



Biorational Management of Pulse Beetle (*Callosobruchus chinensis* L.) on Chickpea Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AH and MH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KA, SS, TUW managed the analyses of the study. Author AMH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The pulse beetle, *Callosobruchus chinensis* is one of the major insect pest in stored pulse (i.e. Chickpea seeds) causing 40-50% losses of pulses in storage. Experiments were conducted to study the efficacy of some selected biorational insecticides on mortality of beetle, weight loss of seeds, fecundity and hatchability of pulse beetle, *Callosobruchus chinensis* under laboratory condition. Among the different botanicals, Neem oil (89.00%) was found the most effective in case of mortality of pulse beetle in direct method followed by Mahogany oil (78.00%) and Karanja oil (62.00%). Among different microbial derivatives, Spinosad was found most effective considering mortality followed by Emamectin benzoate and Abamectin. Among botanicals the highest percentage of weight reduction was observed in Karanja oil (30.18%) and the lowest (22.43%) was in Neem oil while among microbial derivatives the lowest percentage of seed weight reduction was obtained from Spinosad (11.15%) and the highest percentage of weight reduction was obtained

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from Abamectin (20.37%). No. of eggs laid per female was recorded highest in Karanja oil (24.00) and lowest in Spinosad (19.33). The hatchability percentage was highest in Karanja oil (21.73%) and lowest in Neem (12.89%) and in case of chemical treatment lowest in spinosad (6.05%) and highest in Ebamectin (14.42%). Neem oil and Spinosad were found effective against pulse beetle in storage condition. Therefore, Neem oil and Spinosad might be included in the development of IPM packages for the management of pulse beetle in the storage.

Keywords: *Biorational insecticides; pulse beetle (Callosobruchus chinensis); hatchability; chickpea seeds.*

1. INTRODUCTION

Pulses are economically important crops in Bangladesh because of their high protein content (20-40%) and are fairly good sources of vitamins, calcium and iron for the under privileged people, who can't afford animal proteins [1].

A large number of pulses are grown in Bangladesh such as lentil, gram, grasspea, mungbean, cowpea, etc. and the total production of pulse was 2,85,783 metric tons from the area of 5,36,874 acres in 2014-2015 [2]. After harvesting, about 85% of the pulse growers in Bangladesh store pulses throughout the year in their houses [3].

Post-harvest damages by insect pests have been an increasingly important constraint to food legume supplies worldwide. The ways the pulse seeds are stored make them prone to the attack of the insect pests. Pulse beetles, *Callosobruchus spp.* (Coleoptera: Bruchidae) are major storage pests of legume crops grown in the tropics and sub-tropics. Among different species of pulse beetle, *Callosobruchus chinensis (L.)* is the most destructive in Bangladesh and the post-harvest seed losses due to the beetle can reach even 100% during severe period of infestation [4].

Generally pesticides are used for the control of insect pests but due to their hazards, researchers are trying to adopt alternative methods of pest control [5]. In the world, as many as 2400 plant species have been reported that have potential pesticidal properties and biological activity against a wide range of pests [6]. Their main advantage is that they may be easily and cheaply produced by farmers and small-scale industries as crude, or partially purified extracts.

It was reported that when mixed with stored-grains, leaf, bark, seed powder or oil extracts of plants reduce oviposition rate and suppress adult

emergence of bruchids, and also reduced seed damage rate [7,8,9].

The use of locally available indigenous plant materials for the control of pests are an ancient technology used in many parts of the world [10]. The pest controlling efficacy of many plant derivatives has already been proved against several storage pests [11,12].

These are also having less environmental impact in terms of insecticidal hazards and could benefit our agricultural sector.

However, recently, with the increasing consumption and marketing of pulses has developed awareness among the farmers about the crop and the demand for judicious insects control is increasing to meet the immediate needs to save the crop from bruchid damage. The control of storage pests by using synthetic chemicals has become a common practice among farmers and stockholders. It is now widely known that chemical method has several concerns, which include health hazards to the users and grain consumers. It causes residual toxicity, environmental pollution and development of pesticide resistance against bruchids. Sometimes persistent pesticides accumulate in the higher food chain of both wildlife and human and become concentrated by bio magnification [13].

