



Biotoxicity and Eco-Friendly Characterization of Plant Materials as Green Pesticides for the Control of Fish Beetles (*Dermestes Maculatus*)

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ABSTRACT

Biotoxicity of two plant powders; Piper guineense (Schum and Thonn) and Zingiberofficinale was investigated to ascertain their efficacies as sustainable and eco-friendly pesticides against fish beetles, *Dermestes maculatus*. Eight dosages, 0.02, 0.04, 0.06, 0.08, 0.05, 1.0, 1.5 and 2.0 all in grams (g) were used in the assay and larval and adult mortalities monitored in two distinct experiments after 24 hours, 48 hours and 72 hours. Piper guineense powder at 2.0g gave mortalities of 83.33% and 76.67% for larva and adult respectively. In Zingiberofficinale, larval and adult mortalities of 76.67% and 73.33% respectively were obtained at 2.0g. There was 150% and 106.7% larval and adult emergence respectively when the smoked catfish (*Clarias gariepinus*) was treated with P. guineense powder. Similarly, when the fish was treated with Z. officinale, there was 166.7% and 156.7% larval and adult emergence respectively. The percentage weight losses were 0.15% and 0.35% for larvae and adults respectively in fish treated with P. guineense and 0.45% and 0.90% for larvae and adults respectively in fish treated with Z. officinale. The LD₅₀ values were 28.3mg and 130.5mg for larva and adult respectively in fish treated with P. Guineans while the LD₅₀ for larva and adult in Z. officinale treated fish were 66.2mg and 258.8mg, respectively. P. guineense was also observed to more toxic than Z. officinale, thereby ranking higher in storage potentialities. The plant materials could play a sustainable role in protecting the main source of protein in local food menu as well as foster development in bio and socio-economic values of the occupational areas.

Keywords: LD₅₀, Sustainability, Piper guineense and Zingiberofficinale, *Clarias gariepinus*

INTRODUCTION

Fish is a food consumed by many species including humans; fish has been an important source of protein for humans throughout recorded history and contains many vitamins and mineral. Fish protein is known to be the best and cheapest sources of animal protein [1]. It provides about 20% protein and essential amino acids such as lysine and methionine, and it compares favourably with the protein content of egg, milk and meat.

Dried or smoked fish is a highly favoured item of many traditional dishes in Nigeria and it is a condiment that greatly enriches the flavor of various dishes and often a good alternative to fresh fish, which in many places is not readily available. Dried fish especially smoked ones, are often brittle and easily damaged. In the tropics where humidity is constantly high, they absorb moisture and then become susceptible to spoilage by fungi and bacteria and to infestation by dipterous and acarine pest [2].

It has been estimated that post-harvest losses of fish account for between 10% and 35% by weight of the world fish catch as a result of poor handling, processing and storage. Losses in small-scale fisheries are even higher and can sometimes amount to 40 – 50% nation's catch [3]. Dried fish is susceptible to attack by insects especially beetle species of the genera, *Dermestes* and *Necrobia*. The loss of dried fish to its best known pest,

Dermestes maculatus has been variously quantified [2]. Under traditional storage conditions, losses due to beetle infestation for instance, have been estimated at around 50% [3].

Talabu [4] reported that in the Lake Chad area of Nigeria, a lot of fish were sun-dried in the open and may be dipped in 8% formulation of gammalin 20 solution before being sundried. This, it is believed will prevent insect infestation. The direct application of synthetic insecticides in this way is considered potentially dangerous. In Ghana, Bull [5] reported that insecticides applied this way caused blurred vision, dizziness and vomiting in villages where such are consumed. However, as alternatives to these synthetic chemicals a lot has been done by researchers in the use of botanical insecticides. Dale [6] reported a lot of research being undertaken in many countries which include Nigeria, Ghana, Kenya, Egypt, Pakistan, the Phillipines and Japan. Teotia and Terwari [7] noted that insecticides of plant origin, because of their high degree of tolerance by mammals, were particularly valued for application against insect pest of fodder, fruits, vegetable and stored produce. Most of the botanical insecticides that have been tested include guava [8], the African oil palm [9], Eucalyptus [10].

