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* 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 new 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 plants viz. neem, pongamia, Indian privet, *Adathoda, Chrysanthemum,* turmeric, onion, garlic, *Ocimum*, *Cedrus deodara*, *Nicotiana tabacum,* custard apple, ginger, *Citrus* fruits and some others 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 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 a potential insecticide against mosquito larvae [14]. Leaf extracts of *Strychnos nuxvomica* have 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. The bark extract of *Melia azedarach* 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; harvesting of leaves, flowers or fruit should not 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.

The 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 promising 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. The leaves and stalks bear calotropin and calotropagenin [35]. The root bark of the root possesses the phenolics benzoyllineolone, benzoyl isolineolone, madaralban and madar fluavil. The 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.

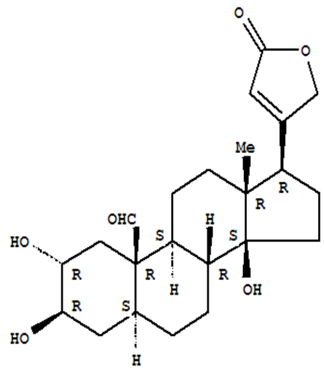

Fig.1(a): Chemical Structure of Calotrapogenin

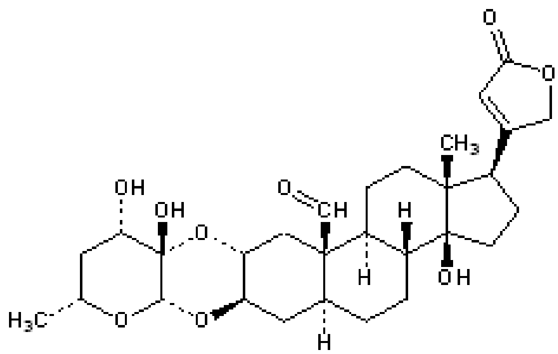

Fig.1(b): Chemical Structure of Calotropin

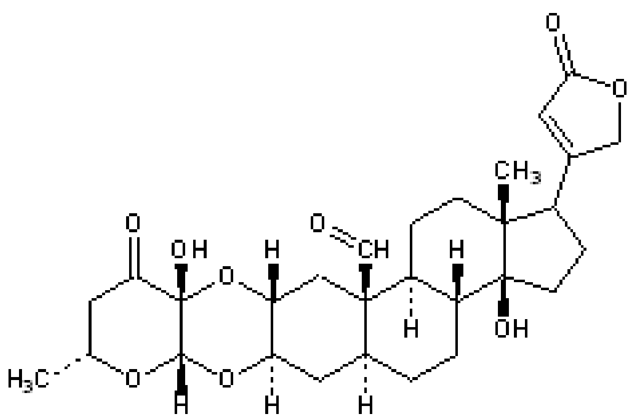

Fig.1(c): Chemical Structure of Uscharidin

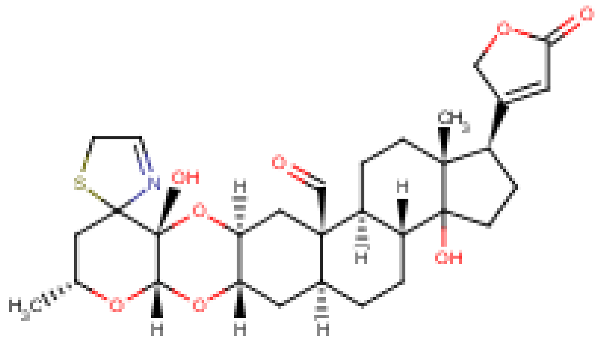

Fig.1(d): Chemical Structure of Uscharin

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 induced synthesis 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 activities. 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].

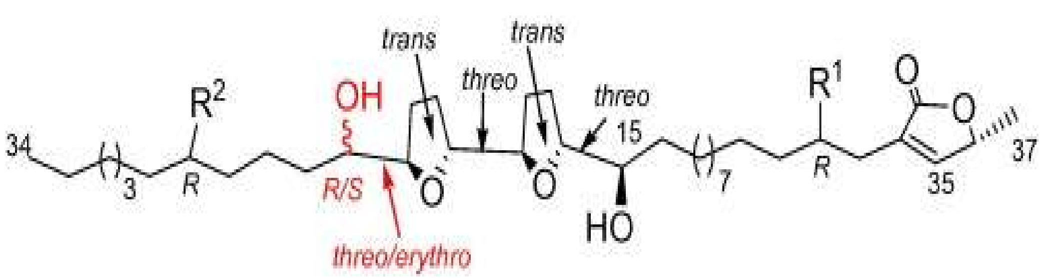

Figure 2. Chemical structure of squamosin

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 and molecular approaches is required. The field trials may be useful in evaluating their suitability as safer, economic and ecofriendly biopesticides.

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