Int. J. Entomol. Res. 03 (01) 2015. 01-07

Available Online at ESci Journals
International Journal of Entomological Research
ISSN: 2310-3906 (Online), 2310-5119 (Print)
http://www.escijournals.net/IJER

LETHAL AND SUB-LETHAL EFFECT OF BAKAIN SEED AQUEOUS EXTRACT ON THE ADULTS OF PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* L. (COLEOPTERA: BRUCHIDAE)

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A B S T R A C T

Pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is a destructive pest of stored chickpea. Laboratory experiments were conducted to find out growth inhibition and toxic effect of aqueous extracts of bakain seed (*Melia azedarach* L.) for the management of *C. chinensis* during storage. Five different concentrations of the extract, viz. 2.5, 2.0, 1.5, 1.0 and 0.5% were mixed with chickpea grains. Results revealed that bakain seed extract significantly reduced oviposition, suppressed adults' emergence, reduced infestation by the pest and decreased weight loss of chickpea grains. The tested concentrations of the extract had no significant effect on adult life span of pulse beetle. Bakain seed extract also showed some degree of toxicity against the pest. Its effectiveness was dose and time dependent. It caused maximum mortality of the pest at the concentration of 2.5 and 2.0% after 72 hours of release of beetles in treated chickpea grains. On the basis of results obtained, it was concluded that aqueous extract of bakain seed could be used effectively for the management of pulse beetle during storage.

Keywords: Pulse beetle, aqueous extracts, *Melia azedarach,* growth inhibition, toxicity.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a rich source of plant protein, certain minerals and vitamins (Deshpande, 1992). Its grains are stored for a long period of time for human consumption and for seed purpose. Many insect pests damage to grains during storage (Obeng - Ofori *et al*., 1997). Infestation is high at farmers and traders level, where storage conditions are usually inadequate to prevent insect attack (Rajapakse *et al*., 1998). *Callosobruchus chinensis* L. is the most destructive pest of stored chickpea, mung bean, peas, cowpeas, lentil, maize, sorghum and cotton (Talukder and Khanam, 2011). It causes qualitative and quantitative losses to stored pulses. The infested seeds lost germination potentials and also become unfit for human consumption (Ramzan *et al.,* 1990). The pest causes damage to stored pulses up to the extent of 10% in temperate and 30% in tropical zones (Nakakita, 1998). The common practice among farmers to protect stored products is through the use synthetic insecticides. Indiscriminate use of synthetic insecticides is hazardous to human health and agro-ecosystems, as these contaminate foodstuffs and kill non-target organisms (Ray & Philip 2000). Natural plant products are desirable as they are safer to non-target organisms, less persistent in the environment and are compatible with other control tactics in an integrated pest management system. 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 a significant reduction in oviposition (2-5 eggs /pair) of pulse beetle with the application of 10% aqueous extract and aqueous suspension of *Solanum surratense* Burm. fruits, while Jayakumar (2010) observed 84.66 and 82.11% oviposition deterrence of *C. maculatus* with the application of *Cassia siamia* Lam and *C. aurantium* Lam respectively. Kiradoo and Srivastava (2010) reported that *Ocimum basilicum* L. was effective in reducing fecundity of *C. chinensis*. Kotkar *et al.* (2001) reported that flavonoids isolated from aqueous extract of *Annona squamosa* Linn at the concentration of 0.07mg

