International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

© International Scientific Organization

Bio-efficacy of latex extracts from plant *species Thevetia nerifolia, and Artocarpus heterophyllus, Ficus glomerata and Calotropis procera* on survival, feeding, development and reproductive behavior of *Spodoptera litura (F.) Noctuidae: Lepidoptera).*

R.K. Upadhyay

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur, 273009 U.P. India

Abstract

Laboratory experiments were conducted to evaluate the bio-insecticidal activity of solvent extracts of latex from, *Thevetia nerifolia (Apocynaceae) , Artocarpus heterophyllus (Moraceae), Ficus glomerata (Moraceae), Calotropis procera (Asclepiadaceae).* on neonate larvae of *Spodopetra litura (Lepidoptera :Noctuidae)* a major pest of Indian agricultural crops. The hexanoic, methanoloic, petroleum ether and chloroform extracts (30-1000µg) of each plant latex have caused very high larval mortality ~90-95%, causing significant reduction in larval weight (10.69-62.56%) at sub-lethal concentration in comparison with the control. Larval weights were found drastically reduced at 7d, and further decreased at 14 d. These effects were found to be dose dependent. Further, sub-lethal doses (20-60% of LD50) of each latex extracts have shown significant repellent action in large number of insect larvae (75-90%), inhibited oviposition (19.01-87.28%) in susceptible adult females of *S. litura* and disallow the emergence of F1 individuals (3.24-39.40%) by blocking their development. Further, plant latex fractions have caused additional pupal mortalities of 7-47%. The present study confirms primarily the insecticidal properties of plant latex, their effect on *S.litura*, and their possible future use in bio-pesticide formulation for safe control of insect pests of agriculture crops in integrated management in environment friendly manner.

Key words: Plant latex, insecticidal properties *Calotropis procera, Thevetia nerifolia, Ficus glomerata and Atrocarpus heterophyllus, Spodoptera litura,*

Full length article Received: 11-09-2013 Revised: 14-10-2013 Accepted: 15-12-2013 Available online: 31-01-2014

*Corresponding Author, e-mail: rkupadhya@yahoo.com

1. Introduction

Plant latex is natural plant polymer secreted from laticiferous tissue. It is a milky fluid mainly flows inside laticifers including stems, roots, leaves and fruits of flowering plants. It is an emulsion like sticky material that exudes from various plant parts after having a small injury. It is a mixture of protein rich multi-component fluid contains alkaloids, starch, sugars, oils, resins, and gums [1]. It is a polymer of micro particles that coagulates on exposure to air. It shows deleterious effects in insects mainly causes high mortality in larvae, inhibition of feeding, egg hatching, larval development and obstruct reproduction [2-4]. Latex from few plants contains an elastic polymer related to rubber [5], and form films without releasing potential organic solvent [6]. It also contains cysteine proteases, profilins and chitin-related proteins that act as catalytic enzymes [7] and provide defense against phytopathogenic fungi and other bacterial infections [8,9]. It serves as defense material and prevents herbivorous insects from feeding[10]. Latex from few plant families such Annonaceae, Solanaceae Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae and Rutaceae, possess phytochemicals which showed insecticidal activity [11-13] against many insect pests such as *Culex quinquefasciatus* [14], *Sarcophaga haemorrhoidalis* [15] and *Musca domestica* [16]. Latex from *Asclepias humistrata* (sandhill milkweed) kills newly hatched monarch butterfly caterpillars by taping [17] while *Calotropis procera* and *Ficus racemosa* were found effective against fourth instar larvae of the lymphatic filariasis vector *Culex quinquefasciatus* (Diptera: Culicidae [18]. Plant latex from the Russian weed *Anabasis aphylla* contain alkaloids like nicotine, anabasine, methyl anabasine and lupinine and kill larvae of *Culex pipiens* Linn., *Cx. territans* Walker, and *Cx. quinquefasciatus* Say [11]. However, Persian poppy (*Papaver bracteatum*) and opium poppy (*Papaver somniferum*) latex contains glycosidase inhibitors 1,4-dideoxy-1,4-imino-d-arabinitol (d-AB1) and 1-deoxynojirimycin (DNJ) which show insecticidal properties [11]. Similarly, cysteine protease in latex of papaya (*Carica papaya*) and wild fig (*Ficus virgatalatex*) have shown high toxicity to caterpillars of herbivorous insects [12]. *Spodoptera litura* belongs to family Noctuidae of the order Lepidoptera. It is a highly destructive major polyphagous pest of many agriculture crops like tobacco, castor, paddy,

maize, wheat, barley, groundnut and sugarcane. The adult female moths laid eggs in an irregular furry mass on the underside of a leaf of a food plant. The larvae feed on a wide range of plants and have been recorded from over 40 mostly dicotyledonous plant families. For control of insect pests, several synthetic insecticides are being used but success remains limited due to the development of resistance in the pest against some insecticides. Synthetic chemicals show residual activity, poison the food chain, generate undesirable effects against humans and environment and results in very high killing of non-target organisms. However, over use of synthetic pesticides is restricted because of emerging resistance to synthetic pesticides in insects and there is a felt a very high demand of botanical insecticides/pesticides to replace these highly toxic synthetic chemicals [19, 20]. Bio-pesticides provide a positive alternative to synthetic pesticides because they have low impact on the environmental, and show low toxicity to humans and have low costs. No doubt plant latex has shown better control of insect vectors of agriculture and medical importance. it is a best insect-plant interaction model system in which both counter parts protect themselves with the help of certain chemical constituents mainly proteins. However, so many plant species have been screened to explore their insecticidal property of latex but no one has shown an overall control on insect life cycle by using latex from *Thevetia nerifolia (Apocynaceae), Artocarpus heterophyllus (Moraceae), Ficus glomerata (Moraceae), Calotropis procera (Asclepiadaceae).* The present study aims to protect agriculture crop plants from *S. litura* infestation under laboratory and field conditions using various plant latexes as well as their extracts prepared in different solvents like hexane, methanol, petroleum ether, chloroform and water.

2. Material and Methods

2.1. Insect rearing

Adult insects (moths) *Spodoptera litura* were collected from the local crop fields and reared in laboratory under controlled temperature 30±2^{o} C and 75±5 RH with a photoperiod of 12:12 (L:D) in B. O. D. All the feeding stages were maintained at castor leaves in plastic jars for initial culture. First generation eggs were surface sterilized with 0.02 per cent sodium hypochlorite, rinsed with distilled water and kept in Petri dishes for hatching. After hatching larvae were transferred to feed on artificial diet containing all essential ingredients. The larvae were separated after third instar due to their cannibalistic tendency and were reared individually till pupation in glass vials (7.5 cm h X 2.5 cm diameter) plugged with cotton, containing 7-9 ml diet. For bioassays de-infected second and third generation early age larvae were used for the experiments.

2.2. Artificial diet used

For mass rearing and bio-evaluations following ingredients were mixed in a definite gradations: Agar (2.4%), Sucrose (4.0), gram flour sterol free (12%), choline chloride (0.1%), vitamins i.e. α-tocopherol acetate (0.05%), linoleic acid (0.10%), linolenic acid (0.05%) and 100% ethanol 1.0 % for solubilizing these ingredients. Distilled water to solublize (5.0 ml); niacinamide 0.003%, thiamine Hcl0.00028, calcium pentothenate 0.008%, riboflavin 0.0005%, pyridoxine HCl 0.004%, folic acid 0.0002%, biotin 0.00008%, vitamin B12 0.000025%, ascorbic acid 0.60%, p-hydroxy benzoic acid methyl ester 0.2%, Streptomycin sulpahte 0.05, sorbic acid 0.15, distilled water 74%.

