EFFECT OF SOME PLANT EXTRACTS ON EGG LAYING, INCUBATION AND ADULT EMERGENCE OF PULSE BEETLE AND PROTECTION OF MUNGBEAN SEEDS IN STORAGE

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CERTIFICATE

This is to certify that thesis entitled, **Effect of some plant extracts on egg laying, incubation and adult emergence of pulse beetle and protection of mungbean seeds in storage** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN ENTOMOLOGY**, embodies the result of a piece of bona fide research work carried out by **Sanjida Ferdowsi**, **Registration No. 12-05244** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: December, 2013
Dhaka, Bangladesh

Prof. Dr. Md. Abdul Latif
Supervisor

EFFECT OF SOME PLANT EXTRACTS ON EGG LAYING, INCUBATION AND ADULT EMERGENCE OF PULSE BEETLE AND PROTECTION OF MUNGBEAN SEEDS IN STORAGE

ABSTRACT

The experiment was conducted in the laboratory under the Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from April to November 2013 to evaluate the effectiveness of some botanicals against the pulse beetle. The experiment comprised five plant materials each with two doses and laid out in Completely Randomized Design (CRD) with three replications. Among the plant materials, Tobacco leaf powder @ 2g/100g mungbean seeds (T_8) showed the best performance against the bruchids regarding minimum days of 100% adult mortality (3 days), and reducing fecundity (83.71%), egg hatching (87.19%), adult emergence (92.05%), number of holes per grain (92.04%), as well as lowest level of grain infestation (21.67% by number) and weight loss (4.10%). Tobacco leaf powder @ 1g/100g seeds (T_7) also gave the similar results. Turmeric powder @ 2.0/100g seeds (T_2) and Red chili powder @ 2.0g/100g seeds (T_4) gave the satisfactory result against *C. chinensis*. Ginger powder also gave intermediate results and neem leaf powder showed the least effectiveness regarding adult mortality, fecundity, egg hatching, adult emergence of bruhcid and grain infestation by them. Tobacco leaf powder may be used for the management of pulse beetle in storage.

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The Author

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CHAPTER 1

INTRODUCTION

Pulses are considered as one of the best sources of plant protein and play an important role in the diet of common people of developing countries including Bangladesh. They are designated as poor man's meat since they are rich in 20-30% protein. Mungbean (*Vigna mungo* L.) is a popular pulse crop in Bangladesh and its cultivated area was 22984.8 ha with annual production of 20177tons (BBS, 2011). This amount is not sufficient to meet up the demand. The storage of pulses is a matter of great concern. Pulse seeds are more difficult to store than cereals as they suffer a great damage during the storage period due to insect pests and microorganisms. In Bangladesh the pulse seeds are mostly stored by the traders. The farmers also store pulse seeds throughout the year for consumption and also these are used as planting material for the next year. Among the pulses, mungbean seeds stored in godown and farmer houses which furnish suitable habitat for growth and multiplication of bruchids. Infestation of pulse beetle causes both qualitative and quantitative losses in legume seeds. The damage in store is more important than in the field.

Mungbean seed is a nutritious component in the human diet as well as in livestock feed. It contains 51% carbohydrate, 26% protein, 4% minerals and 3% vitamins(Yadav *et al*., 1994). Mungbean plant fixes atmospheric nitrogen in symbiosis with soil bacteria to enrich soil fertility as well as it provides useful fodder (Afzal *et al*., 2004). Over the years, pulse production is gradually decreasing (Sarwar *et al*., 1981). Several factors are responsible for this declining trend of which varietal instability, attack pulses of insect pests and diseases are important. Insect pests attack both in field and in storage condition. About 85% of the pulse growers in Bangladesh store pulses in their houses. Unfortunately, in storage condition, pulses suffer enormous losses due to bruchids attack, infestation starts either in the field on the maturing pod and is carried to the stores with the harvested crops or it originated in the storage itself (Fletcher and Ghosh, 1920). Three species of pulse beetles, viz, *Callosobruchus chinensis* Linn.,*C. analis* Fab., and *C. maculates* Fab. have been reported from Bangladesh as the pest of stored pulses (Begum *et al.*, 1984 and Alam, 1971).

Among them *C. chinensis* cause enormous losses to almost all kind of pulses in storage condition. In Bangladesh, C. chinensis commonly called pulse beetle, but in America and Japan it is known as the cowpea weevil or adzuki bean beetle. The degree of damage varies with different kinds of legumes on the basis of exposure time, storage facilities and other factors associated with seeds. Under farmers storage condition, as high as 98.04%, 73.20%, 53.00%, 54.37%, 64.33% grains of mungbean, blackgram, grasspea, lentil and chickpea respectively, were reported to the damage by pulse beetle, *C. chinensis* in Bangladesh (Anon., 1984). The rate of increases or decreases with the duration of storage under normal condition i.e., the longer the duration the higher the damage (Gujar and Yadav, 1978). Control of storage pests by using synthetic chemicals has become a common practice among the farmers and stockholders. It is now widely known that chemical method has several problems, which include health hazards to the users and grain consumers. It causes residual toxicity, environmental pollution and development of pesticide resistance against bruchids. Sometimes persistent pesticides accumulate in the higher food chain of both wildlife and human and become concentrate by biomagnification (Metcalf, 1975). The traditional method of controlling storage pests by sun-drying is safer to human health and environment. But this method is laborious, time consuming, often expensive and requires suitable drying yard, when large volume of stored grain is involved. Moreover, it depends on favorable weather condition. Recently, the use of different plants and their derivatives has appeared as an effective alternative to the poisonous chemical insecticides or the cumbersome traditional methods for the control of various insect pests of crops and storage.

In storage, this pest is controlled by synthetic insecticides, which have got many limitations and undesirable side effects. Chemical pesticides have been used for a long time with serious drawbacks. Indiscriminate use of insecticides to protect pulse beetle in storage may cause serious health hazard and their residual effects remain in the stored grain and also in the environment. In this condition, search for alternative methods of insect control utilizing botanical products is being used in many countries. These botanicals are biodegradable, relatively specific in the mode of action and easy to use. Plant products are environmentally safe, less hazardous, economic and readily available.

In the world, as many as 2400 plant species have been reported that have potential pesticidal properties and biological activity against a wide range of pests (Grainge and Ahmed, 1988). Plant-derived materials are more readily biodegradable, some are less toxic to mammals, may be more selective in action, and may retard the development of resistance. Their main advantage is that they may be easily and cheaply produced by farmers and small-scale industries as crude, or partially purified extracts. It was reported that when mixed with stored-grains, leaf, bark, seed powder or oil extracts of reduce oviposition rate and suppress adult emergence of bruchids, and also reduced seed damage rate (Keita *et al*., 2001; Shaaya *et al*., 1997; Onu and Aliyu, 1995 and Talukder and Howse, 1994). Oils of Neem, Royna and Castor and leaf powder of Biskatali, Marigold and Castor were most effective in preventing the egg in lentil and chickpea(Latif *et al*., 2012). Leaf powder of Biskatali, Marigold, Castor, Nishinda, Dholkolmi, Mahogany, Dhutra, Oleander and Mango were also effective in reducing adult emergence in lentil and chickpea (Bhuiyah, 2001).

Although many reports have been published on the of plant products against pulse beetle hosted on lentil, cowpea, chickpea, greengram, arhar but information on the use of plant products in mugbean are scanty. Therefore, the present study was undertaken to fulfill the following objectives

Objectives

1. To evaluate the effect of some botanicals on adult mortality, oviposition, infestation rate of *C. chinensis*.

2. To find out the effective dose of the plant materials tested against pulse beetle.

3. To determine the protection efficacy of the selected botanicals against *C. chinensis* in storage.

CHAPTER 2

REVIEW OF LITERATURE

The research was carried out to observe the effect of five plants extracts each with two doses on egg laying, incubation and adult emergence of pulse beetle*Callosobruchus chinensis*Linn. Literatures directly related to this aspect are either scanty or non-existent. Therefore, literatures some way linking to the subject of interest from home and abroad are reviewed and out lined below.

2.1. Pulse beetle as a major pest

The pulse beetle, *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae), is a major pest of economically important leguminous grains, such as cowpeas, lentils, green gram, and black gram (Park *et al.*, 2003; Mulatu and Gebremedhin, 2000; Raja *et al*. 2000; Okonkwo and Okoye, 1996; Talukder and Howse, 1994). The larvae bore into the pulse grains, which become unsuitable for human consumption, viability for replanting, or for the production of sprouts. They are important pests of pulse crops in Asia and Africa under storage conditions (Tapondjou *et al*., 2002; Mulatu and Gebremedhin, 2000; Raja e*t al*., 2000; Okonkwo and Okoye, 1996; Ogunwolu and Idowu, 1994).

Serious problems of genetic resistance by insect species, pest resurgence, residual toxicity, photo toxicity, vertebrate toxicity, widespread environmental hazards and increasing costs of application of the presently used synthetic pesticides have directed the need for effective, biodegradable pesticides (Elhag, 2000; Talukder and Howse,1994;Guedes *et al.*, 1997;Glenn *et al*., 1994; Zettler and Cuperus, 1990). This awareness has created worldwide interest in the development of alternative strategies, including the re-examination of using plant derivatives against agriculturally important insect-pests. Plant-derived materials are more readily biodegradable. Some are less toxic to mammals, may be more selective in action, and may retard the development of resistance. Their main advantage is that they may be easily and cheaply produced by farmers and small-scale industries as crude, or partially purified extracts. In the last two decades, considerable efforts have been directed at screening plants in order to develop new botanical insecticides as alternatives to the existing insecticides. It was reported that when mixed with stored-grains, leaf, bark, seed powder, or oil extracts of plants reduce oviposition rate and suppress adult emergence of bruchids, and also reduced seed damage rate (Tapondjou *et al*., 2002; Keita *et al*., 2001; Shaaya *et al*., 1997; Onu and Aliyu, 1995; Talukder and Howse, 1994).

Pulse seeds suffer a great damage during storage due to insect attack (Sharma, 1984). Among the insect pests attacking stored pulses, the pulse beetle, *Callosobruchus chinensis* L. is a serious one (Alam, 1971). This insect has been reported from the area of Phllippines, Japan, Indonesia, Srilanka, Burma, India and Bangladesh. It is a notorious pest of chickpea, mung, cowpea, garden pea, black gram, lentil and arhar. The extent of damage to pulse seeds is very high both qualitatively and quantitatively. There was a 55-69% loss in seed weight and 45.6-66.3% loss in protein content by the pulse beetle on chickpea. About 100% loss of pulse seeds was found due to infestation by the pulse beetle. (Gujar and Yadav, 1978).

2.2 Biology of pulse Beetle

2.2.1 Egg and oviposition

The female oviposited within 24 hours of their emergence and 1 to 3 eggs were laid per pulse grain but as many as were observed in a single grain (Raina, 1970). She also observed that the female laid an average 78 eggs ranging from 63 to 90 over a period of 8 days at 30^0C with 70% RH on mugbean.

Lambrides (2000) reported that the texture, layer present on the seed coat of some mugbean varieties and small grains size might act as oviposition deterrents. The freshly laid eggs were translucent, smooth and shining but become pale yellowish or grayish white with age. The eggs were elongated and oval in shape (Alam, 1971).

2.2.2. Larval Period

Razzak and Pundy (1965) observed that the full grown larvae were curved and the dorsal body was raised into a segmented done like structure. According to Alam (1971) the full grown larva was 6 mm long, flesh strongly wrinkled, perfectly white except brown color at mouth region. Soon after hatching, the young larvae bored into the seeds and started to consume the contents.

