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Insecticidal value of four vegetable oils against *Dermestes maculatus* Degeer on smoked African mud cat fish, *Clarias gariepinus* Burchell

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Abstract
The study focused on the examination of insecticidal value of some edible vegetable oils from four plants: *Vitellaria paradoxa* C.F. Gaetn (Shea tree), *Ricinus communis* Linnaeus (Castor bean), *Carthamus tinctorius* Linnaeus (Safflower) and *Sesamum indicum* Linnaeus (benneseed), against hide beetle, *Dermestes maculatus* Degeer (Coleoptera: Dermestidae) infestation on smoked-dried African mud catfish, *Clarias gariepinus*. Parameters assessed were adult and larval mortality at 1, 3, 5 and 7 Days after Treatment (DAT). Plant oils were separately applied at dosages of 1.0, 1.5 and 2.0 ml per 20 g of dried fish and allowed to dry for 6 hours. Results showed that the vegetable oils evoked significantly (P< 0.05) adult and larval mortality compared to the untreated fish. Similarly, the application of oil significantly (P<0.05) suppressed insect's egg hatchability when compared to the control. The study clearly suggests and confirms insecticidal potential of these plant oils as alternative pest control measure.

Keywords: Smoked cat fish, fish beetle, plant derived oil, protectant

Introduction

There is a growing awareness of the importance of fish and fisheries production in many developing countries [1-3]. It is well accepted that fish is palatable, with high nutritive value and significance in improving human health [4]. Fish is a good source of animal protein and a major source of vitamins and minerals [5]. The high protein content of fish makes them a natural supplement to cereal, root tuber and red meat staples in the human diet. Fish is a renewable form of resource found in wide coverage (most water bodies in the world) which makes it readily accessible and cheap source of food throughout the world [3]. Nigeria is the largest consumer of fish production in Africa with an estimated annual per capita fish consumption of 17.5 kg, and a projected fish demand of 3.61 million metric tonnes [6, 7]. Over the years, Nigeria has relied on importation of fish to meet her ever increasing demand [8]. This trend appears not to have changed, given reports that the country is a net importer of fishing products with total fish imports amounting to about USD 1.2 billion and exports valued at USD 284,390 million in 2013 [9].

The major fish species produced in Nigeria, which include Torpedo-shaped cat fish (*Clarias* and *Heterobranchus* spp.), Tilapias (*Hemichromis oreochromis* spp.), African carps (*Cyprinidae* spp.) and marine fishes (*Osteichthyes* spp.), come from three sources, artisanal (Inland rivers, lakes, coastal brackish water), aquaculture (fish farm) and Industrial fishing [10, 11]. Fish resources are susceptible to both microbiological and chemical deterioration. High levels of moisture, temperature, nutrient content and pH accelerates spoilage and renders fish easily perishable product, often going bad within a short period of time post mortem [12, 13]. In the tropics, the quality and shelf life of fish and fish products are often enhanced by using various curing methods such as drying, salting, pickling and smoking [14]. In Nigeria the African mud cat fish, *Clarias gariepinus* (Burchell), which is the commonest and most widely cultivated fish, has over 45 per cent of the total catch utilized and cured as smoked fish [15, 16]. However, it is reported that inefficient fish curing techniques, handling methods and carriage over long distances make them susceptible to *Dermestes maculatus* Degeer infestation [17]. Under traditional storage conditions, losses due to beetle infestation are estimated at 50 per cent [18, 19]. Although synthetic chemical insecticides have proved effective against *D. maculatus* and other associated stored products pests, their use has been hampered by public suspicion of their potential health hazards; and they are being widely replaced by natural products [20-22]. Extracts containing bioactive compounds isolated from various plant sources have shown remarkable in-vitro antioxidant and antimicrobial activities globally [23-26]. In Nigeria, plant sources *viz*., spices, herbs and vegetables have been employed in the control and management of numerous field and stored products pest [27-29]. The present work was proposed to evaluate the insecticidal efficacies of four vegetable oils commonly used in homes as spices, condiments and medicines in controlling *D. maculatus* on stored smoked cat fish, *C. gariepinus*, with a view of establishing a sustainable management strategy for the beetle.

