Insecticidal effects of *Stephania japonica* L. on pulse beetle (*Callosobruchus chinensis* L.),rice weevil (*Sitophyllus oryzae* L.) and Mosquito larvae

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This Research paper is dedicated to my beloved Parents

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled "**Insecticidal effects of *Stephania japonica* L. on pulse beetle(*Callosobruchus chinensis* L.),rice weevil (*Sitophyllus oryzae* L.) and Mosquito larvae**" is an authentic and genuine research work carried out by me under the guidance of Dr. Shamsun Nahar Khan,Chairman and Associate Professor, Department of Pharmacy, East West University, Dhaka.

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ABSTRACT

An insecticide is a substance used to kill insects.They include ovicides and larvicides used against eggs and larvae,respectively.Insecticides are used in agriculture, medicine, industry and by consumers. Insecticides are claimed to be a major factor behind the increase in agricultural 20th century's productivity. Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans; Insecticides can be classified in two major groups as systemic insecticide which have residual or long term activity and contact insecticides, which have no residual activity.Experiments were conducted in the laboratory to study the efficacy of *Stephania Japonica* L. for the control of rice weevil (*Sitophilus oryzae* L.) in the storage, pulse beetle(*Callosobruchus chinensis* L..*)* in the storage and larvae of mosquito. The efficacy of *Stephania Japonica* was assessed on the basis of Screening test,repellent test, grain soak test, direct toxic and surface contact effects of rice & pulse grains.Extracts were made using solvents such as acetone,ethanol and water. The extract was used in three doses at 1 mg, 5 mg and 10 mg. The higher doses of *Stephania Japonica* L. gave better result. The effect of *Staphania Japonica* on insecticides of direct toxicity, repellency, grain soak and surface contact effects were found directly proportional to the level of concentration. It was observed from the results that the plant extract had significant repellent and screening effect, surface contact effect, and grain soak effect. Mortality percentage was found to vary among different concentrations of plant extracts. The highest mortality was observed with highest concentrations of *Stephenia japonica* L.

Key words: *Staphania Japonica,* rice weevil, *Sitophilus oryzae* L, pulse beetle, *Callosobruchus chinensis* L., larvae of mosquito, Screening, repellent,grain soak , direct toxic and surface contact.

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INTRODUCTION`

1.1 Medicinal Plant

The medicinal use of plants is probably as old as mankind itself. Plants have continued to be a valuable source of natural products for maintaining human health, as studies on natural therapies have intensified. More than 150,000 plant species have been studied, and several of them contain therapeutic substances. The use of plant compounds for pharmaceutical purposes has gradually increased. According to the World Health Organization medicinal plants are probably the best source of a variety of drugs. About 80 % of individuals in developed countries use traditional medicine containing compounds derived from medicinal plants (Varalakshmi, et.al. 2011).

Medicinal plants, defined as plants used for maintaining health and/or treating specific ailments, are used in a plethora of ways in both allopathic and traditional systems of medicine in countries across the world. Even people using only allopathic medicine throughout their lives are likely to be somewhat medicinal plant reliant as 20-25% of drugs prescribed are plant derived (Hall, et.al. 1990).

1.1.1 Definitions of medicinal plants

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. "Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes" (Ghani, 2003).

Herbal medicines have been utilized for many purposes, particularly in medical care as antiasthmatics (86.79 %), anti-rheumatics (62 %), diuretics (60.22 %), antiinflammation (29.62 %), anticancer (9.75 %), antidiabetics (8.33 %), antimicrobials, antifungals, antioxidants, antiallergy, analgesics, anti-obesity and antihypertention. In dental care it has been employed as anticariogenic, analgesic, local anesthetic, wound healing agents, anti-inflammation and recurrent aphthous stomatitis treatment etc (Ghani, 2003).

1.1.2 Importance of Medicinal Plant

Plants are the tremendous source for the discovery of new products with medicinal importance in drug development. Today several distinct chemicals derived from plants are important drugs, which are currently used in one or more countries in the world. Herbal medicines have been utilized for many purposes, particularly in medical care as insecticides (6.7%),antiasthmatics (86.79 %), anti-rheumatics (62 %), diuretics (60.22 %), antiinflammation (29.62 %), anticancer (9.75 %), antidiabetics (8.33 %), antimicrobials, antifungals, antioxidants, antiallergy, analgesics, anti-obesity and antihypertention. In dental care it has been employed as anticariogenic, analgesic, local anesthetic, wound healing agents, anti-inflammation and recurrent aphthous stomatitis treatment etc (Ghani, 2003).

The primary metabolites, in contrast, such as phytosterols, acyl lipids, nucleotides, amino acids, and organic acids, are found in all plants and perform metabolic roles that are essential and usually evident. Although noted for the complexity of their chemical structures and biosynthetic pathways, natural products have been widely perceived as biologically insignificant and have historically received little attention from most plant biologists (Ghani, 2003).

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as secondary metabolites, often are differentially distributed among limited taxonomic groups within the plant kingdom. The secondary metabolites are known to play a major role in the adaptation of plants to their environment and also represent an important source of pharmaceuticals. Their functions, many of which remain unknown, are being elucidated with increasing frequency. Secondary metabolites are economically important as drugs, flavor and fragrances, dye and pigments, pesticides, and food additives. Many of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances (Ghani, 2003).

Based on their biosynthetic origins, plant natural products can be divided into three major groups: the terpenoids, the alkaloids, and the phenolic compounds. All terpenoids, including both primary metabolites and more than 25,000 secondary compounds, are derived from the five-carbon precursor isopentenyl diphosphate (IPP). The 12,000 or so known alkaloids, which contain one or more nitrogen atoms, are biosynthesized principally from amino acids. The 8000 or so phenolic compounds are formed by way of either the shikimic acid pathway or the malonate/acetate pathway (Ghani, 2003).

1.1.3 Medicinal plants & Traditional Medicine Practice in Bangladesh

The plants which are useful for healing several diseases are called medicinal plant. There are 722 medicinal plants in our country. Bangladesh possesses a rich flora of medicinal plants. Out of the estimated 5000 species of different plants growing in this country more than a thousand are regarded as having medicinal properties. Out of them, more than a thousand have been claimed to posses medicinal poisonous properties, of which 546 have recently been enumerated with their medicinal properties and therapeutic uses. In addition to possessing various other medicinal properties, 257 of these medicinal plants have been identified as efficacious remedies for diarrhoeal diseases and 47 for diabetes (Ghani, 2003).

Use of these plants for therapeutic purposes has been in practice in this country since time immemorial. Continuous use of these plants as items of traditional medicine in the treatment and management of various health problems generation after generation has made traditional medicine an integral part of the culture of the people of this country. As a result, even at this age of highly advanced allopathic medicine, a large majority (75-80%) of the population of this country still prefer using traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighbourhood (Hussain, et.al. 2012).

Traditional medical practice among the tribal people is mainly based on the use of plant and animal parts and their various products as items of medicine. The medicaments, prepared from plant materials and other natural products sometimes also include some objectionable substances of animal origin. They are dispensed in a number of dosage forms like infusions, decoctions, pastes, moulded lumps, powders, dried pills, creams and poultices. Diets are strictly regulated. (Hussain, et.al. 2012).

1.2 General Background About Stored Grain Rice

There are many ways to improve the condition of a country. The production of crops is one of them. Losses due to insect infestation are the most serious problems in grain storage, particularly in the cases of developing countries like Bangladesh.

Storing of grains in store houses and to protect them from the attack of insect pests is a problem confronted by every householder, whether he is a cultivator or not. It is reported that about, 5-8% of the food grains, seeds and different stored products are lost annually due to storage pests, and if the losses incurred on farms were included, it would amount to 10% (Alam, 1971). Insect pests cause considerable losses to stored rice, which may affect the food availability of a large number of people, particularly in the developing countries like Bangladesh. As many as 34 species of insects have been reported as pests of stored paddy and clean rice from different countries (Grist and lever, 1969). In Bangladesh 13 insect species have been recorded on stored rice (Alam, 1971). Reliable estimates of over all losses during rice storage are difficult to obtain but these are much greater than generally appreciated. According to some author that about 15 percent rice in storage in Bangladesh is damaged by insect pests (Khan, 1991). Loss is usually lower in the unhusked-unparboiled rice than husked-parboiled rice (Bhuiyan, 1988).

Many preventive and effective control measures have been reported to minimize the loss of stored grains due to insect attack. Among these, many investigators suggested chemical insecticides for their control effectively. But chemical control of insects in storage has been used with serious drawbacks (Sharaby,1988). Synthetic or chemical pesticides which have been used for a long time for controlling insect pests, have got many limitations and undesirable side effects such as- i) development of pesticides resistance in insect pests, ii) toxic residues in food and the environment, iii) resurgence of treated population and outbreak of secondary pests, iv) destruction of natural enemies of pests and non-target organisms, v) heath hazard, vi) environmental population (Husain, 2001, 1993; Metcalf and Luckmass, 1975).

1.2.1 Rice Weevil

Rice is the most important cereal crop and staple food in Bangladesh. It occupies about 10.26 million hectares, which is about 75 percent of total cultivable land of the country (BBS, 1997). The demand for rice is constantly rising in Bangladesh with nearly 2.3 million people being added each year to her population of about 120 million (Anon, 2001). Maintenance of reserve food grain stocks is necessary to ensure a continuous supply at stable price.The rice weevil, *sitophilus oryzae* L. is one of the most important pests of the many common cereals and has a world wide distribution (Gomes,et.al.1983). This insect occurs throughout the tropics and is also found in warm temperature regions, including Bangladesh. Rice weevils can cause losses to grain in storage, either directly through consumption of the grain or indirectly by producing 'hot spots' causing loss of moisture and thereby making grain more suitable for their consumption. This insect infests rice (both husked and unhusked), maize, wheat and sorghum particularly in monsoon. It also causes damage to oats, barely, cotton seed, linseed and cocoa.

All those increasing problems have dictated the need for effective, biodegradable pesticides with greater selectivity (Saxena, 1983). This awareness has created a worldwide interest in the development of alternative strategies, including the search for new types of insecticides and use of age- old traditional botanical pest control agents (Heyda,et.al.1983). In ancient times, Egyptian farmers used to mix the stored grains with fine kitchen ashes .During 1690, tobacco was used as contact insecticides and in 1773 nicotine fumigation was trying.Pyrethrum was first used for insect pest control in the USA in 1858 (Dhaliwal et. al. *1998).* Plants are a rich source of compounds having insecticidal activity (Arnason, et. al. 1989). At present, in many areas of the world locally available plants and plant materials have been widely used to protect stored product against damage by insect attack (Golob and Webley, 1980; Talukder and Howse, 1990).

Indo-Pakistani fanners use biskatalli for the control of stored grain pests, while various Nigerian tribes use roots, stems and leaves of plants (Ahmed and Koppel, 1985; Ahmed and Grainge, 1 The main advantages of botanical are that these can be easily produced by farmers, less expensive, biodegradable, broad spectrum, safe to apply and unique in action. Most of the botanical insecticides are non-hazardous and non-toxic to human. The earlier studies by different authors (Islam, 1987; Talukder and Howse, 1990, 1993, 1994a, 1994b, 1995; Haque and Husain, (1986) also established the successful actions of different plant parts and extracts against different major stored product insect pests of Bangladesh.Few scientific research works have been done to explore locally available plant materials for the management of harmful insect pest in storage. So, the present studies were undertaken to assess direct toxicity and grain soak of plant extracts against rice weevil.

1.2.2 Research objectives of *Stephenia japonica* as a pesticide for rice weevil

The present experiments were undertaken to find out the efficacy of the extracts of *Stephenia Japonica* L. The objectives of the present research were as follows:

i) to determine the direct toxicity of plant extracts;

ii) to evaluate the grain soak of test plant extracts against rice weevil; and

iii) to determine the direct contact toxicity of plant extracts

1.2.3 Pulse Beetle

On the other hand,Pulse is one of the important crops in Bangladesh which is very rich in protein (20-30%) (Sharma, 1984). Pulses have traditionally been called the poor man's meat. They are the cheapest source of protein and amino acids. Pulses are the main sources of plant protein especially in the developing nations, particularly in Bangladesh. Pulses play a significant role in the diet of common people of Asian countries including Bangladesh. In Bangladesh per capita consumption of the animal protein is very low (Saehdeva, 1985). It plays a vital role in the nutrition of the people of Bangladesh. Various species of pulses are grown and consumed in Bangladesh which in order of production are: grass-pea, lentil, chickpea, black-gram and mung-bean. The combined output of all other pulses which include pigeon-pea, cowpea, pea, and other minor crops, do not exceed 90000 tons annually. Recently, pulse production is gradually decreasing day by day. Several factors are responsible for this declining trend of which varietal instability, attack by insect pests and diseases are important.