Therefore, it has become important now to find alternate pest control methods that are not only effective but also environmentally balanced and non-hazardous to men and animals. In these circumstances, botanical insecticides are cheaper, easy to process and the raw materials are available at village level. The use of simple crude botanical oils and seed extracts are important for grain protection by resource limited farmers in developing countries like Bangladesh. The search for alternative methods for pulse beetle control utilizing botanical products and their comparison with existing chemical

insecticides is being pursued now a days. The present experiment was therefore, carried out to evaluate the effects of botanical oils of some indigenous plants against pulse beetle, *C. chinensis* to protect chickpea seeds in storage. The present research was conducted to find out the efficacy of some botanical oils and biorational insecticides on different biological parameters of pulse beetle, *C. chinensis* and determine the effective dose of some botanical oils and biorational insecticides against pulse beetle.

2. MATERIALS AND METHODS

2.1 Experimental Site and Design

The present research work was conducted in the seed science & technology laboratory, Department of seed science & technology, Bangladesh Agricultural University, (BAU), Mymensingh during the period from September to November, 2018. The experiment was conducted in the laboratory, using Completely Randomized Design (CRD).

2.2 Collection of Gram Seed and Oils (Neem, Mahagoni, Karanja)

Seeds of gram *Cicer arietinum* (L.) popularly known as "chhola" were used in this experiment. The gram seeds were bought from the local K & R market of BAU campus, Mymensingh. The oils of Neem, Mahagoni and Karanja were collected from local markets.

2.3 Source and Culture of Pulse Beetles

Gram seeds infested with pulse beetles were collected from the laboratory of the Department of Entomology and from Entomology Division of Bangladesh Institute of Nuclear Agriculture (BINA) to maintain a laboratory culture under 27±2°C temperature and 75±5% relative humidity.

2.4 Systematic Position of Pulse Beetle

Phylum – Arthropoda
Class – Insecta
Sub class- Pterygota
Division- Endopterygota
Order – Coleoptera
Family – Bruchidae
Genus – *Callosobruchus*
Species – *Callosobruchus chinensis* L

2.5 Botanical Species Tested as Botanical Insecticides

Three different rates of three biopesticides were used for the experiment, namely, Mahogany (*Swietenia mahogoni* L - Meliaceae), Neem (*Azadirachta indica* – Meliaceae), Karanja (*Pongamia pinnata* – Fabaceae). Mahogany resists wood rot, making it attractive in boat construction and outdoor decking. The oil of mahogany fruit has insecticidal and antiseptic properties. Neem leaves are dried in Bangladesh and placed in cupboards to prevent insects eating the clothes and also in tins where rice is stored.

Neem oil is used for healthy hair, to improve liver function, and balance blood sugar levels. Juices from karanja plant, as well as the oil, are antiseptic and resistant to pests.

2.6 Treatments of the Experiment

In the present study, three botanical oils and three biorational insecticides as treatments having three doses of each were used against pulse beetles infesting gram in storage condition Viz. Neem oil (0.5, 1.0, 1.50 ml/L), Mahogany oil (0.5, 1.0, 1.50 ml/L), Karanja oil (0.5, 1.0, 1.50 ml/L), Spinosad (Tracer 45 SC) (0.5, 0.75 , 1.0 ml/L), Emamectin benzoate (Suspend 5 SG) (0.5, 0.75, 1.0 ml/L), Abamectin (Ambush 1.8 EC) (0.5, 0.75 , 1.0 ml/L) [14].

2.7 Bioassay and Data Collection Procedures

2.7.1 Preparation of dose solution for botanicals and microbial derivative pesticides

At first three small beakers were taken with 200 ml water. Then, 100, 150 and 200 micro litres (µl) of botanicals (neem, mahogoni and karanja oil) and microbial derivative pesticides (Abamectin and Spinosad) were poured into the beaker to prepare three doses, 0.50, 0.75 and 1.00 ml/L. For Emamectin benzoate, 0.1, 0.15 and 0.2 gm/L of Emamectin benzoate was weighed, poured to prepare 0.50, 0.75 and 1.00 gm/L solution of Emamectin benzoate solutions.

2.7.2 Assessment of toxicity by direct method

About 30 g gram seeds and 10 pulse beetles were taken into Pots previously washed with alcohol. There were three replications of each

pots tested. By the help of spray machine, different doses of botanical (neem, mahogoni and karanja oil) and microbial derivative pesticides (Abamectin, Spinosed and emamectin benzoate) solutions were sprayed in those Petri dishes. Mortality data was calculated after 5 days of treatment.

