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Mosquito-larvicidal efficacy of the extract of Musca domestica maggots against Culex pipiens (Diptera: culidae), an important vector of Filariasis

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Abstract

The intolerably high burdens of mosquito-borne diseases will be reduced sustainably through the development of integral eco-friendly alternative insecticides of natural products origin. The need to broaden the global search for such insecticidal lead-agents, especially , those that will be less vulnerable to resistance, was the reason why this bio-assay study was carried out to test Musca domestica maggots against 4th instar larvae of the mosquito Culex pipiens pipiens. The larvicidal bio-assay followed standard World Health Organisation's protocols for testing the susceptibility of mosquitoes to larvicides. Larvicidal tests were carried out in a series of extract concentrations ranging from 0.25-4.50 mg/ml, in distilled and tap water media. The results showed that maggot extract possesses significant ($P<0.05$) larvicidal activities against the mosquito species, in a way akin to those reported for potent plant extracts. The larvicidal activities of the extract was dose dependent; and extract induced significantly higher larval mortality in tap water bio-assay medium than distilled water, except in the 0.25 mg/ml concentration treatment, where the reverse was the case. While, 100% larval mortality was recorded in extract concentration of 2.50 mg/ml in tap water, it took 4.50 mg/ml to kill all exposed larvae in distilled water bio-assay media. The LC50 values of the extract ranged significantly ($P<0.05$) from 1.57 mg/ml in tap water to 2.26 mg/ml in distilled water. The LC90 equivalents were 2.14 mg/ml and 3.47 mg/ml, respectively. These results suggest that insects may be at-least as promising as the botanicals in our search for eco-friend alternative insecticides.

Key words: Bio-assay media, Insect metabolites, Insecticides, Larval mortality, Lethal Concentration and susceptibility.
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Introduction

Mosquitoes have been adjudged the most important pests in the history of mankind (Larry and Marlin, 2009). Their importance as medical pests continues today, particularly through the vectoring of organisms that cause malaria and other diseases such as *Bancroftian filariasis*, *Japanese encephalitis*, dengue fever, yellow fever etc (Gubler, 1998). These diseases account for millions of human deaths every year (Rahuman *et al.*, 2009; Borah *et al.*, 2010). Filariasis for instance is "considered" endemic in tropical and sub-tropical regions of Asia, Africa, Central, South America and Pacific Island nations, with more than 120 million people infected and one billion people at risk for infection (TCC, 2008). In communities where lymphatic filariasis is endemic, as many as 10 percent of women can be afflicted with swollen limbs, and 50 percent of men can suffer from mutilating genital symptoms (TCC, 2008).

Many approaches have been developed to reduce the burden of mosquito borne diseases. One of such strategies by the World Health Organization is vector control including larviciding interventions (Arivoli *et al.*, 2011); especially as there are no viable vaccine in the horizon for most mosquito borne diseases. For several decades, the four classes recommended by WHO namely, Organophosphates, Organochlorines, Carbonates and Pyrethroids have dominated Universal mosquito-larvicing tools (Yang and Lee, 2002). Those highly effective and hence unsustainable for reasons including , wide-spread development of resistance (Liuh *et. al.*, 2009; Doere and Khadabadi, 2009) and environmental contamination and toxicity to non-target beneficial organisms (Severini *et. al.*, 1993; Lixin *et al.*, 2006; Rawani *et. al.*, 2009). The challenges associated with the continued use of present day insecticides for the control of mosquito –borne diseases have initiated global interests for systematic search for highly efficacious, cost-effective and eco-friendly alternatives (Rawani *et al.*, 2009).The search for alternative insecticide that will combine these attributes have being focused more on natural products, as a result of their inherent rich diversity of bio-active metabolites (Newman and Crag, 2007). Particularly, plant materials have dominated the sources of rich potential insecticidal lead-agents (Sukumar *et. al.*, 1991; Shaalan *et. al.*, 2005), though the expected desired positive results have slow immaterializing as a result of, among others factors, the fear of the vulnerability of plant-based insecticides to resistance by insects generally.

This development, calls for a wider screening of potential natural product sources, especially animal bio-active metabolites, of insecticide lead agents. Since pre-historic times, insects and their products have played prominent role in folklore medicine (Fasoranti, 1997; Conconi and Jose, 1998; Casta-Neto, 2005) and the art of entomotherapy occupies a frontal position in orthodox medicine (Sherman *et. al.*, 2000; Kato and Gopi, 2009). Metabolite extracts from the larvae (i.e, maggots) of housefly (i.e *Musca domestica*), for example have been used effectively in the treatment of Oesteomylitis and inflammation of soft tissues (Sherman *et al.*, 2000). Also, anti-bacterial and immune-sensitivity have been reported for maggot extracts (Bexfield *et al.*, 2004; Lixin *et al.*, 2006; Wang *et al.*, 2012). On the other hand, however, the insecticidal of the extracts of insects have being little investigated, despite the great potentials they hold, judging by their valued contribution to chemo-therapy. This study was, therefore, carried out to evaluate the Larvicidal potentials of methanolic extract of larvae of *Musca domestica* against *Culex pipiens* mosquitoes.

