



LARVAL MORPHOMETRIC AND ADULT MORTALITY OF DERMESTES MACULATUS DEGEER EXPOSED TO POWDERED AND STEAM EXTRACTS PIPER GUINEENSE PRE-TREATED FISH

Ebochuo V.C. & Onwuka C. N.

Department of Fisheries and Marine Technology

Imo State Polytechnic, Umuagwo, Ohaji.

Email: victorebochuo@gmail.com

ABSTRACT

Effect of powdered and steam extracts of Black Pepper (*Piper guineense*) Uziza (Igbo), at doses of 5%, 7.5% and 10% on the Hide beetle (*Dermestes maculatus* Degeer) Exposed To pre-treated fish (*Clarias gariepinus*) was monitored for 21 days. Treated and untreated fish were enclosed in plastic jars covered with muslin to which three pairs of the insect had been introduced and left at ambience. Mortalities were monitored every 24 hours, while instar stages were obtained through the measurement of larvae present at the end of the experiment to the nearest millimeter. Powdered form caused 100% adult mortality at all doses. Steam extracted form resulted in 33.30%, 50.00% and 66.79%, at 5%, 7.30% and 10% spice concentrations respectively. Black pepper powder destroyed perpetuation of Hide beetle in pre-treated fish while specimens placed on spice extract pre-treated fish reproduced. Linear decreases in length of larval instars stages reared on substrate with increasing spice steam extract concentrations were observed. Mean length range for each dose $3.25 \pm 0.7\text{mm}$ - $14.00 \pm 0.00\text{mm}$ for 0.00%; $2.00 \pm 0.04\text{mm}$ - $12.00 \pm 0.09\text{mm}$ for 5%; $5.50 \pm 1.10\text{mm}$ - 12.00mm for 7.3% and $2.00 \pm 0.00\text{mm}$ - 9.00 ± 0.33 for 10%. Five larval instars were found in all replicates of the control while six were found in all replicates of the pre-treated fish. In addition to the traditional usage, *Piper guineense* can further be utilized to preserve and protect smoked dried fish in storage against the hide beetle, "*Dermestes maculatus* Degeer."

INTRODUCTION

Fish are highly perishable foodstuff and demands very quick approach for processing and preservation. Spoilage of fish begins as soon as the fish dies or is caught, if not properly handled. Preservation of fish through smoke drying is a method used to keep the fish in good state so that the changes in sensory parameters are minimized (Clucas Ward, 1996). However, studies have shown that a high proportion of smoke dried fish in Nigeria is usually infested by insect pest, such as *Dermestes maculatus* (Odeyemi et al, 2002). Synthetic insecticides, for example pyrethroid, Deltamethrin and Permethrin have proved to be effective against stored product pest, if used at the right time, quantity and with the correct application (Golob et al 1991). However the use of such chemicals to protect stored fish has been hampered by report of health hazards and high cost of purchase. Odeyemi et al (2000) reported that fish treated with chemical insecticides adversely affected consumers, causing blurred vision, dizziness and vomiting.

In addition Amusan and Okorie (2002) noted that Dermestid larvae and adults, unlike many other beetles are less susceptible to synthetic insecticides that normally attack stored products. Attention is currently being focused on the use of natural preservative materials that are cheap, medicinal, and easily accessible and that have long term protective ability. In this regard, a host of plant materials are presently considered as promising alternatives to synthetic insecticides in the control of pest of fish (Okorie et al, 1990). However, information on the use of plant materials to control insect pest of stored



