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The Efficacy of the Plant Extracts of *Afrostyrax kamerunensis*, *Monodora myristica*, *Moringa oleifera* and *Azadirachta indica* against the Infestation of the Leather Beetle, *Dermestes maculatus* De Geer in Smoked African Mud Catfish, *Clarias gariepinus* Burchell

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Abstract

The information that the leather beetle, *Dermestes maculatus* De Geer is globally less susceptible to chemical insecticides provided the rationale to test some cosmopolitan elite plant materials on the pest. Extracts from four plant materials (country onion, *Afrostyrax kamerunensis* Perkins and Gilg; African nut meg, *Monodora myristica* Dunal; moringa, *Moringa oleifera* Lam; and neem, *Azadirachta indica* A. Juss) at 2.5 and 5.0 mL/ 100 g fish were assessed under tropical storage conditions (temperature: 31.9^{o}C; relative humidity: 68.3 %) to control the leather beetle, *Dermestes maculatus* infesting smoked African mud catfish, *Clarias gariepinus* Burchell. Four important indices in insect pest control were assessed using standard storage entomology procedures. Each of the plant extracts (excluding *M. oleifera*) at 5.0 ml/ 100 g fish caused significantly high mortality in *D. maculatus* adults at third and fourth day exposure periods. Adult beetle emergence was absolutely inhibited in the catfish treated with *A. kamerunensis*, *M. myristica* and *A. indica* at both test concentrations. Weight loss due to insect infestation was suppressed significantly, also when compared with untreated control. The test botanicals were effective in this order, *A. indica* > *M. myristica* > *A. kamerunensis* > *M. oleifera*. *Dermestes maculatus* adults were most sensitive to the repellent action of *A. kamerunensis*. Therefore, the study has identified a plant material that repels *D. maculatus* adults better than neem. These findings revealed that the extracts of *A. indica*, *M. myristica* and *A. kamerunensis* could be incorporated into post-harvest fish management strategies against *D. maculates*, particularly in solving the problem of the development of resistance to chemical insecticides.

Keywords: *Afrostyrax kamerunensis*, Botanicals, *Dermestes maculatus*, Fish management, Repellent, Resistance.

1. Introduction

Fish is highly nutritious and emphasis on the health benefits of fish consumption is on the increase (Nwosu *et al*., 2016). Fish is an important component of several delicacies in Nigeria and other countries of the world. Nowadays, catfish is highly appreciated in Nigeria. Rearing catfish has both aesthetic and economic benefits. It serves as a valuable source of income through self-reliance and employment. Catfish trading alleviates poverty, yields foreign exchange earnings, and provides raw material for the feed industry (Akinwumi *et al*., 2007). Generally, it has been estimated that the annual fish consumption in Nigeria is 1.2 million metric tons (FDF, 2005). The possibility of having fish supply that meets this demand is undermined by insect pest infestations. After harvest, fish is often processed, and sometimes stored for

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inevitable reasons of requiring a longer time to make sales, and to have a stock for consumption for a relatively longer period. Being highly perishable, catfish (like any other fish) must be treated to be relatively durable. One of such important treatments given to fish especially in developing countries is smoking (Nwosu *et al*., 2016). It has been indicated that 45 % of the total fish catch in Nigeria is utilized as smoked fish (FAO, 2002). Unfortunately, even smoked fish is susceptible to both quantitative and qualitative losses. *Dermestes maculatus* infestations contribute substantially (71.5 %) to the heavy losses in smoked catfish (Osuji, 1974; Akinwumi *et al*., 2007) and this calls for quick action.