Lale [3] reported that sprinkling of fish with chilli pepper (*Capsicum frutescens*) powders and lime juice while it is drying prevents infestation by insect pests. According to the author, pulverized dried citrus peels, leaves of *Bosciasenegalensis* and neem seed powder added to dried fish also prevent beetle infestation. In addition, some powders made from fruits, seeds, flowers, leaves, shoots, bark and roots of local medicinal/insecticidal plants have been demonstrated to be effective in protecting stored produce against pest depredation. Examples include powders from *Piper guineense*, schum and Thonn seeds [11], *Zingiberofficinale* [12], dry fruits of *Capsicum* species [13]. Toxicity to adults, reduction of oviposition, ovidical activity and toxicity to immature stages prior or immediately following penetration of plant tissue have been suggested as mechanism of action of insecticidal plant powders [14]. Akinwumi [15] had earlier documented the pesticide potentiality of *Piper guineense* while working on four plant powders (*Dennettatripetala* Baker, *Eugenia aromatic* Hook, *Monodoramyristica* (Dunal) and *Piper guineense*, Schum and Thonn). According to the author, each of the four plant powders caused significantly high ($p < 0.05$) mortality in both the adults and larvae of the fish beetle at all concentrations when compared to the control and was effective in inhibiting progeny development in treated fish.

Egwyunyenga et al. [16] reported the repellency of powders of *Dennettatripetala* to *Dermestes maculatus*. Also, Fasakin and Aberejo [17] showed that pulverized plant materials affected the developmental stages of fish beetle, *Dermestes maculatus* Degeer. Onu and Baba [18] reported that neem kernel powder at the rate of 5g per 35g of fish significantly suppressed oviposition and adult emergence of *D. maculatus*.

However, to ensure food security, substitute synthetic pesticides and sustainable management of fishery resources, this study therefore aims to compare the efficacy of powders of *Piper guineense* and *Zingiberofficinale* for the control of *Dermestes maculatus* on smoked catfish (*Clarias gariepinus*) during storage.

MATERIALS AND METHODS

Collection, Preparation of Fish Samples and Insect Culture

The fish species used for this study was *Clarias gariepinus* procured from "Otuocha" market, Anambra State, Nigeria. The fish were killed and cut into small sizes. They were subsequently dried using local iron processor and stored in an air tight container before being used.

Adults and larvae of *D. maculatus* were cultured in the laboratory of Zoology department, NnamdiAzikiwe University, Awka, Anambra State, Nigeria at laboratory temperature and relative humidity. The adults were collected from fish shops in "EkeAwka" market in Awka, Anambra State and were maintained on a large quantity of fish in a plastic bucket covered with nylon mesh held in place with rubber band, using the Halstead [19] and Stanley and Wilber [20] methods. Freshly emerged adults and late instars of *D. maculatus* larva were then used for the experiment.

Preparation of Plant Powders

Plant materials used in this study were bought from "Eke Awka" market in Awka, Anambra State. These materials were air-dried in a well ventilated area for one week before they were grounded thoroughly in an electric 1.5HP kitchen grinder and sieved through a 40 holes/mm² mesh screen. Each of the plant powders was kept in a separate plastic container with a tightly fitted lid and placed in a cooled incubator at 00C for use in the experiment. The plant materials are listed in the table below.