Materials and Methods

Study Area

The study was conducted between October, 2017 and March, 2018 at the Biology Laboratory of the Cross River University of Technology, Calabar, Nigeria (situated between Latitude 05º 08¹ N and Longitude 8º 14¹ East), under ambient condition; 28 ± 2ºC temperature, 65 ± 5 % relative humidity and average daily hour light / darkness exposure.

Collection of Plants/ Oil extraction

Dry seeds of test plants; Safflower, *Carthamus tinctorius* Linnaeus and Shear tree, *Vitellaria paradoxa* CF Gaertn were purchased from a herbal store in Ewu town in Esan Central Local Government Area of Edo State, Nigeria, situated between Latitude 6º 48¹ 5 N and Longitude 6º 14¹ 28 E. Seeds of Castor bean, *Ricinus communis* Linnaeus and Benneseed, *Sesamum indicum* Linnaeus were obtained from fields in Ogoja, Cross River State, Nigeria, situated between Latitude 6º 39¹ 30.24¹¹ N and Longitude 8º 47¹ 57.23¹¹ E. Plant materials were selected on the basis of their ethno-medical and endemicity, identified and authenticated at the herbarium unit of the Department of Biological Sciences, Cross River University of Technology, Calabar.

Plant parts were washed in clean water to remove any dirt material and dried in an electric oven to 38ºC for 8 hours, thereafter, separately pulverized using a milling machine (All steel, 11.1cm diameter sample input, 17.8cm deep chamber diameter (Corona Mechanical Blender; Landers, Medellin-Columbia). A 63/75 micron sieve shaker was used to obtain powder size. Oil preparation from pulverized seeds was facilitated by soxhlet extraction method using n- hexane as solvent at B.P, 40- 60ºC. Whatman No 1 filter paper was used to filter the extractions which were later kept in clean transparent containers with firm covers and preserved in a refrigerator to maintain their efficacy.

Preparation of Uninfested fish samples and insect rearing

Samples of smoked-dried African mud cat fish, *C. gariepinus* were purchased from Beach market in Calabar. The cured fish samples and bioassay jars were subjected to sterilization using a hot air oven in order to kill any insect pests or contaminants that may be present. An already infested smoked cat fish *C. gariepinus* purchased from the same market formed the initial source of *D. maculatus.*

Cultures were maintained and reared in a Kilner jar covered with muslin cloth at tropical storage conditions of temperature (28 ± 5 ºC) and relative humidity (65 ± 5%) to breed and multiply. New generations were raised by removing newly emerged larvae from the stock culture and placing on fresh disinfested fish and then removing the parent adult after 2-3 weeks oviposition period. Oviposition was however induced by the provision of pieces of water-soaked cotton wool.

Effect of vegetable oil on *D. maculatus* adult and larvae mortality

Twenty grams (20g) of disinfested fish thoroughly and evenly rubbed with different aliquots of vegetable oil (treatments), 1.0, 1.5 and 2.0 ml were placed in 11.0 x 4.50 x 9.40 cm^3 transparent plastic containers. Each container had cover with holes covered with nylon mesh to permit aeration and confinement of insects. Fifteen (15) newly emerged adults (0-24h old) were introduced into each of the test plastic containers containing treatments, set up in a Completely Randomized Design (CRD) under ambient temperature, 23 ± 2 ºC and relative humidity 65± 5 % and kept in open air shelf. Untreated fish, set up in triplicate served as control. Adult beetle mortality was monitored and recorded at 1, 3, 5, and 7 days after treatment and the percentage mortality was calculated thus:

$$\text{Mean \% adult mortality} = \frac{\text{mean number of dead adult insects}}{\text{Mean number of adult insects introduces}} \times 100$$

Similarly, the experiment on the effect of oil on the larva involved the introduction of newly hatched fish instar larva into test plastic containers containing dried fish, separately treated with 1.0, 1.5 and 2.0 ml of oil per fish and replicated three times. The untreated dried fish again served as the control. Percentage larvae mortality was calculated as

$$\text{Mean \% larvae mortality} = \frac{\text{mean number of dead larvae}}{\text{Mean number of adult larvae introduces}} \times 100$$

Effect of vegetable oil on *D. maculatus* eggs hatchability

*Aliqu*ots of 1.0, 1.5 and 2.0 ml of vegetable oil were added to 20g of disinfested fish in transparent test plastic containers and thoroughly mixed with the aid of a glass rod. Fifteen (15) freshly laid eggs were introduced into the test plastic containers. Tests were in triplicate for each treatment and kept in an open air shelf in the laboratory at room temperature. Observation for hatching and recording of the emergent larvae were made at 21 days after treatment and the larvae emergence or hatchability calculated as follows:

$$\text{Mean \% hatchability} = \frac{\text{mean number of eggs hatch}}{\text{Mean number of eggs introduced}} \times 100$$

Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA). Differences between the mean values of the treatments were determined by Duncan new multiple range test (DNMRT) and the significance was defined at 0.05 [30].

