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Evaluation of Insecticidal Efficacy of *Calotropis Procera* and *Annona Squamosa* Ethanol Extracts Against *Musca Domestica*

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Abstract

In the present study, the crude ethanol extracts of *Calotropis procera* and *Annona squamosa* leaves have been screened for their larvicidal activities against *Musca domestica*. The third instar larvae of housefly were treated with the different concentrations of both the extracts by dipping method for 48 h. The LC_{50} values of the extracts of *C. procera* and *A. squamosa* leaves were found to be 282.5 and 550 mgl^{-1}, respectively. The phytochemical analysis of these extracts suggested presence of alkaloids as the major component. The larvae were exposed to 5 and 10% concentrations of the LC_{50} value of each extract along with their control sets to evaluate their effect on metamorphosis, nucleic acid and protein content in different developmental stages. The leaf extract of *C. procera* was found to be more active in terms of insecticidal potential. The data indicate that the leaf extracts of these plants may be utilized as the probable candidates for the development of bioinsecticides to control the population of *Musca domestica* as safer and economic alternatives to the synthetic insecticides.

Keywords: *Musca domestica, Calotropis procera*, *Annona squamosa,* Bioinsecticide, LC_{50}.

Introduction

With a greater awareness of the hazards associated with the use of synthetic organic insecticides, there has been an urgent need to explore suitable alternative products for pest control. Screening of plant extracts for deleterious effect on insects is one of the approaches in the search of novel biological insecticides [1]. Insecticidal activity of many plants against several insects has been demonstrated [2,3]. Seed as well as foliar extracts of several plants have been reported to have toxic and potent growth reducing activity to insects [4]. The deleterious effect of plant extracts or pure natural/ synthetic compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility.

Exposure of toxic agents to animals can cause changes even at the molecular level. Nucleic acids (DNA and RNA) and protein contents are regarded as important biomarkers of the metabolic potential of cells, as they play the main role in regulating the different activities of cells. Changes in the amount of nucleic acid can be used to detect whether toxic agents affect cellular proliferation and cell death [5,6,7].

The common housefly *Musca domestica* (Diptera:Muscidae) is an important mechanical vector of several bacterial and pathogenic organisms of human and animals [8]. Recently some reports have indicated that on prolonged exposure to chemical insecticides worldwide, houseflies have developed resistance to them e.g. spinasod [9], diflubenzuron [10] and synthetic insecticides [11].

Calotropis procera is a member of the plant family, Asclepiadaceae, a shrub widely distributed in West Africa, Asia and other parts of the tropics. The plant is erect, tall, large, branched and perennial with milky latex throughout .A large quantity of latex can be easily collected from its green parts [12]. Local people use it successfully to combat some cutaneous fungal infections. The abundance of latex (containing alkaloids) in the green parts of the plant reinforces the idea that it produced and accumulated latex as a defence strategy against organisms such as virus, fungi and insects [13]. The presence of plant defence related proteins such as hevein, an alpha-amylase inhibitor has been described to occur in the latex secretion of other plants [14]. However there are no reports to indicate that *Calotropis* leaves and seed extracts exhibit insecticidal properties against *Musca domestica.*

The Annonaceae (Custard- apple family) is a large family of almost exclusively tropical trees and shrubs comprising about 130 genera and 2300 species. Some plants of this family have been used traditionally as insecticides [15]. For example, the powdered seeds and leaf juices of *Annona* spp. are used to kill head and body lice, and bark extract of *Goniothalamus macrophyllus* is used as mosquito repellents. Annonaceous acetogenins extracted from the tree leaves, bark and seeds have pesticidal and/or insect antifeedant properties [16]. However there is no report to indicate that *Annona squamosa* extract possess insecticidal activity against housefly.

Keeping these facts in view, the present study was undertaken to investigate the larvicidal activities of ethanolic extracts of *C. procera* and *A. squamosa* leaves under laboratory conditions as well to assess the chemical nature of the active components present in the extracts along with their effect on nucleic acids and protein content in different developmental stages of housefly.

