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RESEARCH ARTICLE

PLANT LATEX: ITS TOXICITY AND DEFENSE AGAINST HERBIVOROUS INSECTS: A REVIEW

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ABSTRACT

Plant latex is a mixture of various chemical constituents which protect plants from herbivorous insects. It contains many chemical substances such as acetogenins, flavonoids, triterpenes alkaloids, lectins, and proteins which show very high insecticidal potential. Latex also contains important other phytochemicals such as glycosides, alkaloids, steroids and resinous substances, tannins and saponins which show diverse biological activities. It also contains enzymatic proteins such as chitinases, proteases, peptidases, plasmins, papain, hevein, lectins and lipases which show enormous insecticidal activity against insects play a critical role in defense against herbivorous insects. Plant show high mortality, inhibit egg hatching and effect post embryonic developmental and survival of insects. Plant latex also showed very high toxicity in mollusks mainly fresh water snail, *Lymnaea acuminata* and *Lymnaea columella*. Plant latex possesses many more advantages over synthetic pesticides. It has better lethal potential than synthetic pesticides and has no side effect on environment because it is biodegradable. No doubt its chemical constituents can be used as a successful pesticide if isolated in pure form.

INTRODUCTION

Plant latex is a protein rich multi-component fluid secreted by laticifer tissues from several plant species. It shows deleterious effects in insects mainly causes high mortality, inhibit feeding, egg hatching and obstruct reproduction in them [1-3]. Nowadays, insect control is usually carried out using chemical insecticides, but emerging insecticide resistance in insects against these chemicals has raised many environment related problems. Over use of synthetic pesticide is poisoning the food chain and killing non-target organisms. Hence, prompted the search for new alternatives of synthetic pesticides. Due to emerging resistance synthetic pesticides in insects there is a very high demand of botanical insecticides/pesticides of plant origin [4-5]. 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 control 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, secondary metabolites isolated from plant latex might be more useful for successful control of insect pests [3].

Plant latex is mixture of secondary metabolites that demonstrate negative effect on insect life mainly on development of larvae, pupae and its survival. Due to presence of many chemical substances such as acetogenins, flavonoids, triterpenes [6], alkaloids [7], lectins [8-9], and proteins [10] plant latex shows very high lethality in insects. In anticipation plants possess a high level of latex-mediated resistance against various insect pest species. Latex also contains important phytochemicals such as glycosides, alkaloids, steroids and resinous substances, tannins and saponins which show diverse biological activities. It also contains enzymatic proteins such as chitinases, proteases, peptidases, plasmins, papain, hevein, lectins and lipases which show enormous insecticidal activity against insects. Similarly, plant latex from *Calatropis procera* [11]. *Heena brassilensis* , *Ficus sp.* [12], *Carica papaya* [13], *Morus alba*, *Anonna squamosa*, *Asclepias humistrata*[14] and Persian poppy (*Papaver breateatum*) has shown strong insecticidal activityand also plays a critical role in defense against herbivorous insects [15].

Few plant families such as Annonaceae, Solanaceae Asteraceae,Euphorbiaceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae and Rutaceae, possess various *phytochemicals* in latex which show very high insecticidal activity [11,13,16]. *Sarcophaga haemorrhoidalis* [17] and *Musca domestica* [18] and affects gonadotrophic cycles of *Aedes aegypti* [19] and shows inhibitory effects on egg hatching and larval development [20]. Latex from *Asclepias humistrata* (sandhill milkweed) kills newly hatched monarch butterfly caterpillars by traping [14]. It was also found effective against fourth instar larvae of the lymphatic filariasis vector *Culex quinquefasciatus* (Diptera: Culicidae) and affect gonadotrophic cycles in *Aedes aegypti*, and show inhibitory effects on egg hatching and larval development [20]. *Calotropis procera* latex possess high mosquitocidal potential [21] against *Culex quinquefasciatus* [22], *Anopheles stefensi* (Singh et al, 2005) and *Musca domestica* [1]. Similarly, latex

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Table 1: List of common and scientific names of certain latex bearing plants and their insecticidal properties

