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EFFECTIVENESS OF INDIGENOUS PLANT POWDERS AS GRAIN PROTECTANT AGAINST *Callosobruchus chinensis* (L.) IN STORED CHICKPEA (*Cicer arietinum*)

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Abstract

The effectiveness of 17 indigenous plant powders as grain protectant were assessed against *Callosobruchus chinensis* (L.). The results indicated that among all the tested plant materials, tobacco leaf powder (TLP) had promising effects on inhibiting oviposition and reducing adult emergence, seed infestation, and weight loss by *C. chinensis*. Tobacco leaf powder offered complete protection of chickpea seeds applied at 20.0 g/kg seeds. Its lower doses exhibited efficacy in dose dependant manner. The lowest number of eggs (24.60), egg bearing seeds (23.40), adult emergence (23.20), seed infestation (8.28%), and weight loss (0.50%) were obtained from the TLP treated at 10.0 g/kg seeds, while the highest of these parameters were in untreated control. In the ovicidal test, TLP showed 100% inhibition at 20.0 g/kg seeds over control. The lowest number of adults (37.20) were emerged when larvae bearing seeds were treated with TLP at 20.0 g/kg seeds along with 59.39% retardation over the control and had no adverse effect on seed germination up to 3 months.

Keywords: Plant powders, chickpea seeds, protectant, *Callosobruchus chinensis*.

Introduction

Pulses play a pivotal role in the diet of common people of third world country including Bangladesh. These are also called “poor man’s meat” since they are rich source of protein (20-40%) and are fairly good sources of thiamin, niacin, calcium, and iron for the under privileged people who cannot afford animal proteins (Sharma, 1984; Bhalla *et al*., 2008). Different types of pulses are grown throughout the winter season in Bangladesh and produce a total 2,05,000 m t of pulses. Among the pulses, chickpea alone occupied 4.06% area of pulse cultivation and contributed 7000 m t of annual production (BBS, 2008). But one of the major limitations for increasing pulses production is losses of seed viability and damage of grains from insect pest infestation in storage. Pulses in developing countries suffer high qualitative and quantitative losses from the attack of pulse beetle, *Callosobruchus chinensis* L., a major pest of pulses in storage (Ahmed *et al*., 2003; Aslam, 2004). They cause damage to pulses both in the field and storage, but infestation is more crucial in stored condition (Rahman

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et al., 1975; Bhalla *et al*., 2008). The adult beetles do not cause damage to the pulse grains by feeding but they mate and oviposite on grains and contaminate by excreta. The larva is solely responsible for the grain damage. The larvae destroy seeds by feeding inside partially or completely and make them unfit for human consumption (Atwal and Dhaliwal, 2005). About 4 to 98% loss of pulse seeds may be observed due to the infestation by the pulse beetle in storage (Mookherjee *et al.*, 1970).

At present, pest control measures mostly rely on synthetic insecticides and fumigants in Bangladesh. But chemical protection measures may be resulted many serious drawbacks (Lee *et al*., 2001). Their extensive and indiscriminate use causes ecological imbalance, resistance of pesticides to pest, pest resurgence and outbreak of secondary pests, creates phytotoxicity, insecticidal residues in foods and feed (Mahmud *et al*., 2002; Ashamo, 2004; Nas, 2004). Moreover, continuous uses of insecticides leads to hazardous effect on pollinators, natural enemies e.g., predators, parasitoids and also caused the environmental pollution (Saxena, 1992; Nagarare and More, 1998; Hossain, 2001). Due to these hazards, globally scientists are trying to adopt alternative methods of pest control. The use of locally available indigenous plant materials in the control of pests are an ancient technology and used in many parts of the world (Roy *et al.*, 2005). Various products of plants have been tried recently by researchers with a high degree of success as grain protectants against pulse beetle to reduce infestation in storage (Umrao and Verma, 2002; Epidi *et al*., 2008; Mahadi and Rahman, 2008). Keeping these views in mind, the present study was conducted to investigate the insecticidal effectiveness of some indigenous botanicals powders as grain protectant against *C. chinensis* on chickpea seeds.