Materials and Methods

Rearing technique

Adult house flies were collected from local areas using a sweep net and reared in the laboratory at 26±2°C, 60±10% RH, photoperiod 12:12

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(L:D). The rearing method described by Kristensen and Jespersen [10] was adopted in the present study. In brief, the adults of *M. domestica* were fed milk and dry sugar. Mixture of wheat flour and milk was prepared at a weight ratio of 1:3 and 35g of this mixture was placed on a Petri dish with wet cotton as an oviposition site.

Collection and processing of plants sample

Fresh leaves of *C. procera* and *A. squamosa* were collected from the Botanical Garden of University of Allahabad. Leaves were properly cleaned and shade dried for 5-7 days at 32-35°C and relative humidity 50-60%. The dried leaves were powdered mechanically using commercial electrical stainless steel blender (Remi Anupam Mixie Ltd., India). The samples were stored in air tight container at room temperature in dark for further analysis.

Extraction of plant extracts

The dried leaves of *C. procera* and *A. squamosa* were extracted with 1 litre of 90% ethanol in a Soxhlet apparatus (Borosil, India) using the method described by Mishra et al.[17] The extracts were concentrated at 50°C and the residue obtained was stored at 4°C.

Phytochemical analysis of the extract

Qualitative phytochemical analysis of leaf extracts of *C. procera* and *A. squamosa* was done by the method of Mishra et al. [17]. In brief, the phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids and phenols/polyphenols were qualitatively determined as following:

Tannins

Twenty mg extract was dissolved in 2 ml distilled water and filtered. Two ml $FeCl_3$ was added to the filtrate, blue-black precipitate indicated the presence of tannins.

Alkaloids

20 mg extract was dissolved in 2 ml distilled water and filtered. To the filtrate, 2–4 drops of 1% HCl was added and steam was passed through it. To the 1 ml of this solution 6 drops of Wagner's reagent was added. Brownish-red precipitate indicated the presence of alkaloids.

Saponins

To 0.5 ml of the filtrate obtained in alkaloids test 5 ml distilled water was added. Frothing persistence indicated the presence of saponins.

Flavonoids

20 mg extract was dissolved in 10 ml ethanol and filtered 0.5 ml conc. HCl and magnesium ribbon were added to 2ml filtrate. Development of pink-tomato red color indicated the presence of flavonoids.

Terpenoids

Salkovski test was performed using a small amount of extract solution. To this solution 5 drops of conc. H_2SO_4 and 1 ml Chloroform were added. Change of yellow colour into red indicated the presence of terpenoids.

Phenols/polyphenols

A small amount of material was extracted in ethanol and evaporated to dryness. Residue was dissolved in distilled water and 0.5 ml Folin-ciocalteau reagent was added followed by 2 ml 20% Na_2CO_3 solution. Development of bluish color indicated the presence of phenols.

Preparation of experimental concentrations: Stock solution was prepared by dissolving 5 mg extract in 10 ml of ethanol as the *Calotropis* extract does not dissolve in water. This solution was used for making further dilutions. The different extract concentrations such as 100, 200, 300, 400 and 500 ppm were used for further study following trial runs with various concentrations of the extract. In case of *A. squamosa*, the extract concentrations were 200, 400, 600, 800 and 1000 ppm.

Method of treatment

Twenty number of late 3rd instar larvae of *M. domestica* identified by shortening and thickening of size and shape, respectively, darker in color and presence of three spiracles at the posterior end of the body [18] were selected for each set of treatment. Seven numbers of glass beakers of 250 ml capacity were taken and labeled for different concentrations in addition to one for check and one for control. In case of control, water and for check, ethanol was added in place of extract. Larvae were dipped into the solution for two minutes and then transferred back in the rearing medium (composition mentioned above). Each experiment was conducted in triplicates along with the control group. Mortality of larvae followed by the exposure was recorded after 24h up to 48h. LC_{50} was calculated using Karber's method [19]. In brief, the mortality due to treatment of 3rd instar larvae with different concentrations of the leaf extracts of the two plants was recorded after 24 and 48 h. The LC_{50} value was determined as following:

$$LC_{50} = LC_{100} - \frac{\Sigma \text{ Mean death X Concentration difference}}{\text{No. of organisms per group}}$$

The experiment was repeated three times on subsequent days. Same method of treatment was applied for both the extracts. Morphological appearance of the resultant pupae and the subsequent emergence of the adults from it were also observed. To evaluate the insecticidal effect of the leaf extracts of *C. procera* and *A. squamosa*, the 3rd instar larvae of *M. domestica* were treated with 5% and 10% (equivalent to 1/20 and 1/10 of LC_{50} value) for 24 and 48h. The control group was exposed only with the equal volume of ethanol, the solvent in which the extract was prepared.