fish is recent and is just growing in view of reports of the poor aesthetics of stored product treated with some plant materials (Boeke et al 2001, Okorie et al, 1990, Amusan and Okorie, 2002 and Onu and Baba, 2003) and because dried fish is often eaten without further processing by most people. Extracts of spices although effective as oils and sprays but are expensive and out of the reach of people involved in the fish processing and distribution chain. Few laboratories if any, around can afford the cost of solvent extraction and distillation process required to elaborate the active ingredients. *Piper guineense* (Uziza), also known as West African black pepper is a member of the family Piperaceae. *P. guineense* is the most pungent and flavourful of all types of pepper. It is used as anticonvulsant (Abila et al 1993). The fruits and leaves are used as spices for preparing soup for post-partum women, powder from the dried fruits or seed mixed with honey acts as carminative and relieves stomach aches (Dewitt, 2006). Extracts of it has been reported to stimulate digestion of foods by stimulating secretion of digestive enzymes, pancreatic amylases, trypsin and chymotrysin (Platel and Srinivan, 2000) and is therefore used for treatment of digestive disorders.

P. guineense is currently being studied for its potency in microbial, parasitic and fungal inhibition, as an antioxidant (Bhandary 1991) and as a protectant against fish and other stored products pests (Amusan and Okorie, 2002) all in an attempt to prolong shelf life of food. Attention is now been paid to several work which are currently in place to ascertain how best the spice, *Piper guineense* among others could be employed effectively in smoke dried fish protection (Ntonifor, 2011) at affordable and safe limits. As we seek to prevent the use of insecticides and other chemicals which are harmful to health in the preservation of dry fish from insect attacks and replace these with cheap and locally available but environmentally friendly spices used both as flavour and medicine by man there is need to find cheap and easy means of treating fish and also follow their impact on *Dermestes maculatus*. Boiling and smoking are known methods of fish preservation. Steaming of spice pre-treated fish is a measure that may elaborate the active ingredients of spice which may protect fish from insect pests. This can be compared with crude powder administration of spice. The aim of this work is to assess the impact of different concentrations of crude powder and steam extracted *Piper guineense* on the mortality of adults and larval instars' morphometries, where survival and perpetuation occurs, of *Dermestes maculatus* Degeer exposed to pre - treated fish.

MATERIAL AND METHODS

The experiment was conducted at the laboratory of the Department of the Fisheries and Marine Technology, Imo State Polytechnic, Umuagwo, South-East Nigeria.

Purchase and Processing of Botanicals

Piper guineense was bought from an herbal store in Eke Ukwu Owerri Market. The dry seeds were pulverised using Q-link grinder (model: QBL-15L40, China). The spices were stored in air tight bottles and kept to conditioned in the laboratory.



Purchase and maintainance of Insect Culture

Fish frass containing the insects, *Demerstes maculatus* at all stages of their life cycle were bought from smoked cured fish mongers at Eke Ukwu Owerri Market. These were brought to the Laboratory and allowed to condition until it is ready for use. Smoked fish was added from time to time to ensure that the culture had enough food for their maintenance. The debris were stored in a large plastic container and covered with Muslin and left in the laboratory under ambient conditions.

Purchase of Fresh Fish Samples.

Fresh catfish (*Clarias gariepinus*) were obtained from the fish Ponds of the Department of Fisheries and Marine Technology of the Polytechnic.

Preparation of Fish Samples

37 pieces of fish weighing 10kg (270.30g/ fish) were sacrificed, degutted, sectioned allowed to drain and weighed on a mini digital pocket balance (capacity: 500g. make: China). Sections of the raw fish were pre-treated with *Piper guineense* at the rate of 2g spice/40g wet fish, 3g spice/40g wet fish and 4g spice/40g wet fish to make up concentrations of 5%, 7.50% and 10% spice pre- treatments. Each treatment was steamed in cooking pot until fish were cooked using its natural moisture. The spiced and steamed fish were then cured with smoke at low flame generated from Ugba wood (*Pentaclethera macrophyla*) in the local mud smoking kiln at a mean temperature of 90° C - 110C for 6 hours. The treated samples were wrapped with polythene bags after cooling and left for one week in the laboratory under ambient conditions before infesting with insects.

Preparation of Fish for Control Experiment

Cleaned, gutted, untreated pieces of fish of about the same size were steamed, smoked and left under ambience to condition in the laboratory before infestation as above.

