RESISTANCE SOURCE(S) AMONG DIFFERENT MUNGBEAN VARIETIES AGAINST PULSE BEETLES AND THEIR ALTERNATIVE MANAGEMENT

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This is to certify that the Thesis entitled, "RESISTANCE SOURCE(S) AMONG DIFFERENT MUNGBEAN VARIETIES AGAINST PULSE BEETLES AND THEIR ALTERNATIVE MANAGEMENT" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh in the partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (M. S.) IN ENTOMOLOGY, embodies the result of a piece of bona fide research work carried out by MOHAMMAD ASADUZZAMAN MIAH bearing Registration No. 02178 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 been duly acknowledged.

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Dated:

Place: Dhaka, Bangladesh

DEDICATED TO MY BELOVED PARENT

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RESISTANCE SOURCE(S) AMONG DIFFERENT MUNGBEAN VARIETIES AGAINST PULSE BEETLES AND THEIR ALTERNATIVE MANAGEMENT

BY

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ABSTRACT

The experiments were conducted in the laboratory under the Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from March, 2007 to October, 2007 to screen some mungbean varieties for resistance to pulse beetles and to find the most effective component(s) among some selected botanicals applied against pulse beetles. Considering the laboratory screening of nine mungbean varieties for resistance against Callosobruchus chinensis Linn. and C. maculatus Fab., in both free choice and no choice tests more or less similar trends of results were found for both the species. In case of Callosobruchus chinensis Linn., the variety BAR1 Mug-6 was highly susceptible; BARI Mug-4, BARI Mug-3 and BARI Mug-5 were susceptible; BARI Mug-2 and BINA Mug-1 were moderately resistant, while BU Mug-2, Barisal local and BU Mug-1 were found to be highly resistant host for C. chinensis in respect of egg deposition, growth and development, adult emergence and grain content loss. But there was no varietal preference on egg hatching. In terms of infestation by C. maculatus Fab., the varieties Barisal local, BARI Mug-3, BARI Mug-4, BARI Mug-5 and BINA Mug-1 were highly susceptible; BARI Mug-6 was moderately susceptible, BU Mug-1 was susceptible, while BARI Mug-2 and BU Mug-2 were found to be moderately resistant to Callosobruchus maculatus in respect of growth and development, adult emergence and grain content loss except egg deposition, but there was no varietal influence on egg hatching. For both free choice and no choice tests, the adult emergence started at 18 and 20 days after release (DAR) of adults of C. chinensis and C. maculatus respectively, then the number of adult emergence increased gradually and maximum adults were emerged at 20-21 DAR and 22 DAR respectively and then decreased gradually. Considering the effectiveness of different botanicals during the management of *C. chinensis* and *C. maculatus*, infesting BARI Mug-6 and Barisal local respectively, the treatment T₃ comprising Camphor @ 2 gm/kg seeds, T₁ comprising Neem oil @ 8 ml/kg seeds and T₂ comprising Castor oil @ 8 ml/kg seeds performed the best results in respect of percent reduction (100%) of larvae and pupae developed internally, damaged seed and grain content loss over control for both the species, while partial reduction occurred by Neem oil and Castor oil in terms of egg deposition, egg hatching. Considering the effectiveness of different botanicals on viability of seeds during the management of pulse beetle, there was no significant effect of Camphor, Neem oil and Castor oil in reducing the viability of seeds, while other treatments (comprising Neem leaf powder 5% w/w, Castor leaf powder 5% w/w and Bankolmi leaf powder 5% w/w) reduced the viability of seeds.



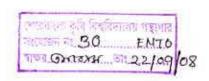
Chapter I Introduction

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CHAPTER I



INTRODUCTION

Pulses serve as one of the main sources of protein and minerals as well as play a vital socio-economic role in the diet of common people of Bangladesh. Among the pulses, mungbean, Vigna radiata (L) Wilezek has come up an important pulse crop in Bangladesh. It contains 51% carbohydrate, 26% protein, 4% minerals, 3% vitamins (Yadav et al., 1994). Its sprout is a high quality vegetable and rich in vitamin-C. Bioavailability of iron in sprouts is twelve-fold high as compared to mungbean as a dhal (soup). A four-fold increase in iron availability of mungbean can be achieved through cooking with vegetables like tomato. 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).

At present only one-forth of the total requirements of pulse are produced in the country. Among the pulses, the cultivated area of mungbean in Bangladesh was 55,000 acres with an annual (2005-2006) production of 17,000 tons and the average production was about 300 kg/acre (BBS, 2006). But as a part of whole pulses, this production of mungbean does not fulfill our total demand.

The traders mostly store the pulses produced in the country. The primary producers also store the pulses at least for few months before they sell it. Almost all growers store the required quantity of pulse seeds in their houses for growing next year. Unfortunately, in storage, pulses suffer enormous losses due to bruchid attack, which infestation starts either in the field on the maturing pod and is carried to the stores with the harvested crops or it originates in the storage itself (Fletcher and Ghosh, 1920). Three species of pulse beetles, viz., Callosobruchus chinensis Linn., C. analis Fab., and C. maculatus Fab. have been reported from Bangladesh as the pest of stored pulses (Begum et al., 1984; Rahman et al., 1981 and Alam, 1971). However, Alam (1971) reported that Callosobruchus chinensis

to cause enormous losses to almost all kind of pulses in storage condition. Rahman (1971) reported 12.5% loss due to pulse beetles infestation in pulses stored in warehouses. Ali et al. (1999) reported that mungbean, Vigna radiata appeared to be the most common and suitable host for C. chinensis in respect of oviposition, egg deposition, adult emergence (66.11-70.29%) and grain content loss (50.37 - 57.58%) but no significant influence on egg hatching (94.33 - 98.50%). Ali and Rahman (2006) also reported that mungbean, Vigna radiata appeared to be the most common and suitable host for the species C. maculatus in respect of oviposition, larvae (58.21-76.31%), pupae (55.35-64.40%), adult emergence (33.18-46.62%) and grain content loss (37.30 - 55.30%). In India, Gujar and Yadav (1978) reported 55-60% loss by seed weight and 45-66% loss in protein content of mungbean by the pulse beetles. According to Chowdury (1961), the extent of damage might be up to 100% in mungbean seed during a period of one year in storage.

Several techniques are used as management approaches for pulse beetles. However, the most effective and environmentally safe method is the planting of tolerant varieties if available. Thus development of resistant varieties is considered to be the best option for the control of bruchid infestation. Many varieties have been released by different research organizations without testing their reaction against pulse beetles. Sison *et al.* (1996) reported among several mungbean entries, TC 1966 was highly resistant while the Pag-asa (1, 3, 5 and 7) was susceptible to *Callosobruchus chinensis*. In Pakistan, Ashraf *et al.* (1991) reported that among different mungbean varieties, Mung-141 (27.2% wt. loss) was relatively susceptible to *C. chinensis*, whereas Mung-1 appeared to be resistant. In Bangladesh, Muhammad *et al.* (1997) reported that among different strains/varieties of mungbean MB-46 and Kanti were found to be highly susceptible, with 13.6% and 13.0% loss in weight of seeds, respectively; strains MB-87, MB-66 were susceptible and strains MB-63 and MB-55 were moderately

susceptible to *C. chinensis*. But there is no bruchid resistant mungbean variety/genotype available for cultivation in Bangladesh.

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 the cumbersome traditional methods for the control of various insect pests of crops and storage.

In the world, as many as 2400 plant species have been recorded that have potential pesticidal properties and biological activity against a wide range of pests (Grainge and Ahmed, 1988). In Bangladesh, as many as 54 plant species have been evaluated for their bio-efficacy against different insect pests, pathogens and weeds (Karim, 1994). 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 (Keita et al., 2001; Shaaya et al., 1997; Onu and Aliyu, 1995 and Talukder and Howse, 1994). A number of botanicals and their derivatives have been tested in Bangladesh and

other developing countries particularly against pulse beetles and have shown promising results (Yadava and Bhatnagar, 1987; Mahadi and Hamoudi, 1984 and Saxena and Yadav, 1983). Bhuiyah (2001) reported that the oils of neem, Royna and Castor at 6 and 8 ml/kg and leaf powder of Biskatali, Marigold and Castor at 5% w/w were most effective in preventing the egg laying in lentil and chickpea and leaf powder of Biskatali, Marigold, Castor and Mango at 5% were most effective in reducing the adult emergence in lentil and chickpea, whereas the adult emergence were nil in pre and post storage release methods. Mummigatti and Ragunathan (1977) reported that castor, mustard and gingerly oils inhibited the multiplication of *C. chinensis* on green gram at 0.3% level, coconut and groundnut oils at 0.5 % level and sunflower oil was ineffective even at 0.5% level. Babu *et al.* (1989) reported that among neem, karanja, mustard, groundnut and castor oil; the karanja (5 and 10 ml/kg) and castor (10 ml/kg) oil effectively reduced oviposition by the bruchid (*C. chinensis*) under conditions of artificial infestation.

Now a day, Camphor (C₁₀H₁₆O), locally named as Karpur in Bangladesh is very new and unexploited approach in this context which occurs in the leaves and wood of Camphor tree (Cinamomum camphora). It is a white transparent waxy solid with a strong penetrating pungent aromatic odor and available at grocery stores, and mostly used in medical purposes (Mann et al., 1994). Abiverdi (1977) reported the insecticidal efficacy of camphor. Chauvin et al. (1994) reported that camphor has fumigation properties and has got a very low mammalian toxicity. Rahman et al. (2001) reported the fumigation action of camphor against pulse beetle, C. chinensis. In Bangladesh, very little study has so far been reported on the efficacy of camphor against pulse beetles.

Therefore, the present study was under taken to find out the resistant or tolerant mungbean variety(s) against pulse beetles and to test the approaches comprising some selected botanicals and/or their derivatives to evaluate their performances in combating these pests considering the following objectives:

OBJECTIVES

- 1. To identify the resistant mungbean variety(s) against Callosobruchus chinensis Linn. and C. maculatus Fab.
- To determine the level of infestation by C. chinensis and C. maculatus among different mungbean varieties.
- 3. To explore the effective component(s) among different alternative management practices against C. chinensis and C. maculatus.

Chapter II Review of literature

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CHAPTER II

REVIEW OF LITERATURE

Bruchids commonly known as pulse beetles are the most serious pests of stored pulses in Bangladesh (Rahman, 1971). The species of pulse beetles so far reported to infest various pulses include Callosobruchs chinensis Linn., C. analis Fab. and C. maculatus Fab. (Begum et al., 1984; Rahman et al., 1981 and Alam, 1971). Results revealed that mungbean, Vigna radiata appeared to be the most common and suitable host for C. chinensis (Ali et al., 1999) and C. maculatus (Ali and Rahman, 2006) in respect of ovipositional preference, egg deposition, internal larvae and pupae developed, adult emergence and grain content loss but no significant influence on egg hatching. But very few works have so far been done for identifying the suitability or resistance source(s) among the available seeds of mungbean varieties and their efficacy against bruchids in Bangladesh. However, voluminous reports are available from studies conducted throughout the world including India, Pakistan, Thailand, Japan, the U.K and the U.S.A. on the susceptibility or resistance of various grain legumes to infestation by bruchids. Redden and McGuire (1983) reported various criteria for the assessment of suitability or resistance of grain legumes to bruchid infestation. These include number of eggs laid per experimental unit, percentage of egg hatching, internal larvae and pupae developed and/or adult emergence over a given period, percentage of weight loss and undamaged seeds.

Literatures on some of such studies relevant to the present study collected from various sources including Bangladesh were reviewed here in brief under the following sub-headings. Prior to this, the biology, identification of male and female, nature and extent of damage, hosts preference, origin and distribution of the pulse beetles pertaining to the identification and evaluation of resistance source(s) among different mungbean varieties against these pests were also reviewed.

