FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 4 No. 2, June, 2020, pp 41 - 45
DOI: https://doi.org/10.33003/fjs-2020-0402-89

PHYTOCHEMICAL SCREENING AND LARVICIDAL EFFECT EVALUATION OF *AZADIRACHTA INDICA* (L) LEAF EXTRACT ON *DERMESTES MACULATUS* (DE GEER, 1774) INFESTATION OF SMOKED *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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ABSTRACT
Dermestes maculatus is a major pest of stored fish in Nigeria, fish is preserved with highly persistent synthetic chemicals. There have been an increasing effort at developing plant-based toxicants that are environmentally friendly. Therefore, this study was conducted to screen the leaf extract of *Azadirachta indica* for phytochemical constituents. The efficacy of the methanolic leaf extract of the plant against *D. maculatus* was also evaluated. The leaves of *A. indica* were obtained, processed and taken to laboratory for methanolic extraction. Phytochemical screening was carried out to identify saponins, flavonoids, alkaloids, tannins, steroids, cardiac glycosides, glycosides, triterpenes and carbohydrates using standard procedure. Both clean and infested samples of smoke-dried *Clarias gariepinus* were purchased from Sabon Gari Market, Zaria. The infested samples were used as initial source of *D. maculatus* larvae. Range finding test was carried out prior to the bioassay. During the bioassay, clean un-infested fish samples were weighed and introduced into kilner jars and respective dosages of 0.20g, 0.40g, 0.60g and 0.80g of extract were added. Another set of fish samples without the extract were maintained as control. The experiment was arranged in Completely Randomized Design with three replicates. Fifteen larvae of *D. maculatus* were introduced into both treated and control containers and covered. Mortality was recorded after 24, 48, 72 and 96 hours. Data obtained revealed an increase in mortality with increasing dose and time. Mortality ranged from 1.67 to 11.00. Therefore, leaf extract of *A. indica* should be incorporated in the development of larvicide against *D. maculatus*.

Keywords: Phytochemical, larvicidal, evaluation, infestation

INTRODUCTION

Fish is one of the major animal protein sources acceptable to all cultures and it is being used increasingly because of its availability and nutritional significance, tropical areas use it as a means to correct protein deficiency in human diets (Ogunduyile, 2015). Consumption of fish provides important nutrients to a large number of people worldwide and thus makes a very significant contribution to nutrition (Fasakin and Aberejo, 2002; Azam *et al*., 2004). Nutritional status will face detrimental effect with decline in fish availability especially in places where fish contributes significantly to the protein intake of the people (Omojowo *et al.,* 2009). According to Olaoye and Oloruntoba (2011), Nigerians consume a lot of fish and offer the largest market for fish and fisheries products in Africa. The annual fish demand in Nigeria is put at about 1.2 million metric tons, and the total domestic fish production is only 511, 700 metric tons meeting only about 50% of the fish demand. Since aquatic resources are finite, although renewable, every effort should be geared towards increased fish production through improved resource management, resource conservation and intensive aquaculture practices. This should be matched with post-harvest fish handling, preservation and processing to prevent spoilage and subsequent loss (Akinneye *et al.,* 2007). Fish is easily perishable in tropical climates and needs to be preserved quickly after capture (Omojowo *et al.*, 2009).

Drying is one of the most popular means of preservation and dried fish is highly favoured in many traditional Nigerian dishes in the third-word countries. To ensure sustained fish availability, it is imperative to process and preserve fish caught during abundance. Most of the dried fish available to fish consumers in the tropics is smoke-dried, salted, fermented and sun dried to extend the shelf-life of the product (Adeyeye and Oyewale, 2016). Dried fish is often a good alternative to fresh fish, and it is readily available in many inland communities because of improved transportation and preservation methods (Adeyeye, 2016). However, preservation, storage and distribution problems of fish are not completely solved by drying, contamination by any foreign material, insects or microorganisms can lower fish quality. Dried fish stored in damp warehouses absorb moisture so rapidly that they become susceptible to infestation by beetles, fungi and mites. Besides, in most cases pest infestation arise due to improper drying of fish (Mohammed and Yusuf, 2001). Insects invade dried fish during storage and feed on it extensively causing up to 50% loss in weight (FAOUN, 2012). Dried fish can suffer considerable loss of weight due to damage caused by insects and mites (Eke *et al.,* 2008).These losses in quantity and quality often lead to lowering or reduction of commercial value.