Insect Infestation of Treated and Control Specimens

Treated and untreated, kiln smoked, uninfested, laboratory conditioned fish were introduced into the plastic (one each to one jar) alongside adult *Dermestes maculatus* beetles on the commencement of the experiments at the rate of three pairs of *Dermestes maculatus* beetles per replicate for three replicates in one treatment.

Experimental Parameters Experiments to Ascertain the Effect of Treated Fish on *Dermestes maculatus* Beetles

This study was carried out for a minimum period of 21 days. Fish treated with 5%, 7.5% and 10% spices were enclosed in the plastic jars with three pairs of *Dermestes maculatus* Beetles. Each dose was made into three replicates for each spice. In the control experiment untreated fish was also introduced into three plastic jars with three pairs of *Dermestes maculatus* beetles. Each jar was covered with Muslin cloth and held with rubber band to prevent escape of the beetle or entry of other insects while allowing aeration for the beetles under ambient conditions. Mortalities were recorded on daily basis for the period that the experiment lasted.



The effect of *P. guineense* on Larvae Instar Sizes

Morphometries of the instar stages of the larvae were obtained through the measurement of the larval instar stages present in the replicates of each dose of the spices to the nearest millimetre. This served as the impact of powdered and steam extracted spices on the larval stages of the beetle.

Statistical Analysis

Mortality differences due to each spice treatment and the control experiment were validated manually using Analysis of Variance (ANOVA). Differences in larval morphometries were evaluated using student T-test. The scores were statistically analyzed using. The differences in the proximate composition of each treatment and control experiment were validated manually using Analysis of Variance (ANOVA). Differences in the scores of the organoleptic tests were validated using Analysis of Variance (ANOVA) and the means were separated according to the methods of Obi (1990).

Result and Discussion

Discussion of Results

At all doses, the steam extracted spices produced significantly higher mortalities of *D. Maculatus* over all replicates of the control experiment. This experiment shows that the highest mortalities achieved with steam extracted spices on *D. Maculatus* was 66.67% (*P. guineense*) for an exposure period of 21 days. This is contrary to the experience with pulverized form of *P. guineense* which resulted in 100% mortalities within 24 hours (Owoade 2008, Akinwumi 2011, etc.) There was no particular order of effectiveness of *P. guineense* with the doses except that the all proved effective significantly ($P < 0.05$)

Table 1: Percent Total Mortalities of *Dermestes Maculatus* Exposed to Fish Treated with powdered and steam mediated spices at concentrations of 5% ,7.5% and 10% for a Period of 21 days.

Dose	<i>Piper guineense</i> (SM)	<i>Piper guineense</i> (P)
0.00%	00.00	00.00
05.00%	33.33 ^a	100.00 ^b
07.30%	50.00 ^{ad}	100.00 ^{bd}
10.00%	66.67 ^{bd}	100.00 ^{ad}

Foot Note: Percent mortalities, the Rows and columns that bear different letters are significantly different while those that bear the same letters are not significantly different ($P < 0.05$, ANOVA)

The percent total mortalities of *Dermestes maculatus* exposed to fish treated with powdered(P) and steam extracted (SM) spices at concentrations of 5% , 7.5% and 10%, for a Period of 21 days are summarized on Table 1. Treatments using powder at all concentrations produced 100% mortalities within 24 hours of exposure to pre - treated fish



($P < 0.05$) over the control experiment. These observations show that the spice, *P. guineense* has high fumigative strength. Adedire et al (2000), Owoade (2008), Amusan and Okorie (2002) have reported that the high fumigative strength of *P. guineense* which involved total kills of *D. Maculatus* exposed to treated fish. The observation with powder treatments were 100% lethal to *D. Maculatus* is in line with results of pre-treatment trials and trials of works done elsewhere (stoll, 2000, Amusan et al 2002, Lale, 1995). Ofuya and Bamgboya (2003) gave the excellent fumigative strength of *P. guineense* on *D. maculatus* to its ability to block the spiracles, penetrate the cuticle, pungency and repellence. Steam extracted *P. guineense* pre-treated substrate produced linear significant ($P < 0.05$) mortalities with increasing concentrations over the control experiment and the deaths recorded in all the replicates spanned the 21 days the experiment lasted and permitting oviposition and eventual perpetuation. The work suggest that steam treatment may have hindered the ability of *P. guineense* to block the spiracles, penetrate the cuticle, repellence and pungency to *D. maculatus* reported by other researchers (Ofuya and Bamgboya 2003) on *D. Maculatus*

