analysis. The experiment was carried out in a complete randomized design replicated three times. All the control batches were treated with 2 ml of pure analytical grade acetone alone. The three essential oils were able to reduce progeny emergence of *T. castaneum* significantly than in the control treatments. Higher number of adults emerged in Ethiopian pepper than in clove and WABP. There was however no significant difference ($p \geq 0.05$) between clove and WABP. The efficacy of the dosage rate was dose dependent as less number of adults emerged in higher concentration than in lower concentrations. The three essential oils evoked classes II - V repellency rates. There was no significant difference ($p \geq 0.05$) between the three oils, but the dosage rates were statistically different ($p \leq 0.05$) from each other. Values for repellency test against adult *T. castaneum* ranged for clove oil between 83.20 – 99.62%, WABP 88.50 – 99.62% and Ethiopian pepper 91.70 – 97.70%; while in the case of repellency for larva, 61 -100% for clove; 25.60 – 100% for WABP and 40.50 – 86.30% for Ethiopian pepper. In the toxicity test, the entire essential oil caused 100% mortality of both adult and larva of *T. castaneum* albeit at higher dosage rates. The LC_{50} for clove was 0.40 (adults), 0.46 (larvae); WABP 0.21 (adults), 0.54 (larvae) and Ethiopian pepper 1.78 (adults), 0.67 (larvae)/ 20 g seed.

Keywords: *Tribolium castaneum*, essential oils, repellency, toxicity, LD_{50}, *Syzgium aromaticum*, *Piper guineense, Xylopia aethiopica.*

INTRODUCTION

Pearl millet, *Pennisetum glaucum* (L.) R. Br., is the most important millet grown in Nigeria and constitutes the major staple diet for the millions of people inhabiting the sahelian parts of tropical Africa and Asia (1). Nigeria produces 4.67 million tones of millet annually (2). Millet is threshed by pounding harvested heads in a mortar with a pestle followed by winnowing. The grains are then stored for varying lengths of time. This method of threshing produces a mixture of broken and

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sound grains. Breakage predisposes millet to infestation by the rust-red flour beetle, *Tribolium castaneum* (Herbst.) (3).

T. castaneum is a serious secondary pest of stored cereals in tropical and subtropical regions of the world requiring prior infestation by an internal feeder, or some form of mechanical damage (Haines, 1991). Both adults and larvae are highly mobile in patches of flour and broken gains (4). Ability of the females to lay more or less continuously throughout their life span result in high population in the substrate it colonizes (5).

The infestation of stored pearls millet by *T. castaneum* results in substantial losses in weight and quality (6). For instance, world annual post harvest losses due to *T. castaneum* range between 25 and 40% (7); and in Nigeria it has been reported that about 10% loss in stored grains each of pearl millet and sorghum are due to infestation by *T. castaneum* (8). These losses thus necessitated control actions so that the resource-poor farmer can continue to have more food and better standard of living. The protection of grains and seeds in storage is an ancient practice (9) in which dry soil and wood ash were mixed with grains causing lethal dehydration of insect and application of some certain plant materials with grains to serve as fumigants (10). However, in the past few decades, application of synthetic broad spectrum pesticides to control stored products pest species has been a standard practice (11). These practices are however attenuated with attendant health and environmental hazards (12). The application of botanical insecticides for the protection of cereals and pulses is currently one of the major foci of research in many developing countries (13). The botanical insecticides causes less damage to human health and environment, degrade rapidly, do not accumulate in the body and environment, while some are very specific and do little or no damage to other organisms (14).

Products from edible spices have been screened for their efficacy in suppressing weevils and bruchids infesting stored cereals and pulses (15; 16). It has been reported that essential oils of botanical origin contain various monoterpenoids which have been explored as repellent and/or antifeedant due to their insecticidal properties (17). Monoterperoids found in essential oils are known neurotoxins (18) and most of them are volatile, thus offering the prospect of their use against stored product pests.

The present study was undertaken to evaluate the effect of three essential oils extracted from clove, *Syzgium aromaticum* (L.) Merril and Percy; West African black pepper (WABP), *Piper guineense* Schum and Thonn. and Ethiopian pepper, *Xylopia aethiopica* (Dun.) A. Rich. as repellant, contact toxicity and as ovicide on the rust-red flour beetle, *Tribolium castaneum* (Herbst.)

