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## BIO-PESTICIDES: A POTENT PHYTOFORMULATION FOR GOOD AGRICULTURAL PRACTICES (GAP)

Gopal V\*, Prakash Yoganandam G, Thamaraikavi K

Department of Pharmacognosy, College of Pharmacy, Mother Theresa Post Graduate & Research Institute of Health Sciences (A Govt. of Puducherry Institution), Puducherry-605006, India.

### ABSTRACT

Chemical pest control agents are extensively used in all countries of the world but they are regarded as ecologically unacceptable. Therefore, there is an increased social pressure to replace them gradually with bio-pesticides which are safe to humans and non-target organisms. The harmful environmental implications of the synthetic chemicals have compelled to search for some alternative methods. This leads to increased development of compounds based on the models of naturally occurring toxins of biological origin, having various biological activities. Bio-pesticides include a broad array of microbial pesticides, bio chemicals derived from micro-organisms and other natural sources and processes involving the genetic modification of plants to express genes encoding insecticidal toxins. This review outlines the current state of knowledge on the potential use of bio-pesticides in global control of pests for good agricultural practices (GAP).

**Keywords:** Microbial pest, Bio-pesticides, Phytoformulation, GAP.

### INTRODUCTION

Agriculture has been facing the destructive activities of numerous pests like fungi, weeds and insects from time immemorial leading to radical decrease in yields. Pests are constantly being introduced to new areas either naturally or accidentally or in some cases, organisms that are intentionally introduced become pests. Global trade has resulted in increased numbers of invasive, non-native pest species being introduced to new areas. Controlling these invasive species presents an unparalleled challenge worldwide. Agriculture and forests form an important resource to sustain global economic, environmental and social system. For this reason, the global challenge is to secure high and quality yields and to make agricultural produce environmentally compatible. Chemical means of plant protection occupy the leading place as regards their total volume of application in integrated pest management and diseases of plants but pesticides cause toxicity to humans and warm-blooded animals.

Despite many years of effective control by conventional agrochemical insecticides, a number of factors are threatening the effectiveness and continued use of these agents. These include the development of insecticide resistance and use-cancellation or de-registration of some insecticides due to human health and environmental concerns.

Therefore, an eco-friendly alternative is the need of the hour. Improvement in pest control strategies represents one method to generate higher quality and greater quantity of agricultural products [1].

Therefore, there is a need to develop bio-pesticides which are effective, biodegradable and do not leave any harmful effect on environment. In this review, it is highlighted three medicinal plants viz., [2] *Annona squamosa*, *Allium sativum* and *Gliciridia sepium* which possess good pesticidal properties for future bio-pesticide preparation based on their ethno medicinal properties.

### CUSTARD APPLE

#### Habit and Habitat

*Annona squamosa* Linn is a small evergreen tree is cultivated throughout India for its fruits, different parts of *Annona squamosa* Linn are used in folkloric medicine for the treatment of various diseases [3]. This plant is commonly called custard apple in English & sharifa in Hindi & sitaphalam in Tamil [4]. *Annona squamosa* Linn is a shrub or small tree 7 m height and is cultivated throughout India. This article intends to provide an overview of the chemical constituents present in various parts of *Annona squamosa* Linn and their pharmacological actions [5-16].

### Taxonomical hierarchy

Kingdom	: Plantae
Order	: Magnoliales
Family	: Annonaceae
Genus	: <i>Annona</i>
Species	: <i>squamosa</i>

Figure 1. Leaves and fruits of *Annona squamosa*



### Phytochemical Evaluation

The plant is reported to Containing glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, protein, phenolic compounds, phytosterols, amino acids. The various chemical constituents isolated from leaves, stems and roots of the plant including anonaine, aporphine, coryline, isocorydine, norcorydine, glaucine. Leaves contains, Anonaine, Benzyl tetrahydroisoquinoline, Borneol, Camphene, Camphor, car-3-ene, Carvone,  $\beta$ -Caryphyllene, Eugenol, Farnesol, Geraniol, 16-Hetriacontanone, Hexacontanol, Higemamine, Isocorydine, Limonine, Linalool acetate, Menthone, Methyl anthranilate, Methylsalicylate, Methylheptenone, p-(hydroxybenzyl)-6,7-(2-hydroxy,4-hydro)isoquinoline, n-Octacosanol, a-Pinene, b-Pinene, Rutin, Stigmasterol,  $\beta$ -Sitosterol, Thymol and n-Triacontanol. Alkaloids, proteins & amino acids are absent in the leaf extract.

