

Insecticidal activity of the seed extracts of *Annona squamosa* (Linn.) against *Callosobruchus analis* in green gram, *Vigna radiata* (L.) R. Vilczek

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I.INTRODUCTION

Agriculture sector is the basic entity in an economy on which the activities of other sectors are determined. Agriculture promotes the economic growth and development, acts as a source of raw material to industries, ensures food and nutritional security, earns foreign exchange, increases the per capita income of the farmers, increases the employment opportunities and national income and alleviates poverty.

When agriculture grows, so does the economy in general, speeding up the reduction of rural and urban poverty. A strong and vibrant agricultural system forms the primary pillar in the strategy of overall economy. In this backdrop, agriculture is beleaguered by challenges like enigmatic weather, uncertainty in rainfall, slumping land area, plummeting water resources, deteriorating soil fertility, growing unrestrainable pests & diseases, increased costs of inputs, residual effects of chemicals, labour scarcity and vacillating market prices. Government of Tamil Nadu is taking sincere efforts to overcome these challenges (Doraikkannu, 2016).

Government of Tamil Nadu which has resolved to usher in a **Second Green Revolution** has formulated multifarious policies and strategies to achieve equitable, competitive and sustainable inclusive growth in agriculture. These include an array of initiatives such as holistic approach for soil and water management, adoption of crop specific innovative technologies to promote Hi-Tech agriculture, Quality input supply management system, farm mechanization, integrated infrastructure development, strengthening farmer – extension system interface, promotion of market led agriculture & value chain management, extensive use of ICT tools and capacity building for better acumen which are being implemented during the **Twelfth Plan period** with an objective to increase the crop productivity and farmers' income(Doraikkannu, 2016)

Second Green Revolution in Tamil Nadu as the state has surpassed 100 L.MT of Food Grain production in 2011-2012, 2013-2014 and 2014-2015. Needless to say, the

fourth advance estimates for the food grain production during 2015-2016 has also been pegged at 130.63 L.MT, which has never been achieved before (Doraikkannu, 2016)

The National Food Security Mission, a 100% central assistance scheme was introduced with an objective to increase the productivity of food grain crops especially rice and pulses. Later, it was extended for coarse cereals and commercial crops (cotton and Sugarcane) from 2014-2015; and the sharing pattern being revised as 60:40 between centre and state from 2015-2016 onwards. The mission focuses on cultivation of food grain crops in rain fed areas; increasing pulses area and production through intercropping, bund cropping & cultivation in rice fallows; besides implementation of improved agronomic practices and cropping system centric interventions.

National Food Security Mission (NFSM) for Pulses is implemented in all districts except Chennai and the Nilgiris. An amount of Rs.138.23 Crore was extended as subsidy for promotion of the scheme during 2011-2012 to 2015-2016. There was a significant impact on area, production and productivity of pulses due to introduction of various innovative components in the districts where NFSM was implemented. (Doraikkannu, 2016)

Even as the global pulse market is awash with large harvests in many origins that is creating a near-glut situation, the ongoing record of high production of 2016 Kharif (autumn harvest) pulse crops in India is being watched with great interest by market participants primarily because of the country's dominant position in production, consumption and trade. There has been a dramatic transformation in market sentiment in the last three months following record planted acreage (14.6 million hectares, an increase of nearly 40 percent from last year) and reasonably well distributed rainfall that has resulted in a new record Kharif season harvest of 8.7 million tons (last year 5.5 million tons). Coming after two years of below-normal rainfall (El Nino effect) and lower harvests, the rebound in overall agricultural production including pulses is set to help raise rural incomes, contain food inflation and spur consumption demand (Chandrashekhar, 2016).

Also, the Minimum Support Price (MSP) for pulse growers has been hiked significantly. MSP for Kharif 2016 pulses is above the threshold level of Indian Rupees 5,000 per 100 kilograms equivalent to approximately US\$ 750 a ton.

Simultaneous large harvest in other origins such as Canada, USA, Australia, Black Sea region (Russia, Ukraine), Myanmar and East Africa has resulted in a collapse of export prices. For example, pigeon pea prices that ruled at the scarcity value of around \$ 1100 a ton until about six months ago are now traded at about \$ 640 a ton. Yellow pea rates are down sharply from around \$ 380 a ton to just about \$ 300 a ton, a throwaway price really (Chandrashekhar, 2016).

The Indian government was acutely embarrassed by the skyrocketing prices of pulses in the last one year. The 'dal shock' as some international media described it, has forced the government to pay more attention to pulses. Procurement of pulses from domestic market and import through government agencies for creation of buffer stock is one of the key measures.

The latest rebound in production and softer prices has come as a big relief to policymakers and consumers alike. However, a huge rebound in production is creating its own problems. In several marketing centres, prices are ruling below the MSP. The government ought to support growers by purchasing at the specified support price; but the efforts leave much to be desired. This can potentially discourage growers. Therefore agricultural growth is essential for poverty alleviation and overall economic development(Chandrashekhar, 2016).

Despite their high production, the pest problems in agriculture are probably as old as agriculture itself. However, under traditional agriculture the pest number were generally low and some losses due to the pest damage were accepted by former as unavoidable. Some of the serious pests were kept in check by culture and mechanical practices developed by the formers largely through trial and error.

The developed countries, the major portion of food grains produced is stored at farm level. Quantitative and qualitative losses during storage occur due to

physical(temperature and moisture),biological (microorganisms, insects), breakdown of the produce and pesticides and engineering (structural and mechanical aspects)factors.

This problem is compounded by the continuous use of infected seeds by the farmers. The result is poor germination due to rotting of seeds and damping off diseases i.e.dead of seedlings after emergence from the soil. These diseases under severe infection will lead to poor establishment and heavy yield losses. Infected seeds become discolored reducing also the market and nutritional value. Seed-borne diseases may perpetuate and cause permanent, sometimes inconspicuous depreciation of yields, or they may initiate devastating epidemics (Patro *et al.*, 2007).

Use of healthy seeds is one of the most important contributing factors for increasing the seed yield per unit area of land in pulses. The pulse beetle *callosobruchus* spp Cause considerable damage to almost all stored pulses and badly affects the quality of the seeds (Patro *et al.*, 2007).

India is a major pulse growing country of the world accounting roughly for the one third of the total world area under pulses and one fourth of the total world production. Mung bean (*Vigna radiata*) is a major source of energy and protein in developing countries, especially for the vegetarian population. The commonly consumed pulses in Indian diets are red kidney beans, Bengal gram, mung bean, cowpea, red gram, moth bean, lentils. Among them, mung bean ranks third in production after Bengal gram and red gram. Among the grain legumes, it is known for its easy digestibility and in addition, it is having high protein content and being a fair source of dietary minerals (Barakoti and Bains, 2007).

Apart from its high value, pulses are most important in Indian agriculture because they are more drought resistant than cereals and it has the capacity to fix the atmospheric nitrogen, which makes a good cover crop and binds the soil particles and also help in preventing soil erosion (kumar *et al.*, 2007).

Green gram (*Vigna radiata*) is annual legume crops grown for their seeds. The green grams could be green,black or yellow in colour. Grams are native crops of India. It is cultivated in several countries of Asia Africa and The Americas (Wikipedia, 2010).

The dried beans are prepared by cooking or milling. They are eaten whole or split. The seed or the flour may enter a variety of dishes like soup porridge, snakes, breads, noodles and even ice cream. Green gram also produces great sprout, which can be sold in health food shop or eaten at home. Crop residues of *Vigna radiata* are useful fodder. Green gram sometime specifically grown for hay, green manure or as a cover crop (Wikipedia, 2010). Green gram nutritionally contains, carbohydrates 62g, sugar, 6.6g, dietary fiber 16g, fat 1.15g, protein 23.86g and vitamin C which are 4.8mg. One of the major problems contributing to low yield of green gram is lack of adequate protection against seed-borne fungal infection (<http://en.m.wikipedia.org>).

The fruit is juicy, acidic, whitish and aromatic with abundant seeds, the average weight of 1000 fresh seeds is 470g and has an average oil content of 24%. When dried for three days in 60°C the average seed weight was 322g and were tolerant of the moisture extraction showing no problems for long-term storage under reasonable conditions. The creamy and delectable flesh of the fruit consists of 80% water, 1% protein, 18% carbohydrates and fair amount of vitamins B, B2 and C, potassium and dietary fiber (Pier, 2008).

Stored grains can have losses in both quantity and quality. Losses occur when the grain is attacked by microorganisms and other organisms including insects, mites, rodents and birds. Among stored grain pests, extracts of custard apple was tested on *Callosobruchus chinensis* Linn. (Tripathy *et al.*, 2001; Kotkar *et al.*, 2002, Misra 2000; Juneja and Patel 1994); *C. maculatus* (Dharmasena *et al.*, 2001); *Rhizopertha dominica* Fab. (Patel and Valand 1994), *Sitophilus oryzae* (L.) (Mishra *et al.* 1992), *Tribolium castaneum* (Herbst) (Malek and Wilkins 1994, 1995, Hussain *et al.* 1995) and *Corcyra cephalonica* Staint. (Ghatak and Bhusan, 1995a).

Nature has been a source of medical agents for thousands of years and the use of plants for prevention and treatment of various health ailments has been in practice from time immemorial. It is estimated that about 25% of drugs prescribed are derived from plants. In recent years, secondary metabolites previously with unknown

pharmacological activities have been extensively investigated as a source of medicinal agents (eady and brown, 2000).

Annonaceae, the custard apple family with about 2500 species and more than 130 genera is a family of flowering plants consisting of trees, shrubs or rarely lianas (PIER, 2008). Biologically active components of the family showed interesting biological properties like anti-HIV, anti-cancer effects, insecticidal properties and useful cytotoxic compounds (Kumar *et al.*, 2010).

All parts of the Graviola (*Annona*) tree are used in natural medicine in the tropic including the bark, leave, root, fruit and seeds. Different properties and uses are attributed to the different parts of the tree. Generally, the fruit and fruit juice are taken to eliminate worms and parasites, cool fever, increase mother's milk after child birth, and is as an astringent for diarrhea and dysentery George, D. and Pamplona, R. (1999). The crushed seeds are used against internal and external parasites, head lice and warms Harbone, J.B. (1973). The dark, leaves are considered sedative and antispasmodic (George, D. and Pamplona, R., 1999).

Due to the potential insecticidal effect of *Annona* on the various field and storage pests, an attempt was made in the laboratory to test the efficacy of *Annona* seed extract against the pulse beetle, *Callosobruchus analis* in green gram. In view of the above facts the present study was undertaken with the following objectives;

1. To rear the test insect, *C.analis* in laboratory condition.
2. To prepare the seed extracts of *Annona* using petroleum benzene, chloroform, acetone and alcohol as solvents based on increasing polarity.
3. To assess the insecticidal potency of *Annona* seed extracts against *C.analis*.
4. To test the germination of treated seeds and vigour of seedlings.
5. To recommend the best solvent to extract *Annona* seed which is used for the management of *C.analis*

II. REVIEW OF LITERATURE

The nutritional value of green gram, infestation of green gram by insect pests under storage, importance of natural protectants to manage the pest population and the pharmacological and insecticidal activity of our test plant *Annona squamosa* are reviewed under the following headings.

In recent years, there is share price in the incidence of a variety of life style disorders. Certain conditions such as cardiovascular diseases, diabetes mellitus etc., are threatening lives and have turned out to being major causes of death. Prevention of occurrence of such diseases has been a major global concern. Green gram principles pulses used as a daily food article, exhibits a potential to act against this. In this regards, ayurveda recommends its use in daily diet and modern research also direct towards the same recommendation. (Kavya *et al.*, 2014).