1. BIOLOGY OF PULSE BEETLES

a. Mating

Adults of bruchids are sexually mature at emergence and ready to mate (Raina, 1970). According to Bhuiyan and Peyara (1978), the mating takes place (Plate 1) immediately after emergence of the adult beetles. In laboratory condition, mating left for 2 to 3 minutes. When a male and female of *Callosobruchus chinensis* Linn. are introduced, the male moves first around rather sluggishly but suddenly beings to move with rapid antennal movement. This sort of behavioral change is called 'Activation' and this activation can not be seen when a male is alone (Nakamura, 1969).

b. Oviposition and incubation

The female oviposits within 24 hours of their emergence and mating. The ovipositional period ranges from 3-8 days depending upon the season of the year. According to Bhuiyan and Peyara (1978), the mean ovipositional period is 6.83 days in winter and 4.00 days in summer. Several eggs may be laid singly on the grain (Alam, 1971). The freshly laid eggs are translucent, smooth and shining (Plate 2) but become pale yellowish or grayish white with age. The eggs are elongated and oval in shape (Alam, 1971). Incubation period lasts from 3-5 days in summer and 7-9 days in winter (Bhuiyan and Peyara, 1978). Govindarajan et al. (1981) observed the ovipositional behavior of Callosobruchus chinensis and C. maculatus in relation to seed size. Laboratory observations on the effect of seed size on mungbean (Vigna radiata) showed that two bruchids preferred to oviposit on seeds weighing more than the average of those used and the number of eggs laid on them was in proportion to seed weight. Raina (1970) reported that on an average 78, 128 and 96 eggs/female were laid on mungbean seeds at 30°C and 70% R.H. by Callosobruchus chinensis, C. maculatus and C. analis respectively. Eggs lasted for 3.5, 4 and 5 days respectively and 94-99% of the eggs hatched.



Plate 1. Mating of pulse beetle

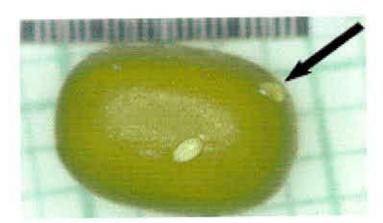


Plate 2. Eggs of pulse beetle on mungbean, singly newly laid egg (Upper arrow) and an old egg (Center)

c. Larval period

According to Alam (1971), the full-grown larva is six mm long, flesh, strongly wrinkled, perfectly white except brown color at mouth region (Plate 3 & Plate 4). Soon after hatching, the young larvae bore into the seeds and start to consume the contents (Bhuiyan and Peyara, 1978). According to Dennis (1990), the larva is scarabeiform having five instars developed in about 20 days.

d. Pupal period

The pupae are exarate type (Plate 5) with a mean pupal duration of 2.5 days in summer and 4.25 days in winter (Bhuiyan and Peyara, 1978). Dennis (1990) reported that the pupation takes place inside the seed in a chamber covered by a thin window of testa prepared by a mature larva.

e. Adult emergence

The adult is 4 mm long and can be distinguished from other species by the elevated ivory like spots near middle of the body (Alam, 1971). The beetle is dark and inconspicuous in colour (Plate 6 & Plate 7) and its body is clothed with hairs. Bhuiyan and Peyara (1978) reported that the head of the beetle is small, hypognathous and provided with short snout. The males are short lived and smaller than females. The antennae of male and female are pectinate and serrate respectively (Dennis, 1990). According to Shukla and Pandey (1977), Raina (1970) and Rajak and Pandey (1965), pectinate antennae of males are elongated with oblong apical segment and curved towards each other. Pectination become prominent from 4th towards the apical segment. The average longevity of the male and female are 4.3 and 5.4 days in summer and 7.4 and 9.2 days in winter respectively. The life cycle of C. chinensis L. completes in 30-32, 20-23 and 40-46 days in early summer, mid summer and winter seasons respectively (Bhuiyan and Peyara, 1978). Raina (1970) reported that the post embryonic development averaged 18.8, 20 and 23.5 days for C. chinensis L., C. maculatus F. and C. analis F. respectively.

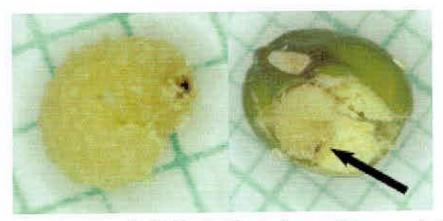


Plate 3. Larvae of pulse beetle. The dark area at the upper right of the isolated larva is the mouth. Alive larva in a mungbean is shown by the arrow



Plate 4. Young larvae within the mungbean seed

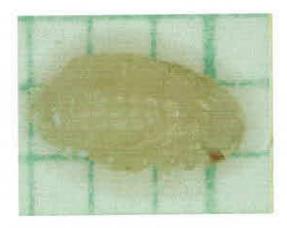


Plate 5. Pupae of pulse beetle



Plate 6. Adult pulse beetle, Callosobruchus chinensis L. (Male)



Plate 7. Adult pulse beetle, Callosobruchus maculatus F. (Female)



f. Identification of male and female bruchids

The distinctive characters of males and females have been well documented by Shukla and Pandey (1977), Raina (1970) and Rajak and Pandey (1965). Antennae of male are pectinate type with elongated and oblong apical segment and curved towards each other (Plate 8 & Plate 9). Pectination in antenna become prominent from the 4th to the apical segment. Antenna of female is straight but serrate type with prominent serration in 5th to the apical segment. The apical segment is some what bluntly rounded or ovate in shape.

Male and female pulse beetles can easily be distinguished from one another by general appearance. The most distinguishing characteristic is the sex specific coloration of the post abdominal plate that's called 'Pygidium'. In the female, the plate is enlarged and is darkly colored on both sides. In the male, the plate is smaller and lacks stripes. In some strains, females are larger in size than males. Also, females are black in coloration and males are brown.

2. NATURE AND EXTENT OF DAMAGE

Begum et al. (1982) stated that in Bangladesh C. chinensis L. is one of the major pests belonging to Callosobruchus spp. causing considerable damage to stored legume grains. Southgate (1979) stated that pulses grown by man have been infested by bruchids since the dawn of agriculture. The larval stage causes only severe damage rendering the seeds unfit for planting and human consumption (Plate 10). In the laboratory study, Rahman (1991) found that the initial presence of 4 larvae or eggs or one pair of Callosobruchus spp. adult could completely damage 10 gm of the pulse grain within 2-4 months depending on the type of the pulses, stage of maturity and species of the beetle. Islam (1977) found that C. chinensis L. is the most serious storage pest in Bangladesh and obtained 10-20% destruction of the stored legume grains every year in the small storehouse of the marginal farmers. Gujar and Yadav (1978) recorded 55-60% loss by seed weight and 45-66% loss in protein content by the pulse beetle.



Plate 8. Dorsal view of adult male (left) and female (right) of C. maculatus. The sex specific coloration of the post abdominal plate (Pygidium) is shown



Plate 9. Dorsal view of adult male (left) and female (right) of C. chinensis showing pectinate and serrate antenna respectively

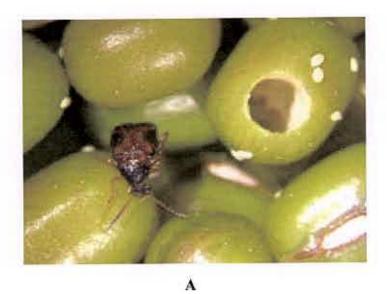




Figure 10. Infested mungbean seeds (A & B) with pulse beetle (Showing eggs on seeds and exit hole)

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 seeds 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 (Chowdury, 1961).

Raja et al. (2004) conducted storage studies for 7 months to evaluate the effect of different levels of bruchid (C. maculatus) infestation in green gram seed revealed that there was significant decrease in seed weight (g), germination (%), vigour index (VI) and protein content (%) with increase in the level of bruchid infestation. A rhythmic decrease in qualitative and quantitative character of seeds was observed with advances in period of storage.

3. ORIGIN AND DISTRIBUTION OF PULSE BEETLES

Callosobruchus chinensis L. was first reported and the 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 breed in every continent except Antarctica. Through the agency of man, bruchids have their cosmopolitan distribution. Most of the species live in the tropical regions of Asia, Africa, Central and South America. Callosobruchus chinensis L. is of Asian origin, where it is still the dominant species (Dennis, 1990). He mentioned that C. maculatus is 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. phaseole (Gyllenhal) in Africa, parts of Asia and South America on Vigna and Dolichos lablab, C. rghodesinus (Pic) in Africa on cowpea, C. sibinnotastus (Pic.) in East Africa on Vigna subterranean and C. theobromae (Linn.) in India on field crops of pigeon pea. Rahman et al. (1942) recovered that the Bruchidae contains more than 100 injurious species distributed over different parts of the world. Among them, 11 injurious species are recorded.

4. OVIPOSITIONAL PREFERENCE, GROWTH AND DEVELOPMENT OF BRUCHIDS

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

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

Gokhale and Srivastava (1977) studied the ovipositional preference of *C. maculatus* on different varieties of moth bean (*Vigna aconitifolia*) and found that oviposition preference was unrelated to the suitability of the seeds for bruchid development. Larval development was not dependent on the amount of food consumed.

Singh et al. (1980) studied in detail the ovipositional preference of C. chinensis and C. maculatus in different pulses. They found that although the pulse beetles did not show any significant variety preference for oviposition, there was a definite inter-pulse response of the oviposition of these pests. The order of preference for C. chinensis was cowpea > blackgram > lentil > pigionpea,

chickpea > mungben > pea, whereas that for *C. maculatus* was chickpea > blackgran > mungbean > cowpea > pigeonpea > pea > lentil. They also concluded that the oviposition preference was independent of larval development.

Dharmasena and Subasinghe (1986) studied with twelve varieties of mungbean (Vigna radiata) for seed characteristics contributing to resistance to Callosobruchus spp. Varieties with small seeds and glossy seed coats were shown to be associated with a higher degree of resistance than large seeds with a dull surface. Female bruchids find it more difficult to lay their eggs on the highly convex and shiny surfaces of small seed.

Piergiovanni et al. (1994) analyzed the seeds of an eight 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 analysis indicated that high levels of both antitrypsic and antiamylasic activity characterize resistant lines. Moreover, high activity of a single inhibitor class is typical of the bruchid (Callosobruchus 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 pest attack. A limiting factor in this breeding strategy is the need to reduce the antiamylastic activity before eating.

5. HOST STATUS OF VARIOUS MUNGBEAN VARIETIES/GENOTYPES FOR BRUCHIDS

Lambrides and Imrie (2000) screened twenty-six mungbean (Vigna radiata) varieties and accessions for resistance to four bruchid species (Coleoptera: Chrysomelidae). On the basis of percentage of seeds damaged, all Australian commercial mungbean varieties tested were highly susceptible to strains of Callosobruchus maculatus and C. chinensis, the 2 species that cause most damage worldwide to mungbean in storage. Three accessions (TC1966, ACC23 and

ACC41) appeared to have bruchid resistance. All 3 varieties are member of the subspecies sublobata, and typically have wild mungbean characteristics of small seed size and the presence of a well-formed texture layer on the seed. These characters may act as oviposition deterrents. Consequently, these assays for determining resistance to bruchid infestation may not be suitable for identifying biochemical resistance of some mungbean genotypes.

Sarwar et al. (2003) directed laboratory investigations by towards identifying mungbean (Phaseolus aureus [Vigna radiata]) host grains resistance against pulse beetle, Callosobruchus analis infestation. Seven genotypes were evaluated for resistances that were obtained from Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan. Results revealed that genotype LI P5/5/89 showed extremes in adults emerged, percent damage, percent weight losses and frass weight, whereas, genotype AEM-96 showed the least limits (1244, 783; 93.17, 80.70; 55.99%, 51.53% and 1.67 gm), 1.06 gm, respectively. The results indicated that the possible presence of certain promising factors in resistant genotypes, which could be transferred to the susceptible genotypes by means of intra-specific crossing.

Liu-Xuming et al. (1998) evaluated both artificial and natural infestation of Callosobruchus chinensis in the identification of resistance source among different mungbean (Vigna radiata) germplasm lines. Some 80 germplasm lines from Asian Vegetable Research and Development Center (AVRDC) and 784 domestic germplasm lines were tested. The rate of damaged mungbean seeds was used as an evaluation index. Some 17 germplasm lines from AVRDC showed moderate resistant to immunity to Callosobruchus chinensis while only 3 landraces from Gangxi showed moderate resistance among domestic germplasm. The greenhouse performance was similar to that in field.

Muhammad et al. (1997) carried out laboratory experiments to evaluate eight different strains/varieties of mungbean [Vigna radiata] (MB-26, MB-48, MB-55,

MB-63, MB-66, MB-87, MB-246 and Kanti) for susceptibility to *Callosobruchus* chinensis, on the basis of the number of eggs laid, duration of development of the immature stages, percentage adult emergence, and weight loss due to damage by the pest. The strains MB-246 and Kanti were found to be highly susceptible, with 13.6 and 13.0% loss in weight of seeds, respectively; strains MB-87, MB-26 and MB-66 were susceptible, and strains MB-63, MB-48 and MB-55 were moderately susceptible. The size, colour and protein content of the seeds had no influence on the susceptibility of mungbean seeds to *C. chinensis*.