Materials and Method

Collection and preparation of plant materials: The study was conducted in the Department of Entomology, Bangladesh Agricultural University (BAU), Mymensingh. Fresh leaves (17 indigenous plants) and seeds (4 plants) (Table 1) were collected from BAU campus and kept in the laboratory for 7 days for air drying followed by one day sun drying before making powder. After drying, the leaves and seeds were made powder separately by an electric grinder in the laboratory and passed through a 60-mesh sieve to get fine powder. Later, each powder was kept separately in air tight plastic pot and stored at room temperature for experimental use.

Collection of chickpea seeds: Healthy chickpea (*Cicer arietinum* L.) seeds was purchased from the local market of Mymensingh town. The seeds were thoroughly cleaned, sun dried, cooled, and stored with 10 ± 2 % moisture content. The seeds were kept in air tight plastic container (25 cm height x 15 cm dia.) at preserved at room temperature for study.

Table 1. List of indigenous plant leaf and seed powders tested against *C. chinensis.*

Serial no.	Common name	Scientific name	Family	Plant part used
1	Akanda	*Calotropis gigantia*	Asclepiadaceae	Leaf
2	Khoksa	*Ficus bengalensis*	Moraceae	Leaf
3	Ghagra	*Xanthium italicum*	Compositae	Leaf
4	Nayantara	*Nerium indicum*	Apocynaceae	Leaf
5	Karabi	*Nerium olender*	Apocynaceae	Leaf
6	Bel	*Aegle marmelos*	Rutaceae	Leaf
7	Tobacco	*Nicotiana tabacum*	Solanaceae	Leaf
8	Alamanda	*Alamanda cathertica*	Apocynaceae	Leaf
9	Dondokalash	*Leucas aspera*	Labiateae	Leaf
10	Marigold	*Tagetes erecta*	Compositae	Leaf
11	Dhutura	*Datura stromonium*	Solanaceae	Leaf
12	Chrysanthemum	*Chrysanthemum segetum*	Compositae	Leaf
13	Karanja	*Pongamia pinnata*	Fabaceae	Leaf
14	Neem	*Azadirachta indica*	Meliaceae	Leaf and Seed
15	Biskatali	*Polygonum hydropiper*	Polygonaceae	Leaf and Seed
16	Ata	*Annona reticulata*	Annonaceae	Leaf and Seed
17	Castor	*Ricinus communis*	Euphorbiaceae	Leaf and Seed

Stock culture of pulse beetle: Pulse beetle was reared in the laboratory at ambient room temperature (30 ± 3^{o}C) in glass jars (47 cm height × 4 cm dia.). Approximately, 200 adults of the collected pulse beetles were released in each jar containing 500 g of chickpea seeds and the mouth was closed with a piece of nylon cloth. The beetles were allowed for free mating and oviposition for 7 days. Then the beetles were separated from the seeds by sieving and seeds along with eggs were kept in the container (8 cm h. x 5 cm dia.) for emergence of next generation. After emergence, the newly emerged adults were collected and again allowed for further mating and oviposition with new seeds in different containers to maintain a series of stock culture of the test insect.

Screening procedure of plant powders: Screening of botanicals as grain protectants against *C. chinensis* was carried out following the primary and secondary screening.