The storage of pulses is a matter of great concern. Pulses are more difficult to store than cereals as these suffer a great damage during the storage due to insect pest and microorganisms. In Bangladesh gram seeds stored in godown's and farmer's houses furnish a suitable habitat for growth and multiplication of storage pests including pulse beetle. Several species of pulse beetle are reported to attack pulses in storage. These are *Callosobruchus chinensis* (L.), *Ccillosobruchus maculates* (F.) and *Callosobruchus analis* (Husain, 1995). The pulse beetles attack pulses both in the field as well as in the storage

The damage in storage is more crucial than in the field (Yamamato, 1990). *C. chinensis* L. is one of the most destructive pests of almost all kind of pulses in storage and also to some extent in the field (Patnaik, 19 Rajapakse, 1989). The insect originally came from East Asia and now has a cosmopolitan nature in distribution. It is more widely spread throughout the tropical and subtropical regions (Mensah, 1986).

Callosobruchus chinensis has been reported as a serious pest of various legumes such as chik pea, lentil, black-gram, cowpea, grass-pea, pigeon-pea, mung-bean, red bean, black bean, yearlong bean, garden pea, etc. They attack pulses in storage causing serious damage (Alam, 1971; Bhuiyan and Peyara, 1978; Singh *etal.*, 1977; Rustamani et.al. 1985; Husain, 1995; Rouf *el ui*, 1996) and also to some extent in the field (Patnaik, 1984; Rajapakse, 1989). The damage to pulses by pulse beetle is very high and quite often each and every seed is infested and as a result the pulse seeds become unlit lor human consumption (Atwal, 1976). It causes a great loss in seed weight and protein content of the seeds. It is reported that the pulse beetle may cause 10-95% loss in seed weight and 45.5-66.3% loss in protein content of the seeds under normal condition and the severity of damage increases with the duration of storage (Gujar and Yadav, 1978). The germination rate of the pulse seed is also reduced to a great extent (Yadav, 1985). The degree of damage varies with different kinds of legumes, duration of exposure time, storage facilities and other factors associated with seeds. Under farmers storage condition, as high as 64.33% grains of chick-pea were recorded to be damaged by pulse beetle, C. *chinensis* in Bangladesh.

In Bangladesh, the pest is chiefly controlled by synthetic insecticides. Though pest control by the use of insecticides is the most potent technology, but it has ultimately fallen into dispute both by the farmers and traders. Continuous and indiscriminate use of synthetic insecticides has got many limitations and undesirable side efleets, sueh, (1) insecticide resistance in pest insects, (2) hazards to human and the environment, (3) destruction of non-target organism, (4), outbreak of secondary pests, and (6) human health hazards. (Rustamani et.al. 1985; Duguet and Wu, 1986; Husain, 1995). Injudicious use of pesticides to protect pulses from pulse beetle in storage may cause serious health hazards (Bhaduri et. al. 1989)

Botanical plant products are environmentally safe, less hazardous and less expensive. The main advantage of botanical insecticides is that they can be easily produced by the farmers in the house and small-scale industries. The farmers of India were reported to save their crop and/or products with herbal substances such as oils, leaves, roots, seeds etc. of different plant instead of synthetic chemical insecticides for a long time (Talukder and Howse, 1993). In Bangladesh, insect pest control technology after 1970 has been based largely on imported synthetic insecticides which are now subject to the constraint of dwindling foreign exchange. Most of the work on the laboratory and field evaluation of botanical pesticides has been reported from South Asian countries. Efforts on research are going on to establish more botanical pesticides as an important pest control tool from different toxic and medicinal plants. Interests on botanical pesticides are gradually increasing because of several distinct advantages. Botanical pesticides are generally much safer to human beings and to the environment than conventionally used synthetic pesticides; thus these are used for millions of years without any ill or adverse effects on ecosystem. However, a very few scientific research works have been done in Bangladesh to explore our locally available plant materials for the control of harmful insect pests in storage and fieldl in minimizing the undesirable side effects of synthetic pesticides (Yadav, 1985).

1.2.4 Research objectives of *Stephenia japonica* as a pesticide for Pulse beetle

The present study was undertaken to study the insecticidal activity of indigenous plant extract, such as *Stephenia japonica* , against the pulse beetles which is given below:

I. To study the insecticidal activities of extract against the pulse beetle used as grain soak of the seed.

II. To study the direct contact toxicity of plants extracts against the pulse beetle

III. To determine the repellent effect of the plants extracts

1.2.5.Mosquito Larve

Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy todeal with themin this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment. Naturalpesticides,especiallythosederivedfromplants,are more promising in this aspect. (Bhaduri et. al. 1989)

Mosquito control manages the population of mosquitoes to reduce their damage to human health, economies, and enjoyment. Mosquito control is a vital public-health practice throughout the world and especially in the tropics because mosquitoes spread many diseases, such as malaria.

Disease organisms transmitted by mosquitoes include West Nile virus, Saint Louis encephalitis virus, Eastern equine encephalomyelitis virus, Everglades virus, Highlands J virus, La Crosse Encephalitis virus in the United States; dengue fever, yellow fever, Ilheus virus, malaria, Zika virus and filariasis in the American tropics; Rift Valley fever, *Wuchereria bancrofti*, Japanese Encephalitis, chikungunya, malaria and filariasis in Africa and Asia; and Murray Valley encephalitis in Australia. (Talukder and Howse, 1993)

Depending on the situation, source reduction, biocontrol, larviciding (killing of larvae), or adulticiding (killing of adults) may be used to manage mosquito populations. These techniques are accomplished using habitat modification, pesticide, biological-control agents, and trapping. The advantage of non-toxic methods of control is they can be used in Conservation Areas (Yadav, 1985).

Figure 1. Mosquito

Mosquitos are generally considered annoying and may also transmit diseases, thus leading to a variety of human efforts to eradicate or reduce their presence.

1.2.6. Research objectives of *Stephenia japonica* as a pesticide for mosquito larva

1. Nuisance mosquitoes bother people around homes or in parks and recreational areas;we can inhibit the grow of larva by using plant extracs as pesticides.

2.Economically important mosquitoes reduce real estate values, adversely affect tourismand related business interests, or negatively impact livestock or poultry production; we can inhibit the grow of larva by using plant extracs as pesticides.

3.Public health is the focus when mosquitoes are vectors, or transmitters, of infectiousdisease. we can inhibit the grow of larva by using plant extracs as pesticides.

REVIEW OF LITERATURE

A number of insect pests seriously attack various kinds of stored grains and causes considerable damage. Now-a-days, control of insect pests by botanicals has drawn special attention all over the world. Several species of insect pests both in field and storage have been reported to be controlled by the application of botanical products such as powder, extract, oil and some botanical pesticides, as potential sources of antifeedant, repellent, and growth inhibitor (Islam, 1987; Talukdar and Howse, 1994 and 1995). Some important literatures about the use of botanical products at home and abroad are cited here.

Sangwan, et. al. (2005) carried out an experiment where, pigeon-pea (cv. Manak) seeds were treated with 11 seed protectants against pulse beetle *(Callosobruchus chinensis).* All the seed protectants, except for sawdust and turmeric powder, recorded significantly higher adult mortality than the control alter the first day of treatment. At 105 days after treatment (DAT), only dung cake ash with sand and wheat husk recorded significant adult mortality. Neem oil was effective (64.33% adult mortality) up to 35 DAT and it was followed by mustard oil + turmeric powder, which recorded only 16.33% adult mortality.

Juneja and Patel (2002) treated one-hundred seeds of green gram with 1, 2, 3, 4 or 5% (w/w) powdered black pepper (*Piper nigrum*) seeds, mint *(Mentha piperita)* leaves, orange (*Citrus reticulate)* peels or neem *(Azadirachta indica)* seed kernels to determine the persistence of the botanicals as protectants of green gram against the pulse beetle *(Callosobruchus chinensis).* The number of eggs decreased with increasing concentrations of the botanicals used, although grain damage increased with the duration of the treatment. Seeds of green gram treated with 1% of powdered black pepper seeds were totally protected from the pulse beetle for up to 5 months.

Mazyad and Soliman (2001) evaluated essential oil of *Eucalyptus globulus* leaves or camphor against the maturation of *Oestrus ovis* larvae under laboratory conditions and reported that camphor at concentrations of 1:0 and 1:1 inflicted 100% mortality. At concentrations of 1:2-1:6, the mortality rate ranged between 45-98%. On the other hand, 38 or 27.5% of the developed pupae emerged to adults but only 36.8% of them were fertile. Camphor was safely used in medicine and was recommended for controlling the zoonotic myiasis agent *O. ovis.*

Oparaeke, et. al. (2000) evaluated the extracts of garlic *(A. sativum)* bulb and African nut-meg *{Monodora myristica)* seeds against some insect pests of cowpea. All treatments exhibited significant protection of cowpea pods from *Maruca vitratu* and pod-sucking Hemiptera damage compared with the untreated control. *A. sativum* and *Monodora myristica* treated plots recorded 37.3 and 35.0% and 36.1 and 42.7% pod damage in 1997 and 1998, respectively, compared with 67.8 and 98.9% pod damage in the untreated control in 1997 and 1998, respectively. *A. sativum* and *M. myristica* treated plots recorded 24.6 and 13.0%, and 30.0 and 17.2% higher grain yields in the 1997 and 1998 seasons, respectively.

Chander et. al. (1999) evaluated acetone extracts of sweet flag *(Acorus calamus*), kut root (*Saussurea lappa),* turmeric *{Curcuma longa),* curry leaf *{Murraya* sp.) and one neem *(Azudirachta indica)* formulation (Nimbicidin) in the laboratory as repellents on jute fabric against the rust red flour beetle, *Tribolium castaneum.* Extracts of sweet flag and turmeric rhizomes were highly effective even at the lowest concentrations of 2.5 and 3.12 mg/25 cm2 jute fabric, respectively. The effective concentrations exhibited repellency even after three months of ageing at room temperature.

Cubillo, et. al. (1999) tested 16 commercial insecticides and plant extracts for their possible repellency or mortality effects on *B. tabaci* adults. The substances were sprayed on tomato plants placed inside sleeve cages, where 100 *B. tabaci* adults were released after 30 minutes. None of the products showed repellency. only those nccm derivatives containing oil did so. Only two of the plant extracts, "anisillo" and the ether fraction of £ *purpurea*, caused mortality, whilst black pepper caused low levels of opposition.

Durrheim and Leggat (1999) reported that aerosolized insecticides, alcohol consumption and garlic were most effective for the control of mosquito.

Thomas and Callaghan (1999) studies the effects of garlic and lemon peel extracts against C. *pipiers* larvae. Both garlic and lemon were found to be toxic to mosquitoes. Garlic was more persistent than lemon, with no significant differences in mortality rate between fresh and 4.5-day-old treatments. The addition of food to the bioassays increased toxicity of both lemon and garlic and represented the situation in the field more closely, where food would be available to the larva.

Behai (1998) studied the effect of some plant oils on the olfactory response of the larvae of rice moth, *Corcyra cephalonica.* Ten days old larvae of *C. cephaloneca* were exposed to oils of Ncem *(A. indica*), Sweet foag *(A. calamus*), Eucalyptus (£ *citriodura),* and Cloves *(S aromaticum).* Sweet Hag oil repelled the larvae at all the concentrations, whereas Clove and Eucalyptus oils were effective at the higher concentrations.

Castillo,et.al. (1998) evaluated the insecticidal activity of ethanol extracts from *Berberis samacana, Berberis saboyana, Nicandra physalodes, Eucalyptus globulus* and *Salpichroa diffusa.* Extract were used at 100, 1000 and 10000 ppm, to determine their effect on the development of *Tecia solanivora.* The variables measured were percentage of pupation, days of pupation and emergence of the adults. The activity of the extracts began at 1000 ppm, with the extracts from *E. globulus* having the greatest effect on the pupation rale.

Khan and Shahjahan (1998) extracted the dried and powdered *Eucalyptus teretocornis* leaves with n-hexane, acetone, ethanol and methanol and the extracts were tested to observe their effects on adults o*t' Sitophilus oryzae* and C. *chinensis.* Results showed that *S. oryzae* was repelled and *C.chinensis* was attracted by all the extracts. The percentages of repulsion for *S. oryzae* were 71.7, 74.7, 69.0, and 63.3 respectively.

Modgil and Samuels (1998) conducted an experiment on wheat grain treated with leaf powder of mint *(Mentha spicata*) and eucalyptus *(E. citriodora).* The seeds were stored for six months and it was observed that mint leaf powder was protective for six months, whereas eucalyptus was effective for five months.

