Calotropis procera and *Annona squamosa*: Potential Alternatives to Chemical Pesticides

Nighat Begum[1], B. Sharma[2] and Ravi S. Pandey[1]*
Departments of Zoology[1] and Biochemistry[2], University of Allahabad;
Allahabad, 211002, India;
*Corresponding author; E-mail:rspandey2004@yahoo.com

ABSTRACT

The control of insect pests in agriculture, forestry, stores, animal husbandry, poultry and in human hygiene are still considered a challenge. Widespread use of chemical pesticides represents a potential risk to human and the environment. Therefore, the search for alternative strategies in pest control is timely to overcome this problem. Desirable are preparations that exhibit new modes of actions and impair processes that are rather specific to the pest in order to be combated. In the last twenty five years, much attention has been devoted to natural pest control agents. One of the most important groups among them are plant based active substances or mixtures of substances commonly known as 'botanicals'. Such natural products typically occur as cocktails of metabolically related compounds with differing activity/spectrum towards different insects. The present paper is a mini review presenting an updated account of biopesticidal properties of extracts from two different plant species, that could be developed as a potential substitute to the chemical pesticides.

Running title: Plant extracts as biopesticides

Key words: Biopesticides, plant extracts, chemical pesticides, toxicity, insects

1. INTRODUCTION

The unrestrained use of synthetic chemicals to control insects has resulted in an increase in resistance by insects to conventional insecticides. Public awareness of environmental and food contamination from pesticides has led environmental protection agencies to ban the use of chemicals like chlorinated, organophosphorus and carbamate insecticides. These synthetic/chemical pesticides have inherent drawbacks like (i) loss of efficacy due to resistance development in insects, (ii) persistence of some active compounds in soil, ground water and lakes, (iii) effects on non-target organisms, (iv) disruption of biological control by natural enemies (v) resurgence of stored product insect pests and (vi) human health concerns [1-4].

For the last two decades scientists have searched for botanical insecticides based on naturally occurring substances as a substitute to synthetic insecticides with an emphasis on their use in integrated pest management (IPM) rather than insect control [5]. The use of botanicals in pest management is useful in suppressing pest population as well as maintaining the sound ecological balance as the non target organisms are less affected.

Though pyrethrin, nicotine and rotenone were recognized as effective insect control agents, the widely accepted pyrethrins obtained from the flower heads of *Tanacetum cinerariaefolium* which are still used in insect control. Despite the relative safety of some well-known botanical insecticides, most of these substances have their drawbacks, hindering large-scale application. Pyrethrins are unstable in the sunlight and are rapidly metabolized thus limiting their potency and application [6]. These limitations gave an impetus for the synthesis of active analogues, termed pyrethroids. Nicotine isolated from a number of *Nicotiana* species is insecticidal, but its use in insect control has dropped steadily because of the high cost of production, disagreeable odour, extreme mammalian toxicity, instability in the environment and limited insecticidal activity [6]. Rotenone is highly toxic to fish. Further, many pest species have developed resistance to pyrethroids [6]. For these reasons, the search for new, safer and more effective insecticides from plants is desirable. However, the research in this area has led to the discovery of compounds with varying insecticidal activities like insect growth regulators / inhibitors and antifeedants. Keeping in mind the importance of application of some environmentally sound plant based molecules as potential substitutes to synthetic pesticides, an endeavour has been made in this paper to present an updated account of biopesticide effects of different plant species in general and *Calotropis procera* and *Annona squamosa* in particular.

2. PLANT PARTS AND THEIR EXTRACTS USED AS BIOPESTICIDES

According to WHO survey 80% populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs and pest control. Exploration of chemical constituents of different parts of the plants and pharmacological screening may provide us the basis for developing the leads for development of novel agents [7]. Botanical insecticides break down readily in soil and are not stored in animal and plant tissues.

Often their effects are not as long lasting as those of synthetic insecticides and some of these products may be very difficult to find. Plant parts used for extraction or assay have included leaves, roots, tubers, fruits, seeds, flowers, bark, sap, pods and wood. The most commonly utilized parts were the leaves (62 species) followed by roots (16 species) and tubers (12 species). The plant families Asteraceae, Annonaceae, Asclepiadaceae, Fabaceae and Euphorbiaceae contain the majority of the insecticidal plant species reported [8].

Recently several other plants viz. neem, pongamia, Indian privet, *Adathoda, Chrysanthemum,* turmeric, onion, garlic, *Ocimum*, *Cedrus deodara*, *Nicotiana tabacum,* custard apple, ginger, *Citrus* fruits and some other plants have been reported to have insecticidal properties and therefore can be used in insecticide preparation [9,10]. Spinosad, a secondary metabolite produced by the fermentation of the fungus *Saccharopolyspora spinosa* and the active principle of the commercial products of the *Naturalyte* class and the azadirachtins - a group of limonoids, obtained from the seeds of the neem tree (*Azadirachta indica*), have shown efficacy in the control of fall webworm (*Hyphantria cunea*) [11].