Table 1. Plants materials evaluated for insecticidal properties

Scientific Name	Common/Local Name	Family	Part to be used
<i>Piper guineense</i> (Schum and Thonn)	Black Pepper	<i>Piperaceae</i>	Seeds
<i>Zingiberofficinale</i>	Ginger	<i>Zingiberaceae</i>	Ginger roots/Rhizomes

Effects of Plant Powders on Larvae and Adults of *D. Maculatus*

Twenty grams (20g) of clean uninfested fish variety were weighed into 60ml plastic vials. To each vials dosages of plant materials added were 0.02g, 0.04g, 0.06g, 0.08g, 0.5g, 1.0g, 1.5g, 2.0g for larva and adult

respectively. A control experiment was set up with no plant material. The experiment was conducted in two ways; fifteen pairs of larva each were introduced to the vials for the experiment one and fifteen pairs of adult each were introduced to the vials for the experiment two as well as the control sample.

The lid of the plastic vials were perforated and secured with a netting material, this is to ensure aeration and avoid entry or exit of insects. The content of the vials were then thoroughly mixed. Each treatment was replicated three times; the experiment was arranged in completely randomized design. The number of dead larvae and adults was counted after 24 hours, 48 hours, and 72 hours to determine the mortality rate of larvae and adult of *D. maculatus*, respectively, oviposition, larval emergence and damage was also assessed in the experiment.

Statistical Analysis

All data obtained were subjected to analysis of variance (ANOVA) model in Genstat 2007 Release 7.2DE. Means were separated after significant F-test ($P < 0.05$) using the least significant difference (LSD). Probit values of corrected mortality were plotted against the corresponding log doses to determine LD50. Data on percentage mortality was corrected using Abott's formula;

$$PT = (PO - PC);$$

Where

$$PT = \text{Corrected mortality (\%)}$$

$$PO = \text{Observed mortality (\%)}$$

$$PC = \text{Control mortality (\%)}$$

Insects subsequently emerging were counted to estimate FI progeny production. Counting was stopped after 10 days to avoid overlapping of generation.

Weight loss was measured by re-weighing the fish after infestation. Percentage weight loss was calculated by dividing the difference over the initial weight multiplied by 100. Damage assessment was carried out on treated and untreated fish by calculating the percentage weight of frass.

RESULTS

The effects of *P. guineense* powder on the biology of fish beetle, *Dermestes maculatus* on the dried catfish are shown on Table 2. The emergence of adult and larva of *D. maculatus* and the losses and damages caused by them were strongly influenced by the plant material, Piper guineense. Significant differences ($p < 0.05$) were observed in the mean emergence of adult and larva of *D. maculatus* and the losses caused on smoked fish. A cursory look at the table shows that adult recorded the lowest number of emergence (250.01 ± 33.6) while to a higher extent larva recorded 340.02 ± 54.0 , although this was not statistically significant ($p > 0.05$) by LSD. The percentage weight loss on fish infested with adult (0.88 ± 0.1) and larva (1.48 ± 0.6) when compared with the control (26.10) and (31.55) respectively shows a clear influence of the plant material on the extent of damage caused by these insects during storage. *D. maculatus* produced a lot of frass by their boring activities but this was suppressed in the treated fish as adult mean percentage frass was 0.12 ± 0.04 and larva mean percentage frass was 0.33 ± 0.1 . This suggests that the larval stage of this beetle is more destructive than the adults. This was significantly ($p < 0.05$) lower when compared with the control (27.42) and (32.25), respectively on *P. guineense* treated fish.

Table 2. Effects of *P. guineense* powder on fish beetle, *Dermestes maculatus* on the dried catfish

Plant concentration (dose in g)	% Larval emergence	% Adult emergence	% Weight loss by larva	% Weight loss by adult	% Frass by larva	% Frass by adult
0.02	626.7	376.7	5.85	1.25	0.460	
0.04	526.7	350	1.55	1.20	0.475	0.195
0.06	326.7	340	1.40	1.05	0.495	0.130
0.08	346.7	250	0.95	0.95	0.565	0.012
0.50	290	250	0.80	0.90	0.250	0.085
1.00	276.7	200	0.60	0.65	0.240	0.065
1.50	176.7	126.7	0.50	0.65	0.150	0.010
2.00	150	106.7	0.15	0.35	0.010	0.005
Mean	340.02 ± 54.0	250.01 ± 33.6	1.48 ± 0.6	0.88 ± 0.1033	0.10 ± 0.12	0.04
Control	1500	2250	31.55	26.10	32.25	27.42