The extent and value of quantitative losses caused to dried fish by *Dermestes* sp. have been assessed by various investigators and estimates range from negligible to 50% weight loss, depending on length of storage, salt content, moisture content, climatic conditions and general hygiene during processing and storage (Fasunwon *et al.,* 2011). According to Fasunwon *et al*. (2011), *D. maculatus* can cause a significant decrease on

nutritional composition of stored commodities especially those containing animal proteins. The physical, economical, and nutritional loss caused by *Dermestes maculatus* infestation are enormous and can cause fish price to increase beyond the purchasing power of the poor (Odeyemi *et al.,* 2000). Interest is however growing fast in the possible role of plants as traditional preservatives of stored products and as alternatives to the use of highly persistent synthetic chemicals with their attendant dangers and high cost (Nwaehujor and Olatunji, 2011). In order to eliminate much of the shortcomings associated with the use of synthetic insecticides and provide an effective storage techniques, in recent years, there have been increasing and concerted effort at developing plant base toxicants that are environmentally friendly (Adesina *et al.,* 2016).

The Neem (*Azadirachta indica*) plant called "Dogonyaro" in Northern Nigeria belongs to the Family meliaceae. As natural insecticide it contains tetranitroterpenoid compounds known as meliatoxins that are highly toxic to insects and mammals (Ascher *et al*., 2006). Therefore, this study aimed at evaluating the phytochemical constituents and larvicidal activity of *A. indica* leaf extract on smoked *Clarias gariepinus* infested with *Dermestes maculatus.*

MATERIALS AND METHODS

Study Area

The experiment was conducted in the 400 level Laboratory, Department of Biology, Ahmadu Bello University Zaria, Nigeria (Latitude $11^0 09^{'} 30.99''$N and longitude $7^0 39' 20.68''$E , altitude of 550-700m above sea level).

Sources of Plant Materials

The leaves of *Azadirachta indica* used for the experiment were obtained from Botanical Garden Ahmadu Bello University Zaria, Nigeria.

Processing of Plant Materials

Leaves of *A. indica* were shade- dried for three weeks after which they were milled into powdery form. The milled sample was sieved and put in a plastic container and the extraction was done using methanol as the solvent in Hydrobiology Laboratory, Department of Biology, Ahmadu Bello University,Zaria.

Phytochemical Screening

Phytochemical screening was carried out on the leaf of *Azadirachta indica* at the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, to determine the active secondary metabolites.

Test for Carbohydrates (Molisch Test)

To a small portion of the extract in a test tube, few drops of Molisch reagent was added and concentrated sulphuric acid was added down the side of the test tube to form a lower layer, a reddish coloured ring at the interphase indicates the presence of carbohydrates (Evans, 1996).

Test for Glycosides (Ferric chloride Test)

To a portion of the extract, 5ml of dilute sulphuric acid was added and boiled on water bath for 10-15mins. This was then cooled and neutralized with 20% KOH. About 3ml of Ferric chloride solution was added, a green to blue colour was produced because of the release of phenolic glycones due to the hydrolysis (Evans, 1996).

Test for Unsaturated Steroids and Triterpene (Liebermam Bucchard Test)

To a portion of the extract, equal volume of acetic acid and anhydride were added and mixed gently; 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple colour at the lower layer indicate the presence of triterpene (Evans, 1996).

Test for Cardiac Glycoside (Keller-kiliani Test)

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observation was done carefully at the interphase for purple-brown ring. This indicates the presence of deoxy sugars and a pale green colour in the upper acid layer indicates the presence of cardiac glycosides (Evans, 1996).

Test for Saponin (Frothing Test)

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates the presence of saponins (Evans, 1996).

Test for Tannins (Ferric chloride Test)

To a portion of the extract, 3-5 drops of ferric chloride solution was added. A greenish-black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish-blue precipitate (Trease and Evans, 2002).

Test for Flavonoids (Sodium hydroxide Test)

Few drops of 10% sodium hydroxide were added to the extract. Yellow coloration indicates presence of flavonoid (Trease and Evans, 2002).

