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# Evaluation of insecticidal and biochemical efficacy of *Ficus nitida* and *Eichhornia crassipes* leaf extracts on *Galleria mellonella* (Lepidoptera: Pyralidae)

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**Abstract:**

The greater wax moth *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) is widely distributed throughout the world. It is an economically important pest of wax combs of the honeybee. The present work was carried out to study the insecticidal and biochemical effects of *Ficus nitida* and *Eichhornia crassipes* leaf extracts by different solvents: petroleum ether, acetone, ethanol, chloroform and water on 4$^{th}$ instar larvae of *G mellonella* . The $LC_{50}$ of *F. nitida* extracts were 5.638, 10.312, 2.440, 13.359 and 4.394 % for petroleum ether, acetone, ethanol, chloroform and water, respectively. And for *E. crassipes* extracts were 4.803, 1.860, 2.550, 12.067 and 3.864 % for the same solvent order, respectively. The data showed that *F. nitida* ethanol extract and *E. crassipes* acetone extract caused reduction in pupal weight and pupation percentage and also increasing in larval duration days compared with control. The data showed that, the two extracts had a significant inhibitory effect on α–amylase, while alkaline phosphatase showed significant increase in activity. The overall effects of *F. nitida* and *E. crassipes* leaf extracts on *G. mellonella* larvae may have led us to a selective natural product that can be employed in integrated pest management strategies.

## Introduction

In recent years, the application of several botanical products has drawn much attention as effective alternatives to the synthetic pesticides and chemical fertilizers. These plant products are reported to be more effective, less expensive, biodegradable and safe for mankind and environment, than their synthetic counterparts, which are environmentally persistent and toxic to non-target organisms including humans eliciting many unidentified diseases after bioaccumulation. Therefore, alternatives to conventional pesticides are required to be developed from the active ingredients of plant origin. These compounds have been shown to affect insect populations by reducing their developmental, survival and reproductive rate. The present study aims to evolve an environmentally safe, economical and effective botanical insecticide for the control of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), it considers as a serious pest of beehives and stored bee wax (Fathy *et al*., 2017). This investigation is expected to yield interesting results

which may be useful for the protection of *G. mellonella*.

The aim of the present work is to study the insecticidal and biochemical effects of *Ficus nitida* and *Eichhornia crassipes* leaf extracts on 4th instar larvae of *G mellonella*

**Materials and methods**

The greater wax moth *G. mellonella* larvae were obtained from infested hives and reared at the laboratory under controlled conditions (25 ± 2 °C, 60 ± 10% RH) according to method of Birah *et al*. (2008) and fed on diet composed according to Metwally *et al*. (2012). *F. nitida* leaves were collected according to the method of Sawidis *et al.* (2011). *E. Crassipes were* collected from the River Nile at the El-Dahab Island (Giza), Egypt.

**1. Extraction method:**

*F. nitida* and *E. crassipes* collected leaves were washed thoroughly with water to remove dirt, if any and chopped into small pieces with a sharp knife. Chopped pieces were crushed into a fine paste with the help of a pestle and mortar. The solvents: petroleum ether, acetone, ethanol, chloroform and water were used at a rate of 500ml/200gm pasted leaves, were soaked for 72 h., was shaken daily for one hour, and its contents were filtered through Whatman no.1 filter paper over anhydrous sodium sulphate. Each solvent extract was evaporated to dryness by a rotary evaporator at 40°C according to method of Rossenthaler (1930). The crude extracts were weighed and kept in deep freezer till used.

**2. Toxicity assay:**

The crude extracts were tested at five different concentrations. Newly active 4th instar larvae were selected and starved for 4 hours before the experiments. Ten larvae were introduced to treated diet in the petri dish with concentrations of 10, 5, 2, 1 and 0.5 % of crude extracts. The control was given an artificial diet only. Five replicates were maintained for each treatment with 10 larvae per replicate. Mortality % were recorded after 48 hrs. Larvae were considered dead if they become immobile and have shown no detectable response to the external stimuli. Mortality data were subjected to Probit analysis according to Finney's method Finney (1971).