2.2.3. Pupal period

Dennis (1990) reported that the pupation took place inside the seed in a chamber covered by a thin window of testa prepared by a mature larva. The pupae were exerate type with a mean pupal duration of 2.5 days in summer and 4.25 days in winter (Bhuiyan and Peyara, 1978).

2.2.4. Adult Emergence

Before emergence, the adult remain a few days inside the pupal chamber. The adult emergence occurred by cutting a window hole and then pushing head and forelegs through it (Razzak and Pundey, 1965). The beetle was dark and incospicuous in color and its body was clothed with hairs. Bhuiyan and Peyara (1978) reported that the head of the beetle is small, hypognathous and provided with short snout. The males were short lived and smaller than females. The adult was 4 mm long and distinguished from other species by the elevated ivory like spots near middle of the body (Alam, 1971).
Arora and singh (1970) found that the adult generally more around the grains and were also capable of making short flights with hind wings. According to Bhuiyan and Peyara (1978), the longevity of the male and female were 4.3 and 5.4 days in summer and 7.4 and 9.2 days in winter, respectively. The life cycle of *C. chinensis* completed in 30-32, 20-23, and 40-46 days in early summer, mid summer and winter seasons, respectively.

2.3 Origin and Distribution of pulse beetle

C. chinensis L. was first reported and described from China in 1958 (Alam, 1971). Though Southgate (1979) has mentioned that the species of bruchidae have their origin in Afro Asian region. According to him, the species of bruchidae in every continent except Antarcticahave their cosmopolitan distribution. Most of the species lived in the tropical regions of Asia, Africa, Central and South America. *C. chinensis*L. is of Asian origin, where it is still the dominant species (Dennis, 1990). He mentioned that *C. maculatus* thought to be African. However, both the species are now widely distributed throughout the warmer parts of the world. Other species of

Callosobruchus recorded as pest include *C. analis* (Fab.) in parts of Asia on *Vigna* species, *C. phaseoli* (Gyllenhal) in Africa, parts of Asia and South America on *Vigna* and *Dolichos lablab*, *C. rghodesinus* in Africa on cowpea *C. sibinnotastus* (Linn.) in East Africa on *V. subterranea* and *C. theobromae* (Linn.) in India on field crops of pigeon pea. Rahman *et al*. (1942) reported that the bruchidae contains more than 100 injurious species distributed over different part of the world. Among them, 11 injurious species were recorded.

2.4. Nature and extent of damage

Begum *et al*. (1982) stated that in Bangladesh *C. chipnensis* L. was one of the major pests belonging to *Callosobruchus spp*. causing considerable damage to stored legume grains. Southgate (1979) stated that pulses grown by man had been infested by bruchids since the dawn of agriculture. The larval stage caused only severe damage the unfit for planting andconsumption. In the laboratory study, (1991) found that the initialpresence of 4 larvae or eggs or one pair of *Callosobruchus spp* adult could completely damage 10 g of the pulse grain within 2-4 month depending on the type of the pulses, stage of maturity and species of the beetle. Gujar and Yadav (1978), recorded 55-60% loss by seed weight and 45-66% loss in protein content by the pulse beetle. Results revealed that 50.37-57.58% (Ali *et al*., 1999) and 37.30-55.30% (Ali and Rahman, 2006). Grain content loss of mungbean seed was occurred by *C. chinensis* and *C. maculatus* respectively. The extent of damage of mungbean seed might be up to 100% during a period of one year storage (Chowdhury, 1961).

2.5. Host preference for oviposition

Lale and Kolo (1998) reported that eight local cultivars of cowpea that have been improved for higher yields and pest tolerance which were recently released for cultivation by the International Institute of Tropical Agriculture (IITA), Ibadah, Nigeria, were compared with respect to their susceptibility to the bruchids *C. maculatus* under laboratory conditions (30-3 5°C and 45-57% RH) Seeds of cultivars Kanannado, T189 KD-391 and Danila with susceptibility indices (SI) OF 0.0, 2.2 and 2.6, respectively and were found to be resistant to bruchid attack and cultivars Babura-4, IT 89 KD 374, Bausse-local, IT89KD- 349 and Alok with SI values of 11.2, 10.9, 7.45, 7.2 and 6.5, respectively, were found to be susceptible. Oviposition and egg viability were significantly reduced on seeds of the resistant cultivars. The total mean numberof eggs laid on seeds of the resistant cultivars Kanannado, 1T89KD-391 and Danila were 16,15 and respectively, while 50, 44, 36, 35 and 26 were laid on seeds of susceptible cultivars Babura-4, IT89KD-374, Bausse-local, Aloka-local and IT89KD respectively. The proportion of unhatched eggs laid on resistant cultivars was 98.2, 81.5 and 50.0 for Kanannado, IT89KD-391 and Danila, respectively.

A study conducted by Chavan *et al*. (1997) on the ovipositional preference of *Callosobruchus chinensis* for 70 cowpea lines and they found that cowpea lines with rough seed surface were less preferred, resulting in a small percentage of grains infect in the viability of eggs were noticed. *C. chinensis* distributed eggs uniformly on grains of different cowpea lines and oviposited a small number of eggs/ grain. Brown, black, grey and red coloured seeds were more preferred than white coloured seeds.

Sharmila and Roy (1994) studied the effects of oviposition and development of the bruchid C. maculats on nine legume seeds under common storage condition in the Bundelhand region of Madhya Pradesh, India. Bengal gram was most preferred both under choice and no choice conditions. However, under no choice condition, bruchids developed on green gram (*Vigna radiata*) cowpeas, lentils and red gram (pigeon peas). The survey results also showed similar trends.

Piergiovanni *et al*. (1994) analyzed the seeds of an 8 lines differing in storage pest resistance for inhibitors of the following enzymes: porcine amylase, Bacillus amylase, bovine chymotrypsin and trypsin. A broad variation was observed among samples for all tested inhibitors. Principal component analysisindicated that high level of both antitrypsic and antiamylasic activitycharacterizes resistant lines. Moreover, high activity of a single inhibitor class is typical of the bruchid (*C. maculatus*) susceptible lines. Hence breeding for high contents of these protein inhibitors could be an effective way of obtaining lines that are naturally resistant to storage to pest attack. A limiting factor in this breeding strategy is the need to reduce the antiamylastic activity before eating.

Shiau *et al*. (1994) studied the oviposition choice of the stored products pest *C. maculatus* by providing females with different ratios of azuki beans (*V. angularis*) and mungbeans (*V. radiata*). The fraction of eggs laid on azuki beans increased with increasing ratio o azuki beans, where as it decreased with a decreasing duration of oviposition. The probability of female encountering azuki beans was significantly higher than for mungbean, but even when the female was provided with the same probability of encounter is still preferred to lay eggs on azuki beans. Females tended to spent more time inspecting azuki than mungbean, but no differences in handling time between the 2 hosts were found.

Ahmed (1992) observed that in all the seasons, lentil and mungbean were highly preferred for oviposition and the emergence of adults were considerably high, while gram and black gram were least preferred for oviposition except high rates of adult emergence for gram and pigeon pea in summer season.

2.6. Management of pulse beetle

Control of storage pests by using synthetic chemicals has become a common practice among the farmers and stockholders. It is now widely known that the chemical method has several problems, which include health hazards to the users and grain consumers. It causes residual toxicity, environmental pollution and development of pesticide resistance against bruchids. On the other hand, the traditional method of controlling storage pests by sun-drying is safer to human health and environment. But this method is laborious, time consuming, often expensive and requires suitable drying yard, when large volume of stored grain is involved. Moreover, it depends on favorable weather condition. Recently, the use of different plants and their derivatives has appeared as an effective alternative to the use of poisonous chemical insecticides or thecumbersome traditional methods for the control of various insect pests of crops and storage.

Literatures on some of such studies relevant to the present study collected from various sources including Bangladesh wire reviewed here in brief.

Latif *et al*. (2012) tested dry leaf powder of nine plants viz., Neem (*Azadirachta indica*), Nishinda (*Vitex nigundo*), Karonda (*Carissa carandas*), Bishkatali (*Polygonum hydropiper*), Nagalingam (*Couropita guianensis*), Mehagany (*Switenia mahogany*), Dholkolmi (*Ipomoea carnea*), Dhutra (*Datura metel*) and Oleandar (*Nerium oleander*) against *C. chinensis* and observed that dholkolmi leaf powder was the most effective against *C. chinensis* by reducing 16.438% egg laying, 29.07% adult emergence, 25.0% seed infestation and 12.42% weight loss over control.

Ahmed *et al*. (2006) conducted an experiment to evaluate the effect of seed containers, indigenous materials and chemicals for the management of pulse beetles in storage. In this experiment, mungbean seeds were stored for two years in different containers with two types of chemicals (napthalene and camphor) and two types of indigenous materials (sand and neem leaf powder). In both years camphor provided better protection than other materials for all the containers and storage period.

Rahman *et al*. (2004) conducted experiments to study the bio-efficacies of different plant/weed derivatives that affect the development of the pulse beetles, *C. maculatus* F. (Coleoptera: Bruchidae) feed on black gram, *V. mungo*, seeds. Plant extracts, powder, ash and oil from nishinda (*Vitex nigundo* L.), eucalyptus (*Eucalyptus globules* Labill), bankalmi (*Ipomoea sepiaria* K.), neem (*Azadirachta indica*), safflower (*Carthamus tinctorius* L.),' sesame (*Sesamum indicum* L.) and babla (*Acacia arabica* L.) were evaluated for their oviposition inhibition, surface protectant, residual toxicity and direct toxicity effects on *C. maculatus*. The results showed that plant oils were effective in checking insect infestation.

In England, Golob and Webley (1980) have complied the information from replies to a questionnaire and from a study of literature. More than 160materials have been listed with the country of use, the sources of information, and a brief description of the use. The various types of natural materials have been grouped together as whole plants or parts of plants, plant extracts, vegetable or citrus oil, ashes or minerals. Some of the information relate to traditional use and some to the use of materials in laboratory or field trials. Pandey and Verma (1977) in their testing (Custard apple) seed powder as a protectant of mung (*Phaseolus radiatus*) against pulse beetle, *C. maculates* (F.) at the rates of 0.5, 1.0 and 2 parts per 100 parts of mung by weight. They found that treated seeds were effectively protected against *C. maculatus* for up to 100 days when used at higher rates of dosages. The protection appeared to of repellent in nature, since the treated seeds produced no mortality in adult *C. maculatus*.

In Bangladesh, Das (1987) investigated the effect of various concentrations of neem (*A. indica*) oil on adult mortality and oviposition of *C. chinensis* in the laboratory at 32.5°C and 83-85% R.H. Ten pairs of newly emerged male and female adults of *C. chinensis* were introduced into pots containing 50g chickpea (*Cicer arietinum*) seed treated at 4, 6, 8 and 10 ml/kg seeds. Adult mortality was significantly greater at all concentrations of treated seeds compared with the untreated seeds. The highest mortality of 100% was observed at 8 and l0ml/kg seeds. The total number of eggs laid on the seeds treated at 6, 8 and l0ml/kg seed significantly lower than the untreated seeds orthose treated at 4ml/kg seed. It is concluded that 8ml of oil/kg seed is the most economic concentration to control *C. chinensis* infestation on chickpea seeds.