2.3. Collection of plant latex

Plant latex was collected from different plant species i.e. *Thevetia nerifolia* (Peeli kaner), *Artocarpus heterophyllus* (Kathal), *Ficus glomerata* (Gular) and *Calotropis procera* (Madar*)* available in the botanical garden of D.D.U Gorakhpur University, Gorakhpur, India and it peripheral areas. Plant species were identified by applying standard taxonomic key specially by observing inflorescence and family formula with the help of a taxonomic expert. Latex was obtained from various plant parts such as stem, flower, bud and unripe fruits in separate aseptic glass vessels by tapping method at a fixed time interval [21]. For this purpose, sharp incisions were be made on tree trunk to open the latex vessels situated in the bark or fruits were used to cut open from its top then slightly squeezed to collect un-conjugated in sterile plastic vessels from different plant species and store at -20^{0}C until used. Fresh latex samples were used to prepare extracts.

2.4. Extraction/fractionation of plant latex

Collected plant latex samples were lyophilized and powdered in vacuum in cold. Lyophilized latex was extracted with different solvents by changing the polarity. Active fractions from the latex were portioned between different solvents on the basis of their polarity. For better fractionation, solvent extraction was performed by using polar and non-polar solvents. Mostly portioning was done between hexane and aqueous methanol, petroleum ether and chloroform. Further a portion of dried latex was extracted with distilled water, 1.5% acetic acid, 1.5% Sodium bicarbonate and 1.5% sulphuric acid and diethyl ether to separate various fractions by fallowing the method of [22]. Extracts were allowed to evaporate in a SpeedVac vacuum concentrators to get residue. It was dried and weighed and re-dissolved in known volume of different solvents. Dissolved residues were stored in cold at 4^{0} C for experimental purpose. The dried seed powder was extracted separately with acetone, chloroform, diethyl ether and water. The solvent was evaporated and the dry extracts were stored in a desiccator till use for bioassay

Bioassays:

2.5. Dose-response determination

To determine dose response relationship of different latex extracts, different doses (w/v) were given to 3rd instar larvae in glass culture tubes separately. The treatments of different extracts were given in the artificial diet. Twenty 3rd instar larvae were released in culture glass tubes (10 cm height X 4 cm diameter). For each extract, five different doses were used and for each doses 6 replicates were set. After 24 hrs of treatment the number of dead larvae was recorded. The LD_{50} values of each extract against different stages of larvae were calculated by probit method in µg/gm body weight of the insect larvae

2.6. Feeding inhibition responses

For observation of feeding inhibition responses in insects known volume of each latex extract was coated on small pieces of castor leaves (1.5 cm^2) coated with different latex extracts separately were offered to the test larvae, and placed in a tri-arm repellency apparatus in the centre from

open side of one arm. Ten third instar larvae were released inside this arm and plugged with cotton. Small pieces of green caster leaves were kept inside from remaining open sides of the two arms. Numbers of repelled larvae in presence of each latex extract were counted after 30 min and five different concentrations ranging from 0.1-10 µg/gm of each latex extract were used. The ED_{50} values that inhibit the transformation of one larval stage to next larval stage by 50% were also calculated.

2.7. Developmental inhibitory activity

To observe the developmental inhibitory effects, 3rd instar larvae were exposed to the sub-lethal doses of different latex extracts separately. The treatments of different latex extracts were given in the artificial diet. After 24 hrs of treatment, all the treated larval forms were transferred to the fresh diet separately. The larval weight, duration of larval period, pupal weight, percent pupation, pupation period and percent adult emergence were recorded.

2.8. Determination of oviposition deterrence

For determination of oviposition deterrence in *S. litura* female adults were provided various sub-lethal doses (20, 40 and 60% of LD_{50}) of each plant latex extract adding with the sugar solution (2%) separately. For this purpose, separate green leaves of castor (1.5 cm^2) were coated with different concentrations of plant latexes and provided to insects, by placing them in the glass jars covered with muslin cloth. Only sugar solution was used to set control. Six replicates were set for each latex extract. The number of eggs laid recorded after 96 hrs and %ODI (Oviposition Deterrence Index) were calculated.

2.9. Statistical analysis

LD_{50} values of each solvent and aqueous extracts were calculated by applying POLO programme [10]. The efficacy of the test stimuli was compared with control on the basis of oviposition deterrence index (ODI). The %ODI of females was calculated as 100 (A- B)/ (A+ B), A and B being the number of eggs in the control and test, respectively. Repellency in various plant extracts was calculated on the basis of insects repelled in presence of each extract. Data was analyzed to have mean ±SE of each concentration used to deter feeding in beetles. Analysis of variance (ANOVA) was performed to test the equality of regression coefficients (Sokal and Rohlf, 1973).

3. Results and Discussion

3.1. Effect on S. litura survival

Data in table 1 demonstrate that plant latexes were found highly toxic to *S. litura* larvae as in each case LD50 values obtained were very low. Here water extract from all four plant species has been reported to be highly toxic as it has shown an LD50 value in a range of 1.100-5.758 µg/gm body weight of *S. litura* larvae. It is due to water solubility of plant latex. In all the cases methanol extract caused significant mortality at a very low dose of 0.818 µg/gm in methanolic extract of *Thevetia nerifolia*, 1.279 µg/gm in *Artocarpus heterophyllus*, 5.27 µg/gm in *Ficus glomerata* and 2.96 µg/gm in case of *Calatropis procera*. Similarly hexanoic, petroleum ether and chloroform extracts have shown high toxicity to *S. larvae*. In comparison, methanolic and water extracts exhibited higher toxicity in comparison to hexnoic, petroleum ether and chloroform fractions because its low doses caused 75-80% mortality of *S, litura* larvae.

3.2. Effect of plant latex on feeding behavior of Spodopetra litura larvae

Data presented in table 2 shows antifeedant activity of various plant latex extracts from different plant species in *S. litura* third instar larvae. *Thevetia nerifolia* methanolic, petroleum ether, chloroform extracts have shown very high antifeednat activity as ED50 values obtained were very low and fall between 2.07-2.88 µg/gm (Table 2). Its hexanoic extract has shown ED50 value 4.72 µg/gm which represents somewhat lower antifeedant activity. Similarly *Atrocarpus heterophyllus* extracts have shown very high antifeedant activity as ED50 values obtained were very low and fall between 1.27-3.23 µg/gm (Table 2). *Ficus glomerata* methanolic latex extract has shown very high antifeedant activity in S. litura larvae as the ED50 value (0.36 µg/gm) obtained was very low in comparison to control (Table 2). Similarly *Calatropis procera* methanolic latex and chloroform extracts have shown very high antifeednat activity i.e. 0.83 and 0.17 µg/gm. Rest of the latex extracts have shown ED50 between in a range of 1.23-3.28 µg/gm in both the cases(Table 2).

3.3. Effect of plant latex on weights of Spodopetra litura larvae

Table 1 showing the mean weights of larvae treated with the crude extracts at 7d. Statistically significant differences occurred between the mean the weight of larvae in the untreated control (0.0187±0.0029) of those of larvae treated with, hexanoic, methanolic, petroleum ether, chloroform fractions which had mean weights of 0.0142±0.000356, 0.0134±0.00038, 0.0115±0.000170, 0.081±0.00422 gm respectively. Larvae treated with latex fractions have shown significantly lower weights than unfed corresponding control insect larvae. Here, water fraction in all the latex have shown significant reduction in larval body weight i.e. 0.0067± 0.000325 after 7 days of treatment (Table 3). Similarly, *Artocarpus heterophyllus, Ficus glomerata, Calatropis procera* solvent extracts have shown significant reduction in larval body weights after at day 7 and 14 after treatment (Table 3).

