GROWTH INHIBITION AND TOXIC EFFECT OF BAKAIN SEED AQUEOUS EXTRACT ON PULSE BEETLE, *Callosobruchus chinensis* L. (COLEOPTERA: BRUCHIDAE).

H. U. Rehman and S. M. Khan

Department of Entomology, Faculty of Agriculture, Gomal University, D. I. Khan.
Corresponding author e-mail: hafeezdik@yahoo.com

ABSTRACT: Laboratory experiments were conducted in Entomology Department, Faculty of Agriculture, Gomal University, D.I. Khan, Pakistan to investigate growth inhibition and toxic effect of bakain (*Melia azedarach* L.) seed aqueous extract on pulse beetle (*Callosobruchus chinensis* L.) infesting chickpea (*Cicer arietinum* L.) during storage. Different concentrations of bakain seed extract, viz. 0.4, 0.3, 0.2, 0.1, 0.05 and 0.025% (v/w) were evaluated. The results showed that bakain seed extract significantly reduced oviposition and suppressed adult emergence of pulse beetle. Consequently, it reduced damage and weight loss of chickpea grains. The effectiveness of bakain seed extract was found to be dose dependent. It exhibited maximum growth inhibition activity at the concentration of 0.4 and 0.3%. The treatments had no significant effect on adult life span of pulse beetle. Aqueous extract of bakain seed also showed some degree of toxicity against the pest. It caused maximum mortality of the pest at the concentration of 0.4 and 0.3% after 72 hours of release in treated chickpea grains. On the basis of these results, it was concluded that bakain seed aqueous extract could be effectively used for the management of pulse beetle during storage.

Key words: Pulse beetle, aqueous extracts, *Melia azedarach*, growth inhibition, toxic effect.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a rich source of vegetable protein, minerals and vitamins. Its grains can be stored for a long period of time for human consumption and for seed purposes. Many insect pests damage to the grains during storage. Gram Dhora (*Callosobruchus chinensis* L.) is the most destructive pest of stored chickpea. In addition to chickpea, it also feeds on mung bean, peas, cowpeas, lentil, sorghum and maize during storage. The infested seeds lose germination potentials and also become unfit for human consumption (Ramzan *et al,* 1990). The pest can cause damage to the grains up to 10% in temperate and 30% in tropical zones (Nakakita, 1998).

The common practice among farmers to protect stored grains is through the use of synthetic insecticides. Indiscriminate use of synthetic insecticides is hazardous to human health as these contaminate foodstuffs. There is a growing need for the use of eco-friendly control tactics to manage insect pests of agricultural importance globally. Plant products are desirable as they are comparatively safer to non target organisms, less persistent and are compatible with other control tactics in an integrated pest management system. Plants derivatives can be used as an alternative approach to synthetic insecticides. (Graing and Ahmad, 1988) stated that many plants extracts and their essential oils constitute a rich source of bio-pesticides. (Srivastava and Gupta, 2007) noted significant reduction in oviposition of pulse beetle with the application of aqueous extract of *Solanum surratense* fruits, while (Jayakumar, 2010) observed 84.66 and 82.11% oviposition deterrence of *C. maculatus* with the application of *Cassia siamia* and *C. aurantium* respectively. (Kiradoo and Srivastava, 2010) found that *Ocimum basilicum* was effective in reducing fecundity of *C. chinensis*. (Kotkar *et al.*, 2001) reported that flavonoids isolated from aqueous extract of *Annona squamosa* @ 0.07mg ml^{-1} cause 80% mortality of *C. chinensis*. (Udo and Epidi, 2009) recorded significant mortality of *C. maculatus* with the application of various fractions of *Ricinodendron heudelotii* leaves extracts. (Denloye *et al*., 2010) reported effectiveness of aqueous extracts of root and bark of *Zanthoxylum zanthoxyloides* against *C. maculatus*. (Adedire *et al*., 2011) reported that steam distillate of cashew kernels was most toxic to *C. maculatus* compared to methanol and ethanol extracts. Aqueous extracts of black pepper, cloves, neem and garlic possess toxic and growth inhibition potential against chickpea beetle (Zia *et al*., 2011).

