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Insecticidal activity of some phytochemical groups of *Calotropis Procera* plant and *Bacillus thuringiensis isrealensis* (HD-1), on the morphology of different developmental stages of *Musca domestica vicina* (Diptera, Muscadae)

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Abstract

The objective of our research is to test the toxicity of LC_{30} of the experimental phytochemical extracted groups from *C. procera;* calactin, calotoxin and calotropin on newly moulted 3rd larval instar, the entomopathogenic effects of *Bacillus thuringensis isrealensis (Bti)* HD-1 strain, LC_{30}, on susceptibility of the resulted adults, as well as their impacts on morphological changes of various developmental stages of *M. domestica*. The results reveled various morphological deformities in all developmental stages. These deformities were in larvae, pupae and adults, like integument abrasions and cuticle bleb in larvae, C-shaped larvae, hyper-pigmentation, constriction in pupal body, under-sized pupae, twisted pupae and poorly developed adults with crumpled legs and curled wings and have short life span than normal ones. The results proved that the calotropin with *(Bti)* is the most efficient in inducing severe morphological abnormalities in various developmental stages of *M. domestica* followed by calotoxin and calactin, respectively. This recommends that the synergism between the tested phytochemical groups of *C. procera* and *(Bti)* is considered as natural insecticides as well as safe to man, animal, plant and their environment.

Keywords: *Musca domestia*, *Calotropis procera*, phytochemical groups, calactin, calotoxin, calotropin, *Bacillus thuringensis isrealensis.*

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1. Introduction

The housefly, *Musca domestica vicina* is considered as one of the most domestic challenges in medical and veterinary fields. *M. domestica* causes various health problems as it accompanies humans and animals during their daily activities in and outdoors. Various control methods are used to bridle the immense hazardous impacts of this insect such as mechanical, chemical, physical, etc. The use of chemical insecticides is currently the most common method, although it's highly risky and very costly. The increasing incidences of insecticides resistance of house fly populations have been reported to most chemical insecticidal groups [1-7]. Recently, resistance to spiasod was conducted by [9]. Also, diflubezuron [10], and synthetic insecticides [11, 12] show high resistance due to prolonged exposure to these chemicals.

Discovering the insecticidal effects of the botanicals with many phytochemicals extracted groups stimulate the investigation to cut down the over usage of manufactured insecticides and looking for eco-friendly alternatives such as those active components that naturally occurred in many plants. *Calotropis. procera* (Family:Asclepiadaceae), a well-known plant with its insecticidal, fungicidal and pesticidal characteristics, is widely distributed in West Africa, Asia, and tropical regions, the plant shows defense strategy against insects, pests, fungi and viruses [13]. Compounds of *C. procera* latex has been analyzed by [14, 15]. Ovicidal characteristics of *C. procera* has been proved against mosquitoes [16]. Regarding *Anopheles* sp., [17] and [18] reported the larvicidal effect of *C. procera* leaf extracts. Also, they proved the toxicity of *C. procera* latex upon egg hatching of *Aedes aegyptei* larvae. As for *M. domestica*, [19] tested the larvicidal tendencies of *C. procera*.

Bacterio-insecticides as an eco-friendly alternative to the synthetic ones were emerged and strongly recommended [20] as they are degradable and completely safe to human, animals, and plants. *Bacillus thuringiensis israelensis*, (*Bti*) gram-positive spores forming bacteria with entomopathogenic properties, is one of the most promising biological solutions.

The proposed scope of the present study is to investigate the insecticidal activity of extracted phytochemical groups of *C. procera* and (*Bti)* strain HD-1 on *Musca domestica* along with their effects on the morphology of different developmental stage of *M. domestica*.