3.4. Oviposition inhibitory responses

The data presented in table 4a-4d indicate that plant latex fractions were significantly effective with respective to reduction in oviposition deterrence was recorded 55.60%, 77.61% and 87.28% in water extract of *Thevetia nerifolia* at dose level 1.151, 2.302 and 3.423 µg/gm (20,40, 60% of LD50 of each latex extract). It was found extremely significant at P<0.0004(F=20.530) (Table 4a). Similarly, in methanolic extract oviposition deterrence (64.09) was found significant at P< 0.0318 at (F=5.910). Here, chloroform extract has shown significant oviposition deterrence 75.03% at P< 0..0154(F=6.498) in *S. litura* at sublethal dose of 60% of LD50 plant latex (Table 4a) while hexanoic and petroleum ether fractions have shown non-significant oviposition deterrence (Table 4a). Similarly in case of *Artocarpus heterophyllus* latex hexanoic fraction was found to be non-significant (P<3.121 at F=0.0880 while methanolic, petroleum ether, chloroform and water extracts have shown significant inhibition (P<0.001,F=12.844) in

oviposition (Table 4b). In case of *Ficus glomerata* hexanoic fraction has shown significant reduction in oviposition deterrence P<0.0482 at (F=4.130) to *S. litura* (Table 4c). In case of *Calatropis procera* latex all fractions have shown significant reduction at P<0.01 to P< 0.0001 level (Table 4d). Here, also water and methanoilc and water fractions have shown significant reduction in ovpoistion (table 4d).

3.5. Effect on F1 emergence and survival of pupae

A significant reduction (F=20.530 P<0.001) was found in F 1 emergence in latex extract treated S. *litura* eggs (39.40%) compared with control. Data presented table 3a-3d showed effect of plant latex on F1 emergence in *S. litura*. Here water extract of *T. nerifolia* showed extremely significant F1 emergence at 20, 40, 60% of LD50 value or sub-lethal concentration F= 20.530 P< 0.0004 (Table 4a). It has shown 27-47% mortality in pupae those which were survived after latex exposure in first treatment. Its methanolic extract has shown significant reduction in F1 emergence and % mortality in pupae 11.44-23.90% (F= 5.910, P<0.0318). It was found to be dose dependent. Here plant latex showed 80-90% ovicidal activity and prevented F emergence in eggs laid by treated female insects. First plant latex has deterred the insect from egg laying, second eggs laid were prevented from F1 emergence and those which were emerged were significantly inhibited from development. Plant latex did slow down on larval development and significantly prolonged the period. Those insects which were transformed into pupae, they were died either during transformation period or after 2nd or 3rd day of pupation. There was observed an overall 14-47% mortality in pupae emerged from the total survived population of larvae (Table 4a-4d). Similarly *Artocarpus heterophyllus* latex water extract has shown very significant reduction in emergence of F1 larvae 3.74-11.47% (F=12.844, P<0.0020) and high % mortality in pupae 14-43% at sub-lethal concentration (20-6-% of LD50 value) (table 4b). Similarly water extract of *Ficus glomerata* has shown significant reduction in F1 emergence 4.92-19.43% (Table 4c) and high % mortality in pupae 21-47% at sub-lethal concentration. Similar activity against larval emergence and % mortality in pupae was obtained in methanolic extract of *Ficus glomerata* (Table 4c). More specifically almost all solvent extracts hexanoic, methanolic, petroleum ether, chloroform and water have shown significant reduction in F1 emergence (3.24-14.14%) and high % mortality in pupae (8-45%) (Table 4d).

3.6. Effect of plant latexes on larval development

Toxicity of water extract of *Thevetia nerifolia* has significantly slow down the development rate of larval stadia when it was added in artificial diet of *S. litura* (Table 5). As latexes from *Thevetia nerifolia* (Peeli kaner), *Artocarpus heterophyllus* (Kathal), *Ficus glomerata* (Gular) and *Calotropis procera* (Madar*)* have prolonged the larval period from days 32.116± 1.195, 33.00±1.290, 32.00±0.966, 33.16± 1.07) in *S. litura* larvae in comparison to control 17.33±0.421 (Table 5). Here choloroform extract of *Thevetia nerifolia* as shown least effect on developmental rate of larval stadia (17.5±0.562) followed by methanolic extract 25.166±1.1420, 20.33±0.945 respectively (Table 5).

4.0. Discussion

In the present investigation various latex extracts prepared from different plant species were found to be highly toxic to *S. litura* larvae and have imposed significant deleterious effects on survival of larvae and pupae, inhibited feeding, oviposition, egg hatching and F1 emergence, reduced the larval weight and delayed larval development. In toxicity bioassays latex extracts from different plant species were proved highly toxic as the LD50 values obtained were very low in each case against *S. litura* 3rd instar larvae. These were found to be dose dependent and significant in comparison to corresponding control. However, methanol extract of plant latexes caused very high mortality (80-95%) in *S. litura* larvae at a very low dose (table 1). The LD50 values shown here are falling in the range between 0.296-1.279. Similarly, other solvent extracts such as hexanoic, petroleum ether and chloroform have also shown almost similar but higher toxicity to *S. litura* 3rd instar larvae and caused 75-80% mortality (table 1). Similarly, water extracts from different plant species were also found highly toxic to *S. litura* 3rd instar larvae as these have shown lower LD50 values i.e. 1.100-5.758 µg/gm body weight (Table 1). It may be due to presence of certain active latex components such as toxins [3], acetogenins [13] [23], flavonoids [24], Triterpenes [25], alkaloids [26, 27], lectins [28, 29], latex proteins [30][4]. Similarly, plant latex from *Asclepias humistrata* (sandhill milkweed) kills newly hatched monarch butterfly caterpillars [17].

Similar insecticidal efficacy is observed in *Annona Squamosa* latex was against third instar larvae of *Musca Domestica* [31,32] at an LC50 of 282.5 and 550 mgl^{-1}, respectively. Similarly, plant latex from *Havea brassilensis* , *Ficus sp*. [12], *Carica papaya* [13], *Asclepias humistrata* [14] and Persian poppy (*Papaver breateatum*), [11], *Goniothalamus macrophyllus* [13] and *Annonaceous acetogenins* [23] have shown strong insecticidal activity against herbivorous insects [10]. *Ficus racemosa* latex contains Gluanol acetate a tetracyclic tri-terpene that shows larvicidal effect in mosquito [18] and also show high mortality in fourth instar larvae of the lymphatic filariasis vector *Culex quinquefasciatus* (Diptera: Culicidae) [18] It also killed 50% of the larval population at a concentration of 0.0062% (V/V) and 0.4796% (V/V) [33] Similarly, *Calotropis procera* was found active against third larvae of *Musca domestica* at a topically dose of 3 µg (5% of the latex) [16]. It contains alkaloids, steroids and resinous substances that display toxicity upon egg hatching and larvae of *Aedes aegypti* [34]. It also shows larvicidal activity [35] against *Anopheles stefensi* and *Culex quinquefasciatus* [36] and oviposition inhibition in females of *Aedes aegypti* [37]. Persian poppy (*Papaver bracteatum*) and opium poppy (*Papaver somniferum*) latex contains glycosidase inhibitors 1, 4- dideoxy-1, 4-imino-d-arabinitol (d-AB1) and 1-deoxynojirimycin (DNJ) that showed insecticidal properties [11].