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ml^{-1} caused 80% mortality of *C. chinensis*. Udo and Epidi, (2009) recorded significant mortality of *C. maculatus* with the application of various fractions of *Ricinodendron heudelotii* (Baill)leaves extracts, using equal volume of hexane, ethyl acetate, chloroform, butanol and water. Denloye *et al*. (2010) found effectiveness of aqueous extracts of root and bark of *Zanthoxylum zanthoxyloides* Lam. against *C. maculatus*. Adedire *et al*. (2011) reported that steam distillate of cashew (*Anacardium occidentale* L.) kernels was most toxic to *C. maculatus* compared to methanol and ethanol extracts. Zia *et al*. (2011) reported effectiveness of aqueous extracts of black pepper (*Piper nigrum* L.) cloves (*Syzygium aromaticum* L), neem (*Azadirachta indica* A. Juss) and garlic (*Alium sativum* (L.) against chickpea beetle during storage. Bakain, *Melia azedarach* L. (family: Meliaceae) is a widely grown ornamental tree in tropical and subtropical countries of the world. It has been reported to possess insecticidal (Defagu *et al*., 2006) and medicinal (Benencia *et al*., 1994) properties. It has a great potential in the pest control because of its repellent, deterrent, antifeedant, morphogenetic defects and toxic properties against a vast range of insect pests (Alouani *et al*., 2009; Maciel *et al*., 2006). Bio-efficacy of *M. azedarach* has been reported against a number of insect pests (Hammad and McAuslane, 2006). A number of organic molecules, viz. terpenoids, flavonoids, steroids, acids, meliantriol, melianone and melianol have been isolated from the fruits of *M azedarach* (Rishi *et al*., 2003). However, chemical composition of *M. azedarach* varies among wild and cultivated states (Carpinella *et al*., 2003). Given the variability in chemical composition of *M. azedarach* depending on the environment, it is imperative to study the variation of its effectiveness in different locations against insect pests. No research work has been reported on insecticidal activity of *M. azedarach* against pulse beetle in environmental conditions of Dera Ismail Khan. Keeping this in view, experiments were designed to determine growth inhibition and toxic effect of bakain seed aqueous extract against pulse beetle under agro climatic conditions of Dera Ismail Khan, Pakistan.

MATERIALS AND METHODS

Research trials were conducted in Entomology Department, Faculty of Agriculture, Gomal University, D. I. Khan to find out growth inhibition and toxic effect of bakain seed aqueous extract against *C. chinensis* infesting stored chickpea. 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 for 30 days. The seed was ground to a 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 g of ground bakain seed powder was kept in 50 ml 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 30 ml of water and were filtered again. The resulting filtrates were combined together. The extract was preserved in sealed bottle in a refrigerator at 5 ^{0}C until required for bioassay. 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 living organism. A small population of pulse beetle was obtained from naturally infested chickpea grains from the farmer's stores and was reared using growth chamber (HPP-260, Memmert) at 30$\pm$2 °C temperature and 70$\pm$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 a piece of muslin cloth and 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 population of pulse beetle. After seven days, the released 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 test the efficacy of different concentrations of bakain seed aqueous extract against pulse beetle. The experiment was laid down in completely randomized design, with 5 replications, each consisting of 6-treatments (including control). Aqueous extract of mixed with 50 g chickpea grains at the concentration of 2.5, 2.0, 1.5, 1.0 and 0.5% (v/w) in a jar of 250 ml capacity. Chickpea grains in control treatment were treated with the same amount of 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 and fastened with rubber bands to ensure ventilation and to prevent entry or escape of insects. 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.

Oviposition Deterrence: Number of eggs per chickpea grain was counted to see the effect of different concentrations of bakain seed aqueous extract on fecundity of female pulse beetle. For this purpose, twenty chickpea grains were selected randomly from each jar (treatment) and the eggs laid by the female on each grain were counted using magnifying glass. The percentage of oviposition deterrence was calculated using the following formula (Elhag, 2000).

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

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

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

$$\text{Percent Inhibition Rate } = \frac{\text{Cn} - \text{Tn}}{\text{Cn}} \text{ X } 100$$

Where: C_n= Number of adults emerged in untreated jar (control); T_n = Number of adults emerged in treated jar.

Adult Life: Data were recorded to see the effect of bakain seed extract on adult life of pulse beetle on the basis of number of days taken from emergence of adults 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 petri dishes containing untreated chickpea grains. The beetles were examined daily and the dead ones were discarded.

Weeviled Grains: Damaged and undamaged chickpea grains were counted in each jar and the percentage of

bakain seed was thoroughly

weeviled grains was calculated using the following standard formula.

