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Contact Action of Spinosad on *Callosobruchus chinensis* (L.) in Three Successive Generations

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Abstract: ***Stored product insect pest's causes substantial economic and quality losses to stored pulses or cereals. Callosobruchus chinensis (L.) is a major insect pest of stored pulses. The beetle infested lentil (Lens esculentus L.) seeds in stored conditions and causes severe damages. Indiscriminate uses of insecticides or fumigants to control the beetle can cause health hazards to human being. This study explores development technology for use in control of C. chinensis which is eco-friendly and biodegradable. Spinosad influenced the progeny production, fertility, adult mortality and sex-ratio of C. chinensis in three successive generations. In the first generation, the concentration was increased (6.2×10^{-5}ppm/µml), the progeny production rate was decreased (male 32.67 and female30.33). In the second and third generations the same was gradually decreased.***

Keywords: Contact action, spinosad, Callosobruchus chinensis

1. Introduction

Stored pulses are primarily attacks by major insect pest particularly the pulse beetle, *Callosobruchus chinensis*. Though chemicals provide effective control of *Callosobruchus* sp., but the efficiency of the chemicals mostly depends on the mode of exposure. The maximum effectiveness is given when the insecticides are provided by gustatory method, but majority of these are toxic to human (Bekele *et al*. 1995). Under such circumstances, alternative methods are needed to be considered (Isman 2008). The alternative solution currently recommended, is the judicious exploitation of our phylogenetic resources and microbial organisms. These two methods are often termed as biological control. Within the framework of the biological fight against such insect attacks, several works have been done by number of researchers (Thiam and Ducommun 1993, Isman 2006, Chiasson *et al.* 2008, Regnault-Roger *et al.* 2008, Allahvaisi *et al*. 2011 and AbdElhady 2012).

Spinosad is a biologically derived insecticide produced via fermentation culture of the actinomycete *Saccharopolyspora spinosa*, a soil bacteria. It is composed of a two members of the chemical class of 12-membered macrocyclic lactones in a unique tetracyclic ring. Spinosyn A and D are identical in structure except for an additional methyl group on the core macrolide of spinosyn D. Commercial formulations contain a spinisyn A to spinosad D ratio of approximately 85:15.

It is commonly known as "reduced-risk" insecticide. The contact action of spinosad has low toxicity (Bert *et al*. 1997). Reports on the effects of contact action of spinosad against the development stages of *Callosobruchus* sp. are scanty. So, the present experiment was designed to determine the contact activities of spinosad on the oviposition, fertility, adult emergence and sex ratio of *C. chinensis* for three successive generations.

2. Materials and Methods

Collection of the insects

C. chinensis was collected from a rearing culture maintained at the Controlled Temperature (30±0.5^{0}C) room, Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, Rajshahi University. Newly emerged adult beetles were collected for sub-culture of the experiments.

Concentrations used

Pilot experiments were conducted with *C. chinensis* released on filter papers treated with different concentrations of spinosad. The concentration for each spinosad doses were determined where adult mortality rate was zero percent. Depending on concentrations, a series of three concentrations for each compound were selected. The doses were 6.2×10^{-5}, 3.1×10^{-5} and 1.5×10^{-5} ppm/µml.

Experimentations

Filter papers (9 cm) were soaked in different concentrations of spinosad solutions and dried at room temperature for an hour. Previously sterilized uninfested 2gm lentil seeds placed in different petri dishes. After that five mated females of *C. chinensis* released separately. The petri dishes were covered and kept until the eggs hatched and develop as adults. The emerged adults were counted and recorded. It was treated as F_1 generation. Again a treated filter paper with same concentrations of spinosad was placed in the petri dish. Previously five mated females of *C. chinensis* from F_1 generation were released in the prepared petri dishes. The experiments have done three successive generations. A similar set of experiment was carried on filter paper soaked with distilled water only, as a control. All the experiments were conducted at 30±1^{0}C in the CT room and replicated three times.

After 1st generation the present experiments were divided into three reproductive combinations or crosses, which are as follows:

- Treated males were paired with untreated females (TU)
- Untreated males were paired with treated females (UT)
- Treated males were paired with treated females (TT)

Each pair of the concentration, spinosad was kept separately in petri dish which was checked. The experiment was conducted and the parameters recorded of the second and third generations, similarly as first generation.