Furthermore, latex extracts have shown very high anti-feedant activity in *S. litura* third instar larvae. *Thevetia nerifolia* methanolic, petroleum ether, chloroform extracts have shown very high antifeednat activity as ED50 values obtained were very low and fall between 2.07-2.88 (Table 2). Its hexanoic extract has shown ED50 value 4.72 µg/gm which represents somewhat lower antifeedant activity. Simailrly *Atrocarpus heterophyllus* extracts have shown

very high antifeedant activity as ED50 values obtained were very low and fall between 1.27-03.23 µg/gm (Table 2). *Ficus glomerata* methanolic latex extract has shown very high anti-feedant activity in *S. litura* larvae as the ED50 value (0.36 µg/gm) obtained was very low in comparison to control (table 2). Similarly *Calatropis procera* methanolic latex and chloroform extracts have shown very high anti-feedant activity i.e. 0.083 and 0.075 µg/gm. Rest of the latex extracts have shown ED50 between in a range of 0.123-0.328 µg/gm in both the cases(table 2). This anti-feedant activity may be due to presence of some distasteful components such as glycosides, alkaloids, resins, tannins and saponins that show diverse biological activities. It also contains enzymatic proteins such as chitinases, proteases, peptidases, plasmins, papain, hevein, lectins and lipases which may be also distasteful or allergic to the insects and might have shown enormous insecticidal activity against insects. Few other botanicals such as glycosidase inhibitors were found active against various insect species [11].

Further, plant latex extract treatments showed statistically significant differences in mean weight of larvae in the untreated or unfed corresponding controls (0.0187±0.0029) and treatment with, hexanoic, methanolic, petroleum ether, chloroform fractions which had mean weights of 0.0142±0.000356, 0.0134±0.00038, 0.0115±0.000170, 0.081±0.00422 gm respectively. Here water fraction in all the latex has shown significant reduction in larval body weight i.e. 0.0067± 0.000325 after 7 days of treatment (table 3). Similarly *Atrocarpus heterophyllus, Ficus glomerata, Calatropis procera* solvent extracts have shown significant reduction in larval body weights after at day 7 and 14 after treatment (Table 3). These results suggest growth inhibitory action action of plant latex components at cellular level which might inhibit or cut down biosynthesis of certain body constituents such proteins.

Similarly plant latex fractions were found to be significantly effective with respective to reduction in oviposition deterrence. As it was recorded 55%, 77.61% and 87.28% in water extract respectively at 20-60% of LD50 of *Thevetia nerifoila* latex extract (Table 4aa). It was found extremely significant at P<0.0004(F=20.530). Here chloroform extract has shown higher oviposition deterrence in *S. litura* at 60% of LD50 of S. 75.03% in case of *Thevetia nerifoila*. (Table 4a). Similarly, methanolic extract was found significant at P< 0.0318 at (F=5.910) in case of *Ficus glomerata* oviposition deterrence in case of chloroform extract was also found significant at P<0.0154(F=6.498) while hexanoic and petroleum ether fractions have shown non-significant oviposition deterrence. Similarly in case of *Atrocarpus heterophyllus* latex, hexanoic fraction was found to be non-significant while methanolic, petroleum ether, chloroform and water extracts have shown significant inhibition in oviposition (Table 4b). In case of *Carica papaya* hexanoic fraction has shown significant reduction in oviposition deterrence P<0.0482 at (F=4.130) to *S. litura* (Table 4c). Here also water and methnoilc and water extracts have shown significant reduction in oviposition (Table 4c). In case of *Calatropis procera* latex all fractions have shown significant reduction at P<0.01 to P< 0.0001 level (Table 4d).

Beside action on insect larvae plant latex also effect egg hatching and embryonic development in insects. Further, there was found a significant reduction (F=20.530 P<0.001) F1 emergence in latex treated S. *litura* eggs compared with corresponding control (Table 4a-4d). Here water extract of *T. nerifolia* showed extremely significant F1 emergence at 20, 40, 60% of LD50 value or sub-lethal concentration F= 20.530 P< 0.0004 (table 3a). It has shown 27-47% mortality in pupae those, who were survived after latex exposure in first treatment. Its methanolic extract has shown significant reduction in F1 emergence and % mortality in pupae 11.44-23.90% (F= 5.910, P<0.0318) (Table 4a). It was found to be dose dependent. Here plant latex showed 80-90% ovicidal activity and prevented F1 emergence in eggs laid by treated female insects of S. *litura*. First plant latex has deterred the insect from egg laying, second eggs laid were prevented from F1 emergence and those which were emerged were significantly inhibited from development. Plant latex did slow down on larval development and significantly prolonged the period. Those insects which were transformed into pupae, they were died either during transformation period or after 2nd or 3rd day of pupation. There was observed an overall 14-47% mortality in pupae emerged from the total survived population of larvae (Table 4a-4d).

Similarly *Artocarpus heterophyllus* latex water extract has shown very significant reduction in emergence of F1 larvae 3.74-11.47% (F=12.844, P<0.0020) and high % mortality in pupae 14-43% at sub-lethal concentration (20-6-% of LD50 value) (Table 4b). Similarly water extract of *Ficus glomerata* has shown significant reduction in F1 emergence 4.92-19.43% (Table 4c) and high % mortality in pupae 21-47% at sub-lethal concentration. Similar activity against larval emergence and % mortality in pupae was obtained in methanolic extract of *Ficus glomerata* (Table 4c). More specifically almost all solvent extracts hexanoic, methanolic, petroleum ether, chloroform and water have shown significant reduction in F1 emergence (3.24-14.14%) and high % mortality in pupae (8-45%) (Table 4d).

Further, plant latex from *T. nerifolia, A. heterophyllus, Ficus glomerata, and C. procera* water extracts have significantly slow down the development rate of larval stadia when it was added in artificial diet of *S. litura* separately (table 5). It has prolonged the larval period from days 32.116± 1.195, 33.00±0.966 to 33.16± 1.07) in *S. litura* larvae in comparison to control17.33±0.421 (Table 5). Here, chloroform extract has shown least effect on developmental rate of larval stadia (17.5±0.562) followed by methanolic extract 25.166±1.1420, 20.33±0.945 respectively (Table 5).

Calotropis procera showed toxic effects upon egg hatching and larval development of *S litura* and causes 100% mortality of 3rd instars within 5 min when treated with water-soluble dialyzable fraction [38]. Similarly, leaf extract of *C procera* inhibit oviposition behavior in *Aedes aegypti* [37]. Similarly, latex of *Calotropis procera* was found active against third larvae of *Musca domestica* at a topically dose of 3 µl (5% of the latex) [16].

Table 1: LD_{50} of values of different solvent extracts of Plant latexes from *Thevetia nerifolia* (Peeli kaner), *Artocarpus hetrophyllus* (Kathal), *Ficus glomerata* (Gular) and *Calotropis procera* (Madar) against *Spodoptera litura 3rd instar larvae*.

