LARVICIDAL EFFECT OF *PHYLLANTHUS MUELLERIANUS* AND *RICINUS COMMUNIS* LEAF EXTRACTS ON *DERMESTES MACULATUS* LARVAE IN SMOKE-DRIED *CLARIAS GARIEPINUS*

[*1]Bawa, S.B., [2]Adepoju, J.A. and [3]Bashir, A.Y.

[1,2,3]Department of Biology, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria
*Author of correspondence: leadfrontiers@gmail.com, aquablends@gmail.com, +2348184032454

Abstract
Fish protein is poised with having advantage over other kinds of proteins which includes high amino acids profile, respectable amount of minerals and vitamins and high amount of polysaturated fatty acids. Insects like D. maculatus have been credited with the reduction in economic and nutritional value of fish. The effects of Phyllanthus muellerianus and Ricinus communis leaf extracts on larvae of Dermestes maculatus was investigated. Extraction from leaves was done via aqueous extraction using distilled water, extracts were screened for secondary metabolites and were used to treat fish samples. Phytochemicals present in the leaves include carbohydrate, cardiac glycosides, flavonoids, steroids, glycosides, tannins and triterpenes. All concentrations of treatment (0.025, 0.050 and 0.075g/g) caused significant mortality of Dermestes larvae. Mortality of larvae increased with time of exposure and ranged from 1.65 – 66.65%. Both plant extracts combined at 0.075 g/g concentration gave the highest rate of mortality. It was concluded that all leaf extracts had larvistatic effect on D. maculatus larvae in smoke-dried C. gariepinus as there was no significant reduction in the weight of the fish.

Keywords: *Clarias gariepinus, Dermestes maculatus, Phyllanthus muellerianus*, *Ricinus communis*, smoke-dried

Introduction

The presence of very high profile of amino acids, notably lysine, methionine and isoleucine in fish protein makes it superior to other animal proteins (Abolagba *et al.*, 2011). Fish protein contains a respectable amount of minerals such as calcium, iodine, phosphorus, iron, magnesium and fluorine (Yem *et al.*, 2006), and it is a good source of vitamins A, B, D, E and K, riboflavin, thiamine. It is also an important factor in the reduction of blood cholesterol level as it is equally high in polysaturated fatty acids (Sahena *et al.,* 2009).

Dermestes maculatus larvae nest on fish as substrate, feeding and metamorphosing into adult to continue the cycle of infestation, diminishing the biochemical quality of the fish. Amusan and Okorie (2002) reported *D. maculatus* to be responsible for about 93% of infestation of dried fish. Insects like *Dermestes maculatus* and *Necrobia rufipes* attack smoke-dried fish in the course of processing, transportation, marketing and storage (Adesina *et al.*, 2012). Physical, economic, and nutritional loss caused by *Dermestes maculatus* infestation are enormous and have been reported to raise the retail value of fish beyond the purchasing power of the poor (Odeyemi *et al.*, 2000).

The use of synthetic insecticides on stored products is not usually safe because accumulated percentage of synthetic pesticides results in different types of food poisoning and pollution in the environment (Mahmood *et al.*, 2015). Plant pathologists have paid attention to the investigation of substitute natural products for the control of pests to avoid the adverse effect of synthetic pesticides on non-targeted organisms. Mkindi *et al.* (2015) reported the use of plant extracts in the control of pests due to their potentials as natural chemicals and as sources of phytotoxic insecticides, attributable to the presence of some active ingredients in them (Akinwumi, 2011).

This study aims to assess the activity of aqueous leaf extracts of *P. muellerianus* and *R. communis* in the control of larvae of *D. maculatus* of smoke-dried fish. This study will provide information on the impact of the extracts of *Phyllanthus muellerianus* and *Ricinus communis* on larvae of *D. maculatus* and the result if favourable, will enhance the use of eco-friendly and cheaper insect-pest control mechanism.

