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Evaluation of Insecticidal Efficacy of Some Selected Plants Leaf- Ethanol Extracts against *Musca domestica* L. [Diptera: Muscidae]

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ABSTRACT

Studies were carried out to determine the toxicidal potentials of leaf-extracts of Azadiracta indica (Juss), Calotropis procera (Aiton), Ocimum basilicum (Lin.) and Argemone mexicana (Lin.) against various developmental stages of housefly, Musca domestica L) in the laboratory. Dipping and feeding methods were employed to evaluate the insecticidal potentials of the plant extracts. Five different concentrations (100ppm, 200ppm, 300 and 400ppm) of the extracts were used in both methods of bioassay tests. All the selected plant extracts were found to affect the pupation and emergence of adults ($p<0.001$). Pupal survival and adult emergence was observed to be inversely proportional to the increase in the concentration of the extracts applied in both methods.

Key words: Dipping method, Efficacy, *Musca domestica*, Plant extracts, Pupation

INTRODUCTION

House flies, *M. domestica* can spread diseases because they feed freely on human and animal feed and filthy matter alike. The flies pick up disease-causing organism while crawling and feeding [1]. Transmission takes place when the fly makes contact with people or their food. Most of the diseases can also be contracted more directly through contamination of food, water, air, hands and person – to – person contact [2].They can also have a negative psychological impact because their presence is considered a sign of unhygienic conditions [3]. They can mechanically transfer them on the hair of their body [4]. They regurgitate them in their vomit, and they can also transfer them in feces through their alimentary tract [2]. The pathogens transfer on the surface of the fly do not multiply and survive for a few hours [5].

There are wide ranges of diseases that can be transmitted by *M. domestica*. Some of these diseases include Typhoid fever (*Salamonella typhi*), Cholera (*Vibrio cholerae), Escherichia coli* and shigellosis which is caused by *shigella* [6]. The diseases that houseflies transmit are intestinal, eye, skin and wound [7] and viral infections e.g. poliomyelitis [4, 7]. Flies can also transmit enteric infections such as dysentery, and certain helminthes infections (WHO, 1997). Outbreaks of diarrheal disease in predominantly developing countries have been associated with the seasonal increase in abundance of flies [4].

Chemical insecticides are being widely used by most people in reducing insect pest of medical importance. However, these chemical insecticides are hazardous to man, his animals and environment at large. The wide spread and massive application of chemical insecticides frequently produce the risk of developing insects resistance and insecticidal residuals for human and environment [8]. These problems coupled with acute neuro-toxicity to man and

his domesticated animals, have stimulated the search for biologically based alternatives [9]. Accordingly, botanical insecticides based on natural compounds from plants, are expected to be a possible alternative. These botanicals tend to have broad-spectrum activity and are respectively specific in their mode of action [10].

Insecticidal activity of many plants against several insects has been demonstrated, Seeds as well as foliar extracts of several plants have been reported to have toxic and potent growth reducing activity to insects [11]. The presence of plant defense related protein such as the vein, an alpha-amylase inhibitor has been described to occur in the latex secretion of other plants [12]. Some plants of Annonaceae family have been used traditionally as insecticides [13]. Powdered seeds and leaf juices of *Goniothalamus macro-phyllis* is used as mosquito repellent, leaf-extract of *A. indica, C. procera, C. aurantifolia* have been tested for larvicidal activity against moquitoes [14]. Use of botanical pesticides, either as crude or formulated extracts is an alternative strategy.

In view of the role of housefly as a vector of different types of pathogenic microorganisms (bacteria, fungi, virus, protozoan and helminthes) and toxicidal hazards of chemical insecticides on the non-target organisms and the environment, the present study has been planned to investigate the toxicidal potentials of ethernolic leaf-extracts of *A. indica*, *C. procera*, *O. basilicum* and *A. mexicana* by assessing their effects on the various developmental stages of *M. domestica.*

MATERIALS AND METHODS

Study Area

The study was conducted at the Department of Biological Sciences, Usmanu Danfodiyo University, and Sokoto, Nigeria. The experiment was conducted between January 2011 to April 2012 and the laboratory condition was 33 – 39^0C and 60 – 70% relative humidity.