Garlic acts as a repellent to various pests and is grown as a border intercrop to prevent pests from infesting the main crop. Extracts and powder preparations of garlic and onion bulbs are used to check pests in fields and grainaries. Similarly, plants like nochi (*Vitex negundo*), pongamia (*Pongamia glabra*), adathoda (*Adathoda vasica*) and sweet flag (*Acorus calamus*) have been found to be effective against various storage pests [12]. Extracts of *Pomoea cornea fistulosa*, *Calotropis gigantea* and *Datura strumarium* contain active principles toxic to many crop pests. Similarly ethyl an acetate extract of *Leucas aspera* leaves was found to be quite effective against the early third instar larvae of the malaria mosquito *Anopheles stephensi* [13].

The data presented by a recent study showed that plant extracts cited by TRAMIL ethnopharmacological surveys have the potential to control the leaf-cutting ant, *Acromyrmex octospinosus*. In particular, a *Mammea americana* extract, with its natural low repellent effect and its high toxicity by ingestion, and *Nerium oleander* extracts, with their natural delayed action, are possibly the best extracts for the control of these ants [14].

The extract of flowers of champak (*Michelia champaca*) is potent against mosquito larvae. Leaf extracts of *Strychnos nuxvomica* had been shown to possess larvicidal efficacy against the filarial vector *Culex quinquefascaitus* [15]. The leaf extracts of lantana (*Lantana camara*), *Citrus* oil, tulsi (*Ocimum basilicum, O. sanctum*) and vetiver (*Vetivera zizanoides*) are useful in controlling leaf miners in potato, beans, brinjal, tomato and chillies, etc. Crushed roots of marigold (*Tagetes erecta*) provide good control of root-knot nematodes when applied to soil in mulberry gardens [16]. The seed extract of custard apple (*Annona squamosa*) and citrus fruit (*Citrus paradisi*) are effective against the diamond back moth and Colorado potato beetle, respectively. Bark extract of *Melia azadiarach* acts as a potential antifeedant against the tobacco caterpillar (*Spodoptera litura*) and gram pod borer (*Heliothis armigera*) [17, 18]. Leaf extracts of lemon grass (*Cymbopagon citratus*), argemone (*Argemone mexicana*), cassia (*Cassia occidentalis*), artemesia (*Artemesia absinthium*) and sigesbekia (*Sieges beckiia orientalis*) are strong antifeedants to caterpillar pests like *Crocidolomia binotalis* [19]. A root extract of drumstick (*Moringa oleifera*) inhibits growth of bacteria [20]. Plant extracts of *Azadirachta indica*, *Garcinia kola*, *Zingiber officinale* and *Allium sativum* have been used for the control of bacterial leaf spot of two varieties of *Solanum* (*S. gilo* and *S. torvum*) caused by *Xanthomonas campestris* [21].

These plant extracts when integrated with other safe methods of pest control like biological control, trap crops and cultural practices etc. can provide eco-friendly and economically viable solutions for pest problems in near future.

4. PROPERTIES OF AN IDEAL INSECTICIDAL PLANT AND THEIR EXTRACTS

An ideal insecticidal plant should be perennial with wide distribution and abundantly present in nature. The plant parts to be used should be removable: harvest of leaves, flowers or fruit and harvesting should damage the plant. The plants should require a modest foodprint, minimal management and little irrigation and should not have a high economic value. The active ingredient should be effective even at lower concentration.

Crude plant extracts are advantageous in terms of efficacy and pest resistance management as the active substances present in them act synergistically [22,23]. Furthermore, they are decomposed in the environment much faster and easier than most synthetic compounds [24]. In the light of differences in geo-climatic zones and biodiversity, the plant kingdom still remains an untapped vast reservoir of new molecules endowed with massive biopesticidal potential. Over the years more than 6000 plant species have been screened and more than 2500 belonging to 235 families have been shown to possess biological activity against various categories of pests [25,26]. Their crude preparations are applied as powders or dusts (for example neem leaf dust, pyrethrum flower dusts etc.) and aqueous or organic solvent extracts [27].

However, deriving new biopesticidal principle(s) from plants remains a complex and time consuming task, because it needs interdisciplinary skills for isolation, purification, characterization, synthesis of standards (new/standard chemicals) and screening for biological effect(s). While plant extracts may afford additive/synergistic action of several weak and strong biopesticidal activities, their purification and structure determination is essential for standardization, and for bioefficacy improvement. In the grim scenario of mounting hazards and cost of synthetic chemical pesticides, natural chemistry of plants shows a ray of hope for sustainable pest management with minimal environmental and health impacts in future. In this regard, leaf and seed extracts of *Calotropis procera* and *Annona squamosa* have shown considerable potential to be as prom ising biopesticides [28-30].