Table 2: Mean length (mm) of Instar stages of *Dermestes maculatus* treated with *P. guineense* for a period of 21 days. The treated media permitted oviposition and development of the beetles. This was not without the initial repellence that has been reported for the spices (Akinwumi et al 2007, stoll 2000) which waned with time.

Treatment & Stages of Development (Length in mm)						
Dosage	1 ST Instar	2 ND Instar	3 RD Instar	4 TH Instar	5 TH Instar	6 TH Instar
0.00%	3.25±0.71 ^a	6.02±0.01 ^a	9.10±0.07	11.00±0.00	14.00±0.00 ^a	-----
5.00%	2.50±0.04 ^a	5.02±0.01 ^b	7.00±0.07 ^a	9.10±0.01 ^a	10.10±0.01 ^b	12.00±0.09 ^a
7.30%	2.41±0.19 ^a	5.50±0.10 ^b	6.27±0.18 ^a	7.10±0.07 ^b	9.00±0.00 ^{bc}	11.88±0.00 ^a
10.00%	2.00±0.00 ^a	3.00±0.00 ^c	5.30±0.03 ^b	6.03±0.04 ^c	8.10±0.00 ^c	9.00±0.33 ^b

Foot Notes: Different Letters a, b and c down the columns indicate significant differences while similar letters in the same direction indicate that results are not different ($P = 0.05$, student t)

Morphometric Studies.

1st Instar Larvae.

Within the *Piper guineense* treatment the highest dosage 10% significantly ($P < 0.05$, student t) repressed growth of larvae when compared to 5% treatment. No first instar larvae was found in all replicates of this 7.3% *Piper guineense* treatment (0%:3.25mm, 5% :2.00mm > 10%: 2.5mm). Zakka et al () noted a range of 1st larvae which varied from 1.62mm to 3.77mm when he reared *D. maculatus* on different substrates. Ezenyanyi and Obaji (2004) reported a first larval instar that measured 3.28 ± 0.71 mm. The range of values observed here are within the observed limits noted by other workers.

2nd Instar larvae

There were linear decreases ($P < 0.05$) in length from control to the highest dosage (0%=6.02±0.01, 5%=5.02±0.01, 7.3% =5.50±1.10 and 10%=3.00±0.00). The highest and least measurements achieved in the second instar stages of Zakka et al's (2009) work



were 6.03 ± 0.56 mm and 3.83 ± 0.09 mm with different substrates while Ezenyanyi and Obaji (2004) recorded 6.06 ± 0.82 mm for this stage.

3rd Instar Larvae

Increasing doses of *Piper guineense* (0%, 5%, 7.3% and 10%) significantly ($P < 0.05$, student t) reduced the growth performance of the 3rd instar larvae (9.10 ± 0.07 , 7.00 ± 0.07 mm, 6.27 ± 0.18 mm and 5.30 ± 0.03 mm) at all levels. On different substrates, Zakka et al (2009) reported a size range of 5.20 ± 0.44 - 7.93 ± 0.69 and Ezenwanyi and Obaji (2004) also measured 8.04 ± 0.75 mm at the 3rd instar stage. The superior performance of the control experiment was due to the conducive condition of the substrate.