Common name	scientific name	Family	Pesticidal activity reported	Effective against life stage
Tut	*Morus alba*	Moraceae	Insecticidal	IV instar larvae of lepidopteran insects
Rubber plant	*Ficus elasica*	Moraceae	Insecticidal	Inhibit egg hatching and larval develoment
Bargad	*Ficus bengalensis*	Moraceae	Insecticidal	Inhibit egg hatching and larval develoment
Chalate	*Ficus insipida*	Moraceae	Insecticidal	Inhibit egg hatching and larval develoment
Ficus	*Ficus racemosa*	Moraceae	Insecticidal	Inhibit egg hatching and larval develoment
Wild fig	*Ficus virgata*	Moraceae	Insecticidal	Inhibit egg hatching and larval develoment
Gazyummaria	*Ficus microcarpa*	Moraceae	Insecticidal	Inhibit egg hatching and larval develoment
Gular	*Ficus glomerata*	Moraceae	Insecticidal	Inhibit egg hatching and larval develoment
Mexican Poppy	*Argemone ochroleuca*	Papaveraceae	Insecticidal	Adults and eggs of Culex sp.
Opium poppy	*Papaver sommeniferum*	Euphorbiaceae	Insecticidal	Eggs and larvae
Spurge	*Euphorbia lacteal*	Euphorbiaceae	Insecticidal	Eggs and larvae
Sudha	*Euphorbia nerrifolia*	Euphorbiaceae	Insecticidal	Eggs, larvae and pupae
Tridhara	*Euphorbia antiqunum*	Euphorbiaceae	Insecticidal	Eggs and larvae
Splendens	*Euphorbia splendens*	Euphorbiaceae	Insecticidal	Post embryonic development of *M. sclaris*
Badi Dudhi	*Euphorbia hirta*	Euphorbiaceae	Insecticidal	Egg hatching and Post embryonic development
Biodiesel plant	*Jatropha curcas*	Euphorbiaceae	Insecticidal	Eggs, larvae and pupae
Hierba mala	*Euphorbia cotimfolia*	Euphorbiaceae	Insecticidal	Eggs, larvae and pupae
Mohan	*Euphorbia rogleana*	Euphorbiaceae	Insecticidal	Eggs, larvae and pupae
Hyaena-poison	*Hyaenanche globosa*	Euphorbiaceae	Insecticidal	Eggs, larvae, pupae and adults
Persian poppy	*Papaver bracteatum*	Euphorbiaceae	Insecticidal	Mosquito and house fly larvae and eggs
Pili kaner	*Thivetia nerrifolia*	Euphorbiaceae	Larvicidal	Eggs and larvae
Rubber tree	*Hevea brasiliensis*	Euphorbiaceae	Insecticidal	Eggs and larvae
Madar	*Calotopis procera*	Asclepiadaceae	Larvicidal,	*Culex quinquefasciatus*
Sandhill milkweed	*Asclepias humistrata*	Asclepiadaceae	Insecticidal	Monarch butterfly
Milkweeds	*Asclepias angustifolia*	Asclepiadaceae	Insecticidal	Herbivorous insects
Milkweeds	*A. barjoniifolia*	Asclepiadaceae	Insecticidal	Herbivorous insects
Milkweeds	*A. fascicularis*	Asclepiadaceae	Insecticidal	Herbivorous insects
Oleander	*Nerium oleander*	Apocynaceae	Insecticidal	Eggs and larvae
Sapthaparna	*Alstonia macrophylla*	Apocynaceae	Insecticidal	Eggs and larvae
Sharifa	*Annona squamosa,*	Annonaceae	Insecticidal	*IV instar larvae of lepidopteran insects*
Papita	*Carica papaya*	Caricaceae	Insecticidal	Eggs and post embryonic development

of *Calotropis procera* and *Ficus racemosa* were found in plant latex from the Russian weed *Anabasis aphylla* contain alkaloids like nicotine, anabasine, methyl anabasine and lupinine and successfully 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]. Similar insecticidal activity is also, screened in *Goniothalamus macrophyllus* [13] and *Annonaceous acetogenins* plants [23].