III.1.Mudga- An Ayurvedic Perspective

The word Mudga in Sanskrit means “*That which brings joy, delight and gladness*” (Radhakantadev and Shabdakalpadruma, 1967). All the pulses are known to produce flatulence with an exception of Mudga. This property makes Mudga complementary to health. There are evidences to show that Mudga was used as a measurement parameter in Ayurvedic practice. It has been used to explain the size of a mass of haemorrhoids (Agnivesha, 2011). It has been also referred to, in determining the outlet lumen of an enema nozzle for children (Agnivesha, 2011) (used in basti or enema therapy) or a horn used in bloodletting therapy (Sushruta *et al.*, 1997). Further Mudga has been used as a reference to describe the shapes of lesions of certain skin diseases [for e.g., Ajagallika (Sushruta *et al.*, 1997), (Diaper dermatitis), Upadamsha (Sushruta *et al.*, 1997) (Chancroid) and Masurika (Sushruta *et al.*, 1997) (Measles)]. These evidences show the familiarity of Mudga to people at large. In Ayurveda, the pharmacokinetics and

pharmacodynamics of a drug are explained in terms of certain attributes viz., Rasa (Taste of the drug), Guna (Properties and effect it has on the body following consumption), Veerya (Potency of the drug, whether it has a catabolic or anabolic effect on the body), Vipaka (Post digestive effect on metabolism). Based on the above, the probable action of the drug can be understood in terms of its effect on the Doshas (Bioforces governing the body whose balance and imbalance determines health and ill health). The pharmacodynamics of mung in Ayurveda has been explained to be that as Madhura (Agnivesha *et al.*, 2011) (Sweet) and Kashaya (Agnivesha *et al.*, 2011) (astringent) in taste, Laghu (light for digestion), ruksha (Dry), sheetaveerya (Agnivesha *et al.*, 2011) (cold in potency), katu vipaka (Agnivesha *et al.*, 2011) (post digestivetransformation into pungency) and it exhibits Kaphapittahara Vatakaratva (Agnivesha *et al.*, 2011) (pacifies kapha and pitta whereas aggravates Vata). It is known to be drushtiprasadaka (Misra *et al.*, 2005) (improves quality of vision). Different varieties of Mudga as Shyama (Black), Harita (Green), Peeta (Yellow), Shwetha (White) and Rakta (Red) (Misra *et al.*, 2005) based on the seed colour are mentioned by the nighantus wherein the green variety is considered to be the best one. Mudga has potential therapeutic, dietary and promotive uses. Mudga has been prescribed as a pathya ahara (favourable interventional diet) in a broad spectrum of disorders like Agnimandya (Loss of appetite), Arochaka (Anorexia), Amlapitta (Hyperacidity), Atisara (Diarrhoea), Pravahika (dysentary), Grahani (Irritable bowel syndrome, Tropical sprue), Chardi (Emesis), Kamala (Jaundice), Sthoulya (Obesity and Dyslipidemia), Prameha (Urinary diseases and Diabetes), Pratishyaya (Rhinitis), Shvasa (Dyspnoea), Kasa (Cough), Rajayakshma (Pthisis), Kushta (Skin diseases), Sheetapitta (Urticaria), Shotha (Inflammatory conditions), Vidradhi (Abscess), Arsha (Haemorrhoids), Bhagandara (Fistula-in-ano), Ashmari (Calculus), Bhagna (Fracture), Galaganda (Diseases of Thyroid), Arbuda (Malignancies), Hrudroga (Cardiac disorders), Pandu (Anaemia), Madatyaya (Alcohol related diseases), Udara (Ascites), Murcha (Altered consciousness), Apasmara (Convulsions) Unmada (psychiatric disorders), and Pradara (Menstrual disturbances). Mudga is told to be apathya (Unfavourable) in some conditions like Navajvara (Acute fever), shula (Gastric and duodenal ulceration), Vatavyadhis (A group of diseases caused due to aggravated Vata dosha or nervous

disorders) viz., Pakshaghata (Hemiplegia), Ardita (Facial palsy), Manyasthambha (Cervical spondylitis), Katishula (Lumbar spondylitis, Intervertebral disc prolapse etc), Apabahuka (Frozen shoulder), Grudhrasi (Sciatica) and Sandhigatavata (Osteo arthritis) (das *et al.*, 2007); (Iakshmipathi, 2007); (Tripathi, 2010). A cursory glance through the above list provides an overview that Mudga could be a potential dietary intervention in a number of lifestyle and life threatening diseases such as cardiovascular diseases, Diabetes mellitus, Obesity, Dyslipidemia and Malignancies etc.,

II.1.1 Botanical Illustration of Mung Bean

The mungbean (*Vigna radiata*) is a member of the legume family Fabaceae and commonly called as green gram. Mungbean originated in the plains of Peninsular India with its botanical origin, area of maximum genetic diversity and location of domestication being South India (Krishna, 2013). Mung bean is an annual, deep rooted herb, 25-100 cm tall with trifoliate leaves and short fine brownish hairs on the stem branches. Planted in early June, the crop begins to flower in 50 to 60 days which continues for few weeks and is ready to be harvested in early to mid-September. The matured pods are glabrous and consist of 8-20 globose seeds per pod (Ismail, 2004). Green gram is cultivated in several countries of Asia, Africa and the America. It grows best at an altitude of 0-1600 m above sea level and under warm climatic conditions (28-30°C). They are well adapted to red sandy loam soils and are drought tolerant giving reasonable yields with as little as 650 mm of yearly rainfall. Heavy rainfall results in increased vegetative growth with reduced pod setting and development (Maiti, 2012). The most important part of mungbean is the seed used in several food products, both as whole seed and in processed form. Like most legumes they are relatively high in proteins, around 25 % of the seed weight. The principal domestic use of mungbean is the production of bean sprouts which is seen commonly in Asian cooking and is used for dhals and soups (Anonymous, 2010).

II.1.2 Nutritional Facts of Mung

Green gram is known for its high nutritional value. 100 g of it produces 334 Kcal of energy (Gopalan *et al.*, 2007). It is rich in carbohydrates (56.7 g/100 g) and is a very

good source for minerals like Potassium (843 mg/100 g), Magnesium (127 mg/100 g), Calcium (124 mg/100 g), Phosphorus (326 mg/100 g) and Iron (4.4 mg/100 g). Vitamins like Carotene, Thiamine, Niacin, Riboflavin, Ascorbic acid and Folic acid are also present in mung bean. It is considered one of the best sources for proteins and constitutes a number of essential amino acids such as Arginine, Histidine, Lysine, Tryptophan, Phenylalanine, Leucine, Isoleucine, Tyrosine, Valine, Threonine, Cystine and Methionine. Mung bean hence is considered to be a substantive source of dietary proteins and carbohydrates. Mungbean provides significant amounts of dietary iron to plant based diets in developing countries where Mungbean is consumed (Nair *et al.*, 2013). Certain chemical components such as flavanoids (Flavones, isoflavones and isoflavonoids), phenolic acids (Gallic acid, Vanillic acid, Caffeic acid, Cinnamic acid, protocatechuic acid, Shikimic acid, β -hydroxybenzoic acid etc.,) and organic acids isolated from Mung in recent years, supports its health promoting action as mentioned in the classics.

III.1.3 Mode of Action of Mudga as per Ayurveda (Pharmacological Effects)

Mudga is known to possess a unique property in initiating physiological effects in the human body. It belongs to the category of substances possessing a distinctive property known as “Vichitra pratyarabdha”. In Sanskrit this term means “*a unity of paradoxes*”. A drug or a substance is known to act at various levels based on its attributes for example milk is a substance with sweet taste known as madhura rasa, possessing guru guna (taking a long time to digest), sheeta veerya (consuming energy while digestion), Madhura vipaka (Post digestive effect being anabolic) and brumhana (bulk promoting or nourishing). But Mudga being a Madhura rasa drug possesses Laghu guna (Takes lesser time for digestion) Katu vipaka (Catabolic post digestive effect) yet is bulk promoting. These paradoxes make Mudga a drug with multifaceted action. Mudga has been known to be an efficient tissue builder and nourisher. It has been described as the most compatible food substance and this property is an attribute of madhura rasa (Agnivesha *et al.*, 2011). The Kashaya rasa (astringent) is useful in wound healing and a very good absorbent (especially to dry up impaired dosage in diseased states) (Agnivesha, 2011). The laghu guna (which gets digested easily in less

time) of Mudga makes it a good dietary candidate. The vishada guna (Agnivesha, 2011) of Mudga makes it a good clearing agent (especially in clearing the obstruction to digestive and metabolic pathways). Further, the sheeta veerya (Cold potency) of Mudga makes it vivifying, nourishing, strength promoting and body growth promoting (Agnivesha, 2011). Thus these properties elaborate the health promoting action of Mudga and also justify its usage as a supportive diet in various diseased conditions. Mudga is known to possess the Rasayana property (antioxidant, anti-ageing and prolonging life). This property makes it a highly beneficial candidate for daily diet in the present scenario wherein numerous lifestyle disorders are affecting people worldwide. Cardio vascular diseases and allied conditions (causal and risk factors) like Diabetes mellitus, Obesity, Dyslipidemia are leading threats to mankind globally. Dietary supplementation both preventive and supportive can help in combating these and usage of Green gram as staple will account for a valuable step. Mungbeans have been tested for several pharmacological activities worldwide. Cao *et al.* (2011) have studied the antioxidant effect of mung bean extracts *in-vivo* wherein vitexin and isovitexin were found to be the major antioxidant components in mung bean. The mung bean extracts were also found to have a potent scavenging activity against pro-oxidant species, including reactive oxygen species and reactive nitrogen species as well as an inhibitory effect on low-density lipoprotein oxidation (Chung *et al.*, 2011). The administration of compound Vitexin isolated from mung bean produced a significantly protective effect in peripheral blood cells and in lymphocyte blast-transformation function in Breast cancer patients undergoing radiotherapy with Co-60 thus establishing the radio protective, antioxidant and detoxifying effects of mung bean (Hien *et al.*, 2002). Regular consumption of Mungbeans can regulate flora of enterobacteria, decrease absorption of toxic substances, reduce risk of hypercholestraemia and coronary heart disease, and prevent cancer (Hien *et al.*, 2002). Mung bean protein isolates improved the plasma lipid profile by normalizing insulin sensitivity and significantly reduced plasma triglyceride level (Tachibana *et al.*, 2013). Mung bean (*Vigna radiata*) has been traditionally used in China both as nutritional food and herbal medicine against a number of inflammatory conditions since the 1050s. This when experimentally tested showed that mung bean extract is protective against lethal sepsis by stimulating

autophagic HMGB1 degradation (Zhu *et al.*, 2012). In the antiglycation assays, vitexin and isovitexin showed significant inhibitory activities against the formation of Advanced Glycation end products induced by glucose or methyl glyoxal with efficacies of over 85 % (Peng *et al.*, 2008). It was found that the mung extracts lowered blood glucose, plasma C-peptide, glucagon, total cholesterol, triglyceride, and BUN levels and at the same time markedly improved glucose tolerance and increased insulin immunoreactive levels suggesting a potent antidiabetic effect (Yao *et al.*, 2008). The above mentioned researches establish the potential of mung bean in preventing the occurrence of certain chronic and life threatening disease conditions on daily consumption and also as a therapy in several diseased conditions.

According to Caraka Samhitas the Varieties of Pulses Mudga (green gram) and Masha (black gram) have been described in samidhanya varga and are called Vaidala (dicotylendons) In Susurta Samhita all these Pulses have been grouped and described under Mudga Vargas. The Species known as Mudga does not excessively generate Vayu in the system but tends to purify and invigorate the organ of vision the green species is the best of all the varieties of Mudga. Mudga is the best as soup is very easily digestible, slightly increase Vata; the green is better than the other one. According to Caraka Samhitas Mudga is best among Pulses utilized in the form of soup. it is Kashya (astringent) and madhur (sweet) in taste, pungent in vaipaka, cold in potency laghu (light) and ruksha (dry). It alleviates the vitiated kapha and pitta (Shital and Yeshwant, 2013).

Mudga or green gram is one of the most commonly used components of Indian cuisine. Majority of the ayurvedic lexicons opine that it is the best among all pulses and advocate its use in everyday diet expects from Ayurveda and recent research based studies showcase is it as potential interventional diet in Cardiovascular and life style disorders. (Kavya *et al.*, 2014)

II.2. Food grain losses due to insect infestation in storage condition

Food grain losses due to insect infestation during storage are a serious problem, particularly in the developing countries (Talukder *et al.*, 2004; Dubey *et al.*, 2008).

Losses caused by insects include not only the direct consumption of kernels, but also accumulation of exuviae, webbing, and cadavers. High levels of the insect detritus may result in grain that is unfit for human consumption and loss of the food commodities, both, in terms of quality and quantity. Insect infestation-induced changes in the storage environment may cause warm moist “hotspots” that provide suitable conditions for storage fungi that cause further losses. It is estimated that more than 20,000 species of field and storage pests destroy approximately one-third of the world’s food production, valued annually at more than \$100 billion among which the highest losses (43%) occurring in the developing world (Jacobson, (1982); Ahmed and Grainge (1986).

The quantitative and qualitative damage to stored grains and grain product from the insect pests may amount to 20–30% in the tropical zone and 5–10% in the temperate zone Talukder (2006); Rajendran and V. Sriranjini (2008). Food grain production in India has reached 250 million tonnes in the year 2010-2011, in which nearly 20–25% food grains are damaged by stored grain insect pests Raja shekar (2010); Rajashekar and Shivanandappa (2010) The efficient control and removal of stored grain pests from food commodities has long been the goal of entomologists throughout the world.

The major pests of stored grain and pulses of the Indian subcontinent are classified in to two groups, namely, primary pests: those which are capable of penetrating and infesting intact kernel of grain and have immature stages develop within kernel of grain and secondary pests which cannot infest the whole grain but feed on as broken kernels, debris, high moisture weed seeds, and grain damaged by primary pests. In general, the immature stages of the secondary pest species are found external to the grain. It is often thought that secondary invaders cannot initiate infestation. The important primary pests are the rice weevil, *Sitophilus oryzae* (L.), granary weevil, *Sitophilus granaries* (L.), (Coleoptera: Curculionidae), lesser grain borer, *Rhyzopertha dominica* (F.), (Coleoptera: Bostrichidae), Khapra beetle, *Trogoderma granarium* (Everts), (Coleoptera: Dermestidae), and the pulse beetle *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). The secondary pests are rust-red flour beetle, *Tribolium castaneum* (Herbst), (Coleoptera: Tenebrionidae), rusty grain beetle, *Cryptolestes*

ferrugineus (L.), (Coleoptera: Cucujidae), sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), (Coleoptera: Silvanidae), mites, (Acarina: Tetranychidae) *Liposcelis corrodens*, and (Psocoptera: Liposcelidae).