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

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

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

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 experiment was laid down in completely randomized design with 6-treatments (including control) and replicated five times. Different concentrations of bakain seed extract, viz. 2.5, 2.0, 1.5, 1.0 and 0.5% were mixed separately with 20 g chickpea grains in pre labeled petri dishes (9 cm diameter). Chickpea grains in control treatment were treated with the same amount of distilled water only. Filter paper was placed on the bottom of petri dishes to facilitate the movement of beetles in the arena. Forty beetles (one-day-old) were released into each petri dish, containing treated chickpea grains. Data on mortality of beetles were recorded after 24, 48 and 72 hours of release of beetles and dead ones were discarded in each data. Percent corrected mortality of beetles was calculated using Abbott's formula (Abbott, 1925).

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

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 (CRD) for growth inhibition effect of bakain seed extracts, 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 the statistical significant difference among treatments.

RESULTS AND DISCUSSION

Growth Inhibition Effect of Bakain Seed Aqueous Extract on Pulse Beetle: Data presented in table 1 shows the effect of different concentrations of bakain seed aqueous extract on oviposition deterrence, adult inhibition, adult life span, weeviled grains and weight loss of chickpea grains by pulse beetle. Results revealed that tested concentrations of bakain seed extract affected normal oviposition of the pest.

Table I. Growth Inhibition Effect of Bakain Seed Aqueous Extract on Pulse Beetle.

Extract Concentration	Oviposition Deterrence (%)	Adult Inhibition (%)	Adult Life (Days)	Weeviled Grains (%)	Weight Loss (%)
2.5%	56.96[a]	68.22[a]	6.24[N.S]	42.20[b]	12.94[c]
2%	52.53[b]	67.91[a]	6.28	42.90[b]	13.02[c]
1.5%	49.75[c]	67.70[a]	6.28	42.85[b]	13.05[c]
1%	49.37[c]	66.25[b]	6.34	42.80[b]	13.08[bc]
0.5%	44.30[d]	65.63[b]	6.34	42.90[b]	13.50[b]
Control	0.00[e]	0.00[c]	6.35	88.04[a]	29.76[a]
LSD	0.83	0.84	0.32	1.25	0.43

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.

Significantly high oviposition deterrence (56.96%) was recorded at the concentration of 2.5%. It was followed by 52.53, 49.75, 49.37 and 44.30% oviposition deterrence at the concentration of 2.0, 1.5, 1.0 and 0.5% respectively. The oviposition deterrence recorded at the concentration of 1.5% was statistically at par with that of 1% concentration of the extract. The tested concentrations of the extract significantly suppressed adults' emergence. Bakain seed extract at the concentration of 2.5% resulted in maximum adults' inhibition (68.22%), which was statistically at par with 67.91 and 67.70% adult inhibition occurred at the concentration of 2.0 and 1.5% respectively. It was followed by 66.25% adults' inhibition at the concentration of 1%, which was non-significantly different from 65.63% adults' inhibition at the concentration of 0.5%. The tested concentrations of the extract had no significant effect on adult life span of pulse beetle. Adult life of pulse beetle ranged from 6.24 to 6.35 days among the treatments. Bakain seed extract at all the tested concentrations reduced infestation by the pest compared to control. Minimum weeviled chickpea grains (42.20%) were recorded at the application rate of 2.5%, which were non-significantly different from rest of the treatments, except for control, where maximum weeviled grains (88.04%) were recorded. As far as weight loss of chickpea grains is concerned, minimum weight loss (12.94%) was recorded at the concentration of 2.5%, which statistically at par with 13.02, 13.05 and 13.08% weight loss noted at the concentrations of 2.0, 1.5 and 1.0% respectively. It was followed by 13.50% weight loss at the concentration of 0.5%. Maximum weight loss of chickpea grains (29.76%) was observed in untreated grains. It was obvious from the research findings that aqueous extract of bakain seed had a strong growth inhibition effect on pulse beetle. It significantly reduced fecundity, adult emergence of pulse beetle and consequently reduced infestation and weight loss of chickpea grains. The tested concentrations of the extracts had no significant effect on adult life span of pulse beetle. Bakain has a great potential in the pest control because of its repellent, deterrent, antifeedant, morphogenetic defects and toxic properties against a vast range of insect pests. (Alouani *et al.,* 2009; Maciel *et al.*, 2006). The insecticidal activity of *M. azedarach* was due to biologically active triterpenoids with an antialimentary effect, i.e. they inhibit the feeding of phytophagous insects cause death and malformations of subsequent generations (Carpinella *et al.*, 2003). Within the triterpenoids in *M. azedarach* seeds, meliacarpine, similar to azadiractine, is also active in the regulation of insect growth. Bio efficacy of *M. azedarach* has long been realized for its highly effective properties against a number of insect pests (Gajmer *et al.*, 2002). Limonoids from this plant are known to exhibit antifeedant, oviposition deterrent, repellent and growth regulatory effects (Carpinella *et al.*, 2003). No literature was found on the efficacy of bakain seed aqueous extracts against pulse beetle. However, some previous workers, viz. Srivastava and Gupta (2007), Jayakumar (2010) and Kiradoo and Srivastava (2010) reported growth inhibition effect of aqueous extracts of different plants against pulse beetle. These researchers observed reduced fecundity and adults' emergence of the pest with the application of aqueous extracts of various plants parts.