### Pharmacological Investigation

#### Traditional applications

In various indigenous and traditional sources of medicinal plants have been extensively used for treatments. Various parts of plants such as the leaves, fruits, the barks, roots and even the seeds are being used for preparation of medicines. *Annona squamosa* is also been extensively used as traditional medicine in various culture. The antibacterial screening by agar cup method indicates that highest zone of inhibition was shown by the methanol extract followed by petroleum ether and aqueous extracts for *Annona squamosa* leaf. Extracts of *Annona squamosa* inhibited the growth of all test strains except *Salmonella typhimurium*.

#### Pesticidal Activity

The pure compound annotemoyin-1 isolated from the chloroform extract of the seeds of *Annona squamosa* Linn was evaluated for its pesticidal activity against both adults and different instars of in *Tribolium castaneum* (Herbst) under laboratory condition

### Other Pharmacological Action

#### Antidiabetic Activity

The observed decreased blood glucose lowering effect of the extract in STZ- induced diabetic rats could also possibly be due to increased peripheral glucose

utilization. It has been reported that using medicinal plant extract to treat STZ-induced diabetic rats results in activation of  $\beta$  cells

#### Anti hyperlipidemic Activity

Treatment with Poly herbal formulation and tolbutamide resulted in a significant reduction of blood glucose and increase in plasma insulin. Poly herbal formulation also resulted in a significant decrease in tissue lipids and lipid peroxide formation.

#### Anti-Head lice effect

The present study focused on the separation and identification of the active compounds against head lice from the hexane extract of *Annona squamosa*. These compounds were found to kill all tested head lice in 49, 11 and 30 minutes respectively. The triglyceride ester can be used as a marker for quantitative analysis of the active compound for quality control of the raw material A. squamosal seed and its extract. This first finding will be useful for quality assessment and the chemical stability of the anti-head lice preparation from this plant.

#### Antimicrobial Activity

Four different solvent extracts of leaves of Custard apple (*Annona squamosa*) were studied for its antibacterial activity. Agar diffusion method was elected to check antibacterial activity. Two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria were selected for screening.

The screening results showed that highest zone of inhibition was observed in methanol extract against *Ps.aeruginosa* (MIC:130 $\mu$ g/ml) followed by petroleum ether extract against *Ps. aeruginosa* (MIC: 165  $\mu$ g/ml) and methanol extract against *E. coli* (MIC: 180  $\mu$ g/ml). Phytochemical studies showed that Linalool, Borneol, Eugenol, Farnesol, and Geraniol present in extracts which provide antibacterial activity.

#### Antioxidant Activity

Free radical scavenging potential of the leaves of *A.squamosa* was studied by using different antioxidant models of screening. The ethanolic extract at 1000  $\mu$ g/ml showed maximum antioxidant models of screening. A study was carried to analyse the antioxidant effect of oral administration of aqueous extract of *A. squamosa* leaf on blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, antioxidant enzymes and lipid peroxidation in liver and kidney to streptozotocin (STZ)-induced diabetic rats.

#### Antitumour Activity

The plant *Annona squamosa* traditionally known ascustard apple possesses potent bioactive principles in all its parts. The effect of aqueous and organic extracts from defatted seeds of *A.squamosa* was studied on a rat histiocytic tumour cell line AK-5. In addition DNA fragmentation and annexin – V staining confirmed that the extracts induced apoptosis in tumour cells through the

oxidative stress. Aqueous extracts of *A.squamosa* seeds possessed significant antitumor activity in vivo against AD-5 tumor.

#### **Insecticidal Activity**

The Insecticidal activity of ethanolic extract of *Annana squamosa*. The preliminary phytochemical investigation was out to identify the various constituents present in the extract pest. The finding of new insecticidal activity is great. The ethanolic *Annana squamosa* extract carried showed potent activity against *Sitophilus oryzae* economic importance both from the agronomic and preventive medicine point of view. The reason for using new natural insecticides.

#### **Antiplasmodial Activity**

The antiplasmodial activity of methanolic extract of plant *A.squamosa* was tested on chloroquine sensitive strain 3D7 and chloroquine resistant strain Dd2 of *P. falciparum*. The methanolic extract of *A.squamosa* leaves showed high anti plasmodial activity with IC50 values of 2 and 30 µg/ml on 3D7 and Dd2, respectively

#### **ii) GARLIC**

##### **Habit and Habitat:-**

Garlic is a narrow flat leaves and bears white small flowers and bulbils. The cultivation of drug is done by planting bulbs generally in the month of September to late in October. It takes about four months for harvesting, it is used in the treatment of pulmonary condition. Garlic is used as carminative, expectorant, stimulant and disinfectant.

