Evaluation of efficiency of neem seed oil against *Dermestes maculatus* and *Necrobia Rufipes* in stored smoked fish; *Clarias gariepinus*

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Abstract: *Laboratory experiment was conducted at Mohamet Lawan College of Agriculture, Maiduguri, Nigeria to evaluate the efficacy of neem seed oil in protecting dried Clarias fish; Clarias gariepinus against infestation by Dermestes maculatus and Necrobia rufipes. The experiment was laid out in a complete randomized design (CRD) with five treatments and untreated control replicated four times. In each block the dried fish C. gariepinus was assigned to the main plots while the botanical; neem seed oil (NSO) was assigned to the sub-plots. Neem seed oil was applied at various concentrations of 0ml (untreated control), 0.5 ml, 0. 7ml, 1.0 ml, 1.2 ml and 1.5 ml. The mortality of adult D. maculatus and adult N. rufipes at 7, 14 and 21 days after treatment were recorded and compared with the untreated control. All concentrations of neem seed oil used recorded higher mortality than the untreated control. However, there was significant difference between the mortality of adult D. maculatus and N. rufipes among the NSO at different concentrations and the control. Higher mortality rates of both study pests were recorded after applying higher doses of NSO at 21days post treatment. NSO concentration at 1.5ml caused significantly the highest mortality of adult D. maculatus and N. rufipes respectively and it is therefore recommended as a protectant on stored, dried fish; C. gariepinus.*

Keywords: *Dermestes maculatus, Necrobia rufipes, Claria gariepinus*, NSO.

Introduction

Fish protein is one of the best and cheapest sources of animal protein (Banie *et al*., 2003; Adewolu and Adeota, 2010). Cured fish with low moisture content provides food for beetles, particularly the larvae and to a lesser extent, the adults of *Dermestes maculatus* and *Necrobia rufipes.* The damage caused by insect infestation is an important cause of economic and physical loss of dried fish in tropical countries (Amusan and Okerie, 2002). Losses by *D. maculatus* and *N. rufipes* occur in two ways: by the actual feeding activity of larvae and adults and by cross-infestation resulting in lowering of the value of other commodities in the store (Anonymous, 2005). About 40% flesh of the dried fish is lost annually during storage (Aderohu and Akpabio, 2009; Adedire and Hajide, 2000). A considerable amount of dried fish is lost in Nigeria due to infestation by hide and copra beetles in storage conditions (Aderolu and Akpabio, 2009). *D. maculatus* and *N. rufipes* are cosmopolitan insect pests causing considerable damage to stored commodities such as copra (dried coconut), cheese, dried fish, ham and other products rich in protein contents (Akunne, 2006). Damage by the feeding of the larvae is a serious problem in the storage of dry fishes for off-season consumption and export purpose (Al-jufiah and Opera, 2006). Their presence on high value commodities such as dried fish, copra, ham or processed meat, etc. can lead to produce reduction and serious losses (Azam *et al.*, 2004; Amusan and Okerie, 2002). Insect infestation with dry fishes 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 (Ayuba and Omeji, 2006). The need to protect smoked fish from insect pests infestation is suggested because dry fish plays a prominent role in the diets of human being. Besides, dry fish commodities are the cheapest and most accessible sources of animal proteins (Akinwumi *et al.*, 2007). Although many synthetic chemicals are effective against the pests of many stored products, the general use of such chemicals to protect stored fish has been hampered due to health hazards, higher costs and development of resistance by dermestid larvae (Amusan and Okorie, 2002; Odeyemi *et al.*, 2000). Based on these problems, botanical insecticides are the alternative to synthetic chemical pesticides since the botanical compounds are biodegradable and less persistent in the environment (Wanyika *et al*., 2009; Michaelraj and Sharma, 2006). Plants are the rich source of insecticidal compounds and the effectiveness of these compounds has been demonstrated against many stored product insects (Sabbour and Shadia E-Abd-El-Aziz, 2007). *Piper guineense* spice powder has been reported to be effective in preventing oviposition in *Callosobruchus maculatus* and *D. maculatus* (Fasakin and Aberejo, 2002), and reducing the longevity of the insect. Similarly, (Rajashekar *et al*., 2010) noted that both the powder and extract of *P. guineense* and *D. tripetela* inhibited adult emergence of *C. maculatus* and *Sitophilus zeamais* completely. *Azadirachta indica*, is known to possess potential insecticidal compounds. Various neem products, oils, cakes, extracts, powder e.t.c. prepared from leaves and seeds are used as protectants against pests in storage. Since there is a pressing need to preserve fish using safer means better than the toxic and unsafe chemical means, this research focusses mainly on the efficacy of NSO on the adult *D. maculatus and adult N. rufipes* infesting *dried fish; C. gariepinus*.

