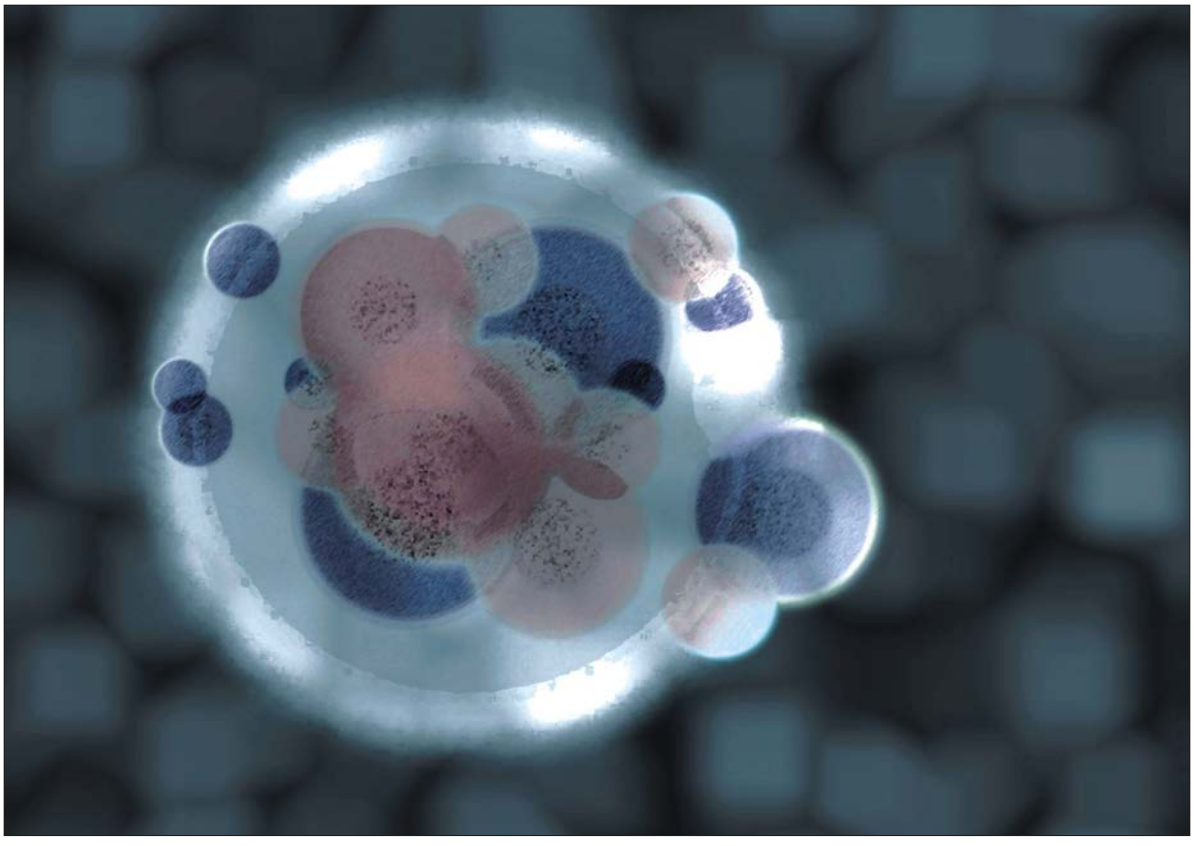

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Progeny Inhibiting Effects of Four Plant Products Against The Leather Beetle and the Copra Beetle on Smoked African Mudfish

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Abstract: The inhibitory performance of four indigenous plant materials, namely: pepper fruit, *Dennettia tripetala* Baker, clove, *Eugenia aromatica* Hook, black pepper, *Piper guineense* (Schum and Thonn) and African nut-meg, *Monodora myristica* (Dunal) on the main developmental stages of two main pests, namely: the leather beetle, *Dermestes maculatus* Degeer and the copra beetle, *Necrobia rufipes* Degeer on smoked African mudfish, *Clarias gariepinus* Burchell was investigated under laboratory conditions. The plant materials were pulverized into powders and also processed into extracts and were applied at 2.50, 5.00, 7.50 and 10.00 g per 100 g fish and 5 mL of 2.50, 5.00, 7.50 and 10.00% per 100g fish respectively. Each of the powdered smoked fish significantly ($p<0.05$) hindered progeny development in *D. maculatus* and *N. rufipes*. Most of the scanty eggs laid, where there was oviposition, remained unviable and the suppression rate of adult emergence was put at 99.75% and 99.60% in *D. maculatus* and *N. rufipes* infestations respectively. Similarly, each of the extract dosages significantly ($p<0.05$) prevented adult emergence in protected fish. The suppression rate of adult emergence in fish protected with the extracts against *D. maculatus* and *N. rufipes* was put at>99.77 and >99.55% respectively. In another study to determine the effect of each plant dosage on the palatability and aesthetic acceptance of protected smoked fish, the treated fish was organoleptically rated excellent.