The eggs laid by females on the surface of the seeds were counted after 24 hours with the help of magnifying glass and their numbers were counted. The hatching was confirmed when the frass was observed by the magnifying glass. The emerged adults were counted and their sexes were recorded. The mortality beetles were observed after 24-, 48- and 72 hours. Adults were considered to be dead when probing with a hot needle failed to produce a response (Yousefinezhad-Irani and Asghar 2007).

Data analysis: The mortality data were corrected by Abbott's formula:

Parameters observed

The following parameters are observed
a) Effects on progeny production
b) Effects on fertility
c) Effects on adult mortality and
d) Effects on the sex ratio

Statistical analysis

The effect of spinosad on the progeny products of various concentrations were tested by ANOVA and Tukey's test. The percent reduction of fertility rate in treatment compared to control (PRC) were calculated by the formula provided by Mian and Mulla (1982).

3. Results and Observation

Effect on progeny products of *C. chinensis*

The progeny productions rate of F_1 generation of *C. chinensis* was found that concentration was increased progeny productions rate were decreased (Table 1). In the highest concentration 6.2×10^{-5} ppm/µml, the male production was 32.67 and the female was 30.33 among the eggs of 72.33. In the lowest concentration 1.5×10^{-5} ppm/µml, the male (40.33) and the female (39.0) progeny produced from the deposited eggs 86.33 whereas in control male progeny production was 41.33 and the female production was 39.67 among the eggs of 90.67.

In the second and third generations the experiment were divided in three pair groups which was described earlier. In the second generation three concentrations of spinosad in TU group the male progeny productions rate respectively were 27.67, 30.00, 31.00 and the female rate 21.67, 24.67, 24.00 among the eggs rate 52.67, 59.67, 61.33. In UT group the progeny productions rate of male 21.00, 22.00, 30.33 and the female rate 16.33, 21.00, 21.33 among the eggs rate 48.33, 52.67, 60.00. Similarly in TT group, the progeny productions rate of male 21.00, 22.00, 21.33 and the female rate 16.00, 18.33, 21.00 among the eggs rate 39.00, 45.67, 51.33 respectively (Table 1).

In the third generation, three concentrations of spinosad in TU group produced male 18.67, 22.33, 20.00 and the female rate 16.67, 18.33, 19.67 respectively among the eggs rate 46.67, 50.00, 55.00. The UT group the progeny products rate of male 16.33, 20.67, 24.67 and the female rate 14.00, 16.33, 18.33 among the eggs rate 36.33, 44.00, 49.67. The TT group the progeny products rate of male 13.67, 19.00, 19.67 and the female rate 11.00, 12.00, 11.67 among the eggs rate 29.33, 35.00, 40.00 respectively (Table 1).

The present experiment noticed that progeny productions rate were reduced drastically in TT group than UT and TU groups. Female progeny productions rate was less than male progeny productions rate. The F_3 generation were showed better result than F_2 and F_1 generations.

Table 1: Effect of pair types with spinosad where treated male or female or both, on the fertility (%) of the egg hatched by *C. chinensis* in three successive generations

Generations	Concentration ppm/µml	Pair type	Number of eggs	Total Hatch	Fertility %	PRC
1st	Control	-	90.67±.0.66	81.00±0.88	89.33	-
	6.2×10^{-5}	-	72.33±1.20	63.00±0.60	87.10	22.22
	3.1×10^{-5}	-	79.67±0.88	72.33±1.13	90.78	10.70
	1.5×10^{-5}	-	86.33±0.88	78.67±0.33	91.12	2.87
2nd	6.2×10^{-5}	TU	52.67±1.20	49.34±1.05	93.63	39.08
		UT	48.33±0.88	37.33±0.57	77.23	53.91
		TT	39.00±1.00	37.00±0.57	94.87	54.32
	3.1×10^{-5}	TU	59.67±0.88	54.67±0.74	91.62	32.50
		UT	52.67±1.20	43.00±0.57	81.64	46.91
		TT	45.67±1.20	40.33±0.72	88.30	50.20
	1.5×10^{-5}	TU	61.33±0.88	55.00±0.57	89.67	32.09
		UT	49.67±1.20	43.00±1.64	86.57	46.91
		TT	40.00±1.73	31.34±1.04	78.35	61.30
3rd	6.2×10^{-5}	TU	46.67±0.88	35.34±0.88	75.72	56.34
		UT	36.33±0.88	30.33±0.90	83.48	62.55
		TT	29.33±0.66	24.67±0.45	84.11	69.54
	3.1×10^{-5}	TU	50.00±1,15	40.66±0.60	81.32	49.80
		UT	44.00±1.73	37.00±0.67	84.09	54.32
		TT	37.00±1.15	31.00±1.52	83.57	61.72
	1.5×10^{-5}	TU	55.00±1.15	41.67±0.94	75.76	48.62
		UT	49.67±1.20	43.00±1.64	86.57	46.91
		TT	40.00±1.73	31.34±1.04	78.35	61.30

