DETERMINATION OF EFFECTIVE DOSE OF CLOVE POWDER FOR THE MANAGEMENT OF PULSE BEETLE (*CALLOSOBRUCHUS CHINENSIS* L.) INFESTING CHICKPEA IN LABORATORY CONDITION

Rony Debnath[1], Md. Abdul Maleque[1]*, A F M Saiful Islam[2], Setu Chowdhury[3] and Mohon Nandi[1]

[1]Department of Entomology, Sylhet Agricultural University, Sylhet 3100. [2]Department of Crop Botany and Tea Production Technology, Sylhet Agricultural University, Sylhet 3100. [3]Department of Entomology, Bangladesh Agricultural University, Mymensingh 2202

ABSTRACT

Pulse beetle, *Callosobruchus chinensis* L. is a major stored grain pest of Bangladesh and it is difficult to manage as infestation starts from the field. A laboratory study was carried out to protect the chickpea seeds in storage against *C. chinensis* by using clove powder as seed protectant at 1, 3, 5, 7, 9, 11 and 13 g kg^{-1} seeds of chickpea on the basis of adult mortality (%), number of eggs laid (%), adult emergence (%) and seed weight loss (%). Treated seeds did not show any egg on their surface and no adult was emerged when they were mixed with 9, 11 and 13 g clove powder kg^{-1} of chickpea seeds. Seed weight loss was lowest at 11 and 13 g powder kg^{-1} seeds, whereas the highest adult mortality (100%) was recorded at 9 and 13 g powder kg^{-1} seeds. In contrast, control seeds had maximum number of eggs laid (229.67), adult emergence (77.86%) and seed weight loss (30.38%) with the minimum adult mortality of 6.7%. It was found that total number of eggs laid, adult emergence (%) and seed weight loss (%) were markedly reduced whereas adult mortality increased with increasing the doses of clove powder. The results reported here that the clove powder could be a potential alternative option for pulse beetle management in chickpea storage.

Keywords: Storage pest, pulse beetle, mortality, adult emergence, weight loss.

INTRODUCTION

Chickpea is a major source of protein for both human and animal as it contains high nutrition and fat content compared to cereals (Bampidis *et al.* 2011). It contains up to 59% carbohydrates and one third portion of protein bearing no major anti-nutritional factor (Hulse 1991). Chickpea contribute 15.1 million tons worldwide production in 2020 where India alone produces around 73% (Alete *et al.* 2012). In

*Corresponding author : maleq.entom@sau.ac.bd

Bangladesh, 8300 ha land of 21 regions is occupied by chickpea and produced 6500 MT per year (Abate 2012). The pulse productivity has been gradually decreasing over the recent years and several factors are considered for the declining trends of pulse production in our country. Seni *et al.* (2022) stated that average pulse crop losses at storage are a 5–10% in the temperate area and 20–30% in the tropical area. One study conducted by Kuotsu *et al.* (2022) revealed that reduction of damage in storage also depend on storage structure and showed that plastic bag had the least effect on weight loss and infestation of stored seed. Dhaker *et al.* (2022) found that chick pea variety also a responsible character for pulse beetle attack in storage and among these varieties JAKI 9218 showed the best result against pulse beetle. However, other factors including weather condition, insect pest infestation, disease, field protection, cultivation tools, seed contamination, seed variety and many more are responsible for seed damage in storage. Among the factors, insect pest infestation has been regarded as a major factor of lower pulse production in the world.

The pulse beetle, *Callosobruchus chinensis* L. spread all over the world and considered as the most devastating pest of stored legumes (Park *et al.* 2003) which also reported in many south Asian countries including Bangladesh (Aslam *et al.* 2002). The invasion of pulse beetle in seed storage causes reduction of both quality and quantity of chickpea seeds by doubling themselves in a short time (IITA 1989). Our farmers store pulses by using local methods and transport them without taking any precautions which make the condition of storage more complicated. Pulse seeds are often attacked by the pulse beetle both field and storage conditions. The damage in storage is more crucial than in field as they stored for yearlong consumption (Husain 1995). In Bangladesh, pulse seeds are often stored in godowns and farmers house which provide suitable habitats for growth and spread of most storage pests including pulse beetle, which make seeds unfit for human consumption (Atwal 1976).

Farmers of Bangladesh prefer to control pest of stored food products and other agricultural commodities from the insect infestation mainly by synthetic insecticides as they give quick response against insect pest. Use of synthetic insecticides has exerted many complications such as development of insecticide resistance in pest insect, hazards to environment and human health, destruction of non-target organisms and outbreaks of secondary pests (Rustamani *et al.* 1985, Duguet and Wu 1986 and Husain 1995). Injudicious use of pesticides to protect pulses from the attack of pulse beetle in storage may cause serious health hazards to the pesticide applicators and stored pulse consumers.