Collection of the Housefly and Culture of the Motherstock

The method described by [15] was used for collection and culture of the houseflies. Four metal frame cages 60 x 30 x 30 cm were constructed and used for rearing. The floor of the cage was made up of plywood fixed at the bottom of the cage, while the remaining five sides were covered with muslin cloth. The front of the cage was half open for the flies to enter, while the rear opening ended in sleeves, serving as opening for the exchange of food, water and collection of eggs. To attract flies, a piece of liver was placed inside the cage. About 300 – 500 flies were observed in the cage. The adult flies collected in the cages were fed with mixture of milk powder + sugar and water provided in slow petri-dish kept on the floor of the cage. Cotton soaked in water was also kept in petri-dish to provide drinking water. Food and water were changed daily. Filter paper soaked in fresh milk was provided in petri dishes for eggs to be laid. Eggs laid on the filter paper were then transferred into plastic bowls containing larva-rearing medium. The adults in the cages were then killed; samples of the flies were taken to the Department of Biological sciences laboratory, Usmanu Danfodiyo University, Sokoto for identification. The bowls containing developing eggs were covered with muslin cloth and left in the cages until hatched.

Preparation of the Medium for Rearing of Larvae

Following the method described by [16], a food composition of guinea corn bran, baker's yeast, powdered milk and water was provided for the larvae. This was prepared by adding 4.0 g of dry baker's yeast, 50 g of powdered milk and 600 ml of warm water, which was stirred until it dissolved. To this solution, 200 g of guinea corn bran was added and the mixture was stirred to mix thoroughly from which a loose texture was obtained. Each rearing container was filled with the prepared medium to about a third of its volume i.e. 50 g. The filter paper containing eggs was cut into pieces, and each containing 30– 40 eggs. Each of the paper containing the eggs was placed on the rearing media for hatching. The larvae were left on the rearing media until they pupated. The rearing media containing the pupae were placed inside the fly rearing cages for adult emergence. The emerged flies were allowed to fly freely inside the cages for copulation among the flies. These flies were fed as their predecessors. Eggs laid on the filter paper were transferred into the larvae rearing medium containing the same food as described above. Third instar larvae were then picked and used for experiments.

Collection of Plant Materials

Fresh leaves of *A. indica, C. procera*, *O. basilicum* and *A. mexicana* were collected at Tudun ma'aji along Bobi-Kasuwan Garba road in Bobi Town, Mariga Local Government Area of Niger state, Nigeria. Taxonomic identification was done by comparing the plant material with the preserved identified materials at the Department of

Biological Sciences, Usmanu Danfodiyo University Sokoto, and Nigeria. The leaves collected were washed and dried in shade for ten days. Thereafter, the dried leaves were crushed to powder using pestle and mortar and sieved with a siever 0.5 mesh diameters. The powders were then kept in brown bottles and taken to the Biological Sciences laboratory, Usmanu Danfodiyo University, Sokoto for the experiments.

Extraction

One hundred grams (100 g) of the leaf powder was submerged in 300ml aqueous 70% ethanol at room temperature. After 24hrs, the supernatants were decanted and filtered through Whatman filter paper No. 5 and dried in a rotary evaporator at 40^0C for 50 – 60 minutes from which crude extracts was obtained. The extract were kept in brown specimen bottles till required for the experimental.

Preparation of Varying Concentrations of Leaf-Extracts

One mill gram (1 mg) of *A. indica* powder was dissolved in 100ml of distilled water, this in turn produced 100 ppm. Also, 2, 3 and 4 mg of the extracts was dissolved independently in 100 ml of distilled water which resulted to 200, 300 and 400 ppm. The same procedure was repeated for other plant extracts in the preparation of different concentrations. The solution was produced in large quantity for use in both dipping and feeding bioassay tests.

Dipping Method

Twenty 3rd instar larvae were collected from the stock culture and dipped into 100 ml of different concentrations (100, 200, 300 and 400 ppm) of *A. indica* ethernolic leaf-extracts. The control was dipped into100 ml of distilled water with a dipping-net. After being dipped, for 30 seconds, the larvae were transferred to the earlier prepared rearing media in separate petri dishes. Pupal duration and emergence of adults were observed and recorded.

Feeding Method

In this method, 10 ml ethernolic leaf-extract of *A. indica* of each concentration prepared (100, 200, 300 and 400 ppm) was mixed with the food in each petri-dish. No extract was mixed with the rearing medium used as control. Twenty 3rd instar larvae were placed on the rearing medium with the help of filter. The paper was wetted and placed inside the larval stock for the larvae to move on; those that climbed the paper were transferred to the rearing media. This was repeated until the required numbers of the larvae were placed on each of the petri-dish. The rearing media and the larvae were then covered with muslin cloth. Similar procedures were repeated using extract of *C. procera, O. basilicum* and *A. mexicana*. The experiments were repeated three times. Pupal duration, morphological deformities and emergence of adults were observed and recorded.