*Means of three replicates ($\pm S.E$)

The percentage mortality of the adult and larva of fish beetle treated with plant powder of *P. guineense* is shown on Table 3. The result shows that the sublethal dose, 0.02g caused 36.67% and 43.33% mortalities on adult and larva of the fish beetle, respectively. Consequently, the lethal dose, 2.00g caused mortalities of 76.67% and 83.33%, respectively on the adult and larva. The table shows that the lower the LD50, the more toxic the plant material. The plot of the probit values of percentage mortality against log.Dose is shown in Figure 1, the lethal dose of the plant powder on adult and larva of *D. maculatus*. The lethal dose of the plant powder of *p. guineense* (2.00g) caused 76.67% and 83.33% mortalities on adult and larva of the beetle, respectively (Table 3) with LD50 values of 130.5mg and 28.3mg (Fig.1 and Table 4) respectively. This suggests that the larva was more susceptible having lower LD50 value. However, the percentage mortality caused by the powder was more on the larvae than the adults. This suggests that the larval stage of this beetle is more susceptible to the treatment than the adult (see Table 4).

Table 3. The percentage mortality of adults and larvae of *D. maculatus* on dried fish treated with *P guineense*

Plant material	Concentration (dose) in x1000mg/20g fish	Log dose	% Mortality	Corrected mortality	Probit value
<i>Piper guineense</i> on adult	0.02	1.301	36.67	36.67	4.64
	0.04	1.622	36.67	36.67	4.64
	0.08	1.778	43.33	43.33	4.82
	0.06	1.903	53.33	53.33	5.08
	0.50	2.698	56.67	56.67	5.15
	1.00	3.000	63.33	63.33	5.33
	1.50	3.176	70.00	70.00	5.52
<i>Piper guineense</i> on larva	0.02	1.301	43.33	43.33	4.82
	0.04	1.602	56.67	56.67	5.15
	0.06	1.778	56.67	56.67	5.15
	0.08	1.903	63.33	63.33	5.33
	0.50	2.698	66.67	66.67	5.41
	1.00	3.000	70.00	70.00	5.52
	1.50	3.176	76.67	76.67	5.71
	2.00	3.301	83.33	83.33	5.95

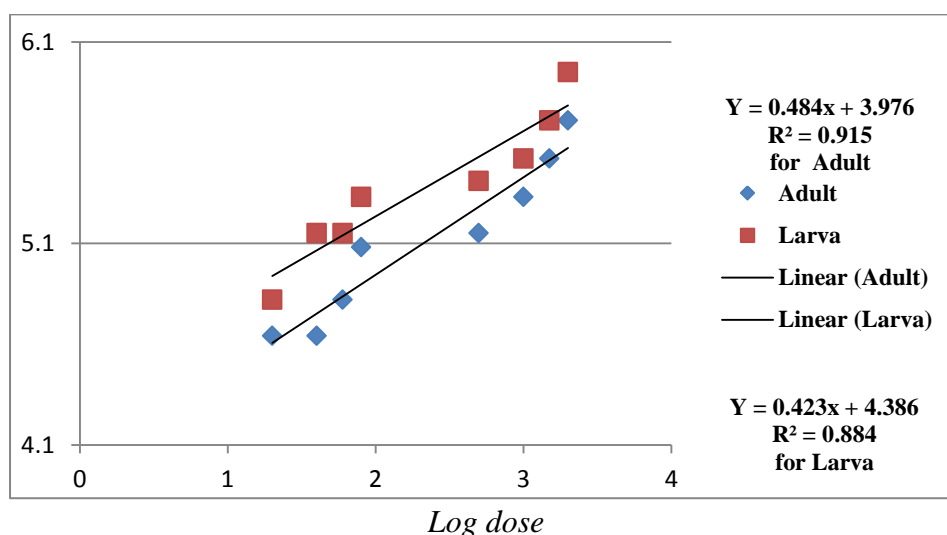


Fig. 1. Probit analysis of mortality (%) of fish beetle treated with *P. guineense* powder