Rouf *et al*. (1996) in the laboratory of the Department of Entomology, Bangladesh Agricultural University (BAU), Mymensingh and found that the leaf powder of neem (*A. indica*), nishinda (*Vitex nigundo*) and biskatali (*Polygonum hydropiper*) alone and in combination of these materials at lower were less effective against the pulse beetle, *C. chinensis*. They recorded that biskatali leaf powder @ 4g/50g lentil seeds was most effective in reducing oviposition and adult emergence of the pulse beetle and its intensity of damage and weight loss to lentil seeds. Germination of lentil seeds was not affected when treated with biskatali leaf powder. Another study was done by Chowdhury *et al*. (1991) on the effect on some indoor containers and they reported that Improved Tin, Traditional Tin and Polythene lined Motka gave full protection to maize seeds up to 13 months of storage against *Sitophilus spp*, and *Sitotroga cerealella* (Ol.) both in periodically open container opened once after the end of the study.

In Africa, Mueke and Apuuli (1987) used vegetable oils and ash for the control of C. maculatus in seeds of cowpea (*V. unguiculata*) and observed that castor (*R. communis*) oil was an outstanding agent. Vegetable oils caused mortality to adult beetles and the eggs that were on the seeds failed to hatch. Ash mixed with cowpea seeds afforded satisfactory control against the bruchid. Ash levelsof 20. and 30% led to 8.96 and 6.18% respectively, as compared with52.59 for no treatment.

A study was conducted in India by Khan (1986) with the residual activity ofrhizome powder of *Acorus calamus* (applied at 0.2%) against *C. chinensis* in stored grains of *Cicer arietinum* was tested in the laboratory at 25°C when the bruchids were introduced with ranges of 0-30 days. After treatment the progeny did not develop and the percentage of infestation was 0-0.04. When the insects were introduced 45-120 days after treatment, the percentage of infestation were 0.26-0.93 compared to 19.59% for the untreated grains. A laboratory study done in USA by Su (1984) on the toxicity of acetone extract of the unripe fruit of *Piper nigrum* (black pepper and green pepper) and maybe the hexane extract of the dry fruit of west African pepper (*P. guineese*) to the stored product insects *C. maculatus*, *Sitophilus oryzae*, *Lasioderma serriicorne* and *Tribolium confusum* showed that black pepper gave the highest contact toxicity to *C. maculatus* and *S. oryzae* with green pepper producing toxicity just slightly lower. Black pepper gave the best protection to black eyed peas (*V. unguiculata*) against *C. maculatus*. Both black pepper and green pepper gave good protection to wheat against infestation by *S. oryzae* even the lowest doses of 250 ppm gave 57.1% protection.

Rajasekaran and Kumaraswami (1985) evaluated the effectiveness of extracts of Karanja (*P. glabrd*) and neem (*A. indica*) for the control of *Sitophilus oryzae* and *C. chinensis* on sorghum and green gram (*V. radiata*) grain. Coating sorghum grain with karjana extract with 0.4% vol/vol or with neem extract at1.0% wt/wt gave complete protection from *S. oryzae*. Coatinggrain with the 2 extracts at 0.8% wt/wt respectively, gave significant protectionfrom *C. chinensis.*

According to Tikku *et al*. (1981), the factors responsible in preventing the multiplication of *C. chinensis* by vegetable oils could be attributed to theprevention of normal exchange of gases, hardening the outer membrane toprevent hatching, interference with water balance. Interference with the waterbalance of the eggs and penetration by the oil of the wax layer under thechorion and consequent contact with the embryo were found the most probablefactors in completion of developmental cycle.

At present, pest control measures in storage rely on the use of synthetic insecticides and fumigants, which is the quickest and surest method of pest control but it is also not advised to mix the insecticides with food grains. Their indiscriminate use in the storage, however, has led to a number of problems including insect resistance, toxic residues in food grains (Fishwick, 1988), environmental pollution (WMO, 1995) and increasing costs of application. In view of these problems together with the upcoming WTO regulations, there is a need to restrict their use globally and implement safe alternatives of conventional insecticides and fumigants to protect stored grains from insect infestations (Yusof and Ho, 1992; Subramanyam and Hagstrum, 1995).

The use of fly ash has been reported for post-harvest preservation/protection of five commonly used pulses including chickpea from infestations of PB for 18 months. No adult PB was found in pulses treated with fly ash even after 12 months of treatment. After 18 months of storage, chickpea was the most infested both in terms of number of insects observed in gunny bags and percent damaged grains. Percentage insect damaged grains were directly proportional to the number of insects observed in gunny bags. There was no effect of fly ash on the nutritional quality and percent germination of pulses. Misra (2000) compared ten different types of plant powders with local treatments of red soil powder, cow-dung ash powder and mustard oil coating for their effect against PB. Among local treatments, only cow-dung ash and mustard oil completely inhibited oviposition. In studies by Singal and Chauhan (1997), coal ash and soft stone were ineffective against PB when mixed in stored grains while according to Sharvale and Borikar (1998), the application of castor oil in grains was more effective than ash and neem leaves. Dhakshinamoorthy and Selvanarayanan (2002) tested the effect of dried leaf powders of neem, nochi, pungam, citrus and thulasi against PB attacking stored grains whereas fly ash, kitchen ash, castor oil, red earth and malathion (as standard control) were also used.

Although effective, such synthetic pesticides causeconsequently residual pollution of the environment and toxicity to consumers. Their repeated use for decades has disrupted biological control by natural enemies and has led to the resurgence of stored product insect pests. Many of these stored product insects have developed resistance to the commonly used chemicals (Srivastava and Singh, 2002;Subramanyam and Hagstrum, 1995). These problems have highlighted the need to develop insect control alternative means.

Many medicinal plants and spices have been used as pest control agents (Yankanchi and Gonugade, 2009). Farmers and researchers often claim the successful use of plant materials in insect pest control, including ash (Ajayi *et al.,* 1987;Ofuya, 1986), vegetable oils (Yankanchi, 2009;Devanand and Usha Rani, 2008; Kazi *et al.*, 1999), plant extracts (Schoovoven, 1978), and botanical powders (Gupta and Srivastav, 2008;Shukla *et al.*, 2007;Abdullahi and Muhammad, 2004). It has been reported that certain plant preparations and tradi-tional methods are much safer than chemical insecticides (Yankanchi and Patil, 2009). Therefore, plant materials should be explored to protectstored products against pest infestation. Grain managers tend to look only at chemical alternativesto control stored-grain insect pests but interest innon-chemical methods of controlling insects in storedgrain is increasing, as consumers become less tolerantof pesticide residues in their food. Consequently, the choice of pesticides for storagepest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food. Furthermore, the continuous use of chemical pesticides for control of stored-grain pests has resulted in serious problems such as insecticide resistance (Mohan *et al*., 2010). Current research and the increasing knowledge about the harm derived from the indiscriminate use of synthetic insecticides have encouraged studies related to novel tactics of pest control like the use of botanical insecticides. Plant materials with insecticidal properties, are one of the most important locally available, biodegradable, and inexpensive methods for control of stored-grain pests. The main advantage of botanicals is that they are easily produced by farmers, small-scale industries and are potentially less expensive. The utilization of botanical insecticides to protect stored products is promising, mainly due to the possibility of controlling environmental conditions inside the storage units, maximizing the insecticidal effect; in these places the natural product can be used as powder, extract and oil. Moreover, the use of plants materials for storage protection is sustainable; they can be continuously propagated year after year; biodegradable; and do not have any negative impact on the environment as long as care is taken to avoid the propagation of plants from foreign ecosystems which might, therefore, become established as weeds (Golob *et al*., 1999).

2.7. Importance of plant material as repellent

The pulse beetle being an internal feeder is hard tocontrol with insecticides. It is also not advisable to mix insecticides with food grains. Fumigation being the most effective method cannot be practiced in our villages because the storage structures are not airtight and are mostly built inside the residential areas. Plant materials which arc being traditionally used by some farmers are quite safe and appear to be the most promising as grain protectants. The use of spices is cheaper; they are easily available and safe.

Spices are dried seed, fruit, root, bark or vegetative substance used in nutritionally insignificant quantities as a food additive for the purpose of flavoring. Many of these substances have other uses, e.g. food preservation, as medicine, in religious rituals, as cosmetics, in perfumery or as vegetables. Pulses have a prominent place in daily diet as a rich source of vegetable protein, minerals and vitamin-B. They are of special significance to the people in developing countries, who can hardly afford animal protein in adequate quantities. Among the pulses black gram, *Phaseolus bengalensis* L. belonging to the family Leguminosae is the most important legume crop in the world. Pulse seeds suffer a great damage during storage due to insect attack (Sharma, 1984).

Among the insect pests attacking stored pulses, the pulse beetle, *C. chinensis* L. is a serious one (Alam, 1971). This insect has been reported from the Philippines, Japan, Indonesia, Srilanka, Burma, India and Bangladesh. It is a notorious pest of chickpea, mung, cowpea, garden pea, black gram, lentil and arhar. The extent of damage to pulse seeds is very high both qualitatively and quantitatively. There was a 55-69% loss in seed weight and 45.6-66.3% loss in protein content by the pulse beetle on chickpea (Gujar and Yadav, 1978). About 100% loss of pulse seeds was found due to infestation by the pulse beetle. The use of plants and minerals as traditional protectants of stored products is an old practice used all over the world. These traditions have been largely neglected by farmers, after the Second World War, with the advent of synthetic or petroleum based insecticides. However, the potential hazards for mammals from synthetic insecticides, the ecological consequences and the increase of insect resistance to pesticides has led to a search for new classes of insecticides with lower mammalian toxicity and a lower persistence in the environment(Golob and Webley, 1980).

Sighamony *et al.* (1984) tested oils of clove, cedarwood *(Juniperus virginiana),* karanja (*Pongamiaglabra)* and an acetone extract of black pepper *(Pipernigrum)* in India by a choice method to determine their repellent effects on adults of *Tribolium castaneum.* Cedarwood, karanja and pepper products were found to be more potent than the standard repellent, dimethylphthalate. Karanja oil and pepper extract were rated as the most repellent at the highest concentration tested (10.38mg/cm3) but were less repellent at the lowest concentration tested (2.59mg/cm3). Karanja oil appeared to retain its repellent effect strongly over the 8 weeks of the experimental period.

Miah *et al.* (1993) reported the effects of several Bangladeshi plant materials against *C. chinensis* on chickpea seeds. Nishinda (*Vitex engundo*) leaf powder was the most effective in reducing numbers of eggs laid, adult emergence and seed weight loss. They also tested powdered spices (flowers of clove. rhizome of ginger and turmeric, fruits of black and chili pepper and bulb of garlic), malathion (1.5%)and powdered stem of the tree *Combrerum imberbe* mixed against *C. maculatus*. Reduction in ovipositionand seed weight loss were recorded 10 and 70 dayspost-treatment respectively. Cloves and black peppergave results, which were not significantly differentfrom those produced by malathion.

M. Aslam (2004) tested six spice powders against *C. chinensis*. Clove and black pepper were good protectants of stored chickpea against the beetle. Kim *etal.* (2003) showed the potent insecticidal activity of extract from cinnamon (*Cinnamomum cassia*) bark and oil, horseradish (*Cocholeria aroracia*) oil, and mustard (*Brassica juncea*) oil against *C. chinensis*.