Protocol of primary screening: Fifty grams of healthy chickpea seeds were weighed by an electric balance, taken in plastic container (300 ml) and mixed it properly with tested leaf and seed powders separately at 20.0 g/kg (w/w) seeds. Five pairs of newly emerged one day old adult beetles of *C. chinensis* obtained from the stock culture were sexed and released in each plastic container along with an untreated control. After release of insects, the mouth of the plastic containers was closed with its porous lid. No plant materials were used in control treatment. Each treatment was replicated thrice. All treated containers were kept at ambient room temperature (30 ± 3°C) in the laboratory for oviposition and development of *C. chinensis.* Dead and alive beetles were removed after 7 days from each container and control. The effectiveness of plant materials as protectant against *C. chinensis* was assessed. For the determination of oviposition, 100 seeds were collected randomly from each plastic container of each treatment and examined under magnifying glass (10 x). The total number of eggs deposited and number of seeds along with eggs (i.e., egg bearing seeds) were counted. After each observation, the grains were returned to the respective containers for the further development. Adults were removed and recorded daily after emergence. Infested and healthy seeds were separated, cleaned, counted, and finally weighed after completion of adult emergence. Seed infestation and weight loss were computed by using the following formulae:

Infestation (%) = $\frac{N_b}{T_n} \times 100$ (Enbakhare and Law-Ogbomo, 2002)

Where, N_b = Number of bored seeds, T_n = Total number of seeds

Weight loss (%) = $\frac{UNd - DNu}{U(Nd + Nu)} \times 100$ (Lal, 1988)

Where, U = Weight of undamaged seeds, D = Weight of damaged seeds, Nu = Number of undamaged seeds, Nd = Number of damaged seeds.

Protocol of secondary screening: From the primary screening, only tobacco leaf powder (TLP) was found very effective to protect chickpea seeds against *C. chinensis* at 20.0 g/kg. On the other hand, TLP inhibited completely the progeny adult emergence. Therefore, TLP was further tested at lower doses of 10.0, 5.0, 2.5, and 1.25 g/kg seeds. Each dose was replicated five times along with an untreated control. The screening protocol and observations were same as followed in the primary screening.

Test on ovicidal and larvicidal effectiveness: To investigate whether tobacco leaf powder possesses ovicidal and larvicidal properties, another study was conducted. For this, 100 chickpea seeds along with one day old eggs (Ovicidal study) and 1-2 days old larvae (Larvicidal study) containing one egg or larva per seed were placed in each container and mixed properly with tobacco leaf powder @ 20.0, 10.0, 5.0, 2.5, and 1.25 g/kg seeds along with an untreated control. After proper mixing, the mouth of the containers were closed with cap and left it undisturbed in the laboratory until adult emergence. The number of adult beetles were counted and recorded daily from the first to the last emergence and removed from the containers. After completion of adult emergence, the inhibition was computed by using the following formula as stated by Shukla *et al*. (2007).

$$\text{Inhibition (\%)} = \frac{\text{Control mean - Treatment mean}}{\text{Control mean}} \times 100$$

Seed germination test: To study the effect of TLP on seed viability and germination of chickpea seeds treated at different dose levels was carried out for a period of 3 months along with untreated control following the procedure with a slight modification as described by Enbakhare & Law-Ogbomo (2002). For this, 100 seeds were placed in each Petridish (120 × 20 mm) containing water soaked blotting paper (Whatman no. 1, UK) at the bottom. The Petridishes were placed in the laboratory under ambient room temperature (30 ± 3^{o}C). Germinated seeds were counted, recorded and worked out the percent germination after incubation.

Statistical analyses: The collected data were analyzed followed by Completely Randomized Design (CRD) and analysis of variance (ANOVA). Data were transformed before analysis. The treatment mean values were compared by Duncan's New Multiple Range Test (DMRT).

Results

Primary screening: The number of eggs, egg bearing seeds, adult emergence, seed infestation and weight after infestation by *C. chinensis* on the chickpea seeds in different treatments differed significantly ($P<0.05$) with applied at 20 g/kg seeds (Table 2). Among all the treatments, the highest number of eggs and egg bearing seeds per 100 seeds were found in the untreated control (97.33 and 77.67) while the lowest (16.33 and 15.0) was with tobacco leaf powder (TLP). Similarly, the highest number of adult emergence (198.33), seed infestation (64.33 %) and weight loss (4.05 %) were found in the untreated control. On the contrary, no adult emergence, seed infestation and weight loss were found when seeds were treated with TLP at 20 g/kg seeds.