Biochemical Analysis

Homogenate preparation

After 48 h of exposure larvae were homogenized (10%, w/v) in 50 mM Tris–HCl buffer (pH 7.5) on ice using Potter-Elvehjam homogenizer fitted with a Teflon-coated pestle (Remi, India). The homogenates were centrifuged at 4°C for 10 min at 15,000 g in a refrigerated centrifuge (Sigma, model-3K30, Germany). The corresponding supernatants were either used fresh or kept frozen at -20°C until further use for determining the concentration of different biomolecules.

Total protein content estimation

Protein levels were estimated following the method of Lowry et al. [20] using bovine serum albumin as standard. In brief, the protein samples (tissue extracts) were mixed with the protein reagent (alkaline copper sulphate solution), incubated for 10 min at room temperature followed by addition of Folin-ciocalteau reagent. The reaction mixture was further incubated at room temperature for 30 min. The absorbance of blue color was monitored at 660 nm using spectrophotometer (Systronics Visiscan 167, India). Simultaneously, a blank was also processed containing all the reagents except the protein. Bovine serum albumin (BSA) was used as a standard.

Concentration (ppm)	No. of live Larvae		% Alive at		%Mortality at	
	24 h	48 h	24 h	48 h	24 h	48 h
0 (control)	20	20	100	100	0	0
0 (check)	20	20	100	100	0	0
100	19	17	95	85	5	15
200	17	14	85	80	15	20
300	12	9	60	45	40	55
400	10	8	50	40	50	60
500	05	0	25	0	75	100

The larvae of *M. domestica* (20 in each set) were treated with different concentrations of ethanolic extract of leaves of C. *procera* for 24 and 48 h as shown in Materials and Methods. The control and check represent larval treatment with water and ethanol, respectively. The experiments were conducted in triplicate

Table 1: Toxicity testing of the ethanol extract of *Calotropis procera* leaves.

Concentration (ppm)	Concentration difference	No. of alive larvae	No. of dead larvae	Mean death	Mean death × Concentration difference
00 (control)	0	20	0	0	0
00 (check)	0	20	0	0	0
100	100	17	3	3.0	300
200	100	14	6	4.5	450
300	100	9	11	8.5	850
400	100	8	12	11.5	1150
500	100	0	20	16.0	1600
Total	4350				

The LC_{50} value of the leaf extract of *Calotropis procera* for 48 h has been determined according to the arithmetic method of Karber (1931). The calculation was done as following: $LC_{50} = LC_{100} - \frac{\Sigma \text{ Mean death} \times \text{Concentration difference}}{\text{No. of organisms per group}}$

$$LC_{50} = 500 - \frac{4350}{20}$$

LC_{50} = 500 – 217.5

LC_{50} = 282.5ppm

Table 2: LC_{50} value of the leaf extract of *Calotropis procera* for 48 h.

Concentration (ppm)	No. of live Larvae		% Alive at		% Mortality at	
	24 h	48 h	24h	48 h	24h	48 h
0 (control)	20	20	100	100	0	0
0 (check)	20	20	100	100	0	0
200	19	16	95	80	5	20
400	17	13	85	65	15	35
600	15	10	75	50	25	50
800	13	6	65	30	35	70
1000	8	0	40	0	60	100

The larvae of *M. domestica* (20 in each set) were treated with different concentrations of ethanolic extract of *A.squamosa* leaves for 24 and 48 h as shown in Materials and Methods. The control and check represent larval treatment with water and ethanol, respectively. The experiments were conducted in triplicate

Table 3: Toxicity testing of ethanol extract of *Annona squamosa* leaves against *M. domestica.*

Concentration (ppm)	Concentration difference	No. of alive larvae	No. of dead larvae	Mean death	Mean death × Concentration difference
0 (control)	0	20	0	0	0
0 (check)	0	20	0	0	0
200	200	16	4	2	400
400	200	13	7	5.5	1100
600	200	10	10	8.5	1700
800	200	06	14	12	2400
1000	200	0	20	17	3400
Total	9,000				