Results

Effect of vegetable oil on adult and larva of *D. maculatus*

The effect of four natural vegetable oils on both adult and larva of *D. maculatus* is presented in Tables 1 and 2. The plant products caused significant differences in the mortality of test insects compared to the control, ($p < 0.05$). The percentage mortality was found to increase with higher dosage of oil which indicates direct relationship between the dose and percentage mortality. Comparatively, *C. tinctorius* was more toxic to adult and larval beetles than the other treatments at all the corresponding dosages. They were relatively lower mortality rates of test insects recorded 1 DAT. Maximum kill of adult and larval insects at the lowest content level were; *C. tinctorius*, 53.35 and 61.65, *V.*

paradoxa, 33.35 and 40.00, *S. indicum*, 15.00 and 32.00 and *R. communis*, 5.00 and 25.00 percent, respectively. Similarly, by 7 DAT percentage mortality showed a record of *C. tinctorius*, 100.00 and 100.00, *V. paradoxa*, 91.65 and 100.00, *S. indicum*, 76.65 and 98.35 and *R. communis*, 58.35 and 86.65 percent, respectively. Also, a total kill (100%) of both adult and larvae, caused by *C. tinctorius*, were recorded at 5 and 7 DAT. The larvae were found to be more susceptible to oil compared to the adult insect and were totally killed by all the oil extracts 7 DAT at the highest oil dosages.

Effect of oil on egg hatchability

Table 3 shows the effect of oil on egg hatchability or larval emergence. The result indicates that all the plant products significantly suppressed larval emergence from the test fish in comparison with the control ($p < 0.05$). It was observed that egg hatchability decreased with higher oil dosages, for instance, with 2.0 ml, *C. tinctorius, V. paradoxa, S. indicum* and *R. communis* showed egg hatchability of 22.22, 48.88, 62.22 and 71.00 percent respectively in comparison to a record of 66.67, 75.55, 80.00 and 82.22 percent respectively observed at the lowest treatment level. It was found that while a relatively high percentage of the emergent larvae died on the treated fish within few hours, a higher number of the emergent larvae in the control (untreated fish sample) survived into adults.

Table 1: Effect of vegetable oil on adult *D. maculatus* at 1,3,5,7 days after treatment (DAT)

Vegetable Oil	Concentration (ml)	Mean % Mortality (±SE) of D. *maculatus*			
		1	3	5	7
Control	0.00	3.35±0.67^{h}	6.65±0.67^{g}	6.65±0.67h	8.35±0.33^{e}
S. *indicum*	1.00	15.00±0.67^{gh}	37.00±1.76^{fg}	58.00±1.20^{fg}	76.65±1.67^{c}
	1.50	32.00±1.20^{f}	55.00±1.00^{cde}	70.00±0.58^{e}	83.35±0.33^{bc}
	2.00	50.00±0.58^{de}	68.50±0.33^{bcd}	81.70±0.33^{cd}	91.65±0.33^{ab}
C. *tinctorius*	1.00	53.35±1.76^{cd}	85.00±1.00^{ab}	98.35±0.33^{a}	100.00±0.00^{a}
	1.50	73.35±1.88^{ab}	93.35±0.67^{a}	100.00±0.00^{a}	100.00±0.00^{a}
	2.00	83.35±0.88^{a}	98.35±0.33^{a}	100.00±0.00^{a}	100.00±0.00^{a}
V. *paradoxa*	1.00	33.35±1.20^{ef}	51.70±0.88^{ef}	71.65±0.67^{de}	91.65±0.33^{ab}
	1.50	60.00±0.58^{bcd}	70.00±0.58^{bc}	86.65±1.33^{bc}	98.35±0.33^{a}
	2.00	70.00±1.73^{abc}	85.00±1.73^{ab}	95.00±1.00^{ab}	100.00±0.00^{a}
R. *communis*	1.00	5.00±0.88^{gh}	23.35±1.20^{g}	50.00±0.58^{g}	58.35±0.88^{d}
	1.50	11.70±1.20^{gh}	41.70±1.45^{ef}	56.65±0.88^{g}	65.00±1.15^{d}
	2.00	25.00±0.58^{fg}	55.00±1.00^{cde}	68.35±0.88^{ef}	76.65±1.20^{c}