Bakain, *Melia azedarach* L. (Meliaceae) is a widely grown ornamental tree in tropical and subtropical countries of the world. It has been reported to possess antimicrobial, insecticidal and nematicidal properties. It has a great potential in pest control because of its repellent, antifeedant and toxic effects against a vast range of insect pests. A number of organic molecules, viz. terpenoids, flavonoids, steroids, acids, meliantriol, melianone and melianol have been isolated from the fruits of *M. azedarach*. Among these, meliantriol has been reported to possess growth disrupting and antifeedant properties against desert locust (*Schistocerca gregaria*). Therefore, it is imperative to study the

variation of its effectiveness in different locations against insect pests. No research work has been reported on growth inhibition and toxic effect of indigenous plants materials against pulse beetle in Dera Ismail Khan.

Keeping in view the potential of plants derivatives in pest management, experiments have been designed to determine growth inhibition and toxic effect of bakain seed aqueous extract on pulse beetle with a view to reduce the problems associated with indiscriminate use of synthetic insecticides.

MATERIALS AND METHODS

Research trials were conducted in Entomology Department, Faculty of Agriculture, Gomal University, D. I. Khan, Pakistan to investigate growth inhibition and toxic effect of bakain seed aqueous extract on pulse beetle, *C. chinensis* infesting chickpea during storage. Bakain seed was collected from various villages of district Dera Ismail Khan. The collected seed was brought to Entomology laboratory, washed with distilled water to remove dust particles and was shade dried. The seed was ground to fine powder with the help of electric grinder (Depose, D 56 750W, Moulinex®). The resulting powder was passed through a 60-mesh sieve to obtain a fine dust. Aqueous extract of bakain seed was prepared following (Talukder and Howse, 1993). Ten grams of ground bakain seed powder was mixed with 50ml of distilled water and stirred for 30 minutes using a mechanical stirrer and was left to stand for two weeks. After two weeks, the mixture was filtered through a filter paper (whatman No.1). The solids were stirred again for 15 minutes in 30ml of distilled water and were filtered again. The resulting filtrates were combined together. The extract was preserved in a sealed bottle in refrigerator at a temperature of 5 ^{0}C until required for bioassay. The seed of most commonly grown chickpea variety (Noor-2009) was obtained from Arid Zone Agriculture Research Institute, Bhakkar. Chickpea grains were sterilized at 120 ^{0}C for 15 minutes using an autoclave (MAC-1200) to kill any already existing organism. A small population of pulse beetle was obtained from naturally infested chickpea grains from the farmer's store and was reared using growth chamber (HPP-260, Memmert) at 30±2°C temperature and 70±5% relative humidity in the laboratory on whole chickpea grains. Mass culture of the test insect was maintained under controlled laboratory conditions. Initially 50 pairs of adult pulse beetles were released on sterilized chickpea grains in a jar of 5 litres capacity. Pairing was done following (Halstead, 1963). Mouth of the jar was covered with muslin cloth, fastened with rubber bands to ensure ventilation and to prevent entry or escape of insects. The beetles were allowed for seven days for mating and oviposition to get maximum adults. After seven days, the beetles were removed from the jar and fresh emerged adults of pulse beetle were released into another jar containing sterilized chickpea grains for 24 hours to get the adults of uniform age. After 24 hours of release, parent stock was removed from the jar and freshly emerged adults (one day old) were used in experiments.

Growth Inhibition Effect of Bakain Seed Aqueous Extract on Pulse Beetle: An experiment was carried out to determine growth inhibition effect of bakain seed aqueous extract on pulse beetle. The experiment was laid down in completely randomized design with 5 replications, each consisting of 7 treatments (including control). Different concentrations of bakain seed extract, viz. 0.4, 0.3, 0.2, 0.1, 0.05 and 0.025% (v/w) were mixed separately with 50g chickpea grains in pre labeled jars of 250ml capacities. Chickpea grains in control treatment were treated with distilled water only. Ten pairs of adult pulse beetles (one day old) were released into each jar. Mouths of the jars were covered with muslin cloth, fastened with rubber bands to ensure ventilation and to prevent entry or escape of insects. The beetles were allowed for seven days for mating and oviposition and then the released beetles were removed from the jars. The following parameters were studied.

i - Oviposition Deterrence: Eggs laid by pulse beetle on chickpea grains were counted to determine anti ovipositional effect of bakain seed aqueous extract on pulse beetle. For this purpose, twenty chickpea grains were selected randomly from each jar (treatment) and the eggs laid by female beetles on each grain were counted using magnifying glass.

