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RESEARCH PAPER

Evaluation of Garlic Products for Protection of Chickpea Seeds against Pulse Beetle *Callosobruchus chinensis* (L.)

Arulraj and Ram Singh*

Department of Entomology, CCS Haryana Agricultural University, Hisar, Haryana, India

*Corresponding author: ramjood2@gmail.com (**ORCID ID:** 0000-0002-9616-6042)

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ABSTRACT

Callosobruchus chinensis (L.) is most destructive pest of chickpea in storage. Among the botanicles, garlic *Allium sativum* (L.) has been observed as promising insecticide in stores. The garlic products/components were evaluated for their efficacy and persistence against pulse beetle *Callosobruchus chinensis* (L.) in chickpea in the Department of Entomology, CCS Haryana Agricultural University, Hisar. The garlic products were mixed with 250g chickpea seeds separately in a round bottom flask (2-liter capacity). There were total of 13 treatments *viz*, garlic oil (0.5 & 1.5 %), methanol, hexane, chloroform and acetone extracts (2 & 3%), garlic powder (2 & 4 %) and, control (treated with acetone alone). The treated seeds (250 g) of each treatment were kept in separate glass jars. Ten pairs of freshly emerged pulse beetles (0 to 24 h old) released in each glass jar. Percent seed damage, number of holes, number of eggs laid and percent egg reduction recorded at monthly interval by drawing a sample of 100 seeds randomly from each treatment in three replications. Among all the treatments, Only garlic oil 1.5% protected the chickpea seeds up to 6 months. In this treatment no seed damage, holes or egg laying were observed up to end of the experiment. Whereas garlic oil 0.5% and methanol extract 3% were effective up to two months. Rest of the treatments were less effective even after one month of treatment.

HIGHLIGHTS

- Garlic oil (0.5 & 1.5%), powder (2 & 4%) and extracts of methanol, Chloroform, hexane and acetone (2 & 3%) were evaluated for protection of chickpea seeds against pulse beetle in storage for 6 months.
- Garlic oil (1.5) proved effective for 6 months in the control of beetle but garlic oil at 0.5% and methanol extract at 3% were effective only for 2 months.
- Seed damage, number of eggs and exit holes per seed were good parameters for assessing efficacy and persistence of insecticidal properties.

Keywords: Garlic oil, Extracts, Chickpea seeds, Pulse beetle, Storage, Persistance

Chickpea is the most important pulse crop worldwide. In India, loss of chickpea seeds in post-harvest storage due to bruchids has been estimated around 8.5% (Agrawal *et al*. 1988), thus is essential to protect the grains from insect-pests during storage. *Callosobruchus chinensis* (L.) is most destructive pest of chickpea in storage. Currently the measure to control pest infestation in grains rely heavily upon the use of synthetic insecticides which have been found very effective for the purpose but these are hazardous due to their residue in food, health hazards and a risk of environmental contamination (White and Leesch, 1995; Pandey and Singh, 1997).

The use of plant materials, vegetable oils and essential oils has acquired an important approach for control of pests of stored grains (Pandey *et al*.

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1976; Rajapakse *et al*. 1997 and Singh *et al*. 2001). Various spices have been used traditionally for protecting food stuff from insects (Chopra *et al*. 1965). Among these spices, garlic *Allium sativum* (L.) has been discovered to act as an insecticide, repellent, antifeedant, bactericide, fungicide and nematicide (Grainge and Ahmed, 1988; Mason and Kinz, 1997). Reena and Singh (2003) reviewed insecticidal properties of garlic and reported wider use and acceptability of garlic as protectant against storage pests in several cereal and pulse crops. The effectiveness and persistence of garlic products/components as protectants need extensive investigations (Dodia *et al*. 2008). Keeping in view garlic products such as oil, powder and various extracts were evaluated for their efficacy and persistence against pulse beetle *Callosobruchus chinensis* (L.) in chickpea.

MATERIALS AND METHODS

Procurement and Extraction

The certified seed of chickpea variety 'HC-5' was procured from the Directorate of Farms, CCS Haryana Agricultural University, Hisar and used in different experiments.

