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Toxic and Repellence Activities of Four Plant Extracts to *Dermestes maculatus* Degeer on Smoked African Mud Catfish, *Clarias gariepinus* Burchell

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Abstract: The efficacy of ethanol extracts of four tropical plant materials: pepper fruit, *Dennettia tripetala* Baker; clove, *Eugenia aromatica* Hook; African nut-meg, *Monodora myristica* (Dunal) and black pepper, *Piper guineense* (Schum and Thonn) was evaluated under tropical storage conditions for the control and repellence of the leather beetle, *Dermestes maculatus*. In separate experiments, adults and larvae of the pest were introduced to disinfested smoked *Clarias gariepinus* treated with plant extracts at 2.5, 5.0, 7.5 and 10.0%. Each of the four plant extracts evoked significant ($p<0.05$) mortalities in both adults and larvae of the fish beetle at all concentrations tested compared with the untreated (control) at 1, 3 and 7 days post-treatment. The extracts also exhibited repellency against *D. maculatus*. This study has revealed that the locally available botanicals could offer effective protection against post-harvest pests of fish and therefore could be incorporated into post-harvest fish management strategies.

Key words: Smoked catfish, *Dermestes maculatus*, plant materials, repellence, mortality

INTRODUCTION

Fish is very rich in essential amino acids, vitamins and minerals (FAO, 2004). Fish is also very important in terms of employment / income generation, poverty alleviation, foreign exchange earnings and provision of raw materials for the animal feed industry. Nigerians are high fish consumers with a total annual consumption figure of 1.2 million metric tons (FDF, 2005). However, fish is highly susceptible to damage by insects and microorganisms as soon as it is caught. BOSTID (1988) estimated post-harvest losses of fish in many developing countries at 50% of landed catch. Fish post harvest losses in Nigeria have been estimated at 30-40% (FAO, 2004). In this regard, a variety of processing methods, such as salting, drying and smoking have been developed to preserve fish.

Smoked fish is one of the most widely distributed and cheapest animal protein products in Nigeria (FDF, 2005; Eyo, 1993). According to FAO (2002), 45% of total fish catch in Nigeria are utilized as smoked fish. However, a large-scale deterioration in quality and losses in quantity of dried fish, due to dermestid infestation, have been reported (Osuji, 1974; Awoyemi, 1989). Osuji (1974) further reported that about 71.5% of dried fish infestation in most of the producing areas was caused by *D. maculatus*. Although, many synthetic chemicals are effective against this implicated principal pest of cured fish, the general use of such chemicals to protect stored fish has been hampered by reports of health hazards and high costs of purchase (Boeke *et al*., 2001). In addition, Amusan and

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Okorie (2002) and Onu and Baba (2003) noted that dermestid larvae and adults, unlike many other beetles, are less susceptible to chemical insecticides that normally attack pests of stored products and that the use of such insecticides renders smoked fish unattractive to fish consumers. Thus, this study sought to search for natural preservation materials that are cheap, easily accessible and consumer friendly. The present work is aimed at investigating the toxicity and repellence of the extracts of four plant materials to *D. maculatus*.

MATERIALS AND METHODS

Preparation of Plant Extracts

Dry fruits of pepper fruit, *Dennettia tripetala,* black pepper, *Piper guineense* and African nut-meg, *Monodora myristica* and dry buds of clove, *Eugenia aromatica* were purchased in herbal stores at Erekesan Market in Akure, Nigeria. Pulverized parts were obtained according to the method described by Adedire and Lajide (2000). The plant materials were dried in an electric oven to a constant weight at 40°C for 8 h, ground thoroughly in an electric 5.0 HP electric grinder and sieved through a 40 holes mm^{-2} mesh screen. To obtain the extract, ten grams of each of ground plant materials was put in a round bottomed flask and 100 mL of absolute ethanol was added and soaked for 24 h. The mixture was boiled at 60°C for 30 min in UNISCOPE SM801A Laboratory Water Bath and the solution was filtered using Whatman No.1 filter paper. The resulting filtrate was kept in a tightly covered dark brown sterile bottle prior to use.