Extracts	hr	LD_{50} Values (µg/gm) ($p<0.05$)	LCL	UCL	t-ratio	Slope Value	Heterogeneity	Chi-test
Thevetia nerifolia								
Hexanoic	24	11.133	9.229	13.027	5.980	2.871	0.775	3.876
Methanolic	24	0.818	0.616	1.019	5.694	2.622	1.1229	5.6145
Petroleum ether	24	3.669	3.027	4.311	5.842	2.769	0.603	3.015
Chloroform	24	6.958	5.768	8.142	5.980	2.871	0.775	3.876
Water	24	5.758	4.866	6.657	6.346	3.168	0.929	4.647
Artocarpus heterophyllus								
Hexanoic	24	6.406	5.183	7.609	5.646	2.582	0.704	3.519
Methanol	24	1.279	1.046	1.504	5.998	2.820	0.771	3.854
Petroleum ether	24	6.406	5.183	7.609	5.646	2.582	0.704	3.519
Chloroform	24	5.466	4.517	6.402	5.987	2.864	0.460	2.302
Water	24	4.113	3.417	4.802	6.077	2.941	0.691	3.456
Ficus glomerata								
Hexane	24	6.224	5.152	7.298	5.909	2.815	0.711	3.553
Methanol	24	0.527	0.429	0.623	5.791	2.680	0.877	4.386
Petroleum ether	24	3.407	2.658	4.191	6.188	2.205	0.622	3.111
Chloroform	24	1.059	0.819	1.293	5.913	2.762	1.006	5.0335
Water	24	1.400	1.166	2.270	6.093	2.949	0.502	2.509
Calotropis procera								
Hexane	24	6.334	5.352	7.323	6.336	3.168	0.929	4.647
Methanol	24	0.296	0.237	0.358	6.147	3.021	1.0422	5.211
Petroleum ether	24	3.518	2.790	4.262	6.365	3.190	1.1759	5.879
Chloroform	24	5.935	5.000	6.892	6.173	3.052	0.834	4.170
Water	24	1.100	0.706	1.483	5.730	2.885	2.35	11.752

[a] LD50 values represents lethal dose that cause 50% mortality in the test insects. [b]LCL and UCL mean lower confidence limit and upper confidence limit

Table 2: Percent repellency obtained in solvent extracts of plant latexes from *Thevetia nerifolia, Artocarpus heterophyllus, Ficus glomerata, Calotropis procera to Spodoptera litura* third instar larvae.

Extracts/ Single fractions Single fractions	Concentration range in µg	Mean no. of Insects repelled	Expected no. of insect repelled	$\chi 2$ value	ED_{50}
Thevetia nerifolia					
Hexanoic	1.38-9.66	10.33	10	1.104	4.72
Methanolic	0.81-5.68	11.83	10	2.080	2.70
Petroleum ether	0.91-6.37	12.66	10	3.087	2.88
Chloroform	0.86-6.02	11.00	10	2.734	2.72
Water	0.71-4.97	10.16	10	7.356	2.07
Artocarpus heterophyllus					
Hexanoic	1.00-7.00	10.33	10	3.553	3.23
Methanolic	0.31-2.17	12.33	10	1.358	1.27
Petroleum ether	0.68-4.76	11.16	10	2.528	2.04
Chloroform	0.42-2.94	10.66	10	3.214	1.24
Water	0.51-3.57	11.33	10	1.261	1.47
Ficus glomerata					
Hexanoic	0.77-5.39	10. 83	10	3.091	2.21
Methanolic	0.10-1.00	12.50	10	2.509	0.36
Petroleum ether	0.85-5.95	10.16	10	4.194	2.23
Chloroform	0.26-1.82	11.16	10	4.297	0.75
Water	0.70-4.90	11.66	10	2.733	1.95
Calatropis procera					
Hexanoic	0.39-2.73	10.83	10	2.734	1.23
Methanolic	0.29-2.03	12.33	10	1.214	0.83
Petroleum ether	0.85-5.95	10.5	10	2.842	2.55
Chloroform	0.37-2.59	11.83	10	2.548	1.77
Water	1.10-0.770	11.00	10	2.391	3.28

a. Not significant as the calculated values of χ^2 were less than the table values at all probability levels (90%, 95% and 99%) .
b. Significant at all probability levels (90%, 95% and 99%)
The data responses lines would fall within 95% confidence limits and thus the model fits the data adequately. UCL-LCL* Upper confidence limit and lower confidence limit.

Table 3: Effect of plant latexes on body weights of *Spodoptera litura* 3rd instar larvae.

Treatment (Plant latexes)	*Sopodoptera litura* larval weight (gm) mean ± SD 7 days	14 days
Thevetia nerifolia		
Control	0.0187±.00029	0.3147± .000766
Hexanoic	0.0142±0.000356	0.226±0.0001161
Methanolic	0.0134 ± 0.00038	0.2447±0.001379
Petroleum ether	0.0115±.000170	0.2208±0.00048
Chloroform	0.081±.000422	0.1639± 0.0008496
Water	0.067±.000325	0.0719±0.00109
Artocarpus heterophyllus		
Hexanoic	0.0127±0.000325	0.2231± 0.0007398
Methanolic	0.0143±0.000395	0.2318±0.003621
Petroleum ether	0.0114±0.000431	0.2217±0.000462
Chloroform	0.070±0.000475	0.1460±0.0013
Water	0.0623±0.000382	0.0709± 0.0000822
Ficus glomerata		
Hexanoic	0.0167±3.117	0.234±0.00090
Methanolic	0.0152±2.713	0.2446±0.0009259
Petroleum ether	0.0132±0.000260	0.2220±0.0005270
Chloroform	0.0786±0.000295	0.1473±0.000591
Water	0.0708±0.0002227	0.0872±0.000853
Calotropis procera		
Hexanoic	0.0164±0.0002512	0. 2047± 0.001257
Methanolic	0.0145±0.0001932	0.1966± 0.0011
Petroleum ether	0.0127±0.00017	0.1640±0.000884
Chloroform	0.07166±0.0003711	0.1333±0.0006824
Water	0.0064±0.000203	0.0745±0.00031162

Table 4a: Efficacy of solvent extracts plant latexes from *Thevetia nerifolia* on oviposition behavior of *Spodoptera litura* females.

Fraction used	Dose applied	Mean no. of eggs laid per insect Mean ± SE	% eggs laid per insect Mean ± SE	%ODI [B]	F1 Emergence	% Mortality of Pupae	F-value [C] At df 3 & 8
Hexanoic	20% of LD_{50}	70.66 ±1.406	58.72±0.983	26.00	36.04	14	F=3.562
	40% of LD_{50}	32.16±0.477	26.73±0.312	57.81	16.56	20	P=0.060
	60% of LD_{50}	28.16±0.600	23.40±0.419	64.22	9.43	29	NQS*
Methanolic	20% of LD_{50}	60.83±1.74	50.50±1.217	32.84	23.90	16	F=5.910
	40% of LD_{50}	35.83±1.558	29.77±1.090	54.10	15.73	26	P=0.0318
	60% of LD_{50}	26.33±0.760	21.88±0.531	64.09	11.44	37	Significant
Petroleum ether	20% of LD_{50}	67.33±0.918	55.95±0.642	28.24	37.23	13	F=4.003
	40% of LD_{50}	30.83±0.703	25.62±0.491	59.20	14.74	18	P=0.0518
	60% of LD_{50}	22.66±1.33	18.83±0.930	68.29	8.32	27	NS**
Chloroform	20% of LD_{50}	54.00±0.856	44.87±0.599	38.04	20.22	19	F=6.498
	40% of LD_{50}	30.50±0.223	25.34±0.156	59.55	11.37	29	P=0.0154
	60% of LD_{50}	17.166±0.654	14.26±0.394	75.03	6.14	37	NS
Water	20% of LD_{50}	34.33±1.173	28.53±0.820	55.60	16.22	27	F=20.530
	40% of LD_{50}	15.166±0.654	12.60±0.457	77.61	6.64	37	P=0.0004
	60% of LD_{50}	8.166±0.401	6.786±0.208	87.28	3.17	47	Extremely Significant

[A]The chemical stimulus was coated on the Whatmann filter paper stripes (1 cm^2) in the oviposition inhibition test. [B] the ODI% was calculated as 100(A-B)/ A+B, where A and B represent the number of eggs laid in the control and in the test respectively. [C] F-values were significant at all probability levels (90, 95 and 99%). NQS*, Not quite significant, NS**=Non significant

Table 4b: Efficacy of solvent extracts plant latexes from *Atrocarpus heterophyllus* on oviposition behavior of *Spodoptera litura* females.