MATERIALS AND METHODS

Insect rearing, oil extraction and pearl millet procurement

The culture of *T. castaneum* was started with an infested batch of millet from the stock of culture in the Agronomy Laboratory of the Faculty of Agriculture, Shabu-Lafia Campus (08.33^0N and 08.32^0E) North Central, Nigeria. The culture was reared on broken millet (cv.Gero) under fluctuating laboratory conditions (29-39^{0}C and 41-58% RH). The study was carried out under these conditions.

Dry seeds of clove, West African black pepper and Ethiopian pepper were obtained in the market in Lafia. 500 g each of the seed were separately ground into coarse powder and steam distilled in a clavenger glass apparatus. Distillation was carried out for five hours in the Chemistry Laboratory of Nasarawa State University, Keffi, Nigeria. The process yielded on the average 0.83% of *P. guineense*, 7.4% *S. aromaticum* and 1.2% *X. aethopica* ml of oil and the distilled oil were collected into a 50 ml glass jar and stored in the refrigerator until ready for use.

One kilogramme of pearl millet (*P. glaucum*) (cv. Gero) used for the study was obtained from harvested millet seeds from the stock of Faculty of Agriculture, Nasarawa State, Nigeria. Pristine seeds were fumigated with aluminum phosphide (Phostoxin® tablet) in an air tight container for 14 days. The seeds were later air dried in the laboratory bench for three days under a screen to allow dissipation of the fumigant. Thereafter, the millet seeds were stored in a double layer black polypropylene bag inside a deep freezer below 0^0C for five days. The seeds were later air dried as described above to dissipate imbibed moisture contents.

Ovicidal effect of essential oils on progeny emergence of *T. castaneum* infesting damaged pearl millet in storage.

20 g of pearl millet containing 20% broken grain (80:20) were weighed into a 50 ml glass jar, and infested with five pairs of 3- to 5-day old unsexed *T. castaneum* adults by means of a pooter. The glass jars were then covered with a lid bearing ten equidistant holes (0.5 mm^2). Female beetles were allowed to lay eggs for 20 days after which all dead or live insects were removed. Thereafter, four rates (0.75, 1.50, 2.25 and 3.00 mg) of clove, WABP and Ethiopian pepper oils dissolved separately in 2 ml analytical grade acetone and admixed with 20 g of pearl millet grains contained in 50 ml glass jar. Stirring with a glass rod to ensure adequate coating of millet with oil was done until the acetone was completely evaporated. The control batches were each treated with 2 ml acetone alone. The glass jars were checked daily for adult emergence and the number emerging in each treatment was recorded for 20 consecutive days following date of first emergence. All adults that emerged were removed from each treatment at the time of recording. Any treatment that did not show adult emergence for 7 consecutive days was adjudged to have entered another generation period. Data were taken for three filial generations. The experiment was set up as a randomized complete block design with a split plot arrangement of treatments which were replicated three times under ambient laboratory conditions.

Repellency of essential oils to adult and larva of *T. castaneum*

The Petri dish chamber test was used to determine the repellency of the three essential oils to *T. castaneum* (19). The test arenas consisted of 9 cm diameter Whatman No.1 filter paper cut into two halves. Four concentrations of the different essential oils were prepared by dissolving 0.75, 1.50, 2.25 and 3.0 mg in 2 ml analytical grade acetone. Each concentration was applied to a half of the filter paper disc as uniformly as possible by means of the pipette. The other filter paper half was left untreated. In the control chambers, half of the paper was treated with 2 ml pure analytical grade acetone and the other half left untreated. The treated and the untreated halve discs were rejoining using clear adhesive tape at the back and placed in a glass Petri dish of 9 cm diameter. Before the introduction of test insects into the Petri dish, the acetone is used as the carrier was allowed to evaporate completely. Thereafter, 30 adult *T. castaneum* that were 2-3 days old were released at the centre of the Petri dish and then covered with the lid. Each treatment and control were replicated tree times, and the treatments were arranged in a randomized complete block design with a split plot lay out. The number of insects present on untreated and treated discs were recorded after 30 min. The same dosage rate was used in determining repellency effect of the three essential oils on larva of *T. castaneum.* Thirty larvae of *T. castaneum* (10 days old) were used for the study, and the procedure for the determination was as described above.