2. Materials and Methods

a. **The tested plant;** The *Calotropis procera* (Family: Asclepiadaceae) plant, obtained from the surrounding area around Benha city, Qaliubia Governorate. The chemical studies of the plant latex revealed the presence of many phytoconsistuents that have, medicinal, insecticidal,

fungicidal…, etc. activities. The pure crystals of the three experimental phytochemical groups; calactin, calatoxin & calotropin are extracted & separated according to [21].

b. **The tested insect;** *Musca domestica* were reared according to [22] standard technique. The rearing of the flies was continued for several generations before starting any experiments to guarantee pure genetic insects for accurate results.

c. The bacterial pathogen: The bacterium, *Bacillus thuringiensis israelensis (Bti)*, strain HD-1, (1600 lU/mg, wettable powder) was produced by The Agricultural Genetic Engineering Research Institute (AGERI) at the Ministry of Agriculture, Giza, Egypt. Subculture from (*Bti)* was grown aerobically at 28 ± 2°C in nutrient broth tubes for 48 hr. to obtain solitary pure colonies. The grown bacteria were harvested by suspending in sterile distilled water. The (*Bti)* suspension was counted by the pour plate count method according to [23].

d. Bioassayes:

I. Toxicity of *Calotropis procera* phytochemical groups on *M. domestica* newly moulted 3rd larval instar

After hatching, groups of 25 newly moulted 3rd larval instars were placed in plastic cups (28 cm in length and 10 cm in diameter). These cups were supplied with a layer of sterilized larval media. Serial concentrations (0.5-2.5 ppm.) of water extracts of the experimental phytochemical groups (calactin, calotoxin and calotropin) were separately applied topically, under bi-nocular microscope, on the dorsal surface of intera segmental thoracic membrane of newly moulted 3rd larval instar (twenty-five larvae) with a dose of 8µl/larva by using Hamilton micro-syringe. Control groups were injected only with equivalent volumes of sterile distilled water**.** The experiment was replicated five times for each *M. domestica* group. All the replicates were held in the same rearing conditions.

II. Susceptibility of *M. domestica* adult groups treated with different experimental phytochemical groups of *C. procera* to the bacterial pathogen; (*Bti),* HD-1:

To determine the effect of the experimental phytochemical groups on the susceptibility level of *M. domestica* adults (2-4 days old) to the bacterium, (*Bti)*, bacterial concentrations were prepared and injected to adult insects. Injection of insects was made with a 10µL Hamilton micro-syringe fitted with a 26-gauge needle according to [24]. Prior to injection, the site of injection was swabbed with 70% ethanol. Injection was made through petroleum jelly, which helped to seal the wound and prevent excessive haemolymph loss following injection. A stock suspension of a concentration of LC_{30} *(Bti)* (that produces 30%mortality); as sub-lethal concentration was injected into the haemocoel of *M. domestica* adults to study the influences and investigate the subsequent consequences. A dosage of 10µL of each bacterial concentration was injected into each insect in the last coxal corium. The needle was inserted laterally and advanced forward parallel to the long axis of the body. After delivering the bacterial dose, the needle was carefully withdrawn to avoid any haemolymph loss from the wound. In case of bleeding or the bacterial suspension discharged during or after injection, the insect will be discarded. Control insects were injected only with equivalent volumes of sterile distilled water**.** Groups of insects (5 replicates), each with 10 individuals in separate cages, were treated with four bacterial concentrations; 1.2 $xl0^4$, 1.2 $xl0^5$, 1.2 $xl0^6$ and 1.2 $xl0^7$ cells/ml (for *M. domestica* adults treated with calactin and calotoxin) and: 2.72 $xl0^4$, 2.72 $xl0^5$, 2.72 $xl0^6$ and 2.72 $xl0^7$ cells/ml (for *M. domestica* adults treated with calotropin). Final mortality percentages were scored at 48 hr. post-treatment to determine the LC_{30}.