The bulbs of garlic (local variety) were crushed using pestle and mortar. The crushed bulbs (one kg) were taken in a round bottom flask (5-liter capacity) and the methanol was added to it in a volume of 2.5 liter. Refluxing was done by fitting the flask with a water condenser and boiling the set using a heating mantle for 6h. The extract was then filtered out of the flask and was concentrated by distillation process. This refluxing and distillation procedure was repeated thrice. Likewise, 20 Kg (5 Kg each) of garlic were extracted using solvents *viz*, methanol, hexane, chloroform, acetone to obtain 20g of methanol, hexane, chloroform and acetone extracts, respectively. These extracts were prepared in the chemistry laboratory, Department of Biochemistry, College of Basic Sciences and Humanities, CCS HAU, Hisar. The garlic oil and garlic powder procured from local market of Hisar (S.K. Products, L-442, Shastri Nagar, Meerut)

Mixing with garlic products

The garlic products (garlic oil, extracts, garlic powder) were mixed with chickpea seeds in a round bottom flask (2-liter capacity). There were total of 13 treatments *viz*, garlic oil (0.5 & 1.5 %), methanol, hexane, chloroform and acetone extracts (2 & 3%), garlic powder (2 & 4 %) and, control (treated with acetone alone)

The required doses of garlic products were mixed with 250 g of chickpea seeds by shaking it manually for five minutes after closing the open end of the flask with a piece of butter paper. This was done to ensure uniform adherence of garlic products with the seeds. The seeds were transferred in the glass jars. Each treatment was replicated three times. Three samples of seeds, 250 g each treated with solvent (acetone alone) were kept as control.

Persistence of garlic products and extracts against pulse beetle

In this part of experiment, the persistence of garlic products and extracts was observed up to six months in various treatments of garlic products and extracts mixed with 250g of chickpea seeds kept separately in different jars. Ten pairs of (0-1 day old) beetles were released in each treatment. After monthly intervals 100 seeds were drawn from each treatment to record number of eggs, number of exit holes and percent damage (Aslam *et al*. 2004).

$$Percent\ damage = \frac{\text{Number of seeds with hole}}{\text{Total number of seeds}} \times 100$$

$$Egg\ reduction\ (\%) = \frac{\text{Eggs in control} - \text{Eggs in treatment}}{\text{Eggs in control}}$$

Statistical analysis

The data were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) to determine critical difference (CD) among treatments. The differences were evaluated at 5% level of significance. Where ever appropriate, data were transformed to angular values or square root transformation (Snedecor and Cochran, 1996).

RESULTS AND DISCUSSION

Persistence action of garlic products and extracts against pulse beetle

1. Effect on seed damage

The seed damage caused by pulse beetle at different

intervals of treatment is presented in Table 1. After one month of storage, no seed damage was observed in garlic oil 0.5% and 1.5% treated seeds. Only 0.3% seed damage was observed in methanol extract 3% treated seeds which was followed by methanol extract 2% (1.0%). Maximum (13.3%) seed damage was recorded in garlic powder 2% treated seeds followed by acetone extract 2% (9.6%) whereas in control 18.6% seed damage was noticed. All the treatments were significantly effective as compared to control. After two months of storage, no seed damage was observed in garlic oil 1.5% treated seeds. Minimum (5.7%) seed damage was observed in garlic oil 0.5% treated seeds followed by methanol extract 3 % (9.6%). Maximum (42.6%) damage was recorded in garlic powder 2% treated seeds followed by acetone extract 2% (42.3%) whereas in the control it was (46.8%).

After 3 months also, no seed damage was observed in garlic oil 1.5% treated seeds. Minimum (20.3%) seed damage was observed in garlic oil 0.5% treated seeds, followed by methanol extract 3 % (27.0%) whereas in control 86% seed damage was noticed.

After four, five and six months of storage, no seed damage was observed in garlic oil (1.5%) treated seeds whereas other treatments were ineffective. The results of present study indicate that the garlic oil 1.5% was significantly effective than other treatments followed by garlic oil 0.5%. Among the extracts, methanol extract 3% was most effective as compared to other treatments. The garlic oil 1.5% completely protected the chickpea seeds for six months, in remaining treatments damage increased with time. After three months of storage both concentration of acetone extract and garlic powder completely fail to protect the seeds from pulse beetle damage. According to Tunwar and Singh (1988), if there is more than 1% visible seed damage, the seed

Table 1: Effect of garlic oil, extracts and garlic powder on seed damage by *Callosobruchus chinensis* (Linnaeus) in chickpea at monthly intervals.