Toxicity of essential oils against adult and larva of *T. castaneum*

Clove, WABP and Ethiopian pepper oils each of 0.75, 1.50, 2.25 and 3.0 mg were dissolved separately in 2 ml of analytical grade acetone and used to treat 20 g of pearl millet containing 20% broken grains contained in a 50 ml glass jar. A glass rod was used for stirring to ensure adequate coating of seeds with the oil; stirring stopped when acetone completely evaporated. The control had the same type and weight of seeds treated with 2 ml acetone, stirred until complete evaporation of the acetone. Thereafter, 30 adults of 2 to 3 days old *T. castaneum* were introduced into seeds by means of a pooter. The three essential oils at the different dosage rates as described above were each carried in the same volume of acetone and treated on 30 larvae in a 50 ml glass jar containing 20 g of 20% broken millet grain. The experiment followed the same procedure as described earlier. The treatments and control were replicated three times and arranged in a completely randomized design. Beetle and larval mortality were taken 24 h after treatment. A beetle or larva were counted as dead if it did not respond to probing with a fine brush bristle (20).

Statistical analysis

The data obtained from ovicidal tests were subjected to analysis of variance using GENSTAT 2005 computer programme. Data obtained from toxicity tests (LC_{50}) were subjected to probit analysis using a United State Environmental Protection Agency Probit Analysis Version 1.5. All percentage data were arc sine transformed before analysis with GENSTAT 2005 computer programme. Data on repellency and classification were analyzed accordingly following the methods of 19 and 21. Differences between means were separated using the least significant difference (P=0.05) statistic.

RESULTS

Table 1 showed that all the three essential oil treatments were significantly different (p≤0.05) from the control treatment during the three generation period. Clove and WABP oils caused significantly (p≤0.05) less number of adult *T. castaneum* to emerged from broken millet when compared with treatment adult mean emergence from treatment with Ethiopian pepper oil at all the dosage rates. From the results as obtained in **Table 1**, the three essential oils completely prevent progeny adult emergence, albeit at higher dosage rates. The effect of the essential oils as shown in **Table 1** showed that the efficacy of the three essential oils were dose dependent. The interaction between the essential oils and the dosage rates was significant (p≤0.05) only at the first filial generation period, while the second and third filial generation periods were not significant (p≥0.05).

Table 1. *Comparative emergence of T. castaneum adults from broken millet grains protected with three essential oils*

Dosage (mg oil/20 g broken grain)	Essential Oil			Dosage Mean	$LSD_{(0.05)}$
	Clove	WABP	Ethiopian pepper		
			1st generation		
0.75	12.00	17.33	34.33	21.22	
1.50	10.00	15.00	16.33	13.78	
2.25	0	0	7.67	2.56	Oil type= 4.27
3.00	0	0	0	0	Dosage= 3.49
Control	93.30	86.00	72.67	84.00	Oil typexDosage= 6.02
Oil type mean	23.07	23.67	26.20		
			2nd generation		
0.75	26.33	35.67	87.33	49.78	
1.50	21.33	21.33	72.33	38.33	Oil type= 14.03
2.25	0	0	40.67	13.56	Dosage= 18.12
3.00	0	0	0	0	Oil type xDosage= NS[a]
Control	158.67	145.33	153.33	152.44	
Oil type mean	41.27	40.47	70.73		
			3rd generation		
0.75	47.33	50.67	124.67	74.22	
1.50	27.00	25.67	118.33	57.00	Oil type= 21.69
2.25	0	0	78.00	26.00	Dosage= 28.00
3.00	0	0	0	0	Oil type xDosage= NS[a]
Control	261.67	261.67	239.33	237.22	
Oil type mean	57.00	67.60	112.07		

*=WABP= West African black pepper
[a]=NS=Non significant

Tables 2 and 3 show the effect of the three essential oils on repellency of adult and larva of *T. castaneum.* Table 2 show that all the three essentials oils were able to show the class V, while the control treatment show the class I in repellent action on adult *T. castaneum.* Table 2 also show, that the use of the essential oil and interaction with the dosage rates were not significantly different (p≥0.05) from each other, and the effects of the essential oils were not significantly different (p≥0.05) from one another. The different dosage rates of clove oil were significantly different (p≤0.05) from each other, except 1.50 and 2.25; and 2.25 and 3.00 mg/30 cm^3 of Whatman No.1 filter paper. WABP oil show the significant differences (p≤0.05) at the dosage rates of 0.75 and 2.25 and 3.00 mg/30 cm^3 Whatman No.1 filter paper. The same trend was also noticed in the repellent action of Ethiopian pepper oil. All the essential oils were significantly different (p≤0.05) from the control treatment. The repellent effect of the essential oils on larva of *T. castaneum* (Table 3) show that clove oil exhibited class IV – V (61.00 – 100.00%), WABP, class II – V (25.60 – 100.00%) and Ethiopian pepper, class III – V (40.50 – 86.30%). The interaction between the different essential oils and the dosage rates was significantly different (p≤0.05) from each other. The repellency action of clove oil was more pronounced than that of WABP and Ethiopian pepper.