The percentage of oviposition deterrence was calculated using the following formula (Elhag, 2000).

$$\text{Percent Oviposition Deterrence} = \frac{NC - NT}{NC} \times 100$$

Where:
NC = Number of eggs laid on untreated grains (control)
NT = Number of eggs laid on treated grains.

ii - Adult Inhibition: Number of adults emerged in each jar (treatment) were counted to determine adult inhibition effect of bakain seeds extract on pulse beetle. Percent inhibition rate in adult emergence was calculated using the following formula (Rahman and Talukder, 2006).

$$\text{Percent Inhibition Rate} = \frac{C_n - T_n}{C_n} \times 100$$

Where:
Cn = Number of adults emerged in untreated jar (control).
Tn = Number of adults emerged in treated jar

iii - Adult Longevity: Data was recorded to see the effect of bakain seed extract on adult life span of pulse beetle on the basis of number of days taken from adult emergence up to mortality in each treatment. For this purpose, adults emerged in each jar (treatment) were collected on daily basis and were released in separate pre

labeled petri dishes containing untreated chickpea grains. The beetles were examined daily and dead ones were discarded in each data.

iv – Weeviled Grains: Damaged and undamaged chickpea grains were counted in each jar and the percentage of weeviled grains was calculated using following standard formula.

$$\text{Percent Weeviled Grains} = \frac{\text{No. damaged grains in a Jar}}{\text{Total no. grains in a jar}} \times 100$$

v - Weight Loss: At the end of the experiment, percent weight loss of chickpea grains was calculated using the following formula.

$$\text{Percent Weight Loss} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight}} \times 100$$

Where
Final Weight = Weight of damaged grains + Weight of undamaged grains

Toxicity of Bakain Seed Aqueous Extract against Pulse Beetle: An experiment was conducted to determine toxicity of bakain seed aqueous extract against pulse beetle. The trial was laid down in completely randomized design with five replications, each consisting of seven treatments (including control). Different concentrations of bakain seeds extract, viz. 0.4, 0.3, 0.2, 0.1, 0.05 and 0.025% (v/w) were mixed separately with 20g chickpea grains and were placed in pre labeled petri dishes (9 cm. dia.). Filter paper was placed on the bottom of petri dishes to facilitate the movement of beetles in the arena. Forty beetles of uniform age (one day old) were released into each petri dish containing treated chickpea grains. The grains in control treatment were treated with distilled water only. Data on mortality of beetles was recorded after 24, 48 and 72 hours of release of beetles and dead ones were discarded in each data.

Percent corrected mortality of pulse beetle was calculated using Abbott's formula (Abbott, 1925).

$$\text{Percent Corrected Mortality} = \frac{\text{\% kill in treated - \% kill in control}}{\text{100 - \% kill in control}} \times 100$$

Statistical Analysis: Data were subjected to analysis of variance technique (Steel *et al*., 1997) using statistical analysis package, MSTAT-C as a one factor completely randomized design for growth inhibition effect of bakain seeds extract, while two factors completely randomized design for toxic effect of the extract. Where significant F value was obtained, Duncan Multiple Range test was applied at 0.05 probability level to detect statistical significant difference among treatments.

