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Evaluation of Toxicity of Ethanolic Extracts of *Piper guineense* and *Capsicum annum* on Dermestid Beetle, *Demestes maculatus* (DeGeer 1774), a Pest on Dried Catfish, *Clarias gariepinus*

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ABSTRACT

The effectiveness of *Piper guineense* and *Capsicum annum* in prevention of *Dermestes maculatus* infestation on sundried catfish, *Clarias gariepinus* was studied. Ethanolic extracts of *P. guineense* and *C. annum* fruits were prepared using Soxhlet extractor. In a split-plot design comprising the two extracts in different concentrations, a total of 63 sundried adult *C. gariepinus* of mean weight 111.47 ± 2.34g were randomized into three treatment groups A – C for each extract and a control D. The treatment groups were in triplicates with each containing three catfish. The groups contained fish infested with 20 larvae, 20 adults and eggs of *D. maculatus*. Groups A – C were exposed to 8, 12 and 15% (w/w) of extract per kilogram of fish respectively. Observation for mortality and larval emergence was made every 3 days and cumulative taking at the end of 28 days. Mortality of adult *D. maculatus* was higher in 8, 12 and 15% (w/w) of *P. guineense* and 15% (w/w) of *C. annum* than control. Both extracts significantly enhanced *D. maculatus* larval and adult mortality ($p < 0.05$). Larval emergence in 8, 12 and 15% (w/w) of *P. guineense* and *C. annum* were significantly reduced than in the control ($p < 0.05$). The three concentrations of *P. guineense* and 15% (w/w) of *C. annum* lowered weight loss of the fish significantly ($p < 0.05$). Ethanolic extracts of *P. guineense* and *C. annum* were moderately effective in protection of dried catfish, *C. gariepinus* against *D. maculatus* infestation.

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Authors' Contribution
FNE, JIN, CMO and GIN conceived the study. IONA and CU performed the experiment. IONA, FNE, CMO and JIN performed statistical analysis. FNE, IONA, CU and GIN wrote the manuscript.

Key words
Piper guineense, Capsicum annum, Dermestes maculatus, Clarias gariepinus, Insecticidal.

INTRODUCTION

Fish supplies a good balance of protein, vitamins and minerals (Ufele *et al*., 2015). Fish protein is known to be the cheapest sources of animal protein (Olayide, 1973). Fish constitute more than 60% of the total protein intake in adults especially in rural areas (Adeleye, 1992). It provides about 20% protein and essential amino acids such as lysine and methionine, and it competes favourably with the protein content of egg, milk and meat. The harvesting, handling, processing and distribution of fish provide livelihood for millions of people as well as providing foreign exchange earnings for many countries (Al-Jufaili and Opara, 2006). Dried fish in particular is one of the highly digestible and respectable sources of proteins and essential minerals in the tropics but it is highly prone to insect pest infestation at storage (Odeyemi *et al*., 2000). It has been estimated that post-harvest losses of between 10% and 35% by weight of the world fish catch result from poor handling, processing and storage (Lale and Alaga, 2002).

Dermestes maculatus is the major pest of smoked fish (Eke *et al*., 2008) and could be controlled by synthesized chemicals (Boeke *et al*., 2001). However in recent years, there have been increasing and concerted efforts directed at developing natural pest management control agents that are relatively cheap, safe and environment friendly as alternatives to synthesized insecticides (Boeke *et al*., 2001; Akinwumi *et al*., 2007; IIyas *et al*., 2017). Botanical extracts being alternatives to the use of insecticide in the control of pests have been to an extent efficient in pest management. Teotia and Terwari (1977) noted that insecticides of plant origin were especially valued for application against insect pest of fodder, fruits, vegetable and stored produce because of their high degree of tolerance by mammals. Most of the botanical insecticides that have been tested include guava (Rehin and Espig, 1991) and eucalyptus (Dakshinamurthy, 1988). However, against this background, the aim of this research was to evaluate two botanicals (*Piper guineense* and *Capiscum annum* seeds) for the control of dermestid beetle (*Dermestes maculatus* De Geer) on dried fish.

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MATERIALS AND METHODS

Collection of dried fish samples

Samples of dried fish were bought from Ogige market in Nsukka, Enugu State, Nigeria. The fish samples used for the project were not infested at the time of purchase. The dried fish samples were taken to the Entomology Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State.

Dermestes maculatus *collection*

Dermestes maculatus were collected from infested stored dried fish by the help of dry fish sellers in Ogige Market, Nsukka, Enugu State. They were all collected into big containers in which the adult males and females were allowed to mate and reproduce. Thereafter, the first generation was collected to infest the pest-free fish samples. The pest used was further confirmed at the Entomology Unit of Zoology and Environmentl Biology, Univeristy of Nigeria, Nsukka as *Dermestes maculatus.*

Collection of plant material and extracts preparation

Piper guineense and *Capiscum annum* seeds were collected from Nsukka in Enugu State. The plant samples were identified at Department of Plant Science and Biotechnology, University of Nigeria, Nsukka where voucher samples were kept.