5. THE BIOPESTICIDE ACTIVITIES OF PLANTS

The biopesticide activities of two known plant species are described:

5.1. *Calotropis procera*

Calotropis procera (Ait.) known as Aak and Madar, is a member of the plant family Asclepiadaceae, a shrub widely distributed in West Africa, Asia and other parts of the tropics [31]. The plant is erect, tall, large, multi-branched perennial with a milky latex throughout. A large quantity of latex can be easily collected from its green parts [31]. The abundance of latex in the green parts of the plant indicates that it is probably produced and accumulated as a defense strategy against organisms such as virus, fungi, insects and larger herbivores [32]. The presence of plant defense related proteins such as hevein, an alpha-amylase inhibitor, has been described from the latex secretion of other plants [33]. Thus it has been found to be used by indigenous people to successfully combat some cutaneous fungal infections.

Despite some reports of toxicity associated with *Calotropis* ingestion in animals, its use in ethnoveterinary medicine is increasing based on empirical evidence in the successful treatment of different ailments. Different plant parts as well as latex of *C. procera* have been reported to have emetic, purgative and anthelminthic effects in traditional medicine. *C. procera* flowers are mostly used as an anthelmintic in small ruminants in the form of decoction and/or crude powder mixed with jaggery (a cane-sugar product) and administered as physic drench/balls [32].

5.1.1 Chemical constituents of *C. procera* extract

The active ingredients of *C. procera* are a number of alkaloids, enzymes and other inorganic elements. Cardenolides, the principal steroidal toxins isolated from *C. procera*, are cardiac poisons reported to inhibit the ubiquitous and essential animal enzyme Na^+/K^+-ATPase. Moreover, only some special sorts of insects are known to feed on cardenolide-containing plants [34]. Coagulum contains resins and caoutchouc. The latex contains caoutchouc, calotropin, uscharin 0.45%, calotoxin 0.15%, calactin (composed of calotropagenin and hexose) 0.15%, trypsin, voruscharin, uzarigenin, syriogenin and proceroside. Leaves and stalks bear calotropin and calotropagenin [35]. Root bark of the root possesses the phenolics benzoyllineolone, benzoyl isolineolone, madaralban and madar fluavil. Flowers contain the anthocyanin cyanidin-3-rhamnoglucoside. The whole plant contains various enzymes such as trypsin, α-calotropeol, β-calotropeol and β- amyrin. Inorganic components such as calcium oxalate, nitrogen and sulphur are also found. The isolated fatty acid composition in the extract of *C. procera* has 7 saturated fatty acids and 11 unsaturated fatty acids. The essential elements such as Al, As, Cu, Ca, Cr, Cd, Fe, K, Mn, Na, Pb, and Zn have been analyzed from the medicinal plant in variable range. The total protein in *C. procera* was 27-32% [36]. The chemical structures of some phytochemicals with biopesticide activities are shown in the Fig.1.

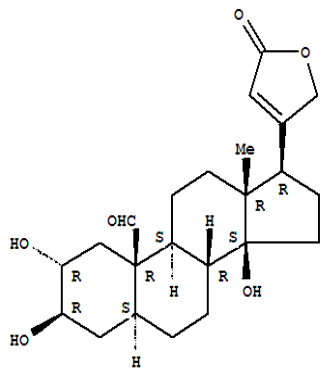

Fig.1(a): Chemical Structure of Calotrapogenin

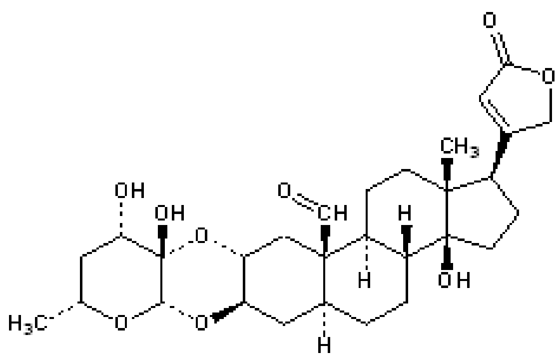

Fig.1(b): Chemical Structure of Calotropin

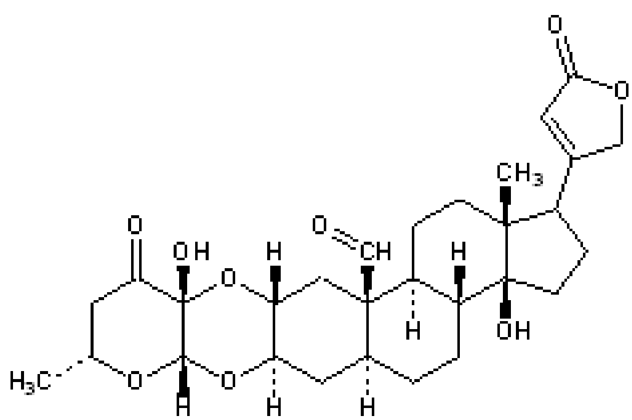

Fig.1(c): Chemical Structure of Uscharidin

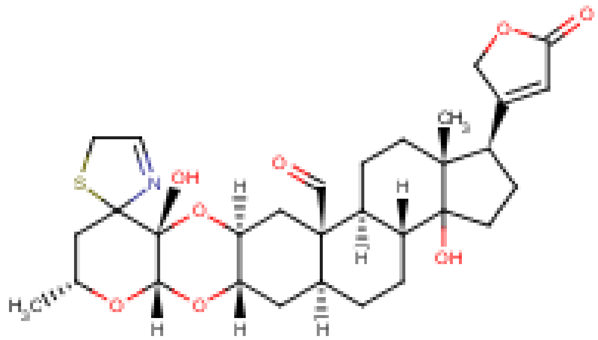

Fig.1(d): Chemical Structure of Uscharin

Source: Hanna et al. [35]