Materials and Methods

Sources of experimental samples

Phyllanthus muellerianus and *Ricinus communis* leaves were collected from the wild around Zaria, properly identified and authenticated at the Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria. Smoked-dried *Clarias gariepinus* were purchased from Sabon-gari market, Zaria, Kaduna State.

Processing of plant samples

Leaves of *P. muellerianus* and *R. communis* were air dried for three weeks, and pulverized using mortar pestle. The pulverized leaves were sieved and stored in pre-labelled new cellophane bags.

Extraction of plant samples

Extraction of both plant samples were done according to the recommendations of Al-Manhel and Niamah (2015). Briefly, distilled water was used for the aqueous extraction of leaves. About 5 grams of each plant leaves powder were mixed in 50 ml of distilled water. The mixture was put in 250 ml sterile conical flasks, plugged with sterile cotton and kept in Shaking Incubator at 200 rpm for 24 hours. The solution was filtered through muslin cloth. This process was repeated three times until clear aqueous extract of the plant was obtained.

Phytochemical screening of the extracts

Phytochemical tests were carried out on the aqueous extracts of *P. muellerianus* and *R. communis* leaves to identify the secondary metabolites that confer pesticidal activity (Trease and Evans, 1996) at the Institute of Agricultural Research, Ahmadu Bello University, Zaria.

Insect culture, maintenance and exposure to extracts

Infested smoke-dried *C. gariepinus* were maintained in a Kilner jar under laboratory condition. New generation of *D. maculatus* were prepared by removing newly emerged (0-72h old) larvae from a stock culture.

Larvicidal bioassay

The purchased smoke-dried fish samples were heat sterilized in an oven at about 10°C for one hour to treat prior infestation and were allowed to cool to room temperature. The fish were weighed and tagged individually. The fishes were treated with aqueous extracts of *P. muellerianus* and *R. communis* leaves (0.025, 0.050 and 0.075 g/g concentrations) separately and in combination, to test for synergetic effects.

The fish treated with the extracts were introduced into separate containers for the larvicidal bioassay. Twenty larvae of *D. maculatus* were introduced into the containers in triplicates for each treatment. Observations were recorded at 24, 48, 72, and 96 hours of exposure. Treated fish samples with a known pesticide (Bextoxin) was put in a container and twenty larvae were also introduced (positive control) while untreated fish species were put in a separate container and twenty larvae were also introduced (negative control).

Data Analysis

All data were subjected to One-way Analyis of Varience (ANOVA) to determine significant difference between treatments using SPSS version 20. $P<0.05$ was considered as statistically significant.

Results

The results of the phytochemical analysis of the aqueous extracts of *P. muellerianus* and *R. communis* showed that flavonoids, triterpenes, and carbohydrates were present (Table 1). Saponin and alkaloids were not detected in *P. muellerianus* leaf extract.

Table 2 shows the effects of *Phyllanthus muellerianus* leaf extract on the mortality of *Dermestes maculatus* larvae. There was significant reduction in the weight of infested smoked dried fish which confirms the presence and activity of *D. maculatus* larvae. All concentrations caused significant mortality ($P<0.05$). Mortality of larvae increased with time of exposure. The highest concentration (0.075 g/g) gave the highest mortality (43.35%) and highest weight of final smoked dried fish (8.00 g, i.e. only a 5% degradation) among the treatments.

The effect of *R. communis* leaf extract on *D. maculatus* is presented in Table 3. All mortality rates varied significantly ($P<0.05$). The positive control gave the highest mortality rate of 100% at 24 hours of exposure while the 0.025 g/g concentration gave highest final weight of smoked dried fish (10.10 g, i.e. only 6.48% degradation).

The effect of the combination of both plant extracts on *D. maculatus* is presented in Table 4. Both plant extracts combined at 0.075 g/g concentration gave the highest rate of mortality (66.65% at 96 hours of exposure) among the treatments, while 0.05 g/g concentration gave the best final weight of smoke-dried fish, i.e. only 3.48% degradation.

