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Larvicidal and Repellent Actions of *Detarium microcarpum* Seed Oil Against the Larvae of *Dermestes lardarius* (Coleoptera: Dermestidae) in Dried *Clarias gariepinus* Fish

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Abstract: Studies were conducted to evaluate the larvicidal and repellency efficacy of the seed oil of the tallow tree *Detarium microcarpum* (Guill. and Perr.) (Fam. Leguminosae: Caesalpinioideae) against late instar larvae of the larder beetle *Dermestes lardarius* Linnaeus, in dried *Clarias gariepinus* Teugels fish under laboratory conditions. Pure seed oil was applied on the dried fish at concentrations of 0.006-0.025 mL g^{-1} and a control, with 30 larder beetle larvae each maintained in triplicates for 24-96 h. The 0.006, 0.010, 0.014, 0.022 and 0.025 mL g^{-1} dosages on treated fish produced 53.33-70, 81.11-92.22, 80-87.78, 82.22-96.67 and 85.56-96.67% mortality of *D. lardarius* larvae after 24-96 h exposure, respectively. The seed oil also repelled 80-96.67% of the larvae from the treated fish within 24 h. Probit analysis of the bioassay data gave an LC_{50} value of 0.023 mL g^{-1} for the seed oil against *D. lardarius* larvae. The studies demonstrate a very high larvicidal and strong repellency of *D. lardarius* by *D. microcarpum* seed oil. These findings indicate a high potential of controlling and protecting dried fish from dermestid beetles infestation using the seed oil.

Key words: *Dermestes lardarius*, *Detarium microcarpum*, seed oil, dried fish

Introduction

Fish production, processing and preservation are valuable industries in virtually all human societies where the products constitute a very rich source of animal protein for humans and livestock feed. There has been large scale deterioration and losses in the quality of processed fish due to the combined effects of insect infestation and other biological agents that flourish under the hot and generally humid conditions of the tropics (Toye, 1970; Osuji, 1974). In Nigeria, as in several other countries, qualitative and quantitative losses of dried fish during storage, transportation and marketing are due mainly to infestation by dermestid beetles including *Dermestes ater* Degeer, *Dermestes frischii* Kugelann, *D. lardarius*, *Dermestes maculatus* Degeer and *Necrobia rufipes* Degeer (Claridae) (Rollings and Hayward, 1963; Osuji, 1974). These insects are also destructive in dried meat, dried bloodmeal, bacon, cheese, leather goods, poultry feeds and allied produce (Osuji, 1973, 1985; Merchant, 2001). Larval stages of dermestid beetles have been found to cause up to 93% infestation of dried fish and up to 62.7% loss in their dry weight (Osuji, 1973, 1974).

Control measures against dermestid infestation of dried fish, amongst several options, include injudicious use of chemical insecticides such as dichlorvos, malathion, agmoxine, endrine and DDT, which are often hazardous to human health, costly and have induced the development of resistance

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in the target pests (Ellis, 1964; Llyod and Dyte, 1965; Proctor, 1972; Mohammed and Yusuf, 2001). Several natural products, including oils derived from plant materials, have been tested as protectants of dried fish from insect infestation. The fruit oil of *Piper guineense* Schum. and Thonn. (Fam. Piperaceae) was found to be effective as protectant of dried fish against larval and adult stages of *D. maculatus* (Amusan and Okorie, 2002). The kernel oil of neem *Azadirachta indica* A. Juss. (Fam. Meliaceae) caused high mortalities in both the larvae and adults of *D. maculatus* in dried fish and suppressed oviposition and adult emergence (Onu and Baba, 2003).