Treatment	Conc (%) v/w	Seed damage (%) at monthly interval after release						
		1	2	3	4	5	6	Mean
Garlic oil	0.5	0.0 (5.7)	5.3 (13.2)	20.3 (26.7)	53.3 (46.8)	68.3 (55.7)	82.3 (65.1)	38.9 (36.9)
Garlic oil	1.5	0.0 (5.7)	0.0 (5.7)	0.0 (5.7)	0.0 (5.7)	0.0 (5.7)	0.0 (5.7)	0.0 (5.7)
Methanol extract	2	1.0 (6.5)	12.3 (20.4)	27.0 (31.2)	64.0 (53.2)	80.6 (63.9)	92.3 (74.2)	46.4 (41.6)
Methanol extract	3	0.3 (5.9)	9.6 (18.0)	27.0 (31.2)	53.3 (46.8)	71.0 (57.4)	88.3 (70.1)	40.8 (37.5)
Hexane extract	2	2.6 (9.2)	37.3 (37.6)	68.3 (55.8)	92.3 (73.9)	100 (90.0)	100 (90.0)	66.7 (59.4)
Hexane extract	3	2.3 (8.7)	28.6 (32.3)	61.3 (51.5)	84.3 (66.6)	100 (90.0)	100 (90.0)	62.7 (56.5)
Chloroform extract	2	2.0 (7.9)	34 (35.6)	65.3 (53.9)	88.3 (70.1)	100 (90.0)	100 (90.0)	64.9 (57.9)
Chloroform extract	3	1.3 (6.5)	13.3 (21.3)	34.6 (36.0)	64.6 (53.5)	100 (90.0)	100 (90.0)	52.3 (49.5)
Acetone extract	2	4.3 (11.2)	42.3 (40.5)	72.3 (58.2)	100 (90.0)	100 (90.0)	100 (90.0)	69.8 (63.3)
Acetone extract	3	4.3 (11.2)	28 (31.9)	57.6 (49.3)	100 (90.0)	100 (90.0)	100 (90.0)	64.9 (61.5)
Garlic Powder	2	13.3 (21.3)	42.6 (40.8)	84 (66.4)	100 (90.0)	100 (90.0)	100 (90.0)	73.3 (66.3)
Garlic Powder	4	9.6 (18.0)	39.6 (39.0)	74.6 (59.7)	100 (90.0)	100 (90.0)	100 (90.0)	70.6 (63.4)
Control (acetone treated)	0	18.6 (25.5)	46.3 (42.8)	86.3 (68.4)	100 (90.0)	100 (90.0)	100 (90.0)	75.2 (67.7)
Mean		4.5 (11.0)	26.0 (29.2)	52.2 (45.7)	76.9 (66.6)	86.1 (76.3)	89.4 (80.0)	
SE (m) ±		(1.4)	(0.8)	(1.2)	(0.9)	(0.9)	(1.6)	
C.D. at 5%		(4.3)	(2.4)	(3.5)	(2.8)	(2.8)	(5.3)	
Factors	DF	SE(m) ±	C.D. at 5%					
Period (months)	5	(0.27)	(0.77)					
Treatment	12	(0.41)	(1.1)					
Period (months) x Treatment	60	(1.0)	(2.8)					

Figures in the parentheses are angular transformation (X+1) values.

lot is to be rejected. Therefore, the garlic oil 0.5 and 1.5%, methanol extract 2 and 3% treatments were below the permissible limit whereas the rest of the treatments were above the permissible limits.

In terms of human and animal consumption, 10% seed damage is acceptable (Aslam, 2004). Based on this the garlic oil 0.5 and 1.5%, methanol extract 3% were below 10% damage after two months of storage whereas the rest of the treatments were unsuitable for human consumption as well as animal consumption. The results of this study are in agreement with those of Jood *et al*. (1996) who reported that garlic powder 1 and 2% treated sorghum seeds protected from attack of *Trogoderma granarium* for three months after that damage increased with time. Present results are also in agreement with those of Pandey *et al*. (1976) who reported that the petroleum ether extract of garlic 3 and 5 parts gave complete protection up to 135 days against *Callosobruchus chinensis* on chickpea seeds.

2. Protection from seed holes

The data (Table 2) showed that after one month of storage no hole was observed in both concentration of garlic oil while minimum number of holes (0.6) was observed in methanol extract 3% which was followed by methanol extract 2% (1.0) whereas maximum number of (13.3) holes observed in garlic powder 2% followed by garlic powder 4% (9.6) and in control it was (18.0) holes. After two months of storage, no hole was observed in garlic oil 1.5%. Minimum number of holes (5.3) was observed in garlic oil 0.5% treatment which was followed by methanol extract 3% (9.6) treatment whereas maximum number of holes (114.0) was observed in garlic powder 2% followed by garlic powder 4% (79.3) and in control it was (117.6) holes. There were significant differences found among the treatments and periods.