Table 2. *Repellency and classification of the essential oils extracted from three edible seeds on adult rust-red Flour Beetle, T. Castaneum*

Dosage (mg/30 cm^3 Whatman No. 1 Filter paper.	Source of Essential Oil						
	Clove	Class*	WABP**	Class	Ethiopian pepper	Class	Mean
0.75	83.20 (65.77)***	V	88.50 (69.51)	V	91.70 (73.21)	V	87.70 (69.49)
1.50	93.20 (74.82)	V	93.20 (74.82)	V	93.20 (74.82)	V	93.20 (74.82)
2.25	96.90 (79.97)	V	97.70 (81.30)	V	97.70 (81.30)	V	97.50 (80.86)
3.00	99.62 (86.45)	V	99.62 (86.45)	V	97.70 (81.30)	V	99.16 (84.74)
Control (0)	4.40 (12.13)	I	2.20 (8.49)	I	6.30 (14.60)	I	4.20 (11.74)
Mean	80.60 (63.83)		80.90 (64.11)		82.20 (65.04)		

SED= 5.55; LSD (p≥0.05) = NS (Interaction).
SED= 2.48; LSD (p≥0.05) = NS (Oil).
SED= 3.20; LSD (p≤0.05) = 5.54 (Dosage).
Class* = Classification is based on the mean percentage.
Class I = 0 – 20%; Class V = 80.1 – 100.00%.
WABP** = West African black pepper.
*** = Figures in parentheses are arc sine values to which SED and LSD are applicable.
NS = Non significant

Table 3. *Repellency and classification of three essential oils against the larva of the rust-red Flour Beetle, T. castaneum*

Dosage (mg/30 cm^3 Whatman No. 1 Filter paper.	Source of Essential Oil						
	Clove	Class*	WABP**	Class	Ethiopian pepper	Class	Mean
0.75	61.00 (51.36)***	IV	25.60 (30.39)	II	40.50 (39.51)	III	42.00 (40.42)
1.50	96.80 (79.70)	V	67.50 (55.26)	IV	47.40 (43.49)	III	74.20 (59.48)
2.25	100.00 (90.00)	V	85.50 (67.63)	V	73.20 (58.84)	IV	90.50 (72.16)
3.00	100.00 (90.00)	V	100.00 (90.00)	V	86.30 (68.29)	V	98.70 (81.57)
Control (0)	1.00 (3.49)	I	2.20 (8.49)	I	1.40 (6.98)	I	1.20 (6.32)
Mean	79.20 (62.90)		58.10 (49.64)		47.30 (43.42)		

SED= 6.32; LSD ($p \leq 0.05$) = 10.75 (Interaction).
SED= 2.82; LSD ($p \leq 0.05$) = 4.80 (Oil).
SED= 3.65; LSD ($p \leq 0.05$) = 6.21 (Dosage).
Class* = Classification is based on the mean percentage.
Class I = 0 – 20%; Class II = 20.1 – 40%; Class III = 40.1 – 60%; Class IV = 60.1 – 80%;
Class V = 80.1 – 100.00%.
WABP** = West African black pepper.
*** = Figures in parentheses are arc sine values to which SED and LSD are applicable.
NS = Non significant

