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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

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ABSTRACT

Various stages of *Dermestes maculatus* Degeer and *Necrobia rufipes* Degeer were placed in Kilner jars containing dried catfish *Clarias* sp. treated separately with 0.25, 0.5, 0.75 and 1.0g of *Dennettia tripetala* Baker f. and *Piper guineense* Schum. and Thonn. seed powders / 25g of fish. *D. tripetala* and *P. guineense* seed powders significantly ($P < 0.05$) inhibited oviposition and achieved 100% adult mortality of the test insects in 7 days. In treated fish, larvae did not develop beyond second instar and 100% of larvae died by day 30 with *D. tripetala* powder being more insecticidally active. Minimal frass was produced in fish treated with both plant powders at 0.25g powder/25g of fish. In experiments under simulated storage conditions, essential oil extracts of *D. tripetala* and *P. guineense* treated at 0.1, 0.2 and 0.3ml / 25g of fish significantly ($P < 0.05$) inhibited oviposition and achieved 100% adult mortality of both *D. maculatus* and *N. rufipes* in 7days, and there was no progeny development by day 30. In treated fish, all the larvae died after 7 days, as compared to fish treated with 0.1 and 0.2ml groundnut oil / 25g and the untreated control. The aesthetic attractiveness of the fish was high. There are prospects for use of *D. tripetala* and *P. guineense* powder and essential oil extracts in the protection of dried fish in storage in Nigeria.

INTRODUCTION

In Nigeria, dried fish is prone to pest infestation during processing, transportation and storage, mainly by the leather beetle, *Dermestes maculatus* Degeer and copra beetle, *Necrobia rufipes* Degeer, resulting in quality deterioration. Haines and Rees (1989) observed that cured fish has low moisture content thereby providing food for beetles, particularly the larvae and, to a lesser extent, the adults of *D. maculatus* and *N. rufipes*. Ezeu (1982) estimated an annual loss of over 30% of dried fish due to infestation by *D. maculatus*. To protect their fish from insect infestation some processors and traders use

synthetic insecticides, which are applied as sprays, dips or in powder form and have been found to be effective against *D. maculatus* and *N. rufipes*. However, insecticide residues are potentially harmful to humans as they can persist in the fish during processing and cooking. Its general use to protect stored fish has been hampered by the interest in insecticides of plant origin, which have been enhanced by the current public suspicion of any chemical of a persistent nature, whether or not evidence is available of any adverse effects (Bell, 1992).

In Nigeria, fish merchants rub groundnut oil or other vegetable oils on dried fish for protective or cosmetic reasons (Don-Pedro 1989, 1990) and in some parts of the country orange peel (containing volatile oils) is burned at night to drive off mosquitoes (Don-Pedro 1985). Studies on the use of vegetable and essential oils against pests of stored animal products have been reported (Don-Pedro 1985, 1989, 1990; Okorie *et al* 1990; Emosairue, 1998; Egwunyenga *et al.* 1998). Ward and Golob (1994) reviewed the plant materials used for control of insect infestation of cured fish.

Although Egwunyenga *et al.* (1998) demonstrated the possible repellency of seed powder and extracts from *D. tripetala* to larvae of *Dermestes maculatus*, limited information is available on the use of *D. tripetala* and *P. guineense* seed powders and the essential oil extracts against insect pests of stored animal products. In response to this dearth of information, this work aimed at determining the action of *D. tripetala* and *P. guineense* seed powders and the extracts with the essential oils against *Dermestes maculatus* and *Necrobia rufipes* on dried fish.

MATERIALS AND METHODS

The experiments were carried out in the laboratories of Nigerian Stored Products Research Institute, Ilorin under $28 \pm 1^\circ\text{C}$ and 72% r.h. between December 1999 and January 2000.

Culture of *D. maculatus* and *N. rufipes*

Culture of *D. maculatus* and *N. rufipes* were maintained in dried (*Clarias* sp.) fish in Kilner jars under these laboratory conditions.

Preparation of seed powders

D. tripetala and *P. guineense* fruits were purchased from a market in Nnewi in Anambra state. The seeds of *D. tripetala* were removed with a scalpel from the soft pericarp, washed thoroughly in running tap water, rinsed in distilled water and dried at 60°C for 96h, while *P. guineense* fruits were washed thoroughly in running tap water, rinsed in distilled water and dried at 60°C for 96h. The dried seeds were ground using a hammer mill and sieved through a 40 holes/mm² mesh sieve to obtain fine powders. The powders were kept in air-tight jars prior to use (Okonkwo and Okoye, 1996 method).