Preparation of Fish Samples and Insect Culture

Samples of African mud catfish, *C. gariepinus* each weighing 100 g were obtained from dried fish markets in Akure, Nigeria. All fish samples and bioassay jars were disinfested by heat treatment in the Gallenkamp oven at 60°C for 1 h and allowed to cool at room temperature. The initial source of *D. maculatus* was obtained from naturally infested smoked catfish and was cultured in a Kilner jar covered with muslin cloth and maintained at tropical storage conditions of temperature (30±2°C) and relative humidity (65±5%). A new generation was obtained by placing adult insects from the stock culture on fresh disinfested fish and then removing the parent adults after 2-3 weeks period. Pieces of water- soaked cotton wool were supplied in the jar to induce oviposition.

Investigation of Extracts on Beetle Mortality and Repellency

An aliquot of 5 mL of each of the plant extracts of 2.5, 5.0, 7.5 and 10% concentration was evenly rubbed onto the body of 100 g disinfested smoked fish sample. The treated fish sample was air-dried for 1-2 h in order to remove traces of the solvent and placed in a plastic jar measuring 80 mm depth and 100 mm in diameter. Six newly emerged adults and six third instar larvae of *D. maculatus* were introduced into separate plastic jars which were then covered with muslin cloth. Each experiment was replicated thrice. A control experiment without ethanol extract was also set up in triplicate. The number of dead insects was assessed and recorded daily up to 7 days and the percentage mortality was calculated. The procedure described by Don-pedro (1985) and Egwunyenga *et al.* (1998) was adapted for the repellency test. 5 mL of 2.5% concentration of each of the plant extracts was rubbed onto the body of disinfested 100 g smoked fish and placed on one side of a plastic chamber, measuring 25×12×10 cm. The same weight of untreated dried fish was then placed on the opposite side of the chamber (10 cm apart). Six adults (2-4 day old) of insect species starved for 48 h were introduced at the centre of each of the triplicate chambers. Daily observations were made for a period of 7 days by counting the number of insects found on or within a 1.0 cm radius of treated and untreated fish in each

chamber. The repellency rate (RR) was calculated by expressing the difference between the number of insects around the untreated and treated fish as a percentage of total number of introduced insects. The RR was assigned a repellency class (RC) as follows:

RC	I	II	III	IV	V	VI
RR (%)	<0.1	0.1-20.0	20.1-40.0	40.1-60.0	60.1-80.0	80.1-100.0

Data obtained were subjected to analysis of variance (ANOVA) and where significant differences existed at 0.05 significance level, the treatment means were separated using Tukey's Test.

RESULTS

Each of the plant extract dosages caused significant difference ($p<0.05$) in mortality of *D. maculatus* adults on smoked fish compared with the control (Table 1). The effect of *D. tripetala* extract dosages proved superior to *E. aromatica, P. guineense* and *M. myristica* in the control of adult insects at 1 and 3 Days After Treatment (DAT). However, there was a total kill of the adults by the extracts of each plant material at 7 DAT. Comparatively, *E. aromatica* was less toxic to adult beetles at all corresponding concentration levels. The result shows a greater devastating effect of plant extracts on larval mortality. Larvae were totally killed by the extracts of *D. tripetala* and *P. guineense* at 3 DAT while there was no survivor in any of the treatments at 7 DAT (Table 2).

The presence of the extracts on dried fish repelled *D. maculatus* adults from smoked fish. Significantly lower number ($p<0.05$) of *D. maculatus* was consistently obtained around treated fish compared with the proportion associated with the control at observation time (Table 3). The repellency test classified *D. tripetala*, *E. aromatica* and *P. guineense* to the Repellency Class $(RC)_{IV}$ with Repellency Rate (RR) 45.65, 46.83 and 48.41%, respectively while *M. myristica* was categorized as RC_{III} with RR 35.32%.