Detarium microcarpum, is one of three species of a tropical African genus of hardwood trees, occurring principally and is widespread in the dry West African savanna, where it grows with a twisted bole for up to 9 m (Keay, 1989). It produces one-seeded drupe-like fruits. It is catalogued as a major African medicinal plant (Iwu, 1993). The root bark extract of *D. microcarpum* inhibited the growth of several microbial agents of diseases (Hassan *et al*., 2004); it was also cytotoxic and showed antifeedant effect on the rust red flour beetle *Tribolium casteneum* Herbst (Hassan *et al*., 2005). It appeared that *D. microcarpum* seed oil or any of its parts thereof has not been investigated on its ability to control dermestid infestation in dried fish. This study therefore presents a laboratory evaluation of the larvicidal and repellency efficacy of the petroleum spirit extract of the seed oil of *D. microcarpum* against late instar larvae of *Dermestes lardarius*, a cosmopolitan commercial cum household insect pest especially of dried fish.

Materials and Methods

Collection and Extraction of Oil from D. microcarpum Seeds

Dried seeds of *D. microcarpum* were purchased from the Sabongari market in Zaria, northern Nigeria. The seeds were pulverized using an electric motorized mill. The oil in the seeds was subjected to solvent extraction in a Soxhlet extractor with petroleum spirit (60-80°C). A rotary evaporator was used to evaporate excess solvent from the oil. The seed oil was stored at room temperature in a labeled vial until used for bioassay.

Maintenance of Laboratory Culture of D. lardarius

Adults of *D. lardarius* were obtained from a heavily infested poultry feed concentrate rich in fish meal (Livestock Feeds Plc., Kaduna, Nigeria). A pure culture of the beetles was maintained on dried fish and wet cotton wool in wire gauze secured Kilner jars at 27.5±2.5°C and 70-80% relative humidity.

Collection and heat sterilization of dried C. gariepinus fish

Dried *C. gariepinus* fish weighing 17-24 g were purchased from the Sabongari market, Zaria, Nigeria. The fish were ascertained to be free of prior insecticidal treatment. The fish were heat sterilized in a Galenkamp IH-150 oven at 60°C for 1 h. to rid them of any prior insect pest infestation.

Bioassay of D. Lardarius Larvae using D. microcarpum Seed Oil

Dried heat sterilized *C. gariepinus* fish in Kilner jars were treated with the seed oil of *D. microcarpum* in the following weight/volume (and equivalent unit) concentrations: 17 g/0.1 mL (0.006 mL g^{-1}), 20 g/0.2 mL (0.010 mL g^{-1}), 21 g/0.3 mL (0.014 mL g^{-1}), 23 g/0.5 mL (0.022 mL g^{-1}) and 24 g/0.6 mL (0.025 mL g^{-1}). Control experiments, using the same weight of fish as the experimentals but devoid of oil treatments, were also set up. Both experimentals and controls were replicated thrice, each replicate containing 30 late instar larvae of *D. lardarius*. The mortality of larvae in the experimentals and controls were determined after 24, 48 and 96 h post exposure. The repellency effect of the seed oil to the larvae was also determined by comparing their number inside and outside the treated and untreated fish after 24 h exposure.

Statistical Analysis

Mortality data obtained with the various oil concentrations used were subjected to probit analysis (Finney, 1964). Control mortality was corrected following Abbott (1925). The probit of mortality was plotted against the natural logarithms of the concentrations on a graph. A line of best fit was obtained and the regression equation for the graph was used to calculate the LC_{50} of the seed oil to the larvae of *D. lardarius*. Students t-test statistic was used to test for significant differences in larval mortality between the experimentals and controls.

Results

The mortality of the larvae in the treated fish increased in a dose and time dependent fashion except for the slight drop in mortality observed with the 0.014 mL g^{-1} dosage. For the least dosage (0.006 mL g^{-1}) mortality ranged from 53.33% at 24 h to 70% at 96 h of exposure. The highest dosage (0.025 mL g^{-1}) produced 85.56-96.67% mortality within 24 and 96 h, respectively (Table 1). Although a maximum of 21% mortality was recorded in the control, the mean mortality in the controls was 5.33%. However, Student's t-test revealed that the mortality of larvae in treated fish was significantly higher than control mortality ($p<0.05$). Table 2 shows the probit analysis of the 24 h mortality data of *D. lardarius* larvae exposed to fish treated with *D. microcarpum* seed oil. The analysis afford the opportunity to adjust the mortality in the control using Abbott (1925) formula. The empirical probit of kill was plotted against the logarithms of concentrations used (Fig. 1). A regression equation $Y = -2.6998X+0.5635$ yielded a median lethal concentration (LC_{50}) of 0.023 mL g^{-1} for the oil against *D. lardarius*.

