

Full Length Research Paper

Contact activities of *Piper guineense* (Schum and Thonn) and *Eugenia aromaticum* (L). (Merril and Perry) extracts against larvae of hide beetles, *Dermestes maculatus* (Degger) (Coleoptera: Dermestidae)

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Contact activities of *Piper guineense* (Schum and Thonn) and *Eugenia aromaticum* (L). (Merril and Perry) extracts in the control of *Dermestes maculatus* larvae infesting stored fish (*Clarias gariepinus*) were investigated under laboratory condition (28±3°C and 65±5% RH). The extracts were tested by application of 2.0 µL each to ten third instars larvae using micro pipette at a concentration of 6.00, 10.00 and 20.00% each of the plant extracts (methanol, n-hexane and ethyl acetate). Mortality was recorded at 1, 2, 3 and 7 days of post treatments. The observed mortality was dose and exposure-dependent. All the extracts significantly enhanced larval mortality (P>0.05) when compared with the control. The n-hexane and ethyl-acetate extracts of *P. guineense* at 20% concentration induced the highest mortality of 86.66%, lowest mortality of 56.66% was observed on methanol fraction treated larvae after 7 days of post treatments. The n-hexane of *E. aromaticum* extracts recorded highest mortality (80.0%), followed by ethyl-acetate (76.66%) and methanolic (7.00%) fractions treated larvae at 20% concentration after 7 days of post-treatments. The results showed strong insecticidal activity in control of larvae of hide beetles infesting dried fish.

Key words: Plant extracts, *Piper guineense*, *Eugenia aromaticum*, *Dermestes maculatus*.

INTRODUCTION

Dermestes maculatus (Hide Beetle, Degeer) (Coleoptera; Dermestidae) is one of the most destructive insect pests of stored smoked-dried fish in Nigeria (Tejumade, 2019). These pests generally infest dried fish during storage, transportation and marketing, thus responsible for extensive damage to marketed fish leading to enormous

weight loss ranging from negligible amount to 50% weight loss (Don-Pedro, 1989; Amadi and Dimkpa, 2018).

Tejumade (2019) reported *D. maculatus* account for about 71.5% of the observed infestation with substantial loss of about 43 to 62.7% in dry weight depending on the length of storage.

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The control of this pest in Nigeria is primarily dependent on repeated application of synthetic chemicals such as chlorpyrifos-methyl, permethrin, cypermethrin, BHC, and "Otapiapia" (locally formulated) onto fish carton for protection against insect pest (Igene et al., 1998; Abolagba et al., 2011). Although many synthetic chemicals are effective the general use of such chemicals to protect stored fish has been hampered by the report of health hazard, high cost of purchase, lack of availability, illiteracy of fish handler for right application and less susceptibility of Dermestid larvae (Booke et al., 2001; Amusan and Okorie, 2002). With these demerits of synthetic chemicals, currently world-wide interest is centered on search for alternative pesticide to stored product by the use of botanical pesticide.

Botanical pesticide tends to have broad spectrum activity relatively specific in their mode of action and easy to process and use (Viglianco et al., 2008). To minimize use of synthetic pesticide, several plants' extracts have been reported as effective against *D. maculatus* on dried fish by several researchers (Fasakin, 2003; Akinwumi et al., 2006, 2007; Akpotu and Adebote, 2013; Olayinka, 2014). These extracts provide a solution to the problem emanating from the use of synthetic chemicals. The present studies have been chosen to investigate the effects of methanolic extracts fractions of *Piper guineense* and *Eugenia aromatica* against *D. maculatus* larvae as an alternative strategy to synthetic chemicals method of pest control.