Discussion of Results

Phytochemical screening

The leaf extract of *P. muellerianus* using distilled water as solvent revealed the presence of glycosides, carbohydrates, cardiac glycosides, steroids and triterpenes, tannins and flavonoids. Further, the leaf extract of *R. communis* using distilled water as solvent shows the presence of secondary metabolites like alkaloids, flavonoid, tannins, steroid and triterpenes, saponin, cardiac glycoside, glycoside and carbohydrates. Presence of these metabolites in high quantity have been of grave concern for human health and safety (Ikani *et al*., 2019). They however have some health benefits which include anti-cancer; anti-human immunodeficiency virus (anti-HIV); and anti-microbial infection potentials and prevention of mucosal atrophy (He *et al*., 2018). Therefore, their presence in low concentration can be beneficial to animals.

Larvicidal bioassay

The use of botanicals for the control of insect pests of stored products is an ancient practice and have been documented by some researchers, especially on *D. maculatus* of smoke-dried fishes (Abdullahi *et al*., 2012; Mufutau, 2012; Ahmed *et al*., 2013). The result obtained from this study revealed that *P. muellerianus* and *R. communis* are effective against larval stage of *D. maculatus*. The study clearly indicates that the higher dosage level application of both treatments was most effective in evoking larvicidal effect compared to the untreated control.

The larvicidal activity of *P. muellerianus* and *R. communis* leaf extracts may be attributed to the presence of bioactive constituents present in the plant. Udoh *et al*. (1999) earlier opined that these bioactive agents could possess among other pharmaceutical properties, a depolarizing neuromuscular blocking action which could result to the death of insect. The study indicates that both plant extracts exhibited toxic effect against larvae of *D. maculatus* causing significant mortality. The study revealed combination of *P. muellerianus* and *R. communis* to give a significantly higher mortality rate. The toxicity effect observed in this study can also be attributed to the various chemical ingredients like triterpenes which has numerous therapeutic applications including treatment of cancer (Rodriguez and Ruiz-Gutierrez, 2010) and have been reported to also show antibacterial activities against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (Hao *et al.*, 2015).

Conclusions

It can be concluded that the combination of both leaf extracts at 0.05 g/g concentration is the best option for the control of *D. maculatus* larvae of smoked dried *C. gariepinus* as there was no significant degradation i.e. appreciable reduction in the weight of the fish; implying a larvistatic effect. The result also proved that these plants can serve as alternative to synthetic chemicals, specifically Bextoxin used in the protection of stored smoke-dried fish. The larvicidal effect of both extracts are dose and time dependent.

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Table 1: Phytochemical constituents of aqueous extract of *Phyllanthus muellerianus* and *Ricinus communis* leaves

Constituents	*P. muellerianus*	*R. communis*
Carbohydrate	+	+
Cardiac glycosides	+	+
Flavonoids	+	+
Saponins	-	+
Alkaloids	-	+
Steroids	+	+
Glycosides	+	+
Tannins	+	+
Triterpenes	+	+

Key: + = Present, - =Absent

Table 2: Effects of aqueous extract of *Phyllanthus muellerianus* on *Dermestes maculatus* larvae

Concentration (g/g)	Weight of fish before infestation	Mortality at 24 hours	Mortality at 48 hours	Mortality at 72 hours	Mortality at 96 hours	Weight of fish after infestation
0.025	7.23 ± 0.09^{c}	0.33 ± 0.33^{b}	1.33 ± 0.33^{c}	2.00 ± 0.58^{c}	2.67 ± 0.33^{cd}	6.83 ± 0.09^{d}
0.050	7.27 ± 0.64^{c}	0.33 ± 0.33^{b}	1.67 ± 0.33^{c}	3.00 ± 0.58^{c}	4.67 ± 0.33^{c}	6.70 ± 0.60^{d}
0.075	8.40 ± 0.15^{c}	0.00 ± 0.00^{b}	3.33 ± 0.67^{b}	6.00 ± 0.58^{b}	8.67 ± 1.33^{b}	8.00 ± 0.26^{c}
+ Control	11.70 ± 0.50^{b}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	9.43 ± 0.09^{b}
- Control	13.67 ± 0.37^{a}	0.33 ± 0.33^{b}	1.33 ± 0.33^{c}	1.67 ± 0.33^{c}	1.67 ± 0.33^{d}	12.53 ± 0.48^{a}
P Value	0.000	0.000	0.000	0.000	0.000	0.000