Chemical insecticides can be used against *D. maculatus*; however, the risks involved outweigh the benefits. Health hazards, eco-toxicity, loss of visual appeal due to impacting influence of chemicals and exorbitant cost prices of these synthetic chemical insecticides are the major problems associated with their general use to suppress infestations and damage by *D. maculatus* in stored catfish (Boeke *et al*., 2001; Akinwumi *et al*., 2007). Notably, it has been reported that unlike other insect pests of stored products, dermestid larvae and adults are less susceptible to chemical insecticides (Amusan and Okorie, 2002; Onu and Baba, 2003). This observation is the major justification for the present testing of widely-available botanicals with a view to identify those that can tackle the problem of resistance to chemical insecticides and thus, effectively protect stored catfish against *D. maculatus* attack. In a previous study by Keeler (1999), neem (*Azadirachta indica*) products (botanicals) were tested for toxic, growth regulatory and antifeedant effects against *D. maculatus*, under similar conditions in storage facility and the result is part of the motivation in this study for testing the four plant materials against *D. maculatus*.

Indeed, for more than a century, plant-derived insecticides such as derris, sabadilla, nicotine, pyrethrum, physostigmine and rotenone have been in use (Adedire and Lajide, 1999). Similarly, a host of plant species in Nigeria have been used as control agents for various pests (Lale, 1995). Information in literature favors the use of plant-derived insecticides to control especially, *D. maculatus* (Amusan and Okorie, 2002; Onu and Baba, 2003). In the present study, parts of some cosmopolitan plant species (readily available) were tested against *D. maculatus*, notorious for devastating stored dried fish (Chris *et al*., 2014).

2. Materials and Methods

2.1. Insect Culture

Population of *D. maculatus* was first obtained from naturally-infested smoked catfish. The leather beetles were cultured in a Kilner jar covered with white muslin cloth and were routinely maintained at laboratory average temperature (30.9 ^{o}C) and relative humidity (68.3 %). To have new progenies for the experiment, adult beetles from the stock colony were placed on fresh disinfested fish for feeding and oviposition. Water-soaked cotton wools were put in the jar to induce oviposition and parent adults were removed after twenty-one days (Akinwumi *et al*., 2007).

2.2. Plant Materials and Extracts

The plant materials used in this study were the bulb of country onion, *Afrostyrax kamerunensis* Perkins and Gilg (Huaceae); seeds of the African nut meg, *Monodora myristica* Dunal (Annonaceae); seeds of moringa, *Moringa oleifera* Lam (Moringaceae) and seeds of neem, *Azadirachta indica* A. Juss (Meliaceae). All the plant materials are available in Nigeria; they have medicinal value, and are unlikely to have adverse effects on human health. The method reported by Akinwumi *et al*. (2007) was employed in the preparation of plant materials and extracts. The botanicals were dried in an electric oven at 40 ^{o}C for a period of eight hours. Thereafter, they were ground thoroughly using an electric blender (5.0 HP), and were made to pass through a-40 holes mm^{-2} mesh screen. Ten g of each of the sieved plant materials were put into a round bottom flask, and 100 ml of absolute ethanol was added and soaked for twenty-four hours. The mixture was boiled at 60 ^{o}C for thirty minutes in the laboratory water bath. The solution was filtered using Whatman no.1 filter paper. The filtrates were kept in separate and tightly-covered bottles prior to use.

2.3. Efficacy Test of Extracts

The samples of smoked African mud catfish, *C. gariepinus* (weighing average of 100 g) used for the assay were obtained from Northbank Market, Makurdi, Benue State, Nigeria. The fish samples and the experimental jars were disinfested by heat treatment in the Gallenkamp oven at 60 ^{o}C for one hour, and were allowed to cool at room temperature prior to commencement of the assay (Akinwumi *et al*., 2007). An aliquot of 2.5 mL of each of the four plant extracts was evenly rubbed separately to four disinfested smoked catfish samples. The treated fish samples were air-dried for two hours in order to eliminate traces of the solvent, and were then placed in four separate plastic jars (depth: 8 cm; diameter: 10 cm). An untreated control was designated. Ten newly emerged adults of *D. maculatus* were introduced into the five different jars, and were covered with muslin nets for ventilation and protection. A similar set-up using 5.0 mL of each of the plant extracts was made. The experimental design was randomized complete block design with four replications by members of the research group in different regions of Nigeria, namely North (Benue State), West (Osun State), South-East (Imo Sate) and South-South (Rivers State). The number of dead *D. maculatus* adults was recorded daily for four days, and the mortality rate was calculated. Beetles were confirmed dead when they failed to respond to probing with a sharp pin at the abdomen. All dead and live beetles were sieved out immediately after mortality count to ensure that the emerging adults were a direct consequence of the number of eggs oviposited in four days (Ileke *et al*., 2012). After mortality check, the set-up was maintained under the same experimental storage conditions until thirty days later when the accumulated emergence of *D. maculatus* in the treated and untreated smoked catfish samples was assessed. On the 30th day (following mortality count), the fish samples were reweighed using a digital balance to evaluate the weight loss resulting from *D. maculatus* infestation.