Essential Oils Extraction

Washed powdered seed was extracted in 25g portions for 16h with 250ml of *n*- hexane (b. p. 40-60°C) in a Soxhlet extractor according to the AOAC (Anon, 1984) method. After the extraction of each essential oil, the solvent was recovered by heating the flasks in a ventilated oven for 45mins at 103±2°C. The oils were stored in glass vials at 5°C. The yields of oil extracted from 25g of the seed powder with 250ml *n* - hexane were *D. tripetala* 0.7ml and *P. guineense* 3.5ml.

Preparation of uninfested dried fish.

Fresh catfish (*Clarias* sp.) was obtained from an Ilorin fish market. The head and intestines were removed and the fish was washed in distilled water. The fish was dried in a Gallenkamp fan-ventilated oven at 60°C for 48h. The dried fish were covered to protect them from insect infestations and allowed to equilibrate under laboratory conditions for 4 days before they were used for the experiment.

Experimental studies

Effect of *D. tripetala* and *P. guineense* powders and oils on the oviposition of *D. maculatus* and *N. rufipes*

Oven dried *Clarias* fish (25g) was dusted with 0.25, 0.5, 0.75 and 1.0g of *D. tripetala* and *P. guineense* powders separately in Kilner jars and covered with muslin material to let in air, but prevent the escape of insects. Twenty (20) 24h-old emerged adults of *D. maculatus* or *N. rufipes* (unsexed) were placed in the jar. The set up was kept in an incubator at 28°C and 72% r. h. There were four replicates of each set of treated fish. Similarly, untreated fish were used as control. Eggs laid on the fish were counted after 18 days. The number of dead adults were noted. Similarly, the effect of essential oil extracts of *D. tripetala* and *P. guineense* on the oviposition of *D. maculatus* or *N. rufipes* were examined. *D. tripetala* and *P. guineense* oils were applied at 0.1, 0.2 and 0.3ml / 25g fish were applied to the fish samples with an Kloehn micro applicator in 700ml Kilner jars. The jars were thoroughly shaken manually to ensure effective coating of the fish with oil. Twenty 24h-old emerged adults of *D. maculatus* or *N. rufipes* (unsexed) were placed in the jar. Similarly, 20 (24h-old) emerged adults of each test insects (unsexed) were placed on fish treated with 0.3ml groundnut oil / 25g of fish which served as baseline control, while untreated fish served as the control. There were four replicates of each set of treated fish. Mortality readings were taken daily but the final analysis was based on mortality data at 7-day exposure periods since these gave the best regression lines in standard probit analysis. The percentage mortalities obtained were subjected to angular transformation (SAS, 1993) and an analysis of variance was applied.

Effect of essential oil extracts of *D. tripetala* and *P. guineense* on immature stages of *D. maculatus* and *N. rufipes*

Twenty newly hatched first instar larvae were placed on dried fish treated with

0.1, 0.2 and 0.3ml *D. tripetala* and *P. guineense* oil /25g of fish and replicated four times. Similarly, 20 newly hatched first instar larvae were placed on dried fish treated with 3ml groundnut oil/25g of fish which served as baseline control, while untreated dried fish served as the control. The insects were allowed to feed on the fish for 30 days, at the end of which, the number of live and dead larvae as well as the progeny development of the insects were monitored. The percentage effectiveness of treatment was calculated as : (Number dead / 20) x 100. The mortalities obtained were subjected to angular transformation before ANOVA tests were carried out on the treatments and comparison means were separated using Fisher's LSD at $P = 0.05$ (SAS, 1993).

Palatability and aesthetic attractiveness of the treated fish

After 30 days of powder and oil treatments, the dried fish were examined for damage due to feeding activities of the larvae. The amount of frass was noted. The number of dead larvae on the fish was counted. Palatability was carried out by a 10-member panel for dried fish before and after boiling subjected to various treatments. The taste was evaluated by a 4-point rating scale modified after Baldwin *et al.* (1972). The fish was tasted before and after boiling with water for 10min.

RESULTS

Effect of *D. tripetala* and *P. guineense* powders and oils on the oviposition of *D. maculatus* and *N. rufipes*.