Sison et al. (1996) screened several mungbean (Vigna radiata) entries for resistance to Callosobruchus chinensis. Results of both free choice and no-choice tests showed that TC 1966 was highly resistant while the Pag-asa series (1, 3, 5 and 7) were susceptible. The life history of C. chinensis was also studied on the susceptible variety Pag-asa 7. It takes about 21-30 days for the bruchid to complete development (egg to adult emergence). The eggs are laid on the seed coat while the larvae develop inside the seed completing four larval instars there. The larvae develop and feed on the seed leaving only the seed coat. Pupation takes place in a cell inside the seed and the adult emerges through the entrance hole made by the larva. Adult life span ranges from 5 to 15 days. Descriptions of the various stages are presented.

Ashraf et al. (1991) studied the effects of different varieties of mung (Phaseolus aureus) [Vigna radiata] (321, 6601, 141, No.1 and 27), mash (P. mungo) [Vigna mungo] (80, 48, 59, 118 and AARIMI 17) and chickpea (86208, CM 72, 86221, 86037and C 44) for their relative resistance against Callosobruchus chinensis under controlled laboratory conditions (25-30°C) in Pakistan. They found that differences in oviposition, percent adult emergence, percent number of grains bored and percent weight loss caused by C. chinensis were significant among varieties. Among varieties, Mung 141 (17.2% wt. loss), Mash AARIMI 17 (27.5% wt. loss) and chickpea C 44 (13.7% wt. loss) were relatively susceptible to C.

chinensis, whereas Mung No. 1, Mash 59 and Gram 86037 appeared to be resistant.

In laboratory studies, Ranganath and Ram (1992) screened the effects of 13 varieties of mungbean [Vigna radiata] for resistance to Callosobruchus maculatus. Lowest feeding damage and adult emergence were recorded with cv. PDM-14.

Brewer and Horber (1984) evaluated 16 varieties of 7 species of grain legumes for their resistance to *C. chinensis* in the laboratory in the U.S.A. They found that mungbean suffered the highest damage while lentil, broad bean, cowpea and one variety of chickpea had the least damage. Pigeonpea, azuki bean (*Vigna angularis*) and most of the chickpeas were intermediate in infestation. Ovipositional antixenosis in the resistant chickpea variety was due rough and almost spinney pericarp. Antibiosis was expressed in lentil, broad bean cowpea.

Kulkarni et al. (1985) studied the damage and losses caused by C. chinensis to different legumes stored in selected containers in India. They found maximum weight loss of seeds in mungbean and dew bean (Vigna aconitifolirs) while adult emergence and percentage damage were minimum in pea.

Khattak et al. (1987) evaluated the susceptibility of different varieties of mungbean against C. maculatus in the laboratory in Pakistan. They found that there were significant differences in the susceptibility among the varieties. Some varieties of mungbean were highly susceptible in terms of adult emergence, percentage weight loss, damage and adult life span and development period while others were less susceptible but none was immune.

Janzen et al. (1977) carried out a detailed set of feeding trails using C. maculatus and found that several non protein amino acids were lethal at concentrations of 0.1%. They also found that alkaloids were generally the most toxic compounds present in the resident legume seeds.

Epino and Regesus (1983) reported that the physical characteristics and chemical components of mungbean seed appeared to be correlated with varietal

susceptibility to bruchid infestation. Large and heavy seeds were more preferred by the bruchids than small seeds. The resistant accessions had lower percentage of fats and starch but a higher percentage of protein than the susceptible accessions.

Modgil and Mehta (1996) observed the effects of Callosobruchus chinensis infestation on the carbohydrate and dietary fibre contents of seeds of chickpea, green gram (Vigna radiata) and pigeon pea at 10, 20, 30, 40, 50 and 60% infestation. With increased level of infestation, levels of energy, starch, total sugars and non-reducing sugars decreased, whereas a significant increase in the amounts of reducing sugars, crude fibre, neutral detergent fibre, acid detergent fibre, hemicellulose, cellulose and lignin was observed.

6. ALTERNATIVE MANAGEMENT OF PULSE BEETLES INFESTING MUNGBEAN

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 the cumbersome 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 were reviewed here in brief.

In the world, as many as 2400 plant species have been recorded that have potential pesticidal properties and biological activity against a wide range of pests (Grainge and Ahmed, 1988). In Bangladesh, as many as 54 plant species have been evaluated for their bio-efficacy against different insect pests, pathogens and weeds (Karim, 1994). A number of botanicals and their derivatives have been tested in Bangladesh and other developing countries particularly against pulse beetles and have shown promising results (Yadava and Bhatnagar, 1987; Mahadi and Hamoudi, 1984 and Saxena and Yadav, 1983).

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 pesticide resistance to insects. 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 reduced 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).

Veer-Singh and Yadav (2003) conducted a laboratory experiment to evaluate the efficacy of six different oils, i.e. neem (Azadirachta indica), mehandi (Lawsonia inermis), castor (Ricinus communis), karanja (Pongamia pinnata), mustard (Brassica juncea) and olive, against C. chinensis in green gram. The seed coating with these oils gave significant protection against C. chinensis compared to the untreated control. The oil coating 6-8 h after treatment gave complete protection at doses of 2.5, 5 and 10 ml/kg seeds, whereas partial to complete protection was observed at 90, 150 and 210 days after treatment. Neem and Mehandi oils at 10 ml/kg seed were effective even beyond 150 days after treatment, and the rest of the oils were effective even after 280 days of oil treatment. Germination test

carried out with the oil-treated seeds at 4 different time intervals (6 to 8 h, 90, 150 and 210 days after treatment) showed significant difference among the treatments and untreated control, but it was beyond 80% in all the treatments; germination in the untreated control ranged from 94.3 to 97.0%. However, the difference became non-significant when the treated seeds were given 8 h exposure to sun.

Dhakshinamoorthy and Selvanarayanan (2002) evaluated the effects of different natural products on the survival of *C. maculatus* infesting stored green gram (*Vigna radiata*). The treatments comprised leaves (as dried powder) of various plants (neem, nochi [*Vitex negundo*], pungam [*Pongamia pinnata*], citrus and thulasi), fly ash, kitchen ash, castor oil, red earth, malathion (as standard control) and untreated control. Treated seeds were kept in plastic containers and 20 adult beetles were introduced into each container and kept covered with muslin cloth. The results revealed that the mortality of the beetle at 7 days after treatment was highest (100%) in castor oil, followed by neem leaf powder (91.66%).

Haque et al. (2002) conducted a comparative study to assess the efficacy of coconut, mustard (Brassica spp.), sesame, castor, olive, safflower, soyabean, neem (Azadirachta indica) oils, and a mixture of the oils, against the pulse beetle (Callosobruchus chinensis) on mungbean under laboratory condition, considering the ovipositional behaviour and adult emergence of the pulse beetle and seed viability of mungbean. No harmful effect was found on oviposition due to oil treatment. However, the percentage of adult emergence was greatly reduced and completely inhibited by the application of oils on the seeds. There was also no adverse effect on the viability of seeds due to different treatments.

Bhuiyah (2001) reported that the oils of Neem, Royna and Castor at 6 and 8 ml/kg and leaf powder of Biskatali, Marigold and Castor @ 5% w/w were most effective in preventing the egg laying in lentil and chickpea and leaf powder of Biskatali, Marigold, Castor and Mango @ 5% were most effective in reducing the adult

emergence in lentil and chickpea, whereas the adult emergence were nil in pre and post storage release methods.

Reddy et al. (1994) tested pre-storage seed treatment of mungbean [Vigna radiata] cv. PS-16 with oils of neem [Azadirachta indica], karanja [Pongamia pinnata], [Indian] mustard, groundnut and castor [Ricinus communis] at 2.5, 5.0 and 10.0 ml/kg seed as a surface protectant against Callosobruchus chinensis. Seeds treated with Thiram 75 WP (2.5 g/kg) were used as a control and the effect on seed viability was tested. Karanja oil, mustard oil and castor oil (10 ml/kg seed) were found effective in halting the embryonic development in C. chinensis and protected the seed over a period of 21 months after treatment followed by neem oil which gave protection for up to 12 months. A significant reduction in germination was noticed among the treatments with neem oil and groundnut oil at 10 ml/kg where seed viability was maintained for up to 6 months, while at the lower dosage (2.5 and 5 ml/kg seed) viability was maintained for up to 18-21 months, respectively. Karanja oil, castor oil and mustard oil even at 10 ml/kg seed did not show any adverse effects on seed viability for up to 18 and 21 months, respectively. There was a progressive increase in moisture content of the seeds with different treatments during storage which was on par with the control.

Babu et al. (1989) studied the effect of pre-storage treatment of mungbean (Vigna radiata) variety PS-16 with neem (Azadirachta indica), karanja (Pongamia glabra [P. pinnata]), mustard, groundnut and castor (Ricinus communis) oils, each at 2.5, 5.0 and 10.0 ml/kg seed, on infestation by the bruchid Callosobruchus chinensis from May 1985 to August 1987. Treatments with karanja oil (5 and 10 ml/kg) and castor oil (10 ml/kg) effectively reduced oviposition by the bruchid under conditions of artificial infestation, while maintaining a high level of germination for over 18 months of storage under ambient conditions. After 24 months of storage, the yield of neem oil treated seed was significantly lower.

Mummigatti and Ragunathan (1977) reported that castor, mustard and gingerly oils inhibited the multiplication of *C. chinensis* on green gram at 0.3% level, coconut and groundnut oils at 0.5 % level and sunflower oil was ineffective even at 0.5% level. Babu *et al.* (1989) reported that among neem, karanja, mustard, groundnut and castor oil; the karanja (5 and 10 ml/kg) and castor (10 ml/kg) oil effectively reduced oviposition by the bruchid (*C. chinensis*) under conditions of artificial infestation.

In India, Pandey et al. (1976) prepared powder from drupes of Thevetia nerifolia, rhizomes of Acorus calamus, leaves of Adhatoda vasica and Ipomea cornea and petroleum ether extracts from bulbs of garlic, Allium sativum, and onion, A. cepa and Neem, Azadirachta indica and these were tested for their repellent properties against C. chinensis infesting grain seed.

As a plant derivative, Camphor (C₁₀H₁₆O) is very new and unexploited component, which derived from the leaves and wood of Camphor tree (Cinamomum camphora). It is a white transparent waxy solid with a strong penetrating pungent aromatic odor and available at grocery stores, and mostly used in medical purposes (Mann et al. 1994). Abiverdi (1977) reported the insecticidal efficacy of camphor. Chauvin et al. (1994) reported that camphor has fumigation properties and has got a very low mammalian toxicity.

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 (naphthalene and camphor) and two types of indigenous materials (sand and neem leaf powder). In both the 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 beetle,

Callosobruchus maculatus F. (Coleoptera: Bruchidae) fed on black gram, Vigna mungo, seeds. Plant extracts, powder, ash and oil from nishinda (Vitex negundo L.), eucalyptus (Eucalyptus globules Labill.), bankalmi (Ipomoea sepiaria K.), neem (Azadirachta indica L.), 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.

Rahman et al. (2001) reported the fumigation action of camphor against pulse beetle, C. chinensis. In Bangladesh, very little study has so far been reported on the efficacy of camphor against pulse beetles.

Latif et al. (2005) conducted a study to evaluate the toxicity of camphor against different stages of rice weevil, Sitophilus oryzae Linn. (Curculionidae: Coleoptera) in parboiled polished rice grains revealed that there exists a very sharp difference among the different stages of the insect and exposure durations n respect of the toxic action of camphor. The LD₅₀ of camphor against adult, egg, larva and pupa was the lowest (1.3316 mg, 1.152 mg, 6.1399 mg and 8.1093 mg respectively) at the 96 h exposure while it was the highest (4.200 mg, 3.7954 mg, 10.6040 mg and 18.9371 mg respectively) at the 24 h exposure.

Latif and Rahman (2000) conducted an experiment to evaluate the efficacy of camphor in protecting maize grains in storage against maize weevil, Sitophilus zeamais Motsch. The experiment comprised 3 treatments of camphor a 2.0 g, 4.0 g and 6.0 g per kg maize grains and an untreated control and was set in CRD. The individual treatment composition had required amount of camphor placed inside the container having 200 g maize grains which was composed each of 7 g of grains infested with eggs, larvae and pupae separately and 179 g de-infested grains and released 5 pairs of adults. After 5 months storage, all the doses kept the infestation 93.03% - 95.57% less than that of the control and offered 90.48%-

93.53% protection of loss. The dose @ 6.0g camphor per kg maize grains was the most effective although the dose 2.0g camphor per kg maize grains provided more than 90% protection.