The number of larvae that penetrated the untreated fish was consistently and significantly higher ($p<0.05$) than those that penetrated the treated fish. This ranged from 35/90 (38.89%) to 79/90 (87.78%) in the untreated and from 3/90 (3.33%) to 18/90 (20.00%) in the oil treated fish (Table 3).

Table 1: Mortality of *D. lardarius* larvae exposed to dried *C. gariepinus* fish treated with various concentrations of *D. microcarpum* seed oil

Seed oil concentration (mL g^{-1})	No. of larvae exposed	No. (Mean±SE) of dead larvae per exposure time			Percentage mortality (%) per exposure time		
		24 h	48 h	96 h	24 h	48 h	96 h
0 (Control)	90	0(0±0)	2(0.70±0.27)	4(1.3±0.72)	0	2.22	4.44
0.006	90	48(16±1.25)	60(20±0.30)	63(21±2.87)	53.33	66.67	70.00
0(Control)	90	1(0.33±0.27)	2(0.70±0.27)	6(2±0.00)	1.11	2.22	6.67
0.010	90	73(24.3±1.19)	81(27±0.74)	83(27.7±0.55)	81.11	90.00	92.22
0(Control)	90	8(2.7±2.18)	12(4±1.89)	19(6.3±2.33)	8.89	13.33	21.11
0.014	90	72(24±3.27)	78(26±2.50)	79(26.3±2.23)	80.00	86.67	87.78
0(Control)	90	0(0±0)	0(0±0)	4(1.3±0.75)	0	0	4.44
0.022	90	74(24.7±0.91)	84(28±0.47)	87(29±0.82)	82.22	93.33	96.67
0(Control)	90	3(1±0.00)	5(1.7±0.55)	6(2±0.75)	3.33	5.56	6.67
0.025	90	77(25.7±1.09)	86(28.7±0.72)	87(29±0.82)	85.56	95.56	96.67

Table 2: Probit analysis of the 24 h mortality of *D. lardarius* larvae exposed to different concentrations of *D. microcarpum* seed oil on dried *C. gariepinus*

Seed oil concentration (mL g^{-1})	Natural logarithm of concentration	No. of exposed larvae	No. of dead larvae	Percentage mortality (%)	Abbott's corrected mortality (%)	Empirical probit
0.025	-1.60	90	77	86	85	6.04
0.022	-1.66	90	74	82	81	5.88
0.014	-1.85	90	72	80	79	5.81
0.010	-2.00	90	73	81	80	5.84
0.006	-2.22	90	48	53	52	5.00
0.00	-	90	2	2	0	0

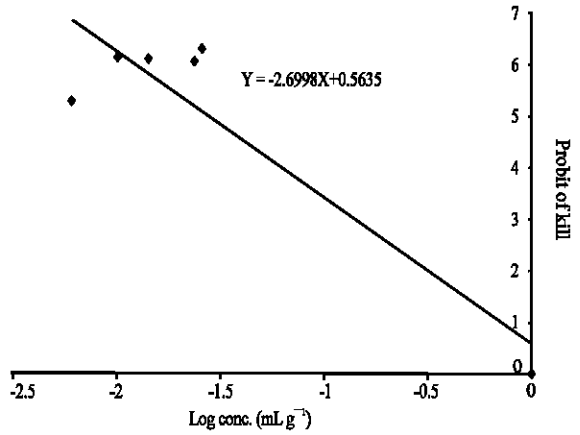

Fig. 1: Showing probit of kill against Log. conc. (ML g^{-1})

Table 3: Repellency effect of dried *C. gariepinus* treated with *D. microcarpum* seed oil on the larvae of *D. lardarius*