2.8. Importance of Neem leaf in storage

since the advent and popularization of broad-spectrum synthetic insecticides, there has been some loss of faith in neem as a protectant for stored products. However, the use of neem can confer significant economic advantage and service to rural areas in tropical developing countries, if reliable recommendations can be made and given to farmers for the protection of stored commodities, especially food grains, animal feed, and seed, from insects. This approach requires on the spot production and field testing of neem-based grain protectants as well as revalidation of previous reports. Some of these are reviewed here with respect to legumes, sorghum and corn, wheat, rice and paddy, and potato.

Neem's efficacy to non-target and beneficial organisms has been documented (Schmutterer, 2002). Many biologically active compounds can be extracted from neem, including triterpenoids, phenolic compounds, carotenoids, steroids and ketones. The tetranortriterpenoid azadirachtin has receivedthe most attention as a pesticide, because it is relatively abundant in neem kernels, and has shown biological activity on a wide range of insects. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin-A to azadirachtin-G with azadirachtin-A being present in the highest quantity and azadirachtin-E regarded as the most effective insect growth regulator. Many other compounds have been isolated that shows antifeedant activity as well as growth regulating activity on insects. This cocktail of compounds significantly reduces the chances of tolerance or resistance developing in any of the affected organisms. However, only four of the compounds in neem have been shown to be highly effective in their activity as pesticides: azadirachtin, salannin, meliantriol, and nimbin (Jacobson, 1990).

In India, green gram, chick pea, cowpea, and pea could be protected from damage by the pulse beetles, *Callosobruchus* spp., for 8-11 months by mixing powdered neem kernel with grains at 1 or 2 to 100 parts (Jotwani and Sircar, 1967). Neem kernel protected the legumes against *C. chinensis* and *C. maculatus* and stopped the development of progeny even 12 months after *C. chinensis* was released on treated lentil seed (Yadav, 1973). Likewise, chick pea and pigeon pea seeds remained undamaged up to 12 months after treatment with 2g neem kernel powder per 100 g seed. Application of 1 to 3 parts of neem oil per 100 parts of Bengal gram rendered complete protection against *C. chinensis* for at least 135 days, without impairing seed germination.

Ketkar (1976) tested the efficacy of neem kernel and oil for protecting bagged leguminous seeds (peas, Bengal gram, *Phaseolus,* and *Vigna* spp.) from pulse beetles during 8 months storage in warehouse trials conducted in Pune, India. Neem oil treatment at 8 ml to 1 kg of grains reduced the infestation to almost zero in Bengal gram and *Phaseolus* (14% in untreated Bengal gram and 26% in untreated *Phaseolus*), and by 50 to 70% in treated peas and *Vigna*; treatment with kernel was less effective. Neem oil did not affect seed viability and unused seeds were fit for animal and even human consumption. Neem oil at 1 ml/100 g seed killed all the pulse beetle grubs and adults, and no eggs were laid on treated seed. On cowpea and bambara groundnut, neem oil at 8 ml/kg seed not only reduced oviposition, but also killed larvae; the activity persisted more than 90 days on cowpea and for 180 days on bambara groundnuts. Green gram was completely protected against *Callosobruchus* spp. when soaked for 20 minutes in a 1% solution of neem oil extractive (Attri and Prasad, 1980). In a warehouse trial conducted in Togo, white cowpea treated with 0.5% neem oil was protected from *C. maculatus* for up to 6 months of storage and even after 10 months of storage only 18% of the initial weight was lost (Zehrer, 1984).

In Ghana, Tanzubil (1987) demonstrated that cowpea treated with neem oil at 0.5%, or mixed with powdered fruit at 10% remained undamaged by *C. maculatus* over a 16 week storage period; mixing neem leaf dust in the grain was less effective, while untreated cowpea had 90% grain damage. In India, sorghum seed mixed with powdered neem kernel in a proportion of 100 to ≥1.5 (wt/wt) remained protected from damage by *Sitophilus oryzae* (Deshpa, 2004). Corn seed soaked for 20 minutes in a 1% solution of neem oil extractive was resistant to attack by *S. oryzae* (Attri and Prasad 1980). In Togo, Adhikary (1981) found that neem treatment of corn stored in sacks or unpeeled corn cobs held in bins was quite simple and effective against *S. zeamais*, *Tribolium* spp., *Rhyzopertha dominica*, and *Cathartus* spp.

Jotwani and Sircar (1965) in India were the first to demonstrate that powdered neem kernel when mixed with wheat seed at a proportion of 1-2 to 100 (wt/wt) parts satisfactorily protected against *S. oryzae, R. dominica*, and *Trogoderma granarium* for 270, 320, and 380 days, respectively. Rahim (1997) found that an ethanolic neem kernel extract, containing azadirachtin, at 75mg/ kg protected stored wheat against *R. dominica*for up to 48 weeks. In warehouse trials, wheat grain treated with neem oil at a proportion of 8 ml to 1 kg grain, prior to storing for 8 months in gunny bags, had 50 to 70% less infestation by *S. oryzae*, *R. dominica*, *T. castaneum*, and *Cryptolestes* spp. (Ketkar 1976). Application of neem oil at a low concentration of 0.1% (wt/wt) to wheat grain reduced egg laying by *Sitotroga cerealella* as effectively as a 5% malathion dust treatment (Verma *et al.*,1985).

In commercial trials conducted in Pakistan, it was demonstrated that paper or cloth grain storage bags treated with water extract of neem leaves at 20% (wt./vol.) or water extract of neem seed at 5% (wt./vol.) checked the penetration of stored grain pests into the bags for 6 months during storage (Malik *et al*., 1976; Jilani, 1981). In an on-farm trial conducted in Sind, Pakistan for 13 months, the application of ethanolic neem seed extract (600 $\mu g/cm^2$) to storage bags or directly to wheat grain controlled more than 80% of the population of *Tribolium castaneum*, *R. dominica*, *S. oryzae*, and *S. cerealella* and prevented grain damage up to 6 months (Jilani and Amir, 1987). The treatments remained effective up to 13 months, providing more than 70% protection; insect infestation and the percentage of weevil attacked grains was much lower than in the untreated control.

In Malaysia, mixing neem leaves with paddy grain in a proportion of 2 to 100 parts (wt/wt), bag treatment with 2% neem leaf water extract (wt./wt.), or placing barriers of neem leaves between bags and storage floor, significantly reduced the infestation by *S. oryzae* and *R. dominica* and damage to paddy grain stored in 40 kg jute bags for 3 months (Muda, 1984). Although it was not clear which treatment was superior, but all treatments had potential for adoption in rural areas.

In a warehouse trial conducted in the Philippines, Jilani *et.al* (1988) evaluated the effectiveness of neem oil alone or in combination with fumigation against five species of major stored grain pests infesting rice and paddy grains. Rice grain treated with 0.05 to 0.1% neem oil or treated with neem oil after fumigation with 'Phostoxin', and stored for 8 months contained significantly less *T. castaneum* adults than in the untreated control. Both kinds of neem treatments were as effective as the bag treatment with 'Actellic' (primiphos-methyl 20 EC) at 25 μg/cm^{2}or grain treatment with 'Actellic' at 0.0005%, and suppressed the pest population by about 60%. The pest population build-up also was reduced when either fumigated or non fumigated rice was stored in bags treated with neem oil at ≥1 mg/cm^2. Other pest species, *R. dominica*, *S. oryzae*, *O. surinamensis*, and *Corcyra cephalonica* were similarly affected by neem treatments alone or in combination with prior grain fumigation. Fumigation with 'Phostoxin' was effective only for a period of about 2 months against *R. dominica*, and for up to 6 months against other pest species. In contrast, neem oil treatments were effective up to 8 months. Compared with the pest damage to untreated or fumigated rice, neem oil treatments significantly reduced the damage to rice grain. At 8 months after storage, weevil attacked grains in neem treatments were 50% of those in the fumigated rice and 25% of those in the untreated rice. *R. dominica*, and for up to 6 months against other pest species. In contrast, neem oil treatments were effective up to 8 months. Compared with the pest damage to untreated or fumigated rice, neem oil treatments significantly reduced the damage to rice grain. At 8 months after storage, weevil attacked grains in neem treatments were 50% of those in the fumigated rice and 25% of those in the untreated rice.

Paddy grain that had been fumigated and then treated with neem oil or, after fumigation, stored in neem oil-treated bags, also had fewer adults of *T. castaneum*, *R. dominica*, *S. oryzae*, and *O. surinamensis*, as compared with the fumigated or the untreated paddy grain. *C. cephalonica* infestation was found in the stored paddy only after 4 months and remained low throughout the trial in treated as well as untreated paddy.

2.9. Importance of tobacco leaf as repellent

All the treatment levels, singly and combined powder of *N. tabacum* and dust of pirimiphos-methyl caused varying degree of mortality in maize weevil, reduced number of emerged adults as well as the weight loss of the grains. Results suggest that the weevils would prefer to avoid maize grains treated with powder and dust. The ability of the plant powder to cause mortality of *S. zeamais* adults on maize grains can be attributed to contact toxicity of the powder on the weevil. (Aslam, 2004) had reported the effectiveness of some plant powders in controlling *S. zeamais* by causing adult mortality of the insect. The treatment with 0.4g *N. tabacum* powder had the highest weevil mortality as well as reduced number of adult emergence than any other treatments. The least weight loss was also recorded 0.4g of *N.tabacum*. Similar effects of plant materials as crop seeds protectants have been observed in the treatment of cowpea and maize weevils. Insecticidal property of any plant material would depend on the active constituents of the plant material. The active constituent in these plant materials appears to be responsible for their insecticidal properties against the maize weevil. *Nicotiana tabacum* has been reported to possess contact, stomach and respiratory poisoning properties attributed to the active constituent nicotine (Lale and Vidal 2003)

CHAPTER 3

MATERIALS AND METHODS

An experiment was conducted to evaluate the effect of five plant extracts on egg laying, incubation and adult emergence of pulse beetle*Callosobruchus chinensis* Linn.on mungbean. In this chapter the materials used, the methodologies followed and the related works done during experimental period are presented.

3.1.Study site and season

Experiment was conducted in the Entomology laboratory of Sher-E-Bangla Agriculture University ,Dhakaduring the period from April 2013 to November 2013.

3.2. Climate and Weathers

The climate of the location was characterized by relatively high temperature and heavy rainfall during kharif or summer season (April to October). Temperature ranged $\pm$ 30^{0}C and humidity was $\pm$ 75%.

3.3. Materials used in the present experiment

Seeds of mungbean were used under the present study as susceptible host to *Callosobruchus chinensis*. Five plant materials with two doses were used against pulse beetle. Common name, Scientific name, Family and plant parts used are given in Table 1.

Table 1. Common name, scientific name, family and plant parts used under present study

Name of plants	Scientific name	Family	Used plant parts
Red chillies	*Capsicum annum* L.	Solanaceae	Fruit
Turmeric	*Curcuma longa* L.	Zingiberaceae	Rhizome
Zinger	*Zingiber officinalis* R.	Zingiberaceae	Rhizome
Tobacco	*Nicotiana tabacum* L.	Solanaceae	Leaf
Neem	*Azadirachta indica* L.	Meliaceae	Leaf

Figure 1. Prepared treatments used in the present study.

3.4. Taxonomy of the insect under experiment

The effect of these plant materials worked upon the insect is pulse beetle.

Classification of pulse beetle

Kingdom: Animalia
Class: Insecta
Order:Coleoptera
Family: Bruchidae
Genus:*Callosobruchus*
Species: *Callosobruchus chinensis* Linn.

3.5. Collection of experimental material:

The infested samples of stored mungbean by *C. chinensis* were collected from different villages, institutes, stores, godowns and research stations.

