Larvicidal Activity of Selected Plant Extracts against the Screwworm Fly Chrysomya Albiceps

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Myiasis is a kind of parasitic disease originating from the invasion of tissues of domestic animals by dipteran larvae. Chrysomya albiceps is a type of screwworm fly spread in the tropical areas and known to cause myiasis among live human and animals leading to health problems and high economic losses to dairy producers. Management and control of this pest is needed to overcome these losses. Nowadays, natural botanical products have been increasingly investigated as controlling agents against insects of medical and veterinary importance. This research was designed to evaluate the larvicidal effect of the total extracts of three plants, Ficus palmate, Juniperus procera and Nerium oleander against screwworm fly Chrysomya albiceps. The plants leaves were extracted with organic solvents mixture methanol : chloroform (1:1) and were tested against the second larval instar of C. albiceps using feeding and dipping methods. The extracts caused larval mortalities in the order of F. palmate > N. oleander > J. procera with IC50 values of 15.97, 33.73 and 37.24, respectively using feeding method and in the order N. oleander > F. palmate > J. procera with IC50 values of 43.12, 47.41 and 73.39, respectively using dipping method. It is concluded that the F. palmate followed by N. oleander and J. procera are candidates to use in controlling the larvae of myiasis-caused fly C. albiceps.

Keywords: Botanical Extracts; Chrysomya Albiceps; Larvicidal Bioassay; Myiasis.
Arabia (KSA)\textsuperscript{10}. 	extit{Wohlfahrtia nuba} (Wied), \textit{C. albiceps} and \textit{C. bezziana} are the major species responsible for inducing cutaneous myiasis in KSA\textsuperscript{11,12}.

Myiasis-caused flies use body tissues and fluids of vertebrate as a source for food.

Myiasis is common parasitic disease that infect animals and causes economic losses in livestock production worldwide\textsuperscript{13}. Dairy farmers face severe economic losses due to myiasis disease that cause mortalities in domestic animals and lead to reduction in productivity. Before the control plan of myiasis-caused screwworm fly, the economic loss in the USA was estimated to be 140 million US dollars per year in livestock industry\textsuperscript{14}. Also the annual average loss in the sheep industry in Australia is estimated to be $280 millions\textsuperscript{15}. Drawbacks associated with wide spread use of conventional insecticides for controlling housefly populations resulted in the development of insect resistance to different insecticides, environmental pollution and negative side effects to non-target organisms including humans. Therefore, there is a need to search for safe alternatives to combat these flies such as insect growth regulators (IGR) and plant-based ingredient\textsuperscript{16,17}. This study aimed to test the larvicidal effect of three botanical extracts against screwworm fly \textit{Chrysomya albiceps} using feeding and dipping methods under laboratory conditions.

**MATERIALS AND METHODS**

**Rearing of flies**

The blowfly, \textit{Chrysomya albiceps} adults were collected from Jeddah slaughterhouse and transferred to insect raring laboratory, King Abdulaziz University, Kingdom of Saudi Arabia (KSA) and maintained for several generations under standard conditions, temperature of (27±2°C), relative humidity (75±5%) and photoperiods (14h light: 10h dark). Collected insects were identified to species by a taxonomist in biological department, King Abdulaziz University using a key\textsuperscript{18}. Flies were reared according to method\textsuperscript{19} with some modifications. Adults were reared in mesh cages (45×45×45cm) with three sides made of the wire and feed on milk powder and sucrose solution. A plastic dish containing fresh cow liver was provided inside the cages for oviposition and as a diet for the larvae. The late 3rd instar larvae were picked up and transferred into plastic pans (25 x 30 x 15 cm) containing 300cm of softwood sawdust and left to pupate and adult emergence.

**Plant's materials**

Three plant species \textit{Ficus palmate}, \textit{Juniperus procera} and \textit{Nerium oleander} were taken from AL-Baha area in the south-west of Saudi Arabia during June 2020. The plants were identified by taxonomy specialist in King Abdulaziz University. Plants leaves were rinsed with distilled water, put in the shade at room temperature until complete dryness, then grounded to a fine powder.

**Preparation of plant extracts**

Nearly 100 gm of the fine powder of selected plants were individually extracted by chloroform-methanol mixture (1:1) under reflux. The supernatant was filtered and evaporated under reduced pressure by rotary evaporator at temperature not exceeding 45°C. the extraction was repeated up to three times. The dry extracts were kept in the refrigerator (4°C) till the bioassay test\textsuperscript{20}.