Secondary screening: From the primary screening, it was found that the TLP showed the best performance among all the tested powders against *C. chinensis*. Hence, it was further assessed at its lower doses in terms of ovicidal and larvicidal effectivity.

Effect of TLP on oviposition: The number of eggs of *C. chinensis* and egg bearing seeds on chickpea seeds treated with TLP differed significantly ($P<0.05$) among all the treatments (Table 3). The highest number of eggs was found in the untreated control (93.60), which was statistically similar to that at 1.25 g/kg TLP treated seeds (84.40). But the lowest number of eggs (24.60) was recorded at 10.0 g/kg seeds. The lowest number of egg bearing seeds per 100 seeds was recorded from TLP treated at 10.0 g/kg seeds (23.40) while the highest in the untreated control (79.60).

Table 2. Effects of indigenous plant powders on oviposition, adult emergence, seed infestation, and weight loss caused by *C. chinensis* on treated chickpea seeds.

Treatments	No. of eggs/100 seeds	No. of egg bearing seeds/100 seed	No. of adult emergence	Seed infestation (%)	Seed wt loss (%)
Akanda leaf	58.00 bc (1.76)	47.00 c-e (1.67)	84.00 c-e (1.92)	27.56 d-f (31.62)	1.65 fg (1.28)
Khoksa leaf	64.00 b (1.81)	52.00 bc (1.71)	128.00 a-c (2.11)	40.34 b (39.11)	2.60 bc (1.61)
Ghagra leaf	75.33 ab (1.88)	63.67 ab (1.80)	122.67 a-c (2.09)	39.84 b (39.12)	2.34 cd (1.53)
Nayantara leaf	29.00 f (1.46)	23.67 lm (1.37)	74.33 c-e (1.87)	23.86 f (29.21)	1.43 gh (1.20)
Neem leaf	22.67 g (1.35)	20.33 m (1.31)	32.67 f (1.52)	11.84 h (19.65)	0.70 jk (0.83)
Karabi leaf	61.33 b (1.79)	47.33 cd (1.67)	91.67 d-e (1.79)	39.45 b (38.89)	2.36 bd (1.54)
Biskatali leaf	36.00 ef (1.55)	31.00 h-k (1.49)	93.67 b-e (1.97)	29.53 (32.89)	1.76 eg (1.32)
Bel leaf	46.33 c-e (1.66)	37.67 e-h (1.57)	116.67 a-c (2.07)	32.81 (34.92)	2.04 (1.42)
Ata leaf	60.67 b (1.78)	48.33 cd (1.68)	96.67 b-e (1.98)	29.56 c-e (32.91)	1.69 e-g (1.30)
Castor leaf	45.00 c-e (1.65)	34.67 g-j (1.54)	91.0 b-e (1.96)	27.19 i (31.41)	1.60 fg (1.27)
Tobacco leaf	16.33 h (1.21)	15.00 n (1.17)	0.00 g (0.00)	0.00 i (0.00)	0.00 k (0.71)
Alamanda leaf	42.33 de (1.62)	36.67 f-i (1.56)	107.0 b-d (2.03)	31.17 cd (33.91)	1.86 ef (1.36)
Dondokalash leaf	65.67 b (1.82)	49.00 cd (1.69)	153.67 ab (2.19)	43.09 b (41.01)	2.76 b (1.66)
Marigold leaf	44.67 ce (1.65)	34.67 g-j (1.54)	96.33 b-e (1.98)	26.93 d-f (31.24)	1.68 fg (1.29)
Dhutura leaf	35.67 ef (1.55)	30.33 h-k (1.48)	85.67 c-e (1.93)	25.82 ef (30.49)	1.58 fg (1.25)
Chrysanthemum leaf	30.67 f (1.48)	26.67 kl (1.42)	60.33 e (1.78)	17.96 g (25.05)	1.06 i (1.03)
Karanja leaf	47.33 cd (1.67)	40.67 d-g (1.61)	128.00 a-c (2.11)	39.16 b (38.71)	2.38 b (1.54)
Neem seed	20.33 gh (1.30)	19.33 m (1.28)	30.67 f (1.48)	11.33 h (20.10)	0.64 jk (0.80)
Ata seed	58.00 bc (1.76)	44.67 c-f (1.65)	92.00 b-e (1.96)	28.51 c-e (32.25)	1.55 f-h (1.24)
Castor seed	35.67 f (1.55)	29.33 i-l (1.46)	82.33 c-e (1.91)	25.44 ef (30.26)	1.29 hi (1.13)
Biskatali seed	31.33 (1.50)	28.00 j-l (1.45)	87.67 b-e (1.91)	28.90 c-e (32.50)	1.60 fg (1.26)
Control	97.33 a (1.98)	77.67 a (1.88)	198.33 a (2.30)	64.33 a (53.33)	4.05 a (2.01)