The LC_{50} value of the leaf extract of *Annona squamosa* for 48 h has been determined according to the arithmetic method of Karber (1931). The calculation was done as following: $LC_{50} = LC_{100} - \frac{\Sigma \text{ Mean death} \times \text{Concentration difference}}{\text{No. of organisms per group}}$

$$LC_{50} = 1000 - \frac{9000}{20}$$

LC_{50} = 1000 – 450

LC_{50} = 550 ppm

Table 4: The LC_{50} value of ethanol extract of *Annona squamosa* leaves for 48 h.

Ethanol extract from plant leaves	Concentration (mg / l)	Percentage of larvae developed into pupae*	Percentage of pupae emerged into adult**
Calotropis procera	0	100% (20/20)	100% (20/20)
	100	85% (17/20)	70.59% (12/17)
	200	80% (14/20)	64.29 % (9/14)
	300	45% (9/20)	44.44% (4/9)
	400	49% (8/20)	12.50% (1/8)
	500	0% (0/20)	0% (0/0)
Annona squamosa	0	100%(20/20)	100% (20/20)
	200	80% (16/20)	62.5% (10/16)
	400	65% (13/20)	53.85% (7/13)
	600	50%(10/20)	40% (4/10)
	800	30%(6/20)	33.33% (2/6)
	1000	0% (0/20)	0% (0/0)

*Each value in parenthesis is the number of total pupae developed from the larvae/ total number of larvae

**Each value in parenthesis is the number of pupae developed to adults/the number of total pupae

Table 5: Effect of *Calotropis procera* and *Annona squamosa* leaf ethanol extracts on the metamorphosis of *Musca domestica.*

Extraction and estimation of nucleic acids

Nucleic acids (DNA and RNA) were extracted using the method described by Schneider [21]. Calf thymus DNA was used as a standard. Total RNA was estimated by the orcinol method [21] using yeast RNA as a standard.

Statistical analysis

All values in the table are given as mean ± standard error of mean (SEM) of three independent experiments. Graph pad software was used to analyze the data.

Results

The results presented in (Tables 1 and 3) exhibit the toxicity of *Calotropis* and *Annona* leaf extracts against *M. domestica* larvae, respectively. The treatment of 3rd instar larvae of *M. domestica* with different concentrations of the leaf extracts of these two plants exhibited relatively lower percent mortality after shorter duration (24h) than that at longer duration (48h). The exposure of the flies to both of the ethanol extracts caused significant mortality in a dose dependent manner. *A. squamosa* leaf extract exhibited similar level of toxicity to the 3rd instar larvae of *M. domestica* at 200 ppm concentration of the leaf extract with that of *C. procera* causing 20% mortality after 48h. The ethanol extract of *C. procera* was found to be quite effective against *M. domestica* larvae as nearly 100% mortality was observed at 500ppm. The mortality curves for the determination of LC_{50} values for the ethanol extracts of *C. procera* and *A. squamosa* are shown in Figures 1 and 3, respectively. The LC_{50} values of *C. procera* and *A. squamosa* extracts were calculated to be 282.5 and 550 ppm, respectively (Tables 2 and 4). Both the extracts showed antifeedant activity leading to weight loss and reduced size of the treated larvae as compared to control ones.

Pupal deformities followed by *C. procera* treatment included dorsoventral compression, reduction in size, collapsed appendages and their failure to metamorphose into adults. Pupae with certain larval characters were also observed (Figure.2).

The morphological changes like reduced size, darkening of the puparium, condensed appendages and failure to metamorphose were recorded due to the treatment of *A. squamosa* leaf extract. Other morphological appearances of the pupae were not much different from that of control excepting that of reduction in size (Figure.4). The results clearly indicated that the mortality of the larvae in both the cases was dose dependent. It was observed that during the course of metamorphosis, the tendency for the development of test larvae to pupae decreased with a rise in the concentration of the ethanol extracts (Table 5). The larvae treated with the extract of *C. procera* leaves at 500 mg/l exhibited 100% mortality. At lower concentration, some larvae could continuously develop. Similarly, no pupa formation was recorded in case of 1000 mg/l concentration of ethanol extract of *A. squamosa* as it caused 100% mortality of the larvae during the exposure period. In the untreated condition, most of these larvae developed to pupae within 5 days but the pupation period got delayed up to 6-7 days due to treatment with these extracts.