Rahman (1998) evaluated the extracts and dust of Urmoi, Neem and Turmeric for their repellency, feeding deterrency, direct toxicity, residual effects and their potentiality against the rice weevil, *S. oryzae* and grain weevil, 5. *granarius.* The three plants had repellency, deterrency and direct toxicity effect on the test insects. The emergence of F| progeny, seed damage rate, percent weight loss reduced significantly in almost all treatments compared to control.

Javaid and Mpotokwane (1997) investigated the insecticidal activities of the powders obtained from *Eucalyptus* sp., *Mclia azedarach* and *Croton gratissimus* against C. *maculatus* with respect to the number of eggs laid and adult emergence and weight loss ofcowpea seeds. All the powders were reported to have some insecticidal activity against the pest.

Singh, et. al. (1996) conducted laboratory studies to determine the effects of extracts of neem, garlic, oranges. *Eucalyptus hybrida, Lantana camara, V. negundo,* against *Rhizoperlha* *dominica.* All of the extracts resulted in lower fecundity per female and adult emergence as well as increased adult mortality and reduced grain damage.

Sarac and Tunc (1995) invested the residual toxicity of the essential oils of *Pimpinella anisum, Eucalyptus camaldulensis, Thymbra spicata* and *Satureja thymbra* against *Tribolium corfusum* and reported that only the essential oil of *P. anisum* had a high residual toxicity against the adults of *Tribolium confusum.*

Shanthi and Janarthanan (1995) conducted laboratory experiment with synthetic and plant origin insect growth regulators to study their effect on reproduction *of Nilaparvatu lugens.* They observed significant reduction in nymphal emergence and the development of males and females through the application with hydropene at 2 ppm and extracts of *Eucalyptus* sp. at 200 ppm.

Subramanya, et. al. (1994) tested the effectiveness of 6 plant species for the control of *C. chinensis* infesting red gram and reported that *Eucalyptus ctriodora* was the most promising. Application of dry leaf powder either as a layer above the grain mass or mixed with the grain gave the highest reduction of the number of eggs laid and adult emergence. Doses of dry leaf powder above 4% (w/w) were the most effective.

Jood, et. al. (1993) used neem oil and powder of leaf and seed kernel, critrus lemon leaf, garlic *(Allium sativum)* bulb, pudina *(Mentha spicata)* leaf on maize kernels at 1 and 2% level (w/w) to control the larvae of *T granarium.* Neem kernel powder and oil provided complete protection to grains for 6 months, whereas substantial insect infestation was noticed after 3 months in other treatment.

Mani, et. al. (1993) observed that the adult females of *Corcyra cephalonica* exposed to the volatile of leaves of tulsi (*Ocimum basilicum),* and eucalyptus *(E. rostrata)* laid reduced number of eggs.

Prakash, et. al. (1993) evaluated 20 plant products of which only 7 products reduced adult populations and weight loss of grain significantly. These were neem seed oil followed by *Piper nigrum* seed powder, leaves of *V. negundo*, leaves of *Andrographis paniculata*, dried mandarin fruit peel, rhizome power of turmeric and seed powder of Cassia fistula.

Srivastava and Krishna (1992) carried out an experiment by exposing the 24-48 hour old first instar nymphs of *Dysdercus koengii* in *Eucalyptus* oil odor for 2 hours and reported that it caused greater mortality of larvae in the third inslar.

Pathak and Krishna (1991) reported that postembryonic development and adult emergence of *Corcyra cephalonica* were adversely affected when individuals were reared in an environment of eucalyptus oil volatiles. A marked decline was observed in the reproductive potential, in terms of egg output and hatchability.

Stamopoulos (1991) studied the effects of vapour from 4 essential oils (*Pelargonium, Cupressus, Eucalyptus* and bitter almond) on the oviposition and fecundity *of Acanthoscelides obtectus.* Choice and nonchoice tests showed that *Eucalyptus* strongly reduced fecundity, decreased egg hatchability and increased neonate larval mortality.

Jilani and Saxena (1990) observed for 8 weeks the repellent effects of turmeric oil, sweet flag oil, neem oil and neem based insecticides against lesser grain borer, *R. duminica.* In a choice test, filter paper strips treated with the test materials repelled the insect. Turmeric oil and sweet flag oils were significantly more repellent during the first two weeks than neem oil.

Srivastava, et. al. (1989) reported that *M. arvensis*, *Cymbopagon martini, Eucalyptus globulus* and C. *winterianus* were most effective for the control of C. *chinensis* on arhar, *Cajanus cajan.*

Jilani, et. al. (1988) treated *Triholium castaneum* in rice grain with 100, 500 or 1000 ppm of turmeric oil, sweet-flag *(Acorus calamus)* and neem *(Azadirachta indica).* The repellency increased with increasing concentration of the oils. In second choice test, filter paper strips treated with turmeric oil at 200, 400 or 800 g/cm repelled insects and thereafter, repellency decreased more rapidly than w ith neem oil. Adults which were fed on wheat flour that had been treated with the test materials at 200 p.p.m. produced fewer and underweight larvae, pupae and adults compared with those fed on untreated flour.

Sharaby (1988) evaluated the toxicity, repellency and LC50 of sun dried guava and eucalyptus leaves against *S. grunarius* and *S. uryzue.* Guava leaves were reported to be more toxic to both species than the eucalyptus leaves. On the other hand. Eucalyptus leaves were reported to be more repellent than guava leaves. Both these test materials at 15g/100g depressed the progeny development of the test insects.

Jilani and Saxena (1987) observed that indigenous plants such as Neem, Turmeric and Sweet flag possessed insect repellent properties. They farther reported that Turmeric, Sweet Hag Neem oil acted as repellent against the lesser grain borer, *R. dominica.*

Mohiuddin, et. al. (1987) tested some plant extracts for their repellency to the *Triholium castaneum.* Seed extracts of neem, *Ocimum hasilicum, Tagetes erecta, Momordica charantia,* celery and garlic were less repellent than *I. bijuga* oil but more repellent than oils from *Cuminum cyminum,* bottle gourd.

Morallo-Rejesus (1987) reported that the extracts of *Piper nigrum* and *Capsicum fruitescens* were effectively toxic *to S. zeamais* and *T. castaneum* when they were topically applied on these insects.

Yadava and Bhatnagar, (1987) assessed the efficacy of some plant products against the bruchid,*Callosobruchus chinensis.* The plant products tested were leaf powders of neem (*Azaclirachta indica*), dhatura *(Datura alba*) and garlic powder, and shell and seed powder of soapnut (*Sapindus trifoliatus).* Mortality of the insect was low initially, but it increased with time until after 1 week. All treatments were significantly superior to the untreated control. After a period of 5 months, the mean percentage of damage to the seeds were 6.9, 7.6, 7.6, and 8.2% for treatments with soapnut shell powder. *C. procera* leaf powder, soap nut seed powder and garlic powder,respectively in comparisons to 2.2 for malathion at 15 ppm. Leaf powders of neem and dhatura were slightly less effective.

Ahmed and Eapen, (1986) investigated the effects of the essential oils screened from *Gaultheria* sp., *Anethum graveolens, Mentha* sp. and *Eucalyptus* sp. against *Musca domestica, S. oryzae,* and *C. chinensis* and *Stegobium paniceum* and found all of them promising.

Sighamony, et. al. (1984) carried out experiment with acetone extracts of black pepper, *P. nigrum*, oils of clove, *S. aromaticum*, Cedar wood oil, *J. virginiana* and Karanja, *P. pinnata* by a choice method to determine their repellent effects on adults of *T. castaneum* and observed that karanja oil had good repellent action.

Jilani and Su (1983) reported that turmeric powder repelled the *S. granarius* and *R. dominica*, whereas its petroleum ether extract was found to be repellent against the *T. castaneum.*

Teotia and singh (1968) found that the wrinkled, depressed or rough seed coat of the seeds were relatively less preferred for oviposition to C. *chinensis*, while that of the smooth and well filled seeds were preferred. They also reported that the bigger the size of the seed, the greater was the no. of eggs laid per seed. The oviposition preference did not seem to have any relation to the suitability of seed for development of the insect.

Girish, et. al. (1974) studied the oviposition preference of *C. maculatus* on seeds of six pulse species in the laboratory in India and found that the no. of eggs laid on seeds of chickpea, pea, soyabean, mungbean, blackgram and pigeonpea were 39.0, 38.3, 36.2, 11.2, 13.7, and 8.2 respectively. They concluded that oviposition preferences were determined by the smoothness of the surface of the seedcoat and the size of the grains. Development of the larvae was not faster on those seeds that were preferred for oviposition and development was not dependent on each other.

Gokhale and Srivastava (1977) studied the ovipositional preference of C. *maculatus* on different varieties moth bean and found that oviposition preference was unrelated to the suitability of the seeds for bruchid development.

Singh, et. al. (1980) studied in detail the ovipositional preference of C. *chinensis* and *C. maculatus* in different pulses. They reported that the pulse beetle did not show any significant varietal preference for oviposition. The order of preference for *C. chinensis* was: cowpea> blackgram> lentil> pigeonpea> chickpea> mungbean> pea, whereas for *C. maculatus* was chickpea> blackgram> mungbean> cowpea> pigeonpea> pea> lentil. They also concluded that the ovipositional preference was independent of larval development.

Dias and Yadav (1988) studied the oviposition preference of *C. maculatus*, *C. chinensis* and *C. analis* on seeds of four legumes. When five different coloured grains of chickpea and pigeon pea were tested, dark-brown pigeon pea seeds were preferred. When these legumes were tested with cowpea and green gram, cowpea seeds were generally preferred.

Parajulee. et. al. (1989) reported that lentil was preferred for oviposition, growth, and development by *C. chinensis.* Although adult females laid more eggs on soybean and kidney bean only a few adults reached maturity.

Fox (1993) reported that females of *Callosobruchus maculatus* mate frequently to replenish depleted sperm supplies, and that females obtain a nutritional contribution from males during copulation (and that this has a positive effect on the female's life history) were tested in the laboratory. There was no difference in lifetime fecundity between females which mated only once and those with access to males throughout their lives. When females were mated at 48-h intervals, however, but were not confined with males, they laid more eggs than females which mated only once. When females were maintained under starvation conditions, multiple mating increased female longevity, but when females had unlimited access to yeast and sugared water, this influence disappeared.

Shiau, et. al. (1994) reported that the oviposition chice of the stored products pest *C. maculatus* was tested by providing females with different rartios of azuki beans and mungbeans. The fraction of eggs laid on azuki beans increased with increasing ratio of azuki beans, whereas it decreased with a decreasing duration of oviposition. The probability of female encountering azuki beans was significantly higher than for mungbeans, but even when the female was provided with the same probability of encounter it still preferred to lay eggs on azuki beans. Females tended to spend more time inspecting azuki than mungbeans, but no differences in handling time between the 2 hosts were found.

Singal (1998) reported that the biology of pulse beetle, *Callosobruchus chinensis* (L.) on cowpea under field and laboratory conditions. The beetls infested 3.4 percent pods in the field in Haryana, India, a female laid 28.6 eggs under field conditions compared to 42.2 eggs in the laboratory conditions. Field biology of the beetle on cowpea showed that it completed its life cycle from egg laying to adult emergence in an average period of 34.5 days compared to 35.5 days under laboratory conditions.

Xu-Weigen and Xu-WG (1999) studied the oviposition activities of C. *maculatus* on *Vigna radiate*, *Vigna angulares*, *Vigna* spp. And *Glycine max* and found that maximum number of eggs were laid on *V radiata* followed by *Vigna* spp., *V angular is* and *G max.*

Anil, et. al. (2001) reported that the orientation, oviposition, development and survival of pulse beetle *C. maculatus* on seeds of 15 cowpea cultivars were studied in the laboratory. The least preferred cultivar for orientation and oviposition was IFC 9907, whice showed an adult orientation level of 3.3 and oviposition rates of 19.3 and 27.0 eggs under free and forced conditions respectively. On the other hand the highest oviposition rates under free (53.2) and forced (53.3) conditions, number of emerged adults (44.1), survival percentage (89.0%), percentage of infested seeds (88.1%), food consumed per grub (27.4mg) and weight loss in seeds (19.1%) were observed in the local cultivar. the number of adults that oriented after 48h was highest (9.7 adults per 50 grains) in IFC 9901. IFC 9901 recorded the highest germination rate of infested seeds (87.9%).