Table 2: Effect of garlic oil, extracts, garlic powder on number of holes produced by *Callosobruchus chinensis* (Linnaeus) on chickpea seeds at different intervals

Treatment	Conc (%) v/w	Mean number of holes produced by *Callosobruchus chinensis* (Linnaeus) months after release						
		1	2	3	4	5	6	Mean
Garlic oil	0.5	0.0 (1.0)	5.3 (2.5)	20.3 (4.7)	123.3 (11.1)	320 (17.9)	396.0 (19.9)	358.0 (10.1)
Garlic oil	1.5	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0 .0 (1.0)	0.0 (1.0)	0.0 (1.0)
Methanol extract	2	1.0 (1.8)	12.3 (3.7)	72.6 (8.6)	136 (11.7)	374.6 (18.9)	426.3 (20.6)	400.4 (10.9)
Methanol extract	3	0.6 (1.2)	9.6 (3.9)	55.3 (7.5)	128.6 (11.2)	356.6 (19.2)	418.3 (20.4)	387.4 (10.2)
Hexane extract	2	2.6 (1.9)	70.6 (8.4)	175 (13.2)	375.6 (19.4)	413.6 (20.2)	413.6 (20.2)	413.6 (14.1)
Hexane extract	3	2.0 (1.7)	57 (7.6)	111.3 (10.5)	244.3 (15.6)	417.3 (20.4)	417.3 (20.4)	417.3 (14.0)
Chloroform extract	2	2.3 (1.8)	76.6 (8.8)	173 (13.1)	390.6 (19.7)	410.6 (20.2)	410.6 (20.2)	410.6 (12.8)
Chloroform extract	3	1.3 (1.5)	13.3 (3.7)	97.0 (9.8)	223.6 (14.9)	400 (20.0)	400.0 (20.0)	400 (11.7)
Acetone extract	2	4.3 (2.2)	102.6 (10.1)	189 (13.7)	422.6 (20.5)	422.6 (20.5)	422.6 (20.5)	422.6 (14.6)
Acetone extract	3	4.3 (2.2)	59.3 (7.7)	174.6 (13.2)	410.0 (20.2)	410.0 (20.2)	410.0 (20.2)	410.0 (14.1)
Garlic Powder	2	13.3 (3.7)	114 (10.7)	191.6 (13.8)	424.0 (20.6)	424.0 (20.6)	424.0 (20.6)	424.0 (14.7)
Garlic Powder	4	9.6 (3.2)	79.3 (8.9)	182.6 (13.5)	419.0 (20.5)	419.0 (20.5)	419.0 (20.5)	419.0 (14.2)
Control (acetone treated)	2	18.0 (4.4)	117.6 (10.9)	467.3 (21.6)	470.0 (21.6)	470.0 (21.6)	470.0 (21.6)	470.0 (17.0)
Mean		4.5 (2.3)	55.1 (6.8)	146.8 (11.1)	303.6 (16.1)	372.1 (18.4)	386.7 (18.8)	
SE (m) ±		(0.20)	0.16)	(0.63)	(0.24)	(0.15)	(0.15)	
C.D. at 5%		(0.58)	(0.49)	(1.86)	(0.70)	(0.46)	(0.52)	
Factors	DF	SE(m) ±	C.D. at 5%					
Period (months)	5	(0.05)	(0.15)					
Treatment	12	(0.08)	(0.22)					
Period (months) × Treatment	60	(0.19)	(0.55)					

Figures in the parentheses are square root transformation values.

After three months of storage, no hole was observed in garlic oil 1.5% while minimum number of holes (20.3) was observed in garlic oil 0.5% which was followed by methanol extract 3% (55.3) whereas maximum number of holes (191.6) was observed in garlic powder 2% followed by garlic powder 4% (182.6) treatment and in control it was(467.3) holes. At this storage period only garlic oil 0.5 and 1.5 % treatments proved highly effective as compared to other treatments and control.

After four, five and six months of storage, no hole was observed on garlic oil 1.5% while other treatments failed to provide satisfactory protection from damage.