Means in a column having the same letters are not significantly different at 5% by DMRT. Figures in the parenthesis are log transformed values.

Effect of TLP on adult emergence: The number of adult emergence differed significantly (P<0.05) among the treatments (Table 3). The highest number of adults were recorded in untreated control (194.60), whereas the lowest (23.20) in TLP treated seeds applied at 10.0 g/kg seeds. The number of adult emergence was recorded 40.80, 71.0 and 154.20 when seeds were treated with TLP at 5.0, 2.5 and 1.25 g/kg seeds, respectively.

Table 3. Effects of tobacco leaf powder on oviposition, adult emergence, seed infestation, and weight loss caused by *C. chinensis* including germination of treated chickpea seeds.

Dose (g/kg seed)	No. of eggs/100 seeds	No. of egg bearing seeds/ 100 seeds	No. of adults emerged	Seed infestation (%)	Seed wt loss (%)	Seed germination (%)
1.25	84.40 a (1.92)	67.20 b (1.83)	154.20 b (2.19)	42.45 b (40.63)	2.58 b (1.61)	91.00 (9.54)
2.5	63.00 b (1.80)	55.60 c (1.75)	71.00 c (1.85)	24.58 c (29.70)	1.52 c (1.23)	90.40 (9.51)
5.0	51.60 c (1.71)	41.20 d (1.61)	40.80 d (1.61)	11.46 d (19.77)	0.70 d (0.84)	89.80 (9.44)
10.0	24.60 d (1.38)	23.40 e (1.36)	23.20 e (1.36)	8.28 e (16.66)	0.50 d (0.71)	89.60 (9.46)
Control	93.60 a (1.97)	79.60 a (1.90)	194.60 a (2.29)	63.55 a (52.86)	4.21 a (2.05)	91.40 (9.56)
CV (%)	3.56	3.19	2.91	4.55	4.56	NS

Means in a column having the same letter are not significantly different at 5% level by DMRT. Figures in the parenthesis are transformed values. NS = Not significant.

Effect of TLP on seed infestation and weight loss: It was observed from the data that the percentage of seed infestation and weight loss differed significantly (P<0.05) among the treatments (Table 3). The highest seed infestation was observed in untreated control (63.55%) and the lowest (8.28%) in TLP treated at 10.0 g/kg seeds. Similarly, the highest seed weight loss was recorded in untreated control (4.21%) and the lowest (0.50%) in TLP applied at 10.0 g/kg seed which was statistically similar to that applied at 5.0 g/kg TLP treated seeds (0.70%).

Effect of TLP on seed germination: The germination percentage of chickpea seeds treated with TLP did not differ significantly among the treatments (Table 3). The germination in different treatments including control ranged from 89.60 to 91.40% with no significant difference among them.

Table 4. Number of adults of *C. chinensis* emerged from 100 eggs and larvae bearing chickpea seeds after treatment with tobacco leaf powder.