Plant defense

Several latex proteins, including cysteine proteases and chitin related proteins have shown to play important role in plant insect interactions. Due to presence of these enzymatic proteins, plant latex is considered as analogous to animal venom because it provides defense against herbivorous insects [15]. Plant possess unique defense molecules mainly chitinases which effect insect skin [24] and operate broad spectrum defense against multiple herbivores [25],[7] Similarly, cysteine proteases isolated from latex of papaya (*Carica papaya*) and wild fig (*Ficus virgatalatex*) have shown high toxicity to caterpillars of herbivorous insects[13]. Mainly cysteine proteases and other plant proteins such as lectins of latex make plants more resistant against insect attack [26][8]. It might be a broader array of proteins that may involve in defense against herbivores [27]. These proteins are toxic and exert their effect by impairing nutrient utilization in insects [25]. Latex of mulberry contains sugar mimic alkaloids which effect sugar metabolism in silk worm *Bombyx mori* [28] while latex of *Calotropis procera* causes significant mortality in insects and impose long-term effects on insect development [29]. A romaine lettuce cultivar, "Valmaine", possesses a high level of latex-mediated resistance against the banded cucumber beetle, *Diabrotica balteata* LeConte (Coleoptera: Chrysomelidae), There may be a change in the chemistry after plant damage due to increased activity of inducible enzymes and act synergistically against *D. balteata* [30]. Similarly, cardenolides are major defenses in milkweeds, *Asclepias angustifolia*, *A. barjoniifolia* and *A. fascicularis* which are proved effective against herbivorous insects. In addition proteases and C/N ratio also integrate in the defense and play an important role in plant defense at intermediate level [31].

Larvicidal activity

Many botanicals such as acetogenins [23], flavonoids [32], triterpenes[6] , alkaloids [7], lectins[8] (Wititsuwannakul et al,1998 and Lam Tzi 2011), latex proteins (Wasano et al, 2009) and other botanicals such as glycosidase inhibitors [11] were found active against various insect species. Similarly, Gluanol acetate a tetracyclic tri-terpene derived from *Ficus racemosa* showed larvicidal effect in mosquito (Abdul Rahman 2008). Crude aqueous extract of the latex of *Calotropis procera* and *Ficus benghalensis* showed insecticidal activity against fourth instar larvae of the lymphatic filariasis vector *Culex quinquefasciatus* (Diptera: Culicidae) and it killed 50% of the larval population at a concentration of 0.0062% (V/V) and 0.4796% (V/V) [33] (Ali and El-Rabba 2010). Similarly, latex of *Calotropis procera* was found active against third larvae of *Musca domestica* at a topically dose of 3 ul (5% of the latex) [17]. It contains alkaloids, steroids and resinous substances and display toxicity upon egg hatching and larvae of *Aedes aegypti*

[20]. It also shows larvicidal efficacy against *Anopheles stefensi* and *Culex quinquefasciatus* [22].(Table 1).

In addition, plant toxins [2] (Carilini and Grossi De 2002) and secondary metabolites such as acetogenins [34] (Castillo et al, 2010) showed insecticidal activity [3] (Champagne et al, 1993). Similarly, leaf extract of *C procera* shows larvicidal potential against mosquito larvae [35] (Singh et al, 2005) and inhibit oviposition behavior in *Aedes aegypti* [19] (Singh et al, 2004). Similar Insecticidal efficacy in plant latex of *Calotropis Procera* and *Annona Squamosa* was found against third instar larvae of *Musca Domestica* at an LC50 of 282.5 and 550 mgl^{-1}, respectively [35,36]. This pesticidal activity in both plants is due to presence of alkaloids which occur as the major component in plant latex [37].

Effect on egg hatching and post embryonic development

Beside action on insect larvae plant latex also effect egg hatching and embryonic development in insects. *Calotropis procera* showed toxic effects upon egg hatching and larval development of *Aedes aegypti* and causes 100% mortality of 3rd instars within 5 min when treated with water-soluble dialyzable fraction [38]. 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] (table 1).