Figure 2. Collected mungbean sample used as experimental material

All the plant materials were collected from fresh natural sources, like the village Srirampur of Patuakhali District and Sher-e-Bangla Agricultural University campus. After collection, all of the samples were sun dried for several days until the moisture content maintained 8-10%. Then the materials were oven dried and then grinded with mechanical grinder. The powders of different plant materials were stored in plastic containers separately.

3.6. Rearing of Pulse beetle

Adult pulse beetle was separated from the collected mungbean by aspirator. Adult bruchids were then kept in three plastic jars each containing 100g mungbean seeds. The mouth of the jars was covered with fine clothes to protect the beetles from outgoing. Then the jars were kept on the table in laboratory for rearing of pulse beetle. After 3 days, mungbean seeds of the jars were observed whether pulse beetle laid eggs or not. Then these jars were kept for 15 days. In the 19^{th}day, new adult pulse beetles emerged from the mungbean seeds and the virgin adults were separated every day in another jar containing 100g mungbean seeds for rearing of beetles to ensure continuous supply of different stages of the insect for the main experiment.

3.6. Treatments of the experiment

There were 10 treatments including control treatments that were applied against pulse beetles infesting in the storage condition. The treatment combinations were as follows**:**

Table 2. Name and dose of botanicals used under the present study

Treatments	Name of botanicals	Dose (g/kg seed)
T_1	Turmeric powder	10 g
T_2	Turmeric powder	20 g
T_3	Red Chili powder	10 g
T_4	Red Chili powder	20 g
T_5	Ginger powder	10 g
T_6	Ginger powder	20 g
T_7	Tobacco leaf powder	10 g

T_8	Tobacco leaf powder	20 g
T_9	Neem leaf powder	10 g
T_{10}	Neem leaf powder	20 g

3.7. Test procedure

Thirty plastic containers marked with ten treatments and 100g fresh mungbean seeds were taken in each plastic jar. Five pairsnewly emerged adult pulse beetles were releasedin each jar.Mouth of the jar was then covered with fine clothes and secured by rim of lid so as to disallow the released beetles as well as any other insect contamination. Each treatment including control was replicated 3 times. The pots were arranged in Completely Randomized Design (CRD) on a wooden table and stored under laboratory condition.

3.8. Data

The effectiveness of plant materials as a protectant of mungbean seeds against pulse beetle was evaluated with some parameters and data were recorded for released adult beetles on 100% mortality (days), egg laying performance (number), egg hatching performance(number), infestation rate (1st and 2nd month in number), adult emergence(number), weight loss (g) in 1st, 2nd and 3rd month from the treated and untreated seeds. The methods employed to record data on the above mentioned parameters are described separately.

3.8.1. Days of 100 percent adult mortality

Effect of plant materials on days to 100% mortality of released adult bruchids was counted to determine the effect of treatments on the life span of its adults. 100g mungbean seeds were treated with different treatments and put separately in jars. Five pairs of newly emerged beetles were released in each jar. After 24hr, 48hrs, 72hrs, 96hrs data was collected. Mortality of the treated beetles was compared with untreated beetles. Data recording was continued until all the released beetles were died. The average days in each replication was calculated to determine the days of 100% mortality of the released beetle.

3.8.2. Fecundity test

Average number of eggs per grain laid by released *C. chinensis* was calculated to check the effect of treatments on its oviposition/fecundity. From treated 100g mungbean seeds of each treatment, twenty grains were randomly selected from each replication and were carefully examined using magnifying glass and the number of eggs glued on the surface of mungbean was counted. At the end, their average was calculated to determine number of eggs per grain in each jar.

3.8.3. Effect on egg hatching

After 7 days of egg laying, 20 grains each with at least one egg of *C. chinensis* was taken from treated 100g mungbean seeds and cut by knife, the larvae inside the grain were carefully observed. The larvae of each treatment were recorded separately and the average number of egg hatching for each treatment was determined.

3.8.4. Effect on adult emergence

For this parameter, 20 mungbean seeds were taken where adult beetles laid eggs earlier. Then these seeds were treated with plant materials. After 20 days adult emergence was started. Number of newly emerged adults in each jar was recorded to observe the inhibition of *C. chinensis* emergence by different treatments. The number of emerged adults was counted daily. After every count, adults were removed from each plastic jar to avoid egg laying. This observation was continued from the first day of adult emergence to last day of emergence. Then average was taken to determine number of adult emerged in each replication.

3.8.5. Number of holes grain^{-1}

Average number of holes per grain was calculated by counting the number of holes made by *C. chinensis* on each grain. For this, twenty grains were randomly selected in each jar from 100g mungbean after adult emergence and number of holes on those grains was counted. Then average was taken to determine number of holes per grain in each replication.

3.8.6. Effect on grain infestation

After one month of treatment application, 20 grains were taken randomly from the treated 100g seeds of each treatment. Healthy and damage grains were separated and counted. After data collection grains were kept in plastic jar of the respective treatments. The extent of damage caused by pulse beetle on mungbean seeds was determined on the basis of the number of seeds eaten by the larvae of the beetle and then percent infestation by number was calculated from it. The weight of total grain was taken separately for each treatment and weight loss of treated mungbean seeds caused by *C. chinensis* was determined. The same data were collected after two months of treatment application.

Infested grain in untreated pot Infested grain in tobacco leaf treated pot

Figure 4. Infested grain in both untreated and treated pot

3.9. Percent inhibition rate (% IR)

Percentage reduction in emergence of adults or inhibition rate was calculated by using the following formula:

$$\% IR = (C_n - T_n) / C_n \times 100$$

Where,

C_n = Number of newly emerged adults in untreated jar (control)

T_n = Number of newly emerged adults in treated jar

3.10. Effect on Infestation rate

Twenty mungbean seeds treated with different treatments and put separately in jars. 5 pairs of beetle were released in each jar. Effect on infestation was calculated by computing infested/unhealthy grain after one and two months.

%infestation was calculated with following formula

$$\% IR = (C_I - T_I) / C_I \times 100$$

Where,

C_I = Number of infested grain in untreated jar

T_I = Number of infested grain in treated jar

3.11. Weight loss (%)

The percent weight loss was calculated at the end of experiment by using the following formula:

$$\text{Weight loss (\%)} = \frac{(A - B)}{A} \times 100$$

Where

A = initial weight

B = weight of Parameters on which data was collected

3.12. Percent reduction of infestation over control

Data recorded from each treated and untreated control pot were used to calculate and the percent reduction over control in terms of rate of oviposition, adult emergence, percent damage infestation, percentage of weight loss and percent number of holes present per grain using the following formula

$$\text{Percent reduction of infestation over control} = \frac{X2 - X1}{X2} \times 100$$

Where , X1 = the mean infestation of the treated pot

X2 = the mean infestation of the untreated pot

3.13. Statistical analysis

The data recorded were subjected to statistical analysis as Completely Randomized Design (CRD) using MSTAT-C and for Windows programs. Duncan's Multiple Range Test (DMRT) was applied to all the means. Moreover, the graphical work was done using Microsoft Excel program.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter comprises the explanation, presentation and interpretation of the results obtained from the study on the effect of five plants extracts each with two dosages on egg laying, incubation and adult emergence of pulse beetle, *C. chinensis* in mungbean. The results of this study have been presented as days of 100% mortality, effect on fecundity, number of holes, effect on adult emergence, effect on infestation rate, weight loss of mungbean seeds in storage.

4.1. Effect on adult mortality

The effect of plant extracts on days of 100% mortality is shown in the Figure 5. The highest result was found in T_8 (Tobacco leaf powder @ 2g/100g seeds), where 100% adult mortality was obtained in 3 days. In T_7, 100% mortality of released beetle was in 4 days. Next result was found in T_2 and T_3 where 100% mortality was obtained in 4.33 days and T_5 gave 100% mortality in 5 days. T_4 (chili @ 2g/100g seeds) and T_6 (Ginger @ 2g/100g seeds) gave 100% mortality at 5.67 days and in T_{10} (Neem @ 2g/100g seeds), 100% mortality occurred in 6.67 days. It required 7 days for 100% mortality of adult in T_1 (Turmeric @ 1g/100g seeds) and T_9 (Neem @ 1g/100g seeds). Lowest result was found in T_{11} (control) where 12.33 days was required for 100% mortality of the released beetle.

The result indicates that tobacco leaf powder @ 2.0g/100g mungbean seeds gave the best result regarding 100% adult mortality of pulse beetle. This result supports the findings of Lajide *et al.* (1998) who reported the effectiveness of some plant powders in controlling *S. zeamais* by causing adult mortality of the insect. The treatment with 0.4g *N. tabacum* powder had the highest weevil mortality than any other treatments.

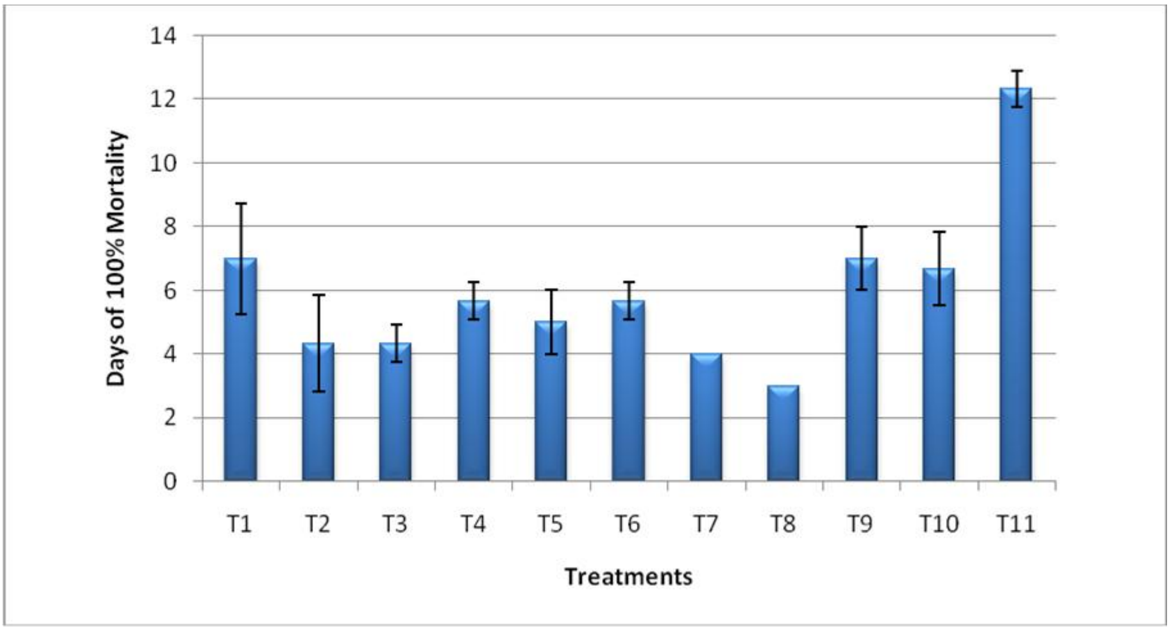

Figure 5. Comparative effects of different plant materials on the days of 100%

mortality of adult*C. chinensis*. T-shaped beams on bar indicate the standard deviation.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

4.2. Effect on fecundity

The mean number of egg laid by released *C. chinensis* is shown in the Table 3. After 24 hours, the lowest number of eggs (2.33) was found in T_8 (Tobacco @ 2g/100g seeds) followed by 3.0 in T_7 (tobacco @ 1g/100g seeds). Next performance is done by T_4 (Chili @ 2g), that was 5.0 and no significant difference was found with T_1 (Turmeric @ 1g/100g seeds), T_2 (Turmeric 2g), T_4 (Chili @ 2g/100g seeds), T_5 (Ginger @ 1g/100g seeds) and T_6 (Ginger @ 2g/100g seeds). The number of egg/20 grains in T_9 (Neem @ 1g/100g seeds), and T_{10} (Neem @ 2g/100g seeds) was 7.67 and 6.67, respectively having no significant difference with T_3 (6.33). T_{11} (control) had the highest number of eggs (15.33) present on grain which was significantly the higher number of eggs found on mungbean seeds than the others treated with plant materials. Furthermore, all the plant materials decreased egg laying of *C. chinensis* but best result was obtained from T_8 (Tobacco @ 2g/100g seeds) which reduced 83.71% egg laying (Table 3) of the pest which was significantly higher than all other plant materials treatments. Tobacco leaf powder @ 1g/100g seeds also gave the similar results in inhibition of egg laying of the pest.