Test for Alkaloids (Dragendoff's Test)

To a portion of the extract, few drops of Dragendoff's reagent were added. A reddish brown precipitate indicates presence of alkaloids (Evans, 1996).

Collections of Smoke-dried Fish and insect maintenance

Smoke-dried *C. gariepinus* samples were purchased from Sabon Gari Market ($11^0 13'$N and $07^0 52'$E) Zaria. Naturally infested smoke-dried *C. gariepinus* was used as the initial source of *D. maculatus* larvae for this study and was maintained in a Kilner jar under laboratory condition.

Pilot Study

Range finding test was conducted to determine the concentration of leaf extract to be used for the definitive tests (bioassay). This was done by applying four nominal concentrations (from 0.20-0.80g/g of fish) of the extract to heat sterilized fish then placing them in kilner jars. Fifteen (15) larvae of *D. maculatus* were then introduced into each kilner jar container. The containers were observed at 24hours, 48hours, 72hours and 96hours to establish total and zero mortality/minimal mortality range. Five concentrations of 0.20g, 0.40g, 0.60g and 0.80g were chosen for the bioassay test based on the high-low mortality range observed (Ogunduyile, 2015). Control was chosen as 0.00g.

Bioassay

Clean un-infested fish samples were weighed into kilner jars. To each of the jar, the respective experimental dosages of 0.20g, 0.40g, 0.60g and 0.80g of extract were added. Another set of fish samples without extract were maintained as control. The initial weights of the experimental fish were recorded. Fifteen larvae of *D. maculatus* were introduced into each treated and control containers containing fish sample and covered. Mortality was observed and recorded after 24, 48, 72 and 96

hours of exposure. Each treatment was replicated three times and arranged in Completely Randomized Design (CRD). Weight loss was determined by re-weighing the fish sample at the end of the experiment (Ogunduyile, 2015; Akpotu *et al*., 2017).

Data Analyses

Data obtained on mortality of *Dermestes maculatus* were subjected to One-way Analysis of Variance (ANOVA) at $p \leq 0.05$ to determine significant difference between treatments. Where Significant, Duncan's Multiple Range Test (DMRT) was used to separate the means.

RESULTS AND DISCUSSION

The use of botanicals for the control of insect pests of stored products is an ancient practice. Several studies have revealed the ovicidal effect, larvicidal effect, effects on adult emergence and adult mortality of plant extracts on insect pests of stored fish and fish products (Shuaibu and Yahya, 2018). Phytochemical analysis conducted on the methanol leaf extract of *A. indica* revealed the presence of saponins, flavonoids, alkaloids, tannins, steroids, cardiac glycosides, glycosides, triterpenes, and carbohydrates (Table 1).

Table 1: Phytochemical constituents of *A. indica* leaf extract

CONSTITUENT	RESULT
Saponnins	+
Flavonoids	+
Alkaloids	+
Tannins	+
Steroids	+
Cardiac glycosides	+
Glycosides	+
Triterpenes	+
Carbohydrates	+

Key: += present

Table 2: Effect of *A. indica* leaf extract on the mortality of *D. maculatus* larvae

Concentration (g/g)	Time (hours)			
	24hr	48hr	72hr	96hr
0.00 (Control)	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
0.20	1.67 ± 0.33^{b}	3.00 ± 0.58^{d}	3.33 ± 0.33^{d}	5.67 ± 0.88^{d}
0.40	2.00 ± 0.00^{b}	4.67 ± 0.33^{c}	6.67 ± 0.33^{c}	7.00 ± 0.58^{c}
0.60	5.00 ± 1.16^{a}	6.33 ± 1.33^{b}	8.00 ± 0.58^{b}	9.67 ± 0.33^{b}
0.80	5.53 ± 0.33^{a}	7.33 ± 0.33^{a}	9.33 ± 0.33^{a}	11.00 ± 0.00^{a}
p-value	0.000	0.000	0.000	0.000

Note: Means with the same superscript along columns are not significantly different ($P \leq 0.05$).

The presence of these compounds may be responsible for larvicidal activities of plant extracts. The larvicidal activity of *A. indica* may be attributed to the presence of bioactive constituents present in the plant. These bioactive agents could possess among other pharmaceutical properties, a depolarizing neuromuscular blocking action which could result to the death of insect (Shaibu and Yahya, 2018). Similar phytochemical constituents were reported by Biu *et al*. (2009) who conducted phytochemical screening on *A. indica* in Maiduguri, Nigeria and opined that the extracts of the plant have strong biological activities against insect pests.