Effect on fertility on *C. chinensis*: All concentrations of spinosad reduced fertility of the deposited eggs in a concentration dependent manner in three successive generations (Table 1). The percentage of fertile eggs in control was found as 89.33. In 1st generation the highest fertility PRC value 22.22 at the concentration of 6.2×10^{-5} but lowest PRC value was 2.87 of 1.5×10^{-5} concentration. In second generation highest PRC value 54.32 in TT pair type where the concentration was 6.2×10^{-5} but lowest PRC value 32.09 in TU pair type in 1.5×10^{-5} concentration. In the same concentration, the highest PRC value was 69.54 in TT pair type in same highest concentration and lowest PRC value was 48.62 in the same lowest concentration in 3rd generation. The present experiment explained the treated and untreated pair types effected egg laying of the female beetles, but showed no significant effect on their viability. Only both treated male and treated female paired showed the fertility of egg significant results.

Though, compared 2nd and 3rd generations among different concentrations and different pair types 3.1×10^{-5} and 1.5×10^{-5} concentrations showed non-significant results. Only 6.2×10^{-5} dose was showed significant result. Moreover, 3rd generation was showed better results than 2nd and 1st generations.

Table 2: Mortality (Mean±SE) of *C. chinensis* at different concentrations of spinosad after three generations time

Gene-rations	Concentrations ppm/µml	Pair type	Number of eggs	Total Hatch	Mortality (Mean ± SE)	PRC
	Control	-	90.67±.0.66c	81.00±0.88	10.66±0.23a	-
1st	6.2×10^{-5}	-	72.33±1.20	63.00±0.60	14.19±0.45b	33.11
	3.1×10^{-5}	-	79.67±0.88	72.33±1.13	13.41±0.27b	25.79
	1.5×10^{-5}	-	86.33±0.88	78.67±0.33	13.27±0.24b	15.72
	6.2×10^{-5}	TU	52.67±1.20	49.34±1.05	16.55±0.47d	55.25
2nd		UT	48.33±0.88	37.33±0.57	16.65±0.49d	56.19
		TT	39.00±1.00	37.00±0.57	17.33±0.28de	62.57
	3.1×10^{-5}	TU	59.67±0.88	54.67±0.74	15.87±0.33cd	48.03
		UT	52.67±1.20	43.00±0.57	16.01±0.30d	50.18
		TT	45.67±1.20	40.33±0.72	16.37±0.13d	53.56
	1.5×10^{-5}	TU	61.33±0.88	55.00±0.57	14.82±0.24c	39.02
		UT	49.67±1.20	43.00±1.64	15.56±0.54c	45.96
		TT	40.00±1.73	31.34±1.04	15.62±0.03cd	46.52
	6.2×10^{-5}	TU	46.67±0.88	35.34±0.88	18.23±0.29ef	71.01
3rd		UT	36.33±0.88	30.33±0.90	18.72±0.10g	75.60
		TT	29.33±0.66	24.67±0.45	19.67±0.40h	84.52
	3.1×10^{-5}	TU	50.00±1,15	40.66±0.60	18.27±0.49ef	71.38
		UT	44.00±1.73	37.00±0.67	18.43±0.26ef	72.88
		TT	35.00±1.15	31.00±1.52	18.68±0.43g	75.23
	1.5×10^{-5}	TU	55.00±1.15	41.67±0.94	17.45±0.33e	63.69
		UT	49.67±1.20	43.00±1.64	17.88±0.36ef	67.72
		TT	40.00±1.73	31.34±1.04	18.00±0.15ef	68.85