Key words: Fish pest control, plant insecticide, post-harvest fish management

INTRODUCTION

Dried fish is a highly relished item of many traditional dishes in Nigeria as food and condiment that greatly enriches the flavour of various dishes. However, studies have shown that a high proportion of dried fish, which most Nigeria's fish consumers rely on to supplement their protein needs, is usually infested by two main insect pests, *Dermestes maculatus* (leather or hide beetle) and *Necrobia rufipes* (copra or red-legged ham beetle) (Ezeu, 1982; Osuji, 1974; Toye, 1970). Although, many synthetic chemicals are effective against pests of many stored products, the general use of such chemicals to protect stored fish has been hampered by health hazards, high costs of purchase and less susceptibility of dermestid larvae (Amusan and Okorie, 2002; Odeyemi *et al*., 2000). The need to protect smoked fish from pests is imperative because dried fish plays a prominent role during the hunger gap period between the first rains and first harvest and also during the active agricultural planting season when it is used to supplement wild indigenous foods in rural communities. Furthermore, dried fish commodities form one of the cheapest and most accessible source of animal proteins in situations of emergency for the displaced, people at war or fleeing from war and the poor in most sub-Saharan African communities. Thus, this study sought to search for natural preservation materials that are not only safe to consume but that are also cheap and easily accessible in protecting one of the highly valuable commodities in the tropics-smoked catfish, *Clarias gariepinus*

MATERIALS AND METHODS

Preparation of plant powders: Dry fruits of *D. tripetala*, *E. aromatica, P. guineense* and *M. myristica* were purchased in local herbal stores at Erekesan Central Market in Akure, Nigeria. Samples are either pepperish or have aromatic flavour or both and are used as food condiments or spice in human diets. Each of the plant materials was washed with clean tap water, dried in laboratory drying cabinet at 40°C for 8 h, ground thoroughly in an electric 1.5 HP kitchen grinder and sieved through a 40 holes per mm^2 mesh screen

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(Adedire and Lajide, 2000). Each of the plant powders was kept in a separate sterile plastic container with a tightly fitted lid and placed in a cooled incubator at 0°C for use in the experiment.

Preparation of plant ethanolic extracts: To 10 g of each of ground plant materials in a round bottom flask, an aliquot 100 mL of absolute ethanol was added and soaked for 24 h. Thereafter, the mixture was boiled at 60°C for 30 min in UNISCOPE SM801A Laboratory Water Bath. 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.

Insect culture and maintenance: The initial source of beetle culture was obtained from naturally infested smoked catfish *C. gariepinus* collected from dried fish markets in Akure, Nigeria. Several males and females of *D. maculatus* and *N. rufipes* were obtained and maintained separately in Kilner jars covered with muslin cloth under laboratory conditions and kept at temperature 30±2 °C and relative humidity 65±5%. All bioassay jars were disinfected using the standard procedure by heat treatment in a Gallenkamp drying cabinets at 70 °C for 1 h and allowed to cool at room temperature. New generations were prepared by removing adults of each insect species from a stock culture, placing them on fresh uninfected fish, then removing the parent adults after 2-3 weeks oviposition period. Water was supplied with pieces of soaked cotton wool.

Effect of plant powders on reproductive performance and immature forms of *D. maculatus* and *N. rufipes*: Each of plant powders at concentration 2.50, 5.00, 7.50 and 10.00 g was uniformly coated to the body of 100 g dried fish and placed in a Kilner jar (300 cm^3). Twenty newly emerged (0-24 h old) adults each of *D. maculatus* and *N. rufipes* were introduced into each jar and covered with muslin cloth. Wet cotton wool was supplied in the jar to induce oviposition. A control experiment consisted of same number of insects exposed to untreated dried fish. Each treatment was in triplicate. Eggs laid on the fish by each of the beetles were counted every 24 h for 18 days. Daily observations were made until adult emergence. The number reaching larval and adult stages was recorded and expressed as percentages.