II.2.1 INFESTATION CONTROL BY PESTICIDES AND THEIR SIDE EFFECTS

Since the 1950s, synthetic insecticides have been used extensively in grain facilities to control stored product insect pests. Fumigants such as methyl bromide, phosphine, cyanogens, ethyl formate, or sulfuryl fluoride rapidly kill all life stages of stored product insects in a commodity or in a storage structure. Fumigation is still one of the most effective methods for the prevention of stored product losses from insect pests. But pests develop resistance, not stored products were showing a slow upsurge in fumigation resistance (Donahaye 2000). Resistance to phosphine is so high in Australia and India, it may cause control failures (Leelaja *et al.*, 2007; Rajashekar *et al.*, 2006). Methyl bromide has been identified as a major contributor to ozone depletion (WMO 1995) and has been banned in developed countries, and developing nations have committed to reducing the use by 20% in 2005 and phase out in 2015. Contact insecticides such as malathion, chlorpyrifos, or deltamethrin are sprayed directly on grain or storage structure for protection from infestation for several months. The incidence of insecticide resistance is a growing problem in stored-product protection. Resistance to one or more insecticides has been reported in at least 500 species of insects and mites (Georghiou *et al.*, 1990). Champ reported that resistance to pesticides used to protect grain and other stored food stuffs is widespread and involves all groups of pesticides and most of the important pests.

Some of the contact insecticides have become ineffective because of widespread resistance in insect population. Resistance to malathion is widespread in Canada, USA and Australia (Subramanyam and Hagstrum 1995). Stored product insects pests were found to be resistant against different insecticides including the cyclodienes, chlorpyrifos, cyanophos, carbamates, carbaryl, cypermethrin, dichlorodiphenyltrichloroethane, deltamethrin, diazinon, dichlorovos, ethylene bromide, ethyl formate organophosphates, permethrin, pyrethrins, and propoxur.

Although chemical insecticides are effective, their repeated use has led to residual toxicity, environmental pollution and an adverse effect on food besides side effect on humans (Dubey *et al.*, 2007; Kumar *et al.*, 2007). Their uninterrupted and indiscriminate use not only has led to the development of resistant strains but also accumulation of toxic residues on food grains used for human consumption that has led to the health hazards. In view of all these problems, several insecticides have either been banned or restricted in their use.

II.2.2 BOTANICALS AS ALTERNATIVE TO SYNTHETIC PESTICIDES

The increasing serious problems of resistance and residue to pesticides and contamination of the biosphere associated with large-scale use of broad spectrum synthetic pesticides have led to the need for effective biodegradable pesticides with greater selectivity. This awareness has created a worldwide interest in the development of alternative strategies, including the discovery of newer insecticides (Heyde *et al.*, 1984; Dayan *et al.*, 2009). However, newer insecticides will have to meet entirely different standards. They must be pest specific, nonphytotoxic, nontoxic to mammals, ecofriendly, less prone to pesticide resistance, relatively less expensive, and locally available (Hermawan *et al.*, 1997).

This has led to re-examination of the century-old practices of protecting stored products using plant-derivatives, which have been known to resist insect attack (Talukder 2006; Lale 1992; Sahayaraj 2008). Plant derived materials are more readily biodegradable, less likely to contaminate the environment and may be less toxic to mammals. There are many examples of very toxic plant compounds. Therefore, today, researchers are seeking new classes of naturally occurring insecticides that might be compatible with newer pest control approaches (Dubey *et al.*, 2008; Talukder and Howse 1995; Yingjuan *et al.*, 2008). Since ancient times, there have been efforts to protect harvest production against pests. The Egyptian and Indian farmers used to mix the stored with fire ashes (Gawad and Khatab 1985; Varma and Dubey 1999). The ancient Romans used false hellebore (*Veratrum album*) as a rodenticide, the Chinese is credited with discovering the insecticidal properties of Derris species, whereas

pyrethrum was used as an insecticide in Persia and China (Ahmed and Grainge 1986). In many parts of the world, locally available plants are currently in wide use to protect stored products against damage caused by insect infestation (Khater, 2012; Hassanalli and Lwande 1989; Tripathi 2009) .

Indian farmers used neem leaves and seed for the control of stored grain pests (Ahmed and Koppel 1985). In northern Cameroon, cowpeas are traditionally mixed with sieved ash after threshing and the mixture put into mud granaries or clay jars (Ahmed and Koppel 1985). In eastern Africa, leaves of the wild shrub *Ocimum suave* and the cloves of *Eugenia aromatic* are traditionally used as stored grain protectants (Powel 1989). In Rwanda, farmers store edible beans in a traditional closed structure (imboho) and whole leaves of *Ocimum canum* are usually added to the stored foodstuff to prevent insect damage within these structures (Weaver *et al.*, 1991). Owusu suggested natural and cheaper methods for the control of stored product pests of cereals, with traditionally useful Ghanaian plant materials.

In some south Asian countries, food grains such as rice or wheat are traditionally stored by mixing with 2% turmeric powder (Saxena *et al.*, 1988; Chatterjee *et al.*, 1990). The use of oils in stored-products pest control is also an ancient practice. Botanical insecticides such as pyrethrum, derris, nicotine, oil of citronella, and other plant extracts have been used for centuries (Park *et al.*, 2003; Sim 2006; Singh and Upadhyay 1993). More than 150 species of forest and roadside trees in India produce oilseeds, which have been mainly used for lighting, medicinal purposes, and also as insecticides from ancient times to early 20th century. Turmeric, garlic, *Vitex negundo*, gliricidia, castor, *Aristolochia*, ginger, *Agave americana*, custard apple, *Datura*, *Calotropis*, *Ipomoea*, and coriander are some of the other widely used botanicals to control and repel crop pests (Golob and Gudrups 1999).

Saxena *et al.*, 1992). Talukder has listed 43 plant species as insect repellents, 21 plants as insect feeding deterrents, 47 plants as insect toxicants, 37 plants as grain protectants, 27 plants as insect reproduction inhibitors, and 7 plants as insect growth and development inhibitors. Eighteen species showed insecticidal with greater

selectivity. This awareness has created a worldwide interest in the development of alternative strategies, including the discovery of newer insecticides. However, newer insecticides will have to meet entirely different standards. They must be pest specific, nonphytotoxic, nontoxic to mammals, ecofriendly, less prone to pesticide resistance, relatively less expensive, and locally available (Hermawan *et al.*, 1997). This has led to re-examination of the century-old practices of protecting stored products using plant-derivatives, which have been known to resist insect attack (Talukder 2006; Lale, 1992; Sahayaraj 2008). Plant derived materials are more readily biodegradable, less likely to contaminate the environment and may be less toxic to mammals. There are many examples of very toxic plant compounds. Therefore, today, researchers are seeking new classes of naturally occurring insecticides that might be compatible with newer pest control approaches (Dubey *et al.*, 2008; Talukder and Howse 1995; Yingjuan *et al.*, 2008). Since ancient times, there have been efforts to protect harvest production against pests. The Egyptian and Indian farmers used to mix the stored with fire ashes (Gawad and Khatab 1985; Varma and Dubey 1999). The ancient Romans used false hellebore (*Veratrum album*) as a rodenticide, the Chinese is credited with discovering the insecticidal properties of Derris species, whereas pyrethrum was used as an insecticide in Persia and China. In many parts of the world, locally available plants are currently in wide use to protect stored products against damage caused by insect infestation (Khater 2012, Hassanalli; Iwande, 1989; Tripathi *et al.*, 2009).

Indian farmers used neem leaves and seed for the control of stored grain pests. In northern Cameroon, cowpeas are traditionally mixed with sieved ash after threshing and the mixture put into mud granaries or clay jars (Wolfson *et al.*, 1991). In eastern Africa, leaves of the wild shrub *Ocimum suave* and the cloves of *Eugenia aromatic* are traditionally used as stored grain protectants (Powell, 1989). In Rwanda, farmers store edible beans in a traditional closed structure (imboho) and whole leaves of *Ocimum canum* are usually added to the stored foodstuff to prevent insect damage within these structures (Weaver, 1991). Owusu (2001) suggested natural and cheaper methods for the control of stored product pests of cereals, with traditionally useful Ghanaian plant materials.

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II.2.3 CLASSIFICATION OF BOTANICAL INSECTICIDES

On the basis of physiological activities on insects, Jacobson (Jacobson, 1982) conventionally classified the plant components into 6 groups, namely, repellents, feeding deterrents/antifeedants, toxicants, growth retardants, chemosterilants, and attractants. Focus on the toxicants and grain protectant on activity of essential oil, extracts, and its constituents has sharpened since the 1980s.

II.2.3.1. Repellents

The repellents are desirable chemicals as they offer protection with minimal impact on the ecosystem, as they drive away the insect pest from the treated materials by stimulating olfactory or other receptors. Repellents from plant origins are considered safe in pest control; minimize pesticide residue; ensure safety of the people, food, and environment (Talukder *et al.*, 2004; Talukder, 2006; Maia and Moore, 2011).

The plant extracts, powders, and essential oil from the different bioactive plants were reported as repellent against stored grain insect pests (Talukder *et al.*, 2004; Owusu, 2001; Boeke *et al.*, 2004). For example, the essential oil of *Artemisia annua* was found as repellent against *Tribolium castaneum* and *Callosobruchus maculatus* (Tripathi *et al.*, 2004).

II.2.3.2. Antifeedants/Feeding Deterrents

Antifeedants, sometimes referred to as “feeding deterrents” are defined as chemicals that inhibit feeding or disrupt insect feeding by rendering the treated materials unattractive or unpalatable (Munakata, 1997; Saxena *et al.*, 1988). Some naturally occurring antifeedants, which have been characterized, include glycosides of steroidal alkaloids, aromatic steroids, hydroxylated steroid meliantriol, triterpene hemiacetal, and others (Jacobson, 1982; Talukder and Howse, 2000). Essential oil constituents such as thymol, citronellal and α -terpineol are effective as feeding deterrent against tobacco cutworm, *Spodoptera litura* synergism, or additive effects of combination of monoterpenoids from essential oils have been reported against *Spodoptera litura* larvae (Hummelbrunner and Isman 2001). The screening of several medicinal herbs showed that root bark of *Dictamnus dasycarpus* possessed significant feeding deterrence against two stored-product insects (Liju *et al.*, 2002).

II.2.3.3. Toxicants

Research on new toxicants of plant origin has not declined in recent years despite the increased research devoted to the discovery of synthetic insecticides (Talukder and Howse, 1995). Worldwide reports on plant derivatives showed that many plant products are toxic to stored product insects (Rajendran and Sriranjini, 2008; Dubey *et al.*, 2007 ; Park *et al.*, 2003 ; Isman, 2006; Ofori and Reichmuth, 1997; owusu, 2001; (Su and Horvat 1987). Talukder (1995) listed the use of 43 plant species expressing toxicant effects of different species of stored-products insects. Pascual-Villalobos and Robledo (Villalobos and Robledo, 1998) carried out screening of plant extracts from 50 different wild plant species of southeastern Spain for insecticidal activity towards *Tribolium castaneum* and reported that four species, namely, *Anabasis*

hispanica, *Senecio lopezii*, *Bellardiatrixago*, and *Asphodelus fistulosus* were found to be promising. Two major constituents of the essential oil of garlic, *Allium sativum*, methyl allyl disulfide and diallyl trisulfide were to be potent toxicant and fumigants against *Sitophilus zeamais* and *Tribolium castaneum* (Huang, 2000). Rahman (1990) reported that nicotine, an active component of *Nicotiana tabacum*, is a strong organic poison which acts as a contact-stomach poison with insecticidal properties. This compound is of course, very toxic to humans as well. The essential oil vapours distilled from anise, cumin, eucalyptus, oregano, and rosemary were also reported as fumigants and caused 100% mortality of the eggs of *Tribolium confusum* and *Ephestia kuehniella* (Tunc *et al.*, 2000). Many species of the genus *Ocimum* oils, extracts, and their bioactive compounds have been reported to have insecticidal activities against various insect species (Ofori *et al.*, 1998; Keita *et al.*, 2001)

II.2.3.4. Natural grain Protectants

Jacobson (1989) noted that the most promising natural grain protectants were generally observed in the plant families, Annonaceae, Asteraceae, Canellaceae, Labiatae, Meliaceae, and Rutaceae. The Indian neem plant is the most well-known example and its various parts, namely, leaves, crushed seeds, powdered fruits, oil, and so forth, have been used to protect stored grain from infestation (Talukder, 2004; Jotwani Sircar, 1964; Devi and Mohandas, 1982). The Neem oil and kernel powder gave effective grain protection against stored grain insect pests like *Sitophilus oryzae*, *Tribolium castaneum*, *Rhyzopertha dominica* and *Callosobruchus chinensis* at the rate of 1 to 2% kernel powder or oil (Pereira and Wohlgemuth, 1982). The neem oil adhered to grain forms uniform coating around the grains against storage pests for a period of 180–330 days (Ahmed, 1994). Yadava and Bhatnagar (Yadava and Bhatnagar, 1987) reported that a dried leaves of *Azadirachta indica* have been mixed with stored grains for protection against insects. Azadirachtin is an active principle from the neem plant, which is an effective grain protectant against insect infestation (Schmutterer, 1990). Rajashekar *et al.* (2010) reported that root powder extracts of *Decalepis hamiltonii* have been mixed with stored grains for protection against various stored grain insect pests. Eighteen species offered protection to wheat up to 9 months without affecting seed

germination (Devi and Devi, 2011). In parts of eastern Africa, leaves of some plants and allelochemicals including azadirachtin, nicotine, and rotenone have traditionally been used as grain protectants (Tulukder, 2005; Hassanali *et al.*, 1990).