Swaroop, et. al. (2001a) reported that the oviposition and larval development of *C. chinensis* on 13 cultivars of chickpea, viz. GNG 663, PG5, K850, C235, BG267, BG362, BG256, BG1003, RSG2, 1186-1, L550, RSG44 and KGD1168 were studied. PG5 was most resistant with a minimum growth index of 1.358 and longest grub development period of 28.33 days. GNG 663 was most susceptible, with maximum growth index of 2.211 and minimum grub development period of 28 days.

Swaroop, et. al. (2001b) reported that the oviposition and development of the pulse beetle C. *chenensis* on 7 cultivars of lentil (L-4076, DPL-61, K-75, K- 303, Sehore-747, DPL-59 and L-4147) were screened. The maximum growth index (2.172) and fastest development (29days) were observed in L- 4076; K-75 had the minimum growth index (1.022) and slowest development period (33.33days).in other cultivars, growth indices ranged Irom 1.638 (L-4147) to 1.064 (Sheore-747), while the development period ranged from 29.33 (DPL-61. 1.-4147) to 32 (K-303) days.

MATERIALS AND METHODS

3.1.The Experimental plants

The test plants namely *Stephania japonica* L. Details description are given below.

Figure 2. ***Stephania Japonica*** **L.**

3.1.1.Scientific classification

Kingdom: Plantae (unranked): Angiosperms (unranked): Eudicots

Order: Ranunculales

Family: Menispermaceae

Genus: *Stephania*

Species: *S. japonica*

Binomial name: ***Stephania japonica* L.** (Varalakshmi, et.al. 2011)

Synonyms: S. hernandifolia Walp., Menispermum japonicum Thunb.

Bengali/Vernacular Name: Akanadi, Nimuka, Maknadi.

Tribal Name: Tung Nah Way, Thaya Nuya (Marma).

English Name: Tape-vine (Anil, et. al. 2001).

3.1.2. Description of the Plant

A slender wiry climber. Leaves peltate, thinly papyraceous, glabrous on both the surfaces, broadly triangular, ovate-acuminate, 3-12 cm long, apex acutely acuminate or obtuse, base rounded, margins entire. Inflorescence axillary, compound, umbelliform cymes, usually single per axil, 3-6 cm long. Flowers small, male flower greenish-white or yellowish. Drupes light yellow to orange red, obovate, glabrous (Anil, et. al. 2001).

3.1.3. Using Information

Leaves and roots are bitter and astringent; used in fever, diarrhoea, urinary diseases and dyspepsia. Leaves are mounted on abscess and kept for bursting. Leaves are mecerated in a glass of water and are taken after mixing with molasses to cure urethritis. Leaves are also given for gastritis in Khagrachari. Root paste is taken for vertigo and dysentery; root tuber mixed with root juice of Flemingia stricta is taken for asthma; root paste is warmed and rubbed in hydrocele.

Ethanolic extract of the leaf possesses wide range of good antibacterial and antifungal properties (Anil, et. al. 2001)

3.1.4.Chemical constituents

Roots, tubers and leaves contain alkaloids, steroids and fats. Stems contain bis-benzylisoquinoline alkaloids, stephasubine and 3′,4′-dihydro-stephasubine, saponins, steroids and fats. Roots contain the alkaloids, fangchinoline, dl-tetrandrine, d-tetrandrine and d-isochondrodendrine. Aknadinine, epistephanine, hernandifoline and magnoflorine have been isolated from aerial parts. Roots and tubers contain alkaloids - aknadinine, aknadine and aknadicine. A new alkaloid-3-O-dimethylhernandifoline also isolated from the plant (Anil, et. al. 2001).

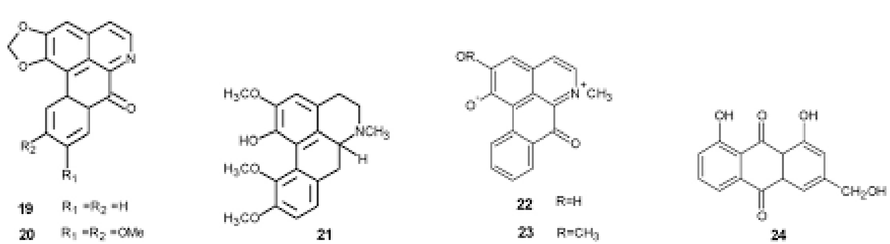

Figure 3 . Chemical constituents of *Stephania japonica* L.

3.1.5. Distribution

Throughout the country.

3.2. The Experimental Insects

3.2.1. *Callosobruchus chinensis* (L.)

The pulse beetle, *Callosobruchus chinensis* (L.) was used as the test insect in the present studies (Figure 3.2). It is a major economic pest, originated in Asia, but is now cosmopolitan in the tropics and subtropics. It is a notorious pest of pulses .(Swaroop, et. al. 2001b)

Figure 4. ***Callosobruchus chinensis*** **(L.)**

3.2.1.1. Scientific Classification

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera

Family: Chrysomelidae

Genus: *Callosobruchus*

Species: *Callosobruchus chinensis* L.

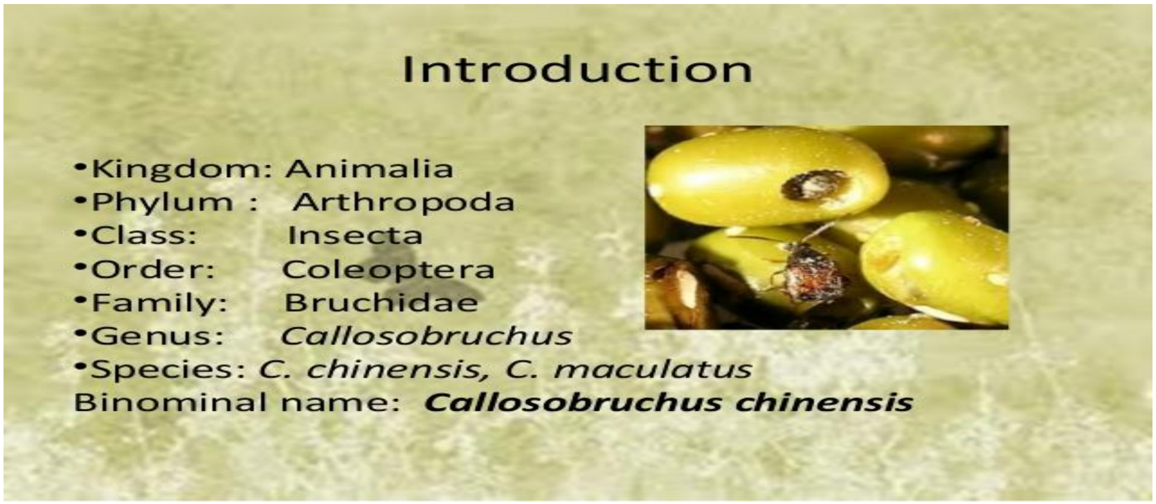

Figure 5. Scientific Classification of *Callosobruchus chinensis* L. (Anil, et. al. 2001)

3.2.1.2. Distribution

C. *chinensis* has been reported from the USA, Mauritius,Formosa, Africa, China, the Philippines, Japan, Srilanka, Myanmar, Bangladesh and India ,that is, throughout the world. In Bangladesh, it is commonly called as the pulse beetle. But in America and Japan, it is known as the cowpea weevil or adzuki bean-seed beetle. (Singal ,1998)

Figure 6. Distribution of C.*chinensis* L.

3.2.1.3. Morphology

Adults small, brownish in colour, 2-3 mm long and rather square in body shape. Antennae pectinate in the male and slightly serrate in the female, the hind femora have a pair of parallel ridges on the ventral edge, each with an apical spine (tooth). The markings on the elytra vary somewhat, but the dark patches can be quite conspicuous. The eyes characteristically emerginate, the elytra do not quite cover the tip of the abdomen. Adults can fly quite well (usually up to one kilometer), but they do not feed on stored products and thus short-lived up to 12 days usually. The following are identifying characteristics of the male and female pulse beetle. (Shiau, et. al. 1994)

Figure 7. Morphology of *Callosobruchus chinensis* L.

Characters	Male	Female
Antenna	Pectinate, Curved towards each other. Apical segment of antenna is elongate and oblong in shape. Pectination becomes prominent from the 4^{th} to the apical segment.	Serrate, straight. Apical segment is somewhat round or ovate. Serration becomes prominent from the 5^{th} to the apical segment.

Table 1 : Characteristics difference of *Callosobruchus chinensis* L. between male & female

(Shiau, et. al. 1994)

3.2.1.4. Biology

Eggs are laid on to the developing pod in the field, or on to the surface of seeds in pods, or on to seeds in store. Upto 100 eggs are laid per female, glued firmly to the seed surface and incubation takes 5-6 days. The larvae after hatching bite through the base of the egg, directly through the testa and into the cotyledons. The larva is scarabaeiform and the final instars develop in about 20 days, the whole time being spent within one seed. Pupation takes place inside the seed in a chamber covered by a thin window ot testa materials, and requires about 7 days to come out as adult. It takes 36 days for the completion of life cycle and 6-7 generations per year are usual. (Parajulee. et. al. 1989)

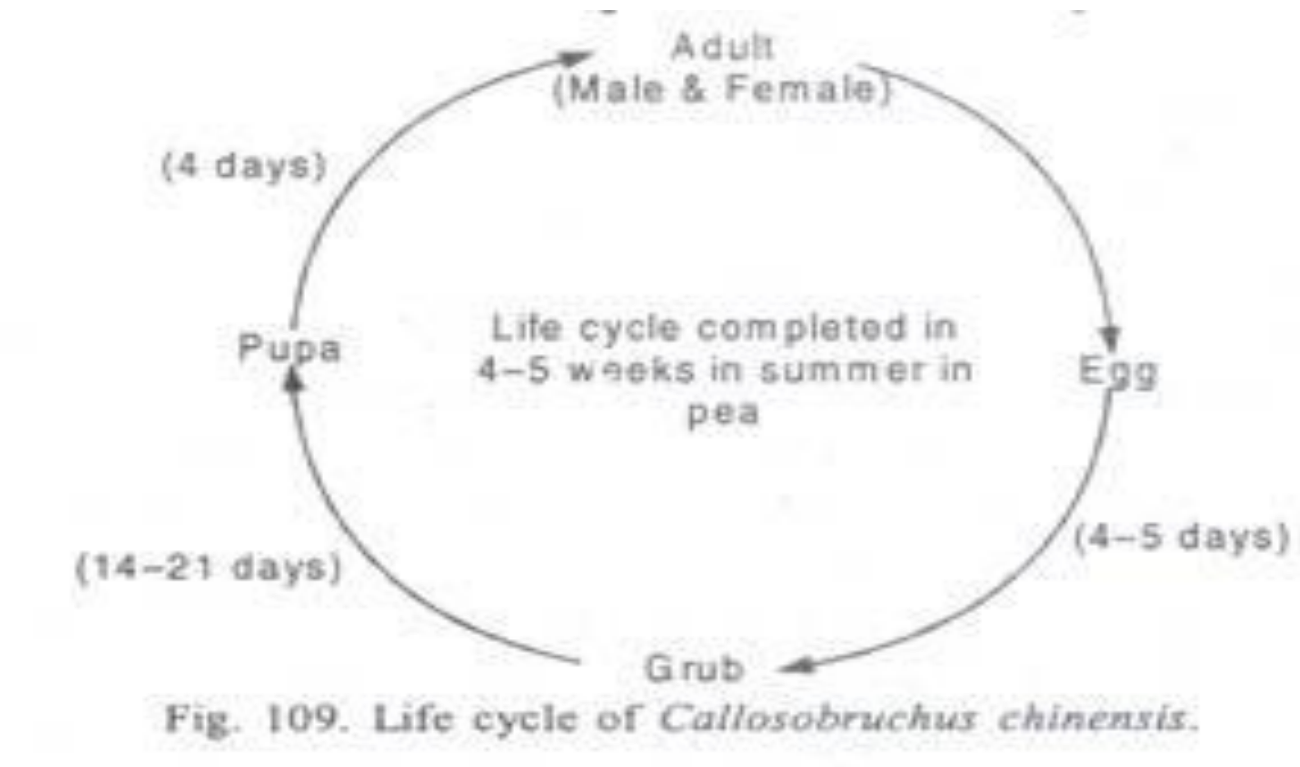

Figure 8 . Life cycle of *Callosobruchus chinensis* L. (Parajulee. et. al. 1989)

3.2.1.5. Ecology

Optimum conditions for development are about 32°C and 90% relative humidity. The life cycle can be completed in 21-23 days in this condition. (Singh, et. al. 1980)

3.2.1.6. Nature of Damage

Larvae bore into the cotyledons and eventually hollow out the seed within the testa, typically 1-3 larvae bore per seed. Infestations start in the field and eggs are laid on the surface of maturing pods, later eggs are laid on the seed surface. They attack all pulses in topical regions like chickpea, *Cicer arietinum*; lentil, *Lens culinaris;* mungbean, *Phaseolus vulgaris* ; green gram, *Vigna radiata*\ adzuki bean, *V. angularis* and cowpea, *V. unguiculate* as well as cotton seed, sorghum and maize.Due to the infestation of pulse beetle, grains become unsuitable for human consumption and loose their viability to germinate, and thus become unfit for sowing in the field. (Girish, et. al. 1974)

3.2.1.7. Insect Culture Maintenance

A stock culture of the insects was maintained in the laboratory at 27-30°C temperature and 70-75% relative humidity. Fifty pairs of adult pulse beetles (about 1-3 days old) were placed in plastic jar containing the rearing material. The jar was then sealed and was allowed for free mating and oviposition for a maximum period of 7 days. The parent stocks were removed and the grain or pulses containing eggs were transferred to preconditioned food material in the breeding jar. The jars were covered with pieces of cloth, fastened with rubber bands to prevent the contamination and escape of insects. Rearing of these insects was being continued for experimental purpose (Talukder and Howse, 1990).