5.1.2 Impact of phytochemicals isolated from *C. procera* showing biopesticide activities against non-target systems

The *Calotropis procera* (Asclepiadaceae) produces abundant latex. Calotropin present in it causes slowing of heart beat and gastroenteritis in frogs. The latex is an irritant to the skin and mucous membranes and may cause blindness. It may rupture the muscles of the intestine and colon and death may occur. The plant may cause severe bullous dermatitis, slowed but stronger heart beat, laboured respiration, increased blood pressure, convulsions and death [37].

The current reports, however, have clearly demonstrated the insect repellent (38) and insecticidal potential of the latex isolated from *C. procera*. A net work of the laticifer cells of this plant is responsible for the synthesis of latex as an endogenous milky fluid under induction. Ramos and coworkers have shown that *C. procera*, latex is rapidly released in response to any incidental biting by insects and pests including caterpillars and beetles. They have described that there is indfuced syntheisis of two key enzymes such as chitinases and proteases in the latex of *C. procera* which act as defensive molecules and are responsible for insecticidal/pesticidal activities (39-40). Though the exact mechanism of induced synthesis of these two defence molecules is not known, but it is quite likely that the cutting/biting of *C. procera* by any insect/pest would be inducing certain genes to initiate the expression of these molecules to protect the plant. However, one of the insects, *Danaus plexippus,* possesses abundance of proteolytic enzymes in its gut which is able to quickly hydrolyse most of the latex proteins of *C. procera.* This ability of the insect makes it resistant to the *C. procera* latex (41).

A recent finding indicates that the root of *C. procera* possesses *in vitro* cytotoxicity against oral and CNS human cancer cell lines [42]. The antimicrobial activities of the organic solvent extracts of stem, leaves and flowers of *C. procera* against *Alternaria alternate, Aspergillus flavus, Asperigellus niger, Bipolaris bicolor, Curvularia lunata, Pencillium expansum, Pseudomonas marginales, Rhizoctonia solani* and *Ustilago* have been reported [43]. In Unani and Ayurvedic medical system, various parts of this plant have been used in curing a number of ailments [34]. The biological properties of different parts of *C. procera* are summarized in Table 1.

Table 1. The biological uses of different parts of *Calotropis procera*

S.No.	**Part used**	**Extract/ fraction**	**Biological activity**	**References**
1.	Flowers	Ethanol	Cytostatic activity, Asthma control, Analgesic activity	44
2.	Latex	Ethanol	Antitermites property, Mosquito control, Anti-inflammatory activity	38
3.	Latex	95% aqueous ethanol	Molluscicidal activity	45
4.	Latex	Petroleum ether	Antimicrobial activity	43
5.	Latex	Dry latex	Anthelminthic activity	32
6.	Leaves	Aqueous	Molluscicidal activity	45
7.	Leaves	95% ethanol	Insecticidal Activity Antifungal activity	28,32
8.	Leaves	Powder mixed with medium	Insecticidal activity	44
9.	Roots	Chloroform	Hepatoprotective effect	44

S.No.	Part used	Extract/ fraction	Biological activity	References
10.	Roots	Chloroform	Antiulcer activity	42

5.2 *Annona squamosa*

The Annonaceae (custard-apple family) is a large family of almost exclusively tropical trees and shrubs comprising about 130 genera and 2300 species. Plant parts of some species of this family have been used traditionally as insecticides. For example, the powdered seeds and leaf juices of *Annona* spp. are used to kill head and body lice, and bark of *Goniothalamus macrophyllus* is used to repel mosquitoes [46].

Annona squamosa L., commonly known as Sitaphal, sweetsop and Custard Apple, is a native of West Indies and is cultivated throughout India, mainly for its edible fruit. The young leaves of *A. squamosa* are used extensively for their antidiabetic activity. The plant contains aporphine alkaloids, carvone, linalool, limonene [47], squamosin [48] and quercetin [49]. Acetogenins, another a characteristic group of compounds isolated from *Annona squamosa* seeds have been suggested to act as potential anti-neoplastic agents [50]. These are also the principal insecticidal constituents of *Annona* seed extracts.

A review article by Saha [51] has indicated various medicinal as well as insecticidal properties of the phytochemicals isolated from A. squamosa. For example the leaves acting as as a vermicide as well as for treating cancerous tumors and insect bites and other skin complaints; the scrapings of root-bark for treatment of toothache; the powdered seeds to kill head-lice and fleas etc. The green fruits, seeds and leaves have effective vermicidal and insecticidal properties. In addition, the phytochemicals isolated from A. squamosa have shown the antimalarial , Antidiabetic, Hepatoprotective , Antitumor , antimicrobial , antiHIV-1 and wound healing activites. Some of these molecules have shown antioxidant, antiulcer, Anthelmintic, Anti-arthritic, anti-inflammatory, analgesic properties and cytotoxic activity against the tumors [51].

