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ANNONA SQUAMOSA: A REVIEW ON ITS LARVICIDAL, OVIPOSITION DETERRENT AND INSECT REPELLENT POTENCY

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ABSTRACT

Insects and pests pose a great problem not only to the humans by transmitting various diseases but also cause agricultural damage. The present review provides an insight on the larvicidal, oviposition deterrent, insect repellent and insecticidal activities shown by various parts of the plant *Annona squamosa* L.

KEYWORDS: *Annona squamosa,* larvicidal activity, insect repellent, plant extract.

INTRODUCTION

Mosquitoes are the main causative agents of various ailments such as malaria, dengue, chikungunya, Japanese encephalitis, filaria, and the list goes on. The commonest among them are *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquifasciatus*.[1] These vectors have been considered as a major obstacle to socioeconomic development of developing countries particularly in the tropical region.[2] Despite considerable efforts in recent years to control vector-borne diseases, malaria alone produces 250 million cases per year and 800,000 deaths including 85% children less than five years.[3]

Owing to the public concerns over safety of synthetic chemicals, interest in botanical repellents has increased lately, and has stimulated a reexamination of the repellent data. Prior to the advent of synthetic repellents, pyrethrum and citronella oil were widely used in repellent lotions, sprays, smokes, and candles. Research has demonstrated the potential of a number of other experimental plant natural products as botanical insecticides including piperamides, acetogenins, thiophenes, and limonoids. The use of plant substances to protect us from insects probably developed since antiquity. Traditional repellents like smoke are still widely used for repelling mosquitoes throughout the rural areas. Thousands of plants have

been tested as potential sources of insect repellents. Plants whose essential oils have been reported to show repellent activity include citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cajiput, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint. Unlike synthetic insect repellents, plant derived repellents have been relatively poorly studied. When tested most of these essential oils gave short lasting protection, usually less than 2 hours.[4]

Annona squamosa L., the plant of Annonaceae family, also known as custard apple, is commonly found in deciduous forests, is a small tree of 3-7 m height. It is mainly grown in gardens for its fruits and ornamental value. It is a native of West Indies; now cultivated throughout India and other tropical countries. This plant is commonly called 'custard apple' in English, 'sharifa' in Hindi and 'sitaphalam' in Telgu in India.[5] Literatures of many research works prove that every parts of *A. squamosa* possess medicinal property.[6] The Annonaceae (Custard apple family) is a large family of almost exclusively tropical trees and shrubs comprising about 130 genera and 2300 species. *Annona squamosa* Linn is a multipurpose tree with edible fruits and is of medicinal and industrial importance. It is used as an antioxidant, anti-diabetics, hepato protective, cytotoxic activity, gene toxicity, anti-tumour activity, anti-lice agent. It is related to contain alkaloids, carbohydrates, fixed oils, tannins and phenolic compounds. Earlier works on the phytochemisrty of *Annona squamosa* leaf, reported the presence of alkaloids, flavonoids, phenols, saponins, glycosides etc in water, methanol, chloroform, and petroleum ether extracts.[5]

Folkloric record reported the use of *Annona squamosa* as an insecticidal, an antitumor agent, anti-diabetic, hyperthyroidism, antioxidant, anti-lipidimic and anti-inflammatory agent which has been characterized due to the presence of the cyclic peptides. An infusion of the leaves is considered efficacious in prolapsusani of children. In addition the crushed leaves were sniffed to overcome the hysteria and fainting spells, and are also applied on the ulcers and wounds. A leaf decoction was taken in the case of dysentery.[7] Leaves are used as poultice over boils and ulcers and also to kill lice. Leaf infusion is efficacious in prolapsus of children. Bruised leaves with salt make a cataplasm to induce suppuration. They are applied for extraction of guinea-worms. The root is considered as a drastic purgative. Roots are employed internally in depression of spirits and spinal diseases. Bark is known to be a powerful astringent. [8] In Ayurveda, fruits are considered as a good tonic; enriches blood, used as expectorant, increases muscular strength; cooling, lessens burning sensation and tendency to biliousness;

sedative to heart and relieves vomiting. The seeds are said to be abortifacient and good to destory lice in hair in Yunani medicine. Seed yields oil and resin which acts as detergent and their powder is mixed with gram-flour which is a good hair wash. Seeds are powerful irritant of conjunctiva and produce ulcers in the eye.[9]