Figure 9. Insect Culture Maintenance

3.2.2. *Sitophyllus oryzae* L.

The present study was conducted on rice weevil, *S. oryzae* L. which is a major stored rice grain pest.

Figure 10 . *Sitophyllus oryzae* L

3.2.2.1. Scientific Classification

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Family: Curculionidae
Subfamily: Dryophthorinae
Genus: *Sitophyllus*
Species: S.*oryzae* L.

Figure 11 . Scientific Classification of *Sitophyllus oryzae* L. (Arnason, et. al. 1989)

3.2.2.2. Distribution

S. oryzae prefers temperate or subtropical climate and is a serious pest of stored grain rice in Bangladesh, India, Pakistan, Srilanka, Bhutan, Nepal and many other countries of the world. (Arnason, et. al. 1989)

3.2.2.3. Morphology of S.*oryzae* L.

The rice weevil, *S. oryzae* L. is somewhat black or reddish brown and measures 3-4 mm in length. Its body is elongate and the head is produced into a well defined snout. The snout of the male is shorter and broader than that of the female. There are two light pale spots on each elytron. The antennae are elbow shaped and the larvae are legless. (Arnason, et. al. 1989)

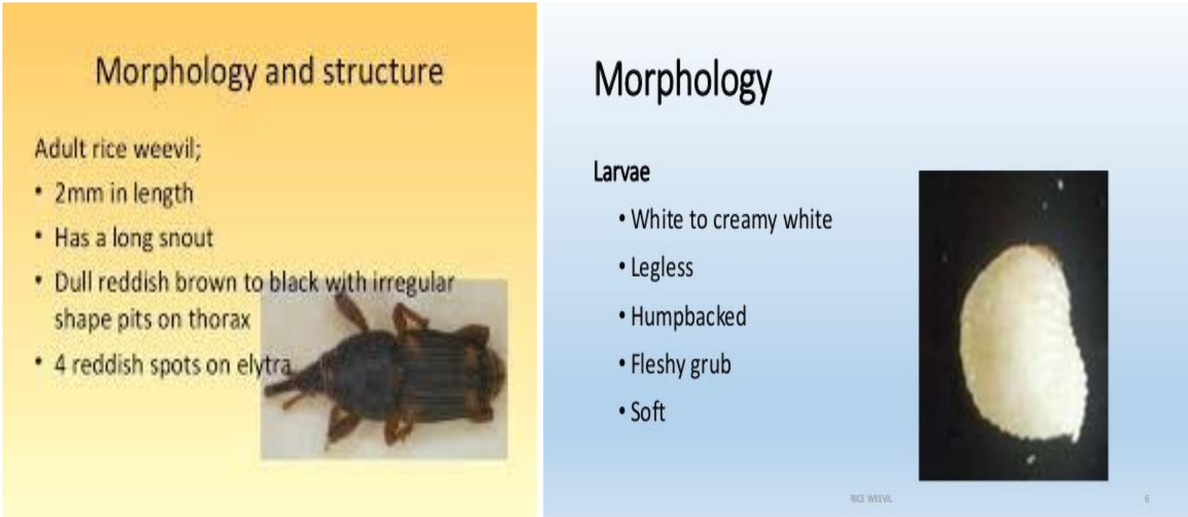

Figure 12 . Morphology of S.*oryzae* L. (Arnason, et. al. 1989)

3.2.2.4. Food habit

Rice weevil is one of the most destructive pests of stored grains. It infests rice (both husked and unhusked), maize and wheat. In fact, it is the most common and serious pest of rice in godown and stores. (Dhaliwal et. al. *1998)*

3.2.2.5. Life cycle

A single female may lay more than 250 eggs. The egg is translucent in color and oval and measures 0.7 mm long, 0.3 mm broad. It hatches in about 4days during summer but in 6-9 days during winter. The grub is white, with small yellowish brown head. They live within the grain and feed on its starchy content. The larval stage lasts about 22 to 33 days. A full grown grub measures about 3mm long and makes a pupal cell inside the grain and pupates after passing 1 or 2 days as a prepupa and the pupa is of exarate type. The pupal stage lasts about 3-7 days depending on the prevailing temperature. The duration of adult life is on an average one and a half month in Bangladesh. There are about 5-7 generations in a year depending on the prevailing temperature. (Dhaliwal et. al. *1998)*

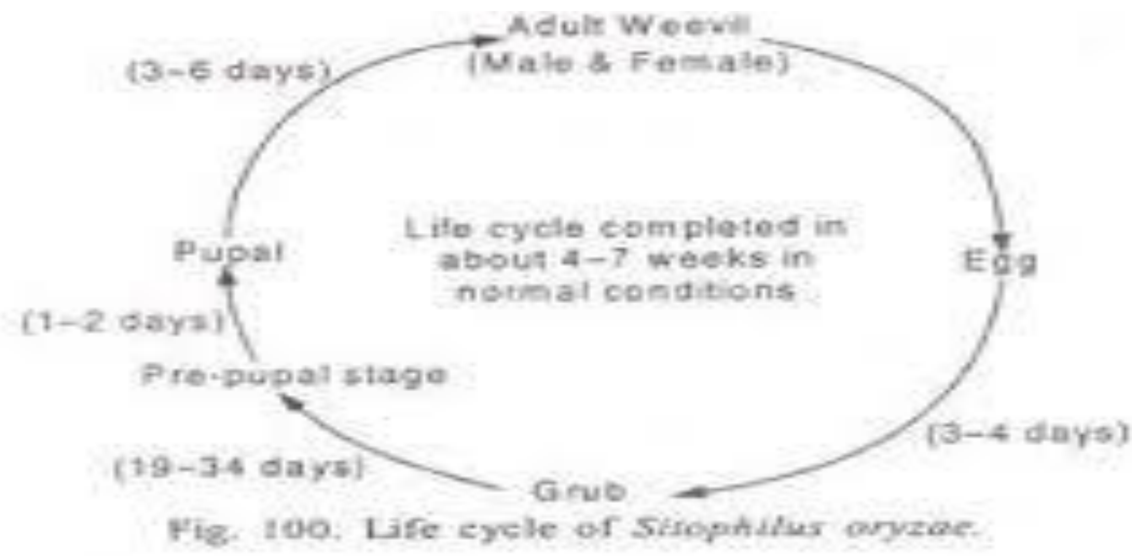

Figure 13 . Life cycle of S.*oryzae* L. (Dhaliwal et. al. *1998)*

3.2.2.6. Nature of damage

Heavy damage may be caused by this pest to rice, wheat, maize and sorghum grains particularly in the monsoons. Both adults and grubs feed the grain, but the apodous larvae is responsible for most of the damage. Wheat losses may be up to 50% of its weight by the time the adult emerges. Besides, the quality and the germinating capacity of the seed is also lowered in severely infested grains (Modgil and Samuels 1998).

3.2.2.7. Collection and rearing of S. *oryzae* L.

Rearing of the weevil was necessary to ensure continuous supply of the test insects during the study. The test insects were collected from Karwan bazaar. The collected adult weevils were separated from the infested grain and transferred to fresh rearing materials for multiplication. Rice grains were kept in an oven at 55°c temperature for 24hr to get insect free rice grain. The collected adult rice weevil were placed in large plastic jar containing rice grain covered by cheese-cloths fastened with rubber bands to prevent contamination and insect escape. The weevils were allowed for free mating and oviposition for a period of 7 days. After 7 days, the released weevils were removed by sieving. The egg containing rice grains were kept in a plastic jar for emergence of the next generation weevils. The newly emerged weevils were separated immediately by sieving and transferred into another jar with fresh rice grains. This process was repeated regularly to get sufficient number of adults for conducting the whole experiment (Talukder and Howse, 1990, 1993, 1994a, 1994b, 1995).

3.2.3. Mosquito

Mosquitoes are small, midge-like flies which comprise the family Culicidae. Females of most species are ectoparasites, whose tube-like mouthparts pierce the hosts' skin to consume blood. The word "mosquito" is Spanish for "little fly" (Rahman 1998)

3.2.3.1. Background about mosquito

Scientific name: Culicidae

Higher classification: Culicoidea

Clutch size: 100 – 200

Rank: Family

Lifespan: Female: 42 – 56 days (Adult), Male: 10 days (Adult) (Rahman 1998)

3.2.3.2. Life cycle of mosquito

Mosquito Egg Raft

Many mosquitoes, such as *Culex quinquefasciatus,* lay their eggs on the surface of fresh or stagnant water. The water may be in tin cans, barrels, horse troughs, ornamental ponds, swimming pools, puddles, creeks, ditches, catch basins or marshy areas. Mosquitoes prefer water sheltered from the wind by grass and weeds (Thomas and Callaghan 1999).

Culex mosquitoes usually lay their eggs at night over a period of time sticking them together to form a raft of from 100 to 300 eggs. A raft of eggs looks like a speck of soot floating on the water and is about 1/4 inch long and 1/8 inch wide. A female mosquito may lay a raft of eggs every third night during its life span (Thomas and Callaghan 1999).

Figure 14 . Mosquito Egg Raft

Anopheles and many other mosquitoes lay their eggs singly on the water surface. *Aedes* and *Ochlerotatus* mosquitoes lay their eggs singly, usually on damp soil. *Aedes* and *Ochlerotatus* eggs are more resistant to drying out (some require complete drying out before the eggs will hatch) and hatch only when flooded with water (salt water high tides, irrigated pastures, treeholes flooded by rains, flooded stream bottoms). *Anopheles, Culex* and *Mansonia* eggs are susceptible to drying out during extended droughts. Tiny mosquito larvae (1st instar) emerge from the eggs within 24 - 48 hours almost in unison (Thomas and Callaghan 1999).

Mosquito Larva

Mosquito larvae, commonly called "wigglers," live in water from 4 to 14 days depending on water temperature (Thomas and Callaghan 1999).

Larvae of almost all species must come to the surface at frequent intervals to obtain oxygen through a breathing tube called a siphon. Larvae of *Coquillettidia* and *Mansonia* possess modified siphons that allow them to pierce the stems of emergent vegetation in water and draw their oxygen from the plant in this process (Thomas and Callaghan 1999).

Figure 15 . Mosquito Larva

Larvae are constantly feeding since maturation requires a huge amount of energy and food. They hang with their heads down and the brushes by their mouths filtering anything small enough to be eaten toward their mouths to nourish the growing larvae. They feed on algae, plankton, fungi and bacteria and other microorganisms. They breath at the water surface with the breathing tube up breaking the water surface tension. The larvae of a few mosquito species are cannibalistic, feeding on larvae of other mosquitoes: *Toxorhynchites* and some *Psorophora*, the largest mosquitoes known, are predators of other mosquito larvae sharing their habitat. Their larvae are much larger than other mosquito larvae (Thomas and Callaghan 1999).

During growth, the larva molts (sheds its skin) four times. The stages between molts are called instars. At the 4th instar, the usual larva reaches a length of almost 1/2 inch and toward the end of this instar ceases feeding. When the 4th instar larvae molts, it becomes a pupa (Thomas and Callaghan 1999).

Mosquito Pupa

Mosquito pupa, commonly called "tumblers," live in water from 1 to 4 days, depending upon species and temperature (Thomas and Callaghan 1999).

Figure 16 . Mosquito Pupa

The pupa is lighter than water and therefore floats at the surface. It takes oxygen through two breathing tubes called "trumpets." The pupa does not eat, but it is not an inactive stage. When disturbed, it dives in a jerking, tumbling motion toward protection and then floats back to the surface (Thomas and Callaghan 1999).

