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# EVALUATION OF INDIGENOUS PLANT POWDER AGAINST PULSE BEETLE (*Callosobruchus chinensis* L.) OF STORED CHICKPEA

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## Abstract

The study was carried out in the Laboratory of Entomology, Faculty of Agriculture, Sylhet Agricultural University, Sylhet during October to December 2014 with a view to assessing the effectiveness of four indigenous plants powder as protectant of stored chickpea seeds from the attack of pulse beetle (*Callosobruchus chinensis* L.). Plant powders were prepared from neem leaf (*Azadirachta indica*), black pepper (*Piper nigrum* L.), clove (*Caryophyllus aromaticus* L.) and methi (*Trigonella foenum graecum* L.). Three doses of all four plant powders @ 5 g, 10 g and 15 g $kg^{-1}$ of chickpea seeds were used to determine the oviposition inhibition, adult emergence (%), adult mortality (%) and seed weight loss (%) due to the attack of pulse beetle. All three doses of clove powder were found to be most effective in protecting chickpea seeds from the attack of pulse beetle, in terms of eggs laid (0), egg bearing seeds (0), adult emergence (0%) and seed weight loss (0%). In respect of above four parameters other treatments had negligible effect having no significant difference with the control. The result indicated that all three doses of clove powder could be used as protectant of chickpea seeds in storage. Low dose (5 g clove powder for 1 kg of chickpea seeds) could be preferable to other two higher doses (10 g, 15 g clove powder for 1 kg of chickpea seeds) in respect of lower cost involvement. Because low dose was found to be effective, year-round experiments could be undertaken to determine the effectiveness of some lower clove powder doses than that of 5 g clove powder $kg^{-1}$ of chickpea seeds.

***Keywords*:** Neem leaf, black pepper, clove, methi, botanicals.

## Introduction

Pulses are leguminous crops under the family Leguminosae and sub-family Papilionaceae. They contain 20 to 30% of protein which is almost three folds higher than cereal crops (Doharey *et al*., 1983). A number of pulses like lentil, gram, blackgram, grasspea, mungbean, cowpea and chickpeaare grown in Bangladesh. However, their per capita consumption is only 12 g in the country (BBS, 1998), whereas World Health Organization (WHO) has recommended an average intake of 45 g per capita per day (BARI, 1998). Among the pulses, chickpea (*Cicer arietinum* L.) is a highly nutritious pulse being cultivated worldwide. It ranks third position in the importance list of food legumes in the world. Chickpea contains 38 to 59% carbohydrate and 25.3 to 28.9% protein without bearing any major anti-nutritional factor (Hulse, 1991).

Despite the above facts the production and productivity of pulses has been gradually decreasing in the country because of varietal instability, insect pest infestation and diseases. A number of pests have been recognized as pests of pulse crops in field and storage conditions. Three species of pulse beetles *viz.*, *Callosobruchus chinensis* L., *Callosobruchus maculates* F. and *Callosobruchus analis* F. have been reported as major pests of stored pulses (Husain, 1995), but the species *Callosobruchus chinensis* L. has been reported to be the most widespread and devastating pest of stored legumes (Park *et al.*, 2003; Aslam, 2004). It causes colossal loss in seed weight and protein content of the pulse seed. It can cause 10-95% loss in seed weight and 45.5-66.3% loss in seed protein under normal condition and damage increases with increasing of storage period (Gujar and Yadav, 1978). The attack of pulse beetle in stores causes reduction in weight, market value and germination of chickpea seeds (IITA, 1989).

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The management of pulse beetle in the world has principally relied on the application of synthetic organophosphate and fumigant insecticides such as methyl bromide and phosphates, which have been regarded as the most effective means of protection for stored food and agricultural commodities from insect infestation (EPA, 2001). Despite the relative effectiveness of these products, their intensive use can cause many problems such as occurrence of insect resistance, accumulation of residues in foods, human health hazards, killing of beneficial insects and environmental contamination (Srivastava and Singh, 2002; Campos *et al.*, 2013). These problems have highlighted the needs of developing an alternative means of controlling insect pests in storage. From this point of view, one alternative to the conventional control is the use of medicinal plants and spices with insecticide properties, whose parts can be prepared and applied as powders, extracts and oils. These products are low cost, easy to produce and use in the farmers' houses and small scale industries. Their application does not require qualified personnel and have no or little impacts on human health and environment (Hernández and Vendramim, 1997; Mazzonetto and Vendramim, 2003; Yankanchi and Gonugade, 2009). Considering the above facts, the study was undertaken to determine the effectiveness of four different plant powders as protectant of stored chickpea seeds.