5.2.1 Chemical constituents of *A. squamosa*

The leaf extracts of this plant are known to contain different types of flavonoids some of which can act as phytoalexins [52]. These are mainly involved with the defense mechanisms of the plant and some are known to possess several antimicrobial and insecticidal properties [53]. Annotemoyin, annotemoyin, squamocin and cholesteryl glucopyranosides are isolated from the seeds of *A. squamosa* [54].

Acetogenin occur in various parts of *A. squamosa* [55]. More than 13 different alkaloids, several terpenes, kauranes were isolated. Antibacterial activity was attributed to terpenes and kauranes. Seeds yielded fixed oil containing hydroxyacids and found to contain anti-inflammatory cyclic peptides. Many pharmacological activities were experimentally reported for extracts of *A. squamosa* L. These include antitumour, cytotoxic, anti-inflammatory, analgesic, antidiabetic, antioxidant, larvicidal, insecticidal, molluscicidal, licicidal, antibacterial, nutritive and antithyroid properties [56].

The seeds are acrid and poisonous. Bark, leaves and seeds contain the alkaloid, anonaine. Six other aporphine alkaloids have been isolated from the leaves and stems: corydine, roemerine, norcorydine, norisocarydine, isocorydine and glaucine. Aporphine, norlaureline and dienone may be present also. A paste of the seed powder has been used to kill head lice but care must be taken to avoid eye contact. If applied to the uterus, it induces abortion. Heat-extracted oil from the seeds has been employed against agricultural pests. Studies have shown the ether extract of the seeds to have no residual toxicity after two days. In Mexico, the leaves are rubbed on floors and put in hen's nests to repel lice [46].

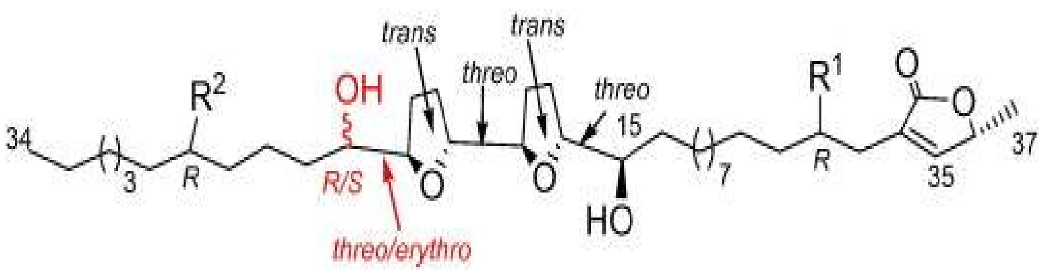

Figure 2. Chemical structure of squamosin

5.2.2 Impact of phytochemicals from *A. squamosa* on non-target systems

Mehra and Hiradher [57] reported larvicidal action of *A. squamosa* against larvae and pupae of *Culex quinquefasciatus*. The seed oil is larvicidal against the rusty grain beetle *Tribolium castaneum* (Herbst) and mosquitoes [58].

Annonaceous acetogenins extracted from tree leaves, bark and seeds have pesticidal and/or insect antifeedant properties [59]. This group of $C_{32/34}$ fatty-acid-derived natural products is among the most potent inhibitors of complex I in the mitochondrial electron transport system [60] which is consistent with the mode-of-action of rotenone. To date, nearly 400 of these compounds have been isolated from the genera *Annona, Asimina, Goniothalamus, Rollinia* and *Uvaria* [61]. Their biological activities include cytotoxicity, and *in vivo* antitumor, antimalarial, parasiticidal and pesticidal effects [62].

Antimicrobial and insecticidal properties of partially purified flavonoids from an aqueous extract of *A. squamosa* have been reported against *Callosobruchus chinensis* [63]. Ethanolic seed extracts of *A. squamosa* from Maluku (Indonesia) were highly inhibitory to larval growth of *Spodoptera litura* [64].

Many plants have been reported for their potential insecticidal actions on larvae and/or adults of house flies [65-67]. They also affect their metamorphosis, emergence, fecundity and/or longevity [68]. The important biological properties of different parts of *A. squamosa* are displayed in the Table 2.

Table 2. The biological uses of different parts of *Annona squamosa*

S. No.	**Part used**	**Extract/ fraction**	**Biological activity**	**References**
1.	Bark	Ethanol	Antimalarial activity	58
2.	Leaves	Petroleum Ether	Antibacterial activity	52
3.	Seeds	Aqueous, methanol	Anthelminthic activity	61
4.	Leaves	Methanol	Antimicrobial Activity	52, 62
5.	Seed	Ethanol	Cytotoxic Activity	55
6.	Leaves	Aqueous	Antioxidant Activity	55

S. No.	Part used	Extract/ fraction	Biological activity	References
7.	Twig	Alcohol	Antiulcer activity	54
8.	Seeds	Ethanol	Licicidal Activity	46
9.	Leaves	Ethanol	Insectticidal activity	28

6. CONCLUSION

The reports citated above clearly indicate the potential of the aforementioned two plants for pest management. Some of the phytochemicals isolated from them are also useful in management of certain diseases. Further validation of the extracts from these plants through multidimensional biochemical is required and molecular approaches is required and field trials may be useful in evaluating their suitability as safer, economic and ecofriendly biopesticides.