Effect of ethanolic extracts on reproductive performance and immature forms of *D. maculatus* and *N. rufipes*: Five millitre of each of plant extracts at concentration 2.50, 5.00, 7.50 and 10.00% was thoroughly rubbed onto the body of 100 g dried fish, air-dried for 1-2 h and placed in a Kilner jar (300 cm^3). Twenty newly emerged adults (0-24 h old) each of *D. maculatus* and *N. rufipes* were introduced into each jar and covered with muslin cloth. A wet cotton wool was introduced into the jar to induce oviposition. A control experiment consisted of same number of insects exposed to untreated dried fish. Tests were in triplicates for each treatment per insect species. Eggs laid on the fish by each of the beetles were counted every 24 h for 18 days. Daily observations were also made until adult emergence. The number reaching larval and adult stages was recorded and expressed as percentages.

Effect of plant materials on the organoleptic properties of smoked fish during storage: Each of plant powders at 2.50, 5.00 and 10.00 g and extracts at 5 mL of 2.50, 5.00 and 10.00% was rubbed onto the body of a 100 g of disinfested smoked fish. The treated fish was placed in a plastic jar, ten insect larvae starved for 48 h were added and the perforated lid covered with muslin cloth and was left on the shelf for 30 days. Disinfested fish without plant treatments served as the control. The protected fish was examined for acceptability by a 3-member panel using a 6-point hedonic scale modified after Clucas (1982) and Okonkwo and Okoye (2001).

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

The effect of each of the powder dosages on progeny development of *D. maculatus* revealed significant differences ($p<0.05$) when compared with the control (Table 1). There was virtually no egg laid on the protected fish and consequently very low numbers of emergent larvae of the beetle developed from the new copulating adults that were exposed to the powders. Each of the powders effectively prevented adult emergence in fish protected against *N. rufipes* (Table 2). The suppression rate of adult emergence in *N. rufipes* ranged between 99.60-100% compared to 5.13-3.05% in the control in each of the plant materials. All the very few new *D. maculatus* and *N. rufipes* larvae died within 2 days after emergence. The effect of plant extracts on number of eggs laid, emergence of larvae and adults when *D. maculatus* were introduced to treated fish is shown in Table 3. The mean number of eggs laid on all protected fish was statistically different ($p<0.05$) from the control. The extracts effectively prevented adult emergence with a progeny reduction of >95%. All the extracts also significantly ($p<0.05$) hindered oviposition in *N. rufipes* in

Table 1: Effect of plant powders on reproductive performance and immature forms of *Dermestes maculatus*

Plant powder	Concentration (g 100 g fish)	Mean No. of eggs laid	Mean (%) larva emergence	Mean (%) adult emergence
D. tripetala	0.00 (Control)	153.67±4.63^{c}	91.76	100.00
	2.50	0.33±0.16b	0.22	0.23
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.67±0.31^{b}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00
E. aromatica	0.00 (Control)	146.67±4.63^{d}	90.22	95.72
	2.50	3.00±0.27^{c}	0.68	0.25
	5.00	0.67±0.31^{ab}	0.00	0.00
	7.50	1.00±0.27^{a}	0.46	0.00
	10.00	0.33±0.16^{b}	0.00	0.00
P. guineense	0.00 (Control)	145.33±1.81^{c}	91.52	95.99
	2.50	0.33±0.15^{b}	0.00	0.00
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.33±0.16^{b}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00
M. myristica	0.00 (Control)	147.33±3.54^{d}	90.73	97.75
	2.50	1.00±0.27c	0.22	0.00
	5.00	0.33±0.16^{b}	0.00	0.00
	7.50	0.00±0.00^{a}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00

Number of adults introduced per replicate = 20. 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 test