Eighteen days after exposure of *D. maculatus* adults to treated dried fish, maximum number of 2 and 4.2 eggs were laid on the fish treated with 0.25g *D. tripetala* and *P. guineense* powders respectively. No eggs were laid on the fish samples treated with 0.5, 0.75 and 1.0g *D. tripetala* and *P. guineense* powders after 7 days. This was significantly different ($P < 0.05$) from the number of eggs oviposited on the untreated control fish; which had a range of 120-125 eggs. Adult insects mortality was significantly higher in the treated dried fish than in the untreated control (Table 1). Similarly, 18 days after exposure of *N. rufipes* adults to treated dried fish, maximum number of 3.5 and 5.3 eggs were laid on the dried fish treated with 0.25g of *D. tripetala* and *P. guineense* powders respectively. This was significantly different ($P < 0.05$) from the number of eggs oviposited on the untreated control which had a range of 112-121 eggs. Adult mortality was significantly higher in the treated fish than in the control (Table 2). *D. tripetala* powder was more insecticidally active to *D. maculatus* and *N. rufipes* larvae than *P. guineense* powder.

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Table 1: Effect of different concentration of plant powders on adult oviposition of *Dermestes maculatus* after 18 days treatment

Treatment	Concentration of plant powder/25g fish	Mean no. of eggs laid	Number of dead adults	% mortality of adult
<i>Dennettia tripetala</i>	0.25	2.0b	8.7a	87.0a
Untreated control	0.50	0.0c	10.0a	100a
CV		0.0c	10.0a	100a
LSD		0.0c	10.0a	100a
<i>Piper guineense</i>	0.75	115a	0.0b	0.0b
	1.00	1.78	0.81	0.50
	0.0	2.04	0.53	0.15
	0.25	4.2b	7.5a	75.0b
	0.50	0.0c	10.0a	100a
	0.75	0.0c	10.0a	100a
	1.00	0.0c	10.0a	100a
Untreated control	0.0	109a	0.0b	0.0c
CV		0.72	0.64	0.33
LSD		0.03	0.50	0.69

Means of four replications. Ten 24h-old emerged adults of *N. rufipes* (unsexed) were used. Means in a column with the same superscript are not significantly different at P = 0.05 level Fisher's LSD. CV = %

Treatment of dried fish with doses of *D. tripetala* and *P. guineense* oils ranging from 0.1 - 0.3ml was effective in preventing oviposition of either *D. maculatus* or *N. rufipes* eggs at 0.2 and 0.3ml / 25g fish. However, 18 days after exposure of *D. maculatus* or *N. rufipes* adults to treated dried fish, maximum number of 5 and 4 eggs were laid on the fish treated with 0.1ml oils of *D. tripetala* and *P. guineense* respectively. This was significantly different from the number of eggs oviposited on fish treated with 1.0 and 2.0ml groundnut oil which had a range of 5.5 - 10.2 eggs and the untreated control which had a range of 102-122 eggs. Adult mortality was significantly higher (P < 0.05) in the treated fish than in the untreated control (Table 3).

Table 2. Effect of different concentration of plant powders on adult oviposition of *Necrobia rufipes* after 18 days treatment.

Treatment	Concentration of plant powder/25g fish	Mean no. of eggs laid	Number of dead adults	% mortality of adult
<i>Dennettia tripetala</i>	0.25	3.5 ^b	9.0 ^a	90.0 ^a
	0.50	0.0 ^c	10.0 ^a	100 ^a
	0.75	0.0 ^c	10.0 ^a	100 ^a
	1.00	0.0 ^c	10.0 ^a	100 ^a
Untreated control	0.0	112 ^a	0.0 ^b	0.0 ^b
CV		0.93	0.64	0.35
LSD		0.04	0.50	0.69
<i>Piper guineense</i>	0.25	6.2 ^b	7.8 ^a	78.0 ^b
	0.50	5.3 ^b	9.0 ^a	90.0 ^a
	0.75	0.0 ^c	10.0 ^a	100 ^a
	1.00	0.0 ^c	10.0 ^a	100 ^a
Untreated control	0.0	121.5 ^a	0.0 ^b	0.0 ^c
CV		0.63	0.24	0.16
LSD		0.11	0.31	0.18

Means of four replications. Ten 24h-old emerged adults of *N. rufipes* (unsexed) were used. Means in a column with the same superscript are not significantly different at P = 0.05 level Fisher's LSD. CV = %

Table 3. Effect of extracts of essential oils of *Dennettia tripetala* and *Piper guineense* on oviposition of *Dermestes maculatus* and *Necrobia rufipes*.