The metamorphosis of the mosquito into an adult is completed within the pupal case. The pupal case thus serves as a factory wherein the mosquito makes an adult out of a larva. The adult mosquito splits the pupal case and emerges to the surface of the water where it rests until its body dries and hardens (Thomas and Callaghan 1999).

Mosquito Adult

Only female mosquitoes require a blood meal and bite animals - warm or cold blooded - and birds. Stimuli that influence biting (blood feeding) include a combination of carbon dioxide, temperature, moisture, smell, color and movement. Male mosquitoes do not bite, but feed on the nectar of flowers or other suitable sugar source. Acquiring a blood meal (protein) is essential for egg production, but mostly both male and female mosquitoes are nectar feeders for their nutrition. Female *Toxorhynchites* actually can't obtain a bloodmeal and are restricted to a nectar diet. Of those female mosquitoes capable of blood feeding, human blood meals are seldom first or second choices. Horses, cattle, smaller mammals and/or birds are preferred (Thomas and Callaghan 1999)

Figure 17 . Mosquito Adult

Aedes and *Ochlerotatus* mosquitoes are painful and persistent biters. They search for a blood meal early in the morning, at dusk (crepuscular feeders) and into the evening. Some are diurnal (daytime biters) especially on cloudy days and in shaded areas. They usually do not enter dwellings, and they prefer to bite mammals like humans. *Aedes* and *Ochlerotatus* mosquitoes are strong fliers and are known to fly many miles from their larval developments sites (Chander, et. al.1999).

Figure 18 . Life cycle of mosquito (Oparaeke, et. al. 2000)

3.2.3.3. Mosquito culture

Mosquito eggs were obtained from human house to start the colony, and larvae were reared in plastic and enamel trays containing tap water. They were maintained, and all the experiments were carried out at 27±2°C and 75–85% relative humidity under a 14:10 light-and-dark cycles. Larvae were fed a diet of Brewers yeast and dog biscuits and algae were collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary (45×45×40 cm) where adults emerged. Adults were maintained in net cages and were continuously provided with 10% sucrose solution in a jar with a cotton wick. On day 5, the adults were given a blood meal from a pigeon placed in resting cages overnight for blood feeding by females (Oparaeke, et. al. 2000)

3.3. Plant Preparation

3.3.1. Collection of plant

The plant was collected from Foridpur district of Bangladesh. A voucher specimen (Accession number: 38479) had been deposited at the Bangladesh National Herbarium. The proper time of harvesting or collecting is particularly important because the nature and the quantity of constituents very gently in some species according to the season.

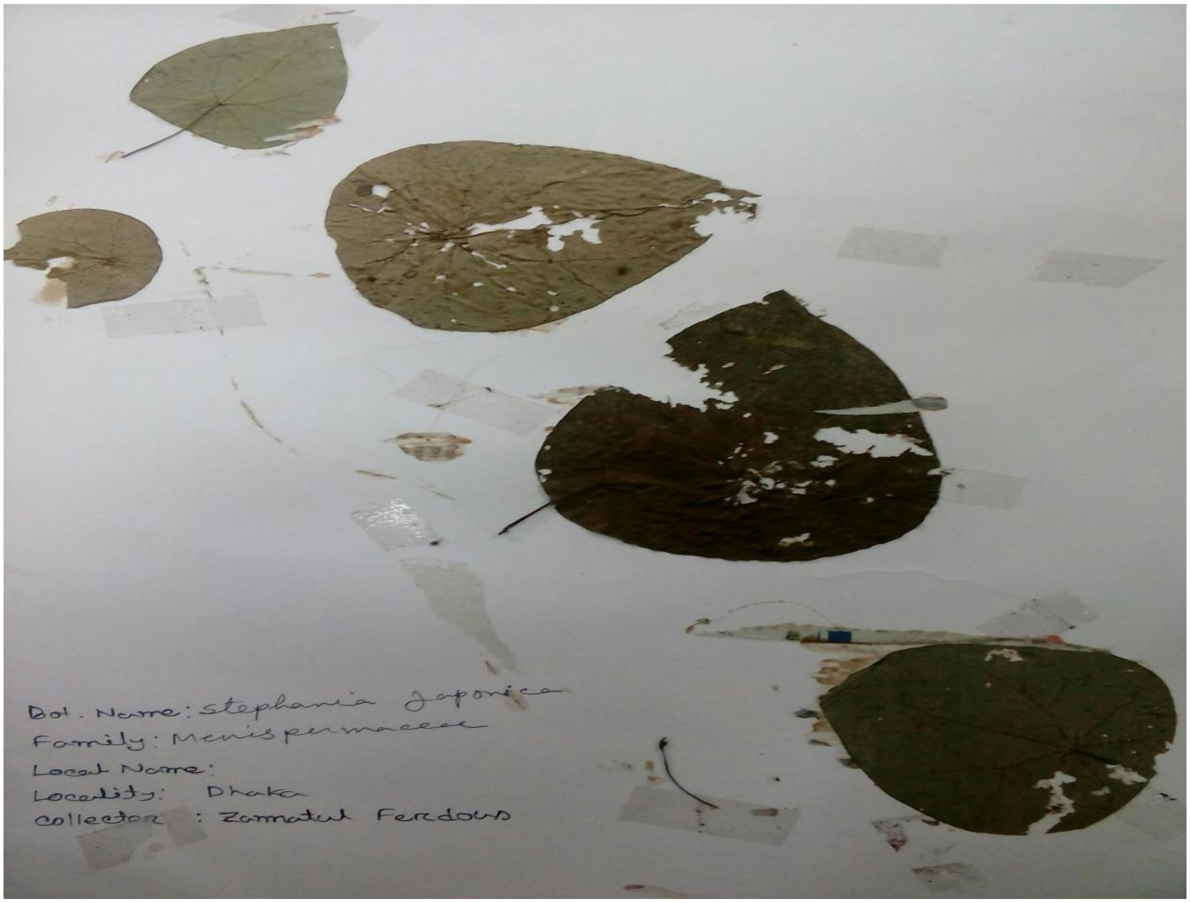

Figure 19 . Herbarium sheet of *Stephania japonica* L.

3.3.2. Drying

Drying is the most common and fundamental method for post- harvest preservation of medicinal plants because it allows for the quick conservation of the medicinal qualities of the plants material in an uncomplicated manner. The plant was dried in room temperature and drying was completed within 2 to 3 weeks. By drying the plant materials one removes sufficient moisture to ensure good keeping qualities and to prevent molding the action of enzyme, the action of bacteria and chemical or other possible changes. Proper and successful drying involved two main principles: control of temperature and regulation of air flow. The plant material can be dried by room temperature.

3.3.3. Cutting, Grinding and Sieving

The stem was cut into small pieces and placed in the grinding machine to make it fine powder. As the stem is too hard, very small amount of pieces were placed in the hopper with an average rotation. The grinded powder was collected very carefully.

3.3.4. Blending

Finally the powder was placed in blender machine in order to get more fine powder. During the entire blending process precautions were taken to avoid all kind of cross contamination and product loss.

3.3.5. Preparation of plant extraction

The whole part of the plant was dried in room temperature for approximately two weeks. Then the dried plants were taken into fine powder by using a grinding machine. Then the extraction process was done. At first 2kg dried plant dust of *Stephania japonica* was soaked in methanol in four bottles. Then it was kept in room temperature for 3 days and everyday it was used to shake properly to ensure the maximum amount of constituents present in the grinded plant become soluble into methanol. After 3 days later, the mixture was filtered. For filtration, white cotton cloth was used. After filtration two parts were obtained.

1. The residue portion over the filter

2. The filtered part

The filtrated part, which contains the substance soluble in methanol, poured into a 1000 round bottle flask, and then the flask was placed in a rotary evaporator. The evaporation was done at 53 degree Celsius temperature. The number of rotation per minute was selected as 125 RPM. The pressure of vacuum pump machine was 6 bars. The water flow through the distillation chamber was also provided in a satisfactory flow rate.

3.3.6. Crystal formation

After completing rotary crystal formation was occurred that was good in amount. These crystals are clear and stable. These crystals are not soluble in polar and not polar solvent and intermediate solvent. Further investigation will be continued to know about these crystals.

Figure 20 . Formation of crystals from crude extract

3.3.7. Liquid-liquid Extraction

The crude extract was subjected to liquid-liquid extraction using three solvent systems, N-hexane, Dichloromethane (DCM) and water system. At first 55 gm of crude extract was mixed with distilled water and methanol. Then added N-hexane to the mixer and mixed properly. Then it poured into a separating funnel. After some time two layers was separated.

- ✓ bottom layer was N-hexane layer
- ✓ Upper layer was Water layer

Then we collected the bottom layer slowly and subjected to evaporation to get N-hexane extract of *Stephania japonica.*

In the upper water layer some distilled water and Dichloromethane (DCM) was added and mixed properly. Then it poured into a separating funnel. After some time two layers was separated.

- ✓ Bottom layer was Water layer
- ✓ Upper layer was Dichloromethane layer

Then we collected the Upper layer slowly and subjected to evaporation to get Dichloromethane extract of *Stephania japonica*.

3.4. Materials

3.4.1. Materials for Insecticidal Test:

-Syringe

-Plactic Jar

-Mosquito net

- Microcapilary tube

3.4.2. Instruments for Insecticidal Test:

-Incubator

-Analytical balance

3.4.3. Reagent for Insecticidal Test:

-Plant extract

-Aceton

-Methanol

-Ethanol

-Distilled water

3.4.4. Preparation of dose

1 mg,5 mg and 10 mg solutions of plant extracts were prepared by dissolving the extract in the acetone prior to insect bioassay.

3.5.Methods

The tests which are conducted in this experiment are

- Screening or direct contact test
- Surface contact by filter paper
- Repellent test
- Grain soak test
- Larvicidal test of mosquito

3.5.1. Screening or direct contact test

The Screening or direct toxicity test was conducted according to the method described by Talukder and Howse (1993). Insects were chilled for a period of 10 minutes. Then the immobilized insects were individually picked up by using a small suction tube. 1ml. solution of different concentrations (1 mg,5 mg,10 mg) were applied to the dorsal surface of the thorax of each insect using a microcapilary tube. 20 insects per replication were treated and each treatment was replicated 3 times. In the control, same number of insects were treated with solvent only. After treatment, the insects were transferred into 9 cm. Diameter petri-dishes (10 insects/petri-dish) containing food.

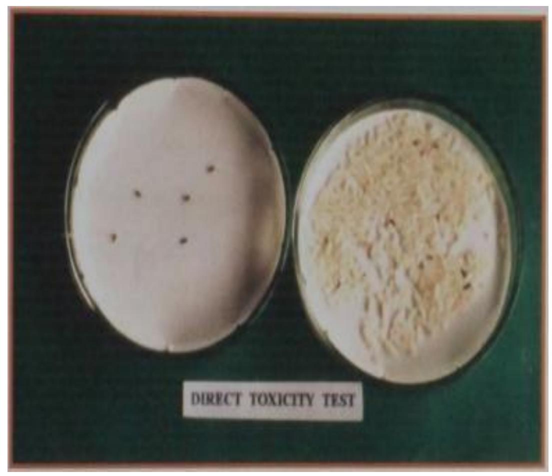

Figure 21 . Screening or direct contact test

3.5.2.Surface contact by filter paper

Surface contact by filter paper test was conducted according to the method of Talukder and Howse (1994a). By the help of a pipette 1 ml solution of each dose was applied to the filter paper The treated filter paper was then air dried.Each filter paper was then placed in a petridish and 60 insects were placed there. 1 mg of grain was placed on the petridish. Control test was also conducted. There were 3 replications for each concentration of plant extracts. Number of dead insects on each petridish were counted for 6 days.


Figure 22 . Surface contact by filter paper

3.5.3. Repellent test:

Repellency test was conducted according to the method of Talukder and Howse (1994a). 9 cm diameter filter papers (Whatman No. 40) were marked into two portion. By the help of a pipette 1 ml solution of each dose was applied to one half of the filter paper The treated half disks was then air dried. Each filter paper was then placed in a petridish and 20 insects were placed there. 1 mg of pulse was placed on the petridish. There were 3 replications for each concentration of plant extracts. Number of insects on each portion were counted at hourly intervals up to the 6^{th} hour.