3. Effect on oviposition

After one month of storage, the number of eggs laid ranged from 0 to 124.2/100 seeds (Table 3) in different treatments as compared to control (716). No egg laying was observed in garlic oil 1.5 % treated seeds while minimum numbers of eggs (14.0) were observed in garlic oil 0.5% treated seeds which was followed by methanol extract 3% (31.6), whereas maximum (124.3) numbers of eggs were recorded in garlic powder 2% treated seeds followed by both concentration of acetone extract (116.6). All treatments were significantly differed from each other.

After two months of storage, there was no egg laying in garlic oil 1.5 % treated seeds. Minimum (146.3) numbers of eggs were observed in garlic oil 0.5% treated seeds followed by methanol extract 3% (197.0) whereas maximum (1832) numbers of eggs were recorded in garlic powder 2% treated seeds followed by acetone extract 2% and garlic powder 4% (1790), while in control 2049 eggs were noticed.

After three months of storage, there was no egg laying in garlic oil 1.5 % treated seeds whereas

Table 3: Effect of garlic oil, extracts and garlic powder on oviposition of *Callosobruchus chinensis* (Linnaeus) released on chickpea seeds at different intervals

Treatment	Conc (%) v/w	Mean number of eggs laid by *Callosobruchus chinensis* on chickpea seeds months after release						
		1	2	3	4	5	6	Mean
Garlic oil	0.5	14 (3.9)	146.3 (12.1)	367 (19.0)	1550.3 (39.3)	2611.3 (51.0)	2975 (53.1)	1273 (30.2)
Garlic oil	1.5	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
Methanol extract	2	41.6 (6.5)	218.3 (14.7)	1357.6 (36.8)	2343.6 (47.4)	3536 (59.4)	4069 (64.1)	1927 (38.6)
Methanol extract	3	31.6 (5.7)	197 (14.05)	970 (31.1)	2249.3 (50.3)	2760 (52.5)	3411 (58.7)	1729 (34.6)
Hexane extract	2	179.3 (13.4)	1276 (35.7)	2809.3 (52.9)	3559 (65.9)	4134.3 (64.2)	4134.3 (64.2)	2682 (48.9)
Hexane extract	3	52 (7.3)	724 (26.9)	2563 (50.6)	3466.3 (65.9)	4003.6 (63.2)	4003.6 (63.2)	2468 (48.0)
Chloroform extract	2	60 (7.8)	1029 (32.0)	1906.6 (43.60	3528.2 (59.6)	4026 (63.4)	4026 (63.4)	2429 (46.9)
Chloroform extract	3	38.6 (6.3)	213.6 (14.7)	1613 (40.1)	3439.6 (65.9)	3961 (62.9)	3961 (62.9)	2204 (41.0)
Acetone extract	2	116.6 (9.8)	1790 (42.2)	3021.2 (55.4)	4298.6 (65.9)	4298.6 (65.9)	4298.6 (65.9)	2970 (51.1)
Acetone extract	3	116.6 (10.8)	1721 (41.47)	2947 (54.2)	4348.6 (65.5)	4348.6 (65.5)	4348.6 (65.5)	2971 (48.2)
Garlic Powder	2	124.3 (11.2)	1832 (42.7)	3280 (56.3)	4370.3 (66.0)	4370.3 (66.0)	4370.3 (66.0)	3057 (50.6)
Garlic Powder	4	79.3 (8.9)	1790 (42.2)	3077.3 (55.8)	4367 (65.9)	4367 (65.9)	4367 (65.9)	3007 (50.3)
Control (acetone treated)	2	716 (26.1)	2049.6 (45.2)	4246.3 (65.1)	4370.4 (66.1)	4370.4 (66.1)	4370.4 (66.1)	3353 (55.7)
Mean		120.7 (9.1)	998.9 (28.1)	2265.6 (43.2)	3222.4 (55.2)	3599 (57.5)	3718 (58.5)	
SE (m) ±		(0.4)	(0.6)	(1.1)	(0.7)	(0.7)	(0.7)	
C.D. at 5%		(1.2)	(2.0)	(3.2)	(2.0)	(2.0)	(2.5)	
Factors	DF	SE(m) ±	C.D at 5%					
Period (months)	5	(0.20)	(0.58)					
Treatment	12	(0.30)	(0.86)					
Period (months) x Treatment	60	(0.75)	(2.1)					

Figures in the parentheses are square root transformation values.