Mortalities of *D. maculatus* were observed in this study to be concentration and time-dependent, there was increase in the mortality with increasing concentration. The mortality of *D. maculatus* larvae on exposure to the various concentrations of the leaf extract also increased in accordance with 24hr, 48hr, 72hr and 96hr exposure (Table 2).

The result obtained from this study revealed that *A. indica* is effective against larval stage *of D. maculatus*. The result is in agreement with the reports by Adebote *et al*. (2006), Abdullahi *et al*. (2012) and Ahmed *et al*. (2013) who confirmed the efficacy of botanicals on suppression of *D. maculatus*

infestation on smoke-dried fish. The study clearly indicated that the higher dosage level of treatment was the most effective in the application rates compared to the untreated control. It was clear that the extract of *A. indica* exhibited toxic effect against larvae of *D. maculatus* causing significant mortality.

CONCLUSIONS

Azadirachta indica leaf extract is a promising bio pesticide against the larvae of *D. maculatus* causing severe damage on smoked fish. It can serve as an alternative to synthetic insecticides that were also found to be effective in controlling insect infestation against smoked fish, the synthetic insecticides were reported to have hazardous effects to humans and domestic animals. On the basis of the result, it can be concluded that the mortality as observed ranged from 1.67to 11.00 and was more effective at 0.80g/g of fish after 96hr exposure (11.00). The larvicidal effect of the extract is dose and time dependent.

RECOMMENDATION

Leaf extract of *Azadirachta indica* should be incorporated in the development of larvicide against *D. maculatus* at 0.8 g/g of fish as an alternative to synthetic insecticides. The infested fish should be exposed to the larvicide for a period of at least 96 hours to ensure high mortality of the larvae.

REFERENCES

Abdullahi, N., Ubayi, S. M. and Babura, S. R. (2012). Determination of the Effect of *Zingiber officinale* and *Allium sativum* Powder on the Mortality of *Dermestid maculatus* Larvae on Treated Dried *Claris gariepinus* Fish. *International Journal of Applied Research and Technology*, **1**(3), 129-133.

Adebote, D. A., Abolude, D. S., Oniye, S. J., Olododo, S. S. and Hassan, M. M. (2006). Larvicidal and Repellent Actions of *Detarium microcarpum* Seed Oil against the Larvae of *Dermestes lardarius* (Coleoptera: Dermestidae) in Dried *Clarias gariepinus*. *Journal of Entomology*, **3**(3):248-253.

Adesina, J. M., Jose, A. R. and Rajashekar, Y. (2016). Bioefficacy of *Clerendrum capitatum* (Willd) Schumachet, Thorn (Lamiales: Verbenaceae) against *Dermestes maculatus* Degeer, 1774 (Coleoptera: Dermestidae) Larvae Infestation on Smoked Catfish *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Claridae). *Brazilian Journal of Biological Sciences*, **3**(5): 37-44.

Adeyeye, S. A. O. (2016). Traditional Fish Processing in Nigeria. A Critical Review. *Nutrition and Food Science*, **46**(3): 321-335.

Adeyeye, S. A. O. and Oyewale, O. B. (2016). An Overview of Traditional Fish Farming in Africa. *Journal of Culinary Science and Technology*, **14**(3):198-2015. doi: 10.1080 /15428052 .2015.1102785.

Ahmed, H., Ahmed, K. N., Khanom and Noor, P. (2013). Damage Potential and Control Measures of *Necrobia rufipes* (De Geer) (Coleoptera: Cleridae) on Dry Fish with Plant Materials. *Bangladesh Journal of Science and Industrial Research*, **48**(1): 19-24.

Akinneye, J. O., Amoo, I. A. and Arannilewa, S. T. (2007). Effect of Drying Methods on the Nutritional Composition of Three Species of Fish (*Bonga* sp., *Sardinella* sp. and *Heterotis niloticus*). *Journal of Fisheries International*, **2**(1): 99-103.