2.4. Repellence Test

A chamber of 25 x12 x 10 cm was constructed for the investigation on repellence (Akinwumi *et al*., 2007). An aliquot of 5.0 mL of each of the plant extracts was thoroughly rubbed separately on the skin of four randomly-selected fish samples weighing about 100 g. An untreated fish (of same weight) was included in the experiment. The treated and untreated samples were placed separately at each edge of the chamber, 10 cm apart. This was immediately followed by the introduction of ten *D. maculatus* adults (2 – 4 days old) at the center. The adult insects used in this segment of the study were also starved for forty-eight hours. Daily observations were made for five days immediately after application and the number of *D. maculatus* adults found on or within a 1.0 cm radius of treated and untreated fish was recorded twice daily at 09:00 hours and 16:00 hours. The average count for each five-day period was expressed as a percentage of repellency and the results (for treated fish only) were assigned to a repellency class using the following scale described by Laudani *et al*. (1955). Class 0, < 0.1 %; class I, 0.1 – 20 %; class II, 20.1 – 40 %; class III, 40.1 – 60 %, class IV, 60.1 – 80 %; class V, 80.1 – 100%. Repellency was re-assessed one month later.

2.5. Statistical Analysis

Data were investigated for normality using both residual and box plots. The assumption for homogeneity of group variance was tested using Levene's test for equality of variances. If data were normally distributed, and the assumption of homogeneity of group variance was met, or the deviation from homogeneity assumption was not sufficiently strong to affect the results (Sulehrie *et al*., 2003), the data were subjected to one-way analysis of variance. When the F-test was significant, treatment means were separated using a more pragmatic multiple comparison test, Honestly Studentized range (HSD). Inference was made at $\alpha = 0.05$. The statistical software was SPSS (Statistical Package for the Social Sciences) (version 19.0).

3. Results

Results of *D. maculatus* mortality due to the treatment with four plant extracts are summarized in Table 1. All the plant species were toxic to the leather beetle. However, the number of *D. maculatus* adults killed by the different plant extracts was statistically different. The extract of *A. indica* was the most toxic to *D. maculatus* after ninety-six hours of exposure. At a dose of 2.5 mL/100 g fish, *A. kamerunensis* and *M. myristica* showed faster action within the first forty-eight hours. At a higher dose of 5.0 ml/100 g fish, *A. indica* recorded the fastest action; killing 74 % of the dermestids in seventy-two hours. The extract of *M. oleifera* was the least toxic to the leather beetles, even at an increased dose of 5.0 mL/100 g fish. In general, mortality increased progressively with increasing application rate and time of exposure. At both test concentrations, it was observed that the extracts of the plant materials (except *M. oleifera*) totally inhibited the emergence of *D. maculatus* adults from smoked catfish within thirty days of storage (Table 2). Inability to prevent adult emergence was significant for *M. oleifera*.