Effect on mortality on *C chinensis*

The tested concentrations of spinosad revealed that mortality of *C. chinensis* was higher in 3rd generations than 2nd and 1st generations (Table 2). The concentrations were found to be potential causing mortality based on the contact compare to control. The average mortality was highest 19.67±0.40 at 6.2×10^{-5} ppm in third generation in treated male and treated female pair type group but lowest was 14.82±0.24 at 1.5×10^{-5} ppm in second generation in treated male and untreated female pair groups. After 1st generation the experiments were divided in three pair groups. Results shows that the first generation was not significant. There were significant differences in mean mortality pair type groups between exposure generation and between concentrations. In addition, the interaction between exposure generation and concentration were significant (Table 2).

Table 3: Effects of spinosad on progeny productions of *C. chinensis* of the F_1 generation (Mean ± SE)

Concentrations ppm/µml	No. of eggs	Hatch	
		Male	Female
Cont.	90.67±. 0.66c	41.33±.088b	39.67±0.88c
6.2×10^{-5}	72.33±1.20a	32.67±0.88a	30.33±0.33a
3.1×10^{-5}	79.67±0.88b	38.33±1.76b	34.00±0.57b
1.5×10^{-5}	86.33±0.88c	40.33±1.20b	39.00±0.57c
In a column means followed by same letter (s) are not significantly different at $p < 0.05$ according to Tukey HSD Test.			

Table 4: Effects of spinosad on progeny productions of *C. chinensis* of the F_2 generation

Concentrations ppm/µml	Treated male and untreated female			Untreated male and treated female			Treated male and treated female		
	No. of egg	Hatch		No. of egg	Hatch		No. of egg	Hatch	
		Male	Female		Male	Female		Male	Female
Control	90.67 ± 0.66c	41.33 ±0.88b	39.67 ±0.88c	90.67 ± 0.66c	41.33 ±0.88c	39.67 ±0.88c	90.67 ±0.66d	41.33 ±0.88b	39.67 ±0.88c
6.2×10- 5	52.67 ±1.20a	27.67 ±1.45a	21.67 ±0.66a	48.33 ±0.88a	21.00 ±0.57a	16.33 ±0.57a	39.00 ±1.00a	21.00 ±0.57a	16.00 ±0.57a
3.1×10-5	59.67 ±0.88b	30.00 ±1.15a	24.67 ±0.33b	52.67 ±1.20a	22.00 ±0.57a	21.00 ±0.57b	45.67 ±1.20b	22.00 ±0.57a	18.33 ±0.88ab
1.5×10-5	61.33 ±0.88b	31.00 ±0.57a	24.00 ±0.57b	60.00 ±1.15b	30.33 ±0.33b	21.33 ±1.20b	51.33 ±0.88c	24.33 ±1.76a	21.00 ±0.57b
In a column means followed by same letter (s) are not significantly different at $p < 0.05$ according to Tukey HSD Test.									

Table 5: Effect of spinosad on progeny productions of *C. chinensis* of the F_3 generation

Concentrations ppm/µml	Treated male and untreated female			Untreated male and treated female			Treated male and treated female		
	No. of egg	Hatch		No. of egg	Hatch		No. of egg	Hatch	
		Male	Female		Male	Female		Male	Female
Control	90.67 ± 0.66c	41.33 ±0.88b	39.67 ±0.88b	90.67 ±0.66d	41.33 ±.088c	39.67 ±0.88c	90.67 ±0.66c	41.33 ±0.88c	39.67 ±0.88b
6.2×10- 5	46.67 ±0.88a	18.67 ±0.88a	16.67 ±0.88a	36.33 ±0.88a	16.33 ±0.66a	14.00 ±1.15a	29.33 ±0.66a	13.67 ±0.33a	11.00 ±0.57a

3.1×10-5	50.00 ±1,15a	22.33 ±0.33a	18.3 ±30.8a	44.00 ±1.73b	20.67 ±1.20ab	16.33 ±0.33ab	35.00 ±1.15b	19.00 ±1.52b	12.00 ±1.52a
1.5×10-5	55.00 ±1.15b	22.00 ±1.00a	19.67 ±0.88a	49.67 ±1.20c	24.67 ±2.40b	18.33 ±0.88b	40.00 ±1.73b	19.67 ±0.88b	11.67 ±1.20a
In a column means followed by same letter (s) are not significantly different at $p < 0.05$ according to Tukey HSD Test.									