The powders of *Rauvolfia serpentina*, *Acorus calamus*, and *Mesua ferrea* are used as a grain protectant against *Rhizoper thadominica* (Tiwari, 1994). In a survey in northern semiarid regions of Ghana only 16 plants were identified as being used as grain protectants (Brice *et al.*, 1996). In Africa, the grain protectant potential of different plant derivatives, including plant oils against major stored-product pests were also found to be very promising and reduced the risks associated with the use of insecticides (Ofori and Reichmuth, 1997; Shaaya *et al.*, 1997). In northern Cameroon, the essential oils of plants *Xylopia aethiopica*, *Vepris heterophylla*, and *Luppia rugosa* are used for protection of stored grains from attack of stored grain insect pests (Ngamo, *et al.*, 2007). The components of citrus peels were used as grain protectant against *Callosobruchus maculatus* (Pedro, 1985). Coconut oil has been found effective against *Callosobruchus chinensis*, for a storage period of six months, when applied to *Vigna radiata* (green gram) at 1% (Doharey, 1990). Formulations of menthol were used as protection of pulse grain from attack of *Callosobruchus chinensis* (Singh and Mehta, 2010). Spinosad, a naturally occurring insecticide from the actinomycete, *Saccharopolyspora spinosa*, has high efficacy, a broad insect pest spectrum, low mammalian toxicity, and minimal environmental profile is unique among existing products currently used for stored-grain protection (Hertlein *et al.*, 2011).

II.2.3.5. Chemosterilants/Reproduction Inhibitors

Lists of 43 plant species have been reported as reproduction inhibitors against stored product insects (Talukder, 1995). Reports have also indicated that plant derivatives including the essential oils caused mortality of insect eggs (Ofori and Reichmuth, 1997). Many ground plant parts, extracts, oils, and vapour also suppress many insects (Rajendran and Sriranjini, 2008; Rajashekar *et al.*, 2010).

II.2.3.6. Insect Growth and Development Inhibitors

Plant extracts showed deleterious effect on the growth and development of insects and reduced larval pupal and adult weight significantly, lengthened the larval and pupal periods, and reduced pupal recovery and adult eclosion (Khanam *et al.*, 1990). Rajasekaran and Kumaraswami (Rajasekaran and Kumaraswami, 1985) reported that grains coated with plant extracts completely inhibited the development of insect like *Sitophilus oryzae*. Plant derivatives also reduce the survival rates of larvae and pupae and adult emergence (Koul *et al.*, 2008). Development of eggs and immature stages inside grain kernel were also inhibited by plant derivatives (Boeke *et al.*, 2004). The crude extract also retarded development and caused mortality of larvae, cuticle melaniation, and high mortality in adults (Jamil *et al.*, 1984).

II.3. Uses of Annona seeds

II.3.1 Traditional uses

Post-cortical anti-fertility activity of *A. squamosa* was reported from studies with the seed extract (Chavan *et al.*, 2010). Seeds, fruits and leaves were found to be effective as an insecticide, fish poison, and as a powerful irritant of the conjunctiva. The roots were found to be effective as a drastic purgative and in the acute dysentery (Rahman *et al.*, 2005).

The plant is attributed with the medicinal properties that include anti-fertility and antitumour activities which were observed in mice and rats. The young leaves of *Annona squamosa* were used extensively due to its anti-diabetic activity by the tribal men who were living in and around the villages of Aligarh district which is located in the state of Uttar Pradesh, India and also by the people of Chotanagpur district which is located in the state of Bihar, India. In Aligarh district, villagers used to consume a mixture of 4–5 newly grown young leaves along with black pepper (*Piper nigrum*) of about five grains, earlier in the morning in the treatment of diabetes, with the

continued therapy that ensures up to 80% of the positive results (Shirwaikar *et al.*, 2004).

II.3.2. Analgesic and anti-inflammatory activity:

Caryophyllene oxide which was isolated from an unsaponified petroleum ether extract from the bark of *Annona squamosa* was studied for its analgesic and anti-inflammatory activity. Caryophyllene oxide at the dosage levels of about 12.5 and 25mg/kg body weight and unsaponified petroleum ether extract at the dosage levels of about 50mg/kg body weight was found to show a significant central as well as the peripheral analgesic and the anti-inflammatory activity. Caryophyllene oxide produced an analgesic effect which is dosage dependent and also considered to be statistically significant. Further pharmacodynamic investigations were required in order to understand the analgesic and anti-inflammatory activity exhibited by the caryophyllene oxide (Chavan *et al.*, 2010).

II.3.3. Anti-bacterial and cytotoxic activity:

The anti-bacterial and anti-fungal activities of the plant compounds such as petroleum ether extract (PE), CHCl₃ extract (CE), EtOH extract (EE), annotemoyin-1, annotemoyin-2, squamocin and cholesteryl glucopyranoside showed maximum inhibition against the gram positive organisms such as *B. subtilis*, *B. cereus*, *B. megaterium*, *Staphylococcus aureus*, *S. b-haemolytica*, *Sarcina lutea* and the gram negative organisms such as *E. coli*, *S. dysenteriae*, *S. shiga*, *S. flexneriae*, *S. sonnei*, *Salmonella typhi*, *P. aeruginosa*, *Klebsiella spp.* The cytotoxicity of the plant extracts was studied by the brine shrimp lethality bioassay and the LC₅₀ values of the petroleum ether and chloroform extracts were calculated (Rahman *et al.*, 2005).

II.3.4. Anti-oxidant and anti-lipidimic activity:

Anti-oxidants are the compounds responsible for the protection of living organism from the damage caused by the abnormal production of reactive oxygen species concomitant lipid peroxidation, protein damages and others including DNA strand breaking etc.. Ethnomedical literature has revealed a large number of plants like

Annona squamosa which can be used against diabetes, in which the free radicals and ROS plays an important role. The effect of the water extract of *Annona squamosa* leaves on the antioxidant enzymes and the lipid profile of animal models of type 2, non-insulin dependent diabetes mellitus (NIDDM) was evaluated. The parameters considered as the measurement of the above activities were the increased activities of the scavenging enzymes, catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione reductase (GR) and glutathione-S-transferase (GST) and the significant decrease in the malondialdehyde levels were expressed in the various tissues. The aqueous extract of the *Annona squamosa* significantly reduced the triglyceride and total cholesterol levels with a gradual increase in the HDL cholesterol level in the treated diabetic rats when compared to that of the untreated diabetic rats (control). These changes were found to be beneficial in the prevention of the diabetic complications and also in the improvement of lipid metabolism in the diabetics (Gupta *et al.*, 2008).

II.3.5. Anti-ulcer activity

Peptic ulcer is a disease that affects a large population throughout the world and it is caused mainly due to the development of gastric lesions, when there is a delicate balance between some of the gastro protective and aggressive factors is being lost. Increased secretion of the gastric acid is found to be a pathological condition, which occurs mainly due to the uncontrolled secretion of hydrochloric acid through the proton pumping H⁺ K⁺-ATPase. Anti-ulcer activity of the plant extract was evaluated against the cold restraint (CRU), pyloric ligation (PL), aspirin (ASP), alcohol (AL) induced gastric ulcer and the histamine (HA) induced duodenal ulcer models and then further confirmed through in vitro assay of H⁺ K⁺-ATPase activity and the plasma gastrin level. The plant and its chloroform and hexane fraction attenuated the formation of ulcer in CRU, PL, HA model and also displayed anti-secretory activity in vivo with the decrease in plasma gastrin level. Cytoprotection of *Annona squamosa* was apparent with protection in AL, ASP models and enhanced mucin level in PL. (+)-O-methylarmepavine, N-methylcorydaldine, lanuginosine, were found to be the active principles of the plant

which may serve as the initial point for the designing of novel semi-synthetic and synthetic compounds as the antiulceragents in the future (Yadav *et al.*, 2011).

II.3.6.Hypoglycemic and anti-diabetic activity

From ancient times, diabetes has been treated orally by using several medicinal plants or their extracts. These herbal remedies produce minimal effect and possess no side effects and also found to be economically low cost than the other synthetic hypoglycemic oral drugs. The ethanolic extract of *Annona squamosa* leaves when administered orally to the normal as well as the streptozotocin (STZ)-induced diabetic rats and alloxan-induced diabetic rabbits at different dose, proved that the dose of 350 mg/kg body weight was found to reduce the fasting blood glucose (FBG) level by 6.0% and the peak blood glucose during the glucose tolerance test (GTT) was also reduced by 17.1% in the normal rats. The same dose of the ethanolic extract has reduced the FBG level by 26.8% and also improved the glucose tolerance by 38.5 and 40.6% during the GTT in alloxan-induced diabetic rabbits. In STZ-diabetic rats, there was a fall of 13.0% in FBG and was an improvement in the glucose tolerance by 37.2 and 60.6% was observed during GTT (Gupta *et al.*, 2005). In the same way, the aqueous extract of the roots of *Annona squamosa* L. At a dose of 250 mg/kg and 500 mg/kg body weight was tested for the antidiabetic activity in the Streptozotocin (STZ) – induced hyperglycaemic rats which cause a reduction in the blood glucose in STZ- induced diabetic rats from 285.52 to 208.81 mg/dl (Mohd *et al.*, 2009).

II.3.7.Vasorelaxant activity:

Vasodilators are the agents that are useful for the treatment of cerebral vasospasm and hypertension, and for the better improvement of peripheral circulation. A cyclic octapeptide, cyclosquamosin B which was isolated from the seeds of *Annona squamosa* was found to show a potential vasorelaxant effect on the rat aorta. The vasorelaxant effect caused by the cyclosquamosin might be attributed significantly to the inhibition of calcium influx from the extracellular space via voltage-dependent calcium channels (Morita *et al.*, 2006)

II.3.8. Anti-tumour activity:

Twelve different acetogenins with diverse stereochemical structures and configurations namely asimicin¹⁸, squamocin¹⁸, squamocin-D¹⁸, desacetyluvaricin¹⁸, Iso desacetyluvaricin¹⁸, squamostatin-D¹⁸, squamostatin-E¹⁸, squamostatin- B¹⁸, squamostatin-A¹⁸, 12,15-cis-squamostatin-A¹⁹, 4-deoxyannoreticuin²⁰, and cis-4-deoxyannoreticuin²⁰ were evaluated for their ability to inhibit the growth of cancer cell lines using MTT method which indicated that the stereo chemical factor is to be considered as a essential factor for the potent activity of the tested compounds irrespective of their bis-adjacent THF ACGs or nonadjacent bis-THF ACGs. The ACGs with S configuration at C-24 possessed more selective cytotoxicity than the ACGs with the R configuration at C-24. ACGs showed the significant inhibitory activities against the growth of various tumor cell lines and also against the multiple drug resistant (MDR) tumor cell lines. Thus, ACGs might be considered as a promising antitumor candidates for the future clinical application (Yang *et al.*, 2009).

II.3.9. Hepatoprotective activity

Natural remedies obtained from the medicinal plants are considered as an effective and safe alternative for the treatment of liver toxicity. The hepatoprotective effect of the alcoholic and water extract of *Annona squamosa* was evaluated in the hepatotoxic induced animals in order to explore its usage for the treatment of hepatotoxicity in the human. The experimental study suggested that the extracts of *Annonasquamosa* were not capable of reverting the hepatic injury completely which was induced by isoniazide and rifampicin, but it was able to limit the effect of these drugs in the liver. The protective role of *Annona squamosa* leaf extracts might be caused due to the antioxidative effect of the flavonoids present in it (Saleem *et al.*, 2008).

The protective effect of the methanolic extract of *Annona squamosa* on isoniazid-rifampicin-induced hepatotoxicity was also evaluated in the rats and was found that they also showed a protective effect against the liver injury (Uduman *et al.*, 2011).

II.3.10.Larvicidal activity:

Mosquitoes possess a great threat to the human health by means of the transmission of the serious diseases (Kaushik and Saini, 2009; Magadula *et al.*, 2009) Development of the resistance, cross-resistance, and also the rising cost as well as the possible toxicity hazards arised due to the synthetic insecticides usage were some of the reasons lead to the interest in the discovery of plant based products in the recent years. The larvicidal and the growth regulating activities of *Annona squamosa* was reported against *Anstephensi* and other mosquitoes. The high potency of *Annona squamosa* as a larvicide against mosquito species was evaluated but the active compound that possess a toxic substance against the larval species has to be identified ((Kaushik and Saini, 2009; Magadula *et al.*, 2009).