**3. Biological activities:**

The effect of the two extracts (*F. nitida* ethanol extract and *E. crassipes* acetone extract) on larval duration, percentage of pupation, pupal weight, and adult emergence of *G. mellonella* carried out. The diets containing $LC_{50}$ of the extracts were put into 9-cm petri dish with 10 freshly molted pre-weighted fourth-instar larvae. The control was given an artificial diet only and the experiment was performed in three replications. After 48hrs. larvae were fed on untreated diet until pupation. Inspections were carried out until adult emergence and larval duration, larval weight, pupation%, pupae weight, and adult emergence % were recorded daily.

**4. Biochemical activities:**

The survived *G. mellonella* larvae after 48 hrs of feeding on artificial diet incorporated with $LC_{50}$ of the two extracts (*F. nitida* ethanol extract and *E. crassipes* acetone extract) were used for various biochemical tests, each experiment was repeated at three times each with ten larvae. A known weight of larvae (whole body) was homogenized in appropriate amount of distilled water using mechanical homogenizer, centrifuged at 10,000g for 5 min at 4 °C and the supernatant was used for tests-freshly or kept in deep freezer until use. The activity of carbohydrate digestive enzymes α–amylase and invertase were measured by the procedure of Ishaaya and Swirski, (1976). Alkaline phosphatase activity was measured by

the method of Bessey *et al*. (1946). The activity of α- and β- esterases were determined according to the method of Van Asperen (1962).

**5. Statistical analysis:**

Probit analysis was done to calculate the median lethal concentrations for all crude extracts and the data were statistically analysed by means of analysis of variance (ANOVA) (Tukey's test) at P<0.05 using IBM® "SPSS" STATISTICS Ver.26 software, most of the results were expressed in percentage, although actual numbers were used for statistical tests. Results were recorded as mean ± standard deviation (SD).

## Results and discussion

**1.Toxicological studies:**

The toxicological effects of *F. nitida* and *E. crassipes* extracts on the 4th instar larvae *G. mellonella* at different concentrations were presented in Table (1) and Figures (1 and 2) , showed that toxicological effect on the 4th instar larvae of G. *mellonella* after 48hrs. of treatment for *F. nitida* extracts (Petroleum ether, acetone, ethanol, chloroform and water) expressed as $LC_{50}$ were 5.638, 10.312, 2.440, 13.359 and 4.394 %, respectively, and for *E. crassipes* extracts for the same solvents were 4.803, 1.860, 2.550, 12.067 and 3.864 %, respectively. From the previous data we can concluded that for *F. nitida* the most effective crude extract was ethanol extract followed by water, petroleum ether, acetone and chloroform, respectively, and for *E. crassipes* the most effective crude extract was acetone extract followed by ethanol, water, petroleum and chloroform extract, respectively.

**Table (1): Effective concentrations of plant extracts on 4th instar larvae of *Galleria mellonella* after 48 hrs. of treatment.**

| Solvent extracts | LC50 | 95% Confidence Limit | | LC90 | 95% Confidence Limit | | Chi square |
|---|---|---|---|---|---|---|---|
| | | Lower | Upper | | Lower | Upper | |
| ***Ficus nitida*** | | | | | | | |
| **Pet. Ether** | 5.638 | 4.348 | 7.936 | .751 | .638 | .900 | .491* |
| **Acetone** | 10.312 | 7.310 | 17.239 | 1.013 | .864 | 1.237 | .945* |
| **Ethanol** | **2.440** | 2.078 | 2.878 | .387 | .318 | .459 | .681* |
| **Chloroform** | 13.359 | 9.101 | 24.296 | 1.126 | .959 | 1.386 | .840* |
| **Water** | 4.394 | 3.514 | 5.768 | .643 | .546 | .761 | .567* |
| ***Eichhornia crassipes*** | | | | | | | |
| **Pet. Ether** | 4.803 | 3.868 | 6.258 | .681 | .587 | .796 | .859[b] |
| **Acetone** | **1.860** | 1.535 | 2.236 | .270 | .186 | .349 | .085[b] |
| **Ethanol** | 2.550 | 2.133 | 3.066 | .406 | .329 | .487 | .126[b] |
| **Chloroform** | 12.067 | 8.528 | 20.337 | 1.082 | .931 | 1.308 | .917[b] |
| **Water** | 3.864 | 3.156 | 4.884 | .587 | .499 | .689 | .940[b] |