III. Treatment of the newly moulted *M. domestica* 3rd larval instar with the phytochemical groups followed by *(Bti)* treatment to the resulted adults:

The extracted phytochemical groups (calactin, calotoxin and calotropin) were separately presented as concentration determined (LC_{30}) and applied topically, under binocular microscope, on the dorsal surface of intera segmental thoracic membrane of newly moulted 3rd larval instar (twenty-five larvae) with a dose of 8 µL/larva by using Hamilton micro-syringe as water extract. The control groups were treated with 8 µL of water only. A stock suspension of LC_{30} *(Bti)*, with a dose of 10µL, was injected into the haemocoel of *M. domestica* adults that previously treated with LC_{30} of (calactin, calotoxin, and calotropin), separately to study the influences the of pathogenic infection of (*Bti)* on the morphology of different developmental stages of the tested insects. The $LC_{30}$was the most logically fitted to induce the immune response of *M. domestica,* yet, it did not induce high mortality rates. Therefore, they were used as sub-lethal concentrations to investigate the subsequent morphological consequences.

The experiment was replicated five times for each *M. domestica* group. All the experiments were held in the same rearing conditions.

3. Results & Discussion

In the present study, extra-efforts were devoted to further investigate and examine the toxicity of calactin, calotoxin and calotropin as experimental phytochemical extracted groups from *Calotropin procera* plant and the entomopathogenic effects of *Bacillus thuringensis isrealensis (Bti)* HD-1 strain bacteria as well against the house fly; *Musca domestica*, that represent one of the most important mechanical pathogens transmitters.

The toxicity of the latex constituents of extracted groups of *C. procera* is shown in table (1) the groups were found to have different LC_{30}, LC_{50}, and LC_{90}. For calactin it is 0.9, 1.2 and 1.8 with slope function (2.0), for calotoxin, it is 0.95, 1.3 and 1.97with slope function (2.01) and for calotropin, it is 0.99, 1.54 and 2.00 with slope function (2.1), respectively.

Table (1): Toxicological impact of the experimental phytochemical groups of *C. procera* against adults of *M. domestica*.

The Phytochemical Groups	Toxicity in ppm.			Slope Function
	LC30	LC 50	LC 90	
Calactin	0.9	1.2	1.8	2.0
Calotoxin	0.95	1.3	1.97	2.01
Calotropin	0.99	1.54	2.00	2.1

The objective of our research is to test the effect of LC_{30} of each experimental phytochemical extracted group of *C. procera* , the susceptibility of *M. domestica* adult that previously treated with the experimental phytochemical groups ,separately, to LC_{30}*(Bti)* as well as their impacts on the morphology of different developmental stages of *M. domestica* and recommending their efficiency as natural insecticide that safe to man, animal ,plant and environment when used as alternative to chemical insecticides in controlling *M. domestica.*

I. Morphology of normal *M. domestica*:

i. Larva, Fully-grown *M. domestica* larvae, also known as maggots, are 12 to 13 mm long and are a yellowish, white color. Their bodies are legless smooth and shiny. They have a pointed anterior end, a blunt posterior end, and two spiracles. A small patch of small spines lies ventrally between abdomen 1 and 7 but is absent on the thoracic segments, Fig (1.a).

ii. Pupa, the pupa of *M. domestica* is hard, brown shells cylindrical, in shape and almost evenly rounded at both ends with very finely transverse striations to protect the inactive developing flies. The size of *M. domestica* pupa is about 5mm in length and 2 mm in width, Fig (1.b).

iii. Adult, the adult of *M. domestica* has short antennae, a gray thorax with four darker longitudinal stripes, and a gray or yellow abdomen with a darker median line and an irregular pale yellowish spot at the anterior lateral margins. The abdomen consists of 8 segments in males and 9 segments in females. In females, the first 5 segments are visible externally. The last 4 segments are normally retracted, but they extend to make the ovipositor when the female lays her eggs. Females are slightly larger than males. Like all flies (Diptera), houseflies have only one pair of wings. The second pair is modified to halteres, which are used for balance. Their wings are translucent and fold back straight at rest. Houseflies are about 4 to 8 mm long, Fig (1.c).