RESULTS AND DISCUSSION

Growth Inhibition Effect of Bakain Seed Aqueous Extract on Pulse Beetle: Data regarding oviposition deterrence, adult inhibition, adult longevity, weeviled grains and weight loss of chickpea grains has been summarized in table-1. The results revealed that bakain seed extract at a concentration of 0.4% caused significant reduction in fecundity of the pest and exhibited 37.47% oviposition deterrence. It was followed by 27.34, 18.67 and 11.86% oviposition deterrence at the concentration of 0.3, 0.2 and 0.1% respectively. A decrease in bioactivity of the extract was observed with a decrease in its concentration. At a concentration of 0.05%, it caused 6.00% oviposition deterrence, which showed a non significant difference from 5.20% oviposition deterrence noted with the application of 0.025% bakain seeds extract. The treatments also suppressed adult emergence of the pest. Maximum adult inhibition (45.05%) was observed at a concentration of 0.4%, which was non significantly different from 44.31% adult inhibition recorded at the concentration of 0.3%. At the concentration of 0.2%, the extract caused 22.94% adult inhibition, which was non significantly different from 22.63, 22.21 and 22.10% adult inhibition noted at the concentration of 0.1, 0.05 and 0.025% respectively. The treatments had no significant effect on adult life span of pulse beetle at 0.05 level of probability. Adult life of pulse beetle ranged from 6.33 to 6.35 days among treatments. The results further revealed that application bakain seed extract reduced damage by the pest compared with control. Minimum weeviled chickpea grains (47.60%) were observed in the samples treated with 0.4% concentration of the extract, which were statistically at par with 48.89% weeviled chickpea grains noted at the concentration of 0.3%. An increase in percentage of weeviled chickpea grains was observed at the concentration of 0.2, 0.1, 0.05 and 0.025%, where 52.54, 52.38, 53.38 and 54.11% weeviled chickpea grains were recorded respectively. Significantly highest weeviled chickpea grains (88.72%) were recorded in untreated samples. Application of bakain seed extract significantly reduced weight loss of chickpea grain compared with control. Minimum weight loss (13.48%) was recorded in the samples treated with 0.4% concentration of the extract, which was statistically at par with 13.54% weight loss recorded in the samples treated with 0.3% concentration of the extract. Significantly highest weight loss of chickpea grains (30.00%) was observed in untreated samples.

The results indicated that bakain seed aqueous extract significantly reduced fecundity, adult emergence of pulse beetle and consequently reduced damage and weight loss of chickpea grains. The tested concentrations of the extracts had no significant effect on adult life span of pulse beetle. No literature was found on the efficacy of bakain seed aqueous extract on pulse beetle. However, some previous workers, viz. (Srivastava and Gupta, 2007, Jayakumar, 2010, Kiradoo and Srivastava, 2010 and Zia *et al*., 2011) have reported growth inhibition effect of aqueous extracts of many other plants on pulse beetle. These researchers observed reduced fecundity and adult emergence of the pest with the application of aqueous extracts of various plants parts. Bioefficacy of *M. azedarach* has long been realized against a number of insect pests. The insecticidal activity of *M. azedarach* has been attributed to biologically active triterpenoids, which inhibit feeding of phytophagous insects, cause death and malformations of subsequent generations. Moreover, Limonoids isolated from this plant have been reported to possess antifeedant, anti ovipositional and growth inhibition effects (Carpinella *et al*., 2003).

Table-1 Showing Growth Inhibition Effect of Bakain Seed Aqueous Extract on Pulse Beetle

Extract Concentrations	Oviposition Deterrence (%)	Adult Inhibition (%)	Adult Longevity (Days)	Weeviled Grains (%)	Weight Loss (%)
0.4%	37.47[a]	45.05[a]	6.33[N.S]	47.60[d]	13.48[e]
0.3%	27.34[b]	44.31[a]	6.33	48.89[d]	13.54[e]
0.2%	18.67[c]	22.94[b]	6.34	52.54[bc]	14.40[d]
0.1%	11.86[d]	22.63[b]	6.35	52.38[c]	15.24[c]
0.05%	6.00[e]	22.21[b]	6.34	53.38[bc]	15.44[c]
0.025%	5.20[e]	22.10[b]	6.35	54.11[b]	16.89[b]
Control	0.00[f]	0.00[c]	6.35	88.72[a]	30.00[a]
$LSD_{0.05}$	1.14	1.39	0.08	1.52	0.72

