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Determination of residual effect of spinosad against the pulse beetle, *C. chinensis* L

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Abstract
The residual toxicity of spinosad against the pulse beetle, *Callosobruchus chinensis* L was evaluated under laboratory condition by treating chickpea seeds stored up to 6 weeks. Mortality, oviposition rate, number of adults emerged, percent seed infestation and weight loss was evaluated by releasing adults of *C. chinensis* after each week of storage. Mortality percentages at one and two weeks of storage periods indicated that the 1000 ppm spinosad possessed the highest residual effect on *C. chinensis* at 96 hours after insect release (HAIR) which remained statistically almost same up to five weeks of storage of chickpea and 76.67% was found after six weeks of storage which was statistically significant. Spinosad also exerted residual effects on oviposition rate, number of adults emerged, percent seed infestation and percent weight loss. It was found that the residual effect of spinosad decreased with the increase of storage period. Considering the above findings, it can be concluded that spinosad could be suggested for the management of *C. chinensis* in storage.

Keywords: biopesticide, toxicity, chickpea, mortality, oviposition

1. Introduction

Pulse serves as one of the main inexpensive sources of dietary protein (20-40%) (Sharma, 1984) [26] and is considered as “poor man’s meat” for the under privileged people who cannot afford animal protein (Bahalla *et al*., 2008) [3] and also provide carbohydrates, vitamins and minerals and does not contain any anti-nutritional component (Chakraborty and Mondal, 2015) [4]. Pulse is attacked by several insect pests including pulse beetle, *Callosobruchus chinensis* L. and it is major pest in storage (Ahmed *et al*., 2003) [2]. A large number of pulses are grown in Bangladesh and after harvesting, about 85% of the pulse growers in Bangladesh store pulses throughout the year in their houses (Ferdowsi, 2013) [11]. The post-harvest seed losses due to the beetle can reach even up to 100% during severe infestation (Srinivasan *et al*., 2010) [29].

About 55-60% seed weight loss and 45.5-66.30% loss of protein content of the chickpea seeds due to damage by pulse beetle and pulse seeds become unfit for human consumption as well as for planting and the severity of damage increases with the duration of storage (Gujar and Yadav, 1978) [14]. Tiwari *et al.* (2012) [32] reported that the weight losses caused by pulse beetle among different pulses (cowpea, green gram, black gram, bengal gram and red gram) ranged from 1.97 to 4.53%. A single larva of *C. chinensis* can destroy several mature seeds. Severe infestation leads to 100% damage thus leaving the seed coat. *C. chinensis* causes 50.37-57.58% grain content loss of mungbean in storage in Bangladesh (Ali *et al.*, 1999) [1].

At present pest control measures in storage rely on liquid insecticides and gases in the form of phosphine and methyl bromide have been widely used as it is cost-effective and simple method (Shaaya *et al.*, 1997; Fields and White, 2002; Lee *et al*., 2004; Islam *et al.*, 2010) [27, 12, 21, 18]. In recent years, spinosad is an eco-friendly biorational insecticide derived from naturally occurring soil actinomycete, *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae) has been found to be effective against stored pests (Vayias *et al*., 2009; Hertlein *et al*., 2011) [35, 15]. The US Environmental Protection Agency has classified spinosad as a reduced risk insecticide due to its low effective use rate and safety to the environment and mammals. Moreover, spinosad gives excellent residual control, which makes it an ideal protectant for stored grain commodities (Fang *et al*., 2002b; Fang and Subramanyam, 2003) [9, 10]. In addition, spinosad has a favorable environmental profile; it is not known to leach, bioaccumulate or volatilize (Thompson *et al.*, 2000; West *et al.*, 2000; Williams *et al*., 2003) [33, 37, 38], and also has no known carcinogenic, teratogenic or mutagenic effects on vertebrates (Legocki *et al*., 2010) [22]. It is currently registered in over sixty countries and is applied to over 200 crop species with 180 in the U.S. alone. It is predicted that the spinosad will be used more widely in many countries for the management of storage pests (Vayias *et al.,* 2009) [37]. So, considering the above situations, the present research was undertaken to study the residual effects of spinosad against the pulse beetle, *C. chinensis* under laboratory condition.

2. Materials and Methods

2.1 Experimental site and design

The experiment was conducted at the laboratory of Department of Entomology, Bangladesh Agricultural University (BAU), Mymensingh. The experiment was laid out in Completely Randomized Design (CRD) having at least four treatments and was conducted from March 2015 to May 2016.