Table 2: Effect of plant powders on reproductive performance and immature

Plant powder	Concentration (g 100 g fish)	Mean No. of eggs laid	Mean (%) larva emergence	Mean (%) adult emergence
D. tripetala	0.00 (Control)	90.33±1.67^{d}	92.63	95.61
	2.50	3.67±0.41^{c}	1.47	0.00
	5.00	3.00±0.82^{c}	0.74	0.00
	7.50	0.67±0.16^{b}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00
E. aromatica	0.00 (Control)	88.67±3.97^{d}	90.60	95.02
	2.50	3.33±0.69^{c}	1.50	0.40
	5.00	1.33±0.42^{b}	0.41	0.00
	7.50	0.67±0.16^{a}	0.41	0.00
	10.00	1.00±0.27^{ab}	0.00	0.00
P. guineense	0.00 (Control)	81.00±6.40^{b}	94.65	96.95
	2.50	0.00±0.00^{a}	0.00	0.00
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.00±0.00^{a}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00
M. myristica	0.00 (Control)	55.33±3.00^{c}	93.98	94.87
	2.50	0.67±0.16^{b}	0.63	0.00
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.00±0.00^{a}	0.00	0.00
	10.00	0.33±0.00^{a}	0.00	0.00

Number of adults introduced per replicate = 20. 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 test

Table 3: Effect of ethanolic extracts on reproductive performance and immature forms of *Dermestes maculatns*

Plant extract	Concentration (5 mL % 100 g fish)	Mean No. of eggs laid	Mean larva emergence (%)	Mean adult larva emergence (%)
D. tripetala	0.00 (Control)	152.67±1.91^{c}	96.94	96.62
	2.50	0.67±0.16^{b}	0.22	0.00
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.00±0.00^{a}	0.00	0.00
	10.00	0.33±0.16^{b}	0.00	0.00
E. aromatica	0.00 (Control)	156.67±5.15^{c}	97.23	96.28
	2.50	7.67±0.69^{b}	1.10	0.23
	5.00	7.00±0.82^{b}	0.66	0.00
	7.50	5.67±0.16^{a}	0.44	0.23
	10.00	5.33±0.31^{a}	0.66	0.00
P. guineense	0.00 (Control)	166.00±2.41^{c}	96.19	96.87
	2.50	0.67±0.31^{b}	0.21	0.00
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.00±0.00^{a}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00

Table 3: Continue

Plant extract	Concentration (5 mL % 100 g fish)	Mean No. of eggs laid	Mean larva emergence (%)	Mean adult larva emergence (%)
M. myristica	0.00 (Control)	153.67±5.38^{b}	96.96	96.42
	2.50	0.00±0.00^{a}	0.00	0.00
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.00±0.00^{a}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00

Number of adults introduced per replicate = 20. 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 test

Table 4: Effect of ethanolic extracts on reproductive performance and immature forms of *Necrobia rufipes*

Plant extract	Concentration (5 mL % 100 g fish)	Mean No. of eggs laid	Mean (%) larva emergence	Mean adult emergence (%)
D. tripetala	0.00 (Control)	94.67±0.96c	93.30	94.34
	2.50	0.00±0.00^{a}	0.00	0.00
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.33±0.16^{b}	0.00	0.00
	10.00	0.33±0.16^{b}	0.00	0.00
E. aromatica	0.00 (Control)	74.67±3.55^{c}	92.85	94.72
	2.50	2.00±0.27^{a}	0.97	0.00
	5.00	1.67±0.42^{a}	0.48	0.00
	7.50	3.00±0.54^{b}	0.97	0.00
	10.00	3.33±0.42^{b}	0.48	0.00
P. guineense	0.00 (Control)	88.00±1.91^{b}	95.46	95.63
	2.50	0.00±0.00^{a}	0.00	0.00
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.00±0.00^{a}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00
M. myristica	0.00 (Control)	79.00±1.63^{c}	97.05	93.25
	2.50	1.67±0.16^{b}	0.85	0.45
	5.00	0.00±0.00^{a}	0.00	0.00
	7.50	0.00±0.00^{a}	0.00	0.00
	10.00	0.00±0.00^{a}	0.00	0.00

Number of adults introduced per replicate = 20. 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 test.