Similarly, mulberry leaves (*Morus spp.)* showed toxicities and defensive activities against herbivorous insects *Bombyx mori* mainly against caterpillar. It contains very high concentrations of alkaloidal sugar-mimic glycosidase inhibitors which protect mulberry leaves from insect attack. These sugar-mimic alkaloids are only toxic to caterpillars because these circumvent the mulberry tree's defense [28]. These latex ingredients play key roles in defense of mulberry tree and of other plants against insect herbivore [40]. Similarly, rubber plant *Hevea brasiliensis* latex heavily deter beetle, *Luprops tristis* and inhibit development and reproductive efficiency of parental adults [41]. 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 microg/mL, 10 microg/mL and 20 microg/mL concentration [42] (Table 1). 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* [17].

Proteolytic activity

The laticifer fluid of *Calotropis procera* is rich in laticifer enzymatic proteins which showed proteolytic activity (Freitas et al, 2007. It is mainly due to four distinct cysteine proteinases, Serine metaloproteinases, aspartic proteinase.and Chitinases (EC: 3.2.1.14). However, presence of these enzymatic proteins in latex from *C. procera* also might have their involvement in resistance to phyto-pathogens and insects [29]. Proteins abundantly accumulated in latex might therefore be involved in the defense system. Similarly Kitezima et al, 2010[15] isolated latex abundant protein a and b (LA-a and LA-b) from mulberry plant (*Morus* sp.) and analyzed their properties. Both proteins showed significant chitinase and chitogenase activity. Similar to other plant chitinases, upon glycosylation both LA-a and LA-b proteins have shown very high insecticidal activities in larvae of *Drosophila melanogaster*. Both LA proteins have a crucial role in defense against herbivorous insects, possibly by hydrolyzing their chitin [15]. Similar laticifer fluids of *Cryptostegia grandiflora* R.Br., *Plumeria rubra* L. and *Euphorbia tirucalli* L. contain proteins such as cysteine proteinases which show endogenous proteolytic activity and exhibit very high larval toxicity to *Aedes aegypti*. Similarly, cystatins, found in tomato (*Solanum lycopersicum*) and cysteine proteases occur in potato plant *Solanum tuberosum* [43] play role in defense insect pests (Table 1). Similar insecticidal activity is also reported in cysteine proteinase (papain) isolated from the latex of *Carica papaya* [44]. Similarly, papain like enzymes from latex of *Asclepias curassavica* [14]. *Carica papaya* (Linn) [44] showed proteoloytic activity against *Aedes aegypti* larvae [38]. *Carica papaya* fruit, latex, leaves and roots of contain cyangenic glucosides and tannins which digest skin of insects [44]. Latex secreting plants such as *Calatropis procera* contains anthroquinones [45], cyanogenic Glucoside [46], and mulberry latex sugar mimic alkaloids [47] which effect insect feeding and integument.

Molluscicidal activity

Plant latex is used as an efficient natural molluscicide, which can be used as an alternative control agent against *L. columella*. [48] . However, Euphorbian latex (*Euphorbia milii*) shows lethal effects on the intermediate host *Biomphalaria* spp., of the human liver parasite *Schistosoma mansoni*. It also contains Milin, a serine protease (up to 0.1mg/l), that significantly reduced the growth and feeding in mollusk at 0.1mg/l dose. Milin also causes high lethality in snails and is responsible for alteration of normal physiological functions in it. It is used to control transmission of the endemic disease schistosomiasis [49]. Similarly, *Euphorbia splendens* var. hislopii (Crown of thorns) showed molluscicidal against *Lymnaea columella* snails, intermediate host of *Fasciola hepatica*, in irrigation ditches. An aqueous solution of the latex containing 5 mg/l concentration showed 100 % mortality in experimental snails. *E. splendens var. hislopii* [48]. Similar molluscicidal activity of the latex of *Euphorbia splendens* var. hislopii on *Melanoides tuberculata* (Thiaridae), is also observed in snail associated with habitats of *Biomphalaria glabrata* (Planorbidae) by [50] at an LD50 of 3.57 mg/l and LD90 6.22 mg/l. Similar molluscidal properties are also found in *Calatropis procera* latex [51]. Similar action of *Euphorbia splendens* var. *hislopii* (E. milli) latex was observed in the species of the genus *Bulinus* and on *Biomphalaria pfeifferi,* intermediate hosts of schistosomiasis in Africa, and the Brazilian snails B. glabrata, *B. tenagophila*, and *B. straminea,* intermediate hosts of schistosomiasis in Brazil. Crude latex showed 90% lethal dose (LD90) ranging from 0.13 ppm for *B. glabrata* and subjected to lyophilized latex to 4.0 ppm for *B. pfeifferi* tested with the natural latex. It has shown high impact on the egg masses and embryos of *B. glabrata*. [52]. The latex of *Euphorbia splendens* var. *hislopii* shows molluscicidal action at low concentration (LD90 less than 1.5 ppm or 1.5