**Testing technique**

The larval bioassay was carried out as described by Singh and Kaur, 2015\textsuperscript{21} with some modifications:

**Dipping method**

The second instar larvae of \textit{Chrysomya albiceps} were exposed to botanical extracts under investigation at five different concentrations. The extracts were prepared by dissolving 1 g in 99.5 ml distilled water and 0.5 ml of the emulsifier triton x100 to obtain complete solubility. Series of concentrations were prepared in plastic jar (6cm x 9cm) using distilled water. the larvae were wrapped in a small cloth bag and gently immersed into extract solutions for 30 second for each concentration, whereas those of the controls were immersed in distilled water. the larvae were transferred to the rearing box containing non-treated food. The experiments were replicated five times for each concentration and the larval mortalities were recorded 24 h post treatment.

**Feeding method**

Larvae were transferred to jars containing treated fresh cow liver after discarding the distilled water completely. In the control experiments, distilled water only was added to the food. Larval mortality was recorded 24h after treatment. The
experiments were replicated five times for each concentration. Twenty larvae were added in each duplicate. Abnormal larvae, pupae and adults were removed and placed in labeled glass vials containing 70% ethanol then photographed under a binocular microscope.

**Statistical analyses**

The percentage of larval mortality were corrected according to abbot’s formula\(^2\). LC50 values (concentration which kill 50% of the larvae) and LC90 values (concentration which kill 90% of the larvae) were calculated by probit model by using Ldp-line program and also compute slope of the toxicity line and Chi square according to Finney\(^2\).

**RESULTS AND DISCUSSION**

The control of insect pests of medical and veterinary importance, including flies, depends mainly on the use of chemical pesticides, but the residues of these pesticides are harmful to the environment, humans and domestic animals.

For this reason, many techniques have emerged that can be used in pest control, such as the use of natural enemies like insects, viruses, bacteria and fungi, as well as the use of natural components of plant origin\(^2\).

Many researchers have reported that some of the metabolites extracted from plants can be used to control insects and thus provide safe alternatives to synthetic chemical pesticides\(^2\).\(^5\).\(^6\).\(^7\).

Alkaloids, terpenes and other plant metabolites have proven their ability to combat insect pests, and the use of these compounds have a promising future due to their desirable environmental and economic properties\(^2\).\(^8\).

The effects of selected botanical extracts on larval survival and development by feeding and dipping methods are demonstrated in (table 1). The toxicity in terms of IC50 and IC90 values with the three extracts applied through the feeding and dipping methods are shown in figure (1, 2 and 3). Figure (4 & 5) compare the toxicity lines between the three extracts applied with the feeding and dipping methods. Figure 6 shows the deformations

| Statical parameters | Feeding bioassay method | Dipping bioassay method | Relative toxicity |
|---------------------|-------------------------|-------------------------|-------------------|
| **J. procera**      |                         |                         |                   |
| IC50 (ppm)          | 37.2409                 | 73.3902                 |                   |
| 95% (F. L.)         | 31.7133- 42.6185        | 65.4985 - 82.4329       |                   |
| IC90 (ppm)          | 136.5096                | 239.5213                |                   |
| 95% (F. L.)         | 113.6238- 174.4432      | 194.5083- 317.8668      | 1.971             |
| Slope               | 2.2718                  | 2.4948                  |                   |
| Tabulated (Chi)\(^2\) | 7.81                    | 7.81                    |                   |
| Calculated (Chi)\(^2\) | 5.6209                  | 4.366                   |                   |
| **N. oleander**     |                         |                         |                   |
| IC50 (ppm)          | 33.7264                 | 43.1236                 |                   |
| 95% (F. L.)         | 29.5912- 37.8548        | 37.4522- 49.5311        |                   |
| IC90 (ppm)          | 113.6993                | 194.702                 |                   |
| 95% (F. L.)         | 93.9919- 147.9467       | 144.4576- 307.0039      | 1.279             |
| Slope               | 2.4282                  | 1.9577                  |                   |
| Tabulated (Chi)\(^2\) | 7.81                    | 7.81                    |                   |
| Calculated (Chi)\(^2\) | 5.99                    | 7.0673                  |                   |
| **F. plamate**      |                         |                         |                   |
| IC50 (ppm)          | 15.9675                 | 47.406                  |                   |
| 95% (F. L.)         | 4.7337 - 21.6508        | 29.8556 - 77.8389       |                   |
| IC90 (ppm)          | 99.5855                 | 208.9072                |                   |
| 95% (F. L.)         | 78.2297- 438.618        | 181.2835 - 1032.0047    | 2.969             |
| Slope               | 1.6122                  | 1.9897                  |                   |
| Tabulated (Chi)\(^2\) | 7.81                    | 7.81                    |                   |
| Calculated (Chi)\(^2\) | 8.7079                  | 9.306                   |                   |
Fig. 1. Toxicity line show the relationship between *J. procera* extract concentrations and larval mortality percentage using feeding and dipping methods.