2.2 Collection of seeds

Fresh and disease-free chickpea (*Cicer arietinum* L.) seeds were used as host for the pulse beetles and purchased from the local ‘K & R Market’ of BAU campus, Mymensingh.

2.3 Collection of pulse beetle

Pulse beetle, *C. chinensis* L. was used as test insect in this study. The insects were collected from a stock culture of the Entomology Department, BAU as well as from Entomology Division of Bangladesh Institute of Nuclear Agriculture (BINA) and then reared in the laboratory as per the following protocol. The average temperature and relative humidity during the study were 27±3°C and 72±3%, respectively.

2.4 Mass culture of pulse beetle

Pulse beetle was mass reared in glass jar and in plastic box also. Approximately 100 adults of the insect were released in each box containing 500 g of chickpea seeds and the mouth being closed with net for proper ventilation. The beetles were allowed for free mating followed by oviposition for a maximum period of 10 days. After oviposition, the beetles were separated from the seeds through sieving and seeds along with eggs were left in the containers to maintain a stock culture of the test insect. The stock culture of test insect was maintained throughout the experimental period and this process was repeated regularly to get sufficient number of adult beetles for conducting the experiments.

2.5 Test biopesticide

Libsen 45SC was used as the source of spinosad. Spinosad is a natural insecticide synthesized by the soil bacterium, *Saccharopolyspora spinosa* Mertz and Yao (Thompson *et al.*, 1997) [33]. Spinosad is relatively fast acting. It has contact activity on all life stages of a pest including egg, larva and adult. The test insect dies within one to two days after ingesting the active ingredient. Spinosad does not persist in the environment. Sunlight and soil microbes break it down into carbon, hydrogen, oxygen and nitrogen. It is classified as an organic substance by the USDA National Organic Standards Board (USDA, 2002) [34].

2.6 Preparation of the serial solutions

Different concentrations of spinosad were considered as experimental treatment. To prepare them, at first 100 ml 10,000 ppm of spinosad was prepared as first stock solution by adding 2.22 ml spinosad with 97.78 ml distilled water. Then, 100 ml 1000 ppm of spinosad as second stock solution (10.00 ml from 1st stock solution + 90.00 ml dH_2O) was prepared. Then the experimental treatments were prepared by taking required amount from second stock solution for each experiment as shown below.

Table 1: Preparation of spinosad serial dilutions

Treatments	Concentration (in ppm*)	Amount of spinosad (ml/20ml in distilled water)	Amount of distilled water
T_1 (Control)	0	0	20
T_2	100	2	18
T_3	500	10	10
T_4	1000	20	0

(*ppm = parts per million)

2.7 Experimental procedures

In this experiment, the residual effect of spinosad was determined to identify how long the spinosad treatment could provide protection of pulses from the infestation of pulse beetle, *C. chinensis*.

2.8 Determination of residual effect of spinosad against the pulse beetle, *C. chinensis* L.

In this experiment, the biotoxicity of spinosad was evaluated through bioassay procedure. Residual toxicity of spinosad was studied by using three concentrations of spinosad (100, 500 and 1000 ppm) along with control were selected and prepared as per required. A total of three kg of chickpea seeds (one kg in each box) was used in three different plastic boxes and the seeds were treated with 20 ml of 100, 500 and 1000 ppm of spinosad, mixed properly by shaking and kept them in laboratory at room temperature to evaluate residual effects. From these treated chickpea seeds, 50 g from each concentration were transferred to three different Petri-dishes and one control was maintained. The experiment was replicated for three times. Five pair of newly emerged adults were released in chickpea seeds treated with spinosad after one week of storage period in each Petri-dish, kept in the laboratory for recording of data Similarly, the procedures were repeated for 2, 3, 4, 5 and 6 weeks of storage after treatment and data on the same parameters were collected.

2.9 Data collection and calculation

2.9.1 Percent mortality

Insect mortality data were recorded at 24, 48, 72 and 96 hrs of post release. Insects that were immobile or unable to move were considered as dead insect. The percent mortality was determined as per following formula:

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

2.10 Oviposition rate

Number of eggs laid by the released beetles, termed as rate of oviposition was recorded after 10 days of post release.