Materials and Methods

Experimental location

The experiment was carried out at Entomology Laboratory of Mohamet Lawan College of Agriculture Maiduguri, Nigeria. Located at latitude 11°51N and longitude 13°15E. All experiments were carried out under ambient environmental condition.

Experimental design

The experiment was laid in complete randomized design (CRD) with five treatments and control replicated four times. In each block the dried fish C. gariepinus was assigned to the main plots while the botanical neem seed oil (NSO) at the concentrations of 0.5ml, 0.7ml, 1.0ml, 1.2ml, 1.5 ml and 0.0 ml (untreated control), were assigned to the sub-plots.

Source of materials

The initial source of culture was obtained from infested smoked *C. gariepinus* collected from a dried fish market in Baga fish market, Maiduguri, Nigeria. Samples of African mud-fish *C. gariepinus* were obtained from tashan baga; a reputable dried fish market in Maiduguri metropolis while The neem seed oil was obtained from previously processed neem seed kernel from the faculty of Agriculture, University of Maiduguri. Borno State, Nigeria.

Methodology

The source of culture (smoked fish infested by *D. maculatus* and *N. rufipes*) of the study pests obtained were maintained separately in Kilner jars covered with muslin cloth under laboratory conditions and kept at a temperature of 34±2°C and a relative humidity of 70±5%. All bioassay jars were disinfected before commencement of the experiments. New generations were prepared by removing adults of the insect species from a stock culture, placing them on fresh uninfected fish, there after the parent adults were removed after three (3) weeks of oviposition period. Samples of African mud-fish *C. gariepinus* were obtained from a reputable dried fish market in Maiduguri metropolis. Smoked fish with broken or damaged parts were not considered in the experiment. Sound, fresh smoked fish were then sterilized by sunning for one week to kill insects before commencement of the experiment. The neem seed oil was obtained from previously processed neem seed kernel which is maintained in the laboratory under good management condition before the commencement of the experiment. Insect species were introduced into each respective bottles and covered with muslin cloth to provide movement of air in and out from the treatment bottles. The Neem seed oil were applied at concentrations of 0.0 ml (untreated control), 0.5, 0.7, 1.0, 1.2 and 1.5 ml, was thoroughly sprayed onto the body of 80g of dry smoked fish and placed into a respective bottles measuring 500ml capacity. All treatments were arranged in laboratory shelf under ambient condition. Mortality counts of *D. maculatus* and *N. rufipes* adult were done at 7, 14 and 21 days post treatment and data were recorded. Dead weevils were removed and discarded after every count.

Data analysis

Data obtained on the mortality counts of the weevils caused by the neem seed oil were subjected to analysis of variance (ANOVA) using Tukey-kramer's HSD Test at $p>0.05$ significant levels of probability.

Results and discussion

Table 1 shows that at 7 days after treatment application, the highest dose of NSO at 1.5ml caused significantly higher mean mortality of adult *N. rufipes* and *D. maculatus* (71.6^{a} and $73.3^{a)}$ when compared with treatment with NSO

at the lowest concentration of 0.5ml and untreated control which were 0.0^{b} and 0.7^{b} respectively.