Toxicity of Bakain Seed Aqueous Extract against Pulse Beetle: Data presented in table 2 revealed the toxicity of tested concentrations of bakain seed extract against pulse beetle. Maximum mortality (34.47%) of the test insect was recorded at the concentration of 2.5%. It was followed by 33.30, 30.98, 20.62 and 19.81% mortality of the pest insect recorded by the concentration of 2.0, 1.5, 1.0 and 0.5% respectively. As far as exposure time is concerned, significantly high mortality (33.24%) was recorded after 72 h of release. A significant decrease in mortality of the pest was observed after 48 and 24 h of exposure time, where 22.90 and 13.45% mortality of the pest was recorded respectively. Data also revealed a significant interaction between extract concentrations and exposure time. Bakain seed extract at the concentration 2.5% registered significantly highest mortality of pulse beetle (48.16%) after 72 h of exposure time in treated chickpea grains. It was followed by 46.72 and 44.01% mortality of the pest after 72 h of exposure time at the concentration of 2.0 and 1.5% respectively. Significantly lowest mortality (10.16%) was recorded 24 h after the release of pulse beetle in chickpea grains treated with 1.0 and 0.5% concentration of the extract. It was obvious from the results that tested concentrations of bakain seed extract showed a varying degree of toxicity against pulse beetle. Its effectiveness was dose dependent; higher the concentration of the extract, higher the mortality of the pest. It caused maximum mortality of the pest insect at a dose of 2.5% after 72 hours of release of beetles in treated chickpea grains. No literature was found on toxicity of bakain seed extract. However, Adedire *et al*. (2011) reported that steam distillate of cashew kernels was most toxic to *C. maculatus* compared to methanol and ethanol extracts. Similarly, Zia *et al*. (2011) effectively controlled chickpea beetle using aqueous extracts of black pepper, cloves, neem and garlic.

Table 2. Toxicity of Bakain Seed Aqueous Extracts against Pulse Beetle.

Extract Concentration	Exposure Time			Mean Mortality
	24 h	48 h	72 h	
2.5%	21.81^{g}	33.42^{d}	48.16^{a}	34.47^{a}
2%	20.81^{g}	32.38^{de}	46.72^{b}	33.30^{b}
1.5%	17.77^{i}	31.16^{ef}	44.01^{c}	30.98^{c}
1%	10.16^{j}	21.06^{g}	30.65^{f}	20.62^{d}
0.5%	10.16^{j}	19.36^{h}	29.91^{f}	19.81^{e}
Control	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{f}
Mean	13.45^{c}	22.90^{b}	33.24^{a}	
LSD: Hours = 0.55, Treatments = 0.78 , Interaction = 1.34				

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 disrupted normal behaviour of pulse beetle and could be used effectively for the management of *C. chinensis* during storage.

ACKNOWLEDGEMENT

The authors are thankful to Higher Education Commission of Pakistan for sponsoring this study.

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