Seed oil concentration (mL g^{-1})	No. of larvae exposed	No. (%) of larvae within fish at 24 h post exposure	No. (%) of larvae outside fish at 24 h post exposure
Untreated (Control)	90	35(38.89)	55(61.11)
0.006	90	5(5.55)	85(94.45)
Untreated (Control)	90	79(87.78)	11(12.22)
0.010	90	4(4.44)	86(95.56)
Untreated (Control)	90	62(68.89)	28(31.11)
0.014	90	18(20.00)	72(80.00)
Untreated (Control)	90	67(74.44)	23(25.56)
0.022	90	3(3.33)	87(96.67)
Untreated (Control)	90	42(46.67)	48(53.33)
0.025	90	3(3.33)	87(96.67)

Conversely, the number of larvae found outside the oil treated fish was consistently and significantly higher ($p<0.05$) than the number found outside the untreated fish. This ranged from 72/90 (80.00%) to 87/90 (96.67%) in the oil treated fish and from 11/90 (12.22%) to 55/90 (61.11%) in the untreated fish. Thus signifying that majority of the larvae on the oil treated fish abandoned the fish and died outside the fish but within the experimental jars. This behaviour was an overt evidence of repellency due to the seed oil.

Discussion

These laboratory bioassays indicate that the seed oil of *D. microcarpum* was larvicidal at low concentrations on late instar larvae of *D. lardarius*. The oil also produced excellent repellency effect on the larvae of the beetle in treated fish. These dual properties are essential in preventing the beetle from initiating a novel attack and to rid infested fish of larvae. The findings of this study has exposed *D. microcarpum* seed oil as a valuable botanical for the control of insect pest of dried fish. The seed oil was suspected to have a contact mode of action on the larvae since mortality was recorded in larvae that had transient contact with the treated fish. Although the active principle responsible for the larvicidal effect of the oil was not determined in this study, toxicity to the larvae could be due to the presence of plant sterols in the oil (Njoku *et al.*, 1999).

This study has added a pest control dimension to the known nutritional properties of *D. microcarpum* seed oil. The low oil content (11.24%) of *D. microcarpum* seeds (Anhwange *et al.*, 2004), will not preclude its commercial application as dermestid larvicide, since it was very effective

at very low concentrations. In addition, the seed oil of *D. microcarpum* has low iodine value (<100), making it a non-drying oil, slow to oxidation and remains as liquid for prolonged duration (Anhwange *et al*., 2004). The oil will therefore be effective as larvicide and repellant on treated fish for a long period. Oil of this nature to be used in protecting fish meant for human consumption should not have any deleterious effect on humans. *Detarium microcarpum* seed oil has been analysed for nutritional and toxicological properties and was adjudged a good oil fit for use in human nutrition (Njoku *et al*., 1999; Anhwange *et al*., 2004). Gossypol and mycotoxins, which could be anti-nutritional, were not found in the oil (Njoku *et al*., 1999). Its incorporation into dried fish to prevent and control dermestid infestation will therefore not introduce materials that are deleterious to human health. In addition, the oil being of low acid value (<15% minimum safe limit) have low deteriorating rate and long storability (Anhwange *et al*., 2004), is thus ideal for treating fish that would be stored for prolonged period. This property will also exclude the development of objectionable flavour and odour in fish treated with the oil (Ekpa and Ekpa, 1996). This characteristic was confirmed by the treated fish not showing any taint in a palatability test conducted in this study. A low peroxide value associated with the seed oil implies that its use to protect fish might prevent oxidative rancidity at room temperature (Anhwange *et al*., 2004).

In conclusion, the seed oil of *D. microcarpum*, while possessing characters of being edible by humans also cause high mortality and repellency to the larvae of *D. lardarius* and should be useful at low concentrations for the protection of dried fish against dermestid attack. It will be worthwhile to investigate the effect of the seed oil on the imago of *D. lardarius* and related species attacking stored fish. This will provide valuable information on the insect pest control potential of the oil on all the stages and species of the beetles that are damaging on dried fish in storage. The active principle responsible for larval mortality needs also to be characterized.

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