## Materials and Methods

The experiment was conducted in the Laboratory of Entomology, Faculty of Agriculture, Sylhet Agricultural University, Sylhet during October to December, 2014. The objectives of the study were to determine the effects of plant powder on the oviposition inhibition, adult emergence (%), adult mortality (%) and seed weight loss (%) against the pulse beetle (*Callosobruchus chinensis* L.) The temperature and relative humidity of the Laboratory of Entomology were 25 ± 3°C and 70 to 80%, respectively during the study.

### Culture of the pulse beetle

Adult male and female pulse beetle (*C. chinensis*) were used to evaluate the effectiveness of indigenous plant powders as protectant of stored chickpea seeds. Male and female pulse beetles were sorted out according to their size, shape and other morphological characters of the body (Bandra and Saxena, 1995). Hundred pairs of the pulse beetle were introduced and allowed for oviposition in a large plastic container having 1000 g of chickpea seeds. Container was then covered with nylon cloth and kept on the laboratory desk for allowing free mating and oviposition for a maximum period of 5 days. Afterwards, living and dead insects were removed by sieving. The seeds with eggs were kept in separate container for emergence of new adults. Newly emerged adults were shifted to different containers. The rearing procedure was repeated in different batches to ensure continuous supply of the test insect.

### Collection and preparation of plant powder

Four different plant powders were prepared from neem leaf (*Azadirachta indica* A. Juss), black pepper (*Piper nigrum* L.), clove (*Caryophyllus aromaticus* L.) and methi (*Trigonella foenum graecum* (Fenugreek) to determine their effectiveness as protectant of stored chickpea seeds. These were collected from the nearest market Sibgonj, Sylhet. The collected plant materials were sun dried for 7 days until they reached to a constant weight suitable for powder preparation. The powders were prepared by pulverizing the sun-dried leaf and seeds with the help of a steel blender. Then the dust was passed through a 25 cm dia sieve to obtain fine and uniform powder.

### Experimental procedures

Each plant powder was mixed with 20 g chickpea seeds @ 5 g, 10 g and 15 g $kg^{-1}$ of seeds in each petri dish (90 cm dia.). Five pairs of newly emerged adult beetles were released into the petri dishes containing treated seeds. Each treatment was replicated thrice including control. The Petri dishes were kept in the Laboratory and wrapped with brown paper to make dark condition with no external disturbances. Then the effectiveness of plant powder against the pulse beetle was evaluated with respect to the following parameters.

### Oviposition inhibition

Oviposition inhibition was evaluated based on the total number of eggs laid by pulse beetle and egg bearing seeds. After seven days, seeds were carefully examined using magnifying glass and seeds with eggs or without eggs were separated. Then total number of egg bearing seeds and total number of eggs per 20 g seed per Petri dish were recorded. After recording data, both seeds with eggs and without eggs were returned to their respective Petri dishes and covered with lid and left undisturbed for further development.

**Adult emergence (%)**

Adult development performance was assessed by recording the number of $F_1$ progeny emerged from eggs. The adults started emerging after 24 days of egg-laying and continued for several days. The emerged adults were counted and removed every day from the Petri dish up to 7 consecutive days.

**Adult mortality (%)**

Data of adult mortality were recorded daily from the 1st day to the 7th day of pulse beetle releases. After seven days total number of dead beetles were calculated and converted to percentage.

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

**Seed weight loss (%)**

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 weighed 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:

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

Where, A= Initial weight, B= Final weight

**Data Analysis**

All data were transformed using square root, log and arcsine data transformation formulae, which were appropriate for each data set. After data transformation, they were subjected to statistical analyses in accordance with one factor Completely Randomized Design (CRD). All statistical analyses were done through a computer software MSTAT-C, a computer based statistical software package (Russel, 1986). The analysis of variance (ANOVA) was calculated for proper interpretation of the results. Treatment means were separated and compared by Tukey's HSD test.

## Results and Discussion

The results showed that clove powder gave the highest protection to stored chickpea seeds with respect to the lowest number of eggs laid by pulse beetle (*C. chinensis* L.) (Fig. 1), the lowest number of egg bearing seeds (Fig. 2), the lowest adult emergence (%) (Fig. 3), the highest adult mortality (%) (Fig. 4) and the lowest seed weight loss (%) (Fig. 5). Compared to control other treatments (neem leaf, black pepper, methi powder) had no significant influence on number of eggs laid by pulse beetle, number of egg bearing seeds, adult emergence (%), adult mortality (%) and seed weight loss (%). There was no significant difference among the doses of all treatments. The highest number of eggs laid (305.00) was observed in control and the lowest (0.0) was observed when chickpea seeds were treated with clove powder at medium dose (Fig. 1). This finding is inconsistent with other previous records that 2% (w/w) black pepper admixed with cowpea significantly reduced oviposition of *C. maculates* after an exposure period of 10 and 70 days (Javaid and Poswal, 1995).