Plant parts of some species of this family have been used traditionally as insecticides. For example, the powdered seed and leaf juices of Annona species are used to kill head and body lice. Annonaceous acetogenins extracted from leaves, bark and seed have pesticidal and/or insect antifeedant properties.The leaves possess insecticidal and pesticidal effects against bugs and lice. Leaves, seeds and unripe fruits have vermicidal and insecticidal properties and are used to kill lice on cattle. [10]

Insect repellent Activity

Crude seed extract of three tropical fruits belonging to the family Annonaceae, viz., sweetsop (*Annona squamosa* L.), soursop (*A. muricata* L.) and biriba (*Rollinia mucosa* Baill.) were investigated for their repellent effects on the Asian subterranean termite *Coptotermes gestroi* Wasmann (Isoptera: *Rhinotermitidae*). The results suggest that *Annona* seed extracts may offer an alternative source of natural insecticide against subterranean termites. Termites showed significant avoidance behavior to filter paper treated with atleast 10% Annona crude seed extract. Soil treated with 5-10% crude extract of the three *Annona* species investigated prevented tunneling and penetration of *C. gestroi*. Termite behavior in both feeding and soil penetration tests indicated that extracts of *A. squamosa*, *A. muricata* and *R. mucosa* seed extracts had anti-feeding or repellent effect on *C. gestroi*.[11]

The skin repellent activity of *A. squamosa* leaf extract against three mosquitoes namely *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* was studied. The highest concentrations of 0.02 ppm provided over 126.2 min protection against *Anopheles stephensi*. Lower concentrations provided 52.4 and 50.4 minutes of protection against *Aedes aegypti* and *Culex quinquefasciatus*. Authors observed that the repellent activity was dose dependent.[12]

Larvicidal activity

The ethanolic and methanolic extracts of *A. squamosa* leaves showed potent larvicidal efficacy against *Aedes albopictus* and *Culex quinquefasciatus* larvae with LC90 values at less than 100 ppm in each case. However, the ethanol extract of leaf of *A. squamosa* was found to have the most promising larvicidal activity against *Cx. quinquefasciatus* larvae. [13] The

larvicidal activity of crude acetone extract of seeds of *A. squamosa* against *Culex quinquefasciatus* larvae is also reported.[14]

Kamaraj et al., 2011 studied the role of larvicidal activities of hexane, chloroform, ethyl acetate, acetone, and methanol dried leaf and bark extracts of *Annona squamosa* L., *Chrysanthemum indicum* L., and *Tridax procumbens* L. against the fourth instar larvae of malaria vector, *Anopheles subpictus* Grassi and Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). All plant extracts showed moderate toxic effect on *An. subpictus* and *Cx. tritaeniorhynchus* after 24 h of exposure at 1000 mg/l; however, the highest mortality was found in bark methanol extract of *A. squamosa* against the larvae of *An. subpictus* (LC50=93.80, LC90=524.90 mg/l) and against the larvae of *Cx. tritaeniorhynchus* (LC50=104.94, LC90=443.79 mg/l), respectively.[15]

Leaf extract of *A. squamosa* was effectively possessing larvicidal activity against the three mosquitoes namely *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. The highest larvicidal potency with LC50 and LC90 value of chloroform extract depicted 219.41 ppm and 394.87 ppm against *Aedes aegypti*, the petroleum ether extract indicated 118.4 ppm and 273.10 ppm of LC50 and LC90 value against *An. stephensi,* and 342.6 ppm and 616.70 ppm of LC50 and LC90 values were observed against *Cx. quinquefasciatus* using acetone extract, respectively.[12]

Das et al., 2007 studied various extracts of herbal plant for finding potent larvicidal activity. The ethanolic and methanolic extracts of *A. squamosa* leaves showed potent larvicidal efficacy against *Ae. albopictus* larvae (LC90 values at less than 100 ppm) and *Cx. quinquefasciatus* larvae (LC90 values at less than 100 ppm).