Effect of botanicals on seed viability

Bato and Sanchez (1972) reported that the larvae of pulse beetle are internal feeders and feed inside the seeds, causing loss of quality, quantity and viability of seeds.

Ahmed et al. (2006) reported that considering the combination of containers and materials, camphor with tin container and polythene lined gunny bag had almost no infestation (< 1.00%) after 270 days of storage and the germination percentage of stored seeds of mungbean was 87.82 - 88.73% against C. chinensis and they suggested that mungbean seeds can be stored up to 270 days using camphor either in tin container or gunny bag lined with polythene.

Haque et al. (2002) also evaluated different plant oils against the pulse beetle (Callosobruchus chinensis) on mungbean and they found that no harmful effect was found on oviposition due to oil treatment. However, the percentage of adult emergence was greatly reduced and completely inhibited by the application of oils on the seeds. There was also no adverse effect on the viability of seeds due to different treatments.



Chapter III Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The study comprising two sets of experiments have been conducted to find out the resistance source(s) among different mungbean varieties against pulse beetles as well as to evaluate some selected alternative management practices against these pests during March, 2007 to October, 2007 in the laboratory under Department of Entomology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.

Other details of the experiments are furnished below:

Experiment 1: Screening of some selected mungbean varieties for resistance against Callosobruchus chinensis Linn. and C. maculatus Fab.

Experiment 2: Evaluation of some selected alternative management practices against pulse beetles infesting mungbean.

Other details of each experiment including their methodologies are described below under the following sub headings:

Rearing of Callosobruchus chinensis and C. maculatus

The homologous stock culture (Plate 11) of Callosobruchus chinensis and C. maculatus were maintained separately on mungbean seeds in plastic jars covered with markin cloth in the same laboratory at ambient temperature of 30°C±0.82 and 88.22 % R.H. Male and female beetles were sorted out under simple microscope by their antennal characteristics, size and shape of the body. One hundred pairs of Callosobruchus chinensis and C. maculatus were introduced into each of the four



Plate 11. Rearing of pulse beetles, Callosobruchus chinensis and C. maculatus under the room conditions

plastic containers (12 cm dia. × 14 cm height) containing 200 gm of de-infested seeds. The mouth of the containers was covered with perforated plastic lids. The insects were allowed to mate and lay eggs for 24 hours. After 24 hours, sieving was done to separate the adult beetles. The mungbean seeds with eggs left on the sieve were kept for adult emergence. One-day-old (0-24 hours) adults were used for study. The rearing procedure was repeated in different batches to ensure continuous supply of the adults with required eggs. Similar rearing procedure was maintained for *Callosobruchus maculatus* on same seeds.

De-infestation of mungbean seeds

Seeds of different mungbean varieties were spread separately on black polythene sheets and sun-dried from 10:00 a.m. to 3.00 p.m. in direct sunlight with air temperature ranging from 32-42 °C for 5 consecutive days. The sun-dried grains were kept in the laboratory for few hours and packed in polythene bags and sealed to avoid future infestation.

Experiment 1: Screening of some selected mungbean varieties for resistance against Callosobruchus chinensis and C. maculatus

The experiment was carried out in the laboratory during March, 2007 to June, 2007. The experiment was laid out in a Completely Randomized Design (CRD) with three replications.

TREATMENTS/VARIETIES OF THE EXPERIMENT

Seeds of nine mungbean varieties were collected from different sources that were used under the present study are given in Table 1 and each of which was considered as an individual treatment.

Table 1. Name and source of mungbean varieties used under present study

Treatment	Variety	Source of availability Bangabandhu Sheikh Mujibur Rahman				
Tı	BU Mug-1					
T ₂	BU Mug-2	Agricultural University, Gazipur, Bangladesh				
T ₃	BARI Mug-2					
T ₄	BARI Mug-3					
T ₅	BARI Mug-4	Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh				
T ₆	BARI Mug-5	(BAKI), Gazipur, Bangiadesii				
T ₇	BARI Mug-6					
T ₈	BINA Mug-1	Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh				
Т9	Barisal local	BARI, Gazipur, Bangladesh				

Two types of tests like free choice test and no choice test were conducted as follows:

Free choice test

Twenty grams of de-infested healthy seeds of each mungbean variety were taken in individual plastic pot (6 cm dia, x 9 cm height). The seed containing pots were placed randomly at equal distances around a central circle in the mosquito netted topped tin cage (60 cm dia, x 90 cm height) (Plate 12). Then 150 pairs of one-day-old (0-24 hours) healthy adults of *Callosobruchus chinensis* from the stock culture were taken in a petridish (90 mm dia.) and placed it in the centre of the cage. The lids of all plastic pots containing mungbean seeds and petridish were removed and covered the cage quickly with mosquito net tightly. The experiment was replicated for three times. After three days (72 hours) of release, both dead and alive insects were removed from the plastic pots. The number of eggs laid on the seeds in each pot was then recorded by using magnifying glass. The pots with the lids were then stored under room conditions. The developmental stages were also observed and





Plate 12. A set of experiment in free choice test arranged in a tin cage (above) and covered with mosquito net (below)

the data were recorded. The number of adults emerged and the weight of the grains were recorded after complete emergence of adult from the laid eggs.

A similar set of experiment was also conducted with the adult of Callosobruchus maculatus as previous experiment.

No choice test

Twenty grams of de-infested healthy seeds of each mungbean variety were taken in individual plastic pot (6 cm dia. x 9 cm height). Then 5 pairs of one-day-old (0-24 hours) healthy adults of *Callosobruchus chinensis* from the stock culture were released in each pot and covered with lids (Plate 13). After three days (72 hours) of release, both dead and alive insects were removed from the pots. The number of eggs laid on the seeds in each pot was then recorded by using magnifying glass. The pots with the lids were then stored under room conditions. Data were recorded as previous parameters.

A similar set of experiment was also conducted with the adult of Callosobruchus maculatus as previous experiment.



Plate 13. A set of experiment in no choice test (Plastic pots covered with lids were arranged in tray)

DATA COLLECTION AND CALCULATION

For recording data from the experiments, 10 seeds for each variety of mungbean were randomly collected from each pot or treatment (variety).

From the experiment, data were calculated on different parameters using the following formula:

Oviposition/egg deposition

The number of eggs laid on the randomly collected seeds was then recorded by using magnifying glass. The total number of eggs was also counted as follows:

Percentage of egg hatching

From the randomly collected seeds after 4th day (Callosobruchus chinensis) or 6th day (C. maculatus) of the insect removal from each pot, the total no. of eggs laid and the number of eggs hatched were recorded and then % egg hatching was calculated using the following formula:

Percentage of internal larvae developed

As previous, from the collected sample, the total number of eggs laid and weight of the sampled seeds was recorded. The larvae then cut open from each sampled seed with the help of sharp blade after 12th day of the insect release and the alive ones were counted and then % internal larvae developed was calculated using the following formula:

% internal larvae developed =
$$\frac{\text{No. of alive larvae in collected sample}}{\text{Total no. of eggs laid in collected sample}} \times 100$$

Percentage of internal pupae developed

The total number of eggs laid and weight of the sampled seeds were recorded. The pupae were then cut open from each sampled seed with the help of sharp blade after 17th day of the insect removal and the alive ones were counted and then % internal pupae developed was calculated using the following formula:

Percentage of adult emergence

Data on the total number of adults emerged from the egg laid were recorded from the first date of adult emergence to last day of emergence and the emerged adults were removed from each pot every day to avoid egg laying and then % adult emergence was calculated using the following formula:

Percentage of grain content loss

After the complete emergence of adults from the laid eggs, the weight of grains for each pot or variety was rerecorded separately. Then the percent grain content loss was measured using the following formula:

Weight loss per pot = (Initial wt. - final weight) of grains per pot.

Infestation severity

Initial weight (Before setting the experiment) and the final weight of the grains for each variety after complete emergence of adults from the laid eggs were recorded separately. Then the percentage of grain content loss was graded as level of resistance as follows:

% grain content loss	Grade / level of resistance	
0-5%	Highly resistant (HR)	
5.1-10%	Moderately resistant (MR)	
10.1-15%	Moderately susceptible (MS)	
15.1-20%	Susceptible (S)	
>20%	Highly susceptible (HS)	

DATA ANALYSIS

Data were analyzed by ANOVA-1 in CRD for each set of test separately and the means were separated by DMRT (Duncan's Multiple Range Test).

Experiment 2: Evaluation of some selected alternative management practices against pulse beetles infesting mungbean.

The experiment was carried out in the laboratory during July, 2007 to October, 2007 by using the procedures described below:

Materials used under the present study

Seeds of BARI Mug-6 and Barisal Local (Local variety) were used under the present study as most susceptible hosts to *C. chinensis* and *C. maculatus* respectively, identified through experiment 1 conducted earlier.

Treatments of the experiment

There are 7 possible treatments that were applied against pulse beetles infesting mungbean in the storage condition given in Table 2.

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

Treatments	Name of the botanicals	Dose
T_1	Neem oil (Azadirachta indica)	8 ml / kg,
T ₂	Castor oil (Ricinus communis)	8 ml / kg,
T ₃	Camphor (Cinamomum camphora)	2 g / kg
T ₄	Neem leaf powder (Azadirachta indica)	5% w/w
T ₅	Castor leaf powder (Ricinus communis)	5% w/w
T ₆	Bankolmi leaf powder (Ipomoea sepiara)	5% w/w
T ₇	Untreated control	

Collection and preparation of the botanicals

Results revealed earlier by different researchers about the biological effectiveness of different botanicals against various field and stored grain pests. The tested botanicals were Neem oil (Azadirachta indica), Castor oil (Ricinus communis), Camphor (Cinamomum camphora), powdered leaves of Neem, Castor and



Plate 14. Different treatments used for controlling pulse beetles such as, (From left side, clock wise) Castor oil - Bankolmi leaf powder -Camphor - Castor leaf powder - Neem oil - Neem leaf powder.

Bankolmi (*Ipomoea sepiara*) (Plate 14). The oils and camphor were bought from the local market whereas the leaves of the tested botanicals were collected from Sher-e-Bangla Agricultural University campus. The collected leaves were dried under ambient room temperature (27°C-34°C). The dried leaves were grinded separately by a hand grinder and sieved through a 60-mesh sieve to get fine powder form.

Design of the experiment

The experiment was laid out in a Completely Randomized Design (CRD) with three replications.

Experiments with Callosobruchus chinensis and C. maculatus

Two sets of experiments were carried out separately to fulfill the objectives. Both the experiments had the same treatments with same dose to determine their efficacy as seed protectants, but the species of bruchids were different. The oils of neem and castor were used @ 8 ml/kg, camphor was used @ 2 g / kg and the leaf powders of the botanicals were used @ 5% w/w i.e. 10 g per 200g mungbean grains. Two hundred gram seeds of BARI Mug-6, which was susceptible to C. chinensis, were taken separately for each treatment in round plastic pot (12 cm dia. × 14 cm height) (Plate 15). Then the seeds of each pot selected for specific treatment was mixed thoroughly with the specific botanicals by hand shaking. The mouth of the each plastic pot was covered with their lids and the treatments were replicated three times. Soon after application of treatment, ten pairs (male and female) of one day old adults of C. chinensis were released in each of the plastic pots and were kept at room temperature (27°C-34°C) in the laboratory. Data on oviposition, egg hatching, internal larvae and pupae developed, extent of grain damage and grain content loss. During each sampling occasion, 10 seeds for each treatment were examined for recording the above data.

A similar set of experiment was conducted with the one day old adult of Callosobruchus maculatus on the Barisal local (local variety of mungbean) as



Plate 15. A set of experiment for management of mungbean seeds against Callosobruchus chinensis



Plate 16. A set of experiment for management of mungbean seeds against Callosobruchus maculatus

the most susceptible variety identified through experiment 1 against C. maculatus as previous experiment (Plate 16).

Determination of seed viability

Germination tests were also done at randomly taken 100 mungbean seeds from each of the treatment at the initial and after completion of the experiment to evaluate the treatment effects on the viability of the seeds and the tests were replicated three times. The seeds were placed in petridishes having blotting paper at the bottom soaked with water. Then the petridishes containing mungbean seeds were incubated at room temperature and germination was estimated by direct count.