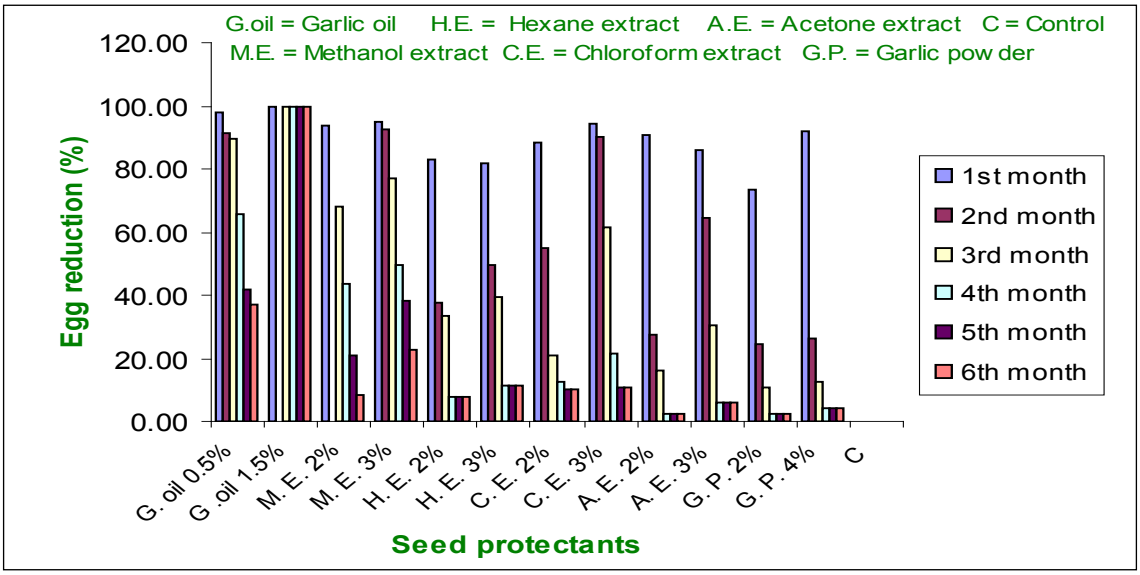

Fig. 1: Effect of garlic oil, extracts and garlic powder on (%) reduction of eggs laid by *Callosobruchus chinensis* (L.) in chickpea seeds at monthly interval

minimum (367) numbers of eggs were observed in garlic oil 0.5% treated seeds which was followed by methanol extract 3% (970). Maximum (3280) numbers of eggs were recorded on garlic powder 2% treated seeds followed by garlic powder 4% (3021), but in control it was 4246.3 eggs. After three months of storage, none of the treatment was significantly effective as compared to garlic oil 1.5% treatment. After four, five and six months of storage, only the garlic oil 1.5% treatment was significantly effective as compared to other treatments and control. Effect of garlic oil, powder and extracts on percentage reduction of egg laid by pulse beetle on chickpea at different intervals are presented in Fig. 1. After one month of storage, the garlic oil 1.5% treated seeds gave 100% reduction of egg laying over the control. Maximum (97.8%) egg reduction found in garlic oil 0.5% treated seeds, which was followed by methanol extract 3% treated seeds (95.2%), whereas minimum (82.8%) egg reduction found in garlic powder 2% treated seeds. All the treatments were significantly differed from each other. After two months of storage, the garlic oil 1.5% treated seeds showed 100% reduction of egg laying over the control. Maximum (91.2%) reduction found in garlic oil 0.5% treated seeds which was followed by methanol extract 3% treated seeds (92.8%) whereas minimum (24.6%) egg reduction found in garlic powder 2% treated seeds. After three months of storage, the garlic oil 1.5% treated seeds gave 100% egg reduction over the control. Maximum (89.5%) reduction found in garlic oil 0.5% treated seeds, which was followed by methanol extract 3% treated seeds (77.0%) whereas minimum (10.5%) egg reduction found in garlic powder 2% treated seeds. At this storage period only garlic oil 1.5% proved highly effective and garlic oil 0.5% up to some extent in reducing the number of eggs laid by pulse beetle. Thereafter, protection of seeds from beetle eggs remained of no significance except garlic oil 1.5% which protected grains up to 6 months.

The results of present studies indicate that garlic oil 1.5% was most effective treatment. Which completely protected the chickpea seeds from egg laying of pulse beetle up to six months, whereas garlic oil 0.5% effective up to three months. It showed that as the time increased reduction of egg laying decreased proportionately.

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