micrograms/ml) against the vector snails of schistosomiasis. In the CHO/cytotoxicity assay, latex was found to have no acute toxicity or mutagenic activity at the concentrations of 10 to 12 micrograms/ml (or ppm) and can be used in the field as molluscicidal [53]. Further, efficacy of E. milii latex as a molluscicide can be modified by factors such as water temperature and concentration of organic materials, and to a lesser extent by snail size [54]. The molluscicidal activity of *E. Cauducifolia* L. latex, against *Biomphalaria glabrata* snails is due to presence of 13-acetoxy-20- O-angeloyl-12 -deoxyphorbol (1), 13- O-[N-(2-aminobenzoyl)]anthraniloyl -20-acetoxy-12-deoxyphorbol (2), 13,20- O-dibezoyl-12 -deoxyphorbol (3), 13,20- O-diangeloyl-12-deoxyphorbol(4), 13- O-angeloyl-20- O-[N-(2-aminobenzoyl)]anthraniloyl-12 -deoxyphorbol (5), 13- O-tigloyl-20- O-[N-(2-aminobenzoyl)] anthraniloyl-12-deoxyphorbol (6), 13- O-benzoyl-20-O-[N -(2-aminobenzoyl)]anthraniloyl-12 deoxyphorbol (7), and 13 -O-hexanoyl-20-O- [N- (2-aminobenzoyl)] anthraniloyl- 12 -deoxyphorbol (8) chemical compounds which were found active against mollusks [55].

Conclusion

Over use of synthetic chemicals in form of pesticides has made serious environmental impact and has hunted non-target organisms in large number, hence there is a mass destruction of non-destructive and non-pest animals. However, 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. These are biodegradable pesticides which can be used to 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, secondary metabolites isolated from plant latex might be more useful for successful control of insect pests. To avoid poisoning of food chain and to stop killing of non-target organisms, bio-pesticides are only option for agriculturists.