MATERIALS AND METHODS

Collection, identification of plant material and preparation of plant powders

The sample of dried fruit of *P. guineense* and *E. aromatica* was obtained from Sokoto Central Market, Nigeria. The plants were identified and authenticated in the Herbarium of Biological Sciences Department of Usman Danfodiyo University, Sokoto. Voucher specimens (UDUH/ANS/0258 and 0221) were deposited. Samples were milled into fine powders using mortar and pestle, sieved with 0.2 mm mesh following the methods of Adedire and Lajide (2000), Akinwumi et al. (2006) and Jose and Adesina (2004). Each of the plant powders was labeled and kept in a separate plastic container and placed in a cool dry place prior to use.

Preparations of feed

The samples of dried fish *Clarias gariepinus* were purchased from fish mongers at Sokoto Central Market, identified and authenticated in Hydrobiology Laboratory, Biological Sciences Department, Usman Danfodiyo University, Sokoto. The fish samples were disinfected by heat treatment in the laboratory-drying cabinet at 60°C for 1 h and allowed to cool at room temperature as adopted by Onu and Baba (2003).

Collection of hide beetle and maintenance of insect culture

Different stages of hide beetle were obtained from naturally infested

fish collected from Sokoto Central Market fish stalls. Several adult pairs of *D. maculatus* were obtained and kept in transparent plastic containers (19.0 cm height and 21.2 cm in diameter) fed with dried fish. The containers were covered with Muslin cloth and tightened with rubber band. Wet cotton wool was supplied in each jar to provide water requirements for oviposition as suggested by Hill (1990). The adult laid eggs were hatched into larvae and changed into pupae, which were picked up and transferred into separate containers to obtain newly emerged adult, which were used for regular supply of larvae for the experiment in line with Akinwumi et al. (2006).

Preparation of methanol extracts and solvent fractionation

Four hundred grams of *E. aromatica* and *P. guineense* were homogenized with 95% methanol (1 L) in plastic container and kept at room temperature for 24 h and filtered. The methanol crude extract was collected and concentrated almost to dryness in drying cabinet at 40°C for 48 h. The dried extracts were stored in freezing medium until used for fractionation (Akinwumi et al., 2006).

The dried methanol crude extract of *E. aromatica* (19.47 g) and *P. guineense* (26.17 g) were suspended in distilled water and then partitioned with 500 ml of n-Hexane, ethyl acetate and water in increasing order of polarity, following the method of Bakele et al. (2016).

Effect of extract fractions on *D. maculatus*

Effects of each plant extract fraction were conducted according to Talukder and Howse (1994). 20% stock solution was prepared for each solvent (methanol, n-hexane and ethyl acetate). Lower concentrations (6 and 10%) were obtained from dilution of the stock solution with distilled water. Ten third instars larvae were chilled for 5 min to immobilize them and then picked up individually by the use of camel hair brush and 2 ul of each of the solution was applied to the dorsal surface of the larvae. Experiments were in three replicates (each replicate contains ten treated larvae). In addition, the same number of larvae (10) was treated with distilled water only as control. After treatment, insects were transferred into transparent plastic containers (19.0 cm height and 21.2 cm in diameter) containing dried fish. Observations were made daily and those that did not move or respond to gentle touch were considered dead. Mortality was recorded at 1st, 2nd, 3rd and 7th days of post treatment.

Data analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using General Linear Model (SPSS, 2019) and means found to be significant were separated using Duncan multiple range test at 5% level of significance ($p < 0.05$).

RESULTS

Effects of methanol fractions on mortality of *D. maculatus* larvae

The effect of *P. guineense* methanolic extracts applied to *D. maculatus* larvae by topical application is presented in Table 1. All the three extracts of *P. guineense* exhibited insecticidal activity against *D. maculatus* larvae as dose and time-dependent variables. At day 1, all the

Table 1. Mortality among *D. maculatus* larvae by topical application with *P. guineense* methanolic fractions.