DATA COLLECTION AND CALCULATION

For recording data from the experiments, 10 seeds for each treatment were randomly collected from each treated pot. From the experiment, data were calculated on oviposition/egg deposition, % egg hatching, % internal larvae and pupae developed and % grain content loss.

Percentage of grain damage

Examination for grain damage by the pulse beetles was done by collecting 100 randomly selected munbean grains for each of the treatment considering the number of bored seeds. Then the percent damaged grain was calculated according to the following formula:

Percentage of seed germination

From the recorded data percent grain germination was calculated by using the following formula:

% grain germination =
$$\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Percent reduction of infestation over control

Data from each treated and untreated control pot were recorded and the percent reduction of infestation over control in terms of oviposion, hatching, internal larvae and pupae developed, damaged seeds and grain content loss were calculated using the following formula:

Percent infestation reduction over control =
$$\frac{X_2 - X_1}{X_2} \times 100$$

Where, X_1 = the mean value of the treated plot X_2 = the mean value of the untreated plot

DATA ANALYSIS

Data were analyzed by ANOVA-1 in CRD for each set of test separately and the means were separated by DMRT (Duncan's Multiple Range Test).

Chapter IV Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

Two sets of experiments were conducted to evaluate different mungbean varieties against pulse beetles for their resistance source(s) as well as to find out the most effective component(s) among some selected alternative management practices against these pests during March, 2007 to October, 2007 in the laboratory under Department of Entomology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh. The results have been presented and discussed, and possible interpretations have been given under the following sub-headings:

Experiment 1: Screening of some selected mungbean varieties for resistance against Callosobruchus chinensis Linn. and C. maculatus Fab.

Evaluation of mungbean varieties against Callosobruchus chinensis Linn.

Significant differences were found among nine mungbean varieties against Callosobruchus chinensis Linn., both in free choice and no choice tests, in respect of egg deposition, internal larvae and pupae developed, adult emergence and grain content loss except egg hatching represented in Table 3 and Table 4.

As depicted in table 3, in free choice test, the number of eggs laid by Callosobruchus chinensis Linn. were the highest (1027) on the variety BARI Mug-6, which was significantly different from all other varieties, followed by Barisal local (970), BARI Mug-3 (936.7), BARI Mug-4 (923.3), BARI Mug-2 (890.0) and BINA Mug-1 (830.0); while it was the lowest (696.7) in the variety BU Mug-2, which was statistically similar with BARI Mug-5 (726.7) followed by BU Mug-1 (756.7). As a result, the order of ovipositional preference was BARI Mug-6 > Barisal local > BARI Mug-3 > BARI Mug-4 > BARI Mug-2 > BINA Mug-1 > BU Mug-1 > BARI Mug-5 > BU Mug-2. But there was no significant

Table 3. Influence of different mungbean varieties on oviposition, developmental stages, adult emergence and food consumption by Callosobruchus chinensis Linn. in free choice test

Mungbean varieties	Egg diposition (No.)	Larvae hatched (%)	Larvae developed (%)	Pupae developed (%)	Adult emergence (No.)	Grain content loss (%)	Level of resistance
BU Mug-1	756.7 f	80,17 a	13.33 c	15.67 c	9.33 e	2.00 c	HR
BU Mug-2	696.7 g	81.67 a	5,92 d	6,86 d	4.67 f	1,50 e	HR
BARI Mug-2	890.0 d	83.67 a	6.7 d	7.17 d	13.33 e	7.67 d	MR
BARI Mug-3	936.7 с	88.42 a	56.83 a	50,00 b	191.7 b	19.17 b	s
BARI Mug-4	923.3 с	86.53 a	50.00 ab	50.00 b	201.7 b	20.00 ab	S
BARI Mug-5	726.7 fg	87.73 a	46.33 b	51.67 b	133.3 c	16.67 c	S
BARI Mug-6	1027a	88,67 a	56.85 a	62.67 a	238.3 a	22,23 a	HS
BINA Mug-1	830.0 e	81.67 a	43.33 b	46,67b	96.67 d	10.00 d	MR
Barisal local	970.0 Ь	80.00 a	10.33 c	10,00 cd	9.17 с	1,83 e	HR

Highly resistant (HR): 0-5% grain content loss; Moderately resistant (MR): 5.1-10% grain content loss; Moderately susceptible (MS): 10.1-15% grain content loss; Susceptible (S): 15.1-20% grain content loss and Highly susceptible (HS): >20% grain content loss.

Figures indicate original means of three replications.

Means followed by same letter(s) are not significantly different (P>0.05) from each other by DMRT

effect on percent egg hatching, ranging from 80.00-88.67%, among different mungbean varieties. The rate of larvae developed was the highest (56.85%) in the variety BARI Mug-6, which was statistically similar with the variety BARI Mug-3 (56.83%) and BARI Mug-4 (50.00%) followed by BARI Mug-5 (46.33%) and BINA Mug-1 (43.33%); while it was the lowest (5.92%) in the variety BU Mug-2, which was statistically similar with the variety BARI Mug-2 (6.47%) followed by Barisal local (10,33%) and BU Mug-1 (13,33%). Similarly, the rate of pupae developed was the highest (62.67%) in the variety BARI Mug-6, which was statistically different from all other varieties used under the present study followed by the variety BARI Mug-3 (50.00%), BARI Mug-4 (50.00%) and BARI Mug-5 (51.67%); while it was the lowest (6.86 %) in BU Mug-2, which was statistically similar with the variety BARI Mug-2 (7.17%) and Barisal local (10.00%) followed by BU Mug-1 (15.67%). As a result, the order in terms of suitability of larvae and pupae was more or less the same, which was BARI Mug-6 > BARI Mug-3 > BARI Mug-4 > BARI Mug-5 > BINA Mug-1 > BU Mug-1 > Barisal local > BARI Mug-2 > BU Mug-2. In terms of adult emergence per 20 gram seeds, the number of adult emergence was the highest (238.3) in the variety BARI Mug-6, which was statistically different from all other varieties followed by the variety BARI Mug-4 (201.7) and BARI Mug-3 (191.7); while it was the lowest (4.67) in BU Mug-2 followed by the variety Barisal local (9.17), which was statistically similar with BU Mug-1 (9.33). Considering the grain content loss after the completion of first life cycle by Callosobruchus chinensis (25 days after release), the rate of grain content loss was also the highest (22.33 %) in the variety BARI Mug-6, which was statistically similar with the variety BARI Mug-4 (20.00%) followed by BARI Mug-3 (19.17%), BARI Mug-5 (16.67%) and BINA Mug-1 (10.00%); while it was the lowest (1.50%) in the variety BU Mug-2, which was statistically similar with Barisal local (1.83%) and BU Mug-1 (2.00%) followed BARI Mug-2 (7.67%). As a result, the order in terms of adult emergence and grain content loss was also almost the same, which was BARI Mug-6 > BARI Mug-4 > BARI Mug-3 > BARI Mug-5 > BINA Mug-1> BARI Mug-2 > BU Mug-1 > Barisal local > BU Mug-2.

In case of no choice test of *Callosobruchus chinensis* Linn, more or less similar trends of results were observed in respect of oviposition, developmental stages, adult emergence and grain content loss that found in free choice test (Table 4).

From these findings it was revealed that BARI Mug-6 was highly susceptible; BARI Mug-4, BARI Mug-3 and BARI Mug-5 were susceptible; BARI Mug-2 and BINA Mug-1 were moderately resistant, while BU Mug-2, Barisal local and BU Mug-1 were found to be highly resistant host for C. chinensis in respect of grain content loss. Besides, more or less similar findings were also revealed by Muhammad et al. (1997) and they reported that out of eight strain/varieties of mungbean MB-26, MB-48, MB-55, MB-63, MB-66, MB-87, MB-246 and Kanti for susceptibility to C. chinensis, on the basis of the number of eggs laid, duration of development of the immature stages, percentage adult emergence, and weight loss due to damage by the pest. The strains MB-246 and Kanti were found to be highly susceptible; strains MB-87, MB-26 and MB-66 were susceptible, and strains MB-63, MB-48 and MB-55 were moderately susceptible. In addition, about similar results were also supported by Liu-Xuming et al. (1998), Sison et al. (1996), Ashraf et al. (1991) and Brewer and Horber (1984). But Ali et al. (1999) revealed that both in free choice and no choice tests, among different pulses mungbean appeared to be the most common and suitable host for C. chinensis in respect of ovipositional preference, egg deposition, internal larvae and pupae developed, adult emergence and grain content loss while no significant varietal influence on egg hatching.

Table 4. Influence of different mungbean varieties on oviposition, developmental stages, adult emergence and food consumption of Callosobruchus chinensis Linn. in no choice test

Mungbean varieties	Egg diposition (No.)	Larvae hatched (%)	Larvae developed (%)	Pupae developed (%)	Adult emergence (No.)	Grain content loss (%)	Level of resistance
BU Mug-1	690.0 g	83.22 ab	4.58 f	6.58 g	6.67 f	2.00 f	HR
BU Mug-2	723.3 f	82.43 abc	3.88 f	2.88 g	4.33 fg	1.00 g	HR
BARI Mug-2	873.3 d	76.67 c	31,67 d	32,33 e	12.00 e	5.5 c	MR
BARI Mug-3	683.3 g	78.33 bc	76.67 ab	74.33 b	138.3 c	17.33 с	s
BARI Mug-4	900.0 cd	84.17 ab	65.33 c	68.33 c	168.7 b	18.00 b	S
BARI Mug-5	920.0 c	76.67 c	60.08 c	56,23 d	105.00 d	16.67 c	S
BARI Mug-6	993.3 b	82,61 abc	82.18 a	84,40 a	182.7 a	24 a	HS
BINA Mug-1	783,3 c	84.33 ab	71,67 b	76,00 b	61.3 c	9.5 d	MR
Barisal local	1040 a	86.67 a	16.23 c	15.67 f	6.33 f	1.33 fg	HR

Highly resistant (HR): 0-5% grain content loss; Moderately resistant (MR): 5.1-10% grain content loss; Moderately susceptible (MS): 10.1-15% grain content loss; Susceptible (S): 15.1-20% grain content loss and Highly susceptible (HS): >20% grain content loss.

Figures indicate original means of three replications.

Means followed by same letter(s) are not significantly different (P>0.05) from each other by DMRT

Considering the duration of adult emergence, for both free choice and no choice test by C. chinensis as depicted in Figure 1, the adults emergence started at 18 days after release (DAR) of adults for both free and no choice tests and gradually increased and the highest number of adults were emerged at 21 DAR and 20 DAR for free and no choice test respectively and then the number of adult emergence decreased gradually for both the tests. More or less similar results were reported by Ali et al. (1999) in their findings.

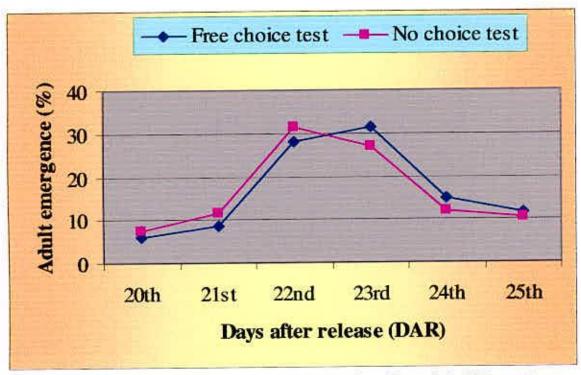


Figure 1. Trend of adult emergence of Callosobruchus chinensis in different days after release in free choice and no choice tests

Evaluation of mungbean varieties against Callosobruchus maculatus Fab.

Significant differences were found among nine mungbean varieties against Callosobruchus maculatus Fab., both in free choice and no choice tests, in respect of egg deposition, internal larvae and pupae developed, adult emergence and grain content loss except egg hatching represented in Table 5 and Table 6.