Table 3. Rate of oviposition by*C. chinensis* on mungbean seeds treated with different plant materials

Treatments	**No. of grain observed**	**No. of eggs/20 grain**	**% decrease of egg laying over control**
T_1	20	6.00 bcd	59.56 c
T_2	20	5.67 bcd	62.81 c
T_3	20	6.33 bc	59.21 c
T_4	20	5.00 bcd	67.25 bc
T_5	20	6.00 bcd	61.43 c
T_6	20	6.00 bcd	60.4 c
T_7	20	3.00 cd	79.74 ab

T_8	20	2.33 d	83.71 a
T_9	20	7.67 b	50.79 c
T_{10}	20	6.67 bc	55.41c
T_{11}	20	15.33 a	--
LSD (0.05)		**3.387**	**15.62**

In a column, means with same letter(s) are not significantly different (P<0.05) by DMRT.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

Similarly, the per cent oviposition rate of *C. chinensis* on mungbean seeds significantly varied among the plant materials treated seeds. The Figure 6 demonstrated that the lowest rate of oviposition (11.65%) was found in T_8 (Tobacco @ 2g/100g seeds) followed by 15% in T_7 (Tobacco @ 1g/100g seeds). In T_4 (Chili @ 2g/100g seeds) rate of oviposition was found 25.0% and 28.3% rate of oviposition was found in T_2 (Turmeric @ 2.0 g/100g seeds). However, same results were shown in T_1(Turmeric @ 1g/100g seeds), T_5 (Ginger @ 1g/100g seeds) and T_6 (Ginger @ 2g/100g seeds) where oviposition rate was 30%. On the other hand, 31.65% rate of oviposition was found in T_3. In T_{10}(Neem @ 2g/100g seeds), this rate was 33.35%. In T_9 (Neem @ 1g/100g seeds), rate of oviposition was 38.3%. The highest rate of oviposition was found in T_{11} (control) which was 76.65%.

The result indicates that tobacco leaf powder @ 2.0g/100g mungbean seeds (T_8) gave the best result regarding oviposition rate of pulse beetle. This result supports the findings of Iqbal and Poswal (1995) who tested powdered spices (flowers of clove. rhizome of ginger and turmeric, fruits of chili and black pepper and bulb of garlic) against *C. maculates* and found best reduction in oviposition with chili powder.

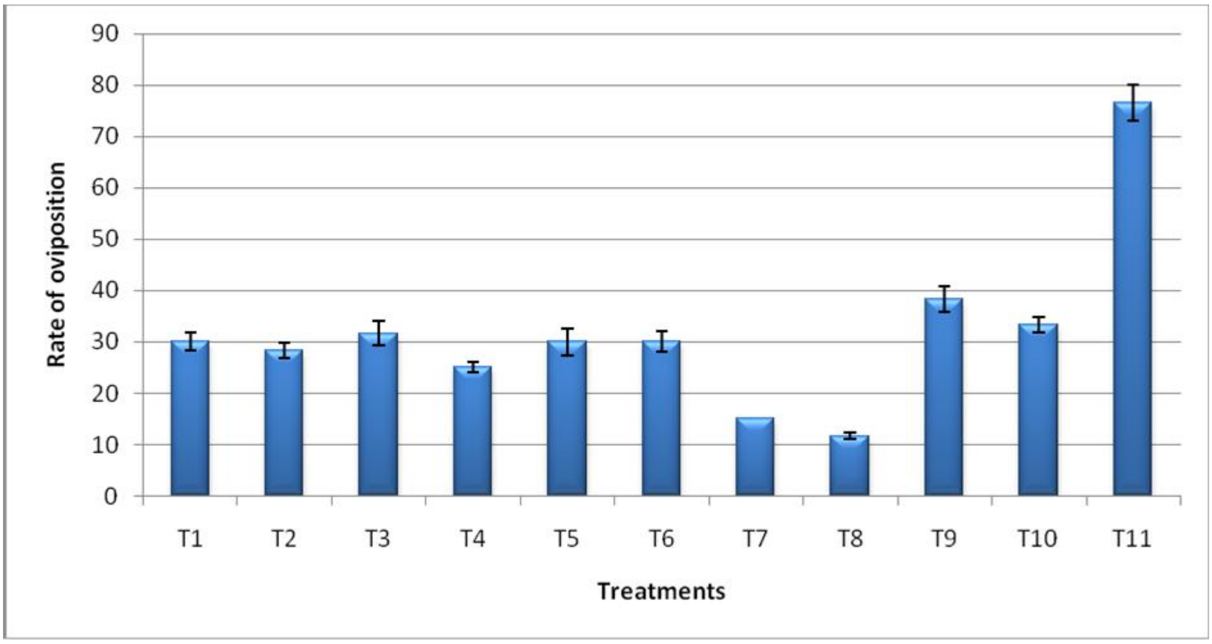

Figure 6. Comparative effects of different plant materials on the rate of oviposition by *C. chinensis* on mugbean. T-shaped beams on bar indicate the standard deviation.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

4.3. Effect on egg hatching

The data on the effect of different plant powders on egg hatching of *C. chinensis* has shown in the Table 5. It was based on number of larvae present inside 20 grain containing at least one egg/grain when it was cut after 7 days of egg laying. The lowest number of egg (2.33) hatched in T_8 (Tobacco @ 2g/100g seeds), followed by 2.67 in T_7 (Tobacco @ 1g/100g seeds), 4.33 in T_1 (Turmeric @ 1.0 g/100g seeds) and T_2 (Turmeric @ 2.0 g/100g seeds, and 5.0 in T_4 (Chili @ 2.0 g/100g seeds) having no significant difference among them. However, significantly higher number of egg hatched in T_9 (Neem leaf @ 1g/100g seeds) and T_{10} (Neem leaf @ 2g/100g seeds) Moreover, the highest number of egg (18.33) hatched in T_{11} (control) which was significantly different from all plant powder treatments.

Table 4. Number of egg of *C. chinensis* hatched from mungbean seeds treated with different plant materials

Treatments	No. of egg hatched/20grain	% decrease of egg hatching over control
T_1	4.33 def	76.45 abc
T_2	4.33 def	76.23 abc
T_3	7.33 cd	59.76 cd
T_4	5.00 def	72.42 abc
T_5	8.00 cd	55.21 cd
T_6	7.00 cde	61.24 bcd
T_7	2.67 ef	85.23 ab
T_8	2.33 f	87.19 a
T_9	10.33 bc	44.02 de
T_{10}	13.00 b	29.99 e
T_{11}	18.33 a	--
LSD(0.05)	**4.158**	**22.61**

In a column, means with same letter(s) are not significantly different ($P<0.05$) by DMRT.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

The data (Table 4) also expressed that both doses of Tobacco leaf powder gave more the 80% reduction of egg hatching over control and Neem leaf powder showed the lowest effectiveness in decreasing egg hatching of the bruchid. The rate of egg hatching was also found lowest (11.65%) in T_8 followed by 13.3% in T_7 (Figure 7). However, same level egg hatching percent (21.65%) was found in T_1 and T_2 which was higher than T_4 (25%). In T_6, 35.0% egg hatching was observed and 36.65% egg hatching was recorded from T_3 and 40% egg hatching occurred in T_5. However, in T_9 and T_{10}, the rate of egg hatching was higher than the other plant powder treatments (51.56% and 65%, respectively). The highest rate of egg hatching (91.65%) was observed in T_{11} (control).

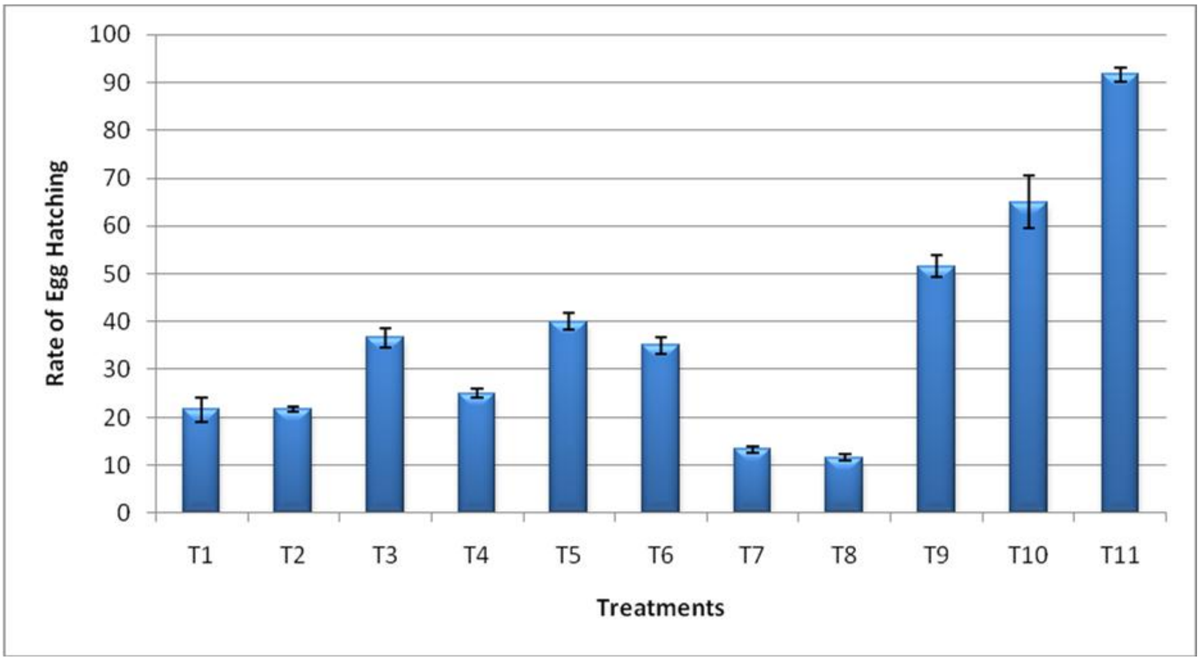

Figure 7.Comparative effects of different plant materials on egg hatching of *C. chinensis* on mugbean. T-shaped beams on bar indicate the standard deviation.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 = Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

4.4. Effect on adult emergence

The number of adult emergence of *C. chinensis* from the 20 grains containing eggs treated with different botanical powders has been presented in the Table 5. The total number of adult emerged from the treated 20 seeds ranged from 1.67 to 21.33 which differed significantly ($P<0.05$) among the treatments. After 18 days, the lowest number of adult (1.67) was emerged from T_8 (Tobacco @ 2g/100g seeds) followed by 2.33 was in T_7 (Tobacco @ 1g/100g seeds). No significant difference was found in T_8, T_7, T_1, T_4 and T_2 regarding adult emergence. But significant difference was observed with other treatments. Again, no significant difference was found in T_5 (7.33) and T_6 (6.33) considering this parameter. The highest number of adult emergence (21.33) was found in T_{11} (control). T_9 (Neem @ 1g/100g seeds) and T_{10} (Neem @ 2g/100g seeds) gave the similar result (9.67 and 13.0 respectively) which was least effectiveness against the bruchid regarding adult emergence. Considering per cent decrease of adult emergence over control T_8, T_7, T_1 and T_4 provided standard effect against the *C.*

chinensis which was more than 80%. Moreover, other botanicals also offered more than 55% decrease of adult emergence over control (Table 5).