**Chi square values are significant at P<0.05 levels.**

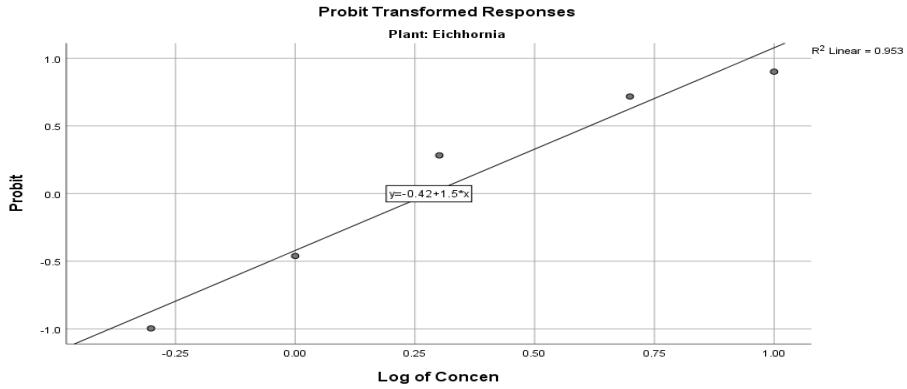


**Figure (1): Mortality curve of *Galleria mellonella* 4th instar larvae for the determination of LC50 of *Eichhornia crassipes* leaf extract.**

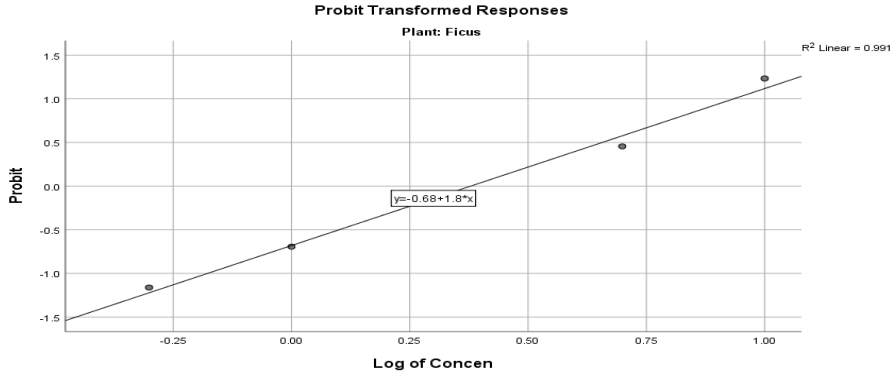


**Figure (2): Mortality curve of *Galleria mellonella* 4th instar larvae for the determination of LC50 of *Ficus nitida* leaf extract.**

## 2. Biological studies:

The effects of the two extracts (*F. nitida* ethanol extract and *E. crassipes* acetone extract) on larval duration, percentage of pupation, pupal weight, and adult emergence of *G. mellonella* were showed in Table (2). Last larval instar weight after treatment with $LC_{50}$ of *E. crassipes* acetone and *Ficus nitida* ethanol extracts were 75 and 60 mg., respectively compared with 146 mg. for the control. Where the larval duration was 25 and 27 days, respectively, while control was 15 days. The pupation percentage was decreased for both extracts, but *F. nitida* ethanol extract was most effective as expressed by the lowest pupation percentage 30%. The weight of the resulted pupae was 80, 70 and 164 mg. for *E. crassipes* acetone, *F. nitida* ethanol extracts and control, respectively.

**Table (2): Biological activities of plant extracts on 4th instar larvae *Galleria mellonella*.**

| Treatments | Last larval instar weight (mg) | Larval duration (days) | Pupation % | Pupal weight (mg) | Adult emergence % |
|---|---|---|---|---|---|
| ***Eichhornia crassipes* acetone extract** | 75 ±3.2b | 25 ±1.5b | 38±4,5b | 80 ±0.2b | 0b |
| ***Ficus nitida* ethanol extract** | 60±1.3b | 27± 0.5b | 30±1.5b | 70±0.01b | 0b |
| **Control** | 146±5.0a | 15 ±4a | 93 ±6a | 164±5.4a | 96 ±7a |