In free choice test as depicted in table 5, the number of eggs laid by Callosobruchus maculatus Linn. were the highest (1043) on the variety BARI Mug-6, which was statistically different from all other varieties followed by BARI Mug-3 (933.30), which was statistically similar with Barisal local (976.7), BARI Mug-2 (973.3) and BARI Mug-4 (960.0); while it was the lowest (803.3) in the variety BU Mug-2, which was statistically similar with BARI Mug-5 (826.7) followed by BU Mug-1 (866.7) and BINA Mug-1 (876.7). As a result, the order of ovipositional preference was BARI Mug-6 > BARI Mug-3 > Barisal local > BARI Mug-2 > BARI Mug-4 > BINA Mug-1 > BU Mug-1 > BARI Mug-5 > BU Mug-2. The percentage of egg hatching, ranging from 82.33-86.00%, was more or less similar in different varieties of mungbean, which did not differ significantly. In terms of internal larvae developed, the rate of larvae developed was the highest (76.67 %) in the variety Barisal local, which was statistically similar with BARI Mug-3 (73.33%) followed by BINA Mug-1 (65.00%), BARI Mug-4 (43.33%), which was statistically similar with BARI Mug-5 (42.33%); while it was the lowest (25.00%) in BARI Mug-2, which was statistically similar with the variety BU Mug-2 (26.67%) and BU Mug-1 (31.67%) followed by BARI Mug-6 (33.33%). Similarly, the rate of pupae developed was the highest (79.33 %) in the variety Barisal local, which was statistically similar with the variety BARI Mug-3 (75,00%) followed by BINA Mug-1 (66,67%), BARI Mug-4 (45,00%), which was statistically similar with BARI Mug-5 (41.67%); while it was the lowest (26.67%) in BARI Mug-2, which was statistically similar with the variety BU Mug-2 (28.33%) and BU Mug-1 (33.33%) followed by BARI Mug-6 (35.00%). As a result, the order in terms of suitability of larvae and pupae was more or less same,

Table 5. Influence of different mungbean varieties on oviposition, developmental stages, adult emergence and food consumption of Callosobruchus maculatus Fab. in free choice test

Mungbean varieties	Egg diposition (No.)	Larvae hatched (%)	Larvae developed (%)	Pupac developed (%)	Adult emergence (No.)	Grain content loss (%)	Level of resistance
BU Mug-1	866.7 cd	83.33 a	31,67 de	33.33 de	75.00 de	16.00 e	s
BU Mug-2	803.3 e	85.00 a	26,67 de	28,33 de	65,00 cf	9.83 cf	MR
BARI Mug-2	973.3 ъ	82.33 a	25,00 e	26.67 c	55.00 f	8.50 f	MR
BARI Mug-3	993.3 b	84.67 a	73.33 a	75,00 a	140,0 b	26,00 b	HS
BARI Mug-4	960.0 ъ	84.33 a	43.33 c	45.00 c	110.0 c	21.50 cd	HS
BARI Mug-5	826.7 de	79.67 a	42,33 c	41.67 c	88.33 d	21.00 cd	HS
BARI Mug-6	1043 a	83.67 a	33.33 d	35.00 d	81.67 dc	19.00 d	MS
BINA Mug-1	876.7 c	85,00 a	65.00 b	66,67 b	120.0 c	23.50 c	HS
Barisal local	976.7 b	86.00 a	76.67 a	79.33 a	223.3 a	29.50 a	HS

Highly resistant (HR): 0-5% grain content loss; Moderately resistant (MR): 5.1-10% grain content loss; Moderately susceptible (MS): 10.1-15% grain content loss; Susceptible (S): 15.1-20% grain content loss and Highly susceptible (HS): >20% grain content loss.

Figures indicate original means of three replications.

Means followed by same letter(s) are not significantly different (P>0.05) from each other by DMRT



which was Barisal local > BARI Mug-3 > BINA Mug-1 > BARI Mug-4 > BARI Mug-5 > BARI Mug-6 > BU Mug-1 > BU Mug-2 > BARI Mug-2. In case of adult emergence, the number of adult emergence was the highest (223.30) in the variety Barisal local, which was statistically different from all other varieties followed by the variety BARI Mug-3 (140.00), BINA Mug-1 (120.00), BARI Mug-4 (110.00); while it was the lowest (55.00) in BARI Mug-2, which was statistically similar with BU Mug-2 (65.00) followed by BU Mug-1 (75.00) and BARI Mug-6 (81.67). Considering the grain content loss after the completion of first life cycle by Callosobruchus chinensis (25 days after release), the rate of grain content loss was the highest (29,50%) in the variety Barisal local, which was statistically different from all other varieties followed by the variety BARI Mug-3 (26.00%), BINA Mug-1 (23.50%), which statistically similar with BARI Mug-4 (21.50%) and BARI Mug-5 (21.00%); while it was the lowest (8.50%) in BARI Mug-2, which was statistically similar with BU Mug-2 (9.83%) followed by BU Mug-1 (16.00%) and BARI Mug-6 (19.00%). As a result, the order in terms of adult emergence and grain content loss was also more or less same, which was Barisal local > BARI Mug-3 > BINA Mug-1 > BARI Mug-4 > BARI Mug-5 > BARI Mug-6 > BU Mug-1 > BU Mug-2 > BARI Mug-2.

In case of no choice test of *Callosobruchus maculatus* Fab., more or less similar trends of results were also observed in respect of oviposition, developmental stages, adult emergence and grain content loss represented in Table 6.

Table 6. Influence of different mungbean varieties on oviposition, developmental stages, adult emergence and food consumption of Callosobruchus maculaus Fab. in no choice test

Mungbean varieties	Egg diposition (No.)	Larvae hatched (%)	Larvae developed (%)	Pupae developed (%)	Adult emergence (No.)	Grain content loss (%)	Level of resistance
BU Mug-1	790.0 cd	88.33 a	43.33 d	46.00 c	103.0 c	14,50 cd	S
BU Mug-2	726.7 c	84.00 a	51.00 cd	53,33 bc	100.3 c	9.83 d	MR
BARI Mug-2	756.7 de	85.00 a	42.00 d	45.00 c	91.67 c	8.50 d	MR
BARI Mug-3	843.3 ab	81.67 a	56,67 c	61.00 b	145.0 b	19.50 b	HS
BARI Mug-4	826.7 abc	85.00 a	58.33 c	61.67 b	103,3 с	18.67b	HS
BARI Mug-5	723.3 e	81.67 a	55,00 c	58.33 b	101.7 c	16,33 c	HS
BARI Mug-6	868.3 a	81067 a	50,00 cd	52.33 bc	101.7 с	16.00 c	MS
BINA Mug-1	806.7 bc	80.00 a	73.33 b	79.33 a	155.0 b	19.00 b	HS
Barisal local	826.7 abc	88.33 a	86.33 a	87.67 a	225.0 a	22.67 a	HS

Highly resistant (HR): 0-5% grain content loss; Moderately resistant (MR): 5.1-10% grain content loss; Moderately susceptible (MS): 10.1-15% grain content loss; Susceptible (S): 15.1-20% grain content loss and Highly susceptible (HS): >20% grain content loss.

Figures indicate original means of three (3) replications.

Means followed by same letter(s) are not significantly different (P>0.05) from each other by DMRT

From these findings it was revealed that the varieties Barisal local, BARI Mug-3, BARI Mug-4, BARI Mug-5 and BINA Mug-1 were highly susceptible; BARI Mug-6 was moderately susceptible; BU Mug-1 was susceptible; while BARI Mug-2 and BU Mug-2 were found to be moderately resistant to Callosobruchus maculatus in terms of percent grain content loss. Besides, more or less similar works had been done by Ranganath and Ram (1992) and they reported that out of 13 varieties of mungbean [Vigna radiata] for resistance to Callosobruchus maculatus, lowest feeding damage and adult emergence were recorded with cv. PDM-14. In addition, about similar results were supported by Khattak et al. (1987) and Janzen et al. (1977). But Ali and Rahman (2006) revealed that both in free choice and no choice tests, among different pulses mungbean appeared to be the most common and suitable host for C. maculatus in respect of ovipositional preference, egg deposition, internal larvae and pupae developed, adult emergence and grain content loss but no significant influence on egg hatching.

Considering the duration of adult emergence, for both free choice and no choice test by C. maculatus as depicted in Figure 2, the adults emergence started at 20 days after release (DAR) of adults for both free and no choice tests and gradually increased and the highest number of adults were emerged at 22 DAR for both the tests and then the number of adult emergence decreased gradually for both the tests also. More or less similar results were reported by Ali and Rahman (2006) in their findings.

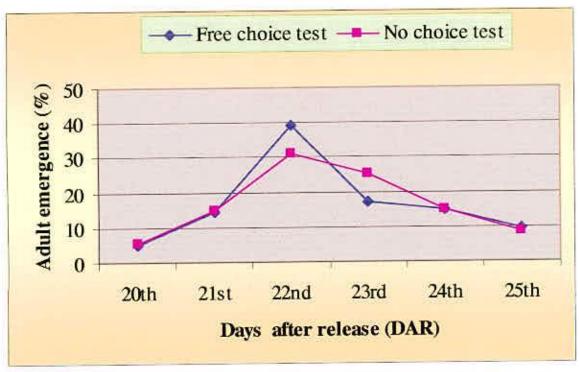


Figure 2. Trend of adult emergence of Callosobruchus maculatus in different days after release in free choice and no choice tests

Comparative effectiveness of Callosobruchus chinensis and C. maculatus on different mungbean varieties

As represented in Figure 3, in terms of the comparative effects of different mungbean varieties on the adult emergence by Callosobruchus chinensis Linn. and C. maculatus Fab. in free choice test, BARI Mug-3, BARI Mug-4, BARI Mug-5 and BARI Mug-6 performed as the common hosts for both the species, while BU Mug-2 was the least preferred host or most resistant for C. chinensis followed by Barisal local, BU Mug-1 and BARI Mug-2, but BARI Mug-6 was the most preferred host. On the other hand, BARI Mug-2 was the least preferred host for C. maculatus followed by BU Mug-2 and BU Mug-1, but Barisal local was the most preferred host.

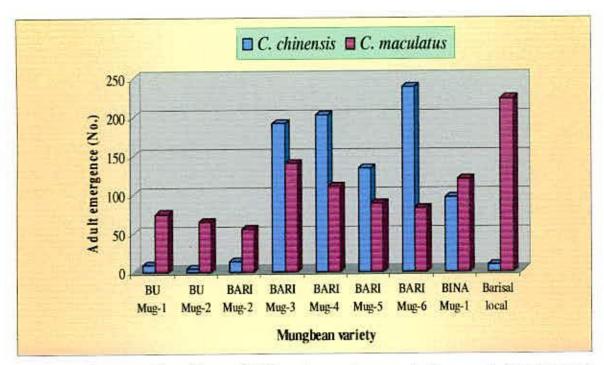


Figure 3. Comparative effects of different mungbean varieties on adult emergence by of C. chinensis and C. maculatus in free choice test

As represented in Figure 4, in terms of the comparative effects of different mungbean varieties on the percent grain content loss by Callosobruchus chinensis Linn. and C. maculatus Fab. in free choice test, BARI Mug-3, BARI Mug-4, BARI Mug-5 and BARI Mug-6 performed as the common hosts for both the species, while BU Mug-1, BU Mug-2 and Barisal local were the least preferred hosts for C. chinensis, but BARI Mug-6 was the most preferred host. On the other hand, BARI Mug-2 was the least preferred host for C. maculatus followed by BU Mug-2 and BU Mug-1, but Barisal local was the most preferred host.

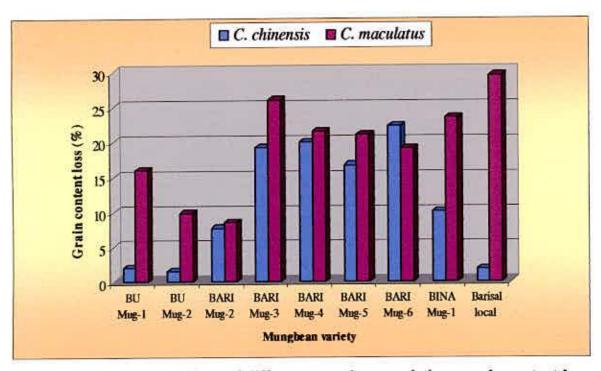


Figure 4. Comparative effects of different mungbean varieties on gain content loss by of C. chinensis and C. maculatus in free choice test

Experiment 2: Evaluation of some selected alternative management practices against pulse beetles infesting mungbean

There were six botanicals and their derivatives named Neem oil @ 8ml/kg, Castor oil @ 8 ml/kg, Camphor @ 2 gm/kg, Neem leaf powder @ 5% w/w, Castor leaf powder @ 5% w/w and Bankolmi leaf powder @ 5% w/w including untreated control treatments evaluated to find out the most effective component(s) against C. chinensis and C. maculatus infesting mungbean. The results were described below considering the following sub-headings:

Evaluation of different botanicals against Callosobruchus chinensis Linn.