Table 1: Effect of ethanol extracts on percentage mortality of *Dermestes maculatus* adults

		% mortality/day post-treatment		
Plant extract	Concentration (5 mL/100 g fish)	1	3	7
D. tripetala	0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2.50	26.67±0.88^{h}	71.67±0.88^{i}	100.00±0.00^{a}
	5.00	26.67±0.67^{h}	75.00±1.16^{k}	100.00±0.00^{a}
	7.50	48.33±0.88^{l}	91.67±0.33^{l}	100.00±0.00^{a}
	10.00	51.67±0.66^{m}	98.33±0.33^{n}	100.00±0.00^{a}
E. aromatica	0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2.50	5.00±0.58^{a}	11.00±0.67^{a}	100.00±0.00^{a}
	5.00	11.67±0.33^{d}	31.67±0.88^{c}	100.00±0.00^{a}
	7.50	10.00±0.58^{c}	35.00±0.58^{d}	100.00±0.00^{a}
	10.00	15.00±0.58^{e}	53.33±1.20^{e}	100.00±0.00^{a}
P. guineense	0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2.50	6.67±0.33^{b}	31.67±0.33^{c}	100.00±0.00^{a}
	5.00	45.00±0.58^{k}	73.33±0.67^{j}	100.00±0.00^{a}
	7.50	38.33±0.33^{j}	76.66±1.45^{k}	100.00±0.00^{a}
	10.00	45.00±0.58^{k}	93.33±0.67^{m}	100.00±0.00^{a}
M. myristica	0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2.50	11.67±0.33^{d}	28.34±0.33^{b}	100.00±0.00^{a}
	5.00	23.33±0.58^{f}	55.00±0.58^{f}	100.00±0.00^{a}
	7.50	25.00±0.33^{g}	63.33±0.33^{g}	100.00±0.00^{a}
	10.00	33.33±0.58^{i}	70.00±0.58^{h}	100.00±0.00^{a}

Values are means of triplicate samples followed by the standard error of means. Means in the same vertical column with different superscripts for each plant material are significantly different ($p<0.05$) by Tukey's Test

Table 2: Effect of ethanol extracts on percentage mortality of *Dermestes maculatus* larvae

Plant extract	Concentration (5 mL/100 g fish)	% mortality/day post-treatment 1	3	7
D. tripetala	0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2.50	60.00±1.16^{e}	100.00±0.00^{h}	100.00±0.00^{a}
	5.00	75.00±0.58^{i}	100.00±0.00^{h}	100.00±0.00^{a}
	7.50	70.00±0.58^{h}	100.00±0.00^{h}	100.00±0.00^{a}
	10.00	81.67±0.88^{j}	100.00±0.00^{h}	100.00±0.00^{a}
E. aromatica	0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2.50	45.00±0.58^{c}	85.00±0.58^{e}	100.00±0.00^{a}
	5.00	48.33±0.33^{d}	93.33±0.88^{f}	100.00±0.00^{a}
	7.50	51.67±0.88^{e}	86.67±0.67^{d}	100.00±0.00^{a}
	10.00	48.33±0.88^{d}	93.33±0.33^{f}	100.00±0.00^{a}
P. guineense	0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2.50	63.33±0.58^{f}	100.00±0.00^{h}	100.00±0.00^{a}
	5.00	68.33±0.33^{g}	100.00±0.00^{h}	100.00±0.00^{a}
	7.50	63.33±0.33^{f}	100.00±0.00^{h}	100.00±0.00^{a}
	10.00	68.33±0.33^{g}	100.00±0.00^{h}	100.00±0.00^{a}
M. myristica	0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2.50	30.00±0.58^{a}	51.67±0.88^{a}	100.00±0.00^{a}
	5.00	31.67±0.33^{b}	68.33±0.67^{b}	100.00±0.00^{a}
	7.50	30.00±0.58^{a}	65.00±0.57^{b}	100.00±0.00^{a}
	10.00	35.00±0.58^{c}	71.67±0.67^{d}	100.00±0.00^{a}

Values are means of triplicate samples followed by the standard error of means. Means in the same vertical column with different superscripts for each plant material are significantly different ($p<0.05$) by Tukey's Test

Table 3: Effect of ethanol extracts on repellence of *Dermestes maculatus* adults on smoked fish