**Values are means ± standard deviation (SD).**

**Means followed by different letters within the same row are significantly different (Tukey's test, $p < 0.05$)**

The present findings showed highly effect in pupal weight, tissue degeneration and preventing adult emergence. These results are confirmed with that obtained by Schloter (1985) who found that the storage of proteins in fat bodies, which is necessary for pupation, did not occur when treated the last larval instar of *E. varivestis* with higher doses of azadirachtin. Larvae take longer to reach the critical weight for ecdysis, which implies an increase of the larval stage duration. Such delays in larval development due to feeding inhibition may result in impaired larval development and unsuccessful emergence of adults. It is likely that this low weight is due to the inhibitory effect of the $LC_{50}$ of the tow extract on α–amylase activity, and at least in part, this contributes to the decrease in growth rate. Alpha-amylase is a major digestive enzyme, that catalyses the hydrolysis of starch into sugars (Nasr *et al.* (2017). As the artificial diet of *G. mellonella* larvae is composed mainly of starch, it obvious that α–amylase plays an essential role in digestion of ingested food. This conclusion could be supported by Batista-Pereira *et al*. (2002) and Abdel-Rahman and Al-Mozini (2007) who mentioned that the mode of action of the *C. procera* as antifeedant may be due to the digestion inhibition through inactivation of digestive enzymes. Also, the results of this study confirmed the suggestion of Upadhyay (2013) that the deleterious effects of *C. procera* latex on insect feeding may be due to presence of α-amylase inhibitors.

**3. Biochemical studies:**

Activities of certain carbohydrate digestive enzymes and detoxification enzymes in *G. mellonella* 4th instar larvae after 48 hrs. feeding on $LC_{50}$ of *E. crassipes* acetone and *F. nitida* ethanol extracts presented in Table (3). Mean activities of α–amylase enzyme was 27.13 ± 0.8 and 18.45±0.5 µg. glucose/min /g protein for larvae fed on *Eichhornia crassipes* acetone and *Ficus nitida* ethanol extracts, respectively and 90.4 ± 2.4 µg. glucose/min /g protein for control. The reduction of α–amylase activity reached more than 3-folds. Also, the activity of alkaline phosphatase for *E. crassipes* acetone extract and *F. nitida* ethanol extract increased significantly compared with the controls 1277.2 ± 23, 1122.1±12 and 909.4 ±25 mg phenol/min/g. protein, respectively. Activities of other detoxifying and carbohydrate hydrolysing enzymes such as α– Esterase, β-Esterase and invertase did not differ significantly than control for both two extracts.

**Table (3): Biochemical activities of plant extracts on 4th instar larvae *Galleria mellonella***

| Treatments | α-amylase (µg glucose/ min /g protein) | Alkaline phosphatase (µg phenol/ min /g protein) | α-esterase (µg α-naphthol / min /g protein) | β -esterase (µg β-naphthol /min /g protein) | Invertase (µg glucose/min /g protein) |
|---|---|---|---|---|---|
| ***Eichhornia crassipes* acetone extract** | 27.13 ± 0.8b | 1277.2 ± 23b | 30.2 ± 1.5a | 20.7 ±1.7a | 20.9 ± 1.5a |
| ***Ficus nitida* ethanol extract** | 18.45±0.5b | 1122.1±12b | 28.05±1.3 a | 17.8 ±1.2a | 18.4±1.4a |
| **Control** | 90.4 ± 2.4a | 909.4 ±25a | 40.2 ± 1.84a | 18.8 ± 1.50a | 20.6 ± 2.3a |

**Values are means ± standard deviation (SD).**

**Means followed by different letters within the same row are significantly different (Tukey's test, $p < 0.05$)**

Esterases (α–EST and β-EST) are important detoxifying enzymes which hydrolyse the esteric bond in toxic chemicals, they have been reported to possess detoxification ability against synthetic and botanical insecticides (Zibaee,2011). The results of this study indicated that feeding *G. mellonella* larvae on artificial diet contains $LC_{50}$ of the tow extracts for 48 hrs did not affect significantly both of α and β esterase activities, it induced an obvious and significantly increase in alkaline phosphatase activity. Increasing alkaline phosphatase activity level indicates that the enzyme is involved in detoxification of certain toxic compounds that could be present in the tow extracts and confirmed the suggestion that portion of the digested food were used by larvae fed on the tow extracts for synthesis of specific detoxification enzymes.

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