In case of number of egg bearing seeds, maximum seed containing eggs (106.0) was found in control whereas opposite was found in case of clove powder (Fig. 2). Juneja and Patel (2002) observed that the number of eggs per 100 seeds decreased with the increase of doses of black pepper although some results showed the performance of plant powder on the oviposition of pulse beetle (*C. chinensis*) was very satisfactory in case of black pepper, but moderately satisfactory in case of neem oil and mustard oil (Thakur and Mandeep, 2013).

Adult emergence was zero (0.0) when chickpea seeds were treated with clove powder at medium dose and it was the highest in control (269.0). In this case other treatment had significant difference with control, suggesting that other treatments are somewhat more effective than control (Fig. 3). There was a different pattern in the results of adult mortality (%). All the treatments with differential doses showed significant differences with control, showing no difference among them. Maximum adult mortality (77%) was recorded in clove at high dose and minimum (23%) in control (Fig. 3). These findings partially agreed with the previous findings that black pepper (*Piper nigrum* L.) was found to be highly effective followed by clove, neem and garlic (Ahmed *et al*., 2011). Although some previous researchers found that the infestation in blackgram with pulse beetle (*C. chinensis*) after 7 days exposure period, black pepper powder, neem oil and mustard oil caused 100% mortality of the pulse beetle, followed by neem leaf powder (86.67%), Chinese chaste tree (*Vitexnegundo* L.) leaf powder (73.33%) and Amlaki (*Emblica officinalis*) fruit and curry tree (*Murraya koenigii*) leaf powder each causing 66.67% mortality (Thakur and Mandeep, 2013). Seed weight loss (%) due to the infestation of pulse beetle (*C. chinensis*) caused variable results. All the treatments differed significantly with control. Moreover significant difference was also found in the treatments having differential doses. The highest seed weight loss (22.41%) was observed in control whereas seeds were unaffected when treated with clove powder at medium dose. A previous study showed minimum seed weight loss (%) in chickpea was found when treated with the extracts of black pepper. Clove, neem and garlic gave significantly different results with control but their performance was similar with black pepper. Moreover, another findings is consistent with the present results in such that the spices clove (*Syzygium aromaticum* L.), black pepper (*Piper nigrum*), ceylon cinnamon (*Cinnamomum zeylanicum*), black cardamom (*Amomum subulatum* Roxb.), turmeric (*Curcuma longa* L.), red pepper (*Capsicum frutescens* L.), cumin (*Cuminum cyminum* Linn.) and bay leaf (*Cinnamomum tamala*) were effective as protectant of stored blackgram (*Phaseolus benghalensis* L.) against the pulse beetle (*Callosobruchusm aculatus* F.) considering days to mortality (100%), number of adults emerged and weight loss (%) where clove and black pepper were found to be most effective (Mahdi and Rahman, 2008).

The highest effectiveness of clove powder as seed protectant may be due to the presence of essential oils (euginol) in clove which have multiple toxic actions on the survival of insect such as acute toxicity, repellence, feeding reduction (deterrence), growth inhibition and limitations in development and reproduction (Coast, 1994). Besides above, certain bioactive compounds such as flavonoids, hexane, methylene chloride, ethanol, thymol, euginol and benzene have been isolated from the clove extracts. These biochemicals have been reported to contain various biochemical properties, including antioxidant, hepato-protective, anti-microbial, and anti-inflammatory properties. Because of the presence of bioactive chemicals in clove, it may also be used as ant repellent. Many unknown underlying causes may be responsible for the effectiveness of clove powder. Nonetheless, clove powder can be used as chickpea seed protectant from the attack of chickpea pulse beetle (*C. chinensis* L.) in seed stores. In this study, three doses of clove powder were used @ 5 g, 10 g and 15 g $kg^{-1}$ chickpea seeds and found effective. Lower doses of clove powder than 5 g $kg^{-1}$ seeds can also be evaluated to find out some effective lower doses of clove powder in managing various pulse pests in storage. If lower doses were found effective, it would be wise to use lower doses due to economic and environmental point of views. Finally as clove powder has been found most effective botanical pesticide against pulse beetle from one season experiment, the year round experiments may be conducted considering other pulses to validate the results of the present study.