Akpotu, O. J., Oniye, S. J., Abolude, D. and Yusuf, A. (2017). Comparative Study of the Toxicity of Oils from Seeds of *Citrullus colocynthis* and *Citrullus vulgaris* on larvae of *Dermestes maculatus*. *Journal of Aquaculture Engineering and Fisheries Research*, **3**(1): 6-12. doi: 10.3153/JAEFR1702.

Ascher, K. R. S., Kllein, M. and Meisner, J. (2006). Azadirachtin, a Neem Formulation, Acts on Nymphs of the Western Flower Tips, *Phytoparasitica*, **20**: 305-306.

Azam, K., Ali, M. Y., Asaduzzaman, M., Basher, M. Z. and Hossain, M. M. (2004). Biochemical Assessment of Selected Fresh Fish. *Journal of Biological Science*, **4**: 9-10.

Biu, A. A., Yusufu, S. D. and Rabo, J. S. (2009). Phytochemical screening of *Azadirachta indica* (Neem) (Meliaceae) in Maiduguri, Nigeria. *Bioscience Research Communications*, **21**(6): 281-283.

Eke, F. N., Ekechukwu, N. E. and Onah, I. (2008). Arthropod Pests of Dried Fish and Fish By-product in a Tropical Urban Community Market. *Animal Research International*, **5**(3): 900-903.

Evans, J. D. (1996). *Straightforward Statistics for the Behavioral Sciences*. Brooks/Cole Publishing; Pacific Grove, Calif.: 1996.

FAOUN (2012). Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations. Pp.3-24.

Fasakin, E. A. and Aberejo, B. A. (2002). Effect of Smoked Pulverized Plant Material on the Developmental Stages of Fish Beetle, *Dermestes maculatus* Degeer in Smoked Catfish (*Clarias gariepinus*) During Storage. *Bioscience Technology*, **85**: 173-177.

Fasunwon, B. T., Banjo, A. D. and Jemine, T. A. (2011). Effects of *Dermestes maculatus* on the Nutritional Qualities of Two Edible Insects (*Oryctes boas* and *Rhynchophorus phoenicis*). *African Journal of Food, Agriculture, Nutrition and Development*, **7**(7). 5601-5613.

Mohammed, A. A. K. and Yusuf, S. A. K. (2001). Insect Infestation and Preventive Measures in Dry Fish of Chittagong Bangladesh. *Online Journal of Biological Sciences*, **1**(10): 963-965.

Nwaehujor, I. U. and Olatunji, G. A. (2011). Orange Peel Powder and its Extracts as Preservative against *Callosobruchus maculatus* (coleoptera:bruchidae) Attack in Cowpea. *Journal of Science and Technology*, **31**(2): 21-26.

Odeyemi O. O., Owode, R. A. and Akinkurolere, A. (2000). Toxicity and Population Suppression Effects of *Parkia clappertoniana* on Dried Fish Pests (*Dermestes maculatus* and *Necrobia rufipes*). *Global Journal of Pure and Applied Sciences*, **6**(2): 191-195.

Ogunduyile F. D. (2015). An Assessment of the Effects of Some Plant Oils on *Dermestes maculatus* (Degeer) Larvae in Smoke-dried *protopterus annexes* (owen). Ahmadu Bello University Zaria (M.Sc. Fisheries) - Unpublished.

Olaoye, O. J. and Oloruntoba, A. (2011). Determinants of Aquaculture Technologies Adoption among Fish Farmers in Obafemi-Owode Local Government Area of Ogun State, Nigeria. *Journal of Humanities, Social Sciences and Creative Arts*, **5**(1): 37-48.

Omojowo, F. S., Olokor, J. O. and Ihuahi, J. A. (2009). Microbial Qualities of Potassium Sorbate on Treated Smoked Tilapia (*Oreochromis niloticus*). *World Journal of Biological Research*, **2**: 1-4.

Shuaibu, T. and Yahya, M. A. (2018). A review on the Insecticidal Properties of Some Plant Materials Used against *Dermestes maculatus* Infestation on Stored Smoked Fish in Nigeria. *Dutse Journal of Pure and Applied Sciences* (DUJOPAS), **4**(2): 394-402.

Trease, G. E. and Evans, W. C. (2002). *Pharmacognosy*. 15th Edition. London: Saunders Publishers: pp. 42-44.