II.3.11.Insecticidal activity

The common housefly *Musca domestica* (Diptera: Muscidae) which is an important mechanical vector of many bacterial and pathogenicmicrobes 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 bio insecticides in order to control the population of *Musca domestica* for safer and economic alternative to the synthetic insecticides(Begum *et al.*,2010). 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 (Leatemia and Isman, 2004). Similar results were also observed by (Gajalakshmi *et al.*, 2011).

III. MATERIALS AND METHOD

An investigation was carried out in the laboratory to test the efficacy of *Annona squamosa* L. seed extract against the pulse beetle, *Callosobruchus analis* in green gram, *Vigna radiata* Wilezck. The methodologies used in present studies are described under the following headings.

III.1 Preparation of green gram

Green gram was purchased from the local market, cleaned manually to remove infested seeds and other debris and sun dried for 2 hours. The dried seeds were transferred to clean glass jars of 1000ml capacity and closed with perforated lids for good circulation. This is kept at ($28 \pm 30^{\circ}\text{C}$ Temperature $70 \pm 80\%$ RH) for about 3 weeks to remove all prior infestation by the pest before bioassay studies.

III.2 Collection and maintenance of *Callosobruchus analis* culture

The insect used in the present study was obtained from the Department of pulses, Tamil Nadu Agricultural University (TNAU), Coimbatore and a healthy culture was maintained in the BOD incubator at $28 \pm 2^{\circ}\text{C}$ and 70-75% RH in clean glass jars of 500ml capacity containing green gram. The glass jars were covered with perforated lids for good aeration. Adults that emerged from the culture were used for the bioassay within 24 hours of emergence. The remaining insects were used to start a new culture so that cultures with emerging adults were made available continually.

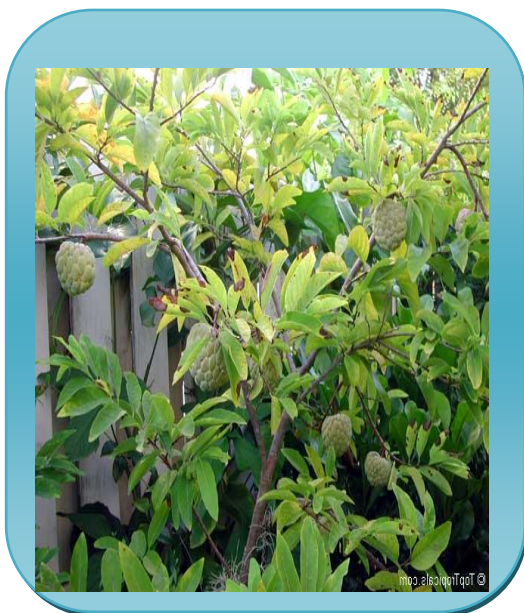
III.3 Preparation of seed extracts

Fresh seeds of *Annona* fruit were collected locally. The seeds were dried under laboratory conditions, ground to fine powder and packed in polythene bags until required. 10g of seed powder was subjected to soxhlet extraction using Petroleum

benzene, chloroform, acetone and alcohol as solvents based on increasing order of polarity. The leaf extracts were distilled and the residues were kept in a water bath below 40°C for solvent evaporation. The residue obtained was stored for future use.

PLATE I

A. ANNONA SQUAMOSA –ENTIRE PLANT



B. ANNONA FRUIT WITH SEEDS



C. SEED OF ANNONA FRUIT

D. ANNONA SEED POWDER



III. 4 Bioassay Studies

A laboratory experiment was laid in a completely Randomized Design (RD).

25g of green gram was taken in small polythene bags and mixed with 5 ml of different concentrations of each seed extract. The untreated and acetone treated controls were also maintained. All the treatments and controls were replicated three times. In each replicate 5 pairs of newly emerged adults were released after the evaporation of solvents. The polythene bags were tightly closed. The various parameters studied to find out the efficiency of seed extracts included adult mortality, adult emergence, weight loss in grains, seed germination and vigor index.

III.5 Observations

a. Percentage mortality of Green gram

Observations on the mortality of insects were recorded for each treatment and control at every exposure time. The dead and moribund insects which were taken as

dead were removed from grain at the time of counting. Percentage mortality was calculated using the following formula

$$\text{Percentage mortality of insects} = \frac{\text{Dead insects}}{\text{Insects introduced}} \times 100$$

PLATE II

A. HARVESTED GREEN GRAM



B. EGG-LADEN GREEN GRAM





b. Adult emergence

F1 adult emergence of green gram was recorded on the 30th Day After Treatment (DAT). The emerged insects were separated from the seed using a grain sieve (2 mm diameter).

c. Percentage weight loss

To calculate the weight loss of the green gram, control and treated grains were sieved to remove all the dust and insects and weighed using an electronic balance (Denver, 2010). The percent weight loss was calculated by the formula suggested by Haris and Linblad (1978).

$$\text{Percentage weight loss} = \frac{\text{OW-CW}}{\text{OW}} \times 100$$



Where,

OW = Original weight on dry weight basis

CW = Current weight on dry weight basis

PLATE III

A. BIO ASSAY STUDIES

EXPERIMENTAL SETUP WITH 1%



EXPERIMENTAL SETUP WITH 3%



EXPERIMENTAL SETUP WITH 5%



EXPERIMENTAL SETUP WITH 1000PPM



EXPERIMENTAL SETUP WITH 5000PPM



EXPERIMENTAL SETUP WITH 1000PPM

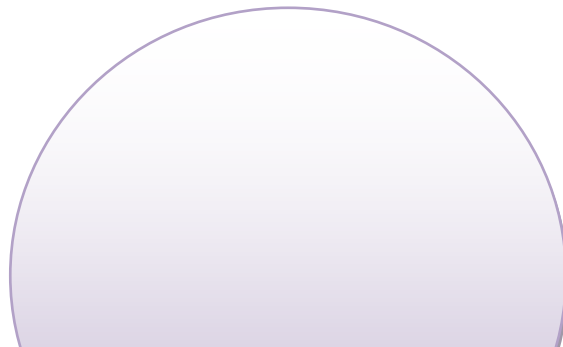


d. Germination Percentage and vigour index

For evaluating the impact of seed extracts on seedling growth, the seeds of 30 DAT (Days After Treatment) were germinated in germination towels following the wet towel method (ISTA, 1966). Five seeds from every treatment and control were taken and soaked in distilled water for 3 hours. The water was decanted and the seeds were arranged in germination towels and rolled (Plate-IV). After one week, the numbers of germinating seeds of green gram were counted and the seedling parameters such as root length and shoot length were also measured. The vigour index of the seedling was calculated using the following formula.

PLATE V

GERMINATION OF GREEN GRAM SEED

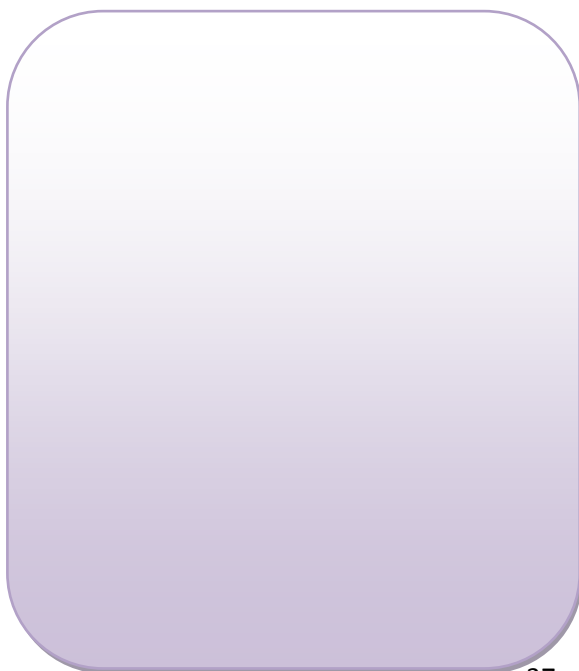


Vigour index = Germination percentage X Mean length of root + Mean length of shoot seedling

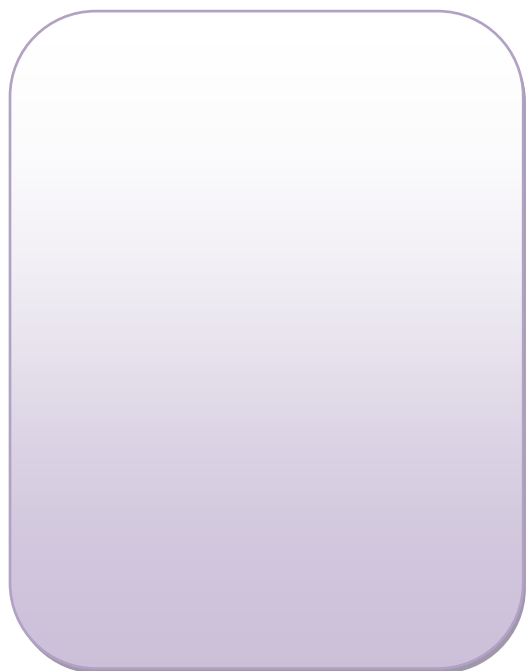


SEEDLING OF GREEN GRAM

1000ppm



5000ppm





1000ppm



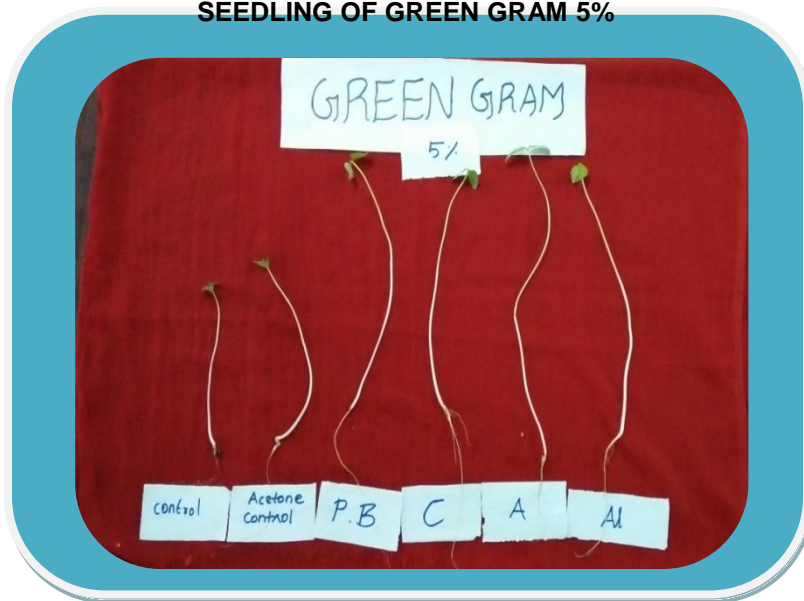
SEEDLING OF GREEN GRAM 1%



SEEDLING OF GREEN GRAM 3%



SEEDLING OF GREEN GRAM 5%



III.6 Phytochemical Screening

Phytochemical analysis of the seed extracts were carried out simultaneously for different solvents following standard procedures. Described by Trease and Evans 1989 and Sofowora 1993. Tannins, saponins, reducing sugars, alkaloids, terpenoids, flavonoids, cardiac glycosides and anthraquinones were estimated following standard methods.

A. Qualitative Analysis

a. Tannins

The extract was (0.5g) dissolved in 10 ml of distilled water, then a few drops of 1% ferric chloride solution was added to obtain a brownish green or blue black precipitate, which confirms the presence of tannin.

b. Saponins

The extract was (0.5g) dissolved in 5 ml distilled water. The mixture was shaken vigorously. Formation of stable persistent froth shows the presence of saponins. A further addition of 6 drops of olive oil while shaking forms an emulsion, confirming the presence of saponins.

c. Alkaloids

The extract was (6ml) mixed with 6 ml of 1% HCl in steam bath, then it was filtered. 1 ml of Mayer's reagent was added. Presence of turbidity shows the presence of alkaloids. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.

d. Terpenoids

The extract was (0.5g) dissolved in 2 ml of chloroform then 3 ml concentrated sulfuric acid was added, a reddish brown colour in interphase indicates the presence of terpenoids.

e. Flavonoids

5 ml dilute ammonia was added to 5 ml extract and then 5 ml concentrated sulfuric acid was added. Formation of yellow colour shows the presence of flavonoids

f. Cardiac glycosides

The extract was (2.5g) added to 2.5 ml distilled water. 1 ml glacial acetic acid containing a few drops of ferric chloride was added then 0.5 ml of concentrated sulfuric acid was added. Presence of brown ring at the interphase indicates the presence of deoxy sugar. A violet ring below the brown ring was observed, while a greenish ring also appears above the brown ring, confirming the presence of Cardiac glycosides.

g. Anthraquinones

The extract was (2.5) dissolved in 5 ml of conc. sulfuric acid and filtered. The filtrate was dissolved in 2.5 ml of chloroform. Chloroform layer was pipetted into a tube and 0.5 ml of 10% diluted ammonia was added. Formation of pink red or violet colour shows the presence of anthraquinones.

h. Phenols

The extract was (2ml) dissolved in 4 ml of distilled water and added few drops of 10% FeCl_3 . Appearance of blue or green colour indicates presence of phenols.

i. Detection of carbohydrates

Molisch's test: To two ml of filtrate, two drops of alcoholic solution of α naphthol was added and the mixture was shaken well. 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. Formation of violet ring indicates the presence of carbohydrates.