Materials and Methods

Collection, Identification and Processing of Maggots

Matured Larvae (i.e, maggots) of *Musca domestica* were harvested from a poultry wastes dump site, made receptacle to adult houseflies for oviposition. The identity of the larvae was authenticated by an Entomologist, and voucher specimens deposited in the Laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. Collected Larvae were washed gently in distilled water to remove associated debris and then killed by soaking in Saline Solution. After killing, the larvae were washed again in distilled water to get rid of any trace of salt, from the saline solution, in them. Thereafter, the larvae were dried outdoor (Odesanya *et al.*, 2011), for a week and pulverized using an electrical blending machine. The Pulverized maggot material was preserved in an air-tight container till needed for extraction.

Extraction of the Maggot Material

Preparation of the extract of the maggot material followed the techniques of Adebayo *et al.* (2003). A 200g of the maggot material was percolated in 1600ml of absolute methanol for 48 hours, after which the content of the flask was filtered through No. 1 Whatman filter paper. The filtrate was dried by exposure to the atmosphere at room temperature (26.00±2.00°C), for about 48 hours. The crude extract obtained was transferred to an air tight bottle and stored at -4°C till used for bioassay.

Source of Mosquito Larvae for Bio-assay

The 4th instar larvae of *Culex pipiens pipiens* mosquito used in this study were obtained from a colony maintained in the laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. The mosquito colony was maintained following Standard protocols (Olayemi and Ande, 2008), under ambient Laboratory conditions of 26.00±2.00°C, 60.00±10.00 relative humidity and 12 hr light: 12 hr darkness photoperiod.

Larvicidal Bio-assay

Preparation of test-extract concentrations and procedures for larvicidal tests was according to the standard WHO protocols for testing the Susceptibility of mosquitoes to larvicides (WHO, 2005). Following this procedure, a series of test extract concentration, ranging from 0.25-4.50 mg/ml, were prepared. Then batches of 25 healthy 4th instar larvae of the mosquito species were introduced into 100 ml of each test-extract concentration, with four replicates per treatment. A Control experiment was set-up similar to those of the test extract , except that the 100 ml water contained only 1 ml of the solvent (Methanol), i.e., no extract was added. Bio-assayed experiment was carried out in both tap and distilled water media. The larvae were subsequently monitored for mortality at the end of 24 hrs post-exposure to the extract. The whole experiment was repeated within one week of the termination of the first.

Data Analysis

Larva mortality data were corrected using Abbot's formula (Abbot, 1925), and subsequently processed as Mean±SE. The processed data were statistically analysed using SPSS (Version 20.0), and the mean were compared for significance using Paired sample T-test, at 95% confidence limit and P>0.05. The LC_{50} and LC_{90} of the extract against the mosquito larvae in both tap and distilled water bio-assay were determined using Probit Regression Analysis.

Results

The mortalities induced by increasing concentrations of the extract of *Musca domestica* maggots against 4th instar larvae of *Cx. p. pipiens* mosquito, as well as, the influence of bio-assay media (i.e, tap and distilled water) on such mortalities are presented in table 1. For both bio-assay media, larval mortality increased significantly ($P<0.05$) with rising extract concentration. However, on the whole, larval mortality induced by individual extract concentration was significantly higher in tap water than distilled water bio-assays, expect in the 0.50 mg/ml extract-concentration, where the reverse was the case.

Table 1: Larvicidal activities of house fly maggot crude methanolic extract with tap and distilled water media against *Culex pipiens pipiens* after 24 hours exposure period.

Concentration (mg/ml)	Water Media: Tap	Water Media: Distilled
Control	0.00 ± 0.00^{a}*(0.00)**	$.00\pm0.00^{a}$* (0.00)**
0.50	0.25 ± 0.25^{a} (4.00)	1.75 ± 0.48 (7.00)
1.00	8.50 ± 0.64^{b} (34.00)	4.25 ± 0.47^{a} (17.00)
1.50	15.00 ± 0.71^{b} (60.00)	5.75 ± 0.63^{a} (23.00)
2.00	17.50 ± 0.86^{b}(70.00)	10.25 ± 0.95^{a} (41.00)
2.50	21.50 ± 0.65^{b} (85.00)	13.75 ± 1.31^{a} (55.00)
3.00	25.00 ± 0.00^{b}(100.00)	20.75 ± 0.85^{a} (83.00)
3.50	25.00 ± 0.00^{b}(100.00)	23.50 ± 0.65^{a} (94.00)
4.00	25.00 ± 0.00^{b}(100.00)	25.00 ± 0.00^{b} (100.00)

*Values followed by same superscript alphabets in a column are not significantly different at $P>0.05$ **Values in parentheses are the percentage mortality of their respective doses.