2.7.3 Calculation of percent mortality

Number of dead and alive insects were regularly observed and percent mortality was calculated by using the following formula:

$$\text{Percent mortality} = \frac{\text{No. of dead pulse beetle}}{\text{Total no. of released puls beetle}} \times 100$$

2.7.4 Percent weight loss

Percent weight loss and percentage protection of seed weight over control were calculated with the following formulae.

$$\text{Percent weight loss} = \frac{\text{Weight loss per pot}}{\text{Initial weight of seed grains per pot}} \times 100$$

After 20 days weight loss was measured

Where, Weight loss per pot = (Initial weight- final weight) of grains per pot.

Data collection on adult longevity and fecundity (no. of eggs laid, percent of hatchability, larval duration, pupal period) of pulse beetle) were evaluated by the same bioassay procedure. The number of emerged adults were counted daily starting from 7 days after the release of insects. After every count, adults were removed from each plastic jar to avoid egg laying.

2.8 Statistical Analysis

Analysis of variance was done with the help of a computer statistical package R Statistics Software version 3.5.3. The mean differences among the treatments were adjudged with Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) when necessary.

3. RESULTS AND DISCUSSION

3.1 Effect of Some Botanical Oils and Bio-rational Insecticides on the Mortality of Pulse Beetle

The mean numbers of adult mortality on chickpea seeds treated with different botanical oils and bio-rational insecticides at different rate at laboratory condition are presented in Table 1.

For botanical oil, it was revealed that lowest number of adult mortality from untreated seeds was 8.43% compared to all other treatments. The number of adult mortality was higher at higher ratio of all botanical oils. Among the treated seeds, the highest number of adult mortality 89% was found from seed treated with neem oil @ 1.5 ml/L followed by neem oil @1.0 ml/L and mahogoni oil @1.5 ml/L (78%). The lowest number of mortality of 32% was from seed treated with karanja oil @ 0.5 ml/L followed by mehagoni oil @ 0.5 ml/L (41%). Our observations are in consistent with the previous findings of Singh and Pandey, [15] that evaluated the efficacy of karanja (*Pongamia pinnata*) oil against *C. chinensis* in green gram and found it was very effective for the protection of pulse beetle. Moreover, this result was similar to the findings reported by Tapondjou [16] indicating that the adult mortality was more when stored seeds were treated with some indigenous plant powder and oils. Karanja oil also show the highest mortality at higher dose and the lower doses of Karanja oil showed the lower mortality among all the treatment and the order of effectiveness among the botanical oils was Neem oil>Mahogany oil>Karanja oil. Grainge and Ahmed [6] observed that, under storage condition the botanicals like neem kernel powder (*Azadirachta indica*) was most effective against pulse beetle.

Efficacy of some 3rd generation insecticides was determined through the mortality test of adult pulse beetle. The highest mortality was found by using Spinosad treated seeds followed by Emamectin benzoate treated seeds.

The highest mortality was about 100% with the higher dose of spinosad. The mortality rate for Spinosad at the doses 0.75 ml/L, 1.0 ml/L and for emamectin benzoate at the dose 1.0 ml/L was 92.00%, 100% and 90.00% respectively (Table 1). But in this case Abamectin showed the lower efficacy comparing it with other two insecticides. The order of effectiveness among the treatments was Spinosad>Emamectin>Abamectin.

3.2 Efficacy of Some Selected Botanical Oils and Bio-rational Insecticides on the Oviposition Rate of Pulse Beetle, *C. chinensis* L.

The following graph showed that the higher efficacy of fecundity of pulse beetle by using Neem oil. The lowest number of egg laid at the

dose of 1.5 ml/L Neem oil is 12.89. In case of Mahagoni oil and Karaja oil the oviposition of pulse beetle is about to same and it is between 14.55 to 21.73. But the control treatment showed higher difference in no. of egg laid which was 62.55 (Fig. 1).