Fehling's test: One ml of the filtrate was boiled in water bath with 1ml each of Fehling's solution A and B. Brick red precipitate was formed, that indicates the presence of sugar.

j.Detection of protein and amino acids

Millon's test: To two ml of filtrate, few drops of Millon's reagent was added. A white precipitate indicates the presence of proteins.

Biuret' test: Two ml of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by 2-3 potassium hydroxide pellets. Pink colour in the ethanol layer indicates the presence of proteins.

Ninhydrin test: Two drops of ninhydrin solution was added to two ml of aqueous filtrate. A characteristic purple colour indicated the presence of amino acids.

III.6 Statistical analysis

The data on percentage adult mortality and percentage weight loss were transformed into ARC sign values before subjected to ANOVA. Two way Analysis of Variance was used to analyze the collected data. Germination percentage and vigour index were analysed by One Way Analysis of Variance. Mean difference of each variable was further analyzed by using Duncan's Multiple Range Test (DMRT).

IV. RESULTS

IV.1 Impact of Annona seed extract on the mortality percentage of *C.analis*

The mortality percentage of the seed extracts are given in table number I. Significant variation was noticed between the control and different treatment. Due to moderate mortality variation between control and different treatment, the dosage level of each extract has been increased from ppm to percentage level. Therefore, the dosages selected for the present study included lower concentration of 1000, 5000 and 10,000 ppm and higher concentration of 1%, 3% and 5%. However, the mortality percentage recorded for higher concentration of 1%, 3% and 5% were 100% on the 1st Day After Treatment (DAT). Therefore, the results pertaining to the lower concentration were tabulated.

Analysis of the mean values of the mortality on different days indicated that increase in mortality percentage from 1st day - 4th Day After Treatment (13.71% – 26.39%) and the percentage declined from 4th day onwards up to 8th day (26.39%- 2.93%). Therefore the peak mortality range was obtained on 4th Day After Treatment (26.39%).

Highest mortality of 30.00% was recorded for 10,000ppm Petroleum benzene while, the control and Acetone treated control recorded no mortality on 1st day of observation. Chloroform extract of 1000 and 5000ppm concentration levels registered highest mortality on 2nd Day After Treatment (26.67%) However the control recorded no mortality on this day.

Natural death of insects were observed in control from 3rd day onwards (10.00%) and all the treatment recorded the mortality percentage ranging from 10.00%- 26.67%. From 4th day onwards the mortality percentage of control and Acetone control (18.43%-33.21%) recorded the maximum death in comparison with other treatment (6.14 - 30.99%). The **mean mortality value attained peak** on this day of treatment (20.71%).

From 5th day onwards the mortality percentage of treatment either attained cent percent level (10,000 dose of Petroleum benzene, Alcohol and Chloroform) or minimum mortality (3.33-20.00%). Impact of Annona seed extract on *C. analis* on 6th 7th and 8th Day After Treatment recorded gradual decrease in mean mortality percentage from 8.81%, 9.28% and 2.14 respectively.

Overall data confirmed that, the solvent used extraction of Annona seed (petroleum benzene, chloroform, acetone and alcohol) were effective in bringing out the cent percent mortality on the 1st day itself at higher concentration levels (1%,3%, and 5%). Moderate variation of mortality was noticed between control, Acetone treated control & the treatments of the lower concentration (1000, 5000 and 10,000 ppm).

IV.2 Impact of Annona seed extract on the number of adult emergence in Green Gram

Analysis of the data on number of adult emergence in the treatment and control showed the significant variation among them. Dose dependent variation of adult emergence was noticed for chloroform, acetone and alcohol extract

The adult emergence of Annona seed extract at 1%, 3% and 5% concentration of all the solvent (petroleum benzene, chloroform, acetone, and alcohol) were greatly reduced due to their cent percent mortality of introduced insects on the 1st day of observation and the number of adult emerged were recorded minimum (1-2 numbers) or nil. Although, the moderate variation of mortality was observed between the treatment and control, the adult emergence in the treatment were reduced to a maximum number in Acetone (10-33 numbers) followed by alcohol (75-148 numbers) and chloroform extracts (109-191 numbers). However, the lower concentration of 1000, 5000 and 10,000 ppm of petroleum benzene Annona extract recorded the highest adult emergence 360.33, 319.30 and 213.00 respectively while the control and acetone treated control registered 513.33 and 492.66 numbers respectively.

The overall result on adult emergence of Annona seed extract revealed the efficiency of Annona seed extract (petroleum benzene, chloroform, acetone and

Figure-I
PERCENTAGE MORTALITY OF *C.analis* IN ANNONA SEED EXTRACT
TREATED GREEN GRAM

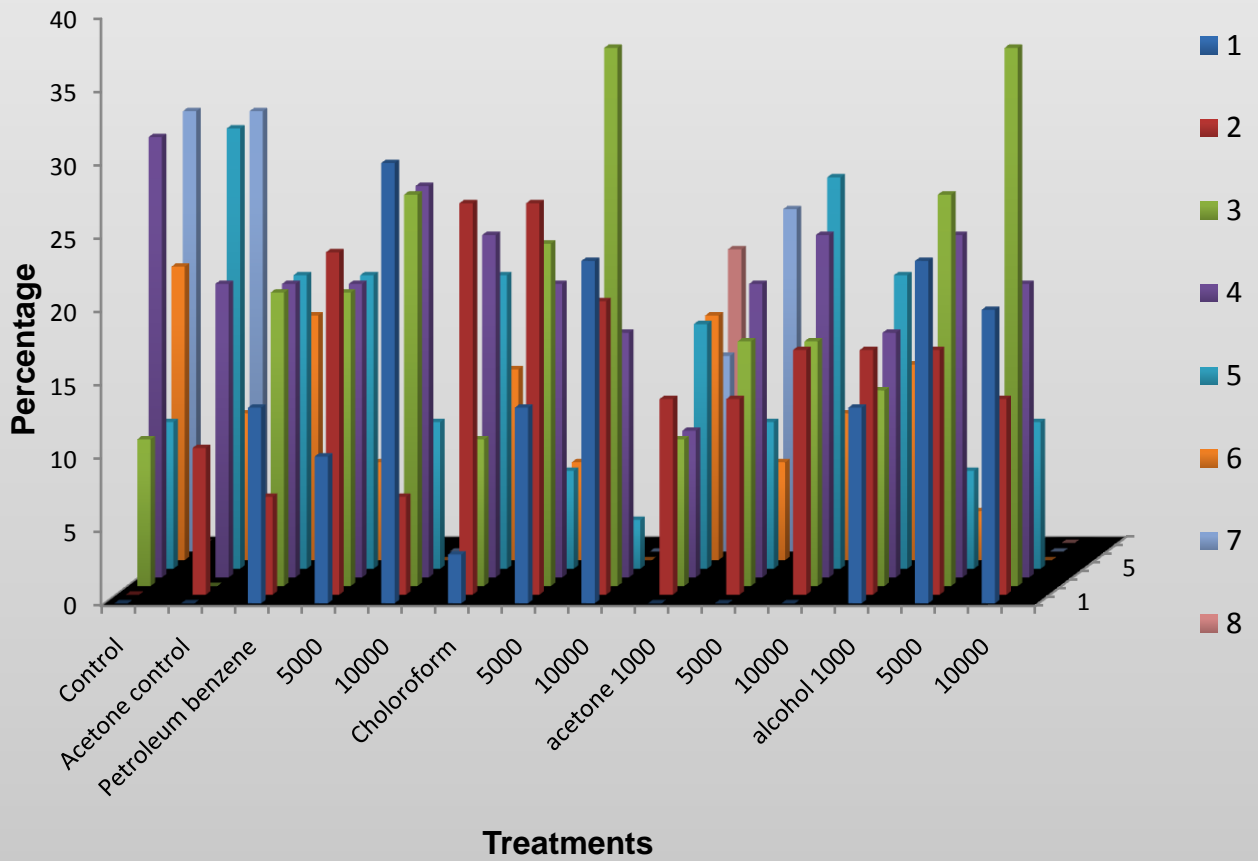


TABLE I PERCENTAGE MORTALITY OF *C.analis* IN GREEN GRAM TREATED WITH ANNONA SEED EXTRACTS

EXTRACT	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY	8 DAY	MEAN
Control	0.00c (0.00d)	0.00c (0.00d)	10.00a (18.43bc)	30.00a (33.21a)	10.00 b (18.43bcd)	20.00ab (26.57a)	30.00a (33.21a)	0.00c (0.00b)	12.50 (16.23)
Acetone control	0.00c (0.00d)	10.00b (18.43abc)	0.00c (0.00d)	20.00ab (26.57ab)	30.00a (33.21a)	10.00b (18.43abc)	30.00a (33.21a)	0.00c (0.00b)	12.50 (16.23)
Petroleum benzene 1000	13.33ab (21.14ab)	6.67bc (12.28bcd)	20.00a (26.07abc)	20.00a (26.56ab)	20.00a (26.67abc)	16.67ab (23.85ab)	3.33c (6.14c)	0.00c (0.00b)	12.50 (17.76)
5000	10.00b (15.00bc)	23.33a (28.79a)	20.00ab (26.07abc)	20.00ab (26.07ab)	20.00ab (26.07abc)	6.67cd (12.29bcd)	0.00d (0.00c)	0.00d (0.00b)	12.50 (16.79)
10000	30.00a (32.21a)	6.67bc (8.85cd)	26.67a (30.99ab)	26.67a (0.79a)	10.00b (15.00cd)	0.00c (0.00d)	0.00c (0.00c)	0.00c (0.00b)	12.50 (14.73)
Chloroform 1000	3.33cd (6.14cd)	26.67a (30.79a)	10.00bc (15.00c)	23.33a (28.79ab)	20.00ab (25.37abc)	13.00ab (21.14ab)	3.33cd (6.14c)	0.00d (0.00b)	12.50 (16.67)
5000	13.33bc (17.21BC)	26.67a (30.99)	23.33ab (28.79abc)	20.00ab (26.07ab)	6.67cd (12.29cd)	6.67cd (12.29bcd)	3.33cd (6.14c)	0.00d (0.00b)	12.50 (16.72)
10000	23.33ab (20.07ab)	20.00ab (26.07ab)	36.67a (37.22a)	16.67b (23.37ab)	3.33c (6.14d)	0.00c (0.00d)	0.00c (0.00c)	0.00c (0.00b)	12.50 (15.10)
Acetone 1000	0.00b (0.00d)	13.33a (21.14abc)	10.00a (15.00c)	10.00a (15.00b)	16.67a (23.36abc)	16.67a (23.85ab)	13.33a (21.14ab)	20.00a (26.67a)	12.50 (18.19)
5000	0.00c (0.00d)	13.33ab (21.14abc)	16.67ab (19.92bc)	20.00ab (26.07ab)	10.00ab (18.43bcd)	6.67bc (12.29bcd)	23.33a (28.29a)	10..ab (15.00a)	12.50 (17.21)
10000	0.00c (0.00d)	16.67ab (23.85ab)	16.67ab (23.85abc)	23.33a (28.29ab)	26.67a (30.99ab)	10.00ab (18.43abc)	6.67bc (12.29bc)	0.00c (0.00b)	12.50 (17.21)
Alcohol 1000	13.33a (17.21bc)	16.67a (23.86ab)	13.33a (17.21bc)	16.67a (23.85ab)	20.00a (26.07abc)	13.33a (21.14ab)	13.33a (21.14ab)	0.00b (0.00b)	13.33 (18.81)
5000	23.33a (28.79ab)	16.67ab (23.37ab)	26.67a (30.99ab)	23.33a (28.79ab)	6.67bc (12.29cd)	3.33c (6.14cd)	3.33c (6.14c)	0.00c (0.00b)	12.91 (17.06)
10000	20.00ab (26.07ab)	13.33b (17.71abc)	36.67a (36.84a)	20.00ab (26.07ab)	10.00b (15.00cd)	0.00c (0.00b)	0.00c (0.00c)	0.00c (0.00b)	12.50 (15.21)
MEAN	10.71 (13.70)	15.00 (20.52)	19.04 (23.31)	20.71 (26.39)	15.00 (20.62)	8.81 (14.03)	9.28 (12.41)	2.14 (2.93)	12.58 (16.74)

	SED	CD (0.05)	CD (0.01)
Days	1.68681	3.32749	4.38339
Treatment	2.23144	4.40186	5.79868
Days x Treatment	6.31146	12.45033	16.40116