Table 4. 72 hour LD50 mortality response for *P. guineense* on *D. maculatus*

<i>P. guineense</i>	72hr LD ₅₀ (mg)
Adult	130.5
Larva	28.3

In comparative addition, the effects of *Z. officinale* on the adult and larvae fish beetles are portrayed on Table 5. Empirically, *Z. officinale* was also effective in suppressing adult emergence percentage (573.76±130.1)

and percentage larva emergence (429.19±90.0) compared with the control (2250) and (1500) for adult and larva, respectively. The emergence of adult and larva of *D. maculatus* and the losses and damages caused by them were strongly influenced by the plant material, *Z. officinale* (Table 5). Significant differences ($p < 0.05$) were observed in the mean emergence of adult and larva of *D. maculatus* and the losses caused on smoked catfish. The percentage weight loss on fish infested with adult (1.49±0.2) and larva (1.28±0.2, respectively shows a clear influence of the plant material on the extent of damage caused by these insect during storage. *D. maculatus* produced a lot of frass by their boring activities but this was suppressed in the treated as adult mean percentage frass was 0.43±0.1 and larval mean percentage frass was 0.59±0.1. This also suggests the larval stage of this beetle to be more destructive than the adults. This was significantly ($p < 0.05$) lower when compared with the control (27.42) and (32.25), respectively on *Z. officinale*.

Table 5. Effects of *Z. officinale* powder on fish beetle, *Dermestes maculatus* on the dried catfish

Plant Concentration (dose in g)	% Larval emergence	%Adult emergence	%Weight loss by larva	% Weight loss by adult	%Frass by larva	%Frass adult
0.02	716.7	1300	2.10	2.30	0.870	0.815
0.04	890	900	1.50	1.85	0.845	0.575
0.06	520	670	1.40	1.70	0.575	0.655
0.08	336.7	670	1.40	1.65	0.570	0.460
0.50	320	436.7	1.35	1.40	0.590	0.295
1.00	256.7	236.7	1.05	1.10	0.550	0.295
1.50	226.7	220	0.95	1.05	0.485	0.055
2.00	166.7	156.7	0.45	0.90	0.215	0.020
Mean	429.19 ±90.0	573.76± 130.1	1.28±0.2	1.49±0.0	0.59±0.1	0.43± 0.1
Control	1500	2250	31.55	26.10	33.25	27.42

Means of three replicates ($\pm S. E$)

The percentage mortality of the adult and larva of fish beetle treated with *Z officinale* powder is shown in Table 6. The result shows that the sublethal dose, 0.02g caused 30% and 40% mortalities on adult and larva of the fish beetle respectively. However, the lethal dose, 2.00g caused mortalities of 73.33% and 76.67% respectively on the adult and larva. Probit analysis of percentage mortality of fish beetle treated with *Z. officinale* powder is shown in fig 2. From the graph, the LD50 value for adult treated with *Z. officinale* powder was 258. 8mg while that of larva was 66.2mg (Fig. 2 and Table 7), the lower the LD50, the more toxic the plant material. However, the percentage mortality caused by the powder was more on the larva than the adult suggesting that the larva stage of this beetle is more susceptible to the treatment than the adult synonymous to the above toxicity of *P. guineense* (Table 7).