Effect of sex ratio of *C. chinensis* in three successive generations:

The results of sex ratio of 1st generation of *C. chinensis* in mated condition with different concentrations of spinosad. These ratio represented from female to male. It is striking to note that the number of progeny production is always lower in higher concentration 6.2×10^{-5} compared to the number of progeny produced in lower concentrations 3.1×10^{-5} and1.5×10^{-5}.

It is found that, in 1st generation of *C. chinensis* progeny production of male and females were very same ratio. It was also showed very similar results in the control concentration. After 1st generation, the experiments were divided into three pair groups. It is also obvious that the TT pair type of *C. chinensis* always higher than other two pairs in the tested concentrations. In second (Table 3) and third generations (Table 5), female progeny production was lower than male progeny production. After generations to generations different concentrations of spinosad and different pair types of both sexes was decreased remarkably. The highest concentration 6.2×10^{-5}, in the TT pair type there was very low female progeny production of *C. chinensis* and it also noticed that other pair types of sex ratio were very remarkable. These results were so significant than the control.

In 1st generation, the female or male ratio was recorded as 1.21 to 1 of *C. chinensis* in the highest concentration of spinosad. The ratios were 1.11 to 1 and 1.05 to 1 respectively in other two 3.1×10^{-5} and 1.5×10^{-5} concentrations. In 2nd generation, the highest concentrations of TU, UT and TT pair groups, the sex ratio were 1.27 to 1, 128 to 1 and 1.31 to 1 respectively. In 3.1×10^{-5} dose, the sex ratio of *C. chinensis* is 1.21 to 1, 1.04 to 1 and 1.20 to 1 respectively TU, UT and TT pair groups. In 1.5×10^{-5} dose, the sex ratio of the same beetles were 1.29 to 1, 1.34 to 1 and 1.68 to 1 respectively TU, UT and TT pair groups. Finally in 3rd generation the highest doses and similar pair groups the sex ratio were 1.11to 1, 1.16 to 1 and 1.24 to 1 respectively. These showed very significant results because the progeny produced were reduced generation to generations. These sex ratio was not much difference from among the concentrations and among pair groups.

4. Discussion

The present experiments revealed that there was significant impacts of the contact activity of different concentrations of spinosad and exposure generations against egg hatchability of *C. chinensis.* Egg hatchability, fertility and mortality were found to be dependent on the concentrations and successive generations. Similar effects of spinosad and other extraction have been reported on other stored product insects (Tabu *et al.* 2012, Ratnasekera and Rajapakse 2012 and Fatiha *et al.* 2014).

The biopesticide Spinosad controls many insect pests of stored-food products reported Niango *et al.* (2010). Laboratory and field trials were carried out to determine the efficacy of this pesticide against *Callosobruchus maculatus*, the main storage pest of cowpea, *Vigna unguiculata*, Walp, in West Africa. Spinosad caused high mortality of adult *C. maculatus* and decreased the number of eggs laid by females. Spinosad, however, was less toxic in the 24 h treatment to *C. maculatus* than deltamethrin, an insecticide commonly used in Burkina Faso to control this insect. In "on-farm" experiments, Spinosad was effective in controlling *C. maculatus*. After 6 months of storage, the number of insects emerging from cowpeas seeds was reduced by >80% by coating seeds with Spinosad but only by 43% by coating with deltamethrin. Less than 20% of the seeds were perforated in the Spinosad treatment compared with 29% for deltamethrin. Spinosad controlled *C. maculatus* throughout the 6 mo of cowpea storage whereas deltamethrin failed to control *C. maculatus* after 3 mo of storage. Spinosad has the potential to be more effective in controlling *C. maculatus* than deltamethrin. The present results are similar to the above findings.