The superiority of the tap water bio-assay, with respect to induced larval mortality was further made manifest by the fact that while 100% larva mortality was attained from the 3.00mg/ml extract concentration treatment in tap water bio-assay, it took the highest concentration of extract tested (i.e; 4.00 mg/ml) to achieve the same level of mortality in distilled water.

Table 2: LC_{50} and LC_{90} (mg/ ml) of methanolic extract of the Larvae of *Musca domestica*, against 4[th] instar Larvae of *Culex pipiens pipiens* mosquito, bio-assay in both tap and distilled water media.

Bio-assay media	LC_{50} mg/ml (Confidence limit)	LC_{20} mg/ml (Confidence limit)
Tap water	1.57(1.31-1.83)	2.74(2.19-2.85)
Distilled water	2.26(2.03-2.47)	3.47(3.19-3.63)

Table 2 highlights the LC_{50} and LC_{90} of the extract against larvae of the mosquito in both tap and distilled water bio-assay media. The LC_{50} values ranged significantly ($P<0.05$) from 1.57 mg/ml in tap water to 2.26 mg/ml in distilled water. LC_{90} equivalents of the LC_{50} values were 2.14 and 3.47 mg/ml, respectively; and were also significantly different.

Discussion

The result of this bio-assay revealed that methanolic extract of *Musca domestica* maggot possesses significant larvicidal activities against *Cx. p. pipiens* mosquitoes, and such activities are dose dependent. Results similar to these have been obtained from bio-assay studies of plant extracts against mosquito larvae including those of *Culex pipiens* (Borah *et al.*, 2010; Arivoli *et al.*, 2011). These similarities in mosquito larvicidal activities of plant extracts and those of the extract of maggots, as obtained in this study suggest that insect's secondary metabolites may be as promising as potential sources of insecticidal lead-agent, as their plant counterparts. The larvicidal activities demonstrated by maggot extract in this study, may be due to the presence of certain bio-active compound such as allantoin, prophenoloxidase, protein peptides, etc, in the extract (Lemon and Terra, 1999; Wang *et al.*, 2012). This is more so as these maggot secondary metabolites were found to possess significant anti-bacterial and immune-sensitivity activities.

Larva mortalities were consistently and significantly higher in tap water bio-assay media than distilled water. Yet, larval mortalities in the control group of mosquitoes maintained in tap water plus solvent only (i.e; no addition of extract) was less than 2%? This therefore means that certain chemicals especially chlorine, used for treatment of public pipe-borne water supply in the area and, thus present in the tap water used for bio-assay in this study, probably acted as synergist for the larvicidal secondary metabolites in the maggot extract. According to Pfadt (1985), certain chemicals that are not necessary insecticidal in nature, may act as synergists to increase the toxicity of insecticidal agents. This potential is a complementary insecticidal attributes for the extract of *M. domestica* maggots; and coupled with the fact that insect product-based insecticides may not be vulnerable to resistance (Hetru *et al.*, 1998), stand the extract of maggot out as a viable source of mosquito larvicidal lead-agent. The LC_{50} and LC_{90} values of the extract against *Cx. p. pipiens* larvae were 2.26 and 3.47 mg/ml, respectively. Though, the death of published information on mosquito larvicidal efficacy of insect extract precludes relative comparison with the lethal concentration values obtained in this study, they never-the-less compare favourably and in some cases better than those of certain plant extracts bio-assayed against larvae of different mosquito species (LC_{50} range = 3.00 to >100 mg/ml and LC_{90} range = 15.00 to >300 mg/ml) (Krishnappa *et al.*, 2012; Ravi *et al.*, 2012)

Conclusion

The extract of *M. domestica* maggots possesses significant mosquito larvicidal efficacy against *Cx. p. pipiens*, probably due to the inherent secondary metabolites that have been credited with therapeutic properties in human medicine. The pathern of larvicidal activities of maggot is similar to those of plant extracts while, the leather concentration values were much better, thus suggesting that insects may be as promising as the botanicals, as sources of eco-friendly alternative insecticides.

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