Table 6. The percentage mortality of adult and larva of *D. maculatus* on dried fish treated with *Z. officinale*

Plant Material	Concentration (dose) in x1000ng/20fish	Log dose	% mortality	Corrected mortality	Probit value
<i>Z. officinale</i> on adult	0.02	1.301	30	30	4.48
	0.04	1.602	33.33	33.33	4.56
	0.06	1.778	36.67	36.67	4.64
	0.08	1.903	43.33	43.33	4.82
	0.50	2.698	50	50	5.00
	1.00	3.000	60	60	5.25
	1.50	3.176	66.67	66.67	5.41
	2.00	3.301	73.33	73.33	5.61
<i>Z. officinale</i> on larva	0.02	1.301	40	40	4.75
	0.04	1.602	46.67	46.67	4.90
	0.06	1.778	50	50	5.00
	0.08	1.903	56.67	56.67	5.15
	0.50	2.698	63.33	63.33	5.33
	1.00	3.00	66.67	66.67	5.41
	1.50	3.176	70	70	5.52
	2.00	3.301	76.67	76.67	5.71

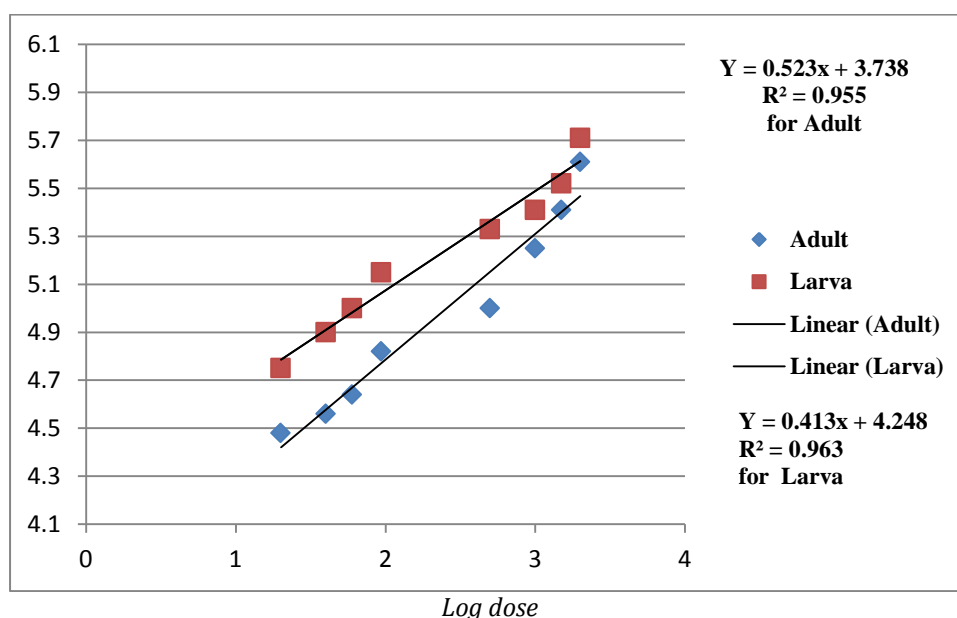


Fig 2. Probit analysis of mortalities (%) of fish beetle treated with *Z. officinale* powder

Table 7. 72 hour LD50 mortality response for *Z. officinale* on *D. maculatus*

<i>Z. officinale</i>	72hr LD ₅₀ (mg)
Adult	258.8
Larva	66.2

Generally, the plant materials were observed to be more toxic on the larva with corresponding high larval mortality than the adults. The two plant powders significantly ($p < 0.05$) reduced the number of live larvae of the pest when compared with the untreated fish samples. Each of the powder treatments showed a good activity against both the adults and larvae of the fish pest. Though, *P. guineense* powder relatively caused more mortality than *Z. officinale*.