Means are presented. Means with the same superscripts along columns do not vary significantly (P>0.05).

Note: + Control = Positive control (Bextoxin), - Control = Negative control (No Pesticide)

Table 3: Effects of aqueous extract of *Ricinus comunnis* on *Dermestes maculatus* larvae

Concentration (g/g)	Weight of fish before infestation	Mortality at 24 hours	Mortality at 48 hours	Mortality at 72 hours	Mortality at 96 hours	Weight of fish after infestation
0.025	10.80 ± 0.41^{bc}	0.67 ± 0.33^{b}	2.00 ± 0.58^{bc}	3.00 ± 0.58^{b}	3.00 ± 0.58^{d}	10.10 ± 0.52^{b}
0.050	10.13 ± 0.35^{c}	1.00 ± 0.58^{b}	3.00 ± 0.58^{b}	3.33 ± 0.33^{b}	4.33 ± 0.33^{c}	9.60 ± 0.32^{b}
0.075	8.27 ± 0.56^{d}	0.67 ± 0.33^{b}	2.67 ± 0.33^{bc}	4.00 ± 0.58^{b}	6.00 ± 0.58^{b}	7.63 ± 0.68^{c}
+ Control	11.70 ± 0.50^{b}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	9.43 ± 0.09^{b}
- Control	13.67 ± 0.37^{a}	0.33 ± 0.33^{b}	1.33 ± 0.33^{c}	1.67 ± 0.33^{c}	1.67 ± 0.33^{e}	12.53 ± 0.48^{a}
P Value	0.000	0.000	0.000	0.000	0.000	0.000

Means are presented. Means with the same superscripts along columns do not vary significantly (P>0.05).

Note: + Control = Positive control (Bextoxin), - Control = Negative control (No Pesticide)

Table 4: Effects of aqueous extract of *Phyllanthus muellerianus* and *Ricinus communis* on *Dermestes maculatus* larvae

Concentration (g/g)	Weight of fish before infestation	Mortality at 24 hours	Mortality at 48 hours	Mortality at 72 hours	Mortality at 96 hours	Weight of fish after infestation
0.025	7.77 ± 0.75^{c}	0.67 ± 0.33^{bc}	2.67 ± 0.33^{c}	3.00 ± 0.00^{cd}	4.33 ± 0.67^{cd}	7.13 ± 0.85^{d}
0.05	11.50 ± 0.75^{b}	1.00 ± 0.58^{bc}	4.67 ± 0.88^{b}	6.00 ± 1.73^{bc}	7.00 ± 1.73^{c}	11.10 ± 0.66^{ab}
0.075	8.53 ± 0.38^{c}	2.00 ± 0.58^{b}	4.67 ± 0.33^{b}	8.33 ± 1.20^{b}	13.33 ± 2.19^{b}	7.83 ± 0.71^{cd}
+ Control	11.70 ± 0.50^{b}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	9.43 ± 0.09^{bc}
- Control	13.67 ± 0.37^{a}	0.33 ± 0.33^{c}	1.33 ± 0.33^{c}	1.67 ± 0.33^{d}	1.67 ± 0.33^{d}	12.53 ± 0.48^{a}
P Value	0.000	0.000	0.000	0.000	0.000	0.001

Means are presented. Means with the same superscripts along columns do not vary significantly (P>0.05).
Note: + Control = Positive control (Bextoxin), - Control = Negative control (No Pesticide)