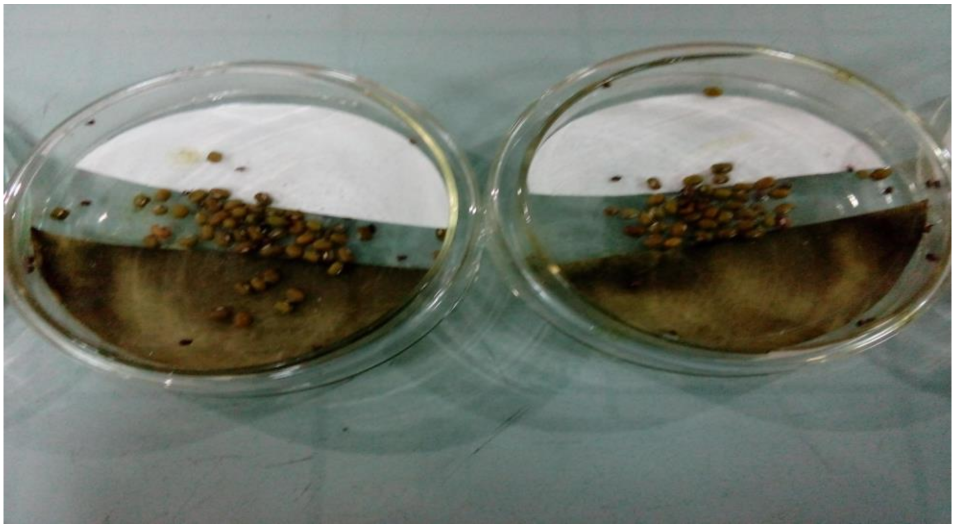

Figure 23 . Repellent test

3.5.4. Grain soak test

In this method,we took grain and soak it in each 1 ml of three concentrations(1 mg,5 mg,10 mg). After air drying,we took 1 mg of soak grain in each petridish. 20/60 insects were placed in each petridish. Control test was also conducted. There were 3 replications for each concentration of plant extracts. Number of dead insects on each petridish were counted for 4 days (for pulse beetle) and 30 days (for rice weevil) **(**Talukder and Howse 1994a).

Figure 24 . Grain soak test

3.5.5. Larvicidal test of mosquito

In this test, we used 95 ml distilled water,1 ml nutrients,3 ml mosquito liveable water and 1 ml dose (dose is prepared with 100 mg extract,1 ml ethanol ,2 ml aceton and 7 ml water & control dose is prepared with 1 ml ethanol, 2 ml aceton,7 ml water). Three concentrations (1 mg,5 mg,10 mg) dose was given.20 mosquito larvae was placed in each cup. Control test was also conducted. There were 3 replications for each concentration of plant extracts. Number of dead larvae on each cup were counted for 5 days (Sangwan, et. al. 2005).

Figure 25 . Larvicidal test of mosquito

RESULT AND DISCUSSION

The results of various experiments conducted on the effects of *Stephenia japonica* against the pulse bettle, *Callosobruchus chinensis* (L.),rice weevil, sitophyllus aryaze L. and mosquito larve at different concentrations are presented and discussed in this chapter.

4.1.Screening test of *Callosobruchus chinensis* L.

	Control	Dose 1 mg	Dose 5 mg	Dose 10 mg
Death rate/20	0	4	12	18

Table 2: Screening test of *Callosobruchus chinensis* L.

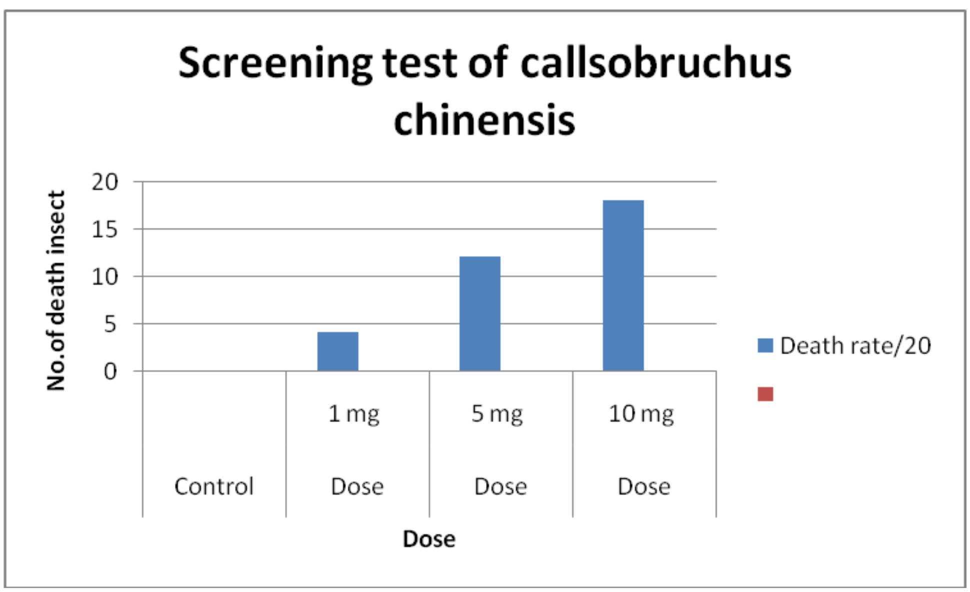

Figure 26 .Graphical representation of Screening test of *Callosobruchus chinensis* L.

In Screening test of *Callosobruchus chinensis*,we can see that when the concentration of dose increases,the rate of death insect also increases. At dose 5 mg,it is achieved 50% death of test insect.

4.2.Screening test of *Sitophyllus oryzae* L.

	Control	Dose 1 mg	Dose 5 mg	Dose 10 mg
Death Rate/20	0	0	2	6

Table 3: Screening test of *Sitophyllus oryzae* L.

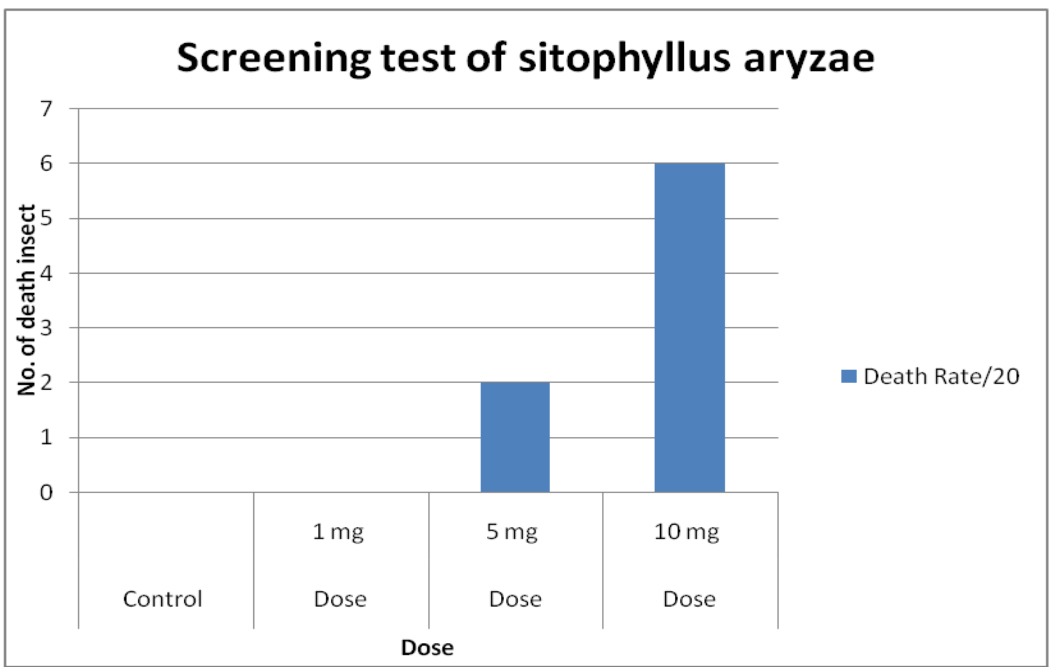

Figure 27 . Graphical presentation of Screening test of *Sitophyllus oryzae* L.

In Screening test of *Sitophyllus oryzae* L. We can see that when the concentration of dose increases,the rate of death insect also increases but the dose does not achieve 50% death of test insects.So *Stephania japonica* L. is not effective against Sitophyllus oryzae L.

4.3. Surface contact by filter paper of *Callosobruchus chinensis* L.

Day	Control	Dose 1 mg	Dose 5 mg	Dose 10 mg
01 Death rate/60	1	15	18	29
02 Death rate/60	2	22	25	36
03 Death rate/60	2	26	36	40
04 Death rate/60	3	32	40	44
05 Death rate/60	7	41	45	48
06 Death rate/60	10	44	49	50

Table 4: Surface contact by filter paper of *Callosobruchus chinensis* L.

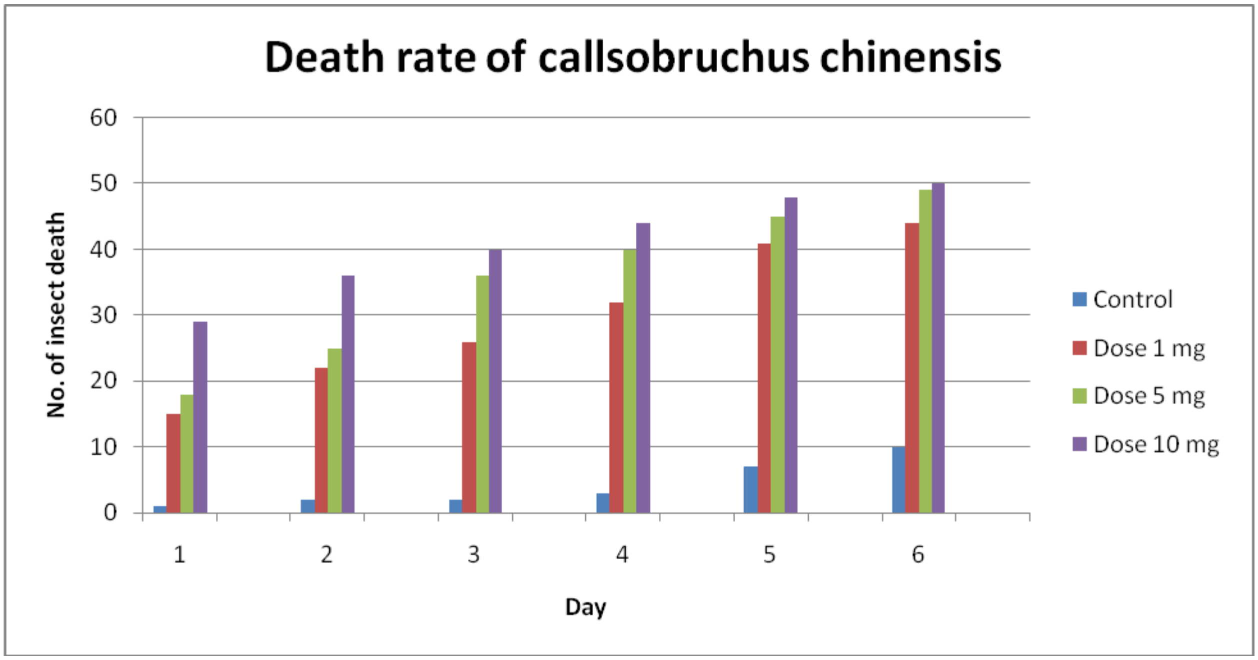

Figure 28 . Graphical presentation of Surface contact by filter paper of *Callosobruchus chinensis* L.

In surface contact by filter paper test of *Callosobruchus chinensis* L.,we can see that when the concentration of dose increases, the rate of death insect also increases. we also can see that in second day of obsevation,dose 10 mg achieve 50% death of test insect.

4.4.Repellent Test of *Callosobruchus chinensis* L.

Hour	Control	Dose 1 mg	Dose 5 mg	Dose 10 mg
01	19	11	7	4
02	16	13	10	3
03	19	15	11	4
04	16	14	14	6
05	15	15	10	3
06	14	9	7	2

Table 5. Repellent Test of *Callosobruchus chinensis* L.