REFERENCES

1. Singh D, Jain DC: Relative toxicity of various organic solvents generally used in screeaning plant product for insecticidal activity against house fly (Musca domestica L.). *Ind J Exp Biol.,* 1987, 25: 560-570.
2. Carlini CR, Grossi-de Sa MF: Plant toxic proteins with insecticidal properties- a review on the potentialities as bioinsecticides. *Toxicon.,* 2002, 40: 1515-39.
3. Champagne DE, Ismam MB, Downum KR, Towers GHN: Insecticidal and growth reducing activity of foliar extracts from Meliaceae. *Chemoecology*, 1993, 4: 165-173.
4. Alkofahi A, Rupprecht JK, Anderson J E, McLaughlin JL, Mikolajczak KL: A Search for new pesticides from higher plants .Insecticides of plant origin ,ACS symposium series387.Washington, D.C1987, 24-25.
5. Ismam MB: Leads and prospects for the development of new botanical insecticides in: Roe, R.M. Khur, R.J. (Eds) Reviews in Pesticide Toxicology, 1955, 3: 1-20.
6. Mazoir N, Benharref A, Bailén M, Reina M, González-Coloma A:. Bioactive triterpene derivatives from latex of two *Euphorbia* species. *Phytochemistry*, 2008, 69(6):1328-38.
7. Oppel CB, Dussourd DE, Garimella U., Visualizing a plant defense and insect counter play: alkaloid distribution in Lobelia leaves trenched by a plusiine caterpillar. *J Chem Ecol.,* 2009, 35(6):625-34.
8. Wititsuwannakul R, Wititsuwannakul D, Sakulborirug C: A lectin from the bark of the rubber tree (*Hevea brasilliensis*). *Phytochem.,* 1998, 47: 183-187.
9. Lam KS, Tzi BN Lectins: production and practical applications Appl Microbiol Biotechnol. 2011, 89(1): 45–55.
10. Wasano N, Konno K, Nakamura M, Hirayama C, Hattori M, Tateishi K: A unique latex protein, MLX56, defends mulberry trees from insects. Phytochemistry, 2009, 70(7):880-8.
11. Sukumar K, Perich MJ, Boobar LR: Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc.,* 1991, 7(2): 10-37.
12. Abdul Rahuman A, Venkatesan P, Geetha K, Gopalakrishnan G, Bagavan A, Kamaraj C: Mosquito larvicidal activity of gluanol acetate a tetracyclic triterpenes derived from *Ficus racemosa* Linn. 2008, 103(2): 333-9 .
13. Shaalan EAS, Canyon D, Younesc MWF, Abdel-Wahab H, Mansoura AH: A review of botanical phytochemicals with mosquitocidal potential. *Env Int.,* 2005, 31: 1149-66.
14. Obregón WD, Liggieri CS, Trejo SA, Avilés FX, Vairo-Cavalli SE, Priolo NS:. Characterization of papain-like isoenzymes from latex of *Asclepias curassavica* by molecular biology validated by proteomic approach. *Biochimie*., 2009, 91(11-12): 1457-64.
15. Kitajima S, Kaeko K, Shigeru T, Masamitsu Y, Fumiko K, Aino K, and Yoshihiro I: Two chitinase-like proteins abundantly accumulated in latex of mulberry show insecticidal activity. *BMC Biochem.,* 2010; 11: 6
16. Castillo LE, Jiménez JJ, Delgado MA: Secondary metabolites of the Annonaceae. Solanaceae and meliaceae families used as biological control of the insects. *Trop Subtrop Agroecosystems*, 2010, 12: 445-62.
17. Moursy LE: Insecticidal activity of *Calotropis procera* extracts on the flesh fly *Sarcophaga haemorrhoidalis* Fallen. *J Egyptian Soc Parasitol.,* 1997, 2: 505-14.
18. Morsy TA, Rahem MA, Allam KA: Control of *Musca domestica* third instar larvae by the latex of *Calotropis procera* (Family: Asclepiadaceae). *J Egyptian Soc Parasitol.,* 2001, 31(1): 107-10.
19. Singhi M, Joshi V, Sharma RC, Sharma K:. Ovipositioning behaviour of *Aedes aegypti* in different concentrations of latex of *Calotropis procera*: studies on refractory behaviour and its sustenance across gonotrophic cycles. *Dengue Bull.,* 2004, 28: 184-8.
20. Ramos MV, Bandeira GP, de Freitas CDT, Nogueira NAP, Alencar NMN, de Sousa PAS, Carvalho AFU:. Latex constituents from *Calotropis procera* (R. Br.) display toxicity upon egg hatching and larvae of *Aedes aegypti* (Linn). *Mem Instit Oswaldo Cruz*., 2006, 101(5): 503-10.