7. REFERENCES

1. Denholm I, Pickett JA, Devonshire AL. (Eds) Insecticide resistance: from mechanisms to management. IACR-Rothamsted, Harpenden;1999.

2. Nivsarkar M, Cherian B, Padh H. Alphaterthienyl: A plant-derived new generation insecticide. Current Science. 2001;81:667-72.

3. Taskin V, Kence M, GöÇmen B. Determination of malathion and diazinon resistance by sequencing the Md□E7 gene from Guatemala, Colombia, Manhattan, and Thailand housefly (*Musca domestica* L.). Russian J Genetics. 2004;40:377-80.

4. Ramoutar D, Alm SR, Cowles RS. Pyrethroid resistance in populations of *Listronotus* maculicollis (Coleoptera: Curculionidae) from southern New England golf courses. J Econ Entomol. 2009;102(1):388-92.

5. Etebari K, Matindoost L, Singh RN. Decision tools for mulberry thrips *Pseudodendrothrips mori* (Niwa, 1908) management in sericultural regions; An overview. Entomologia Sinica. 2004;11:243-58.

6. Isman MB. Botanical insecticides, deterrents and repellents in modern agriculture and in increasingly regulated world. Ann Rev Ent. 2006;51;44-56.

7. Pandey N, Dushyant B. Phytochemical and pharmacological review on Annona squamosa Linn. Int J Res Pharma Biomed Sci. 2011;2(4):1404-12.

8. Boulogne I, Petit P. Insecticidal and antifungal chemicals produced by plants: a review. Environ Chem Lett, 2012; 10(4): 325-47.

9. Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res. 2009;104:1365–372.

10. Osipitan AA, Oseyemi AE. Evaluation of the Bio-insecticidal Potential of Some Tropical Plant Extracts Against Termite (Termitidae:Isoptera) in Ogun State, Nigeria. J Entomol. 2012;9:257-65.

11. Brudea V, Rîşca I, Enea C, Tomescu C. Efficacy of some biopesticides and plant secondary metabolites against fall webworm *Hyphantria Cunea* Drury (*F. Arctiidae-Lepidoptera*) in the Lab Conditions. Cercetari Agronomice in Moldova. 2012;45(1):73–80.

12. Sadek MM. Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). J Appl Ent, 2003;127:396–404.

13. Arivoli S, Ravindran KJ, Tennyson S. Larvicidal efficacy of some plant extracts against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). World J Med Sci. 2012;7(2):77-80.

14. Boulogne I, Germosen-Robineau L, Ozier-Lafontaine H, Jacoby-Koaly C, Aurela L, Loranger-Merciris G. *Acromyrmex octospinosus* (Hymenoptera: Formicidae) management. Part 1: Effects of TRAMIL's insecticidal plant extracts. Pest Manag Sci. 2012;68(2):313–20.

15. Arivoli S, Samuel T. Larvicidal efficacy of *Strychnos nuxvomica* Linn. (Loganiaceae) leaf extracts against the filarial vector *Culex quinquefascaitus* Say. (Diptera: Culicidae). World J Med Sci. 2012; 7(1): 6-11.

16. Chitwood DJ. Phytochemical based strategies for nematode control. Annu Rev Phytopathol, 2002;40: 221–49.

17. Wheeler DA, Isman MB, Sanchez-Vindas PE, Arnason JT. Screening of Costa Rican *Trichilia* species for biological activity against the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). Biochem Syst Ecol. 2001; 29:347–58.

18. Nathan SS. Effects of *Melia azedarach* on nutritional physiology and enzyme activities of the rice leaffolder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), Pestic Biochem Physiol. 2006; 84:98–108.

19. Abdelgaleil SAM, Abbassy MA, Belal AH, Abdel Rasoul MAA. Bioactivity of two major constituents isolated from the essential oil of *Artemisia judaica* L. Biores Tech. 2008; 99 (13): 5947-950.

20. Fahey JW. *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part1. Trees for Life J. 2005;1:5-10.

21. Opara EU, Obani FT. Performance of Some Plant Extracts and Pesticides in the Control of Bacterial Spot Diseases of *Solanum*. Agri Journal.2010;5(2):45-49.

22. Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann Rev Entomol.1990;35:271-97.

23. Vo¨llinger M, Schmutterer H. Development of resistance to azadirachtin and other neem ingredients. In: Schmutterer, H. (Ed) The neem tree, 2nd edn. Neem Foundation, Mumbai. 2002; 598–606.

24. Ujvary I. Transforming natural products into natural pesticides- experience and expectations. Phytoparasitica. 2002;30:1–4.