FIGURE - II
ADULT EMERGENCE OF *C.analis* in GREEN GRAM TREATED
WITH ANNONA SEED EXTRACTS

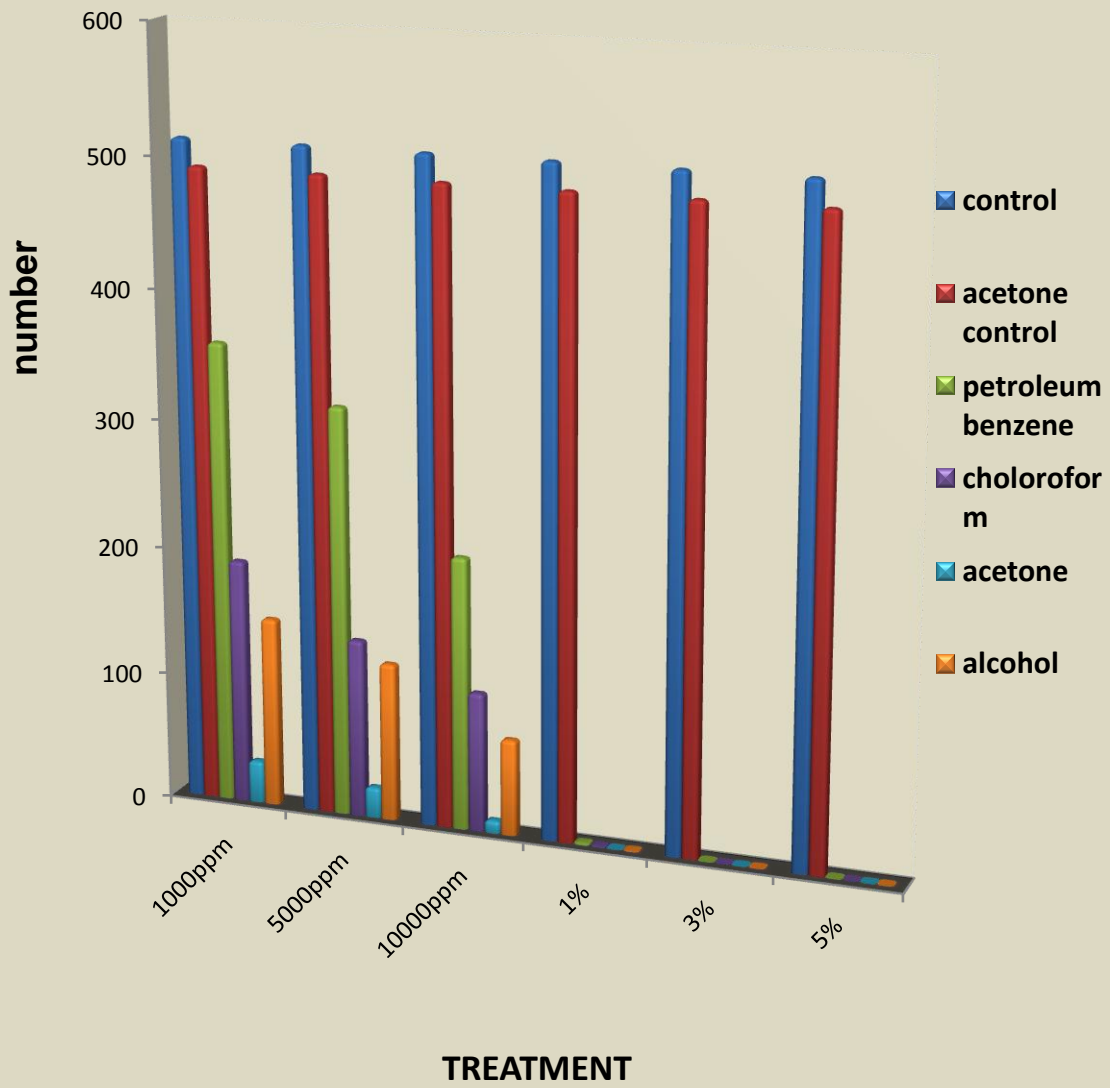


TABLE II

ADULT EMERGENCE IN GREEN GRAM TREATED WITH ANNONA SEED EXTRACTS

EXTRACT							
TREAT (T)	1000ppm	5000ppm	10,000ppm	1%	3%	5%	T-Mean
Control	513.33a	513.33a	513.33a	513.33a	513.33a	513.33a	513.33a
Acetone control	492.66a	492.66a	492.66a	492.66a	492.66a	492.66a	492.66a
Petroleum benzene	360.33b	319.33b	213.00b	2.00b	0.00b	0.00b	149.11
Chloroform	191.66c	139.33c	109.00c	1.00b	0.00b	0.00b	73.50
Acetone	33.00d	24.00d	10.00d	0.00b	0.00b	0.00b	11.16
Alcohol	148.33c	123.66c	75.33c	0.66b	0.00b	0.00b	58.00
E-Mean	289.88	268.72	235.55	168.27	167.66	167.66	216.29

	SED	CD (0.05)	CD (0.01)
Days	9.54	19.03	25.27
Treatment	9.54	19.03	25.27
Days x Treatment	2.0623.38	46.63	61.91

alcohol) as an effective botanical protectant in arresting the pulse beetle population at higher concentration levels (1%, 3%, 5%). Among the solvent used for seed extraction of Annona, the Acetone extract minimized the beetle population even at minimum concentration (10-33 numbers). Moderate insecticidal effect was noticed for alcohol and chloroform extract as evidenced from 75-191 numbers of adult emergence.

IV.3 Impact of Annona seed extract on percentage weight loss of Green Gram by *C.analis*

Similar trend of observation were noticed for percentage weight loss as seen in adult emergence. Dose dependent variations among the treatment were also noticed in this parameter as like that of adult emergence.

Minimum percentage of weight loss of green gram were recorded for higher dose levels of all the solvent of Annona extracts (0.04 - 0.01%).The weight loss of the grain was also reduced to a minimum level in Acetone extract at lower concentration (0.60%-2.08%).This was followed by Alcohol (4.73% -8.01%) and Chloroform (8.84%-21.78%) of moderate loss in weight. The 1000 and 5000 ppm of Petroleum benzene annona extract recorded the highest adult emergence among the treatment (51.84- 55.00%) which were on par each that of control and acetone treated control.

From this table it was evident that the higher concentration of Annona seed extract possesses the potential insecticidal activity and thereby reducing the weight loss at minimum level (0.04-0.11%).Among the different solvents used, the acetone ranks first as evidenced from minimum adult emergence and weight loss even at lower concentration (1000, 5000 and 10000 ppm) followed by alcohol and chloroform.

IV.4 Impact of Annona seed extract on germination percentage and vigour index in green gram

Germination percentage between control, acetone treated control and treatments varied significantly. Therefore the 100% was recorded for all the treatments both at lower and higher concentration (7.33 – 83.33), except petroleum benzene at lower concentration. As the insect infestation in control and acetone treated control recorded maximum, the germination percentage also get reduced (66.67%).

**FIGURE III
PERCENTAGE WEIGHT LOSS OF *C.analis* IN GREEN GRAM**

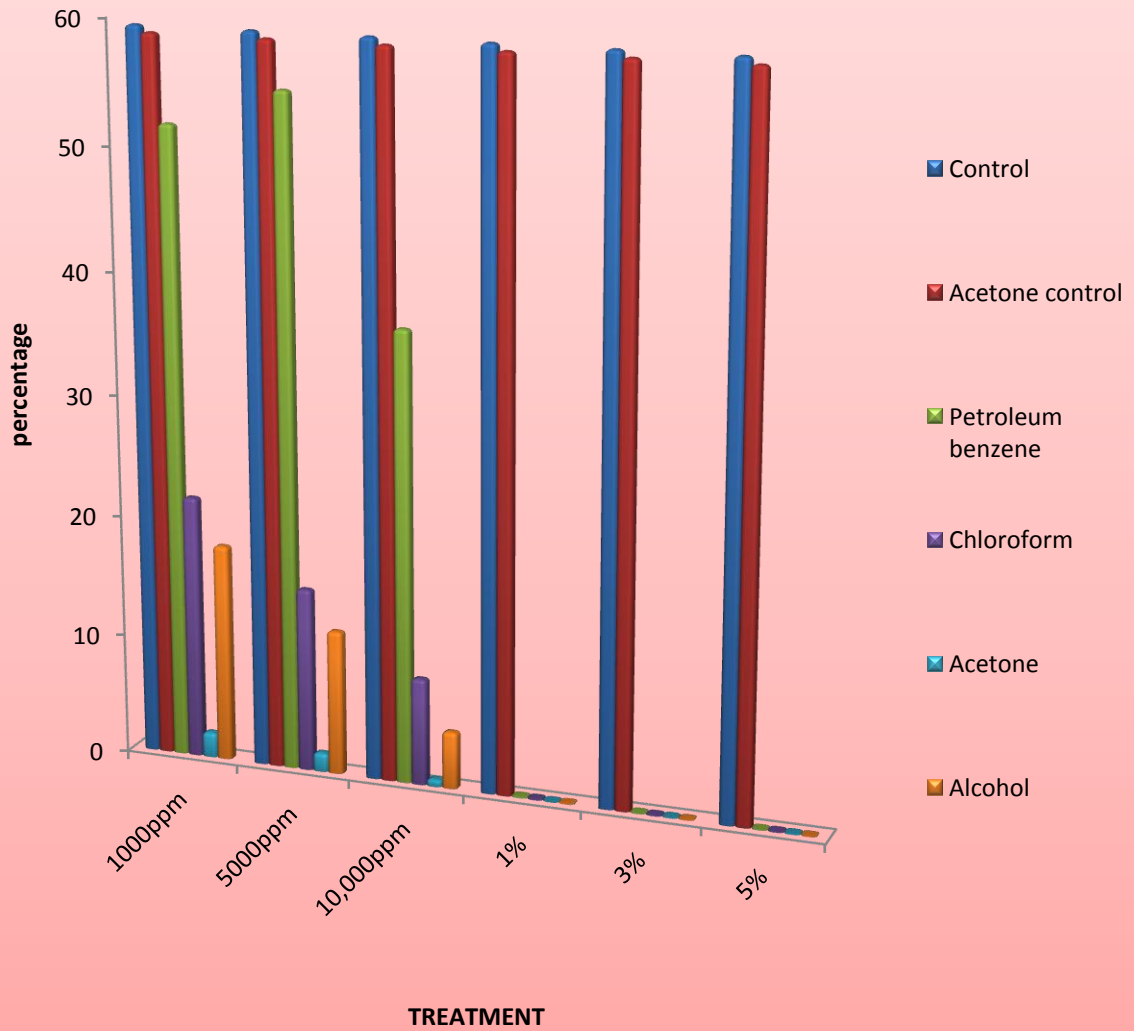


TABLE III

PERCENTAGE WEIGT LOSS OF GREEN GRAM TREATED WITH ANNONA SEED EXTRACTS

Concentrations							
TREATMENT	1000ppm	5000ppm	10,000ppm	1%	3%	5%	T-Mean
Control	59.43 a (50.43)a	59.43 a (50.43) a	59.43 a (50.43) a	59.43 a (50.43) a	59.43 a (50.43)a	59.43 a (50.43)a	59.43 (50.43)
Acetone control	58.87a (50.11)ab	58.87a (50.11) a	58.87a (50.11)a	58.87a (50.11) a	58.87a (50.11) a	58.87a (50.11)a	58.87 (50.11)
Petroleum benzene	51.84 a (46.05 b	55.00 a (47.88) a	37.05 b (37.44)b	0.11 c (1.86)b	0.05 c (1.30) b	0.04 c (1.15) b	24.01 (22.62)
Chloroform	21.78 a (27.42)c	15.16b (22.28) b	8.84 c (16.48)c	0.10 d (1.81)b	0.05 d (1.30) b	0.04 d (1.15) b	7.66 (11.74)
Acetone	2.08 a (8.12)d	1.57 a (7.17) c	0.60 ab (4.27) d	0.04 b (1.15)b	0.06 b (1.46) b	0.04 b (1.15)b	0.733 (3.89)
Alcohol	18.01 a (24.93)c	11.95 b (20.01) b	4.73 c (12.52) c	0.07d (1.46)b	0.07 d (1.46) b	0.04 d (1.15) b	5.81 (10.26)
E-Mean	35.34 (34.51)	33.66 (32.98)	28.25 (28.55)	19.77 (17.80)	19.76 (17.68)	19.74 (17.52)	26.08 (24.84)

	SED	CD (0.05)	CD (0.01)
Days	0.84	1.68	2.23
Treatment	0.84	1.68	2.23
Days x Treatment	2.06	4.12	5.47

IV.4 Impact of Annona seed extract on germination percentage and vigour index in green gram

Germination percentage between control, acetone treated control and treatments varied significantly. Therefore the 100% was recorded for all the treatments both at lower and higher concentration (7.33 – 83.33), except petroleum benzene at lower concentration. As the insect infestation in control and acetone treated control recorded maximum, the germination percentage also get reduced (66.67%).

As the germination percentage was high for all the treatments both at lower and higher concentration, the vigor of seedlings also high. The vigor index was reduced to a greater extent control and acetone treated control.