Per cent Pupation and adult emergence were calculated according to [17] as follows:

%Pupation = A/B x 100

Where:

A = number of pupae emerged; and

B = number of tested larvae.

There were three replications in all the experiments.

RESULTS

It was observed that during the larval development, tendency for the treated larvae to pupate decreased with an increase in the concentration of the extracts used in both methods (Tables 1 – 4). In the experiments treated with *A. indica* extract at 100 ppm, 73.3 and 71.7% of the larvae pupated when treated with dipping and feeding method respectively (Table 1). There was no significance difference between the treatments. Almost similar rate of pupation (71.7 and 85%) was observed in both methods when treated with *C. procera* (Table 2). Observations from the two methods in *O. basilicum* treatment at the same concentration were recorded as 75% pupation (Table 3). *A. mexicana* has the highest pupation (91.7%) at 100ppm in dipping, while in feeding it was 71.7% (Table 4). At 400 ppm, 20.0, 21.7, 31.7 and 25.0% larvae pupated when treated by dipping method was the result observed in the above extract respectively when dipping method , while pupation in feeding method was 21.7, 23.3, and 16.7 and 25.0%. When the results of the two methods of treatments were compared, it revealed that there was no significance difference ($p>0.999$) in the rate of pupation of the larvae, but a very significant difference ($p<0.001$) was observed between the treatments and the controls at all levels of concentrations of each extracts.

Table 1: Effects of *A. indica* ethernolic leaf extracts on the pupation and adult emergence of *M. domestica*

Conc.(ppm)		Mean No. of Pupae Observed	Mean No. of Adult Emerged	Pupal Duration (Days)	Pupal Survival (Per cent)	Adult Emergence (Per cent)
Control	Dipping	20.0±0.00^{a}	19.6±0.00^{a}	3.0±0.00^{a}	98.3±2.89^{a}	98.3±2.89^{a}
	Feeding	19.7±5.77^{a}	19.76±0.58^{a}	3.0±0.00^{a}	98.3±2.89^{a}	98.3±2.89^{a}
100	Dipping	14.7±0.58^{b}	14.6±0.58^{b}	4.0±0.00a	73±2.89^{b}	73.3± 2.89^{b}
	Feeding	14.0±1.00^{b}	14.6±1.00^{b}	3.0±0.00^{a}	71.7±5.77^{b}	70.0± 5.00^{c}
200	Dipping	12.3±0.58^{b}	11.3±1.53^{c}	4.0±0.00^{a}	61.6±2.89^{c}	56.7 ±7.64^{d}
	Feeding	12.6±0.58^{b}	11.3±1.53^{c}	4.3±0.58^{a}	63.3±2.89^{c}	56.7 ±5.78^{d}
300	Dipping	7.6±2.52^{c}	11.3±1.53^{c}	5.0 ±0.00^{a}	38.3±2.89^{e}	33.3±14.43^{f}
	Feeding	11.0±1.00^{b}	11.3±53^{c}	4.0 ±0.00^{a}	55.0±5.00^{d}	48.3±2.89^{e}
400	Dipping	4.0±2.00^{e}	3.6±2.09^{d}	5.0± 0.00^{a}	20±10.00^{f}	18.3±10.41^{g}
	Feeding	4.3±1.53^{e}	3.6±2.09^{d}	4.0± 0.00^{a}	21.7±7.64^{f}	11.7±5.77^{h}

Values followed by different letters on the same columns are significantly different at p<0.05.

Table 2: Effects of *C. procera* ethanolic leaf extracts on the pupation and adult emergence of *M. domestica*