The percent adult emergence of *C. chinensis* from 20 mungbean seeds treated with different plant materials is shown in Figure 8. The lowest result (8.35%) was obtained from T_8 (Tobacco @ 2g/100g seeds) followed by 11.65%, 18.35%, 20% and 28.35% from T_7 (Tobacco @ 1g/100g seeds), T_1 (Turmeric powder @ 1g/100g seeds), T_4 (Chili powder @ 2g/100g seeds) and T_2 (Turmeric powder @ 2g/100g seeds), respectively. Moreover, 31.65% adult emergence occurred in T_6 (Ginger @ 2g/100g seeds). In T_5 (Ginger @ 1g/100g seeds),the adult emergence rate was 36.65% and 65% adult was emerged from T_{10}. The highest rate of adult emergence (106.65%) was found in T_{11} (control) which indicated that more than one adult was emerged from single grain.

Table 5. Adult emergence of *C. chinensis* from mugbean seeds treated with different plant materials

Treatments	**Adult emergence/20grain**	**% decrease of adult emergence over control**
T_1	3.67 def	82.48 abc
T_2	5.67 cdef	72.93 abcd
T_3	8.33 c	60.79 d
T_4	4.00 def	81.05 abc
T_5	7.33 cd	64.78 cd
T_6	6.33 cde	70.27 bcd
T_7	2.33 ef	88.93 ab
T_8	1.67 f	92.04 a
T_9	9.67 bc	55.13 de
T_{10}	13.00 b	39.31 e
T_{11}	21.33 a	**--**
LSD(0.05)	**3.646**	**17.81**

In a column, means with same letter(s) are not significantly different (P<0.05) by DMRT.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

The result on effects of plant materials on adult emergence of *C. chinensis* indicates that tobacco leaf powder @ 2.0g/100g or @ 1.0g/100g mungbean seeds gave the best in reducing adult emergence of pulse beetle from treated seeds and both doses of neem leaf powder showed the least effectiveness among all plant powders. This result supports the report of (Aslam, 2004) who found that 0.4g *N. tabacum* powder gave the highest weevil mortality (*S. zeamais*) as well as reduced number of adult emergence than any other treatments.

Figure 8. Comparative effects of different plant materials on adultemergence of *C. chinensis* on mungbean. T-shaped beams on bar indicate the standard deviation.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

4.5. Effect on holes per grain

The number of holes by *C. chinensis* is shown in the Table 6. The lowest number of holes (0.08/grain) was found in T_8 (Tobacco @ 2g/100g seeds) followed by 0.12 in T_7. No significant difference was observed among T_8, T_7, T_1, T_4 and T_2. In contrast, the highest number of holes (1.07) was found in T_{11}(control) which was significantly higher than all other treatments. Similarly, all the plant materials treatments decreased the number of holes/grain and best result was found for T_8 which reduced 92.04% holes/grain. More than 80% reduction was found in case of T_8, T_7, T_1 and T_4 (Table 8).

Table 6. Number of holes/grain caused by *C. chinensis* on mugbean seeds treated with different plant materials

Treatments	No. of grain observed	No. of holes/grain	% decrease of holes over control
T_1	20	0.18 def	82.48 abc
T_2	20	0.28 cdef	72.93 abc
T_3	20	0.42 c	60.79 bcd
T_4	20	0.20 def	81.05 abc
T_5	20	0.37 cd	64.78 abcd
T_6	20	0.32 cde	70.27 abc
T_7	20	0.12 ef	88.93 ab
T_8	20	0.08 f	92.04 a
T_9	20	0.48 bc	55.13 cd
T_{10}	20	0.65 b	39.31 d
T_{11}	20	1.07 a	--
LSD(0.05)		**0.186**	**6.04**

In a column, means with same letter(s) are not significantly different ($P<0.05$) by DMRT.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

The rate of holes of *C. chinensis* on mungbean treated with different plant material is showed in Figure 9. The lowest result (8.35%) was found in T_8 (Tobacco 2g) followed by 11.65% in T_7. In T_1, rate of holes was 18.35%. 20% rate of holes was found in T_4(Chili 2g). 28.35% rate of holes was seen in T_2 (Turmeric 2g). However, T_6 showed 31.65% rate of holes and in T_5, the rate of holes was 36.65%. In T_3 and T_9, nearer rate of holes was shown by them and these was (41.65% and 48.35%). In T_{10}, rate of holes was 65%. The highest rate of holes was found in T_{11} (control). The rate was 106.65% which indicates that more than one number of insect emerged from per seed.

The result indicates that Tobacco leaf powder @2.0g/100g or 1g/100g mungbean seeds gave the best result regarding number of holes on mungbean seeds treated with different plant material attacked by *C. chinensis*. This result support the findings of Singh (2006) who reported that plant materials treatment lowered percent holed on cowpea seeds compared with the control. Tobacco leaf powder was the most effective with 11.0% holed cowpea seeds, followed by Zinger powder (15.8%). Hot pepper powder (HPP) was the least effective in protecting cowpea seeds against damage by *C. chinensis* with 39% holed cowpea seeds.

Figure 9.Comparative effects of different plant materials on the rate of holes of *C. chinensis* on mugbean.T-shaped beams on bar indicate the standard deviation.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

4.6. Effect on grain infestation by number

The effect of plant materials on grain infestation by *C. chinensis* of treated mugbean seeds is shown in the Table 7. The extent of damage caused by pulse beetle on mungbean seeds was determined on the basis of the number of seeds eaten by the larvae of the beetle. After one month, the lowest number of infested grain (2.33) was observed in T_8 (Tobacco @ 2g/100g seeds) and followed by 2.67, 4.33, 5.0 in T_7 (Tobacco @ 1g/100g), T_1 (Turmeric @ 1g/100g) and T_4 (Chili @ 2g/100 seeds) respectively. Grain infestation in number was equal 4.33 in T_1 and T_2. Therefore no significant difference was observed among T_8, T_7, T_1, T_2 and T_4. Low performance among all treatments was shown by T_9 (Neem @ 1g/100g) and T_{10} (Neem @ 2g/100g) where grain infestation by number was 10.33 and 13.0 respectively. However, no significant difference was observed among T_3(Chili @ 1g/100g), T_5(Ginger @ 1g/100g) and T_6(Ginger @ 2g/100g).The highest grain infestation by number (18.33) was found in T_{11} (control), which was significantly higher than any other treatments.

After two month, similar type of result found again but the infestation number was higher than previous. Lowest result was found in T_8 (4.33) and followed by 4.67 in T_7.However, no significant difference was observed among T_2 (Turmeric @ 2g/100g), T_7 and T_8. The grain infestation by number was equal (7.0) in T_1 (Turmeric @ 1g/100g) and T_6 (Ginger @ 2g/100g). Therefore, no significant difference was observed among T_3 (Chili @ 1g/100g), T_4 (Chili @ 2g/100g) and T_5 (Ginger @ 1g/100g). On the other hand, T_9 (Neem @ 1g/100g) and T_{10} (Neem @ 2g/100g) showed the lowest performance among the treatments where number of infestation was found 9.0 and 10.33. The highest number of infestation value was 19.0 which was observed in T_{11} (control).

Grain infestation after two months by treated *C. chinensis* is shown in the Figure 10. After 2 months, the lowest infestation 21.67% was observed in T_8(Tobacco @ 2g/100g). The rate of infestation was 23.33% in T_7(Tobacco @ 1g/100g). Infestation was nearly equal in T_1 (Turmeric @ 1g/100g) and T_4 (Chili @ 2g/100g) and those were 36.67% and 38.33%. Again T_3(Chili @ 1g/100g) and T_9 (Neem @ 1g/100g) showed equal result (45%) and followed by 41.67% in T_5 (Ginger @ 1g/100g) . The highest infestation was observed in T_{11} (control) and that was 95%.

Table 7. Number of grain infestation by *C. chinensis* on mungbean treated with different plant material

Treatments	No. of grain observed	No. of infested grain after 1month	No. of infested grain after 2month
T_1	20	4.33 def	7.00 de
T_2	20	4.33 def	6.00 ef
T_3	20	7.33 cd	9.00 bc
T_4	20	5.00 def	7.67 cde
T_5	20	8.00 cd	8.33 cd
T_6	20	7.00 cde	7.00 de
T_7	20	2.67 ef	4.67 ef
T_8	20	2.33 f	4.33 f
T_9	20	10.33 bc	9.00 bc
T_{10}	20	13.00 b	10.33 b
T_{11}	20	18.33 a	19.00 a
LSD(0.05)		**1.817**	**9.085**

In a column, means with same letter(s) are not significantly different ($P<0.05$) by DMRT.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

The result on effects of plant materials on adult emergence of *C. chinensis* indicates that tobacco leaf powder @ 1.0g/100g or @ 2.0g/100g mungbean seeds gave the best in reducing grain infestation on mungbean by pulse beetle treated with different plant material and both doses of neem leaf powder showed the least effectiveness among all plant powders.

This result contradicts with the findings of Su (1984). Who reported that, both black pepper and green pepper gave good protection to stored product insects against *C. maculatus* and *Tribolium confusum*. Even the lowest doses of 250 ppm gave 57.1% protection.

Figure 10. Comparative effects of different plant materials on the percent grain infestation by *C. chinensis* on mungbean. T-shaped beams on bar indicate the standard deviation.

T_1= Turmeric powder (1g), T_2= Turmeric powder (2g), T_3= Red chili powder (1g), T_4= Red chili powder (2g), T_5= Ginger powder (1g), T_6=Ginger powder (2g), T_7= Tobacco leaf powder (1g), T_8= Tobacco leaf powder (2g), T_9= Neem leaf powder (1g), T_{10}= Neem leaf powder (2g) and T_{11}= Untreated control.

4.7. Effect on weight loss

The weight loss by the attack of *C. chinensis* is shown in the Table 8.The amount of weight loss of treated mugbean seeds caused by the pulse beetle ranged from 2.47g to 9.47g at first month. The lowest weight loss of grain 2.47g was observed in T_8 (Tobacco @ 2g/100g) and followed by 3.03g in T_7.The weight loss showed no significant difference in T_9 (Neem @ 1g/100g) and T_{10} (Neem @ 2g/100g). Besides, these are the lowest performance among the plant materials used in this test. T_4 (Chili

@ 2g/100g), T_7 (Tobacco @ 1g/100g) showed equal weight loss 3.03g and followed by 3.77g in T1 (Turmeric @ 1g/100g). However, no significant difference was observed among T_2 (Turmeric @ 2g/100g), T_3 (Chili @ 1g/100g), T_5 (Ginger @ 1g/100g) and T_6 (Ginger @ 2g/100g). The highest weight loss 9.47g after 1 month was observed in T_{11}(control).