Significant differences were found on the effects of different botanicals applied against *Callosobruchus chinensis* Linn. infesting BARI Mug-6, most susceptible variety identified in the earlier experiment 1, in respect of egg deposition, egg hatching, internal larvae and pupae developed, damaged seed and grain content loss represented in Table 7.

As depicted in table 7, the number of eggs laid on mungbean seeds by Callosobruchus chinensis Linn. were the highest (18,400) in untreated control treatment (T_7), which was significantly different from all other treatments, followed by T_6 (16,500) treatment comprising Bankolmi leaf powder, T_5 (15,400) treatment comprising Castor leaf powder and T_4 (12,100) treatment comprising Neem leaf powder; while no eggs were laid in treatment T_3 followed by T_2 (500) treatment consisting castor oil and T_1 (450) treatment comprising Neem oil. That is the highest percent reduction of egg deposition over control was found in treatment T_3 comprising Camphor (100%) and the lowest in T_6 treatment comprising Bankolmi leaf powder (10.32%) and the order of the effectiveness among the treatments was Camphor > Neem oil > Castor oil > Neem leaf powder > Castor leaf powder > Bankolmi leaf powder. The rate of egg hatching was the highest (88.0%) in untreated control treatment (T_7), which was significantly

Table 7. Effect of different botanicals on oviposition, infestation and food consumption by Callosobruchus chinensis Linn. infesting BARI Mug-6 in the laboratory

Treatments	Egg diposition (No.)	% Egg diposition reduction over control	Hatch -ing (%)	% hatch -ing reduction over control	larva developed (%)	% larva develop -ed reducti- on over control	pupae develop -ed (%)	% pupae develop -ed reduction over control	(%) damage	(%) damage reducti -on over control	Grain content loss (%)	(%) Grain content loss reduction over control
T ₁ =Neem oil @8ml/kg	450 d	97.55	8 c	87.90	0 d	100.00	0 d	100.00	0.00 e	100.00	0.00 d	100,00
T ₂ =Castor oil @8ml/kg	500 d	97.28	9 c	88.50	0 d	100,00	0 d	100.00	0.00 e	100.00	0.00 d	100,00
T₃=Camphor @2g/kg	0 e	100.00	0 d	100.00	0 d	100.00	0 d	100.00	0.00 e	100.00	0.00 d	100.00
T ₄ =Neem leaf powder @5%w/w	12,100 c	34.24	80 b	9.09	30 c	62.50	31 c	62.19	41,67 d	50.97	9,00 c	64.00
T ₅ =Castor leaf powder @5%w/w	15,400 b	36.60	83 b	5.68	40 b	50.00	40 Ь	51.21	51.67 c	39.21	995 с	60.20
T ₆ =Bankolmi leaf powder @5%w/w	16,500 b	10.32	85 b	3.40	45 b	43.75	45 Ъ	45.12	71.67 b	15.68	11.89 Б	52.44
T ₇ =Untreated Control	18,400 a	(F)	88 a	9369	80 a	(*)	82 a	3522	85,00 a		25,00 a	i.

Figures indicate original means of three replications
Means followed by same letter(s) are not significantly different (P>0.05) from each other by DMRT

different from all other treatments, followed by T6 (85.00%), T5 (83.00%) and T4 (80.00%) treatments; while no eggs were hatched in treatment T₃ (0.00%) followed by T1 (8.00%) treatment and T2 (9.00%) treatment. That is the highest percent reduction of egg hatching over control was found in treatment T₃ (100%) and the lowest in T6 treatment (3.40%). In case of internal larvae and pupae developed, the highest (80.00% and 82.00%) larvae and pupae development were observed respectively in untreated control treatment (T7) followed by T6 (45.00% and 45.00%), T₅ (40.00% and 40.00%) and T₄ (30.00% and 31.00%) treatments respectively; while no larvae and pupae were developed in treatment T3, T2 and T1 treatments. That is the highest (100%) reduction of larvae and pupae development over control was found in treatment T1, T2 and T3, while it was the lowest in T6 treatment (43.5% and 45.12%). Considering the presence of bored seed and grain content loss after the completion of experiment, the rate of damaged seed and grain content loss by the C. chinensis, the highest (85.00% and 25.00%) values were observed respectively in untreated control treatment (T7) followed by T6 (71.67% and 11.89%), T₅ (51.67% and 9.95%) and T₄ (41.67% and 9.00%) treatments; while no damaged seeds and grain content loss were found in treatment T₃, T₂ and T₁ treatments. That is the highest (100%) reduction of damaged seeds and grain content loss over control were found in treatment T1. T2 and T3, while it was the lowest in T6 treatment (15.68% and 52.44%) (Table 7).

From these findings it was revealed that T_3 (Camphor @ 2 gm/kg seeds), T_1 (Neem oil @ 8 ml/kg seeds) and T_2 (Castor oil @ 8 ml/kg seeds) performed the best results in considering the percent reduction of egg deposition, egg hatching, internal larvae and pupae developed, damaged seed and grain content loss applied against *Callosobruchus chinensis* Linn. infesting BAR1 Mug-6. As a result, the order of results considering the effectiveness among the treatments is T_3 (Camphor @ 2 gm/kg seeds) $> T_1$ (Neem oil @ 8 ml/kg seeds) $> T_2$ (Castor oil @ 8 ml/kg seeds) $> T_4$ (Neem leaf powder @ 5% w/w) $> T_5$ (Castor leaf powder @ 5% w/w) $> T_6$ (Bankolmi leaf powder @ 5% w/w) $> T_7$ (Untreated control) (Table 7). More

or less similar works had also been done by Veer-Singh and Yadav (2003) and they reported that six different plant oils applied against *C. chinensis* in green gram, the seed coating with these oils gave significant protection against *C. chinensis* compared to the untreated control. The oil coating 6-8 h after treatment gave complete protection at doses of 2.5, 5 and 10 ml/kg seeds, whereas partial to complete protection was observed at 90, 150 and 210 days after treatment. In addition, Haque *et al.* (2002) evaluated different plant oils against the pulse beetle (*Callosobruchus chinensis*) on mungbean and they found that no harmful effect was found on oviposition due to oil treatment. However, the percentage of adult emergence was greatly reduced and completely inhibited by the application of oils on the seeds. There was also no adverse effect on the viability of seeds due to different treatments. Besides, Rahman *et al.* (2001) reported the fumigation action of camphor against pulse beetle, *C. chinensis*.

Evaluation of different botanicals against Callosobruchus maculatus Fab.

Significant differences were found on the effects of different botanicals applied against *Callosobruchus maculatus* Fab. infesting Barisal Local (local variety of mungbean), most susceptible variety identified in the earlier experiment 1, in respect of egg deposition, egg hatching, internal larvae and pupae developed, damaged seed and grain content loss represented in Table 8.

As depicted in table 8, the number of eggs laid on mungbean seeds by Callosobruchus maculatus Fab. were the highest (17,300) in untreated control treatment (T₇), which was significantly different from all other treatments, followed by T₆ (15,400) treatment comprising Castor leaf powder, T₅ (13,500) treatment comprising Bankalmi leaf powder and T₄ (10,200) treatment comprising Neem leaf powder; while no eggs were laid in treatment T₃ followed by T₂ (450) treatment consisting castor oil and T₁ (400) treatment comprising Neem oil. That is the highest percent reduction of egg deposition over control was found in treatment T₃ comprising Camphor (100%) followed by T₁ (97.68%) and T₂

Table 8. Effect of different botanicals on oviposition, infestation and food consumption by Callosobruchus maculatus Linn. infesting Barisal local in the laboratory

Treatments	Egg diposition (No.)	% Egg diposition reduction over control	Hatch -ing (%)	% hatching reduction over control	larva develop -ed (%)	% larva developed reduction over control	pupae develop -ed (%)	pupae develop -ed reduction over control	(%) damage	(%) damage reducti -on over control	Grain content loss (%)	(%) Grain content loss reduction over control
T ₁ =Neem oil @8ml/kg	400 d	97,68	8 c	87,90	0 d	100.00	0 d	100.00	0.00. d	100.00	0.00 d	100.00
T ₂ =Castor oil @8ml/kg	450 d	97.39	10 c	88.63	0 d	100.00	0 d	100.00	0.00 d	100.00	0.00 d	100.00
T ₃ =Camphor @2g/kg	0.00 e	100.00	0 d	100.00	0 d	100.00	0 d	100,00	0,00 d	100.00	0.00 d	100.00
T ₄ =Neem leaf powder @5%w/w	10,200 c	41.04	80 b	9.09	30 с	65.11	32 c	63.21	35,67 c	55.96	8.33 c	67.96
T ₅ =Castor leaf powder @5%w/w	13,500 ь	21.96	82 b	6.80	35 с	66.27	37 c	57.47	49.67 b	38.67	11.89 b	54.26
T ₆ =Bankolmi leaf powder @5%w/w	15,400 b	10.98	84 b	4.55	40 b	53.28	45 b	48.27	55.67 b	31.27	13.50 b	48.07
T ₇ =Untreated Control	17,300 a	3	88 a	-	86 a	×	87 a	7890	81.00 a	2	26 a	-

Figures indicate original means of three replications

Means followed by same letter(s) are not significantly different (P>0.05) from each other by DMRT

(97.39%); while it was the lowest in T₅ (10.98%) treatment comprising Castor leaf powder. The rate of egg hatching was the highest (88.0%) in untreated control treatment (T7), which was significantly different from all other treatments, followed by T₆ (84.00%), T₅ (82.00%) and T₄ (80.00%) treatments; while no eggs were hatched in treatment T₃ (0.00%) followed by T₁ (8.00%) treatment and T₂ (10.00%) treatment. That is the highest percent reduction of egg hatching over control was found in treatment T₃ (100%) followed by T₁ (87.90%) treatment and T₂ (88.63%) treatment; while it was the lowest in T₅ treatment (4.55%). However, in case of internal larvae and pupae developed, the highest (86.00% and 87.00%) larvae and pupae development were observed respectively in untreated control treatment (T₇) followed by T₆ (40.00% and 45.00%), T₅ (35.00% and 37.00%) and T4 (30.00% and 32.00%) treatments respectively; while no larvae and pupae were developed in treatment T₃, T₂ and T₁ treatments. That is the highest (100%) percent reduction of larvae and pupae development over control was found in treatment T1, T2 and T3, while it was the lowest in T6 treatment (53.28% and 48.20%). Considering the percentage of bored seed by number and grain content loss by weight after the completion of experiment, the highest (81.00% and 26.00%) damaged seed and grain content loss were found respectively in untreated control treatment (T₇) followed by T₆ (55.67% and 13.50%), T₅ (49.67% and 11.89%) and T₄ (35.67% and 8.33%) treatments respectively; while no damaged seed (0.00%) and grain content loss (0.00%) were found in treatment T3, T2 and T1 treatments. That is the highest (100%) percent reduction of damaged seed and grain content loss over control were found in treatment T1, T2 and T3, while it was the lowest in T₆ treatment (31.27% and 48.07%) respectively.

From these findings it was revealed that T₃, T₁ and T₂ performed the best results in considering the percent reduction of egg deposition, egg hatching, internal larvae and pupae developed damaged seed and grain content loss applied against Callosobruchus maculatus Fab. infesting the variety Barisal local. As a result,

more or less similar trend of the results were found when treated C. chinensis considering the effectiveness among the treatments. Thus the trend is T_3 (Camphor @ 2 gm/kg seeds) > T_1 (Neem oil @ 8 ml/kg seeds) > T_2 (Castor oil @ 8 ml/kg seeds) > T_4 (Neem leaf powder @ 5% w/w) > T_5 (Castor leaf powder @ 5% w/w) > T_6 (Bankolmi leaf powder @ 5% w/w) > T_7 (Untreated control) (Table 8). Almost similar works had been done by Dhakshinamoorthy and Selvanarayanan (2002). They evaluated the effects of different natural products on the survival of C. maculatus infesting stored green gram (Vigna radiata) and the results revealed that the mortality of the beetle at 7 days after treatment was the highest (100%) in Castor oil, followed by Neem leaf powder (91.66%). Besides, Rahman et al. (2001) reported the fumigation action of camphor against pulse beetle, C. chinensis.

As depicted in Figure 5, in terms of the comparative effects of different botanicals on mungbean seeds in terms of grain content loss during the management of *C. chinensis* and *C. maculatus* in the laboratory. No grain content was lost (0.00%) in T₃, T₂ and T₁ treatments for both the species of pulse beetles. But a significant loss of grain content were found in T₇ (25.00% and 26.00%), T₆ (11.89% and 13.50%), T₅ (9.95% and 11.89%) and T₄ (9.00% and 8.33%) treatments for both *C. chinensis* and *C. maculatus* respectively.