The leaf ethanolic extract and leaf powders of *Annona squamosa* procured from Brachystegia Julbernadia Savanna Woodlands of Tanzania eco-zone showed the highest larvicidal activity with LC50 value of 0.0066 mg/ml and 0.0860 mg/ml against *Culex quinquefasciatus* while activity of the same sample against *An. gambiae* was 0.0252 mg/ml and 0.0599 mg/ml respectively after 24 h of exposure.[16]

Cytotoxicity and larvicidal properties of the leaf extracts of 3 Annonaceous plants, *Annona muricata* L., *A. senegalensis* Pers and *A. squamosa* L. were tested against brine shrimp larva and the late 3 rd instar of *Culex quinquefascintus* Say. The larval mortality was observed 24 h

post-exposure. Based on the probit analysis, the LC 50 value for crude extracts of *A. muricata*, *A. senegalensis* and *A. squamosa* were observed to be 20.87, 0.67 and 0.64 µg/mL respectively for shrimp larvae and 56.47, 23.42 and 11.01 µg/mL respectively for *C. quinquefascintus.*

Bioassay-guided fractionation of crude extracts indicated high activity of the ethyl acetate fractions with mortality of 90% at 50 µg/mL for both *A. senegalensis* and *A. squamosa*. Two known aporphine alkaloids, (-)-roemerine and annonaine were identified as active principles from the ethyl acetate extracts of *A. senegalensis* and *A. squamosa* respectively. [17]

Oviposition deterrent activity

Following the oviposition deterrent tests, the leaf extract of *A. squamosa* greatly reduced the number of eggs deposited by gravid *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* at several concentrations. At the highest concentrations, the extract reduced egg laying by 99.6% against *Anopheles stephensi* followed by lower oviposition deterrent activity recorded in *Culex quinquefasciatus* (92.4%) and *Aedes aegypti* (92.4%), respectively.[12]

Insecticidal activity

The common housefly *Musca domestica* (Diptera: Muscidae) which is an important mechanical vector of many bacterial and pathogenic microbes of human and animals have become resistant to the chemical insecticides. *Annonaceous acetogenins* which were extracted from the tree leaves, bark and seeds possess the insect anti-feedant properties. The larvicidal activities of the ethanolic extracts of *A. squamosa* leaves against the *Musca domestica* was evaluated and was found that the LC50 values of the extract was found to be of around 282.5 and 550 mg/l. The data obtained suggested that the leaf extracts of the above plant can be utilized as the probable candidates in the development of bioinsecticides in order to control the population of *Musca domestica* for safer and economic alternative to the synthetic insecticides. [18] Crude ethanolic seed extracts of *A. squamosa* was also screened for their inhibition of larval growth against the polyphagous lepidopteran *Spodoptera litura* (Noctuidae) in which the extracts significantly showed more active (20-fold) insecticidal effect.[10]

Egunyomi A et al., 2010 investigated ten herbal plants from Nigeria for potent repellent activity. Seven out of ten plant extracts showed promising repellent activity against

Anopheles stephensi, i.e. *C. citratus, Annona squamosa, Lantana camara, Citrus sinensis Solanum nigrum, Tridax procumbens* and *Azadirachta indica*. Low repellency was observed in *O. gratissimum, A. conyzoides* and *H. suaveolens*. Hexane plant extracts were found to be more effective than methanol plant extracts indicating that the active compounds were more soluble in hexane. Increased repellency was observed at 2mg/ml extract concentration. Lower concentration showed no repellency and concentration higher than 2mg/ml eventually killed some of the mosquitoes that came into physical contact with the treated guinea pigs. This indicated that the extracts also possess adulticidal properties.[19]