Use of synthetic organic pesticides with stored pulses beyond the permissible level of concentration for the control of pulse beetle has prompted the researchers to look for some less or non-toxic pulse protectants as alternative to toxic chemical pesticides. The integration of natural insecticidal products from locally available plants for use in storage by the farmers in developing countries appear to be quite safe and promising (Jillani *et al.* 1988). Phadtare *et al.* (2023) found that neem oil + APSA 80 can protect the storage seed up to 270 days without causing any seed damage and weight loss caused by pulse beetle. Ahmed *et al.* (2016) has evaluated the effects of clove and others natural powders on the oviposition inhibition, adult emergence, adult mortality and seed weight loss by the pulse beetle. He found that clove powder gave excellent protection to chickpea seeds from the attack of pulse beetle in the laboratory. Considering the above factors, the present study was designed to evaluate the minimum applied doses of clove powder in laboratory condition for managing chickpea seeds in storage condition.

MATERIALS AND METHODS

The present study was carried out to develop effective lower dose(s) of clove powder on adult mortality, egg laying capacity, adult emergence, and seed weight loss against pulse beetle, *Callosobruchus chinensis* L. at the laboratory, Department of Entomology, Sylhet Agricultural University, Sylhet, Bangladesh during April to August 2016. The average temperature was around 30°C and relative humidity was around 80%. The experiment was set at ambient condition of the laboratory and laid out in a Completely Randomized Design (CRD). Three replications were used in this experiment and a total of 24 Petri dishes were used to carry out the experiment.

Collection and mass rearing of pulse beetle *C. Chinensis* L. : The samples of stored chickpea infested by *C. chinensis* were collected from different households, stores and godowns of local Sylhet areas. Adult pulse beetles were separated from the collected chickpea with an aspirator. Hundred pairs of male and female pulse beetles were introduced and allowed to oviposit in a large plastic container having 1kg of chickpea seeds and kept on the laboratory desk. Jar mouth was covered with fine clothes to protect the beetles from escaping. Then insects were allowed for free mating and oviposition for a maximum period of 5 days. The seeds with eggs were kept for 48 days to emergence of new adults. Newly emerged adults were kept in different containers. The rearing procedure was repeated in different batches to ensure continuous supply of test insects.

Preparation of clove powder : Clove (*Caryophyllus aromaticus* L.) was collected from the nearest market and sun dried for 7 days until they reached to a constant weight suitable for powder preparation (Fig. 1). Clove powder was prepared by pulverizing the sun-dried clove with the help of mortar and pestle. Then the dust was passed through a sieve to obtain fine and uniform powder. Clove powders of 1 g, 3 g, 5 g, 7 g, 9 g, 11 g, and 13 g kg^{-1} of chickpea seeds were used as seven different doses. Chickpea seeds were not treated with clove powder in the control observation.



Fig. 1. Sun-dried clove

Collection of chickpea seed : The seeds of chickpea (*Cicer arietinum* L.) commonly known as "Chola" were used in the experiment and collected from the nearest market (Fig. 2). The collected seeds were separated from broken parts, other crop seeds and any inert particles and then sun dried for 7 days until they reached to a constant weight. Seeds were matured, fresh and completely free from insects, eggs and other micro-organisms. When seeds were stored, no insecticide was used.



Fig. 2. Healthy chickpea seeds

Treatment of chickpea seed with clove powder: Clove powder was mixed with 20 g chickpea seeds at 1, 3, 5, 7, 9, 11 and 13 g powder kg^{-1} chickpea seeds in separate Petri dishes. Five pairs of newly emerged adult beetles and each pair containing a male and a female was released into the Petri dishes containing treated seeds. Each of the doses was replicated thrice with the control. The Petri dishes were kept in the laboratory and wrapped with brown paper to maintain dark condition without any disturbances which facilitate mating and oviposition. The efficacy of clove powder against the pulse beetle was evaluated considering adult mortality, egg laying capacity, adult emergence and seed weight loss from treated and untreated seeds. The methods employed to record data on the above-mentioned parameters are described separately.