Fig. 2. Toxicity line show the relationship between *N. oleander* extract concentrations and larval mortality percentage using feeding and dipping methods.
Fig. 3. Toxicity line show the relationship between F. palmate extract concentrations and larval mortality percentage using feeding and dipping methods.

Fig. 4. Toxicity line show the relationship between the three extracts concentrations and larval mortality percentage using feeding method.
Fig. 5. Toxicity line show the relationship between the three extracts concentrations and larval mortality percentage using dipping method.

A- Control larvae. B- Pigmentation. C– Dead deformed larvae partially exuviated. D – Control Adult. E – Twisting wings. F- Dead Adult with shrunk appearance (Segment Body Contraction)

Fig. 6 Morphological abnormalities resulting from treatment with plants extracts.
in the larvae down to the full insect stage. The mortality percentage of larvae increased as the extract concentration increased. The mortality rate in control experiments did not exceed 4%. The efficacy of the plant extracts was expressed by IC50 values. The results demonstrated that F. plamate (IC50=15.9675) was 2.3 and 2.1 times more effective than J. procera (IC50=37.2409) and N. oleander (IC50=33.7264) Extracts (Table 1).

Generally, the three extracts proved larvicidal effect in both the techniques, where in the feeding method, Ficus palmate extract revealed the highest larvalcidal activity followed by N. oleander and J. procera extracts with IC50 values 15.97, 33.73 and 37.24 ppm, respectively whereas in the dipping application method, N. oleander extract recorded the highest mortalities followed by Ficus palmate extract then J. procera extract with IC50 values 43.12, 47.41 and 73.39 ppm, respectively.

Some active ingredients of the plant have the ability to penetrate the body of the larva by direct ingestion if the feeding method is applied or enter through the skin during the application of the dipping technique. Studies have demonstrated that Four wild plants components of Artemisia herba-alba, Artemisia monosperma, Euphorbia aegyptiaca and Francoeuria crispa can penetrate the abdomen of the larva, which leads to damage to the epithelial lining, causing it to die or change its feeding behavior. In this field, histological analysis of the intestines of larvae treated with some extracts of a wild medicinal plants showed damage of the epithelial lining in the dead larvae.

The properties of botanical natural products make it suitable for use to combat insect pest. They affect their life cycle and alter their developmental process leading to inhibition of adults emergence. Botanical compounds such as azadirachtin, salamin and nimbin in Azadirachta indica plant exhibited insecticidal, antifeedant and insect growth inhibitor activities. Compounds such as azadirachtin belong to the class of tetracentriciterpenoids compounds and is similar in its chemical composition to the insect growth regulator called ecysone. This hormone affects and inhibits the developmental process of the insect.

Researchers have reported insecticidal effect for the tested extracts in this study against different insects, for examples J. procera extract had insecticidal effect against Anopheles mosquito Anopheles arabiensis larvae.

Also the essential oil content of J. procera exhibited larvicidal effect against Anopheles arabiensis with LC50=14.4 mg/L and LC90=24.65 mg/L.

The extracts of the three types of Nerium oleander flower, (pink, red and white) had larvicidal effect against Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti with LC50 values of 94.60, 101.21 and 121.79 mg/L, respectively. Additionally, the Ficus benghalensis showed lethal effect on early second, third and fourth instar larvae of Culex quinquefasciatus, Aedes Aegypti and Anopheles stephensi by LC50 41.4, 58.2 and 74.3 ppm, 56.5, 70.3 and 80.6 ppm and 60.4, 76.4 and 89.6 ppm respectively.

Morphological changes of treated larvae
Deformities were observed in the dead individuals as a result of the treatment with the three plant extracts (Fig. 6). The deformities included pigmentation and the dead larvae appeared partially exuviated as well as deformities of dead adults included small sized, twisting wings, shrunk appearance (segment body contraction) in addition to poorly developed and deformed wing and legs.

CONCLUSION

The plant extracts tested in this study have proven their effectiveness in controlling the screwworm fly Chrysomya albiceps, and this makes them suitable as better alternative to the chemical insecticides used in the current control programs, which have significant collateral damage in terms of their accumulation in the food chain and the negative effects on humans, animals and the environment. In this study, the individual components of the extracts were not investigated, but the evaluation of the total extracts of plants was done. Therefore, we recommend further studies to investigate the active components present in the extracts.

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