Table 5: Percentage acceptability of fish treated with plant extracts at 30 days post-treatment

Concentration (%) (5 mL 100 g fish)	Acceptability of fish / plant extract admixture (%)			
	D. tripetala	*E. aromatica*	*P. guineense*	*M myristica*
0	24.07±1.16$^{a*\wedge}$	23.12±0.44^{a*}	28.70±1.15^{a*}	25.00±0.76$^{a\wedge}$
2.5	75.93±1.59^{b*}	75.00±0.76^{d*}	70.37±0.86^{b}	71.27±0.88^{b}
5.0	75.93±0.88^{b}	73.13±0.44^{c}	72.20±0.76^{c}	73.13±0.44^{c}
10.0	73.13±1.16^{b}	66.67±0.75^{b*}	73.13±1.16^{c}	71.27±0.44$^{b\wedge}$

Values are means of triplicate samples followed by the standard error of means. Means in the same column with different superscripts are significantly different ($p<0.05$) by Tukey Test. Means in the same row with different subscripts are significantly different ($p<0.05$) by Tukey test

comparison with the control, thus there was virtually no larval and adult emergence (Table 4). In general, the emergent larvae were very few in fish protected against *N. rufipes* infestation when compared with the control. However, no F_1 progeny was sustained because all new adults died within 24 h of emergence. The organoleptic rating of the treated fish at 30 days post-treatment was superior and significantly higher ($p<0.05$) when compared with the unprotected fish during storage (Table 5). Irrespective of extract concentration, smoked fish treated with each of the plant materials was generally ranked excellent by fish consumers.

DISCUSSION

The results obtained in this study show that the powders and extracts of *D. tripetala, E. aromatica, P. guineense* and *M. myristica* were effective surface protectants against *D. maculatus* and *N. rufipes*. The few number of eggs oviposited by each of the beetles on treated fish and the consequent low larva and adult emergence reported in this study, could be as a result of high adult mortality caused by the treatments thereby disrupting mating and sexual communications or that the plant materials reduced the viability of the eggs laid. The results of this study are also in agreement with many other works on the use of plant products against stored product insects. Olaifa and Erhun (1988) and Fasakin and Aberejo (2002) observed that *P. guineense* powder prevented oviposition on *Callosobruchus maculatus* and *D. maculatus* respectively. Similarly Okonkwo and Okoye (1996) noted that both the powder and extract of *P. guineense* and *D. tripetala* inhibited adult emergence of *C. maculatus* and *Sitophilus zeamais*completely. Amusan and Okorie (2002) found *P. guineense* extract to be effective in the prevention of

growth and development of *D. maculatus*. While Adedire and Lajide (1999) implicated *P. guineense* extract as the most effective agent against oviposition on *C. maculatus*, Ofuya *et al.* (1992) reported that *M. myristica* had ovicidal, larvicidal and anti-ovipositional effect on *C. maculatus*. The reduced emergence of adults has been attributed to the contact effect of the plant material while they are gnawing their way out of the treated produce (Boeke *et al*., 2001).

The insecticidal activity of members of the Family Piperaceae, which *P. guineense* belongs, has been attributed to the presence of chavicine, piperine, camphene, limonene and beta-pinene (Lale, 1995; Su, 1977; Golob *et al*., 1999). Ossisiogu and Agbakwuru (1978) identified the bioactive component of *D. tripetala* to be 2, nitroethyl benzene. While terpenes, linoleic acid and oleic are the main toxic chemicals in *E. aromatica*, the bioactive constituents found in *M. myristica* include eugenol, limonene, tannic acid, asarone and citral (Golob *et al*., 1999). These bioactive agents could possess among other pharmacological properties, a depolarizing neuromuscular blocking action which could result to the death of insects (Udoh *et al*., 1999).

The protected fish were accepted by fish consumers as there was no adverse evidence of taint, smell or change in taste, texture or flavour of fish. Sowunmi (1982) reported that insect infestation caused an increase in the anti-nutritional factors, such as phytic acid, trypsin inhibitor activity and crude fibre as well as a decrease in starch and protein contents of stored produce. It was evident that the application of the studied plant materials prevented the unwholesomeness of the smoked fish during storage and thus its acceptance by fish consumers. On the other hand, the unprotected fish (control) became unhygienic and therefore unfit for human consumption, thus its low hedonic rating. The unfit nature of such a spoilt fish could be due to the presence of high amounts of uric acid. The results obtained in the study are indicative of the potentials of the four plant materials in preserving the quantity and quality of smoked fish.

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REFERENCES

Adedire, C.O. and L. Lajide, 1999. Toxicity and deposition deterrency of some plant extracts on cowpea storage bruchid, *Callosobruchus maculatus* Fabricius. J. Plant Dis. Protect., 106: 647-653.