Means followed by the same letter(s) are not significantly different ($P < 0.05$ from each other using Duncan's multiple range test (DMRT).

Table 2: Effect of vegetable oil on larvae of *D. maculatus* at 1, 3, 5 and 7 Days after Treatment (DAT)

Vegetable Oil	Concentration (ml)	Mean % Mortality (±SE) of *D. maculatus*			
		1	3	5	7
Control	0.00	0.00±0.00^{h}	0.00±0.00^{d}	8.35±0.88^{d}	10.00±1.00^{c}
S. indicum	1.00	32.00±1.20^{fg}	53.35±1.76^{abc}	70.00±0.58^{c}	98.35±0.33^{a}
	1.50	50.00±0.58^{de}	65.00±1.15^{abc}	81.70±0.33^{b}	100.00±0.00^{a}
	2.00	71.65±0.67^{bc}	81.70±9.67^{cd}	93.35±0.67^{a}	100.00±0.00^{a}
C. tinctorius	1.00	61.65±0.88^{cd}	85.00±0.58^{ab}	100.00±0.00^{a}	100.00±0.00^{a}
	1.50	68.35±0.33^{bc}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
	2.00	96.65±0.33^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
V. paradoxa	1.00	40.00±1.53^{ef}	65.00±1.15^{abc}	81.70±0.33^{b}	100.00±0.00^{a}
	1.50	60.00±1.53^{cd}	83.35±0.88^{ab}	95.00±1.00^{a}	100.00±0.00^{a}
	2.00	81.65±0.33^{b}	96.65±0.67^{a}	100.00±0.00^{a}	100.00±0.00^{a}
R. communis	1.00	25.00±0.58^{g}	41.70±1.45^{bcd}	66.65±0.33^{c}	86.65±0.88^{b}
	1.50	41.70±1.45^{ef}	55.00±1.00^{abc}	76.65±0.67^{b}	95.00±0.58^{a}
	2.00	60.00±0.82^{cd}	68.35±0.88^{abc}	85.00±0.58^{b}	100.00±0.00^{a}

Means followed by the same letter(s) are not significantly different ($P < 0.05$ from each other using Duncan's multiple range test (DMRT)

Table 3: Egg hatchability of *D. maculatus* at 21 days post treatment.

Vegetable Oil	Mean % Concentration	Egg Hatchability
Control	0.00	95.55±0.33^{e}
S. indicum	1.00	80.00±1.15^{de}
	1.50	68.88±1.76^{bcde}
	2.00	62.22±1.20^{bcd}
C. tinctorius	1.00	66.67±1.15^{bcde}
	1.50	46.67±2.33^{bcd}
	2.00	22.22±1.45^{abc}
V. paradoxa	1.00	75.55±1.15^{bcde}
	1.50	55.56±1.15^{ab}
	2.00	48.88±1.33^{a}
R. communis	1.00	82.22±1.20^{de}
	1.50	77.77±0.88^{cde}
	2.00	71.00±1.20^{bcde}

Means followed by the same letter(s) are not significantly different ($P < 0.05$ from each other using Duncan's multiple range test (DMRT)

Discussion

The study indicated the ability of four vegetable oils from dry seeds of *C. tinctorius, V. paradoxa, S. indicum and R. communis* to cause significant ($p < 0.05$) mortality and suppression of egg hatching of *D. maculatus* on dry fish. This is in line with earlier report of insecticidal efficacy of castor plant oil for control of termites on the wood of *Mangira indica* and *Pinus longifolia* [31]. Also, a similar study reported the insecticidal superiority of castor oil over soya bean in an experiment which recorded rates of 80.83 and 86.66% mortalities of *Callosobruchus maculatus* and *C. phaseoli* in stored chick pea, with both oils inhibiting population growth of the two species [32]. Furthermore, this study confirms the findings of Hidayat and co-workers, who observed that safflower oil treatment at 2.5 and 5.0 m^{-1} significantly reduced the number of queens and fruit fly, *Bactrocera tryoni* punctures on treated fruits and also reduced the number of fly landings and eggs laid [33].