The ethanol extract of *Annona squamosa* produced significant "Knockdown" (KD50) in the concentration 1% w/v and 5% w/v tested 23.1 min and 11.4 min for respectively. The mortality (100%) was achieved at 39.6±1.4 and 14.5±1.1min for 1% w/v and 5% w/v concentration respectively. No mortality of the insects was found in any of the controls up to 100hours. The ethanolic *Annona squamosa* extract showed potent activity against *Sitophilus oryzae* pest.[20]

Mst. Shahnaj Parvin et al., 2003 evaluated the pure compound annotemoyin-1 isolated from the chloroform extract of the seeds of *Annona squamosa* Linn. for its pesticidal activity against both adults and different instars of *Tribolium castaneum* (Herbst); which is a major pest of stored grains under laboratory condition. The LD50 values of the pure compound were determined for adults and different instars of larvae. The LD50 values of the compounds ranged from 191.70 to 920.54. The authors found out that the earlier instars were more sensitive to the compound than those of late instars as well as adults. [21]

Evaluation methods

After the collection and authentification of the required plant part, the dry drug sample is taken. Extracts of the powdered plant are taken in different suitable solvents using Soxhlet apparatus which are later on subjected to different phytochemical tests.

Phytochemical screening

After the extract has been taken, they are tested for phytochemical screening of powdered plant samples are tested for the presence of alkaloids, tannins, saponins, anthraquinones, steroids and phenols, using different methods prescribed in literature.

Repellency test

Standard cages (20 × 20 × 20 cm) were used to test the repellent activity of plant extracts. Repellency test is defined as the ability of the test material to keep away mosquitoes from landing in order to take a successful blood meal. In each mosquito cage, one guinea pig was placed for one test concentration and the other guinea pig applied with only ethanol served as control. The control and treated guinea pigs were introduced simultaneously into the cage. Before each test, the readiness of the mosquitoes to bite was confirmed by inserting the untreated guinea pig into the test cage. Once five mosquito landings were observed on the untreated guinea pig, it was removed from the cage and the test guinea pig was inserted into the cage. The first test of each repellent was conducted by inserting the treated guinea pig into a test cage for one full minute every three minutes. If it was not bitten within six minutes, then the guinea pig was reinserted for three full minutes every 10 minutes, until the first bite occurred.

On the basis of this initial complete-protection time, the guinea pig's next tests of that particular repellent were conducted as follows: if the repellent had initially worked for less than 10 minutes, the subject was placed in the cage for 1, 2 and 3 minutes every 5 minutes; if the repellent had initially worked for 10 – 15 minutes, the subject was placed in the cage for 1, 2, and 3 minutes every 15 minutes; and if the repellent had initially worked for more than 20 minutes, the subject was placed in the cage for 1, 2, and 3 minutes every 30 minutes (up to 1 hour). If it was observed at any point during testing, that mosquitoes were landing but not biting (a behavior that typically occurs when the efficacy of a repellent begins to wane), the intervals between insertions were decreased to 5 minutes. [19] The mosquito repellency of different extract was measured on the basis of the number of mosquitoes that fed within a specified time (minute), that is, the accurate documentation of the duration of exposure and the time of the first bite was recorded and the elapsed time to the first bite was then calculated and recorded as the "complete-protection time" for the guinea pig in that particular test. [22]