Adedire, C.O. and L. Lajide, 2000. Effect of pulverized plant materials on fish damage and growth performance of the fish beetle *Dermestes maculatus* (Degeer). Entomol. Soci. Nig. Occassional Publication, 32: 215-221.

Amusan, A.A.S. and T.G. Okorie, 2002. The use of piper guineense fruit oil (PFO) as proctectant of dried fish against *Dermestes maculatus* (Degeer) infestation. Global J. Pure Applied Sci., 8: 197-201.

Boeke, S.J., J.J.A. van Lon, A. van Huis, D.K. Kossou and M. Dicke, 2001. The use of plant materials to protect stored leguminous seeds against seed beetles: A Review. The Netherlands, Backhuys Publishers, pp: 108.

Clucas, J.J., 1982. Fish handling preservation and processing in the tropics. Part 2. Report of the Tropical Products Institute, G 145, 8: 144.

Ezeu, C.E., 1982. Pests of dried fish in Nigerian markets. Nig. Insti. Stored Prod. Res., 2: 6-15.

Fasakin, E.A. and B.A. Aberejo, 2002. Effect of some pulverized plant materials on the developmental stages of fish beetle, *Dermestes maculatus* Degeer in smoked catfish (*Clarias gariepinus*) during storage. Biosci. Technol., 85: 173-177.

Golob, P., C. Moss, M. Dales, A. Fidgen, J. Evans and I. Gudrups, 1999. The use of spices and medicinals as bioactive protectants for grains. Food Agric. Org. Agric. Services Bull., pp: 137.

Lale, N.E.S., 1995. An overview of the use of plant products in the management of stored product Coleoptera in the products. Post Harvest News Inform., 6: 69N-75N.

Odeyemi, O.O., R.A. Owoade and R. Akinkurolere, 2000. Toxicity and population suppression effects of *Parkia clappertoniana* on dried fish pests (*Dermestes maculatus* and *Necrobia rufipes*). Global J. Pure Applied Sci., 6: 191-195.

Ofuya, T.I., B.C. Okoye and A.S. Olola, 1992. Efficacy of a crude extract from seeds of *Monodora myristica* (Gaertn) Dunal as surface protectant against *Callosobruchus maculatus* (F) attacking legume seeds in storage. J. Plant Dis. Protect., 99: 528-532.

Okonkwo, E.O. and W.I. Okoye, 1996. The efficacy of four seed powders and the essential oils as protectants of cowpea and maize grains against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Nigeria. Intl. J. Pest Manage., 42: 143-146.

Okonkwo, E.O. and W.I. Okoye, 2001. Insecticidal activity of *Dennettia tripetala* Baker f. and *Piper guineense* Schum. and Thonn against *Dermestes maculatus* Degeer (Coleoptera: Dermestidae) and *Necrobia rufipes* Degeer (Coleoptera: Cleridae) on dried fish. Nig. J. Entomol., 18: 109-117.

Olaifa, J.I. and W.O. Erhun, 1988. Laboratory evaluation of *Piper guineense* for the protection of cowpea against Callosobruchus maculatus. Insect Sci. Applic., 9: 55-59.

Ossisiogu, I.U.P. and E.O.P. Agbakwuru, 1978. Insecticides of Nigeria vegetable origin I: *Dennettia off*. A new seed preservative. Nig. J. Sci., 12: 477-485.

Osuji, F.N.C., 1974. Beetle infestation of dried fish purchased from a Nigerian market, with special reference to *Dermestes maculatus* Degeer. Nig. J. Entomol., 1: 69-79.

Sowunmi, E.O., 1982. Effect of storage length and insecticide treatment on cowpea (*Vigna unguiculata* L. Walp). Trop. Grain Legumes Bull., 25: 21-27.

Su, H.C.F., 1977. Insecticidal properties of black pepper to rice weevils and cowpea weevils. J. Econ. Entomol., 70: 18-21.

Toye, S.A., 1970. Studies on the humidity and temperature reactions of *Dermestes maculatus* Deg. (Coleoptera: Dermestidae) with reference to infestation in dried fish in Nigeria. Bull. Zool. Res., 60: 23-31.

Udoh, F.V., T.Y. Lot and V.B. Braide, 1999. Effects of extracts of seed and leaf of *Piper guineense* on skeletal muscle activity in rat and frog. Phytother Res., 13: 106-110.