Table 3 presents the effect of the plant extracts on the weight loss of the treated fish. Decrease in weight loss was not consistently recorded when fish was treated with a higher concentration of 5.0 mL. Each of the plant materials allowed a certain level of weight loss among the treated specimens; inclusive of extracts that completely inhibited adult emergence. Extracts of *A. indica* and *M. myristica* were best at disallowing loss in the quantity of stored smoked catfish infested by leather beetles. Table 4 shows the spectrum of repellence against adult *D. maculatus* offered by extracts of different plants. *Afrostyrax kamerunensis* is the best repellant, followed by the seed extracts of *M. myristica* and *A. indica*, which came second in repelling adult leather beetles from infesting smoked catfish. Meanwhile, *D. maculatus* adults were the least sensitive to the repellent action of the seed extract of *M. oleifera* under the tropical storage conditions investigated.

Table 1. Effects of four plant extracts on the mortality of adult *D. maculatus* De Geer infesting smoked cat fish, *C. gariepinus* Burchell (in all cases, df = 4,15).

Plant materials	% mortality at 2.5 mL/100 g fish				% mortality at 5.0 mL/100 g fish			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
A.kamerunensis	13.33±4.70^{a}	33.33±0.05^{a}	36.67±0.43^{a}	60.00±0.00^{a}	16.67±0.50^{a}	33.34±0.01^{a}	56.67±0.41^{a}	86.67±2.67^{a}
M. myristica	16.67±0.66^{a}	40.00±0.00^{a}	46.67±0.02^{a}	66.67±0.11^{a}	30±0.00^{b}	43.33±0.29^{a}	73.33±0.67^{b}	96.66±0.45^{a}
M. oleifera	6.67±0.10^{b}	10.00±0.00^{b}	16.67±0.55^{b}	30.00±0.00^{b}	10.00±0.00^{a}	13.33±3.90^{b}	36.66±0.01^{c}	49.99±0.01^{b}
A. indica	3.33±0.10^{b}	20.00±0.01^{c}	46.66±0.30^{a}	86.66±0.01^{c}	16.67±0.33^{a}	43.34±0.89^{a}	74.34±2.50^{b}	97.67±0.02^{a}
Control	0.00±0.00^{b}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}
F	5.3	22.1	10.7	19.0	14.5	12.9	32.2	18.9
P	0.03	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Data are means ± standard error of the means of four replications

Means in a column followed by the same letter are not significantly different by HSD ($\alpha = 0.05$)

Table 2. Effects of four plant extracts on the adult emergence of *D. maculatus* De Geer infesting smoked cat fish, *C. gariepinus* Burchell (in all cases, df = 4,15) within 30 days.

Plant materials	Number of emerged adults (at 2.5 mL/100 g fish)	Number of emerged adults (at 5.0 mL/100 g fish)
A. kamerunensis	0.00±0.00[a]	0.00±0.00[a]
M. myristica	0.00±0.00[a]	0.00±0.00[a]
M. oleifera	37.53±7.10[b]	41.96±3.20[b]
A. indica	0.00±0.00[a]	0.00±0.00[a]
Control	93.18±10.20[c]	93.18±10.20[c]
F	45.2	38.3
P	< 0.01	< 0.01

Data are means ± standard error of the means of four replications

Means in a column followed by the same letter are not significantly different by HSD (α = 0.05)

Table 3. Effects of four plant extracts on the weight loss caused by *D. maculatus* De Geer infesting smoked cat fish, *C. gariepinus* Burchell (in all cases, df = 4,15).

Plant materials	% weight loss (at 2.5 mL/100 g fish)	% weight loss (at 5.0 mL/100 g fish)
A. kamerunensis	0.63±0.37[a]	0.30±0.00[a]
M. myristica	0.07±0.50[b]	0.25±0.01[a]
M. oleifera	3.07±0.10[c]	3.90±1.20[b]
A. indica	0.04±0.00[b]	0.27±0.00[a]
Control	4.01±0.20[c]	4.01±0.20[b]
F	8.6	5.5
P	< 0.02	< 0.02

Data are means ± standard error of the means of four replication

Means in a column followed by the same letter are not significantly different by HSD (α = 0.05)

Table 4 Repellent action of four plant extracts (5.0 mL/100 g fish) on adults of *D. maculatus* De Geer infesting stored catfish, *C. gariepinus* Burchell