Fig (1): a. Normal *M. domestica* 3rd larval instar (25X).

b. Normal *M.domestica* pupa (25X).

c. Normal *M. domestica* adult (25X).

II. The developmental abnormalities and deformities in *M. domestica*:

The topical application of the experimental phytochemical groups of *C. procera* and (*Bti*) on the early 3rd larval instar of *M. domestica* resulted in various morphological deformations in all developmental stages. The effect of latex active phyto-constituents is enzymatic in nature, which causes unbalance in total carbohydrates, total protein and total lipids [25]. Most of the treated larvae are able to pupate; yet failure in successful metamorphosis, hyper-pigmentation, and cuticle bleb are clearly shown in treated larvae, (Fig.2). Under-sized pupae failed to develop into adults were observed. Pupal intermediates were also detected. Incomplete adults enclosed in the puparium were the most observed. Partially emerged adults were poorly developed with crumpled and curled wings attaching with exuvia were most common, (Figs.3-6). The results proved that the calotropin as a phytochemical group, that extracted from *C.procera*, with *(Bti)* is the most efficient in inducing severe malformations and morphological abnormalities in various developmental stages of *M. domestica* followed by calotoxin and calactin, respectively. [26], investigated the insecticidal `performance of *Sorghumbicolor* and *Verium oleander* against the gray flesh fly; *Parasarcophaga aegyrostoma* larvae. His results clearly indicated

malformations in the survival larval, pupal and adult stages that resulted from treated larvae. [27] studied the effect of *C. procera* extracts against *Cx. pipiens* and *An. multicolor* larvae, results indicated some morphological abnormalities in both mosquitoes. Larva-pupa and pupa-adult intermediates were detected. Also, [28] studied the ultrastructure of blowfly *Chrysoma megacephala* larvae that dipped for 30 sec. in the eucalyptol oil, resulted in aberrant appearances, some parts of the body looked corroded with remarkable swelling in the integument, varied size of bleb formation and deformed spines. The same findings were obtained by several authors; [29-36].

i. In the larval stage:

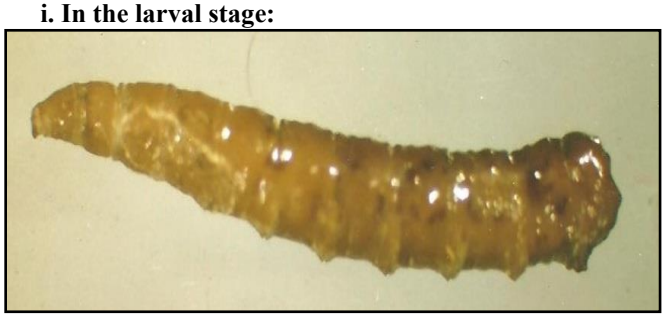
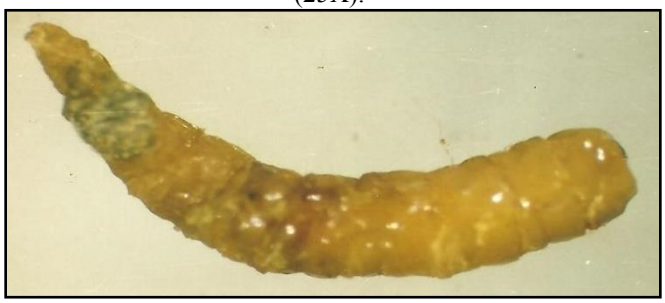


Fig (2): a. *M. domestica* larva with hyper pigmented brown batched integument as treated with calactin and LC_{30} (*Bti)*. (25X).



b. *M. domestica* larva with integumental bleb and dark brown spots as treated with calotoxin and LC_{30} (*Bti)*. (25X).

ii. In the pupal stage:

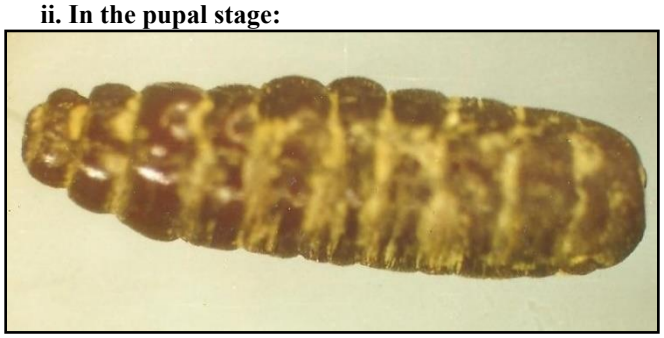


Fig (3): a. *M. domestica* larva-pupal intermediate with the segmented last larval instar skin as treated with calotoxin and LC_{30} *(Bti).* (25X).

b. *M. domestica* hyper pigmented larva-pupal intermediate with the segmented last larval instar skin as treated with calotropin and LC_{30} *(Bti).* (25X)

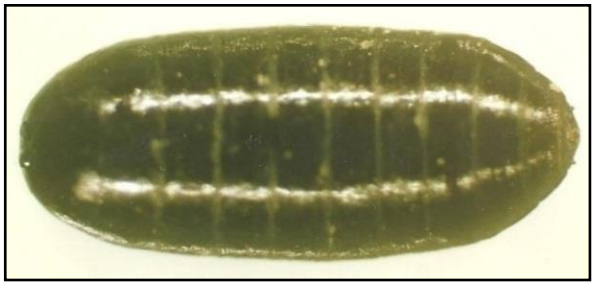


Fig (4): a. *M. domestica* hyper pigmented pupa as treated with calactin and LC_{30} *(Bti)* (25X).

b. *M. domestica* undersized pupa as treated with calotoxin and LC_{30} *(Bti)* (25X).

c. *M. domestica* hyper pigmented and constricted pupa as treated with calotropin and LC_{30} *(Bti).* (25X).

Fig (5): a. *M. domestica* pupa-adult intermediate with a poorly developed adult head as treated with calactin and LC_{30} *(Bti).* (25X).

b. *M. domestica* pupa-adult intermediate with larval form pupal skin as treated with calotoxin and LC_{30} *(Bti).* (25X).

iii. In the adult stage:

Fig (6): a. *M. domestica* malformed adult attached with the puperium at as treated with calactin and LC_{30} *(Bti).* (25X).

b. *M. domestica* malformed adult with a wing attached with the puperium as treated with calotoxin and LC_{30} *(Bti).* (25X).

4. Summary

In the present study, extra-efforts were devoted to further investigate and examine the toxicity of calactin, calotoxin and calotropin as experimental phytochemical extracted groups from *Calotropis procera* (Family: Asclepiadaceae) plant and the entomopathogenic effects of *Bacillus thuringensis isrealensis (Bti)* HD-1 strain bacteria against the house fly; *Musca domestica*, that represent one of the most important mechanical pathogens transmitters. The objective of our research is to test the effect of LC_{30} of each experimental phytochemical extracted group of *C.procera*, the susceptibility of *M.domestica* adult that previously treated with the experimental phytochemical groups ,separately, to LC_{30}*(Bti)* as well as the synergistic impacts of *(Bti)* and each experimental phytochemical extracted group of *C.procera* on the morphological developmental of *M. domestica* and recommend the most efficient that act as natural insecticide as well as safe to man, animal ,plant and environment when used as alternative to chemical insecticides in controlling *M.domestica.*The results showed various morphological deformities and clear impact on the morphology of all developmental stages. Most of the treated larvae are able to pupate, yet failure in the successful metamorphosis by most candidates are reported. Hyper pigmentation and cuticular bleb are clearly detected. In the pupal stage, C-shaped larvae and larval-pupal intermediates with the last larval instar segmented skin. Are observed. Hyperpigmented, under-sized, constricted body shaped pupae and deformed pupae were reported which failed to emerge successfully to adults. This varied from a partial to complete enclosed adults with legs or wings that still attached to the puparium.

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