The rate of emergence of adults was also significantly inhibited due to the treatment of both of these extracts and the impact was concentration

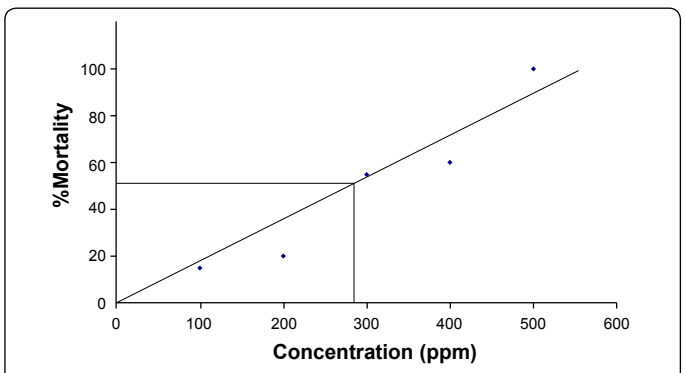

Figure 1: Mortality curve of *Musca domestica* larvae for the determination of LC_{50} of *Calotropis procera* leaf extract for 48 h. The graph was plotted using the data presented in Table 1a.

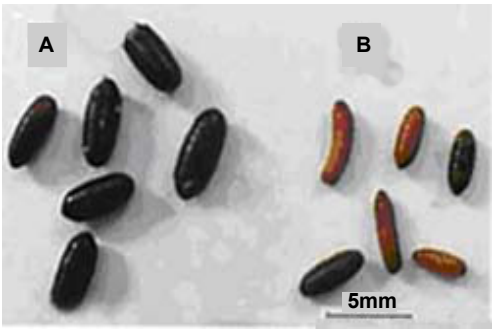

Figure 2: *Musca domestica* pupae showing the morphological changes due to the treatment with the leaf extract of *Calotropis procera* under experimental conditions as mentioned in the Materials and Methods. a –Treated with water (Control) or ethanol (check) for 2 min daily for two days b –Treated with *C. procera* leaf extract daily for two days.

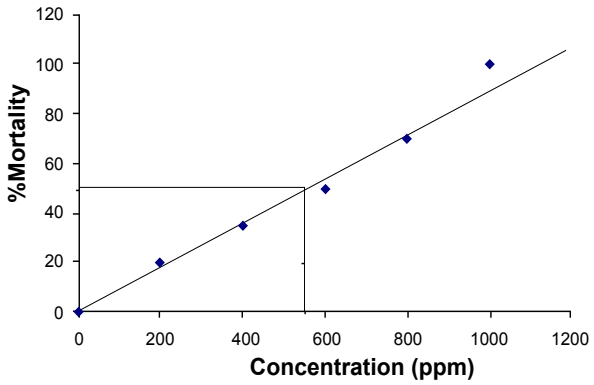

Figure 3: Mortality curve of *Musca domestica* larvae for the determination of LC_{50} of *Annona squamosa* leaf extract. The graph was plotted using the data presented in Table 3a.

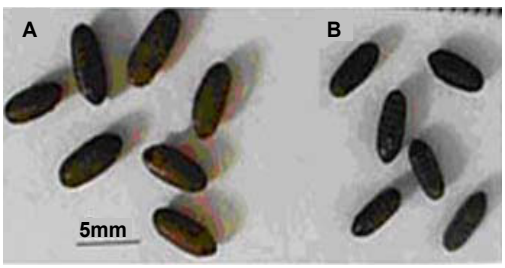

Figure 4: *Musca domestica* pupae showing the morphological changes due to the treatment with *Annona squamosa* leaf extract under experimental conditions as mentioned in the Materials and Methods. a –Treated with water (Control) or ethanol (check) for 2 min daily for two days b –Treated with *Annona squamosa* leaf extract daily for two days.