21. Giridhar G, Deval K, Mittal PK, Vasudevan P: Mosquito control by *Calotropis procera* latex. *Pesticides*, 1984, 18: 26-9.
22. Shahi M, Hanafi-Bojd AA, Iranshahi M, Vatandoost H, Hanafi-Bojd M:. Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against Culex quinquefasciatus and *Anopheles stephensi* (Diptera: Culicidae). *J Vector Borne Dis.,* 2010, 47(3):185-8.
23. Rupprecht JK, Hui YH, McLaughlin JL: Annonaceous acetogenins: Review. *J Nat Prod.,* 1990, 53: 237-276.
24. Diaz-pearles *et al.,* 1998. 14. Diaz-Perales A, Collada C, Blanco C, Sanchez-Monge R, Carrillo T, Aragoncillo C, Salcedo G. Class I chitinases with hevein-like domain but not class II enzymes are relevant chestnut and avocado allergens. *J Allergy Clin Immunol.,* 1998; 102: 127-33.
25. Zhu-Salzman K, Luthe DS, Felton GW : Arthropod-Inducible Proteins: Broad Spectrum Defenses against Multiple Herbivores. *Plant Physiol.,* 2008, 146(3): 852–858.
26. Ding X, Gopalakrishnan B, Johnson LB, White FF, Wang X, Morgan TD, Kramer KJ, Muthukrishnan S: Insect resistance of transgenic tobacco expressing an insect chitinase gene. *Transgenic Res.,* 1998, 7:77–84.
27. Felton GW**.** Indigestion is a plant's best defense. *Proc Natl Acad Sci.,* USA, 2005, 102: 18771–18772.
28. Hirayama C, Konno K, Wasano N, Nakamura M:. 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 Biochem Mol Biol.,* 2007, 37(12):1348-58.
29. Freitas CD, Oliveira JS, Miranda MR, Macedo NM, Sales MP, Villas-Boas LA, Ramos MV: Enzymatic activities and protein profile of latex from *Calotropis procera. Plant Physiol. Biochem.,* 2007, 45(10-11):781-9.
30. Sethi A, McAuslane HJ, Rathinasabapathi B, Nuessly GS, Nagata RT. Enzyme induction as a possible mechanism for latex-mediated insect resistance in romaine lettuce. *J. Chem. Ecol.,* 2009 Feb;35(2):190-200. Epub 2009 Jan 28.
31. Rasmann S, Johnson MD, Agrawal AA. Induced responses to herbivory and jasmonate in three milkweed species. *J Chem Ecol.,* 2009 Nov;35(11): 1326-34. Epub 2009 Dec 11.
32. Seetharaman TR: Flavonoids from the leaves of *Annona squamosa* and *Polyalthia longifolia*. Fitoterapia 1986, 57: 189-198.
33. Ali NO, El-Rabaa FM: Larvicidal activity of some plant extracts to larvae of the mosquito *Culex quinquefasciatus* (Say 1823). *Eur Rev Med Pharmacol., Sci.* 2010, 14(11):925-33.
34. Singh RK, Mittal PK, Dhiman RC: Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family-Asclepiadaceae) against mosquito larvae. J Commun Dis*., 2005, 37: 109-113.*
35. *Sripongpun* G: Contact toxicity of the crude extract of Chinese star anise fruits to house fly larvae and their development. *Songklanakarin J Sci Technol.,* 2008, 30: 667-672.
36. Begum N Sharma B, Pandey R.S. 2010. Evaluation of Insecticidal Efficacy of *Calotropis Procera* and *Annona Squamosa* Ethanol Extracts Against *Musca Domestica***.** *J Biofertil Biopestici.,* 1:101-105
37. Kotkar HM, Mendki PS, Sadan, SV, Jha SR, Upasani SM: Antimicrobial and pesticidal activity of partially purifed flavonoids of *Annona squamos*a. *Pest Manag Sc.,* 2002, 58: 33-37.
38. Ramos MV, Pereira DA, Souza DP, Araújo ES, Freitas CD, Cavalheiro MG, Matos MP, Carvalho AF: Potential of laticifer fluids for inhibiting *Aedes aegypti* larval development: evidence for the involvement of proteolytic activity. *Mem Inst Oswaldo Cruz.,* 2009, 104(6):805-12.
39. Mendonça PM, Lima MG, Albuquerque LR, Carvalho MG, Queiroz MM: Effects of latex from "*Amapazeiro"Parahancornia amapa* (Apocynaceae) on blowfly *Chrysomya megacephala* (Diptera: Calliphoridae) post-embryonic development. *Vet Parasitol.,* 2011,178(3-4):379-82. Epub 2011 Jan 18.
40. Konno K, Hirayama C, Nakamura M, Tateishi K, Tamura Y, Hattori M, Kohno K, Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. *Plant J.,* 2004; 37: 370-8.
41. Thomas KS,Vinod KV: 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***.** *J Insect Sci.,* 2009, 9: 56
42. Mello Rda S, Ferreira AR, Queiroz MM: Bioactivity of latex from Euphorbia splendens var. hislopii (Euphorbiaceae) on post-embryonic development of *Megaselia scalaris* (Phoridae).*Vet Parasitol.,* 2010, 172(1-2):100-4.
43. Marie-Claire G, Cindy D, Louis-Philippe V, Moustafa K, Amine MB, Andreja P, Marc-Olivier D, Charles G, Conrad C, and Dominique M: Tailoring the Specificity of a Plant Cystatin toward Herbivorous Insect Digestive Cysteine Proteases by Single Mutations at Positively Selected Amino Acid Sites. *Plant Physiol.,* 2008, 146(3): 1010–1019
44. Thomas TG, Raghavendra K, Lal S, Saxena VK: .Mosquito larvicidal properties of latex from unripe fruits of *Carica papaya* linn. (Caricaceae).J Commun Dis. 2004, 36(4):290-2. No abstract available.
45. Khirstova P, Tissot M: Soda-Anthroquinone pulping of *Hibiscus sabdariff* (Karkadesh) and *Calotropis procera* from Sudan. *Bioresource Tech.,* 1995, 53: 670- 672.
46. Kirsten J, Søren B, Peter Kamp B, Charlotte S, Carl EO, Puonti-Kaerlas J, and Birger LM, Cassava: Plants with a Depleted Cyanogenic Glucoside Content in Leaves and Tubers. Distribution of Cyanogenic Glucosides, Their Site of Synthesis and Transport, and Blockage of the Biosynthesis by RNA Interference. *Technology Plant Physiol.,* 2005, 139(1): 363–374.
47. Konno K, Hiroshi O, Nakamura M, Tateishi K, Hirayama C, Tamura Y, Hattori M, Koyama A, and Kohno K: Mulberry latex rich in antidiabetic sugar-mimic alkaloids forces dieting on caterpillars Proc Natl Acad Sci., U S A 2006, 31; 103(5): 1337–1341.
48. de Vasconcellos MC, de Amorim A. Activity of *Euphorbia splendens* var. hislopii N.E.B. (Euphorbiaceae) latex against *Lymnaea columella* (Say,