##### **Taxonomical hiarachy**

Kingdom : Plantae  
Family : Lilliaceae  
Genus: *Allium*  
species : *Sativum*

**Figure 2. Bulb of *Allium sativum***



##### **Phytochemical Evalution**

Intact garlic cloves contain only a few medicinally active compounds. The main chemical constituent of intact garlic is the amino acid alliin, an alkyl derivative of cysteine alkyl sulfoxide, which may varies from 0.2 to 2.0% fresh weight .The biological activity of an extract of garlic depends on the mode of its preparation. The extract prepared at room temperature contains mainly allicin, which has a powerful antibacterial property and a strong smell. Besides allicin, small amounts of several other

thiosulfinates and complex sulphinyl components, including the antithrombotic ajoenes, are also present. The enzyme allinase responsible for converting alliin to allicin is inactivated by heat.

#### **Pharmacological Investication**

##### **Pesticide activity**

The major bioactive compound derived from garlic, showed a potent leishmanicidal activity in *in vitro* against *Leishmania mexicana* and *L. donovani*. The 50% inhibitory concentration (IC50) for lysis was about 2 µM. They reported that leishmanicidal activity of ajoene is due to the morphological alteration of the mitochondrial membrane and nuclear envelope, as well as the formation of large autophagic vacuoles. Gamboa-Leon *et al* suggest that garlic extract has mild protective effect against *Leishmania donovani*. This might be due to unspecific enhancement of interferon-gamma secretions. The homopteran sucking insect *Lipaphis erysimi* (mustardaphid) causes severe damage to various crops. This pest not only affects plants but it also transmits single-stranded RNA luteoviruses while feeding, which cause disease and damage in the crop. The mannose-binding garlic leaf lectin has been Control of nematodes in ground nuts.

Six treatments with plant extracts including garlic and a synthetic pesticide were compared with respect to the control of nematode caused root-knot in groundnuts. While the best results were obtained from the synthetic nematocide, the garlic extract showed good effect in promoting plant growth and suppressing the nematode.

##### **Control of western flower thrips (WFT) in strawberry**

Experiments were conducted to assess the efficiency of four plant extracts including garlic for the control of WFT in greenhouse strawberry. The study is demonstrated the suitability of garlic extract, as botanical insecticide, for inclusion in WFT integrated pest management programs.

##### **Activity of garlic extract against plant pathogenic fungi**

In an *in vitro* experiment showed that the growth of *Alternaria*, *Botrytis*, *Magnaporthe* and *Fusarium* was inhibited by application of garlic extract (allicin) placed on agar plates seeded with fungal spores. Garlic treated rice seedlings showed greater resistance to attack by rice blast disease (*M.grisea*) with treated plants showing fewer symptoms. In further work significant effects on the development and pathological symptoms *Phyophthra infestans* (potato blight) were observed following exposure to allicin.

##### **Other Pharmacological Action**

##### **Antihypertensive Effect**

Meta-analysis by Reinhart et al showed that garlic reduced blood pressure only in those patients with elevated systolic blood pressure. It reduced systolic blood pressure by 16.3 mm Hg (95% confidence interval 6.2 to 26.5) and diastolic blood pressure by 9.3 mmHg (95% confidence interval 5.3 to 13.3) compared to placebo.

### Hypolipidaemic effect

Based on animal data, Allicin decreased blood cholesterol and triglycerides and caused a significant reduction in the hepatic cholesterol storage<sup>12</sup>. Meta analyse based on clinical trials suggest that regardless of garlic preparation and compared to placebo garlic reduces total blood cholesterol (7.2 mg/dl, 95% CI: 1.2-13.5) and low density cholesterol (However 6.2 mg/dl, 95% CI: 0.77-11.6) levels though there are variations in the findings.

### Antiplatelet effect

Studies have been shown that there is significant reduction in platelet aggregation after garlic treatment. Garlic also reduces epinephrine induced platelet aggregation<sup>14</sup>. Patients taking oral anticoagulants like warfarin may experience increased bleeding episodes. It is advisable to stop garlic administration one week prior to any surgical intervention.

### Hypoglycaemic effect

Short term clinical trials have shown significant reduction in the blood glucose level in non-diabetic persons compared to placebo. Other reports on human studies have also shown the hypoglycemic effects of garlic. The antidiabetic effects of garlic are mainly due to the volatile sulfur compounds. Garlic has been shown to be effective in reducing insulin resistance as well.

### Antineoplastic Effect

Animal studies have shown that garlic inhibit spirocarcinogens for esophageal, stomach, colon, lung and breast cancer. Human clinical and epidemiological studies have also the evidence of chemoprotective effects of garlic.

### Effect on post-menopausal osteoporosis

Garlic may decrease postmenopausal osteoporosis in the females by modulation proinflammatory cytokins IL-1, IL-6 and TNF- $\alpha$ . However, more research is required.

### Neuroprotective effect

Data on animal study have shown that allicin may cause neuro protection in ischemia reperfusion injury to the spinal cord by improving mitochondrial function.