Chemicals	*Calotropis procera*	*Annona squamosa*
Tannins	_	_
Flavonoids	_	+
Tarpenoids	_	+
Phenols	+ +	+
Alkaloids	+ + +	+ +
Saponins	_	_

The phytochemicals in the ethanol extracts of the *Calotropis procera* and *Annona squamosa* leaves were determined as described in Materials and Methods.
- absent; + moderately present; ++ highly present; +++ very highly present

Table 6: Phytochemical analysis of ethanol extracts of *Calotropis procera* and *Annona squamosa* leaves.

Stage	DNA mg/g wet wt. of tissue			RNA mg/g wet wt. of tissue			Protein mg/g wet wt. of tissue		
	C	5%	10%	C	5%	10%	C	5%	10%
Larva	0.382 ±0.0007	1.911** ±0.00 (-49.98)	0.564* ±0.0015 (-85.71)	0.241 ±0.002	0.225** ±0.0030 (-6.64)	0.182** ±0.0019 (-24.49)	415.22 ± 0.00	291.51** ±0.002 (-29.8)	290.35** ± 0.002 (-30.1)
Pupa	1.446 ±0.002	0.873* ±0.0015 (-39.63)	0.573** ±0.0035 (-60.38)	0.168 ±0.00	0.163^{ns} ±0.0045 (-2.98)	0.108** ±0.0115 (-35.72)	309.63 ±0.0064	261.37** ±0.0075 (-15.59)	240.50** ±0.0035 (-22.31)
Adult	1.719 ±0.0015	1.416^{ns} ± 0.002 (-17.63)	1.392** ±0.00 (-19.03)	0.159 ±0.0015	0.102* ±0.0075 (-35.85)	0.089** ±0.0455 (-44.03)	299.12 ±0.0065	147.75** ±0.0075 (-50.61)	136.75** ±0.0035 (-54.29)

Data were analyzed by Graphpad software,, values given as mean ± SEM Values in parentheses are percent change over control. Significance (*) of data is shown in superscripts, ns P>0.05(non significant), *P<0.05(significant), **P<0.01(very significant), C-Control (0%), 5% & 10% are 1/20 and 1/10 of LC_{50}

Table 7: Effect of ethanol extract of *Calotropis. procera* leaf on the level of nucleic acids and protein after 48 h exposure of *Musca domestica.*

Stage	DNA mg/g wet wt. of tissue			RNA mg/g wet wt. of tissue			Protein mg/g wet wt. of tissue		
	C	5%	10%	C	5%	10%	C	5%	10%
Larva	0.382 ±0.0007	0.213* ±0.0017 (-35.06)	0.134* ±0.002 (-59.15)	0.241 ±0.002	0.237* ±0.0017 (-1.65)	0.211** ±0.0023 (-12.45)	415.22 ± 0.00	332.70* ±0.002 (-19.92)	311.56** ± 0.0019 (-24.97)
Pupa	1.446 ± 0.002	0.935* ±0.0017 (-39.63)	0.611** ±0.003 (-60.38)	0.168 ±0.00	0.165^{ns} ±0.0024 (-1.79)	0.135* ±0.003 (-19.64)	309.63 ±0.0064	280.32^{ns} ±0.0052 (-9.47)	263.54* ±0.0039 (-14.89)
Adult	1.719 ±0.0015	1.493^{ns} ± 0.002 (-17.63)	1.429* ±0.001 (-16.87)	0.159 ±0.0015	0.135^{ns} ±0.0041 (-15.09)	0.117* ±0.0027 (-26.42)	299.12 ±0.0065	173.79** ±0.0059 (-42.03)	166.05** ±0.0048 (-49.49)

Data were analyzed by Graphpad software,, values given as mean ± SEM Values in parentheses are percent change over control. Significance(*) of data is shown in superscripts, ns P>0.05(non significant), *P<0.05(significant), **P<0.01(very significant), C-Control (0%), 5% & 10% are 1/20 and 1/10 of LC_{50}

Table 8: Effect of ethanol extract of *Annona squamosa* leaf on the level of nucleic acids and protein after 48 h exposure of *Musca domestica.*

dependent. The results suggest inhibition of adult emergence by 12.50% due to the treatment of *C. procera* extract (400 mg/l), whereas 33.33% emergence was recorded at 800mg/l concentration of *A. squamosa* extract. However, at highest concentrations (500 and 1000 mg/l, respectively) of *C. procera* and *A. squamosa* extracts, no emergence was observed (Table 5). In the control set of experiments, the adults emerged from pupae within eight days but in case of treated groups the pupation period got delayed resulting in emergence of the adults on 10-11th day.