The lowest weight loss (3.77g) after two month was observed in T_8 (Tobacco @ 2g/100g) followed by 5.70g in T_7 (Tobacco @ 1g/100g). Therefore, no significant difference in weight loss was observed among T_2 (Turmeric @ 2g/100g), T_4 (Chili @ 2g/100g) and T_5 (Ginger @ 1g/100g). Higher weight loss among the treatments was observed in T_9 (Neem @ 1g/100g) followed by 8.67g and 8.47g in T_{10} (Neem @ 2g/100g) and T_1 (Turmeric @ 1g/100g). However, they have no difference significantly. Highest weight loss was observed in T_{11}(control), where weight loss was recorded 19.90g.

Weight loss of mungbean seeds was finally recorded after three months where lowest result was recorded in T_8 (Tobacco @ 2g/100g) and that was 4.10g. However, it was observed 5.97g weight loss in T_7 (Tobacco @ 1g/100g). No significance difference was observed among T_1 (Turmeric @ 1g/100g), T_2 (Turmeric @ 2g/100g), T_4 (Chili @ 2g/100g) and T_5 (Ginger @ 1g/100g). In case of T_9 (Neem @ 1g/100g) and T_{10} (Neem @ 2g/100g), weight loss was higher than the other treatments (10.30g and 9.50g). The highest weight loss (20.33g) was observed in T_{11} (control). Though, many variations were found in the results but all the treatments decreased the weight loss significantly.

The percent weight loss of mungbean treated with different plant material against *C. chinensis* after three months is showed in Figure 11. The lowest percent loss of weight (4.10%) was observed in T_8(Tobacco @ 2g/100g). However, in T_7 (Tobacco @ 1g/100g), the nearer weight loss (5.97%) was occurred. In T_3 (Chili @ 1g/100g), percent weight loss was 8.20%, followed by 8.43% and 8.47% in T_5 (Ginger @ 1g/100g) and T_2(Turmeric @ 2g/100g). Though they have different value but they are bearing same value statistically. In T_1 (Turmeric @ 1g/100g), the percent weight loss is 9.17% and followed by 9.50% in T_{10} (Neem @ 2g/100g). Again, in T_6 (Ginger @

2g/100g), percent weight loss was 9.83%. Percent weight loss was observed as 10.30% In T_{10} (Neem @ 2g/100g). The highest percent weight loss was in T_{11} (control) and that was 20.33%. All the treatments reduced the weight loss of mungbean by reduction of infestation of pulse beetle.

The result indicates that Tobacco leaf powder @ 2.0g/100g or 1g/100g mungbean seeds gave the best result regarding weight loss of mungbean seeds treated with different plant material attacked by *C. chinensis*. This result contradicts with the findings of Jilani and Amir (1987). Who reported that, application of neem leaf powder can control *C. chinensis*more than 80% attacked on mungbean seeds and the treatments remained effective up to 13 months.

However the result support the findings of Dixit and Saxena (1990) reported that various plant products (powder, extracts and oils) are reported to be effective against the pulse in terms of weight loss.

Table 8. Weight loss of mungbean seeds treated with different plant material due to damage caused by *C. chinensis*

Treatments	weight loss after 1 month (g)	weight loss after 2 months (g)	weight loss after 3 months (g)
T_1	3.77 cde	8.47 cd	9.17 def
T_2	4.20 bcd	7.93 de	8.47 ef
T_3	4.43 bc	10.10 b	10.70 b
T_4	3.03 de	7.03 e	8.20 f
T_5	4.93 bc	8.03 de	8.43 ef
T_6	5.37 b	9.10 bcd	9.83 bcd
T_7	3.03 de	5.70 f	5.97 g
T_8	2.57 e	3.77 g	4.10 h
T_9	4.00 cd	9.53 bc	10.30 bc
T_{10}	3.93 cd	8.67 cd	9.50 cde
T_{11}	9.47 a	19.90 a	20.33 a
LSD(0.05)	**1.189**	**1.188**	**0.991**

In a column, means with same letter(s) are not significantly different ($P<0.05$) by DMRT.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 =

Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

Figure 11. Comparative effects of different plant materials on the percent weight loss of *C. chinensis* on mugbeanT-shaped beams on bar indicate the standard deviation.

T_1 = Turmeric powder (1g), T_2 = Turmeric powder (2g), T_3 = Red chili powder (1g), T_4 = Red chili powder (2g), T_5 = Ginger powder (1g), T_6 =Ginger powder (2g), T_7 = Tobacco leaf powder (1g), T_8 = Tobacco leaf powder (2g), T_9 = Neem leaf powder (1g), T_{10} = Neem leaf powder (2g) and T_{11} = Untreated control.

CHAPTER 5

SUMMARY AND CONCLUSION

The present investigation was conducted at the Entomology lab of the Sher-e-Bangla Agricultural University, Dhaka, during the period from April 2013 to November 2013. The experiment consisted of eleven different treatments viz., T_1 (Turmeric powder @ 1g/100g), T_2 (Turmeric powder@ 2g/100g), T_3 (Chili powder @ 1g/100g), T_4 (Chili powder @ 2g/100g), T_5 (Ginger powder @ 1g/100g), T_6 (Ginger powder @ 2g/100g), T_7 (Tobacco leaf powder @ 1g/100g), T_8 (Tobacco leaf powder @ 2g/100g), T_9 (Neem leaf powder @ 1g/100g), T_{10} (Neem leaf powder @ 2g/100g) and T_{11} (Untreated control). Each treatment was replicated three times in a Completely Randomized Design (CRD). Firstly all treatments were applied in 100g seed. Data were collected in respect to oviposition rate, egg hatching, adult emergence, percent grain infestation, percent weight loss and percent number of holes done by pulse beetle. Effect of five plant extracts on these parameters is summarized below.

The effectiveness of different plant materials against *Callosobruchus chinensis* in terms of days of 100% mortality differed significantly. The highest result was found in T_8 (Tobacco leaf powder @ 2g/100g), where 100% adult mortality was obtained in 3 days. In T_7, 100% mortality of released beetle was in 4 days. The next result was in T_2 and T_3 where 100% mortality was obtained in 4.33 days. Then T_5 having the 100% mortality In 5 days. T_4 (chilli @ 2g/100g) and T_6 (Ginger @ 2g/100g) obtained 100% mortality at 5.67 days. In T_{10} (Neem @ 2g/100g), 100% mortality occurred in 6.67 days. It required 7 days for 100% mortality of adult in T_1 (Turmeric @1g/100g) and T_9 (Neem@ 1g/100g). Lowest result was found in T_{11} (control) where 12.33 days was required for 100% mortality of the released beetle.

Considering the rate of oviposition, the lowest rate of oviposition (11.65%) was found in T_8 (Tobacco @ 2g/100g). In T_7, oviposition rate is found 15%. In T_4 (Chilli @ 2g/100g) rate of oviposition was found 25%. 28.3% rate of oviposition was found in

T_2. However, same results were shown in T_1, T_5 and T_6 where oviposition rate was 30%. On the other hand, 31.65% rate of oviposition was found in T_3. In T_{10}, this rate was 33.35%. In T_9 (Neem @ 1g/100g), rate of oviposition was 38.3%. The highest rate of oviposition was found in T_{11} (control) which was 76.65%.

Considering the rate of adult emergence of*C. chinensis* on mugbean treated with different plant material, the lowest result of adult emergence was found in T_8 (Tobacco @ 2g/100g) which was 8.35%. Then the nearest lowest result was in T_7 (11.65%). In T_1, adult emergence rate was 18.35%. 20% adult emergence rate was found in T_4 (Chilli @ 2g/100g). Adult emergence was seen 28.35% in T_2 (Turmeric @ 2g/100g). However, T_6 showed 31.65% adult emergence where Ginger @ 2g/100g was used as treatment. In T_5, the adult emergence rate was 36.65%. In T_3 and T_9, nearer adult emergence rate was shown by them and these was (41.65% and 48.35%). In T_{10}, adult emergence rate was 65%. The highest rate of adult emergence was found in T_{11} (control). The rate was 106.65% which indicates that more than one number of insect emerged from per seed.

The effectiveness of different plant materials on therate of holes of *C. chinensis* on mugbean was identical. The lowest result of rate of holes was found in T_8 (Tobacco 2g) which was 8.35%. Then the nearest lowest result was in T_7 (11.65%). In T_1, rate of holes was 18.35%. In T_4(Chilli 2g), rate of holes was found as 20% . In T_2 (Turmeric 2g), rate of holes was seen as 28.35%. However, T_6 showed 31.65% rate of holes where Ginger 2g was used as treatment. In T_5, the rate of holes was 36.65%. In T_3 and T_9, nearer rate of holes was shown by them and these was (41.65% and 48.35%). In T_{10}, rate of holes was 65%. The highest rate of holes (106.65%) was found in T_{11} (control).

Considering the rate of egg hatching of *C. chinensis* on mugbean treated with different plant material, the lowest rate of egg hatching was recorded in T_8 (Tobacco @ 2g/100g) where the rate was was 11.65%. Then the nearest result was found in T_7 (13.3%). However same result was found in the rate of egg hatching in T_1 and T_2 (21.65%). Then the next rate was found in T_4 (25%). In T_6, 35% rate of egg hatching was observed. 36.65% rate of egg hatching was observed in T_3. In T_5, the rate of egg hatching was 40%. However, in T_9 and T_{10}, the rate of egg hatching was higher than the other treatments (51.56% and 65%). The highest rate of egg hatching was observed in T_{11} (control) and that was 91.65%.

In case of grain infestation after two months by treated *C. chinensis*, the lowest infestation 21.67% was recorded in T_8(Tobacco @ 2g/100g). The rate of infestation was observed 23.33% in T_7. Again T_3 and T_9 showed equal result and that infestation rate was 45%. T_5 had the nearer result to them followed by 41.67%. In T_2, 30% seed infested by *C. chinensis* after two months and T_6 followed by 35%. In T_9, the rate of infestation was 45%. T_{10} showed nearer result 51.67% . In the untreated control (T_{11}) the rate of infestation was very high and that was followed by (95%).

Considering the rate of weight loss of mugbean treated with different plant material after three months, the lowest percent loss of weight (4.10%) was observed in T_8(Tobacco @ 2g/100g). In T_7, the nearer lowest weight loss (5.97%) was occurred. In T_3, percent weight loss was 8.20% and followed by 8.43% and 8.47% in T_5 and T_2. Though they have different value but they are bearing same value statistically. In T_1, the percent weight loss is 9.17% and followed by 9.50% weight lost in T_{10}. In T_6, percent weight loss was 9.83%. In T_{10} (Neem 2g) , percent weight loss was 10.30%. The highest percent weight loss was in T_{11}(Control) and that was 20.33%. All the treatments reduced the weight loss of mungbean by reduction of infestation of pulse beetle.

With all the observation the best performance was found in T_8 (Tobacco @ 2g/100g). Satisfactory result was found in T_7 (Tobacco @ 1g/100g), T_2 (Turmeric @ 2g/100g) and T_4 (Chilli @ 2g/100g). Mainly Tobacco leaf but other three also can be used as a protectant of *C. chinensis* for mugbean in storage condition. Other treatments also showed significantly nearer responses. Some of them can show different results in other weather condition. Further study will clear the dissimilar attitude of the treatments in the same condition. However, further studies in the following areas are suggested:

1. Other botanicals may be included in the further study.
2. Proper dose of botanicals should be determind.

3. Environmental factors should be considered.

CHAPTER 6

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