### iii. SEEMAI AGATHI

#### Habit and Habitat

*Gliricidia sepium* is a medium-sized tree and can grow to from 10 to 12 meters high. The bark is smooth and its color can range from a whitish gray to deep red-brown. It has composite leaves that can be 30 cm long. Each leaf is composed of leaflets that are about 2 to 7 cm long and 1 to 3 cm wide. The flowers are located on the end of branches that have no leaves. These flowers have a bright pink to lilac color that is tinged with white. A pale yellow spot is usually at the flower's base. The tree's fruit is a pod which is about 10 to 15 cm in length. It is green when unripe and becomes yellow-brown when it reaches maturity. The pod produces 4 to 10 round brown seeds. The tree grows well in acidic soils with a pH of 4.5-6.2. The tree is found on

volcanic soils in its native range in Central America and Mexico.

#### Taxonomical hierarchy

Kingdom : Plantae  
Family : Fabaceae  
Genus : *Gliricidia*  
Species : *Sepium*

Figure 3. Leaves and flowers of *Gliricidia sepium*



#### Phytochemical Evaluation

*G. sepium* leaves are rich in protein and highly digestible, and low in fibre and tannin. There is evidence of improved animal production (both milk and meat) in large and small ruminants when *G. sepium* is used as a supplement. Goats on *G. sepium* gained weight and maintained a positive N balance. However, non-ruminants fed on *G. sepium* have shown clear signs of poisoning. The leaves contain 3-4% dry weight of nitrogen and small amount of phosphorus, potassium, calcium and magnesium, so they can be used as excellent green manure and fodder. The present studies were focused on the ability of the plant or root to decrease soil nematodes population and control insects and fungi. Researches have been conducted on both the antifungal and antimicrobial properties of *Gliricidia* plant extract.

#### Pharmacological Investigation

##### Mosquito's repellent activities

The duration of protection provided by each product was tested by means of arm-in-cage studies, in which volunteers insert their repellent treated arms in to a cage with a fixed number of unfed mosquitoes as a The described by Granett & Starnes. 0.2 ml of the *Gliricidia* leaves ethanolic extract was applied evenly over a hand and a part of a forearm so as not leave any area uncovered. The treated area of a hand was then exposed for 5 minutes at half an hour intervals in a cage containing 50 unfed mosquitoes, *Aedes aegypti* of 4-5 days old. The time elapsing between the treatment and the first confirmed bite was determined as the maximum effective repellent time. Results obtained with the *Gliricidia* extract were compared with the standard repellent Citronella oil.

##### Nematicidal activity

Measured quantities of plant leaves extract was dissolved in water to make different dilution, G/1, G/2, G/3, G/4 and control. To determinate the nematicidal effect of plant extract 100 freshly hatched second stage juveniles of *Meloidogyne* spp, were transferred to different Petri dishes. Measured amount of all dilution were added and only distilled water was taken as control. Each treatment was replicated thrice. The Petri dishes were kept at room

temperature 28±2°C for 45 hours. The number of active and inactive nematodes was confirmed, by keeping them in distilled water for 24 hrs, percentage mortality was calculated.

#### Antibacterial activity

The essential oils from leaves and flowers of *G. sepium* were extracted by steam distillation and analysed by GC-MS 17 (table 1 and table 2). These oil samples were examined for their antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Enterobacter faecalis* and *Streptococcus faecalis* and Gram-negative bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Serratia marcescens*.

#### CONCLUSION

The extensive survey literature reviewed that *Annona squamosa* Linn is an important medicinal plant with diverse pharmacological spectrum. Few novel chemical constituent isolated from the *A. squamosa* showed anti-cancer, anti-HIV and anti-diabetic (type 2 diabetic) properties too. Further evaluation need to be carried out on

*A. squamosa* in order to explore concealed areas and their practical clinical application, which can be used for the welfare of the mankind use of fresh garlic juice as a bio-pesticide against *S. zeamais*. A search of the leading electronic database, Scopus, returned no hits for each of the search terms “garlic juice weevil”, “garlic juice *Sitophilus*” and “garlic juice *Sitophilus zeamais*”.

Therefore, this work most likely represents a remarkable shift from the conventional approach to studying the use of biologically active compounds from garlic in crop protection. The antimicrobial activity of *Gliciridia sepium* (Leaf ethanol extract) was assayed in-vitro by well diffusion method against clinical isolates of *E. coli*, *S. aureus*, *Pseudomonas*, *S. typhi*, *Klebsiella* sp. Summarized the microbial growth inhibition by ethanol extract. The plant extract was particularly active against *E. coli*. Ethanol leaf extract of *G. sepium* was tested for its nematocidal property at different concentration against *Meloidogyne incognita* nematodes.

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#### CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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