The results are presented in **Table 4** show that WABP caused mortality of adult *T. castaneum* at relatively low concentration (0.21 mg/20 g of seed) compared either to clove (0.40 mg/20 g of seed) or Ethiopian pepper (1.78 mg/20 g of seed). Essential oil from clove performed relatively better (0.46 mg/20g of seed) when treated against larva of *T. castaneum* when compared either to WABP (0.54 mg/20 g of seed) or Ethiopian Pepper (0.67 mg/20 g of seed). The tree oil tested caused 100% mortality of the Tenebrionids within 24 h at the highest dosage rate when compared to the lower dosage rates. Comparisons of the 24 h LC_{50} values test on adults and their corresponding 95% Fiducial limits for the three essential oils in Table 3 showed that there was no significant differences in the LC_{50} of clove (0.40 mg/20 g of seed), WABP (0.21 mg/20 g of seed) oils (overlap in 95% Fiducial limits), while Ethiopian pepper oil was significantly less toxic than either of the two test oils (no overlap in 95% Fiducial limits) The LC_{50} values tests on the Tenebrionids larvae and their corresponding 95% Fiducial limits for the three oils showed that there was no significant differences in the LC_{50} values of the three oils (0.46, 0.54, 0.67 mg/20 g of seed) for clove, WABP and Ethiopian pepper, respectively because of the overlapping in their Fiducial limits.

Table 4. *Acute toxicity (LC_{50}) of three essential oils against larva and adult of T. castaneum*

	Clove		WABP*		Ethiopian pepper	
	Adult	Larva	Adult	Larva	Adult	Larva
LC_{50} (mg/20 g of 20% broken millet)	0.04	0.46	0.21	0.54	1.78	0.67
95% Fiducial Limits	0.04-0.70	0.11-0.72	0.01-0.57	0.11-0.84	1.45-2.16	0.19-0.99
Slope values	2.08	2.64	1.36	2.03	3.28	1.92

* = WABP= West African black pepper

DISCUSSION

In this study, the three essential oils had significant effect on the emergence of *T. castaneum*. It has been reported that plant oils contained monoterpenes such as 1,8-cineole, eugenol and camphor that can elicit mortality and inhibition of progeny production (22). The seed oil obtained from clove, (*S. aromaticum*) contains sesquiterpene, eugenol and caryophylline as major constituents (23). *Piper* species have been reported to contain piperricide, dihydropipericide and guineesine (24) and *Xyplopia aethiopica* contains ß-phelladrene, α-pinene and eucalyptol (25).

The study has also shown that application of these oils can significantly reduced infestation of pearl millet grains by *T. castaneum*. Reduction of beetle development in treated grains may have been achieved through the toxic effects of deposits of the essential oils against a large proportion of eggs that were laid. Female *T. castaneum* lay their eggs loosely on the surface of the produce (6; 26) and this should bring them into direct contact with the oil deposits. It is now well known that plant oils have the ability to penetrate the chorion of insects' eggs via the micropyle and cause the death of developing embryos through asphyxiation (27; 28). Essential oils have been reported to have low vapour density than fatty oils; hence, they are readily volatilized (25). This could be the reason why most of the eggs that might have hatched could not survive the volatility effects of the three essential oils especially as the dosage of the oils increase. In some other reports, the use of clove and WABP has been shown to completely suppress the development of *T. castaneum* adults and larvae in millet grains (16). It has also been reported that edible fatty oils of *Moringa oleifera, Sesamum indicum* and *Olea europaea* completely suppress progeny emergence of *T. castaneum* in broken millet (26).

The repellant action of the essential oils may be due to the different active principles in the plant oils. For instance, repellency of class IV has been recorded against *T. castaneum* with the use of clove oil (19). A repellency class of 94.4% (class V) and 97.4% (Class V) has been reported with the use of clove and WABP oils in a study again *Callosobruchus maculatus* (29). The toxicity of clove and WABP oils as observed in this study is in agreement with an earlier findings which showed that clove and WABP oils caused 100% mortality of the cowpea bruchid, *C. maculatus* on cowpea, *Vigna unguiculata*, when applied at the rate of 250 mg/10 g seed (29). *X. aethiopica* essential oil at a concentration of 1 ml per 100 g maize seeds has been reported to cause 100% *Sitophilus zeamais* mortality in 24 h (30).

Essential oils generally contain a blend of volatile components of chemicals which cause a reversible reaction in herbivores when attacking plant species (31). This blend of volatile oils can exert toxic, deterrent, antifeedant and repellent effects on insect herbivores (32; 33). The three essential oils seem to possess quadruple effects (ovicidal–

and emergence–reducing, repellency and toxicity) which should render them effective protectants of stored millet grains. One important advantage of these plant products is that they can be afforded easily by resource-poor farmers and they already constitute an important component of the diet of Nigerians, most Africans and Asians and they have form part of the international trade on spices. Hence, this would facilitate their adoption as grain protectants since the hazard of residues in stored food commodities usually associated with insecticides would be eliminated.

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