Table 2 shows the effect of different concentrations of NSO on the mortality of *N. rufipes* and *D. maculatus* adults, 14 days after treatment. The results obtained also revealed that adult mortality is dose dependent, increasing with increase in concentration of the NSO. Significantly higher mean mortality of the two adult insects were recorded after treatment with NSO at 1.50ml which were 93.3^{a} and 100^{a} respectively, while significantly lower mean mortality of the adult insects was recorded at the lowest dose of NSO and untreated control which were 1.0^{b} and 0.3^{b} respectively. The result further revealed that *D. maculatus* is more susceptible NSO than *N. rufipes* because treatment by NSO at the rate of 1.2ml caused 100% mortality of *D. maculatus* while at same rate of NSO (1.2 ml), 86% mean mortality was recorded for *N. rufipes*.

Results on the effect of different concentrations of NSO after 21 days of exposure period was presented in table 3. The data obtained showed that increasing the concentration of NSO and duration of storage significantly increased the mortality of the adult weevils. Significantly higher mean mortality was recorded for *N. rufipes* after treatment with NSO at 1.0 ml, 1.2 ml and 1.5 ml which were 93.3^{a}, 96.7^{a} and 100^{a} respectively. Similarly, significantly higher mean mortality of adult *D. maculatus* was recorded after exposure for 21 days and treatment with 1.0 ml, 1.2 ml and 1.5 ml which was 100^{a} % for all the three doses. The results obtained from the present study showed that the efficacy of the NSO against the two adult weevils is dose dependent, in which the mortality of both study insects increase with increase in concentration of the botanical, NSO. Treatment by NSO caused significantly higher mortality of *D. maculatus* and *N. rufipes* (adult) than the control. However, even at the lowest concentration and at 21 days post treatment was capable of evoking more than 60% mortality in the adult of both species.

The results obtained in this study is in agreement with the work of (Okorie *et al*., 1990, Egwunyenga *et al*., 1998; Okonkwo and Okoye, 2001) who reported that 93% of *D. maculatus* larvae were killed and total mortality of all adults was recorded when treatment was done with 2g of neem seed powder per 25g Tilapia species. Similarly, Fasakin and Aberejo, (2002) reported that pulverized plant materials from *P. guineense* inhibited egg hatchability and adult emergence of *D. maculatus* Degeer in smoked catfish (*Clarias gariepinus*) during storage. Baba *et. al.*, (2014) also reported that after the application of neem kernel extract to control *D. maculatus* the emergence of F1 generation (Adult) from the late instar larvae was highly suppressed by the effect of NKO and NKP at 1ml and 5g respectively per 35g dried fish than NLP (5g/35g dried fish) after 8weeks PTA. Mufutau (2012) reported that after the application of neem seed oil to protect dried fish against infestation by *D. maculatus*, the fish were protected for 6 months against *D. maculatus* and the efficacy of the neem seed oil as a treatments was found to be dosage dependent, the LD50 was 0.125 while 0.275ml significantly halted hatchability and development of the pest. He recorded 100% adult mortality at high doses within the first 10 days.

This work supports other researches on the use of plant materials as bioinsecticides in protecting stored products. Essential Plant oils produced by different plant genera have been reported to be biologically active and are endowed with insecticidal, antimicrobial and bio regulatory properties (Ahmed *et al*., 2009; Kumar *et al*., 2007; 2008; Swella and Mushobozy, 2007). The advantage of insecticides of plants origin is that they are easy to apply (Parugrug and Roxas,