The fecundity of pulse beetle was determined through some chemical pesticides. The Fig. 2 indicates the higher efficacy of Spinosad at the dose 1.0 ml/L and the number of eggs laid was 6.05. The Abamectin and Emamectin benzoate had no significant difference on fecundity of pulse beetle. The control condition showed the highest no. of eggs which was 56.75. The pulse beetle caused damage to gram seeds in the petri dish. The larvae fed on the internal contents of seeds for their growth and development. Pupae formation takes place inside the seeds and finally emerged as adult from the seed creating hole(s) on gram seeds. The effect of different ratios of

botanical oil and bio-rational insecticides on percent seed weight loss by the attack of pulse beetle was statistically significant at 1% level of probability at Table 2. The Highest weight loss was recorded from untreated seeds (14.08%). Among the plant oil, the lowest weight loss (22.43%) was recorded from the seeds treated with neem oil @1.5 ml/L followed by neem oil @ 1 ml/L (24.13%), mehagoni oil @ 1 ml/L (25.95%) and karanja oil @ 1.5 ml/L (26.4%). Among the seeds treated with plant oil, the highest weight loss (30.18%) was recorded from the seeds treated with karanja oil @ 0.5 ml/L (Table 2). The weight loss for Spinosad at the doses 0.75 ml/L, 1.0 ml/L and for Emamectin benzoate at the dose 1.0 ml/L was 13.04%, 11.15% and 13.55% respectively (Table 2). But at this case Abamectin showed the lower efficacy comparing with other two insecticides. The order of effectiveness among the treatments was Spinosad>Emamectin benzoate> Abamectin.

Table 1. Effect of some botanical oils and bio-rational insecticides on the mortality of pulse beetle (title should be on the top)

Treatments	Doses (ml/L)	No. of insects before spray	No. of dead insect	Mortality (%)
Neem oil	0.5	10	5.13	51.33 g
	1.0	10	8.33	83.33 b
	1.5	10	8.90	89.00 a
Mahagoni oil	0.5	10	4.10	41.00 h
	1.0	10	6.90	69.00 d
	1.5	10	7.80	78.00 c
Karanja oil	0.5	10	3.20	32.00 i
	1.0	10	5.50	55.00 f
	1.5	10	6.20	62.00 e
Control	-	10	0.84	8.43 j
Level of significance		NS	*	*
CV (%)		-	8.83	6.85
LSD		-	0.17	1.8
Spinosad (Tracer 45 SC)	0.5	10	6.80	68.0 f
	0.75	10	9.20	92.0 b
	1.0	10	10.00	100.0 a
Emamectin benzoate (Suspend 5 SG)	0.5	10	4.60	46.0 h
	0.75	10	8.00	80.0 d
	1.0	10	9.00	90.0 c
Abamectin (Ambush 1.8 EC)	0.5	10	3.65	36.5 i
	0.75	10	6.31	63.1 g
	1.0	10	7.50	75.0 e
Control	-	10	0.0	0.00 j
Level of significance		NS	***	***
CV (%)		-	8.56	7.96
LSD		-	0.17	0.20

*In column, means followed by different letters are significantly different.
 In column, means followed by same letters are not significantly different.
 *means at 5% level of probability
 NS means non-significance*

It was noted from the results of present investigation that the number of adult emergence was greatly reduced by the application of

botanical oil and bio-rational insecticides on the seeds (Table 2).

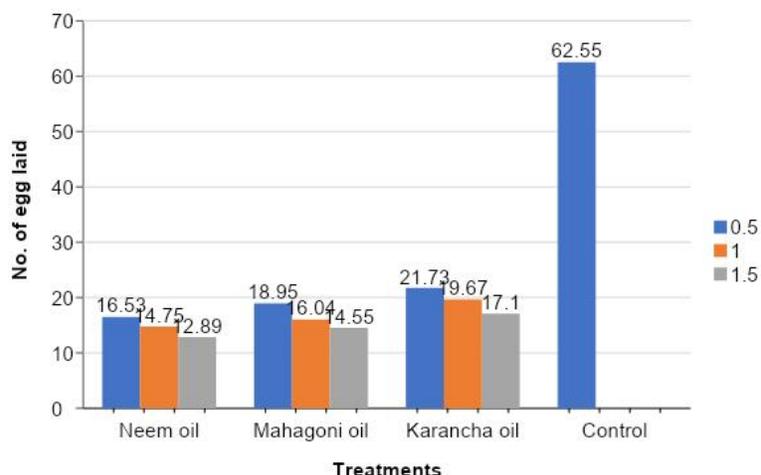


Fig. 1. Efficacy of some selected botanical oils on the oviposition rate of adult pulse beetle, *C. chinensis* L.