Dose (g/kg seed)	Egg bearing seed		Larvae bearing seed	
	No. of adults emerged	Inhibition (%)	No. of adults emerged	Inhibition (%)
1.25	84.80 a (1.94)	6.61	88.00 ab (1.94)	3.93
2.5	65.00 b (1.81)	28.41	79.00 b (1.90)	13.76
5.0	38.00 c (1.58)	58.15	64.00 c (1.80)	30.13
10.0	17.80 d (1.24)	80.40	55.50 d (1.74)	39.41
20.0	0.00 e (0.00)	100.0	37.20 e (1.57)	59.39
Control	90.80 a (1.96)	-	91.60 a (1.96)	-
CV (%)	3.71	-	2.46	-

Means in a column having the same letters are not significantly different at 5% level by DMRT. Figures in the parenthesis are log transformed values.

Discussion

The results of the present study revealed that among all the tested plant materials, tobacco leaf powder (TLP) had promising effects on the inhibition of oviposition and decreasing effect on adult emergence, seed infestation, and weight loss caused by *C. chinensis*. The TLP offered complete protection of chickpea seeds by applying the highest dose of 20.0 g/kg seeds although few eggs (16.33) were deposited but failed to develop as subsequent adults (Table 2). While TLP at lower doses exerted its effectiveness in dose dependant manner. The lowest seed infestation and weight loss were found 8.28 % and 0.50 %, respectively, when seeds were treated with TLP at 10.0 g/kg seeds. The grain protectant properties of TLP might be attributed to its antiovipositional, ovicidal, and larvicidal properties. The results of the present findings are in close proximity with those of Govindan and Nelson (2008). They treated pulse seeds with ten botanicals and found that tobacco leaf powder (*Nicotiana tabacum*) along with *Lictifers isora* attributed the lowest number of eggs against the *Callosobruchus maculatus*. The present study showed that complete inhibition of adult emergence was found when egg bearing chickpea seeds were exposed to TLP treated at 20.0 g/kg seed, but 59.39% inhibition was occurred when larvae bearing chickpea seeds were treated with the same dose of TLP (Table 4). These may be due to the mortality of eggs and/or larvae, or reduction of egg hatching. Bamaiyai *et al*. (2007) also opined that the ovicidal properties of botanical powders suppressed the emergence of pulse beetle. Chickpea seeds mixed with TLP have no adverse side effect on seed germination (Table 3) up to 3 months of storage. The present results are comparable to those of Paneru and Shivakoti (2001). The biological activity of TLP can be credited to its alkaloid contents, such as nicotine, nornicotine, neonicotine, anabasine, nicotyrine, metanicotine, etc. having deterrent action to insect (Prakash and Rao, 1996). Moreover, nicotine, a colourless liquid acts as fumigant and able to penetrate directly through insect integument. It also acts as non-persistent contact insecticide against various pests (Cremlyn, 1978). The semiochemical nature and pungent smell of TLP might alter the behaviour adversely and physiology of beetles markedly and thus preventing from oviposition. Moreover, fine powder of TLP could block the spiracles of the beetles, thereby, impairing respiration and leading to death.

Among other tested botanicals, neem leaf and neem seed powder significantly reduced the pulse beetle infestation and weight loss of chickpea seeds but failed to prevent to damage completely. None of the other tested powders applied at 20.0 g/kg showed significant inhibition of adult emergence, reduction of seed infestation and weight loss of chickpea seeds. Varied effectiveness of different tested botanical powders indicated that the pest suppressing properties are not uniformly distributed among the tested plant powders. Significant level of success in the suppression of *Callosobruchus* sp. was reported by various authors with leaf powder of different botanicals including neem leaf and seed powders (Singh, 2003; Gundannavar and Deshpande, 2006; Sharma and Rathore, 2006; Lakshmi and Venugopal, 2007). However, with the finding of the present study, it may be opined that for eco-friendly management of *C. chinensis*, the TLP may be considered at farmer's level as it is cheaper, easily available, processable, and usable.

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