DISCUSSION

The toxicological studies conducted to compare the toxicities of *P. guineense* and *Z. officinale* powders against the adults and larvae of fish beetle, *D. maculatus* on smoked catfish, *Clarias gariepinus* showed varying degree of toxicity/ pesticidal efficacies on the fish beetles and its acceptance as a sustainable way of fish storage compared to the harmful use of insecticides. The resultant high mortality of larvae and adults of the beetle observed on dried fish treated with two plant materials could be due to high toxic effect of these products on both development stages. From the result (probit analysis) obtained, *P. guineense* has a lower LD₅₀ showing that it is more effective than *Z. officinale* in controlling *D. maculatus*. Insecticidal property of any plant material would depend on the active constituents of the plant material. Okonkwo and Okoye [21] reported that *P. guineense* contains piperine and chavicine, which are insecticidal while Lale [14], included piperidine and alkaloids as the major active components in *P. guineense* seeds which may be the cause of its high mortality observed.

Alternatively, *Z. officinale* contains zingeroneshogols and gingerols as the major active constituents contributing to its pungent taste [22]. However, the toxicity has been attributed by various authors to their pungent and pepperish taste which could asphyxiate insects by blocking the spiracle [21] and the presence of bioactive ingredients such as alpha-pinene, limonene and Linalool in *P. guineense* [22].

The current observation thus supports the assertion earlier made that these plant materials to have a high toxic effect on the adult mortality of *Sitophilus zeamais*. The insecticidal activity of members of the family Piperaceae, which *P. guineense* belongs, has been attributed to the presence of chavicine [23] and piperine [24]. Similar observations have also been made by Adedire et al. (1998) that plant materials within that family (Piperaceae) have been reported to possess some form of insecticidal properties against eggs of cowpea storage, Bruchid, and also capable of suppressing various developmental instars of *Callosobruchus maculatus*.

Odeyemi et al. [12] observed that cases of high mortality reported in the larvae were partly because of their inability to detoxify plant toxins during feeding activity, especially at 1st – 4th larval stage. According to the authors, larvae are voracious eaters because of their growth requirement in contrast to adult insect, which tend to

have a reduced feeding habit. In the present study, high mortality was observed more in *P. guineense* than in *Z. officinale* treated fish, ranking the former higher on the toxicity scale and for better use in storage.

The observed low emergence of F1 generation of *D. maculatus* on dried fish treated with *P. guineense* and *Z. officinale* could be due to high mortality of the late instar larvae resulting from toxic effects of the two products. However, the formation of moribund F1 generation and some active adults of *D. maculatus* from late instar larvae on dried fish treated with the two plant materials suggest that the products were not effective in outright killing of the instar larvae, but were effective in disrupting the normal development of these larvae. Consequently, observations have been reported by some authors on the disruptive effect of *P. guineense*. Fasakin and Aberejo [17] reported that pulverized plant materials from *P. guineense* inhibited egg hatchability and adult emergence of *D. maculatus* Degeer in smoked catfish (*Clarias gariepinus*) during storage.

Results obtained from these studies demonstrate the attractive potentials of *P. guineense* and *Z. officinale* as plant derived insecticides against fish beetle, *D. maculatus* in Nigeria. These protective and toxic effects of the powders in addition to their local availability make them attractive materials in upgrading traditional post-harvest protection practices. They can easily be obtained with no specialized technology involved in their processing. The effectiveness of *P. guineense* in reducing damage and controlling *D. maculatus* infestation in smoked fish during storage could be encouraging and a possible means of ensuring a steady supply of good quality dried fish as against *Z. officinale*. This simple technology can readily be adopted by dried fish traders for the control of Dermestid beetles during processing, storage, transportation and marketing of dried fish.

However, the two spices used in this experiment especially *P. guineense* is worth exploiting as protectants of dried fish and other stored products against their insect pest and therefore can serve as an affordable substitute for synthetic insecticide. Further investigation is therefore advocated to identify and determine other local plant products having insecticidal properties for effective control of this insect on dried fish with minimum technology and optimal consumer acceptability.

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