TABLE IV

TREATMENTS	RANKS	MEANS
CONTROL	5	573.95 e
ACETONE CONTROL	6	463.30 f
PETROLEUM BENZENE	4	702.94 d
CHOLOROFORM	3	1732.60 c
ACETONE	1	2042.20 a
ALCOHOL	2	1880.00 b

IV.5. Phytochemical screening of Annona seed Extract

The result on phytochemical screening of Annona seed extract were tabulated in table no VI. Phytochemical screening was carried out for petroleum benzene, chloroform, acetone and alcohol extracts. Alkaloids, flavonoids, terpenoids, saponins, tannin, anthocyanin, glycoside, phenol, carbohydrate and protein were analysed in all the extracts. The compounds carbohydrate, protein, saponins and phenol were present in all the extract. The numbers of compounds were maximum in alcohol extract, (alkaloids, flavanoids, anthocyanine, terpenoids, saponins, Tannins, phenol, carbohydrate) followed by acetone extract (Alkaloids, terpenoids, saponins, tannin, anthocyanin, glycoside, phenol, carbohydrate and protein). In comparision with alcohol and acetone extract the choloroform and petrolrum benzene extracts were found to possess the minimum compounds (Alkaloids, saponins, phenol, carbohydrate and protein) and (Alkaloids, terpenoids, saponins, tannin, anthocyanin, phenol, carbohydrate and protein) respectively.

TABLE V**PHYTOCHEMICAL SCREENING OF ANNONA SEED EXTRACTS**

COMPOUNDS	PETROLIUM BENZENE	CHLOROFORM	ACETONE	ALCOHOL
ALKALOIDS				
A.WAGER'S TEST	+	-	-	+
B.MAYER'S TEST	-	+	+	-
C.DRAGONDEAF	+	+	+	-
FLAVANOIDS	-	-	-	+
SAPONINS	+	+	+	+
ANTHOCYANIN	-	+	+	+
TANNINS	-	-	+	+
TERPENOIDS	-	-	+	+
PHENOL	+	+	+	+
GLYCOSIDE	+	-	-	+
CARBOHYDRATE				
A.MOLISCH'S TEST	+	+	+	+
B.BENDITES TEST	+	+	-	+
PROTEINS	+	+	+	+

V.DISCUSSION

Use of plant bioproducts became substitute of synthetic pesticides, protecting environment from pesticidal pollution (Prakash *et al.*, 1990; Tiwari *et al.*, 1990). The efforts have been made by many workers, and the efficacies of botanicals have been found against most of the stored grain pests (Rao *et al.*,1990) .Recently, an attempt has been made for developing and evaluating botanical insecticides in view of their relative safety to the environment (Schmutterer *et al.*,1990). Since, pulses are the major source of protein and their storage is more difficult. Therefore, the present work has been undertaken to observe the efficacy of Annona seed extract against *Callosobruchus analis* in order to protect the environment from hazardous effect of synthetic pesticides.

Custard apple (*Annona squamosa* L.) extracts have shown promise for pest control against a range of insect pests. Laboratory and field tests showed that extracts from custard apple kernels were effective against crop pests like spotted stem borer, *Chilo partellus* (Swin.); leafhopper, *Nilaparvata lugens* (Stal.), *Spodopteralitura* (Fab.), *S. frugiperda*, *Helicoverpa armigera* (Hubner), hairy caterpillar, *Spilosoma obliqua* (Wk.); Brinjal spotted leaf beetle, *Henosepilachna vigintioctopunctata* (Fabr.); cotton boll worm, *Dysdercus koenigii* (Fab.); semi-looper *Achaea janata* Linn. and Aphids (Saito *et al.* 1989; Rao *et al.*, 1990; Bhagawan, *et al.*, 1992; Ghatak and Bhusan 1995b; Hiremath *et al.*, 1997; Bhatnagar and Sharma 1997; Mathew *et al.* (1999), Raman *et al.*, 2000; Sonkamble *et al.*, 2000; Bhuiyan *et al.*, 2001).

Among stored grain pests extracts of custard apple was tested on *Callosobruchus chinensis* Linn. (Tripathy *et al.* 2001, Kotkar *et al.*, 2002, Misra 2000); *C. analis* (Juneja and Patel 1994); *C. maculatus* (Dharmasena *etal.* 2001); *Rhizopertha dominica* Fab. (Patel and Valand 1994), *Sitophilus oryza*(L.) (Mishra *et al.*, 1992), *Tribolium castaneum* (Herbst) (Malek and Wilkins 1994,1995, Hussain *et al.*, 1995) and *Corcyra cephalonica* Staint. (Ghatak and Bhusan 1995a).

Gupta *et al.*, (2015) observed the efficacy of six plant materials namely bhilawa (*Semecarpus anacardium* L.), black gram flour (*Vigna mungo* L.), custard apple seed powder (*Anona squamosa* L.), neem leaf powder (*Azadirachta indica* A. Juss.), neem

seed kernel powder and tobacco leaf powder (*Nicotiana tobaccum*) at two doses 0.5 mg and 0.25 mg per 100 g seeds (w/w) against stored grain pest, *Callosobruchus maculatus* (Fab.) and noticed the effectiveness of grain damage by *C. maculatus* was in the order of custard apple > neem seed kernel > tobacco leaf > neem leaf > black gram flour > bhilawa. Therefore Gupta *et al.*, 2015 ranked the custard apple as first in the series.

In the present investigation, seed extracts of Annona were tested against *C. analis* and the result revealed that higher concentration of all the solvents of Annona (1%, 3% and 5%) extract resulted in 100% mortality on the 1st DAT itself. Similar observation were made by Sonkamble *et al.* (2000) who recorded seed extract of Annona at 1.5 per cent concentration produced highest mortality in *H. armigera* (43.33%) and 36.66 per cent mortality at 1% concentration in *S. litura*.

On the 1st Day of observation of mortality in our study, 10,000ppm petroleum ether extract of Annona recorded higher percentage of mortality (30%) This is in accordance with the findings of Kaushik and Saini (2009) who reported that Petroleum ether leaf extracted from leaves of *A. squamosa* were reported to possess an insecticidal and growth regulating activities on three mosquito species namely *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*.

Among the solvents tested for its efficacy in our study, acetone extract recorded minimum adult emergence and percentage weight loss. The efficacy of acetone extract was also proved by Dharmasena *et al.* (2001) who reported that Acetone extracts from fresh and stored leaves of Annona were toxic to adult of *C. maculatus*, whereas the ethanol extracts were not active.

Extracts of *A. squamosa* seed had repellent and antioviposition properties when applied to *Ceratitis capitata* (Epinò and Chang 1993). In laboratory studies, topically applied seed extract of caused a steady decline of all free amino acids in freshly emerged 5th-instar larvae of *Dysdercus koenigii* (Reddy *et al.*, 1993). The depletion in free amino acids had an adverse effect on protein turnover, resulting in delayed

metamorphosis (Bhagawan *et al.*, 1992). Kawazu *et al.* (1989) isolated two compounds from the seed, which were found to be toxic to fruit fly.

Phytochemical screening of Annona seed extract in our study showed that the number of compounds were maximum in alcohol extract (alkaloids, flavonoids saponins, terpenoids, anthocyanin, phenol, glycoside, carbohydrate and protein) followed by acetone extract (alkaloids, saponins, terpenoids, anthocyanin, phenol, carbohydrate and protein). Phytochemical screening of Annona was carried out by many researchers.

Annonaceae are empirically known to elicit insecticidal activities (Grzybowski *et al.*, 2011; Das *et al.*, 2007). Plant species in this family contain an array of toxic compounds such as acetogenins, alkaloids, flavonoids that confer to these plants their insecticidal properties (Grzybowski *et al.*, 2011). Both seeds of *A. squamosa* and *A. muricata* contain a great (Das *et al.*, 2007) amount of acetogenins. Some alkaloids were found in *A. muricata*. Extracts of *A. muricata* not only affect the mortality rate of pupal and adult stages of mosquitoes but also reduce the reproductive success of surviving adults by decreasing fecundity and egg hatchability (Kaushik *et al.*, 2007). Flavonoids isolated from aqueous extracts showed antimicrobial activity against all the common microbial contaminants of pulses and 80% insecticidal activity against *C. chinensis* at a concentration of 0.07 mg ml⁻¹ (Kotkar *et al.* 2002). Adults of *T. castaneum* were repelled by contact with food medium treated with 2 and 5g custard apple leaf dust/10 g flour (Hussain *et al.* 1995). The petroleum ether extract of the seeds of this species yielded 13 known adjacent, and 4 non-adjacent bistetrahydrofuranic acetogenins, and the compounds squamocin and squamostatin a (Sahai *et al.*, 1994). Four non-adjacent bis-tetrahydrofuranic acetogenins, named squamostatins-B to -E, were isolated from the petroleum ether extract of *A. squamosa* seeds by Fujimoto *et al.* (1994).

Investigation on the evaluation of insecticidal activity of Annona seed extract revealed that higher concentration (1%, 3% and 5%) of all the solvents were effective in bringing out the cent percent mortality, number of adult emergence and percent weight loss and also good germination and vigour index. Therefore, all the solvents can be used to extract Annona and each extract can be used as protectant as these extracts

were equally potential. This study is helpful to store and protect the greengram from insecticidal activities of pests and thus become important from agricultural point of view to make the environment free from pesticide pollution and for betterment of human health.

VI. SUMMARY AND CONCLUSION

- An attempt was made in the laboratory to test the efficiency of indigenous Annona seed extracts for the management of pulse beetles, *Callosobruchus analis*.
- The test insect, *Callosobruchus analis* was collected from the pulses Department of TNAU, Coimbatore.
- The green gram for the presence study was procured from the local market, cleaned manually to remove dust, infested grains and stored. The insect were cultured in the laboratory $28 \pm 2^{\circ}\text{C}$ and 70-75% RH under glass of 500ml capacity containing cowpea seeds and kept in BOD incubator. Newly emerged adults within 24 hours of emergence were used for bioassay studies.
- The seeds of ANNONA (*Annona squamosa*) were collected from the local fruit stalls dried and ground into fine power. The powders were subjected to soxlet extraction using petroleum benzene, chloroform, acetone and alcohol on the basis of increasing polarity.
- The residue obtained after evaporation of solvent in water below 40°c and refrigerated for further use. From this residue, the test solutions of different concentration (1%, 3% and 5% doses) were prepared.
- The test solutions were mixed with 25g of green gram in separated polythene bags. 5pairs of test insects were introduced after evaporation of the solvents for 3hours. 3 replications were kept for the each concentration. Control and Acetone treated control with 3 replications were also maintained simultaneously.
- Observations were made on the adult mortality, adult emergence, and weight loss of seeds, germination and vigour index of seedlings.
- The result on the mean values of mortality of *C.analis* in Annona treated green gram showed cent percent mortality on the first day itself at higher concentration levels (1%, 3%, 5%). Moderate variation of mortality was noticed between control and the treatments of lower concentration (1000, 5000, 10000ppm).

- The adult emergence reduced to a maximum number in acetone (10-33 numbers) followed by chloroform extracts (109-191 numbers). Adult emergence 30 day after treatment in higher concentration of all the extracts (1%, 3%, 5%) recorded minimum (1-2 numbers) or no adult emergence. Which prove the potential use of this extract as effective insecticides. Among the lower concentration progeny suppression was greatly reduced in acetone extracts (10-33 numbers).
- Weight loss of green gram was recorded minimum in higher dose levels of all solvents of Annona extracts (0.04% – 0.01%). Weight loss of grain was also reduced to minimum level in acetone extracts at lower concentration (0.60% - 2.08%).
- Germination percentage between control, acetone treated control and treatments varied significantly. Therefore the 100% was recorded for all the treatments both at lower and higher concentration (7.33-83.33), except petroleum benzene at lower concentration. As the insect infestation in control and acetone treated control recorded maximum, the germination percentage also get reduced (66.67%).
- As the germination percentage was high for all the treatments both at lower and higher concentration, the vigor of seedlings also high. The vigor index was reduced to a greater extent control and acetone treated control.
- Phytochemical analysis of petroleum benzene, chloroform, acetone and alcohol extracts of Annona revealed that the presence of many compounds in alcohol (alkaloids, flavanoids, anthocyanine, terpenoids, saponins, Tannins, phenol, carbohydrate) extract, followed by acetone extracts (Alkaloids, terpenoids, saponins, tannin, anthocyanin, glycoside, phenol, carbohydrate and protein).
- From this study it was obvious that the higher concentration of all the extracts of Annona and lower concentration acetone produced maximum mortality, minimum adult or no emergence and weight loss higher germination percentage and vigor index forced as to recommend. These bioprotectant to the rural farmersto keep the grain health. as the test plant, *A. squamosa* have a wide

geographical distribution, integrating and promoting their use as an alternative plant based method in stored pest control advocated especially for farmers and traders. If used properly at an effective dose, the extracts of Annona were revealed to be a low cost and powerful environmental friendly trend thereby avoiding accumulation and negative impact of non-target endofauna.

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