Adult mortality: To carry out the study, only the clove powder was kept at the amount of 0.02, 0.06, 0.10, 0.14, 0.18, 0.22 and 0.26 g powder (at the rate of 1, 3, 5, 7, 9, 11 and 13 g kg^{-1} chickpea seeds) in separate Petri dishes. Ten newly emerged adult beetles were released into the Petri dishes containing clove powder at different doses. Each of the doses was replicated thrice with the control. Data on the adult mortality were recorded daily from the 1st day to the 7th day of release of

the pulse beetles. After seven days total number of dead beetles were calculated and converted to percentages.

$$\text{Mortality (\%)} = \frac{\text{Total number of dead pulse beetle}}{\text{Total number of pulse beetle released}} \times 100$$

Egg laying capacity: Egg laying capacity was evaluated based on the total number of eggs laid per 20 g of chickpea seeds. After seven days, seeds were carefully examined using magnifying glass (Fig. 3). Then total number of eggs per 20 g seed per Petri dish was recorded. After recording data, seeds with eggs were returned to their respective Petri dishes and covered with lid and left undisturbed for further development.



Fig. 3. Eggs of pulse beetle laid on chickpea seeds

Adult emergence: After completion of larval development inside the seed, adult was started emerging after 24 days of egg-laying and continued for several days (Fig. 4). The emerged beetles were counted and removed every day from the Petri dishes up to 7 consecutive days. After seven days total number of emerged adult beetles were calculated and converted to percentages.

$$\text{Adult emergence (\%)} = \frac{\text{Total number of emerged adultpulse beetle}}{\text{Total number of eggs of pulse beetle}} \times 100$$

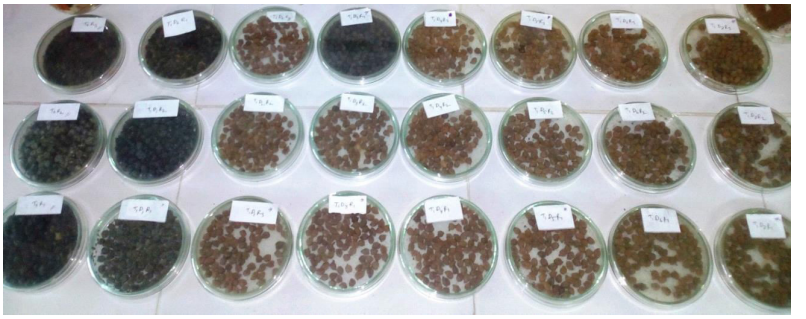


Fig. 4. Showing adult emergence test in the laboratory

Seed weight loss: After hatching, the larvae entered the seeds and fed on the cotyledons where they pupated and afterwards, they came out as adults making exit holes on the seed (Fig. 5). The chickpea seeds were separated from dust particles and dead bodies of pulse beetles by sieving and winnowing. The cleaned infested seeds in each Petri dish were weighted separately. The seed weight losses were calculated subtracting the final weight from the initial weight in each Petri dish.

The seed weight losses were converted to percentage using the following formula:

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

Where, A= Initial weight, B= Final weight



Fig. 5. Damage symptom on chickpea seeds

Statistical Analysis: Adult mortality data were transformed into percentage. Data of egg laying capacity (Total number of eggs laid), adult emergence (%) and seed weight loss (%) were transformed using SQRT (X+0.5) transformation formula. After transformation, data were subjected to statistical analyses in accordance with one factor Completely Randomized Design (CRD). All statistical analysis was done using statistical software (R ver. 4.1.0, R Core Team 2021). Means were compared with LSD value.

RESULTS AND DISCUSSION

Effect of clove powder on the adult mortality: It was observed that there was a significant difference between the treated seed and untreated control seed (Table 1). The lowest adult mortality (6.7%) was recorded from control. It was also found that the adult mortality increased with increasing the doses of clove powder. The adult mortality was higher (100%) at two doses (i.e., 9 and 13 g powder per kg chickpea seeds) among all the treatments. Besides, 10.0%, 60.0%, 76.7%, 90.0% and 96.7% adult mortality were recorded when the doses of clove powder were used at the rate of 1, 3, 5, 7 and 11 g powder per kg of chickpea seeds, respectively. The findings of the present study revealed that adult mortality is dose depended and mortality increase with the increasing of clove powder dose. The results are closely fitted with Mahdi and Rahman (2008) who found clove powder as the most effective, showing 100% adult mortality within 3 days of application against pulse beetle. Sajane *et al.* (2022) tested a bioassay against red flour beetle with some botanicals and clove power (83.33%) found a promising management tool after neem-azal (100%) and acorus oil (90%). Furthermore, Mario *et al.* (2023) stated that clove powder fumigation has the highest mortality rate over adzuki bean weevil, *C. chinensis*.