The seeds of the plants were collected in different baskets. They were exposed for seven days to dry at room temperature to avoid the loss of its active compounds. After drying, they were taken to the Department of Crop Science, University of Nigeria, Nsukka for grinding. The powdered form was put in air-tight bottles and stored in refrigerator. Ethanolic extracts of *P. guineense* and *C. annum* seeds were prepared using a Sohxlet apparatus.

Phytochemical analysis

Phytochemical analysis was done in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences of the University of Nigeria, Nsukka. Phytochemicals tested for were alkaloids, saponings, glycosides, tannins, steroid, terpenoid, flavonoids and phenols.

Experimental design

In a split-plot design comprising the two extracts at different concentrations, 63 sun-dried adult *C. gariepinus* catfish of mean eight 111.47 ± 2.34g were randomized into three groups A – C for each extract and a single control D. The treatment groups were replicated three times with each containing three catfish. The groups contained fish infested with 20 larvae, 20 adults and eggs of *D. maculatus*. Groups A – C were exposed to 8, 12 and 15% (w/w) of extract per kilogram of fish, respectively. Observation for mortality and larval emergence was made every 3 days and cumulative taking at the end of 28 days.

Evaluation of insecticidal activity

Numbers of dead *D. maculatus* in the treatment groups were recorded every 3 days for the duration of the study. All the dead insects were removed every 3 days. The insecticidal activity of *P. guineense* and *C. annum* seed extracts were evaluated as percentage *D. maculatus* mortality using the formula:

$$\text{Mortality (\%)} = \frac{\text{No. of dead } C.\,maculatus}{\text{Total No.of } C.\,maculatus \text{ introduced}} \times 100$$

Evaluation of larvae emergency

At the end of 28 days the total number of larvae that emerged was counted. Percentage emergence was calculated using the formula:

$$\text{Emergence (\%)} = \frac{\text{No. of larvae that emerged}}{\text{No. of eggs laid}} \times 100$$

Weight loss

The weight of the fish in each replicate at the commencement (initial weight) and at the end of 28 days (final weight) treatment was taken. From which weight loss was calculated using the formula:

$$\text{Weight loss (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Data analysis

Data was analysed using Statplus Pro Version v 5.8.9 (AnalystSoft Inc., Walnut, Canada) and Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp., Armonk, New York). Student T-test and two-way Analysis of Variance (ANOVA) was used to compare concentration and duration associated activity of the extract against *D. maculatus.* Level of significance was set at $p < 0.05$.

RESULTS

Mortality

Mortality of larvae *D. maculatus* was enhanced by *P. guineense* and *C. annum* ethanolic extracts (Table I). The performance of *P. guinnense* was not improved by increase in concentration from 8 to 15% (w/w). Only 15% (w/w) of *C. annum* extract significantly improved *D. maculatus* mortality compared to control. There was no significant difference between equal concentrations of the two extracts in causing mortality of *D. maculatus* larvae. Mortality of

adult *D. maculatus* was similarly enhanced on exposure to ethanolic extracts of *P. guineense* and *C. annum* (Table I). The activity of *P. guineense* appears to be concentration dependent; 8% *P. guineense* performance was not different from that of control significantly ($p > 0.05$). There was no significant difference in the performance of *P. guineense* compared to equal concentration of *C. annum* extract ($p > 0.05$).

Table I.- Effect of *Piper guineense* and *Capsicum annum* extracts on mortality of larvae and adult *Dermestes maculatus*.

Concentration (%w/w)	*P. guineense*	*C. annum*	P – value*
Larvae (n = 10)			
Control (0)	15.67 ± 4.04^{b}	15.67 ± 4.04^{b}	-
8	32.38 ± 5.72^{a}	19.63 ± 1.59^{b}	0.098
12	35.83 ± 8.74^{a}	49.79 ± 22.80^{b}	0.598
15	49.21 ± 7.96^{a}	55.00 ± 14.40^{a}	0.650
Adult (n = 10)			
Control (0)	15.00 ± 5.77^{b}	15.00 ± 5.77^{b}	-
8	26.67 ± 6.01^{ab}	40.00 ± 7.64^{a}	0.152
12	38.33 ± 1.67^{a}	36.67 ± 3.26^{a}	0.670
15	40.00 ± 10.00^{a}	31.67 ± 4.01^{a}	0.191

Values as mean ± SE. Values with different alphabet superscript in a column were significantly different from DMRT. *P – value from Student t – test.

Table II.- Effect of *Piper guineense* and *Capsicum annum* extracts on larvae emergence (%) of *Dermestes maculatus*.