Treatment	Concentration in ml/25g of fish	<i>D. maculatus</i>		<i>N. rufipes</i>			
		mean no. of eggs	No. of dead adults	(%) adult mortality	mean no. of eggs	No. of dead adults	(%) adult mortality
<i>Dennettia tripetala</i>	0.1	4 ^c	20 ^a	100 ^a	5.0 ^c	20 ^a	100 ^a
	0.2	0 ^d	20 ^a	100 ^a	0 ^d	20 ^a	100 ^a
	0.3	0 ^d	20 ^a	100 ^a	0 ^d	20 ^a	100 ^a
<i>Piper guineense</i>	0.1	2.0 ^a	20a	100 ^a	4 ^c	20 ^a	100 ^a
	0.2	0 ^d	20 ^a	100 ^a	0 ^d	20 ^a	100 ^a
	0.3	0 ^d	20 ^a	100 ^a	0 ^d	20 ^a	100 ^a
Groundnut oil	1.0	7.0 ^b	12.5 ^b	62.5 ^b	10.2 ^b	12 ^b	60 ^b
	2.0	5.5 ^b	15.5 ^b	77.5 ^{ab}	7.5 ^b	18 ^a	90 ^a
	3.0	0 ^d	20 ^a	100 ^a	0 ^d	20 ^a	100 ^a
Untreated control	0.0	102 ^a	1.8 ^e	8 ^e	121 ^a	2 ^e	10 ^e
CV		9.05	9.15	27.30	9.62	8.62	9.90
LSD		0.20	0.15	0.98	1.74	0.98	0.0

* Number dead x 100

20

Means of four replications. Twenty newly hatched first instar larvae were used. Means in a column with the same superscript are not significantly different at P = 0.05 level Fisher's LSD. CV = %

Larvicidal activity of *D. tripetala* and *P. guineense* powders and oils on *D. maculatus* and *N. rufipes*

Table 4 showed that out of the 20 first instar larvae introduced on dried fish treated with *D. tripetala* and *P. guineense* powders at 0.75 and 1.0g/25 fish, only 1.5 and 4.5 were found alive after 30 days. Untreated control experiment showed 98.5% larvae were found alive after 30 days. The average effectiveness was found to be in a range of 77.5 - 100% for powder treated fish infested with *D. maculatus* and a range of 66.5 - 97.5% for powder treated fish infested with *N. rufipes* larvae; and 0 - 6.5% for untreated control fish. *D. tripetala* powder was more toxic to *D. maculatus* and *N. rufipes* larvae than *P. guineense*.

Table 4. Larvicidal effect of plant powders on *Dermestes maculatus* and *Necrobia rufipes*

Treatment	Concentration of plant powder/25g fish	No. of dead		% effectiveness * of treatment	No. of dead		% effectiveness * of treatment
		Adult	Larvae		Adult	Larvae	
<i>Dennettia tripetala</i>	0.75	-	18.5 ^a	92.5 ^a	-	16.5 ^a	82.5 ^a
	1.00	-	20 ^a	100 ^a	-	19.5 ^a	97.5 ^a
Untreated control	0.0	-	0.9 ^b	4.5 ^b	-	0 ^b	0 ^b
	CV		1.55	5.01		6.77	6.51
<i>Piper guineense</i>	0.75	-	15.5 ^b	77.5 ^b	-	13.5 ^a	66.5 ^b
	1.00	-	20 ^a	100 ^a	-	18 ^a	90 ^a
Untreated control	0.0	-	1.5 ^b	7.5 ^c	-	1.3 ^b	6.5 ^c
	CV		9.15	9.45		1.60	7.30
LSD		-	30.16	7.08	-	0.77	7.94

* Number dead x 100

20

Means of four replications. Twenty newly hatched first instar larvae were used. Means in a column with the same superscript are not significantly different at P = 0.05 level Fisher's LSD. CV = %

Table 5 showed that all the first instar larvae placed in dried fish treated with *D. tripetala* and *P. guineense* oils at 0.3ml/25g fish were dead after 7 days, except for fish treated with groundnut oil at 0.1 and 0.2ml / 25g of fish. The average effectiveness was found to be 100, 100, 87.5 and 8% for *D. tripetala* and *P. guineense* oils, groundnut oil treated fish and for untreated fish respectively.