Plant materials	1 - 5 days after application		1 month after application	
	% Repellence	Repellence class	% Repellence	Repellence class
A. kamerunensis	50.45±2.90	III	50.01±0.01	III
M. myristica	39.67±0.12	II	30.52±0.34	II
M. oleifera	8.77±5.34	I	9.02±0.10	I
A. indica	37.64±0.01	II	36.33±2.61	II

Class 0, < 0.1 %; Class I, 0.1 – 20 %; Class II, 20.1 – 40 %; Class III, 40.1 – 60 %, Class IV, 60.1 – 80 %; Class V, 80.1 – 100%.

4. Discussion

The results of this investigation revealed that the extracts of neem, *Azadirachta indica* is the best bio-insecticide that controlled *D. maculatus* adults because it killed the highest number of adult leather beetles at the end of the exposure period. The analyses of results further showed that the extracts of *A. indica*, *M. myristica* and *A. kamerunensis* inhibited adult emergence totally, while the extracts of *A. indica* and *M. myristica* allowed the lowest quantitative fish loss. It was also revealed that the extracts of *A. indica* and *M. myristica* were next to *A. kamerunensis* in repelling the insects. The best performance of neem in terms of causing mortality of *D. maculatus* adults is in agreement with the findings of Keeler (1999). According to the literature, at various test concentrations of neem products, the botanical caused high mortality of *D. maculatus*, had antifeedant effect on the insect larvae which consequently failed to develop to the pupal stage, and the adult emergence was totally inhibited. According to the information in the literature, the repellence property of neem is not as strong as the toxic and antifeedant properties. However, repellence has been reported as a major mechanism by which plant materials evoke control on stored product insect pests (Akunwumi *et al*., 2007). This is supported by the findings of this study. The active ingredient in *A. indica* is largely *azadirachtin* (Xie *et al*., 1995). In other words, azadirachtin was largely responsible for the toxic (physiological), inhibitory (physiological) and repellent (behavioural) actions of neem on *D. maculatus*. From the analyses of results, the seed extract of *M. myristica* was also effective in managing *D. maculatus* infestations of smoked catfish. The biological activity of *M. myristica* is strongly attributed to terpenes and linoleic acids (Akinwumi *et al*., 2007). On the whole, plant extracts are highly lipophilic (Lale, 1995) and thus, they have the capacity to penetrate the insect integument. Previous studies reported the effectiveness of *M. myristica* and *A. indica* in the control of some stored product insect pests (Fasakin, 2003, Akinwumi *et al*., 2007). The poor insecticidal activity of the extract of *M. oleifera* tallied with the information in the current literature. Irikannu *et al*. (2015) observed that the seed oil extract of *M. oleifera* had no obvious insecticidal effect on some stored product insect pests which was also observed in this study.

5. Conclusion

In conclusion, although the medicinal value of *M. oleifera* has been reported (Lakshmipriya *et al*., 2016), in this study, it failed to control *D. maculatus* infesting smoked catfish. At the application rates examined, it can be concluded that the seed extract of *M. oleifera* is not recommended for use in the protection of smoked catfish against *D. maculatus* infestation under tropical storage conditions. This study has included *D. maculatus* in the range of stored-product insect pests controllable by the extracts of *A. indica* and *M. myristica* seeds. Thus, they can substantially be of help in the bio-rational management of *D. maculatus* in stored smoked catfish. It is encouraging to note that the two effective botanicals possess medicinal values and do not have adverse effects on human health. Therefore, it can be inferred that the topical applications of these plant materials will remedy the problem of resistance associated with the use of chemical insecticides in *D.*

maculatus control, and prevent the health and environmental risks also associated with chemical insecticides. The effects of *A. kamerunensis* and *M. myristica* were more noticeable than the effect of *A. indica* within the first forty-eight hours; however, they were soon overtaken by *A. indica* which ultimately killed more dermestids. In terms of repellence, *A. kamerunensis* (with a cumulative lower mortality effect) ranked first and that is an edge over neem.

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