Spinosad's performance was evaluated on four classes of wheat by Fang *et al.* (2002) (hard red winter, hard red spring, soft red winter, and durum wheats) against adults *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst) and larvae of *Plodia interpunctella* (Hübner). Beetle adults (25) or *P. interpunctella* eggs (50) were exposed to untreated wheat and wheat treated with spinosad at 0.1 and 1 mg (AI)/kg of grain. On all untreated wheat classes, adult beetle mortality ranged from 0 to 6%, and *P. interpunctella* larval mortality ranged from 10 to 19%. The effects of spinosad on *R. dominica* and *P. interpunctella* were consistent across all wheat classes. Spinosad killed all exposed *R. dominica* adults and significantly suppressed progeny production (84–100%) and kernel damage (66–100%) at both rates compared with untreated wheat. Spinosad was extremely effective against *P. interpunctella* on all wheat classes at 1 mg/kg, based on larval mortality (97.6–99.6%), suppression of egg-to-adult emergence (93–100%), and kernel damage (95–100%), relative to similar effects on untreated wheats. The effects of spinosad on *S. oryzae* varied among wheat classes and between spinosad rates. Spinosad was effective against *S. oryzae, O. surinamensis* and *T. castaneum* only on durum wheat at 1 mg/kg. Our results suggest spinosad to be a potential grain protectant for *R. dominica* and *P. interpunctella* management in stored wheat.

In another study Spinosad performance were recorded against *Plodia interpunctella* (Hübner), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.), and *Cryptolestes ferrugineus* (Stephens), winter wheat stored on farms in northeastern Kansas by Huang *et al.* (2004). Fifty eggs of *P. interpunctella* and 25 beetle adults of each species were exposed to 100 g of untreated wheat or wheat treated with various rates of spinosad, to determine susceptibility of the field and corresponding insecticide-susceptible laboratory strains. Mortality of beetle adults and *P. interpunctella* larvae was assessed after 7 and 21 d post infestation, respectively. Field strains of *P. interpunctella*, *C. ferrugineus*, and *T. castaneum* were less susceptible to spinosad than the corresponding laboratory strains. The LD_{50} and LD_{95} values for *P. interpunctella* and *C. ferrugineus* field strains were 1.7–2.5 times greater than values for corresponding laboratory strains. Adults of both laboratory and field strains of *T. castaneum* were tolerant to spinosad, resulting in <88% mortality at 8 mg/kg. The LD_{50} and LD_{95} values for the field strains of *T. castaneum* were 2.0–7.5 times greater compared with similar values for the laboratory strain. The field and laboratory strains of *R. dominica* were highly susceptible to spinosad, and one of the field strains was relatively less susceptible to spinosad than the laboratory strain. Our results confirm a range of biological variability in field populations, which is consistent with findings for other compounds, and underscores the need to adopt resistance management programs with stored grain insect pests. The baseline data generated on the susceptibility of the four insect species to spinosad will be useful for monitoring resistance development and for setting field rates.

Chakraborty and Mondal (2014) observed the hatching of eggs laid by*C. chinensis* was 92% and 95.5% in two successive generations in cowpea. The increment of the egg laying percentage shows that cowpea is preferable host for deposition of more eggs. Tabu *et al.* (2012) observed the mean average percentage of eggs hatched were 91.6 % with a range of 91 to 93% which was in close agreement with the report of Teshome (1991), who reported that the average egg hatchability of *C. chinensis* on chickpea was 89.6% with a range of 83 to 96.4%.

The edible oil of *G. abyssinica*, *B. juncea* and *L. usitatissimum* (at the highest concentration) showed significantly the highest mortality when compared to the untreated check. It was even effective at the lowest concentration although the efficacy is gradual in killing the adult bruchids. In general, these results suggested that it is possible to control *C. chinensis* effectively by using edible oils though there appeared variation of efficacy due to concentration and exposure period. According to Inge (2004), oil may also kill the insect eggs. When the egg is already present at the surface of the seed or inside the seed, the oil coating prevents gaseous exchanges. So the larvae inside the egg or the kernel will die due to lack of oxygen. Abraham (2003) compared oils of maize, sunflower, *G. abyssinica* and *A. indica* against the maize weevil at Bako, Ethiopia. He further obtained effective control of the maize weevil with all oils at the rates ranging from 5 to 10 ml kg-1. Similarly, Talekar (1976) also examined that, coating seeds with 5-10 ml of vegetable oils per kg of seeds, protect stored seeds from bruchid infestation. Mixing of soya bean or ground nut oil at the rate of 2-3 ml/100 g seeds gave mung bean considerable in bruchid infestation for up to two months.

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