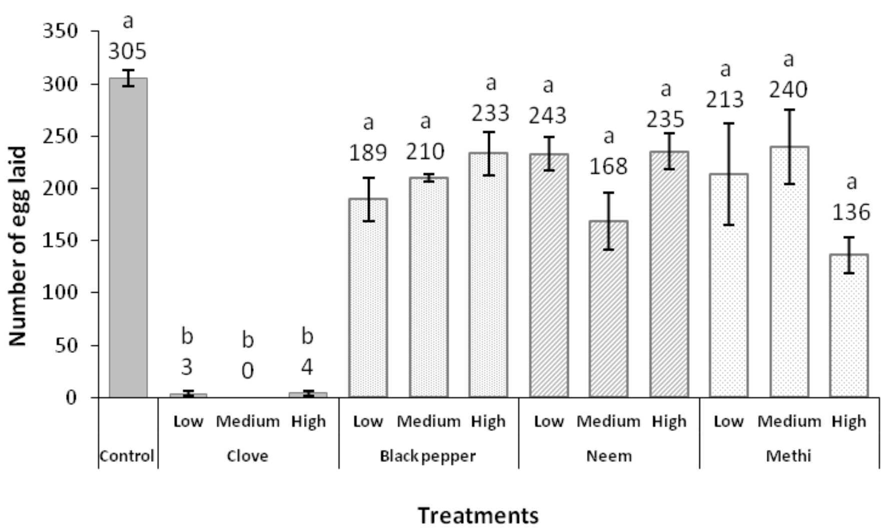


**Fig. 1. Effect of plant powders on the number of eggs laid by five pairs of pulse beetle. In each category, columns with different letters are significantly different ($p$<0.05)**

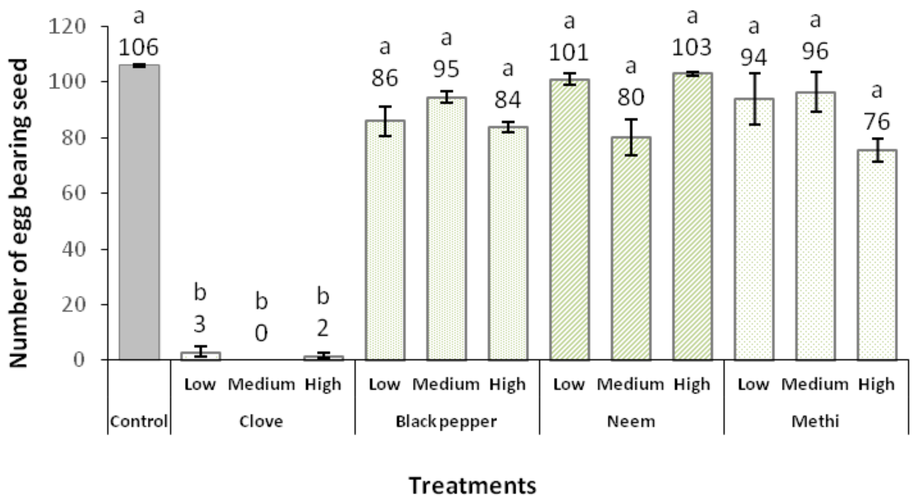


**Fig. 2. Effect of plant powders on the number of egg bearing chickpea seeds by five pairs of pulse beetle. In each category, columns with different letters are significantly different (*p*<0.05)**