Conc.(ppm)		Mean No. of Pupae Observed	Mean No. of Adult Emerged	Pupal Duration (Days)	Pupal Survival (Per cent)	Adult Emergence (Per cent)
Control	Dipping	20±0.00^{a}	20±0.00^{a}	3.0±0.58^{a}	100±0.00^{a}	100±2.89^{a}
	Feeding	20±0.00^{a}	20±0.00^{a}	3.0±0.58^{a}	100±0.00^{a}	100±0.00^{a}
100	Dipping	14.0±1.00^{c}	14.6±1.00^{c}	3.0±0.00^{a}	71.7±5.77^{c}	70± 5.00^{d}
	Feeding	17±1.00b	17.0±1.00^{b}	3.0±0.00^{a}	85±5.00^{b}	85± 5.00b
200	Dipping	12.6±0.58^{c}	11.3±1.16^{d}	4.3±0.58^{a}	63.3±2.89^{d}	56.7±5.78^{c}
	Feeding	13±2.69^{c}	13±2.65^{c}	3.3±0.58^{a}	65±13.23^{d}	78.3±10.41^{c}
300	Dipping	11.0±1.00b^{c}	9.7±0.58^{d}	4.0±0.00^{a}	55.0±5.00^{d}	48.3±2.89^{e}
	Feeding	9.3±1.16b^{c}	7.7±2.08^{d}	3.3 ±0.58^{a}	46.7±5.77^{e}	38.3±10.41^{f}
400	Dipping	4.3±1.53^{d}	3.0±2.00^{e}	4.0±0.00^{a}	21.7±7. 64^{f}	11.7±5.77^{h}
	Feeding	4.7±1.53^{d}	3.7±2.08^{e}	3.6± 0.58^{a}	23.3±7.64^{f}	18.3±10.41^{g}

Values followed by different letters on the same columns are significantly different at p<0.05.

Table 3: Effects of *O. basilicum* ethernolic leaf extracts on the pupation and adult emergence of *M. domestica*

Conc.(ppm)		Mean No. of Pupae Observed	No. of Adult Emerged	Pupal Duration (Days)	Pupal Survival (Per cent)	Adult Emergence (Per cent)
Control	Dipping	20±0.00^{a}	20.0±0.00^{a}	3.0±0.00^{a}	100±0.00^{a}	100±0.00^{a}
	Feeding	20±0.00^{a}	20.0±0.00^{a}	3.0±0.00^{a}	100±0.00^{a}	100±0.00^{a}
100	Dipping	15±2.00^{b}	15.0±2.00^{b}	3.0±0.00^{a}	75±10.00^{b}	75.0± 10.00^{b}
	Feeding	15±3.00^{b}	14.3±2.08^{b}	3.0±0.00^{a}	75±15.00^{b}	71.7± 10.41^{bc}
200	Dipping	11±1.53^{c}	11.3±1.53^{c}	3.0±0.00^{a}	56.7±7.64^{d}	56.7 ±7.64^{d}
	Feeding	14.7±1.53^{b}	14.7±1.53^{b}	4.0±0.00^{a}	73.3±7.64^{c}	73.37 ±7.64^{b}
300	Dipping	9.0±1.00^{bc}	9.0±1.00^{c}	3.0 ±0.00^{a}	45.0±5.00^{e}	45.0±5.00^{e}
	Feeding	8.3±1.15^{bc}	5.7±1.16^{d}	3.7 ±0.577^{a}	41.7±5.77^{e}	28.3±5.77^{f}
400	Dipping	6.3±1.53^{bc}	5.7±0.58^{d}	3.3± 0.58^{a}	31.7±7.64^{g}	26.7±2.89^{f}
	Feeding	3.3±2.2^{d}	2.0±1.47^{e}	4.0± 0.00^{a}	16.7±12.5^{h}	10.0±7.07^{g}

Values followed by different letters on the same columns are significantly different at p<0.05.

Adult emergence was also affected by the extracts at all levels of concentrations. The results of adult emergence at 100 ppm in the dipping method significantly ($p<0.05$) varied with that of feeding. In dipping method *A. mexicana* had the highest (91.7%) adult emergence while *C. procera* resulted in the least (70.0%) adult emergence (Tables 2 and 4). In feeding method the adult emergence was higher (85.0%) in *C. procera* than in *A. indica* which resulted in 70.0%, it was similar (71.7%) in both *O. basilicum* and *A. mexicana* treatments (Tables 1, 2, 3 and 4). As the concentration increased to 200 ppm, the number of adult emerged decreased as well, therefore, 56.7, 56.7, 56.7 and 73.4% was recorded in the dipping method for *A. indica, C. procera O.basilicum and A. mexicana* respectively and in feeding method, 56.7, 78.3, 73.4 and 58.3% was obtained for *A. indica, C. procera O.basilicum and A. mexicana* respectively. An extreme significant ($p<0.0001$) difference was observed when the results of 400 ppm was compared to that obtained from 100, 200 and 300 ppm dipping. Even in the feeding method the result was similar when percentage adult emergence was compared within the treatment and that of the control (Tables 1, 2, 3 and 4).