Concentration (% w/w)	*P. guineense*	*C. annuum*	P – value*
Control (0.0)	60.66 ± 6.02^{a}	60.66 ± 6.02^{a}	
8	25.85 ± 1.10^{bc}	30.07 ± 1.40^{b}	0.076
12	30.33 ± 8.36^{b}	37.51 ± 5.64^{b}	0.683
15	7.43 ± 8.43^{c}	38.33 ± 9.66^{b}	0.073

Values as mean±SE. Values with different alphabet superscript in a column were significantly different from DMRT. *P – value from Student t – test.

Larvae emergence

Emergence of larval *D. maculatus* was inhibited by ethanolic extracts of *P. guineense* and *C. annum* (Table II). Percentage larval emergence of *D. maculatus* was significantly suppressed by 8, 12 and 15% (w/w) of *P. guineense* and *C. annum* extracts compared to control ($p < 0.05$). Comparatively, *P. guineense* had better *D. maculatus* larval emergence inhibitory effects than *C. annum*. The *D. maculatus* larvae emergence inhibitory effect of *P. guineense* was, however, not significantly different from *C. annum* performance at $p < 0.05$.

Weight Loss

Percentage weight loss of dried *C. gariepinus* on infestation with *D. maculatus* for 28 days was minimal (< 12%). Treatment of infested sundried fish with *P. guineense* and *C. annum* lowered weight loss (Fig. 1). *P. guineense* caused significant reduction in fish weight loss at 8 and 15% w/w; only at 15% w/w was the performance of *C. annum* against weight loss in fish significant ($p < 0.05$). Weight loss was not significantly different between same concentrations of the extracts ($p > 0.05$).

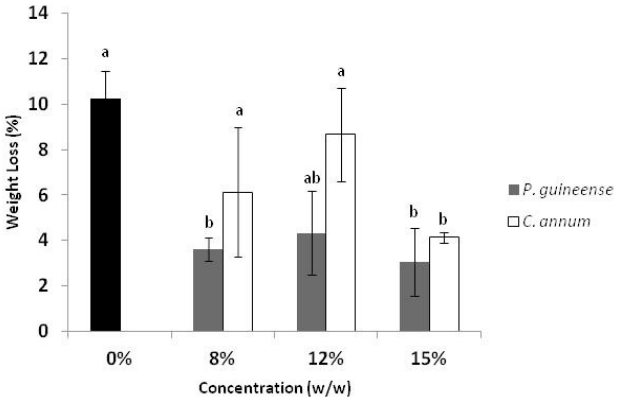

Fig. 1. Weight loss of dried fish after treatment of *Dermestes maculatus* infestation by *P. guineense* and *C. annum* extracts. Compared to the control (0%) percentage weight loss with different alphabet label was significantly different ($p < 0.05$).

Proximate composition of sundried fish

The proximate composition of sundried *C. gariepinus* before and after treatment with the ethanolic extracts of *P. guineense* and *C. annum* are presented in Table III. Initial proximate composition before infestation and after infestation with *D. maculatus* and treatment with concentration of the extracts are given.

Phytochemical composition of Piper guineense *and* Capsicum annum

The phytochemicals tannin, flavonoids, alkaloids, steroids, saponin, terpenoids and cardiac glycosides were isolated from *P. guineense* (Table IV). Steriods and cardiac glycosides were abundant while tannin, flavonoids, alkaloids and terpenoids were in moderate concentrations. Less number of phytochemicals namely tannin, flavonoids, alkaloids, saponin and terpenoids were isolated from *C. annum*. Only alkaloid and saponin were in moderate concentration in *C. annum*.

Table III.- Effect of *Piper guineense* and *Capsicum annum* extracts on proximate composition of the dried fish before and after treatment.