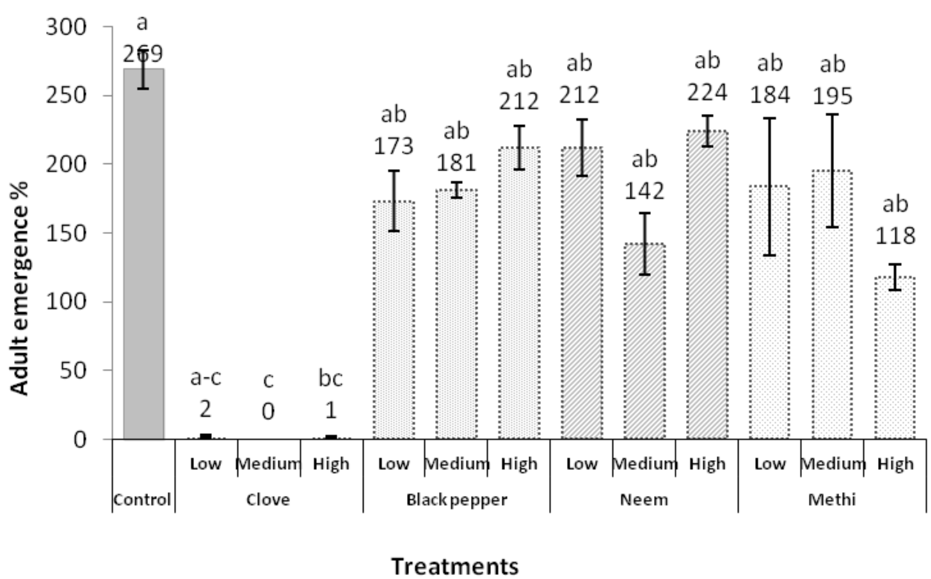


**Fig. 3. Effect of plant powders on adult emergence (%) of pulse beetle. In each category, columns with different letters are significantly different (*p*<0.05)**

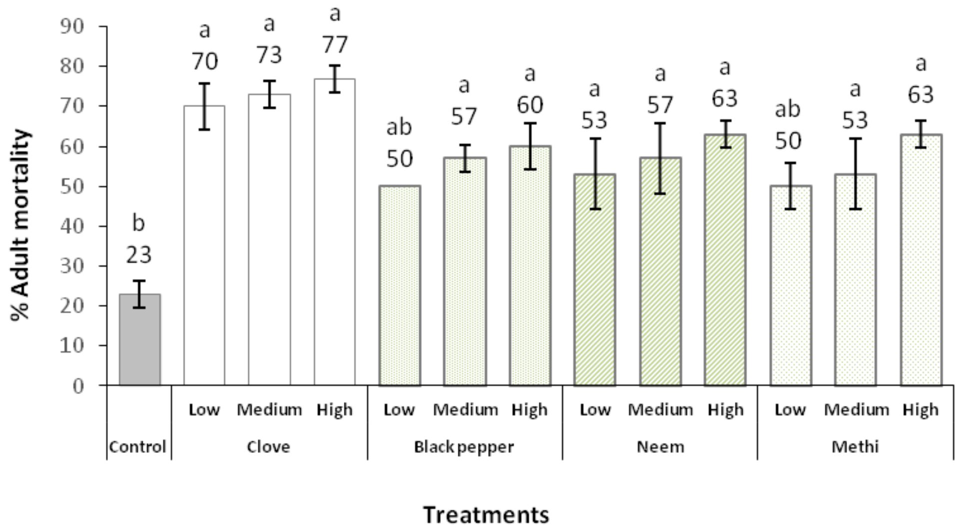


**Fig. 4. Effect of plant powders on adult mortality (%) of pulse beetle. In each category, columns with different letters are significantly different (p<0.05)**

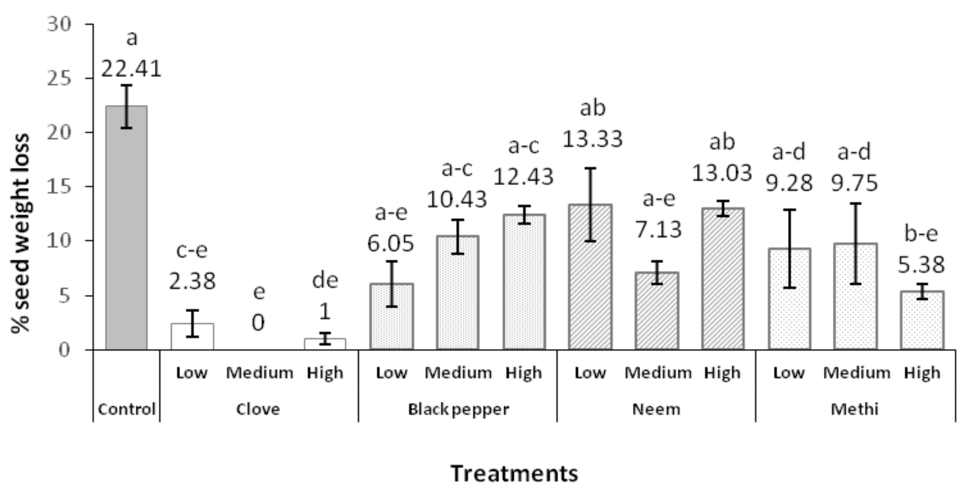


**Fig. 5. Effect of plant powders on seed weight loss (%). In each category, column with different letters are significantly different (*p*<0.05)**

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