Table 4: Effects of *A. Mexicana* ethanolic leaf- extracts on the pupation and adult emergence of *M. domestica*

Conc.(ppm)		Mean No. of Pupae Observed	No. of Adult Emerged	Pupal Duration (Days)	Pupal Survival (Per cent)	Adult Emergence (Per cent)
Control	Dipping	20.0±0.00^{a}	20.0±0.00^{a}	3.0±0.00^{a}	100±0.00^{a}	100±0.00^{a}
	Feeding	20±0.00^{a}	20±0.00^{a}	3.0±0.00^{a}	100±0.00^{a}	100±0.00^{a}
100	Dipping	18.3±2.08^{b}	18.3±2.08^{a}	3.0±0.00^{a}	91.7±10.41^{b}	91.7± 10.4^{b}
	Feeding	14.3±1.53^{c}	14.3±1.53^{b}	3.0±0.00^{a}	71.7±7.64^{c}	71.7± 7.4^{c}
200	Dipping	14.0±1.73^{c}	14.0±1.73^{b}	3.3±0.58^{a}	70.3±8.66^{c}	70.0 ±8.66^{c}
	Feeding	12.0±1.00^{c}	11.7±1.53^{c}	3.3±0.58^{a}	60.3±5.00^{d}	58.3 ±7.64^{c}
300	Dipping	10.3±2.52b^{c}	10±12.52^{c}	3.3 ±0.58^{a}	51.6±12.58^{e}	51.7±12.58^{d}
	Feeding	11.0±3.61b^{c}	10.3±2.52^{c}	4.0 ±0.00^{a}	55±18.03^{f}	57±12.58^{c}
400	Dipping	5.0±2.6^{d}	3.0±1.00^{d}	3.7± 1.00^{a}	25.0±13.23^{g}	15.0±5.00^{f}
	Feeding	5.0±2.65^{d}	3.0±1.00^{d}	3.7± 1.00^{a}	25±13.23^{g}	11.7±3.54^{g}

Values followed by different letters on the same columns are significantly different at p<0.05.

DISCUSSION

M. domestica has been extensively utilized as a test organism to screen candidate insecticides, chemosterilant and insect growth regulations by scientists in public or private research institutions. In the present study, the toxicity of ethanolic extracts of the leaves of *A. indica, C. procera, O. basilicum* and *A. mexicana* were found quite effective against the various developmental stages of housefly especially on the larval development, pupation and the emergence of adult. The effects were in dose dependent manner. All the extracts tested have shown potential toxicidal activity, the active constituent of all the plant species may have contact, stomach poison or neurotoxic actions against the various developmental stages of *M. domestica*.

All the plant materials screened hindered larval-pupal transformation, and adult emergence. Results of the study revealed that there was a severe decrease in adult emergence of *M. domestica* when larvae were treated with different concentration of the extracts. This decrease in the adult emergence could be due to the fact that the extracts block the maturation of imaginal discs which are the primordial of many adult integumentary structures in endopterygote insects or due to deformation of adult chitin, or this may be due to the effects of some active ingredients present in the extracts which exhibit potentials to cause interference into the normal metabolism of the insects. This is in agreement with the findings of [18, 19, 20, 21, 22] who reported the same results, that the active constituents may transform the alcohol present into the insect body to the corresponding esters and causes alteration in the normal metabolism of the insects which may result to failure in pupation and adult emergence.

All the tested extracts have resulted in more than 50% decrease in pupation, which is in agreement with the findings of [23]. Investigations on the plant *Annonaceous acetogenin*, of family Annonaceae, have shown that it may have pesticidal or antifeedant properties. It has been reported that *C. procera* caused alteration in the rate of pupation and adult emergence of *M. domestica* [24, 25, 26].

CONCLUSION

The result of present investigation reveals the insecticidal potentials of the tested botanical extract against the larvae of *M. domestica*. The leaf extract of *A. indica, C. procera, O. basilicum* and *A. mexicana* were very promising, furthermore, all these plant materials can be easily collected from the natural vegetation. Therefore, plant originated insecticides can be used as sustainable larvicide in a housefly control programme. In the present study, all extracts used act as larvicidal and possess growth and emergence inhibition against *M domestica*. These findings have emphasized the need to explore the possibility of using plant based larvicides and reduce the chemical hazards in the environment. Further studies on these plants, including mode of action, synergism with the biocides under field condition are needed. Also isolation of the active compounds from these plants and further trial assay in the field are required.

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