Plant material	Concentration (5 mL/%/ 100 g fish)	Repelled insects (in number) at days post-treatment 1st	2nd	3rd	4th	5th	6th	7th	RR(%)	RC
D. tripetala	0.00 (Untreated)	5.00±0.00^{b}	4.67±0.16^{b}	5.67±0.16^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	45.65	IV
	2.50	0.33±0.16^{a}	0.00±0.00^{a}	0.33±0.16^{a}	0.33±0.16^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}		
E. aromatica	0.00 (Untreated)	4.67±0.16^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	46.83	IV
	2.50	1.33±0.16^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}		
P. guineense	0.00 (Untreated)	5.33±0.16^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	48.41	IV
	2.50	0.67±0.16^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}		
M. myristica	0.00 (Untreated)	3.67±0.16^{b}	4.33±0.16^{b}	4.00±0.27^{b}	5.67±0.16^{b}	6.00±0.00^{b}	6.00±0.00^{b}	6.00±0.00^{b}	35.32	III
	2.50	2.00±0.00^{a}	1.67±0.16^{a}	3.00±0.27^{a}	0.33±0.16^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}		

Values are means of triplicate samples followed by the standard error of means. Means in the same column with different superscripts for each plant material are significantly different ($p<0.05$) by Tukey's test RR: Repellency Rate, RC: Repellency Class

DISCUSSION

In this study, ethanol extracts of *D. tripetala*, *P. guineense*, *M. myristica* and *E. aromatica* significantly increased adult and larval mortality and were repellent to the main pest of the highly relished smoked catfish. The results obtained in this study are similar to the findings of Ofuya and Dawodu (2001) and Adedire and Lajide (2000) who reported the susceptibility of different ages of *Callosobruchus maculatus* and *D. maculatus* respectively to *P. guineense* powder at all rates of application. While Okorie *et al.* (1990) reported a 93% kill for *D. maculatus* larvae and total mortality of all adults when exposed to 2 g of neem seed powder / 25 g *Tilapia* species, Ofuya and Bamigbola (1991) and Fasakin (2003) reported the effectiveness of the crude extract of *M. myristica* against *C. maculatus* and *D. maculatus*, respectively.

In this study, the larvae were especially rapidly killed by the extracts. Lale (1995) reported that plant extracts are highly lipophilic and could penetrate the cuticle of insects. The result obtained in the study is in line with Aku *et al.* (1998) who reported that extract of *Anonna senegalensis* was more effective than the powder in the control of *C. maculatus*. Similarly, Okonkwo and Okoye (2001) reported 100% kill of larvae of *D. maculatus* when treated with extracts of *D. tripetala* and *P. guineense* at dosages lower than the powders. Odeyemi *et al.* (2000) observed that cases of high mortality occur in larvae partly because of their inability to detoxify plant toxins when feeding

actively, especially at the 1st-4th instar larval stage. According to the authors, larvae are voracious eaters because of growth requirements, in contrast to the adult insects, which tend to have a reduced feeding habit.

Stoll (2000) reported repellence as a major mechanism by which plant products control insect damage to stored produce. This view has been largely supported by the significant repellent effect of the ethanol extracts of the four plant materials investigated in this study. The rating of the extracts as promising repellents in this study is partly in agreement with Egwunyenga *et al*. (1998) who also attributed the repellence of *D. maculatus* and *N. rufipes* from admixed fish to olfactory and gustory sensations.

The toxicity properties of *P. guineense* and *D. tripetala* have been attributed to their pungent and pepperish taste which could asphyxiate insects by blocking the spiracles (Amusan and Okorie, 2002) and the presence of bioactive ingredients, such as, alpha-pinene, limonene and linalool in *P. guineense* (Golob *et al*., 1999), 2, terpenes and linoleic acid in *M. myristica* and eugenol, tannic acid and asarone in *E. aromatica* (Golob *et al*., 1999).

The use of the studied plant materials could be desirable in protecting cured fish in the tropics, especially as organoleptic assessment has shown that treated fish do not exhibit adverse evidence of taint, smell or change in taste, texture or flavour (Akinwumi *et al*., 2006).

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