4 instar Larvae:

All doses of *Piper guineense* 5%, 7.30% and 10% treatment significantly ($P < 0.05$) reduced growth (9.10 ± 0.07 mm, 7.10 ± 0.07 mm and 6.03 ± 0.04 mm) when compared to control experiment (11.00 ± 0.00). There is attendant significant ($P > 0.05$ student t) reduction of growth with increasing concentrations of *Piper guineense*. In validation of the instar size found in this work, Zakka et al (2009) observed a range of 8.15 ± 0.19 - 12.58 ± 0.16 mm while Ezenwanyi and Obaji (2004) also measured 10.83 ± 0.98 mm at the 4th instar stage.

5th Instar Larvae:

Increasing doses of *Piper guineense* significantly ($P < 0.05$, student t) reduced the growth of the 5th instar larvae so treated. 5%, 7.3% and 10% *Piper guineense* treatments produced 10.10 ± 0.01 mm, 9.00 ± 0.00 mm and 8.10 ± 0.00 mm respectively. Control (0.00%) produced 14.00 ± 0.00 mm which by far exceeded all pre-treatments. This finding did not conform to the observation of Samish *et al.* (1992) who recorded 16 mm as the highest measurement. Here again the substrate has played a role. While the substrate used was untreated *C. gariepinus*, for the control, Samish *et al.* (1992) observation was made on poultry where they became pest of live Turkey. Note that the control had five larval instars in all replicates.

6th Instar Larvae

Sixth instar was not found in control experiment. Linear significant decreases in instar sizes resulted with increasing doses. This stage may have been bypassed due to the suitability of the substrate and general ambience of rearing medium. The same reason is advanced with the decreases in size with increasing doses. These explanations are in tune with the observations of Ezenwanyi and Obaji, (2004) on number of instar stages and substrate influence posited by Zakka et al (2009).

5 stages of larva instar developmental stages were identified in the control replicates while 6 stages were noted in the spice pre-treated substrates. Zakka et al (2009) reported six larval instars in *D. maculatus* reared on different fish substrates with variable instar sizes. Hines and Rees (1989) reported 5-11 instar stages with the number of instars increasing with unfavorable conditions. Ezenwanyi and Obaji, (2004) noted five larva



Instars in a developmental period of 16-21 days. . Okorie et al (1990) reported 7 larval instar stages with a mean length of 7.7mm and range of 5.5mm -15.8mm on Tilapia substrate treated with *Azadirachta indica*. It is possible to extend or shorten the period of development and the number of larval instar stages, depending on prevailing conditions.

In the control experiment, five instars posting very favourable measurements were seen. What would form the 6th instar was absent in the three replicates of control and the size of the instar stages were greater ($P < 0.05$) than the size of the complimentary stages in the different doses of treated media (Table 2). The absence of the 6th instar in the control may be attributed to an attainment reached due to favourable conditions in the treatment media while the linear decrease in size of instar stages with increasing treatment dose are dictated by harsh environments. The size of the hide beetles' instar stages reared on control experiment especially at 5th stage recorded 14.00mm. The highest by Zakka et al (2009) is 12.95mm. This is due to a favourable ambience and good food supply. Owoade (2008) noted that *P. guineense* not only repressed growth of *D. maculatus* exposed to treated media but also slowed down the developmental period. Okorie et al (1990) reported 7 larval instar stages with a mean length of 7.7mm and range of 5.5-15.8mm on Tilapia species treated with *A. indica*. In all cases *P. guineense* significantly ($P < 0.05$) reduced the growth of all instar stages when compare to the control experiment.

CONCLUSION

From these observations and literature cited, powdered black pepper has very high fumigative strength even at very small doses bringing about total 100% hide beetle mortality in 24 hours. Steam mediation of the spice impedes the potency of this spice through a diminish in its ability to block the spiracles, exert repellence, penetrate the cuticle and a reduction in potency. Though steam mediation of spice on substrate can kill pests substantially, those which survive oviposit and perpetuate. Increasing concentrations brings about reduction in the size of larval instars residual significantly. The powdered form although has a poor aesthetic appeal, its potency is effective in protecting dry fish kept in storage.

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