	Extracts concentration		
	8%	**12%**	**15%**
Protein			
Initial (%)	69.18 ± 1.41	74.20 ± 1.81	60.36 ± 1.30
Pg Final (%)	67.16 ± 1.95	65.18 ± 0.68	69.18 ± 1.35
Cpa Final (%)	63.31 ±0.42	70.32 ± 0.43	54.05 ± 0.19
Carbohydrate			
Initial (%)	3.78 ± 0.50	0.73 ± 0.55	7.86 ± 0.72
Pg Final (%)	1.79 ± 0.22	6.43 ± 0.38	11.17 ± 0.39
Cpa Final (%)	4.81 ± 0.35	0.59 ± 0.48	5.87 ± 0.91
Fat			
Initial (%)	0.67 ± 1.73	0.68 ± 0.34	0.42 ± 0.18
Pg Final (%)	4.34 ± 0.23	5.64 ± 0.53	1.78 ± 0.43
Cpa Final (%)	0.64 ± 1.71	0.65 ± 1.03	0.44 ± 0.39
Fibre			
Initial (%)	0.71 ± 0.32	0.48 ± 0.33	0.56 ± 0.41
Pg Final (%)	3.27 ± 0.34	3.27 ± 0.34	1.56 ± 0.16
Cpa Final (%)	0.63 ± 0.21	0.58 ± 0.11	0.40 ± 0.37
Ash			
Initial (%)	5.25 ± 0.37	5.35 ± 0.39	5.78 ± 0.33
Pg Final (%)	14.86 ± 1.79	9.92 ± 0.73	10.47 ± 0.56
Cpa Final (%)	22.41 ± 0.44	14.38 ± 0.98	19.05 ± 0.90
Moisture			
Initial (%)	16.10 ± 0.84	19.17 ± 2.14	31.14 ± 1.01
Pg Final (%)	10.18 ± 0.19	10.78 ± 0.65	10.79 ± 0.53
Cpa Final (%)	13.50 ± 2.33	16.34 ± 1.82	18.26 ± 0.12

Pg, *Piper guineense*; Cpa, *Capsicum annum.*

Table IV.- Phytochemical analysis of *Piper guineense* and *Capsicum annum.*

Phytochemical	***Piper guineense***	***Capsicum annum***
Tannin	++	+
Flavonoid	++	+
Alkaloid	++	++
Steriods	+++	-
Saponin	+	++
Terpenoids	++	+
Cardiac glycosides	+++	-

+++, abundant; ++, moderate; +, little; -, absent.

DISCUSSION

The efficacy of plant materials and other chemicals as insect control agents are measured directly in terms of abilities to cause mortality of insects, reduction in insect reproductive efficiency (such as reduction in larvae emergence from eggs, and reduction of imago emergence from larvae or pupa), and insect repellency. Indirectly, reduction in damage to infested material measured as number of holes borne into the material, weight or nutrient loss by the material, *etc*. may be used. The plant materials used had disparate activities in the aspects considered. Both plant extracts performed moderately as insecticidal against larva and adult *D. maculatus*. Insecticidal activities were recorded against larvae and adult *D. maculatus*.

Toxicity of *P. guineense* has been attributed by various authors to their pungent and peppery taste which could asphyxiate insects by blocking the spiracles (Ufele *et al*., 2015). The presence of bioactive ingredients such as alpha-pinene, limonene and Linalool in *P. guineense* is a major contributor to their toxic properties against insects (Golob *et al*., 1999). Similar observation have also been made by Adedire and Lajide (1998) that plant materials within the family *Piperaceae* to which *P. guineense* belongs have been reported to possess some form of insecticidal properties against eggs of cowpea storage pest, *Bruchid*, and also capable of suppressing various developmental instars of *Callosobruchus maculatus*. *C. annum* seed have been reported to possess insecticidal activities against adults *C. maculatus* and *Sitophilus zeamais* (Oni, 2010; Mailafiya *et al*., 2014), larvae *Anopheles stephensi* and *Culex quinquefasciatus* (Madhumathy *et al*., 2007). The insect control performance of *C. annum* and other *Capsicum* (Solanacae) is due to the presence of seven capsinoids including capsaicin and hydrocapsain (Madhumathy *et al*., 2007; Oni, 2010).

The low emergence of larvae *D. maculatus* from sundried *C. gariepinus* treated with *P. guineense* and *C. annum* could be due to the presence of alkaloid, steroid and cardiac galacosides groups. Similar observations have been reported by some authors on the disruptive effect of *P. guineense* and *C. annum*. Akinwumi *et al*. (2007) reported that pulverized plant materials from *P. guineense* inhibited egg hatchability and adult emergence of *D. maculatus* in smoked catfish (*C. gariepinus*) during storage. The resultant decreased emergence observed on dried fish treated with the *P. guineense* could be due to high toxic effect of these products on both egg, larval and adult stages of *D. maculatus*. At the end of four weeks, appreciable difference in weight loss by the dried catfish noticed between the groups treated with the extracts and those untreated may have resulted from the reduction in the number of *D. maculatus* infestation in the infested fish. The short duration of treatment probably caused the minimal observed difference in weight loss generally. Longer duration of treatment would probably have caused more appreciable weight reduction untreated dried catfish than the treated.

CONCLUSION

P. guineense and *C. annum* have insecticidal potential against adult *D. maculatus*. The protective and toxic effects of *P. guineense* and *C. annum* in addition to local availability make them attractive material in upgrading traditional post-harvest protection practices. The effectiveness of *P. guineense* and *C. annum* in reducing damage and controlling *D. maculatus* infestation in sundried catfish, *C. gariepinus* during storage could be employed as a possible means of ensuring a steady supply of good quality dried fish.

Statement of conflict of interest

Authors have declared no conflict of interest.

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