25. Copping LG, Menn, JJ. Biopesticides:a review of their action, applications and efficacy. Pest Manag Sci. 2000; 56:651–76.

26. Walia S, Koul O. Exploring plant biodiversity for botanical insecticides. In: Sustainable crop protection, Biopesticide stratergies, Kalyani Publishers, New Delhi. 2008;191-206.

27. George DR, Guy JH, Arkle S, Harrington D, De Luna C, Okello EJ, Shiel RS, Port G, Sparagano O.A. Use of plant-derived products to control arthropods of veterinary importance: a review. Ann N Y Acad Sci. 2008;1149:23-6.

28. Begum N, Sharma B, Pandey RS. Evaluation of Insecticidal efficacy of *Calotropis procera* and *Annona squamosa* ethanol extracts against *Musca domestica.* J Biofertil Biopestici. 2010; 1:1,.doi.org/10.4172/2155-6202.1000101.

29. Begum N, Sharma B, Pandey RS. Toxicity potential and anti AChE activity of some plant extracts in *Musca domestica.* J Biofertil Biopestici. 2011; 2:2,.doi.org/10.4172/2155-6202.1000108.

30. Begum N, Sharma B, Pandey RS. Insecticidal potential of *Calotropis procera* and *Annona squamosa* ethanol extracts against *Musca domestica*. Natural Products – An Indian journal. 2012;7:5.

31. Irvine FR. Woody plants of Ghana. Oxford University Press, London. 1961;48-50.

32. Larhsini M, Bousad M, Lazrek, JM, Amarouch H. Evaluation of antifungal and molluscicidal properties of extracts of *Calotropis procera.* Fitoterapia. 1997;68:371-73.

33. Wititsuwannakul D, Sakulborirug C, Wititsuwannakul RA. Lectin from the bark of the rubber tree (*Hevea brasilliensis*). Phytochem. 1998;47:183-87.

34. Agrawal AA, Petschenka G, Bingham RA, Weber MG, Rasmann S. Toxic cardenolides: chemical ecology and coevolution of specialized plant–herbivore interactions. New Phytologist, 2012;194: 28–45.

35. Hanna AG, Shalaby NMM, Morsy NAM, Simon A, Tóth G, Malik S, Duddeck, H. Structure of a calotropagenin-derived artifact from *Calotropis procera*. Magnetic Resonance in Chemistry. 2002;40:599–602.

36. Khanzada SK, Shaikh W, Kazi TG, Sofia S, Kabir A, Kandhro AA. (Analysis of fatty acid, elemental and total protein of *Calotropis procera* medicinal plant from Sindh, Pakistan. Pak J Bot. 2008; 40(5):1913-921.

37. Duke JA. CRC Handbook of Medicinal Herbs, CRC Press, Inc. Raton Florida, USA; 1986;292-95.

38. Ramos MV, Araújo ES, Oliveira RSB, Teixeira FM, Pereira DA, Cavalheiro M G, Souza DP, Oliveira JS, Freitas CDT. Latex fluids are endowed with insect repellent activity not specifically related to their proteins or volatile substances. Braz J Plant Physiol. 2011;23(1):57-66.

39. Ramos MV, Grangeiro TB, Freire EA, Sales MP, Souza DP, Araújo ES, Freitas CDT. The defensive role of latex in plants: detrimental effects on insects. Arthropod-Plant Interactions. 2010;4(1):57 – 67.

40. Ramos MV, Freitas CDT, Stanisçuaski F, Macedo LLP, Sales MP, Sousa DP, Carlini CR. Performance of distinct crop pests reared on diets enriched with latex proteins from *Calotropis procera*: Role of laticifer proteins in plant defense. Plant Sci. 2007;173(3):349-57.

41. Pereira DA, Ramos MV, Souza DP, Portela TCL, Guimarães JA, Madeira SVF, Freitas CDT. Digestibility of defense proteins in latex of milkweeds by digestive proteases of Monarch butterflies, *Danaus plexippus* L.: A potential determinant of plant–herbivore interactions. Plant Sci. 2010;179(4):348-55.

42. Bhagat M, Arora JS, Saxena, AK. *In vitro* cytotoxicity of extracts and fractions of *Calotropis procera* (Ait.) roots against human cancer cell lines. Int J Green Pharmacy. 2010;4(1):36-40.

43. Varahalarao V, Chandrashekar KN. *In vitro* bioactivity of Indian medicinal plant *Calotropis procera* (Ait). J Glob Pharma Technol. 2010;2(2):43-45.

44. Sharma P, Sharma JD. Evaluation of *in vitro* schizontocidal activity of plant parts of Calotropis procera—an ethnobotanical approach. J Ethnopharmacol.1999;68:83–95.

45. Al-Sarar A, Hussein H, Abobakr Y, Bayoumi A. Molluscicidal activity of methomyl and cardenolide extracts from Calotropis procera and Adenium arabicum against the land snail Monacha cantiana. Molecules. 2012;17(5):5310-18.