Fraction	No. of Larvae introduced (n)	Concentration (%)	Mean larval mortality \pm SE				Mortality (%)
			Period of exposure (in days)				
			1 st	2 nd	3 rd	7 th	
Methanol	10	20.00	2.33 \pm 0.88 ^{bcd}	3.33 \pm 0.88 ^{bc}	4.33 \pm 0.33 ^{cd}	5.66 \pm 0.88 ^{cd}	56.60
	10	10.00	2.33 \pm 0.33 ^{bcd}	3.33 \pm 0.88 ^{bc}	4.33 \pm 1.20 ^{cde}	5.66 \pm 0.33 ^{cde}	56.60
	10	6.00	1.33 \pm 0.66 ^{cde}	1.66 \pm 0.88 ^{cd}	2.00 \pm 1.73 ^{ef}	3.33 \pm 1.20 ^{ef}	33.30
N-hexane	10	20.00	4.66 \pm 0.33 ^b	6.33 \pm 0.33 ^{ab}	8.00 \pm 0.57 ^{ab}	8.66 \pm 0.66 ^{bc}	86.60
	10	10.00	4.33 \pm 1.45 ^b	6.00 \pm 2.08 ^{abc}	7.00 \pm 2.08 ^{abc}	8.00 \pm 2.00 ^{abc}	80.00
	10	6.00	1.00 \pm 0.57 ^{cd}	2.00 \pm 0.57 ^{cd}	3.66 \pm 0.33 ^{cde}	5.66 \pm 0.33 ^{de}	56.60
Ethyl-acetate	10	20.00	6.67 \pm 0.88 ^a	8.00 \pm 0.57 ^a	8.33 \pm 0.66 ^{ab}	8.66 \pm 0.33 ^{bc}	86.60
	10	10.00	4.33 \pm 0.33 ^b	5.66 \pm 0.33 ^{ab}	5.66 \pm 0.33 ^{bcd}	7.33 \pm 0.33 ^{abc}	73.30
	10	6.00	3.66 \pm 0.88 ^{bc}	4.00 \pm 1.15 ^{bc}	5.66 \pm 0.33 ^{bcd}	6.33 ^{bc}	63.30
Cypermethrin	10	0.05	3.00 \pm 0.57 ^{bcd}	6.33 \pm 0.33 ^{ab}	9.33 \pm 0.33 ^a	9.66 \pm 0.33 ^a	96.60
Control	10		0.00 \pm 0.00 ^e	0.33 \pm 0.46 ^d	1.33 \pm 0.33 ^e	1.33 \pm 0.33 ^e	13.30

Means that have the same super script within a column are not significantly different at 5% level using Duncan's multiple range test.

Source: Authors.

three extracts of *P. guineense* showed less than 50% mortality of larvae except ethyl-acetate extract at highest concentration (20%) which caused 66.70% mortality of larvae. At 2nd and 3rd days, mortalities in all the treatment at all concentrations increased as compared to day 1 of exposure. The ethyl-acetate extract at 20% concentration remains the highest (80%) mortality of larvae. However, at the 7th day of exposure, all the three extracts at all concentrations except 6% concentration of methanol showed a significant ($p < 0.05$) mortality of larvae when compared with the control. The ethyl-acetate extract recorded the highest mortality range of 63.30 to 86.66%, followed by n-Hexane (56.60-86.6%) and methanol extracts (33.30-56.60%).

The contact activity of *E. aromatica* methanolic extracts fractions applied *D. maculatus* larvae are presented in Table 2. All treatments except 6% concentration of methanol and 10 and 6% of n-Hexane were significantly more toxic than control at 1st day of exposure. Efficacy was dosage-dependent with significant higher mortality occurring with increase in dosage. No mortality occurred in the control (0.00%). Highest mortality was recorded in ethyl-acetate extract at 20% concentration with a percentage mortality of 63.30%. At the 2nd day of exposure, only 6.0% concentration of methanol extract was statistically similar ($p > 0.05$) with the control; all other treatments showed significant mortality of larvae when compared with the control. Highest mortality was recorded in ethyl-acetate (63.33%) followed by n-Hexane (56.60%) and methanol (53.30%). However, at the 3rd day of exposure, mortality in all the treatments followed a similar trend with the 2nd day of exposure with ethyl-

acetate extract which was the highest with a mortality range of 50 to 70%. At the 7th day of exposure, the highest mortality of 80.00% was recorded from n-Hexane fraction at 20% concentration. Other concentration of n-Hexane also showed higher mortality of larvae of 53.30 and 46.66%. In addition, the ethyl acetate and methanol extract fraction recorded mortality of larvae ranging from 53.30 to 76.60% and 43.30 to 70.00%, respectively.