Table 2. Effect of some botanical oils and bio-rational insecticides on the weight loss and adult emergence of pulse beetle

Treatments	Doses (ml/L)	Weight loss (%)	Adult emergence (%)
Neem oil	0.5	26.23 e	12.97 f
	1.0	24.13 h	11.19 h
	1.5	22.43 i	9.33 j
Mahagoni oil	0.5	28.36 c	15.72 d
	1.0	25.95 g	12.48 g
	1.5	24.15 h	10.85 i
Karanja oil	0.5	30.18 b	18.17 b
	1.0	27.03 d	16.11 c
	1.5	26.04 f	13.54 e
Control	-	86.75 a	58.99 a
Level of significance		**	**
CV (%)		9.09	7.00
LSD		0.05	0.30
Spinosad (Tracer 45 SC)	0.5	16.24 e	7.60 e
	0.75	13.04 i	5.28 h
	1.0	11.15 j	3.10 j
Emamectin benzoate (Suspend 5 SG)	0.5	18.82 c	9.20 d
	0.75	15.03 g	7.52 f
	1.0	13.55 h	5.20 i
Abamectin (Ambush 1.8 EC)	0.5	20.37 b	11.47 b
	0.75	17.12 d	9.40 c
	1.0	15.75 f	7.40 g
Control	-	89.95 a	53.80 a
Level of significance		**	*
CV (%)		8.14	7.30
LSD		0.05	0.06

In column, means followed by different letters are significantly different.
 In column, means followed by same letters are not significantly different.
 *means at 5% level of probability, NS means non-significance

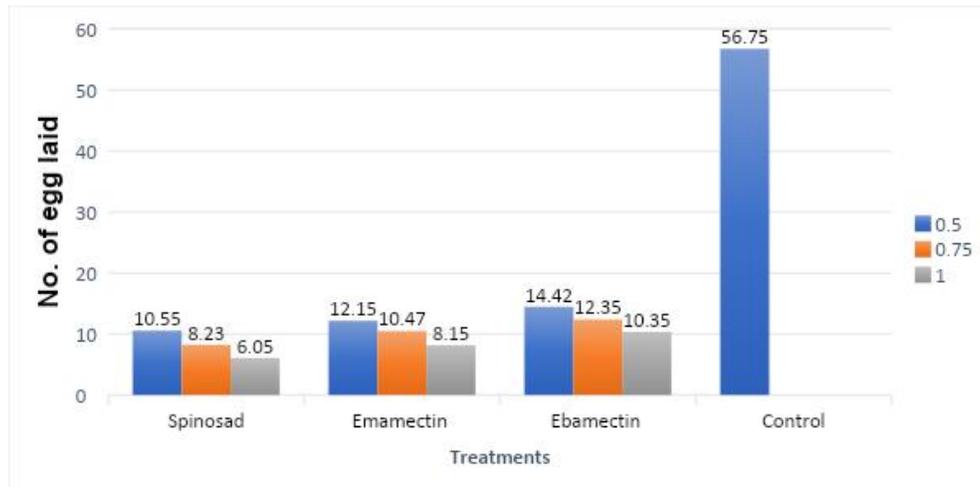


Fig. 2. Efficacy of some selected bio-rational insecticides on the oviposition rate of adult pulse beetle, *C. chinensis* L

Among the treated seeds with botanical oil, the highest number of adult emergence (18.17%) from seeds treated with karanja oil @ 0.5 ml/L. The lowest number of adult emerged (9.33%) from seeds treated with neem oil @1.5 ml/L which was followed by mahagoni oil @ 1.5 ml/L (10.85%) and neem oil @ 1.0 ml/L (11.19%).

4. CONCLUSION

The above results indicated that Karnaja oil, Neem oil, Mahogany oil, Spinosad, Abamectin and Emamectin were effective biorational insecticides for the management of pulse beetle in storage. However, Neem oil and Spinosad were found more effective against pulse beetle in storage condition than others. Therefore, Neem oil and Spinosad might be included in the development of IPM packages for the management of pulse beetle in the storage. Yet, further studies in the following areas are suggested: Other botanicals and biorationals may be included in the future study and proper dose of biorationals should be determined.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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