Fraction used	Dose applied	Mean no. of eggs laid per insect Mean ± SE	% eggs laid per insect Mean ± SE	%ODI B	F1 Emergence	% Mortality of Pupae	F-value [C] At df 3 & 8
Hexanoic	20% of LD50 40% of LD50 60% of LD50	79.83±2.181 43.83±1.013 26.50±0.881	66.34±1.52 36.42±0.708 22.02±0.616	20.23 46.59 67.71	25.77 17.52 9.52	19 28 36	F=3.121 P=0.0880 NQS*
Methanolic	20% of LD50 40% of LD50 60% of LD50	51.33±0.760 25.83±0.600 19.66±0.802	42.66±0.531 21.46±0.419 16.34±0.561	40.194 64.65 71.90	23.56 13.47 7.43	15 23 37	F=9.143 P=0.0058 Very Significant
Petroleum ether	20% of LD50 40% of LD50 60% of LD50	65.00±1.527 33.83±1.249 21.83±0.600	54.01±1.068 28.11±0.874 18.14±0.419	29.85 56.108 69.28	34.07 21.82 11.32	12 19 31	F=4.229 P=0.057 Considered Significant
Chloroform	20% of LD50 40% of LD50 60% of LD50	69.16±1.447 37.5±0.763 22.16±0.600	57.47±1.013 31.16±0.533 18.42±0.419	35.40 52.48 68.88	37.14 20.08 9.12	7 12 23	F=4.556 P=0.0384 Considered Significant
Water	20% of LD50 40% of LD50 60% of LD50	41.5±1.08 19.16±0.601 10.66±0.666	34.48±0.755 15.92±0.421 8.86±0.466	48.71 72.52 83.71	11.47 6.35 3.74	14 24 43	F=12.844 P=0.0020 Very Significant

[A]The chemical stimulus was coated on the Whatmann filter paper stripes (1 cm^2) in the oviposition inhibition test. [B] the ODI% was calculated as 100(A-B)/ A+B, where A and B represent the number of eggs laid in the control and in the test respectively. [C] F-values were significant at all probability levels (90, 95 and 99%). NQS*, Not quite significant, NS**=Non significant

Table-4c: Efficacy of solvent extracts plant latexes from *Ficus glomerata* on oviposition behavior of *Spodoptera litura* females.

Fraction used	*Dose applied*	*Mean no. of eggs laid per insect Mean ± SE*	*% eggs laid per insect Mean ± SE*	*%ODI B*	*F1 Emergence*	*% Mortality of Pupae*	*F-value C At df 3 & 8*
Hexanoic	20% of LD50 40% of LD50 60% of LD50	63.00±0.577 38.00±0.856 17.66±0.307	52.35±0.403 31.57±0.599 14.68±0.214	32.14 52.00 74.39	31.24 19.16 7.72	13 18 29	F=4.130 P=0.0482 Significant
Methanolic	20% of LD50 40% of LD50 60% of LD50	44.66±1.256 23.00±0.365 11.16±0.307	37.11±0.878 19.11±0.249 9.27±0.214	45.65 67.90 83.01	20.55 10.12 4.77	17 32 43	F=10.558 P=0.00307 Very Significant
Petroleum ether	20% of LD50 40% of LD50 60% of LD50	65.33±0.760 30.66±0.557 14.33±1.406	54.29±0.531 25.48±0.389 11.91±0.983	29.02 59.38 78.71	39.40 14.34 6.37	12 20 27	F=431 P=0.0725 NQS
Chloroform	20% of LD50 40% of LD50 60% of LD50	74.00±0.894 32.00±0.577 16.33±0.614	61.49±0.625 26.59±0.403 13.57±0.429	23.84 57.98 76.09	38.11 14.44 8.04	19 17 24	F=2.724 P=0.1143 NS*
Water	20% of LD50 40% of LD50 60% of LD50	43.33±1.115 21.66±0.333 10.50±0.428	36.01±0.780 18.00±0.233 8.725±0.299	47.14 69.48 83.94	19.43 8.66 4.92	21 43 47	F=11.389 P=0. 0029 Very Significant

[A]The chemical stimulus was coated on the Whatmann filter paper stripes (1 cm^2) in the oviposition inhibition test. [B] the ODI% was calculated as 100(A-B)/ A+B, where A and B represent the number of eggs laid in the control and in the test respectively. [C] F-values were significant at all probability levels (90, 95 and 99%). NQS*, Not quite significant, NS**=Non significant

Table-4d: Efficacy of solvent extracts plant latexes from *Calatropis procera* on oviposition behavior of *Spodoptera litura* females.

Fraction used	Dose applied	Mean no. of eggs laid per insect Mean ± SE	% eggs laid per insect Mean ± SE	%ODI [B]	F1 Emergence	% Mortality of Pupae	F-value [C] At df 3 & 8
Hexanoic	20% of LD_{50} 40% of LD_{50} 60% of LD_{50}	30.16±0.307 21.16±0.477 16.83±0.307	68.04 47074 37.96	19.01 35.36 44.95	14.14 7.43 4.32	14 23 33	F=10.755 P=0.0035 Very significant
Methanol	20% of LD_{50} 40% of LD_{50} 60% of LD_{50}	24.5±0.562 16.33±0.333 10.16±0.307	55.26 36.82 22.93	28.81 46.15 62.69	9.57 7.22 4.77	17 31 40	F=8.495 P=0.0072 Very significant
Petroleum ether	20% of LD_{50} 40% of LD_{50} 60% of LD_{50}	28.0±0.365 18.33±0.333 12.5±0.223	63.15 41.35 28.19	22.58 41.48 56.01	13.65 8.25 4.38	9 16 29	F=7.176 P=0.0117 significant
Chloroform	20% of LD_{50} 40% of LD_{50} 60% of LD_{50}	29.0±0.632 18.83±0.307 9.5±0.223	65.41 42.48 21.42	20.90 40.36 64.70	11.24 9.04 4.38	8 17 31	F=4.339 P=0.0431 Significant
Water	20% of LD_{50} 40% of LD_{50} 60% of LD_{50}	24.33±0.333 15.16±0.307 8.16±0.307	54.88 34.21 18.42	29.12 49.01 68.88	11.20 7.22 3.24	20 36 45	F=6.938 P=0.0129 significant

[A]The chemical stimulus was coated on the Whatmann filter paper stripes (1 cm^2) in the oviposition inhibition test. [B] the ODI% was calculated as 100(A-B)/ A+B, where A and B represent the number of eggs laid in the control and in the test respectively. [C] F-values were significant at all probability levels (90, 95 and 99%). NQS*, Not quite significant, NS**=Non significant

Table 5: Effect of plant latex fractions incorporated in the artificial diet on the duration of larval development of *Spodoptera litura* larvae.