2008). Plant materials have been used successfully in suppressing the population of storage pests (Rajapakse, 2006; Parurug and Roxas, 2008; Asawalam and Emosairue, 2006; Akunne *et al*., 2014). Similarly, neem seed oil and powder has been reported to be effective in preventing oviposition on *C. maculatus* and *D. maculatus* (Rupp, 2006). Arong *et al*., (2011) also reported that neem seed oil has shown to possess phytochemicals that confer on it significant insect repellent and insecticidal value (Watt, 2010). The results obtained from this study imply that for better results, higher concentrations of neem seed oil should be used against adult of *D. maculatus* and *N. rufipes*. Time of exposure of *D. maculatus* and *N. rufipes* to plant oils of neem is a factor to be considered in the control of *D. maculatus* and *N. rufipes*. The highest concentration (1.5ml) used caused more adult mortality. Neem seed oil are safer as control measures and should be used in preserving dry fish. However, the dosage of 1.5ml oil concentration per 80g of dried fish is recommended in the control of adult of both *D. maculatus* and *N. rufipes.* Neem seed oil should be renewed in wider scope for effective control of stored product insect pests. Protein supply is lower than its demand, therefore to boost animal protein (especially fish) proper control measures should be taken against insect pests' infestation at the larval and adult stage.

Table 1: Mean (%± SE) of adult mortality of *Necrobia rufipes* and *Dermestes maculatus* after 7 days exposure to NSO to protect *C. gariepinus*

Treatment (ml)	***N. rufipes***	***D. maculatus***
0.0	0.0 ± 0.0^{b}	0.7 ± 0.3^{b}
0.5	35.7 ± 6.7^{ab}	46.0 ± 11.5^{a}
0.7	46.7 ± 6.7^{a}	53.3 ± 11.5^{a}
1.0	53.3 ± 13.3^{a}	60.6 ± 6.11^{a}
1.2	60.0 ± 11.5^{a}	66.7 ± 6.7^{a}
1.5	71.6 ± 6.7^{a}	73.3 ± 6.7^{a}

Means followed by the same letter are not significantly different from each other at p>0.05

Table 2: Mean (%± SE) of adult mortality of *Necrobia rufipes* and *Dermestes maculates* after 14 days exposure to NSO to protect *C. gariepinus*

Treatment	***N. rufipes***	***D. maculatus***
0.0	1.0 ± 0.6^{b}	0.3 ± 0.3^{b}
0.5	46.7 ± 0.6^{b}	60.0 ± 11.5^{ab}

0.7	63.3±13.7^{ab}	66.7±6.7^{ab}
1.0	73.3±6.7^{ab}	93.3±6.7^{a}
1.2	86.3±3.7^{a}	100.0±0.0^{a}
1.5	93.3±6.7^{a}	100.0±0.0^{a}

Means followed by same letter are significantly different from each other p>0.05

Table 3: Mean (%± SE) of adult mortality of *N. rufipes* and *D. maculatus* after 21 days exposure to NSO to protect to protect *C. gariepinus*.

Treatment	***N. rufipes***	***D. maculatus***
0.0	2.0±0.0^{c}	1.0±0.6^{b}
0.5	73.3±17.6^{b}	86.7±6.79^{a}
0.7	86.7±13.3^{b}	96.7±6.79^{a}
1.0	93.3±6.7^{a}	100.0±0.0^{a}
1.2	96.7±6.7^{a}	100.0±0.0^{a}
1.5	100.0±0.0^{a}	100.0±0.0^{a}

Mean followed by same letter are not significantly different from each other at P>0.05

Conclusion

In view of the high mortality rates caused by NSO on both the adults of *D. maculatus* and *N. rufipes*, it can be concluded from this work that NSO is as effective as synthetic insecticides in suppressing these beetle's population and providing maximum dry fish protection. It is therefore recommended for fish farmers and dry fish sellers to adopt and practice the use of neem seed oil in protecting stored dried Clarias and other dry fishes against infestation by the beetles; *D. maculatus* and *N. rufipes* since NSO has shown to be effective, cheap and safe for human consumption. Moreover, the use of synthetic insecticides has several disadvantages such as high cost of procurement, development of resistance by pests, introduction of toxic residues into the food of man and other mammals.

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