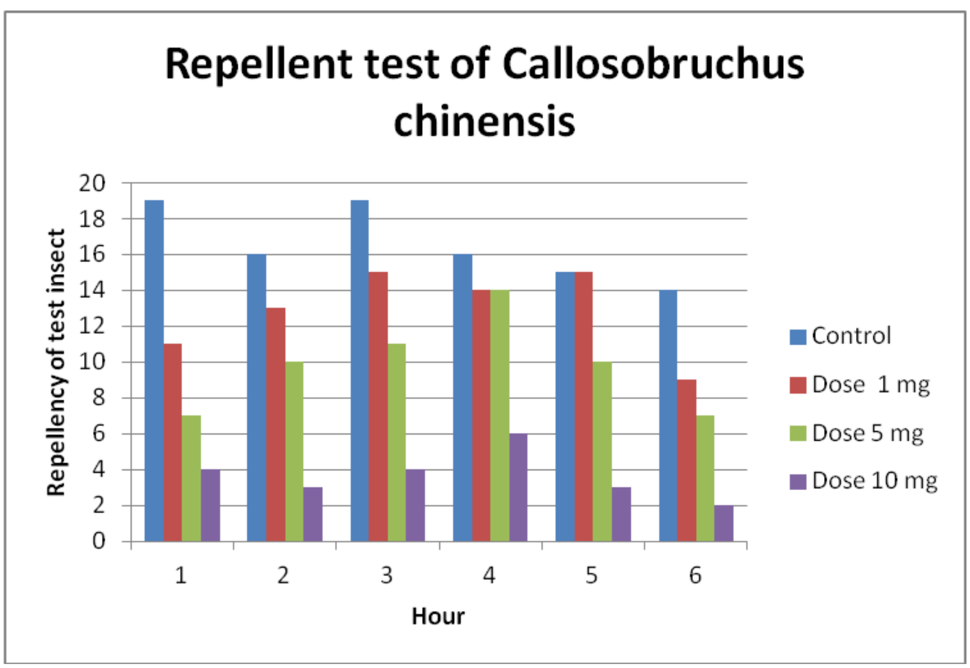

Figure 29 . Graphical representation of Repellent Test of *Callosobruchus chinensis* L.

In repellent test of *Callosobruchus chinensis* L.,we can see that when the concentration of dose increases, the repellency of insect also decreases.So we can say that the plant *Stephania japonica* L. has toxic effect on *Callosobruchus chinensis* L.

4.5. Grain soak test of *Callosobruchus chinensis* L.

Day	Control	Dose 1 mg	Dose 5 mg	Dose 10 mg
01	5	15	23	29
02	6	16	28	35
03	11	20	33	40
04	13	55	58	60

Table 6 . Grain soak test of *Callosobruchus chinensis* L.

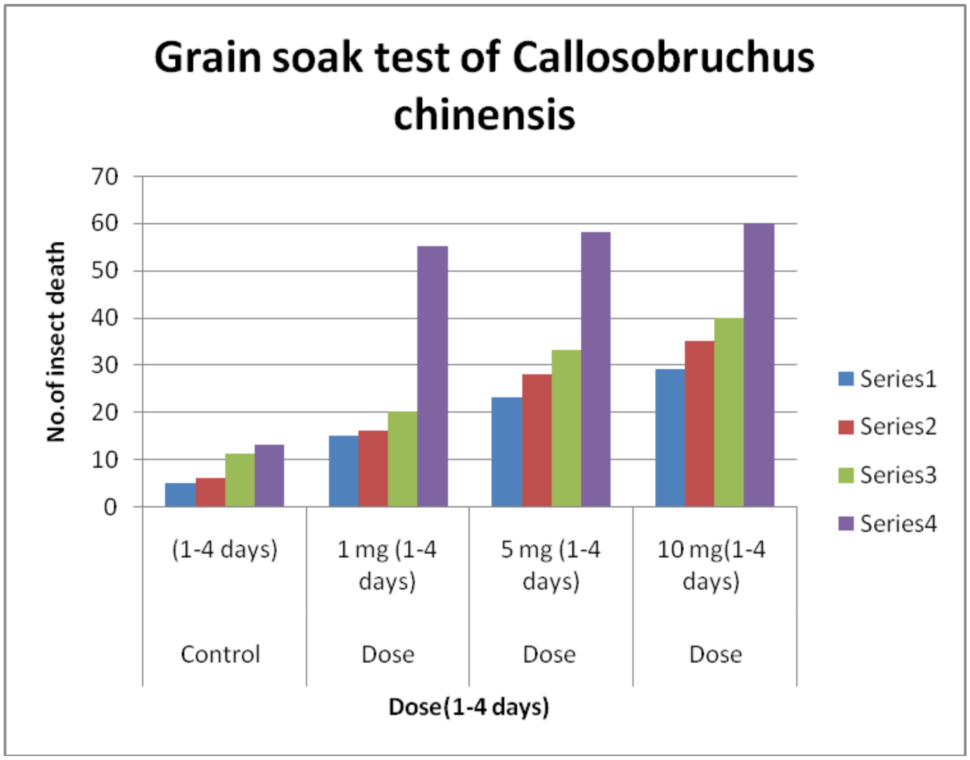

Figure 30 . Graphical representation of Grain soak test of *Callosobruchus chinensis* L.

In grain soak test of *Callosobruchus chinensis* L.,we can see that when the concentration of dose increases,the rate of death insect also increases.we also can see that in third day of obsevation,dose 5 mg achieve 50% death of test insect.

4.6. Grain soak test of *Sitophyllus oryzae* L.

Day	Control	Dose 1 mg	Dose 5 mg	Dose 10 mg
01	0	0	0	0
02	0	0	0	0
03	0	0	0	0
04	0	0	0	0
05	0	0	0	1

06	0	0	0	1
07	0	0	0	1
08	0	0	1	1
09	0	0	1	2
10	0	0	1	2
11	0	0	1	2
12	0	0	1	2
13	0	1	1	2
14	1	1	1	3
15	1	1	2	3
16	1	1	2	3
17	1	1	2	4
18	1	2	2	4
19	1	2	4	4
20	1	2	4	6
21	1	2	4	6
22	1	2	4	6
23	1	3	6	7
24	2	3	6	7
25	2	3	6	8
26	2	3	6	8
27	2	5	8	10
28	2	6	9	10

29	3	8	11	13
30	3	8	12	14

Table 7. Grain soak test of *Sitophyllus oryzae* L.

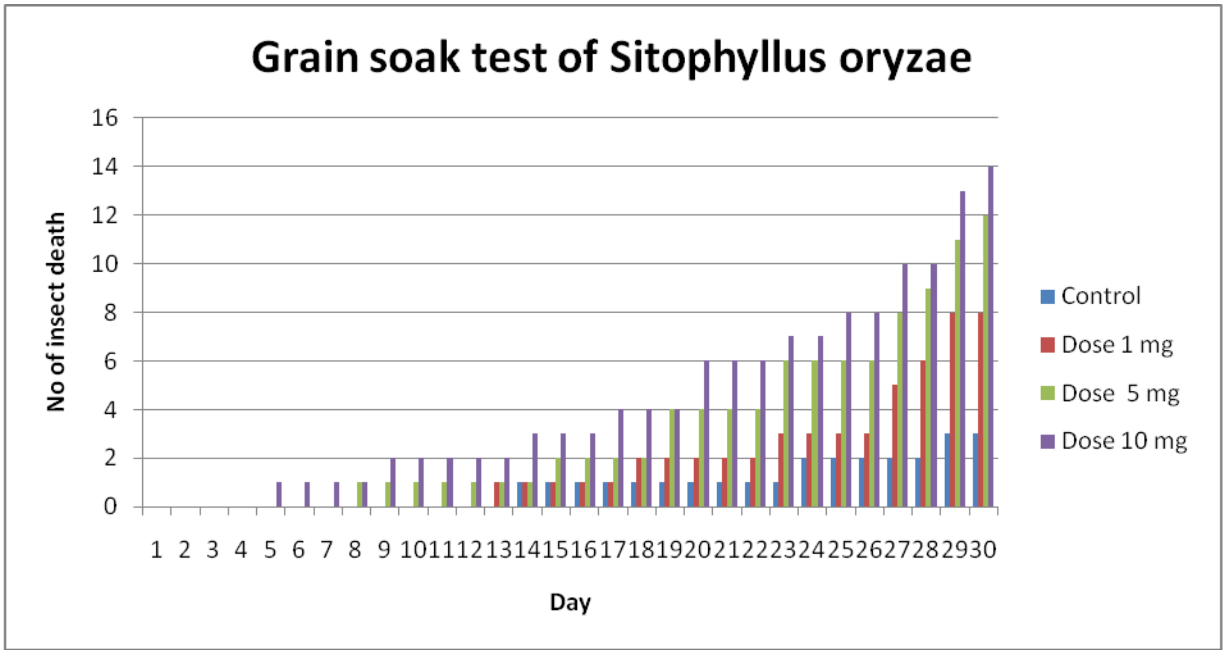

Figure 31 . Graphical representation of Grain soak test of *Sitophyllus oryzae* L.

In grain soak test of *Sitophyllus oryzae* L.,we obseved the experiment for 30 days.In day 27, we can see that dose 10 mg achieve 50% death of test insect.

4.7. Larvicidal effect of *Stephania japonica* L.

Day	Control	Dose 1 mg	Dose 5 mg	Dose 10 mg
01	0	2	3	6
02	1	3	4	6
03	3	5	8	11
04	4	7	8	17
05	8	11	13	19

Table 8. Larvicidal effect of *Stephania japonica* L.

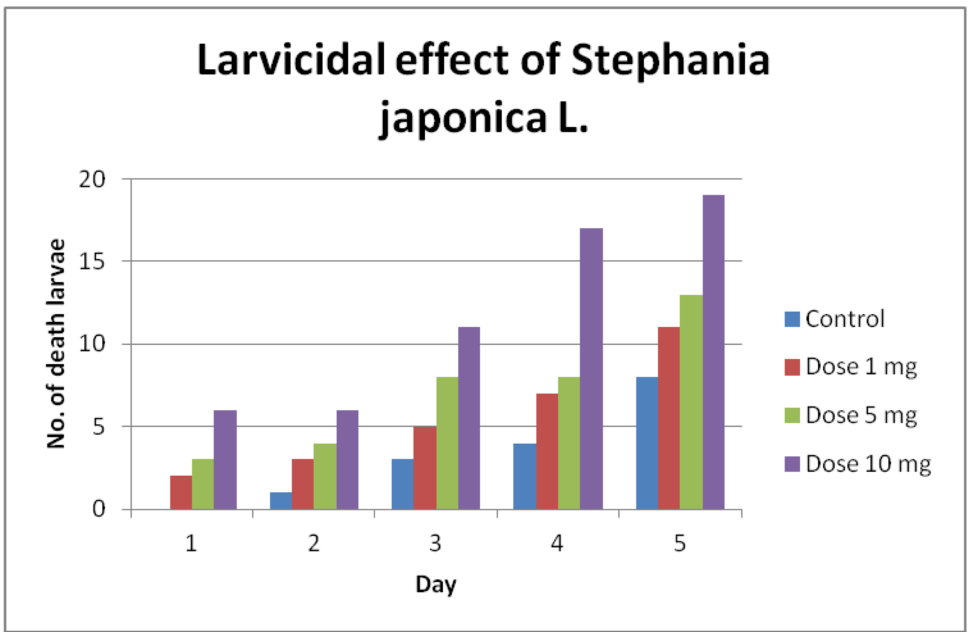

Figure 32. Graphical representation of larvicidal effect of Stephania japonica L.

In larvicidal test of *Stephania japonica* L.,we can observe that when the dose increases,the rate of death larvae also increases.We can see that in day 3, dose 10 mg achieve 50% death of test larvae.

SUMMARY AND CONCLUSION

Laboratory experiments were carried out to determine the surface contact action, screening(direct contact effect),grain soak effect and repellent effect of *Stephenia japonica* against the pulse beetle, rice weevil and mosquito larve. Experimental results revealed that plant material was effective against the pulse beetle, *Callosobruchus chinensis* (L.),rice weevil, *Sitophyllus oryzae* (L.) and mosquito larvae.

It was observed from the results that the plant extract had significant repellent and screening effect, surface contact effect, and grain soak effect. Mortality percentage was found to vary among different concentrations of plant extracts. The highest mortality was observed with highest concentrations of *Stephenia japonica* L.

The extracts of *Stephenia japonica* L. had good grain soak effect in rice weevil rather than other procedure. The highest repellency was observed in pulse beetle. The results also indicated that repellent effect increased proportionally with the increase of concentrations of plant extract.

In this experiment, indigenous plant was evaluated against pulse beetle,rice weevil and mosquito larvae. The results showed that Stephenia japonica is greatly effective against the pulse beetle, *Callosobruchus chinensis* (L.),rice weevil,*Sitophyllus oryzae* (L.) and mosquito larvae.

Pesticides are essential input in agriculture. Use of indigenous plants as insecticides will benefit our agricultural sector, as these substances are not only cheaper, but also environmentally friendly and do not leave any hazardous residues on the environment and food. Therefore, the application of plant products in our country will be highly effective against stored product insects. Finally, these initial efficacy tests of this present experiment will be helpful to identify the potential of botanical pesticides for controlling stored grain pests. These plant products will reduce our dependency on dangerous synthetic insecticides and will act as one of the effective tools for integrated pest management.

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