Treatment	*Mean duration of larval stadia (days prior to pupation)*			
	Thevetia nerifolia	*Atrocarpus heterophyllus*	*Ficus glomerata*	*Calatropis procera*
Control	17.33±.0.421	21.7±1.01	18.2±0.444	20.66±0.557
Hexanoic	32.33±1.208	29.833±.1.301	24.00±.1.460	26.5 ± 1.118
Methanolic	25.166 ± 1.470	28.833 ± 1.447	18.00 ± 1.095	29.833±0.601
Ethyl acetate	20.33±.0.954	16.166±1.04	21.5±0.763	26.666±1.1737
Chloroform	17.50±.0.562	18.66± 0.666	18.36 ± 0.872	19.66±0.608
Water	32.166±1.194	33.00±1.290	32.00±.0.966	33.16±1.077

It also affects gonadotrophic cycles in *Aedes aegypti*, and show inhibitory effects on egg hatching and larval development [34].. It contains alkaloids, steroids and resinous substances and display toxicity upon egg hatching and larvae [16] of *Aedes aegypti* [34]. Similarly, *Parahancornia amapa* (Huber) Ducke (Apocynaceae) lyophilized latex affect the post embryonic development of *Chrysomya megacephala* (F.) (Diptera: Calliphoridae). Its 1.0% (w/v) dose has made shorter post embryonic development period of larvae, pupae and newly hatched larvae to adult whereas 3.0% latex provoked a prolongation of these periods [39]. Similarly Mulberry leaves latex showed very high toxicity to *B. mori* when added to artificial diet [40]. It also protect plants from herbivore insects [41]. Rubber plant *Hevea brasiliensis* latex heavily deter beetle, *Luprops tristis* and inhibit development and reproductive efficiency of parental adults [42]. Similarly crude latex from *Euphorbia splendens* var. *hislopi*i (Euphorbiaceae) effect post-embryonic development time and viability of *M. scalaris* under laboratory conditions at 5 µg/mL, 10 µg/mL and 20 µg/mL concentration [43]. Similar effects of latex from *Amapazeiro para hancornia amapa* (Apoynaceae) were found on post embryonic development of blowfly *Chrysomya megacephala* (Diptera : Calliphoridae) and fleshly *Sarcophaga heamorrhoidalis.*

The results obtained from various bioassays demonstrated very strong anti-insect efficacy of plant latexes from *T. nerifolia, Atrocarpus heterophyllus, Ficus glomerata* and *Calatropis procera.* However, sub-lethal dose caused maximum mortality in third instar larvae, slow down the larval development and made the pupal duration longer, and caused significant reduction in body weight of larvae. The major adverse effects related to survival, larval weight reduction and delayed post embryonic development of *S. litura* larvae which may be due to obstructions in the insect body metabolism mainly synthesis of biomolecules, hormonal disturbances and failures in gene regulatory mechanisms. Further, The biochemical effecicay of certain latex components have forced the female insects to be deterred from oviposition. The dose at which plant latex proved fatal, causing 100% mortality and completely inhibiting F 1 emergence by ovicidal activity determined in a range of 0.296 µg/gm- in case of all plant species. The wider toxicity of plant latex to different life stages of *S. litura* could be ascribed due to presence of certain active constituents in latexes, these might be growth inhibitory toxic proteins, enzymes and other biomolecules.

Though previous studies have also demonstrated deleterious effects of latexes on insect pests mainly on development of larvae, pupae and its survival confirms presence of some active principles or different classes of bio-organic compounds in above plant species. As in the literature, few major components, such as terpenes, glycosides and acetogenins, have been reported which showed very high antifeedant, phytodeterrent and toxic effects on herbivorous insects. Moreover, the previous findings and results obtained in present study, confirmed very high insecticidal potential of plant latexes on surviving population of *S. litura* larvae and metamorphic pupae. Further, each extract caused additional high mortality in the pupae ranging 18-47%. Mode of action was assessed as an antifeedant and insecticidal action due to which high mortality was occurred both in larvae and pupae. The findings of present investigation based on laboratory experiments can therefore be recommended for potential exploitation of plant latex from *Thevetia nerifolia* (Peeli kaner), *Atrocarpus hetrophyllus* (Kathal), *Ficus glomerata* (Gular) and *Calotropis procera* (Madar) for production of bio-organic pesticides for safe control of insects. Latex based pesticide formulations can work more efficaciously than any synthetic chemical to be used for control of insect pests. Furthermore, present study will be continued to have confirmations on active phytochemicals and to find exact mode of action of these ingredients, so then, their effects on non-target organisms might be explored.

References:

[1] J.M. Hagel, E.C. Yeung, P.J. Facchini. Got milk? (2008). The secret life of laticifers. Trends Plant Science. 13: 631-639.

[2] D. Singh, D.C. Jain. (1987). Relative toxicity of various organic solvents generally used in screeaning plant product for insecticidal activity against house fly (*Musca domestica* L.)" Indian Journal of Experimental Biology. 25:560-570.

[3] C.R. Carlini, Grossi-de M.F. Sa. (2002). Plant toxic proteins with insecticidal properties– a review on the potentialities as bioinsecticides. Toxicon. 40:1515- 1539.

[4] D.E. Champagne, M.B. Ismam, K.R. Downum, G.H.N. Towers. (1993). Insecticidal and growth

reducing activity of foliar extracts from Meliaceae. Chemoecology. 4:165-173.

[5] T. Yagami, N.M. Sato, M. Shono. (1994). One of the rubber latex allergens has lysozyme activity. International Archives of Allergy and Immunology. 104:307-311.

[6] W.F. Pickard. (2008). Laticifers and secretory ducts: two other tube systems in plants. New Phytology. 177: 877-888.

[7] A. Domsalla, M.F. Melzig. (2008). Occurrence and properties of proteases in plant latices. Planta Medica. 74(7) 699-711.

[8] T. Yagami M. Sato, A .Nakamura, T. Komiyama, K. Kitagawa, A Akasawa, Z Ikezawa. (1998). Plant defense-related enzymes as latex antigens. Journal of Allergy and Clinical Immunology. 101: 379-85.

[9] B.J. Sequira, M.J. Vital, A.M. Pohlit, I.C. Pararols Cauper GSB. (2009). Antibacterial and antifungal activity of extracts and exudates of the Amazonian medicinal tree *Himatanthus articulates* (Vahl) Woodson (Common neme: *sucuba*). Memorias do Instituto Oswaldo Cruz. 104(4) 34-39.

[10] S. Kitajima, K. Kamei, S. Taketani, M. Yamaguchi, F. Kawai, A. Komatsu, Y. Inukai. (2010).Two chitinase-like proteins abundantly accumulated in latex of mulberry show insecticidal activity. BMC Biochemistry. 11: 6-11.

[11] K. Sukumar, M.J. Perich, L.R. Boobar. (1991). Botanical derivatives in mosquito control: a review. Journal of the American Mosquito Control Association. 7(2)10-37.

[12] E.A.S. Shaalan, D. Canyon, M.W.F. Younesc, H. Abdel-Wahab, A.H. Mansoura. (2005). A review of botanical phytochemicals with mosquitocidal potential. Environment International, 31:1149-1166.

[13] L.E. Castillo, J.J. Jiménez, M.A. Delgado. (2010).Secondary metabolites of the Annonaceae. Solanaceae and meliaceae families used as biological control of the insects. Trop Subtrop Agroecosystems. 12:445-462.

[14] G. Giridhar, K. Deval, P.K. Mittal, P. Vasudevan. (1984). Mosquito control by *Calotropis procera* latex. Pesticides. 18: 26-29.

[15] L.E. Moursy. (1997). Insecticidal activity of *Calotropis procera* extracts on the flesh fly *Sarcophaga haemorrhoidalis* Fallen. Journal of the Egyptian Society of Parasitology. 2: 505-514.