The phytochemical analysis of the extract (Table 6) showed the presence of alkaloids in maximum amount in ethanol extract of *C. procera* leaves, which was higher than that in *A. squamosa*. The phenolic contents were also found but in lower quantity in both the extracts. In addition, the flavonoids and terpenoids were also found to be present in ethanol extract of *A. squamosa* in traces. These two compounds were found to be missing from *C. procera* extract as determined by the method used in this study. Tannins and Saponins could not be detected in both of these extracts.

DNA, RNA and protein levels were reduced significantly ($p<0.5$) by both plant extracts in the high concentration after 48 h of exposure for stages (larva, pupa, adult) of *M. domestica* (Tables 7, 8).

Discussion

In the present study, the ethanol extracts of the leaves of the plants, *C. procera* and *A. squamosa,* were quite effective against the housefly larvae. These extracts drastically affected the pupation and emergence of the adults from pupae in dose dependent manner.

Previous investigations on annonaceous acetogenin, the bioactive principle of the plant family Annonaceae, have shown that it may have pesticidal or antifeedant properties [22, 23]. Seed oil of *A. squamosa* has been reported to reduce survival of leaf hopper, *Nephotettix virescens* (Hemiptera: Cicadellidae) and transmission of rice tungro virus [24, 25].

Though reports on nematicidal [26], antimicrobial and antihelminthic [27] activities of *C. procera* extract and its use in the treatment of toothache, cough and subcutaneous diseases [28] exist, there is no report at all regarding the LC_{50} for the alcoholic extract of *C. procera* leaf against *M. domestica.* The laboratory study on larvicidal properties of leaf extract of *C. procera* against mosquito larvae is known [29]. The results from the present study indicate the antifeedant property of the *Calotropis* extract which may be due to the different compounds present in the extract possessing different bioactivities.

As shown in (Table 5), the alterations in the rate of pupation and adult emergence due to treatment of the plant extracts were dose dependent. This may be due to the effect of some active ingredients present in the extracts which exhibit potential to cause interference into the normal metabolism of the insects [30].

The phytochemical analysis (Table 6) of the two extracts revealed the presence of alkaloids and phenols only in *C. procera* leaf extract whereas in *A. squamosa,* the presence of alkaloids, phenols, flavonoids and tarpenoids was detected. These phytochemicals may be responsible for the insecticidal nature of the extracts. Previously from the leaves of *A. squamosa*, several flavonoids [31] and a tetrahydroisoquinoline alkaloid with cardiotonic activity [32] have been isolated. Partially purified flavonoids of aqueous *A. squamosa* leaves extract have been

demonstrated to possess antimicrobial and insecticidal activity [33]. Similarly, the alkaloids reported to be present in the latex of *C. procera* have been shown to contain insecticidal properties [13].

Similarly, when 6th instar larvae of *Tribolium castaneum* were treated with higher doses of fenpropathrin, RNA and DNA contents were reduced by 21% and 20%, respectively [34].

Nucleic acids and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating the different activities of cells. Since insects have very little carbohydrate, protein is used to meet the increased energy demand. Proteins are mainly involved in the architecture of the cell which is the chief source of nitrogenous metabolism.

The decreases in total protein level in the different developing stages suggest the high protein hydrolytic activity due to elevation of protease activity. Inhibition of DNA synthesis, thus, might affect both protein as well as protein synthesis machinery. The results of this study suggest that the extracted compound (s) is a potent inhibitor of DNA synthesis, which in turn results in the reduction of RNA level. Carbohydrates are the primary and immediate sources of energy. In stress condition, carbohydrate reserve is depleted to meet energy demand.

The data obtained from the present study clearly indicate that *C. procera* and *A. squamosa* leaf extracts were quite effective as larvicides for providing a better and excellent alternate for the control of *M. domestica.* However, isolation of the active compounds from these plants and further trial assay in the field conditions is underway to explore its suitability for such application.

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