DISCUSSION

In this study, the three extracts of *P. guineense* demonstrated contact efficacy to *D. maculatus* larvae. The results indicated that insecticidal activity of *P. guineense* extract fraction varied depending on the organic solvent used for extraction, concentration and exposure time. Among the extracts applied, ethyl-acetate fractions were the most toxic against *D. maculatus* larvae followed by n-Hexane and methanolic fractions. The efficacy of the ethyl acetate and n-Hexane could be attributed to their oily appearance, in contrast to the solid methanolic extracts, which is similar to the finding of Ajayi and Peter (2016) who reported extract of *P. guineense* yielded oil which has been reported to be very effective in the control of stored product pest. The use of plant extract and other forms of plant materials as insect pest control and management of stored food products have been reported by several researchers (Fasakin, 2003; Adebote et al., 2006; Akinwumi et al., 2007; Olayinka, 2014; Fasakin, 2003) and it has been shown that oil extracts obtained from *P. guineense*, *Monodora myristica*,

Table 2. Mortality among *D. maculatus* larvae by topical application of *E. aromaticum* methanolic fractions.

Fractions	No. of Larvae introduced (n)	Mean larval mortality ± SE					Mortality (%)
		Concentration (%)	Period of exposure (days)				
			1st	2nd	3rd	7th	
Methanol	10	20	3.33±0.33 ^{ed}	5.33±0.33 ^{abc}	6.00±1.15 ^b	7.00±1.15 ^{bcd}	70.00
	10	10	4.44±0.66 ^{bc}	4.00±0.57 ^{bcd}	5.66±0.33 ^b	6.33±0.66 ^{bcd}	63.30
	10	6.0	1.33±0.33 ^{efg}	1.33±0.33 ^{ef}	2.66±0.66 ^{cd}	4.33±0.66 ^e	43.30
N-Hexane	10	20	5.33±0.33 ^{ab}	5.66±0.33 ^{ab}	6.33±0.33 ^b	8.00±1.00 ^{ab}	80.00
	10	10	1.00±0.66 ^{fg}	3.33±0.66 ^{cd}	4.66±0.66 ^{bc}	5.33±0.88 ^{cde}	53.30
	10	6.0	1.66±0.88 ^{defg}	2.33±0.88 ^{de}	2.33±0.88 ^d	4.66±0.33 ^{de}	46.60
Ethyl-acetate	10	20	6.33±0.66 ^a	6.33±0.57 ^{ab}	7.0±1.15 ^b	7.66±0.57 ^{abc}	76.66
	10	10	4.33±1.20 ^{bc}	6.00±0.57 ^{ab}	6.33±0.33 ^b	7.00±0.33 ^{bcd}	70.00
	10	6.0	2.00±0.57 ^{def}	4.33±1.20 ^{abcd}	5.00±1.15 ^b	5.33±0.88 ^{cde}	53.30
Cypermethrin	10	0.05	3.00±0.57 ^{cde}	6.33±0.33 ^a	9.66±0.33 ^a	9.66±0.33 ^a	96.60
Control	10	0.00	0.00±0.00 ^g	0.33±0.33 ^f	0.66±0.33 ^d	1.33±0.33 ^f	13.30

Means that have the same super script within a column are not significantly different at 5% level using Duncan's multiple range test. Source: Authors.