Table 5. Larvicidal effect of extracts of essential oils on *Dermestes maculatus* and *Necrobia rufipes*

Treatment	Concentration of oil in ml/25g fish	No. of dead		% effectiveness* of treatment	No. of dead		% effectiveness* of treatment
		Adult	Larvae		<i>D. maculatus</i>	<i>N. rufipes</i>	
<i>Dennettia tripetala</i>	0.3	-	20 ^a	100 ^a	-	20 ^a	100 ^a
<i>Piper guineense</i>	0.3	-	20 ^a	100 ^a	-	20 ^a	100 ^a
Groundnut oil	3.0	-	17.5 ^a	87.5 ^a	-	8 ^b	33 ^b
Untreated control	0.0	-	1.5 ^b	7.5 ^b	-	2 ^b	8 ^b
CV	-	-	9.05	9.15	-	8.60	27.30
LSD	-	-	0.15	0.98	-	0.77	1.34

* Number dead x 100

20.

Means of four replications. Twenty newly hatched first instar larvae were used. Means in a column with the same superscript are not significantly different at P = 0.05 level Fisher's LSD. CV = %

Palatability and aesthetic attractiveness of the treated and untreated fish

The treated fish was undamaged after 30 days with very little frass for *D. tripetala* and *P. guineense* powder treatments at 0.25g. The taste of the fish was generally very good and fish treated with *D. tripetala* and *P. guineense* oils had better aesthetic appearance than fish treated with powder. The untreated fish had been damaged by the larvae, with a large amount of frass in the specimen jars due to feeding activities of the larvae. The taste of the untreated fish was not palatable before and after boiling.

DISCUSSION

Dennettia tripetala and *P. guineense* powders significantly reduced the rate of oviposition of *D. maculatus* or *N. rufipes*, an affect that has also been observed by Okonkwo and Ewete (1999) who stated that *D. tripetala* and *P. guineense* powders treated with cowpea or maize at 0.25g/25g achieved 89.4% adult mortality of *Callosobruchus maculatus* and *Sitophilus zeamais*. The powders had ovicidal effect on both insects, the incubation of freshly laid eggs did not produce progeny. Ninety seven and a half percent of *D. maculatus* larvae and 92.5% of *N. rufipes* larvae were killed on treated fish. The remaining 2.5 or 7.5% were still in the second instar larval stage after 30 days of exposure to either *D. tripetala* or *P. guineense* powders. This is in agreement to Okorie *et al.* (1990) who reported in their experiments that 2g of neem seed powder/25g *Tilapia* fish significantly inhibited oviposition and 93% of *D. maculatus* larvae were killed on treated fish. Osuji (1975) stated that the larval stages usually account for about 93.2% of the beetles. This study shows that *D. tripetala* and *P. guineense* powder treatments are effective in controlling either *D. maculatus* or *N. rufipes* larvae. At a concentration of 1g powder/25g dried fish, the botanicals killed off all adult insects within 14 days. This is also consistent with the observation of Okorie *et al.* (1990) where the neemseed killed all *D. maculatus* adults.

Dennettia tripetala and *P. guineense* essential oil extracts were effective in controlling *D. maculatus* or *N. rufipes* larvae at a concentration of 0.1 - 0.3ml/25g dried fish. This study shows that the botanical oil treatments are very effective in controlling the test insects, contrary to observation made by Don-Pedro(1989) who stated that application of fixed vegetable oils at 14 and 28ml/kg of dried trout (*Salmo gairdnerii*) had no apparent effect against larval emergence and adult progeny development of *D. maculatus* 18 days after treatment.

Dennettia tripetala and *P. guineense* powders and oils therefore afforded protection to dried fish against *D. maculatus* and *N. rufipes* infestation. The fish remained very attractive, as there was minimal frass production. These botanicals are easily available to people in rural areas and therefore, is a promising technology for small scale preservation of fish at the village level by subsistent farmers. In terms of efficacy *D. tripetala* is preferred to *P. guineense* and the fruits are masticatories without any health risks and are already a part of human diet.

In conclusion, the use of *D. tripetala* and *P. guineense* as protectants of dried fish is feasible. The treated fish will be highly accepted by consumers as there is no evidence of taint or smell on the treated fish and there is no effect on the flavour of fish

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