Larvicidal Activity

In order to study the toxicity of the concerned plant extracts, the tested material of the ethanolic extracts was dissolved in 0.1 ml of 70% ethanol, while the tested material of petroleum extracts was dissolved in 2 drops of Tween 80 as emulsifier to facilitate the dissolving of tested material in water. Different range of concentrations of each concerned extract was prepared in order to detect mortalities. All tested materials were performed in 100

ml of dechloronated tap water contained in 200 ml plastic cups. Then, twenty 2nd instar larvae were put immediately into plastic cups containing different concentrations of extracts. At least, three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions at temperature of 27 ± 2°C, relative humidity 70 ± 10% and 12-12 light-dark regime. Control larvae received 0.1 ml of 70% ethanol or 2 drops of Tween 80 in 100 ml water. Mortality was recorded daily and dead larvae and pupae removed until adult emergence. Abnormal larvae, pupae and adults were removed daily and placed in labelled glass vials containing 70% ethanol then photographed under binocular microscope. The larvae were observed daily until pupation and adult emergence to estimate the following parameters: [23]

Larvicidal activity.

Larval mortality percent was estimated by using the following equation**:** Larval mortality % = A – B / A × 100, where A = number of tested larvae and B = number of tested pupa.

***Pupation rate*.**

The pupation percent was estimated by using the following equation:

Pupation % = A / B × 100, where A = number of pupae and B = number of tested larvae.

Pupal mortality.

The pupal mortality percent was estimated by using the following equation: Pupal mortality % = A – B / A × 100, where A = number of produced pupae and B = number of observed adults.

Adult emergence.

The emerged males and females adults were counted and the adult emergence percent was calculated by using the following equation:

Adult emergence % = A / B × 100, where A = number of emerged adults and B = number of tested pupae.

Malformative effects.

Any abnormal shape, size, color or failure to pupate indicated as malformation of larvae. All malformed larvae died after a short time, they were counted and removed immediately.

Larval malformation percent was estimated by using the following equation: Larval malformation % = C / A × 100, where C = number of malformed larvae and A = number of tested larvae.

Pupal malformation was estimated by any change in color, size, shape or failure to develop to adult stage (pupal adult intermediate). All malformed pupae were counted and removed immediately. The pupal malformation percent was calculated by using the following equation: Pupal malformation % = C / A × 100, where C = number of malformed pupae and A = number of tested pupae.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC50, LC90, and other statistics at 95 per cent fiducial limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated by using various softwares.

Oviposition deterrent activity

The oviposition deterrent test of various solvent extract of *A. squamosa* could be performed using the method of Xue et al. 2001.[24] Fifteen gravid female mosquitoes *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* were (6 days old, 4 days after blood feeding) transferred to each mosquito cage (45x38x38 cm) and separately covered a plastic screen, with a glass top and a muslin sleeve for access. A 10% sucrose solution was available at all times. Serial dilutions of leaf extract of each test plant were made in solvent medium used. Enamel bowls containing 100 ml of water treated with leaf extract to obtain test solutions of 0.01, 0.025, 0.050, 0.075 and 1.0%. Two enamel bowls holding 100 ml of water were placed in the opposite corners of each cage, one treated with the test material and the other with a solvent control (1 ml). The positions of the bowls were alternated between the different replicates so as to nullify any effect on oviposition. Five replicates for each concentration were run, with cages placed side by side for each bioassay. All experiments were run at ambient temperature (27 ± 2°C) with a relative humidity of 70-80%. After 24 hr the number of eggs laid in treated and control bowls were recorded. The per cent effective repellency for each plant leaf extract concentration was calculated using the following formula: [25, 26]

ER(%) = [(NC- NT)/NC] ×100 (%)

where, ER = Per cent effective repellency, NC = Number of eggs in control.

CONCLUSION

Long before the advent of synthetic insecticides, plants and their derivatives were used to kill pest of agriculture, veterinary and public health. The herbal plants can be used alone or combined for effective protection against mosquitoes. They can also be used for control of mosquito breeding under integrated disease vector control programme in various situations. They also offer safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. However toxicity tests of the active plants need to be done to ascertain their safety in administration. Larvicidal activities of the plant extracts vary according to the plant species, the parts of the plant, the geographical location where the plants were grown and the application method. Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals for control of insect vectors.

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