The physiological mechanism surrounding exposure of insects to oil and their subsequent death is still a subject of disagreement among researchers. While some reported that oils had no effect on mortality or longevity of adult bruchids [34], others maintained that insect death caused by oil is due to interference in normal respiration resulting in suffocation [35]. However, it is also suggested that oils could act as anti-feed ants and subsequently discourage feeding and penetration [36]. In this study, although all the plant oils demonstrated insecticidal abilities, *C. tinctorius* showed more potency than others. At its highest dosage, it caused 83.35 % and 100.00 % mortalities 1 and 7 DAT, respectively, while for the same time-point, the least effective treatment, *R. communis* influenced 25.00 and 76.65% insect deaths. The high potency demonstrated by *C. tinctorius* is not surprising considering the unique features it possess, having a wide spectrum of latent medicinal and insecticidal properties [37, 38].

It was also observed from the results that the toxic action of plant oils increased with ascending exposure period and resulted in subsequent increases in the mortality rates at 3, 5 and 7 DAT. The reason for this may not be unconnected with the fact that the exposure time is crucial for the effectiveness of the oils, because insects' movement increases the contact of the cuticle with treatments [39]. *C. tinctorius* and *V. paradoxa* both induced complete larval mortality from 5 DAT at all the dosages by the former, but at only the highest dosage by the latter. This result is similar to previous findings that plant oil significantly reduced the survival of adult and larval *D. maculatus* and *D. rufipes* after 7 days of exposure [40]. Also, the percentage insect mortality in this work was found to increase with higher dosage of oil which indicates direct relationship between the dose and percentage mortality. This observation confirms the findings of Stoll, who reported that a relatively larger amount of oil per unit weight of stored product is required to achieve insect control in fish because of the absorbent nature of fish muscles [41]. Similarly, it was also observed from the study that the presence of plant oil suppressed insect egg hatching. This result corroborates similar findings which showed that pulverized plant materials from *Piper guineese* inhibited egg hatchability and adult emergence of *D. maculatus* in smoked fish, *C. gariepinus* [21, 42]. A study in Chile showed that 2% concentration (w/w) powdered leaves of *C. ambrosoides* and boldo, *Peumus boldus* Mol. (Monimiaceae) exhibited 90.1% and 98.8% mortality, respectively of *S. zeamais* after 24h exposure, and suppressed progeny production by 13% [43].

It has been stated that the insecticidal property of any plant product would depend on the bioactive constituents of the plant materials [20, 44]. The presence of secondary metabolites like alkaloids, saponins, tannins, phenols, terpenes, sterols oils, resins, lignins, etc. have been reported to exhibit physiological activities in man, animals and microorganisms [45, 46, 22, 26]; and there are indications that alkaloids inhibit oxidative phosphorylation, block mitochondria enzymes and impair fish's oxygen consumption [47]. Similarly, Obomanu and co-workers observed in that the restlessness and mortalities of test fish might be due to the effect of flavonoids, alkaloids and saponins present in the extraction [48]. Over the years, considerable attention has been directed at exploring the potentials of plant substances as alternatives to synthetic insecticides [49]. The findings from this study if properly exploited by the local fish farmers and traders would no doubt assist in protecting harvested fish against beetle infestation, and can further contribute substantially to reducing post-harvest losses as well as help fight food insecurity.

Conclusion

The use of *S. indicum*, *C. tinctorius*, *V. paradoxa* and *R. communis* to protect dried fish is feasible and should be advocated for the control of *D. maculatus* infestation among resource fisher farmers and traders during processing, storage, transportation and marketing of smoked dried fish. These botanicals are without any known health risks, are very easily available to people in rural areas, used among the rural folks for their ethno-medical importance; and therefore, a promising technology for small scale preservation of fish at the village level by subsistence farmers. Further investigation needs to be carried out to examine the mode of action of the oil toxicity effects as well as the isolation of the chemical constituents. Success of this could result in the likely formulation of better herbal insecticide.

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