[16] T.A. Morsy, M.A. Rahem, K.A. Allam. (2001).Control of *Musca domestica* third instar larvae by the latex of *Calotropis procera* (Family: Asclepiadaceae). Journal of the Egyptian Society of Parasitology. 31(1)107-110.

[17] W.D Obregón, C.S. Liggieri, S.A. Trejo, F.X. Avilés, S.E. Vairo-Cavalli, N.S. Priolo. (2009). Characterization of papain-like isoenzymes from latex of *Asclepias curassavica* by molecular biology validated by proteomic approach. Biochimie. 91(11-12) 1457-1464.

[18] A. Abdul Rahuman, P. Venkatesan, K. Geetha, G. Gopalakrishnan, A. Bagavan, C. Kamaraj. (2008). Mosquito larvicidal activity of gluanol acetate a tetracyclic triterpenes derived from *Ficus racemosa* Linn. Parasitology Research. 103(2)333-339.

[19] A. Alkofahi, J.K. Rupprecht, J. E. Anderson, J.L. McLaughlin, K.L. Mikolajczak. (1987). A Search for new pesticides from higher plants .Insecticides of plant origin, ACS symposium series 387. Washington, D.C. pp. 24-25.

[20] M.B. Ismam. (1955). Leads and prospects for the development of new botanical insecticides in. Roe, R.M. Khur, R.J. (Eds) Reviews in Pesticide. Toxicology. 3:1-20.

[21] A.U. Buranov. (2010). B.J. Elmuradov Extraction and Characterization of Latex and Natural Rubber from Rubber-bearing Plants. Journal of Agricultural and Food Chemistry. 58 (2)734–743.

[22] S. McCay and P. Mahlberg. (1973). Study of Antibacterial Activity and Bacteriology of Latex from *Asclepias syriaca* L. Antimicrobial Agents Chemother, 3(2)247–253.

[23] J.K. Rupprecht, Y.H. Hui, McLaughlin J.L. (1990).Annonaceous acetogenins. Review. Journal of Natural Products. 53: 237-276.

[24] T.R. Seetharaman. (1986). Flavonoids from the leaves of *Annona squamosa* and *Polyalthia longifolia*. Fitoterapia. 57:189-198.

[25] N. Mazoir, A. Benharref, M. Bailén, M. Reina, A. González-Coloma. (2008). Bioactive triterpene derivatives from latex of two *Euphorbia* species Phytochemistry. 69(6)1328-1338.

[26] H.M. Kotkar, P.S. Mendki, S.V. Sadan, , S.R. Jha, S.M. Upasani. (2002). Antimicrobial and pesticidal activity of partially purifed flavonoids of *Annona squamos*a. Pest Management Science. 58:33-37.

[27] C.B. Oppel, D.E. Dussourd, U. Garimella. (2009). Visualizing a plant defense and insect counter play: alkaloid distribution in Lobelia leaves trenched by a plusiine caterpillar. Journal of Chemical Ecology. 35(6) 625-634.

[28] R. Wititsuwannakul, D. Wititsuwannakul, C. Sakulborirug. (1998).A lectin from the bark of the rubber tree (*Hevea brasilliensis.* Phytochemistry. 47:183-187.

[29] K.S. Lam, B.N. Tzi. (2011). Lectins. production and practical applications. Applied Microbiology Biotechnology. 89(1) 45–55.

[30] N. Wasano, K. Konno, M. Nakamura, C. Hirayama, M. Hattori, K. Tateishi. (2009). A unique latex protein, MLX56, defends mulberry trees from insects. Phytochemistry. 70(7):880-888.

[31] G. Sripongpun. (2008). Contact toxicity of the crude extract of Chinese star anise fruits to house fly larvae and their development. Songklanakarin Journal of Science and Technology. 30:667-672.

[32] N. Begum B. Sharma, R.S. Pandey. (2010). Evaluation of Insecticidal Efficacy of *Calotropis Procera* and *Annona Squamosa* Ethanol Extracts Against *Musca Domestica*. Journal of Biofertilizers and Biopesticides. 1:101-105.

[33] N.O. Ali, F.M. El-Rabaa. (2010). Larvicidal activity of some plant extracts to larvae of the mosquito *Culex quinquefasciatus* (Say 1823). European Review for Medical and Pharmacological Sciences. 14(11) 925-933.

[34] M.V. Ramos, G.P. Bandeira, C.D.T. de Freitas, Nogueira N.A.P, Alencar N.M.N, de Sousa P.A.S, Carvalho A.F.U. (2006).Latex constituents from *Calotropis procera* (R. Br.) display toxicity upon egg hatching and larvae of *Aedes aegypti* (Linn) " The Memórias do Instituto Oswaldo Cruz. 101(5) 503-510.

[35] R.K. Singh, P.K. Mittal, R.C. Dhiman. (2005).Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family-Asclepiadaceae) against mosquito larvae. Journal of Communicable Diseases. 37:109-113.

[36] M. Shahi, A.A. Hanafi-Bojd, M. Iranshahi, H. Vatandoost, M. Hanafi-Bojd. (2010). Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). Journal of Vector Borne Diseases. 47(3) 185-188.

[37] M. Singh, V. Joshi, R.C. Sharma, K. Sharma. (2004). Ovipositioning behaviour of *Aedes aegypti* in different concentrations of latex of *Calotropis procera*: studies on refractory behaviour and its sustenance across gonotrophic cycles. Dengue Bulletin. 28, 184-188.

[38] M.V. Ramos, D.A. Pereira, D.P. Souza, E.S. Araújo, C.D. Freitas, M.G. Cavalheiro, M.P. Matos, A.F. Carvalho. (2009). Potential of laticifer fluids for inhibiting *Aedes aegypti* larval development: evidence for the involvement of proteolytic activity. The Memórias do Instituto Oswaldo Cruz. 104(6) 805-812.

[39] P.M. Mendonça, M.G. Lima, L.R. Albuquerque, M.G. Carvalho, M.M. Queiroz. (2011). Effects of latex from *Amapazeiro"Parahancornia amapa* (Apocynaceae) on blowfly *Chrysomya megacephala* (Diptera: Calliphoridae) post-embryonic development. Veterinary Parasitology. 178(3-4) 379-382.

[40] C. Hirayama, K. Konno, N. Wasano, M. Nakamura. (2007). Differential effects of sugar-mimic alkaloids in mulberry latex on sugar metabolism and disaccharidases of Eri and domesticated silkworms: enzymatic adaptation of *Bombyx mori* to mulberry defense. Insect Biochemistry and molecular Biology. 37:1348-1358.

[41] K. Konno, O. Hiroshi, M. Nakamura, K. Tateishi, C. Hirayama, Y. Tamura, M. Hattori, A. Koyama, and K. Kohno. (2006). Mulberry latex rich in antidiabetic sugar-mimic alkaloids forces dieting on caterpillars. Proceedings of National Academy of Sciences USA. 31; 103(5) 1337–1341.

[42] K. Thomas, Sabu and K.V. Vinod. (2009) Population Dynamics of the Rubber Plantation Litter Beetle *Luprops tristis*, in Relation to Annual Cycle of Foliage Phenology of Its Host, the Para Rubber Tree, *Hevea brasiliensis.* Journal of Insect Science. 9: 56,

[43] S. Mello Rda, A.R. Ferreira, M.M. Queiroz. (2010). Bioactivity of latex from *Euphorbia splendens* var. *hislopii* (Euphorbiaceae) on post-embryonic development of *Megaselia scalaris* (Phoridae). Veterinary Parasitology. 172 (1-2) 100-104.