Effect of clove powder on the egg laying capacity of pulse beetle : The number of eggs laid varied significantly when chickpeas were treated with clove powder at seven different doses (i.e., 1, 3, 5, 7, 9, 11 and 13 g powder kg^{-1} of chickpea seeds) as compared to untreated control (Table 2). The lowest number of eggs laid was only 0.00 when chickpea seeds were treated with clove powder at the rate of 9, 11 and 13 g kg^{-1} of chickpea seeds and untreated seeds showed higher numbers of eggs (229.67). It was observed that the egg laying capacity of *C. chinensis* decreased with increasing the doses of clove powder. Besides, 176.67, 39.00, 61.33 and 3.00 eggs were laid when the doses of clove powder were used at the rate of 1, 3, 5, and 7 g powder kg^{-1} of chickpea seeds, respectively.

Table 1. The effect of clove powder on the percent adult mortality of pulse beetle

Clove powder (g kg^{-1})	Adult mortality (%)
Control (untreated)	6.7 d
1	6.7 d
3	60.0 c
5	76.7 bc
7	90.0 ab
9	100.0 a
11	96.7 a
13	100.0 a
LSD (0.05)	1.76
CV (%)	15.21

Footnote: *Means followed by the same letter are significantly different (P<0.05)*

Table 2. The effect of clove powder on the number of eggs laid by pulse beetle

Clove powder (g kg^{-1})	Number of eggs laid
Control (untreated)	229.67 (15.09) a
1	176.67 (13.29) a
3	39.00 (6.11) b
5	61.33 (6.55) b
7	3.00 (1.71) c
9	0.00 (0.71) c
11	0.00 (0.71) c
13	0.00 (0.71) c
LSD (0.05)	3.73
CV (%)	38.42

Footnote: *Means followed by the same letter are significantly different (P<0.05). Values in the parentheses are square root transformed.*

Kumar *et al.* (2018) found that clove oil gave the minimum number of egg-laying and least number of adult emergences on black gram seeds. The previous workers found that the effectiveness of black pepper powder on the oviposition of *C. chinensis* was very satisfactory, but moderately satisfactory in case of neem oil and mustard oil (Thakur and Mandeep 2013). Several treatments including comprising of clove powder (3g kg^{-1} grain), pepper powder (3g kg^{-1}), *Acorus calamus* rhizome powder (10g kg^{-1}) and sesame oil (5ml kg^{-1}) were used against adult pulse beetle on green gram seed and found all the treatment has a negative effect on egg laying capacity of pulse beetle (Rathod *et al.* 2019).

Effect of clove powder on the adult emergence (%): The percentage of adult emergence significantly differed among the doses under laboratory condition (Table 3). Chickpea grains treated with clove powder at the rate of 9, 11 and 13g kg^{-1} of chickpea seeds showed lowest adult emergence (0%) suggesting that clove powder was strongly effective protectant against the pulse beetle. In contrast, the highest adult emergence (77.87%) was found on untreated seeds. The percentages of intermediate levels of adult emergence were 57.02%, 31.43%, 40.63% and 22.22%, respectively when chickpea seeds were treated with clove powder at 1, 3, 5 and 7 g kg^{-1} of chickpea seeds.

This result is in line with the previous experiments and showed effective action on many stored grain pests. All these experiments were dose and environmental condition depended. El-sawy *et al.* (2022) examined many plants powder and extract against *C. maculates*. Among them, clove powder exhibits the lowest adult emergence over clove extract against *C. maculates.* Javaid and Poswal (1995) reported that 2% (w/w) black pepper admixed with cowpea significantly reduced oviposition and adult emergence of *C. maculates* after an exposure period of 10 and 70 days respectively. Similarly, Su (1983) also reported that black pepper oil extracted in hexane applied @ 1000 mg kg^{-1} had reduced 96% and 97% adult emergence of *C. maculates* and *S. oryzae*, respectively, on cowpea and wheat.

Table 3. The effect of clove powder on the adult emergence (%) of pulse beetle

Clove powder (g kg^{-1})	Adult emergence (%)
Control (untreated)	77.87 (8.84) a
1	57.02 (7.53) ab
3	31.43 (5.64) bc
5	40.63 (6.27) bc
7	22.22 (4.12) c
9	0.00 (0.71) d
11	0.00 (0.71) d
13	0.00 (0.71) d
LSD (0.05)	2.21
CV (%)	29.71

Footnote: *Means followed by the same letter are significantly different (P<0.05). Values in the parentheses are square root transformed.*