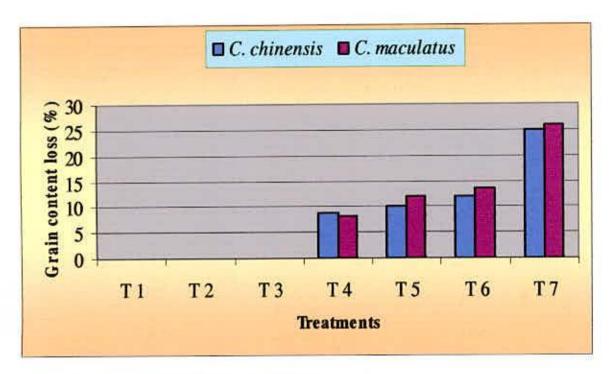


Figure 5. Comparative effects of different botanicals on the grain content loss by C. chinensis and C. maculatus

T₁ = Seeds treated with Neem oil @ 8 ml/kg, T₂ = Seeds treated with Castor oil @ 8 ml/kg

T₃ = Seeds treated with Camphor @ 2 g/kg, T₄= Seeds treated with Neem leaf powder @ 5%w/w

T5=Seeds treated with Castor leaf powder @ 5%w/w, T6=Seeds treated with Bankolmi leaf powder

(a) 5%w/w; T_7 = Untreated control

Effect of different botanicals on the viability of mungbean seeds

As depicted in Figure 6, in terms of the comparative effects of different botanicals on the viability of mungbean seeds during the management of pulse beetle (C. maculatus Fab.) in the laboratory. To evaluate the seed viability, germination tests were done at initial and after the completion of two life cycles of insects (50 DAR). At the initial, percent germination ranged from 90.00 to 91.80%, while after completion of the experiment, the highest percent germination ranged from 90.00 to 90.50% were recorded in T₃ (90.50%) treatment comprising Camphor @ 2 gm/kg seeds, T₁ (90.00%) treatment comprising Neem oil @ 8 ml/kg seeds and T₂ (90.00%) treatment comprising Castor oil @ 8 ml/kg seeds. On the other hand, the lowest percent germination was recorded in T7 (7.33%) comprising untreated control followed by T₆ (12.00%) treatment comprising Bankolmi leaf powder @ 5% w/w, T₅ (14.67%) treatment comprising Castor leaf powder @ 5% w/w and T₄ (16.33%) treatment comprising Neem leaf powder @ 5% w/w. From these findings it was revealed that there were no significant effects of Camphor, Neem oil and Castor oil in reducing the viability of mungbean seeds. However, more or less similar works had been done by Ahmed et al. (2006) and they reported that Camphor did not reduce the viability of mungbean seeds when applied against C. chinensis and the viability retained (87.82 - 88.73% germination) up to 270 days after application of the camphor. Besides, Haque et al. (2002) evaluated different plant oils against the pulse beetle (Callosobruchus chinensis) on mungbean and they found that there was also no adverse effect on the viability of seeds due to different treatments.

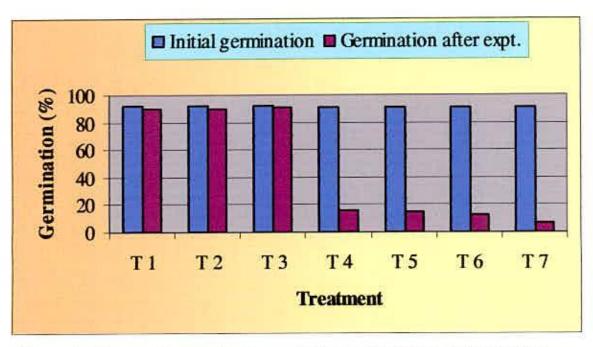


Figure 6. Comparative germination percentages of mungbean with different botanicals between initial and after completion of experiment

T₁ = Seeds treated with Neem oil @ 8 ml/kg, T₂ = Seeds treated with Castor oil @ 8 ml/kg

T₃ = Seeds treated with Camphor @ 2 g/kg, T₄= Seeds treated with Neem leaf powder @ 5%w/w

 T_5 =Seeds treated with Castor leaf powder @ 5%w/w, T_6 =Seeds treated with Bankolmi leaf powder

@5%w/w; $T_7 = Untreated control$

Chapter V Summary

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CHAPTER V

SUMMARY

Two sets of experiments were conducted under this study to identify the resistant source(s) among different mungbean varieties and to find out the most effective component(s) among different alternative management practices against Callosobruchus chinensis and C. maculatus in the laboratory during the period from March, 2007 to October, 2007. First set of experiment considered the screening of nine mungbean varieties for resistance against pulse beetles (C. chinensis and C. maculatus). Second set of experiment considered the alternative management practices consisting two plant oils, one derivative, two dried leaf powder and one untreated control as treatments. The experiment was laid out in the one factor Completely Randomized Design (CRD) with three replications. Data were collected in respect of the egg deposition, egg hatching, internal larvae and pupae developed, adult emergence and grain content loss. The obtained data of different parameters were statistically analyzed to find out the significance level of the treatment. Germination test was also done to evaluate the viability of the seeds before and after the completion of the experiment in case of management practices.

Considering the screening of different mungbean varieties against pulse beetles, there was a significant influence of different varieties of mungbean on the oviposition, growth and development, adult emergence and food consumption by *C. chinensis* and *C. maculatus* in both free choice and no choice tests were observed.

In free choice test for *C. chinensis*, the order of ovipositional preference was BARI Mug-6 > Barisal local > BARI Mug-3 > BARI Mug-4 > BARI Mug-2 > BINA Mug-1 > BU Mug-1 > BARI Mug-5 > BU Mug-2. The rates of egg

hatching were more or less similar in all the mungbean varieties. The order in terms of suitability of larvae and pupae was more or less same, which was BARI Mug-6 > BARI Mug-3 > BARI Mug-4 > BARI Mug-5 > BINA Mug-1 > BU Mug-1 > Barisal local > BARI Mug-2 > BU Mug-2. The order in terms of adult emergence and grain content loss was also more or less same, which was BARI Mug-6 > BARI Mug-4 > BARI Mug-3 > BARI Mug-5 > BINA Mug-1 > BARI Mug-2 > BU Mug-2 > BU Mug-1 > BARI Mug-2 > BU Mug-2 > BU Mug-1 > BARI Mug-2 > BU Mug-2 > BU

In case of no choice test for *C. chinensis*, the order of host suitability among different mungbean varieties in terms of ovipositional preference, growth and development, adult emergence and food consumption were more or less similar that found in free choice test.

In case of free choice and no choice test for *C. chinensis*, the adult emergence started at 18 days after release (DAR) and gradually increased and maximum adults were emerged at 21 DAR in free choice test and 20 DAR in no choice test and then decreased gradually.

Considering the percent adult emergence and grain content loss by C. chinensis Linn. and C. maculatus Fab. in free choice test, BARI Mug-3, BARI Mug-4, BARI Mug-5 and BARI Mug-6 performed as the common hosts for both the species, while BU Mug-1, BU Mug-2 and Barisal local were the least preferred hosts for C. chinensis, but BARI Mug-6 was the most preferred host. On the other hand, BARI Mug-2 was the least preferred host for C. maculatus followed BU Mug-2 and BU Mug-1, but Barisal local was the most preferred host.

Again, in free choice test for *C. maculatus*, the order of ovipositional preference was BARI Mug-6 > BARI Mug-3 > Barisal local > BARI Mug-2 > BARI Mug-4 > BINA Mug-1 > BU Mug-1 > BARI Mug-5 > BU Mug-2. The rates of larvae hatch from eggs were more or less similar in all the mungbean varieties. The order in terms of suitability of larvae and pupae was more or less same, which was Barisal local > BARI Mug-3 > BINA Mug-1 > BARI Mug-4 > BARI Mug-5 >

BARI Mug-6 > BU Mug-1 > BU Mug-2 > BARI Mug-2. The order in terms of adult emergence and grain content loss was also more or less same, which was Barisal local > BARI Mug-3 > BINA Mug-1 > BARI Mug-4 > BARI Mug-5 > BARI Mug-6 > BU Mug-1 > BU Mug-2 > BARI Mug-2.

In case of no choice test for *C. maculatus*, the order of host suitability among different mungbean varieties in terms of ovipositional preference, growth and development, adult emergence and food consumption were more or less similar that found in free choice test.

In case of free choice and no choice test for *C. maculatus* the adult emergence started at 20 days after release (DAR) and gradually increased and maximum adults were emerged at 22 DAR in both tests and then decreased gradually.

Considering the evaluation of different botanicals applied against pulse beetles, there was a significant influence of different botanicals on the oviposition, growth and development and food consumption by *Callosobruchus chinensis* and *C. maculatus* were observed.

The effectiveness of different treatments applied against both *C. chinensis* and *C. maculatus* was more or less similar. The performance of the treatments in terms of oviposition differed significantly and the order of the effectiveness among the treatments was Camphor > Neem oil > Castor oil > Neem leaf powder > Castor leaf powder > Bankolmi leaf powder. In terms of percent egg hatching, internal larvae and pupae developed, percent damaged seeds and grain content loss, the order of the performance of the different treatments was also more or less similar and the order was also Camphor > Neem oil > Castor oil > Neem leaf powder > Castor leaf powder > Bankolmi leaf powder.

Considering the comparative effects of different botanicals in terms of grain content loss during the management of *C. chinensis* and *C. maculatus*, no grain content was lost (0.00%) in T₃, T₂ and T₁ treatments for both the species. But a significant loss of grain content were found in T₇ (25.00% and 26.00%), T₆

(11.89% and 13.50%), T₅ (9.95% and 11.89%) and T₄ (9.00% and 8.33%) treatments for both C. chinensis and C. maculatus respectively.

Considering the comparative effects of different botanicals on viability of mungbean seeds during the management of pulse beetle (*C. maculatus* Fab.), there was no significant effect of Camphor, Neem oil and Castor oil in reducing the viability of seeds, while other treatments (comprising Neem leaf powder 5% w/w, Castor leaf powder 5% w/w and Bankolmi leaf powder 5% w/w) reduced the viability of seeds.

Chapter VI Conclusion

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CHAPTER VI

CONCLUSION

The following conclusions are drawn from the findings of experiments for further evaluation on a large scale.

- Different varieties of mungbean had significant influence on the oviposition, growth and development, adult emergence and food consumption by Callosobruchus chinensis and C. maculatus for both free choice and no choice tests.
- Among the mungbean varieties BARI Mug-6 was the most suitable host for C.
 chinensis and Barisal local for C. maculatus, whereas BARI Mug-4, BARI
 Mug-5, BARI Mug-6 appeared to be most common and suitable host for both
 the species in respect of oviposition, growth and development, adult
 emergence and food consumption.
- In case of C. chinensis Linn., the variety BARI Mug-6 was highly susceptible; BARI Mug-4, BARI Mug-3 and BARI Mug-5 were susceptible; BARI Mug-2 and BINA Mug-1 were moderately resistant, while BU Mug-2, Barisal local and BU Mug-1 were found to be highly resistant host in respect of grain content loss.
- In terms of infestations by C. maculatus Fab., the varieties Barisal local, BARI Mug-3, BARI Mug-4, BARI Mug-5 and BINA Mug-1 were highly susceptible; BARI Mug-6 was moderately susceptible, BU Mug-1 was susceptible, while BARI Mug-2 and BU Mug-2 were found to be moderately resistant in respect of grain content loss.
- There was no significant influence of different mungbean varieties on egg hatching for both the species.
- The developmental period of C. chinensis and C. maculatus were not so influenced by different mungbean varieties.



- Different botanicals for the management of C. chinensis and C. maculatus had significant influence on their oviposition, growth and development, adult emergence and food consumption.
- Among the botanicals, camphor appeared as the most effective components
 followed by Neem oil and Castor oil for both the species in reducing (100%)
 growth and development and food consumption by them, but the partial
 reduction were occurred by Neem oil and Castor oil in terms of egg deposition
 and egg hatching.
- Among different botanicals there was no significant effect of Camphor, Neem
 oil and Castor oil in reducing the viability of seeds. But other treatments
 comprising Neem leaf powder, Castor leaf powder and Bankolmi leaf powder
 affected the viability of seeds.

Chapter VII References

CHAPTER VII

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