1817) (Pulmonata: Lymnaeidae), intermediate host of *Fasciola hepatica*, Linnaeus, 1758 (Trematoda: Fasciolidae). 2: limited field-testing. *Mem Inst Oswaldo Cruz.,* 2003 Oct;98(7):981-5. Epub 2004 Jan 7.

49. Yadav SC, Jagannadham MV. Physiological changes and molluscicidal effects of crude latex and Milin on Biomphalaria glabrata. Chemosphere. 2008 Apr;71(7):1295-300. Epub 2008 Feb 11.

50. Giovanelli A, da Silva CL, Medeiros L, de Vasconcellos MC. The molluscicidal activity of the latex of *Euphorbia splendens var. hislopii* on *Melanoides tuberculata* (Thiaridae), a snail associated with habitats of *Biomphalaria glabrata* (Planorbidae). *Mem Inst Oswaldo Cruz.,* 2001 Jan;96(1):123-5.

51. Larhsini M, Bousad M, Lazrek HB, Jana M, Amarouch H: Evaluation of antifungal and molluscicidal properties of extracts of C*alotropis procera*. *Fitoterapia,* 1997, 68: 371-373.

52. Schall VT, de Vasconcellos MC, de Souza CP, Baptista DF. The molluscicidal activity of Crown of Christ (*Euphorbia splendens* var. hislopii) latex on snails acting as intermediate hosts of *Schistosoma mansoni* and *Schistosoma haematobium. Am J Trop Med Hyg.,* 1998 Jan; 58(1):7-10.

53. Schall VT, Vasconcellos MC, Valent GU, Sato MI, Furlan EV, Sanchez PS. Evaluation of the genotoxic activity and acute toxicity of *Euphorbia splendens* latex, a molluscicide for the control of schistosomiasis. *Braz J Med Biol Res.,* 1991;24(6):573-82.

54. Oliveira-Filho EC, De-Carvalho RR, Paumgartten FJ. The influence of environmental factors on the molluscicidal activity of *Euphorbia milii* latex. *J Environ Sci Health B.,* 1999 Mar;34(2):289-303.

55. Baloch IB, Baloch MK, Baloch AK. Schistosomiasis suppressing deoxyphorbol esters from *Euphorbia caudicifolia* L. latex. *Planta Med.,* 2010 May; 76(8):809-14.

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