46. Morton J. (1987). Sugar Apple. In: Fruits of warm climates. Julia FM, Miami FL (Ed) p. 69-72.

47. Ekundayo O. A review of the volatiles of the Annonaceae. J Essent Oil Res. 1989;1: 223.

48. Yu JG, Luo, XZ, Sun L, Li DY, Huang WH, Liu CY. Chemical constituents from the seeds of *Annona squamosa*. Yao Xue Xue Bao. 2005;40(2):153-58.

49. Panda S, Kar A. *Annona squamosa* seed extract in the regulation of hyperthyroidism and lipidperoxidation in mice: Possible involvement of quercetin. J Plant Biochem. 2007;14(12):799-805.

50. Yuan SSF, Chang HL, Chen HW, Yeh YT, Kao YH, Lin KH, Wu YC, Su JH. Annonacin, a mono-tetrahydrofuran acetogenin, arrests cancer cells at the G1 phase and causes cytotoxicity in a Bax and caspase-3-related pathway. Life Sci. 2003;72(25):2853-861.

51. Rajsekhar S, Pharmacognosy and pharmacology of Annona squamosa: A review. Int J Pharm Life Sci. 2011;2:1183-89.

52. Chaterjee A, Pakrashi SC, editors. *Annona squamosa* in the treatise of Indian medicinal plants Publication and Information Directorate, New Delhi, 1995;130.

53. Padmavati M, Reddy AR. Flavonoid biosynthetic pathway and cereal defence response: An emerging trend in biotechnology. J Plant Biochem Biotechnol.1999;8:15-20.

54. Mukhlesur RM. Antimicrobial and cytotoxic constituents from the seeds of *Annona squamosa*. Fitoterpia. 2005;7:484-89.

55. Yang H, Zhang N, Li X, Chen J, Cai B. Structure–activity relationships of diverse annonaceous acetogenins against human tumor cells. Bioorg. Med. Chem. Lett. 2009;19(8):2199-202.

56. Jagtap NS, Nalamwar VP, Khadabadi SS, Pratapwar AS. Phytochemical and Pharmacological Profile of *Annona squamosa* Linn: A Review. Res J Pharmac phytochem. 2009;1(3):139.

57. Mehra BK, Hiradhar PK. Effect of crude acetone extract of seeds of *Annona squamosa* Linn. (Family: Annonaceae) on possible control potential against larvae of *Culex quinquefasciatus* Say. J Entomol Res. 2000;24(2):141–46.

58. Malek MA, Wilkins RM. Effects of *Annona squamosa* L. seed oil on the pupae and adults of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Bangladesh. J Entomol.1994;4:25-31.

59. González MC, Lavaud C, Gallardo T, Zafra-pollo MC, Cortes D. New Method for the determination of the absolute Stereochemistry of Antitumoral Annonaceous Acetogenins. Tetrahedron. 1998;54(22):6079-88.

60. Zafra-Polo MC, Gonzales MC, Estornell E, Sahpaz S, Cortes D. Acetogenins from Annonaceae: inhibitors of mitochondrial complex 1. Phytochem. 1996;42:253-71.

61. Johnson HA, Oberlies NH, Alali FQ, McLaughlin JL. Thwarting resistance: annonaceous acetogenins as new pesticidal and antitumor agents, *in:* Cutler, S.J. and Cutler, H.G. [Eds.] Biologically Active Natural Products. Pharmaceuticals. CRC Press, Washington, DC. 2000;173-83.

62. Asmanizar AD, Idris AB. Evaluation of *Jatropha curcas* and *Annona muricata* seed crude extracts against *Sitophilus zeamais* infesting stored rice. J Entomol. 2012; 9:13-22.

63. Kotkar HM, Mendki PS, Sadan SVG, Jha SR, Upasani SM, Maheshwari VL. Antimicrobial and pesticidalactivity of partially purified flavonoids of *Annona squamosa*. Pest Manag Sci. 2002;58: 33-37.

64. Leatemia, J.A., Isman, M.B. Insecticidal activity of crude seed extracts of *Annona* spp. (Annonaceae), *Lansium domesticum* and *Sandoricum koetjape* (Meliaceae) against lepidopteran larvae. Phytoparasitica. 2004;32:32-37.

65. Morsy TA, Rahem MM, Allam KA. Control of *Musca domestica* third instar larvae by the latex of *Calotropis procera* (Family: Asclepiadaceae). J Eqypt Soc of Parasitol. 2001;31:107-10.

66. Sukontason KL, Boonchu N, Sukontason K, Choochote W. Effects of eucalyptol on housefly (Diptera: Muscidae) and blow fly (Diptera: Calliphoridae). Rev Inst Med Trop S Paulo. 2004;46: 97-101.

67. Abdel Halim AS, Morsy TA. Efficacy of *Trigonella foenum-graecum* (fenugreek) on third stage larvae and adult fecundity of *Musca domestica*. J Eqypt Soc Parasitol. 2006; 36: 329-34.

68. Abdel Halim AS, Morsy TA. The insecticidal activity of *Eucalyptus globulus* oil on the development of *Musca domestica* third stage larvae. J Eqypt Soc Parasitol. 2005; 35: 631-36.