Effect of clove powder on the seed weight loss (%): Damage symptoms of pulse beetle to chickpea seeds in Petri dish are presented in Fig. 5. The larvae fed on the seed's internal contents for their growth and development. Pupae formation takes place inside the seeds and finally emerged as adults from the seed creating hole on chickpea. The seed weight (0%) loss by the attack of pulse beetle on chickpea treated with clove powder at various doses under laboratory condition is presented in Table 4. The percent seed weight loss differed significantly among the treatments. Chickpea grains treated with clove powder had lowest seed weight loss (0%) at 11 and 13 g powder kg^{-1} of chickpea seeds, suggesting that clove powder is a strong effective protectant against the pulse beetle. In contrast, the highest seed weight loss (30.38%) was found from the untreated seeds. The percentages of intermediate levels of seed weight losses were 22.53%, 9.82%, 8.32%, 0.72% and 0.03%, respectively, when chickpea seeds were treated with clove powder at 1, 3, 5, 7 and 9 g per kg of chickpea seeds. This result also showed that the percentage of seed weight loss decreased gradually with increasing the doses of clove powder.

Table 4. The effect of clove powder on the seed weight loss (%) of pulse beetle

Clove powder (g kg^{-1})	Seed weight loss (%)
Control (untreated)	30.38 (5.55) a
1	22.53 (4.79) a
3	9.82 (3.18) b
5	8.32 (2.66) b
7	0.72 (1.06) c
9	0.03 (0.73) c
11	0.00 (0.71) c
13	0.00 (0.71) c
LSD (0.05)	1.10
CV (%)	26.34

Footnote: *Means followed by the same letter are significantly different ($P<0.05$). Values in the parentheses are square root transformed.*

Treatment on seed weight depends on various conditions including active ingredient of plant product and the condition of the seeds. An experiment conducted on wheat seed during storage for 18 months based on package type and some plant

product treatment revealed that polyethylene packages with copper nanoparticles showed the best protectant against seed weight loss compared to clove powder in storage condition (El-Seidy *et. al.* 2022). Thakur and Mandeep (2013) have recently found that black pepper fruit powder, neem oil and mustard oil minimize the weight loss of seeds due to the attack of pulse beetle at 0%, 0.22% and 0.45% respectively while as high as 38.33% in the untreated control.

In place of using synthetic pesticide, the study designed to manage pulse beetle with nature based plant product (clove powder) in the laboratory. Clove has many wonderful activities against insect pest and plays a game changer role to deter the pest in storage. Its pungent odor acts as an excellent repellent against many insects in both field and storage. Eugenol the active ingredient of clove oil is actually playing the vital role in agriculture sector by acting as a repellent, antifeedant, growth inhibitor and causing acute toxicity on pests where synthetic pesticides develop resistant against mites, ticks, spider, and fungal diseases. Bandara and Senevirathne (2021) conducted a study and found other than clove buds stems and leaves extracts exhibit contact repellent against *S. oryzae*. The phytochemical ingredient of clove is volatile and its fume when come into contact with the insect olfactory cause an odor sensation, which induced changes in insect behavior and repellence (Gitahi *et al.* 2021, Maia and Moore 2011). In this study, seven doses (1, 3, 5, 7, 9, 11 and 13 g clove powder kg^{-1} of chickpea seeds) were used and only three doses (9, 11 and 13 g clove powder kg^{-1} of chickpea seeds) were found as the most effective against all parameters such as reduced adult mortality (%) and decreased the number of eggs laid and adult emergence (%) and seed weight loss (%) in laboratory condition. Other doses also effective but may need further consideration to use in storage as these doses showed some damage on chickpea seed due to insect infestation.

CONCLUSION AND RECOMMENDATIONS

The present results clearly indicate that clove powder is an excellent protectant against the pulse beetle in storage in respect of increasing adult mortality, reducing egg laying capacity, adult emergence and seed weight loss. Having few exceptions, all the seven doses (1, 3, 5, 7, 9, 11 and 13 g clove powder kg^{-1} of chickpea seeds) used in the study gradually increased the adult mortality (%) and decreased the number of eggs laid, adult emergence (%) and seed weight loss (%). Since clove powder showed effectiveness in controlling pulse beetle on chickpea seeds, it may

be recommended that further study using several hosts of pulse beetle e.g., lentil, cowpea, mungbean etc. should be undertaken to ascertain the present results. As the experiment was conducted during the summer-rainy season only, further research should be conducted covering both winter and rainy seasons to know the seasonal variation in the effectiveness of clove powder as seed protectant in storage conditions. As the price of clove is high the marginal benefit cost ratio (MBCR) should also be evaluated to determine the cost-effectiveness of the results.

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