Means followed by different letter (s) in a respective column are statistically different at 5% probability level.
N.S = Non-Significant, LSD = Least Significance Difference

Toxicity of Bakain Seed Aqueous Extract against Pulse Beetle: Toxicity of bakain seed extract against pulse beetle has been summarized in table-2. The results revealed that tested concentrations of the extract exhibited a varied degree of toxicity against pulse beetle. Maximum mortality of the test insect (18.20%) was recorded at a concentration of 0.4%, which was statistically at par with 18.17% mortality of the pest observed at a concentration of 0.3%. A decrease in mortality of the test insect was observed by lowering the concentration of the extract. 8.68, 7.42 and 5.61% mortality of the pest was observed at a concentration of 0.2, 0.1 and 0.05% respectively. Minimum mortality of the pest (2.72%) was observed at a concentration of 0.025%. The results further revealed that toxicity of the extract was directly proportional to the exposure period of pulse beetle in treated chickpea grains. 5.34% mortality of the pest was observed after 24 hours of release of beetles in treated chickpea grains. An increase in mortality of the pest (8.99 and 11.72%) was noted after 48 and 72 hours of release of beetles in treated chickpea grains. As far as interaction of extract concentrations and exposure period were concerned, significantly highest mortality of the pest (28.29%) was recorded at the concentration of 0.4 after 72 hours of release of pulse beetle in treated grains, which was statistically at par with 28.22% mortality of the pest recorded at a concentration of 0.3% after the same exposure period. Bakain seeds extract at these concentrations caused 18.72 and 18.71% mortality of the pest after 48 hours of the exposure time. Both the values were statistically at par with each other at 5% level of probability. Minimum mortality of the pest (2.52%) was recorded at the concentration of 0.025% after 24 hours of exposure, which was non significantly different from 2.73 and 2.91% mortality of the pest recorded at the same concentration of the extract after 48 and 72 hours of exposure time respectively.

It was investigated from the results that tested concentrations of bakain seed aqueous extract showed a varying degree of toxicity against pulse beetle. Its effectiveness was found to be dose dependent; higher the concentration of the extract, higher the mortality of the pest. It was further observed that bakain seed extract caused maximum mortality of the pest after 72 hours of the exposure to treated grains. No literature was found on toxic potential of bakain seed aqueous extract against pulse beetle. However, (Adedire *et al*., 2011) found that steam distillate of cashew kernels was more toxic to *C. maculatus* compared with methanol and ethanol extracts. Similarly, (Zia *et al*., 2011) observed significant mortality of chickpea beetle with the application of aqueous extract of black pepper.

Table-2 Showing Toxicity of Bakain Seed Aqueous Extracts against Pulse Beetle

Percent Mortality of Pulse Beetle after Different Exposure Time (Hours)				
Extract Concentrations	**Exposure Time**			**Mean**
	24 hours	**48 hours**	**72 hours**	
0.4%	7.58^{de}	18.72^{b}	28.29^{a}	18.20^{a}
0.3%	7.58^{de}	18.71^{b}	28.22^{a}	18.17^{a}
0.2%	7.58^{de}	8.63^{cd}	9.82^{c}	8.68^{b}
0.1%	7.06^{e}	8.57^{cd}	6.62^{ef}	7.42^{c}
0.05%	5.05^{g}	5.60^{fg}	6.17^{efg}	5.61^{d}
0.025%	2.52^{h}	2.73^{h}	2.91^{h}	2.72^{e}
Control	0.00^{i}	0.00^{i}	0.00^{i}	0.00^{f}
Mean	5.34^{c}	8.99^{b}	11.72^{a}	
$LSD_{0.05}$: Hours = 0.47 , Treatments = 0.72 , Interaction = 1.25				

Means followed by different letter (s) in a respective column / rows are statistically different at 5% probability level.
LSD = Least Significance Difference

Conclusion: It was concluded from the results that bakain seed aqueous extract has potential to protect stored chickpea due to its growth inhibition and toxic effect on pulse beetle and could be used effectively for the management of this pest during storage.

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