and *African melegueta* were highly effective in controlling various stages of *D. maculatus* on smoked fish during storage. The oil extracts of these plants were 100% effective when compared with 16.7% in untreated (control) smoked fish. Adebote et al. (2006) observed that 0.025 ml g⁻¹ of oil from *Detarium microcarpum* seed produced 85.56 to 96.67 mortality of *Dermestes* larvae within 24 and 96 h of post treatment. Okonkwo and Okoye (2001) reported 100% mortality of larvae of *D. maculatus* when treated with extracts of *Dennettia tripetala* and *P. guineense* at a dosage lower than powder. Akinwumi et al. (2007) reported that ethanol extract of *D. tripetala* and *P. guineense* resulted in 100% mortality of *D. maculatus* larvae after 3 days of post treatment. Ajayi (2015) stated that acetone extract is more effective in reducing oviposition and adult emergence of *Callosobrunchus maculatus* than methanolic and ethanolic extracts of the same plant, while methanolic and ethanolic extracts were significantly more effective than aqueous extract.

In the current study, higher activity of ethyl acetate fraction observed might be due to the presence of polar and no polar bioactive component against larval stage of *D. maculatus*, as ethyl acetate is a semi polar solvent that had the ability to extract polar and nonpolar compounds in the extract of *P. guineense*. Variation in the bioactivities of different solvent fractions observed in the study confirmed the finding of Sun et al. (2001). That crude extract that was screened with ethyl acetate, n-butyl alcohol and water fractions of alcoholic extract of leaves and stem of *Vanilla fragrans* against *Culex pipiens* larvae showed that n-butyl alcohol and ethyl-acetate

fractions were active in the bioassay, while the water fraction appeared to contain no substance that inhibited the larval growth (Overgaard et al., 2014). The mortality rates of mosquitoes declined with increasing polarity of the solvent, the water extract of *Zanthoxylum heitzii* (Rutaceae) produced the lowest adult mortalities whereas its ethyl-acetate and hexane extracts produce high mortalities against *Anopheles gambiae*.

Furthermore, phytochemical compound contained in the fractions might be responsible for insecticidal actions. Lale and Alaga (2000) reported *P. guineense* extract is known to contain atleast three different alkaloids responsible for its insecticidal activity (piperine, chavicine and piperidine).

The result of the study also revealed the efficacy of *E. aromatica* extracts in which all three extract fractions gave high mortalities of larvae which could be due to its important secondary metabolite such as terpenes, linoleic acid and oleic identified as the main active compound in *E. aromatica* (Golob et al., 1999). This supports the finding of Akinwumi (2010) who reported 100% mortality of *D. maculatus* adults when 1 ml of oil is mixed with 10.00 g of powder of *E. aromaticum* after seven days of post treatment. The finding also supports the work of Ajayi (2015) who reported that clove and west African black pepper were significantly more toxic to adult of *Tribolium castaneum* than ginger at dosage of 100 mg/50 of seed. Clove and West African black pepper and ginger oil caused 96.3, 100 and 13.2% adult mortalities, respectively and 65.7 and 9.6 larval mortalities, respectively. Akinwumi et al. (2007) also reported 0.5 g of *E. aromatica* recorded 50.00% larval mortality and

51.67% adult mortality and concentration of 1.0, 2.0 and 2.5 g recorded 100% larval and adult mortalities.

Conclusion

The study demonstrated the contact toxicity of *P. guineense* and *E. aromatica* against *D. maculatus* (larvae). The maximum mortality was recorded at the highest dose of ethyl acetate fractions of *P. guineense* and *E. aromatica*. This is followed by n-Hexane, while methanolic extract recorded the least activity. Hence, ethyl-acetate extract of both plants has potential insecticidal activity against *D. maculatus* larvae.

Recommendations

The finding revealed that both methanolic fractions of *P. guineense* and *E. aromatica* could be used as fish protectant against *D. maculatus* infestations.

Therefore, the use of these extracts is recommended as potential dry fish ant protector in the control of *D. maculatus* infestation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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