2.11 Adult emergence

The emerged *C. chinensis* adults were counted daily from the beginning of the first insect emergence started at 20 days after post insect release and continued up to 3 weeks as per criteria described by Ouedraogo *et al.* (1996) [23].

2.12 Percent seed infestation

The data was recorded at 25 and 45 days after insect release (DAIR). Then the percent seed infestation was calculated according to the following formula stated by Enobakhare and Law-Ogbomo (2002) [7].

$$\text{Percent grain damage} = \frac{\text{Number of bored seeds}}{\text{Total no. of seeds observed}} \text{ X } 100$$

2.13 Percent seed weight loss

Data recorded at 45 DAIR and then the percent weight loss was measured using the following formula as stated by Lal (1988) [20].

$$\text{Percent weight loss} = \frac{\text{Weight loss per Petri-dish}}{\text{Initial weight of seed grains per Petri-dish}} \text{ X } 100$$

Weight loss per Petri-dish = (Initial weight- final weight) of grains per Petri-dish.

2.14 Percent inhibition

The inhibition of oviposition rate, adult emergence, seed infestation and seed weight loss was computed by using the following formula as stated by Shukla *et al.* (2007) [28].

$$\text{Percent inhibition} = \frac{\text{Control mean} - \text{Treatment mean}}{\text{Control mean}} \times 100$$

2.15 Statistical analysis

All collected data were analyzed in Completely Randomized Design (CRD) by using Analysis of Variance (ANOVA) with two factors. The treatment means were separated by using the Duncan's Multiple Range Test (DMRT) (Duncan, 1951) [6] and Least Significant Difference (LSD). Data analysis was carried out using the computer package MSTAT-C and graphical works through Microsoft Excel program in a computer.

3. Results and discussion

3.1 Residual effect of spinosad against the pulse beetle, *C. chinensis* L.

The effect of residual toxicity of spinosad against pulse beetle, *C. chinensis* was examined by releasing five pair of insects at different storage periods viz., 1, 2, 3, 4, 5 and 6 weeks of chickpea seeds treated with spinosad by indirect application method. The results are described at the following sections.

3.2 Residual effect of spinosad on percent mortality of pulse beetle, *C. chinensis*

Average mortality percentage of *C. chinensis* at 24, 48, 72 and 96 hours after release (HAIR) indicated that the highest residual activity of spinosad was observed at maximum dose (1000 ppm) (Table 2). An increasing trend of mortality with the progress of hours after insect release was observed. On the other hand, the mortality of *C. chinensis* showed a gradually decreasing trend with increasing storage period (Table 3). The lowest mortality was found after six weeks of storage period of chickpea and it was 15.83, 29.17, 38.33 and 60.00% at 24, 48, 72 and 96 HAIR, respectively. While maximum mortality was observed after one week of storage period and it was estimated 27.50, 60.83 and 78.33 percent at 24, 72 and 96 HAIR, respectively (Table 3). This indicated that the residual effect of spinosad gradually reduced over storage time.

Table 2: Mean mortality percentages of pulse beetle, *C. chinensis* treated with different doses of spinosad by indirect application method at different HAIR (Interaction of doses and observation time)

Dose (ppm)	Percent Mortality			
	24 HAIR*	48 HAIR	72 HAIR	96 HAIR
Control	10.56b	17.78d	27.78c	40.00c
100	16.67b	30.00c	49.44b	68.33b
500	27.22a	46.67b	62.22a	75.56b
1000	31.67a	59.44a	72.78a	88.89a
LSD	8.941	9.996	11.25	11.25
Level of significance	0.01	0.01	0.01	0.01
CV (%)	18.96	11.86	9.68	7.53

*HAIR=Hours after insect release, Different letters in a column differ significantly

Table 3: Effect of different storage periods of chickpea seeds treated with spinosad by indirect application method on the mortality of pulse beetle, *C. chinensis* at different HAIR (Interaction of storage periods and observation time)

Storage period	Percent Mortality			
	24 HAIR*	48 HAIR	72 HAIR	96 HAIR
1 Week	27.50a	44.17ab	60.83a	78.33a
2 Week	21.67ab	37.50abc	55.00ab	73.33ab
3 Week	22.50ab	35.00bc	48.33bc	66.67bc
4 Week	22.50ab	39.17ab	55.83ab	62.50bc
5 Week	19.17ab	45.83a	60.00a	68.33abc
6 Week	15.83b	29.17c	38.33c	60.00c
LSD	8.941	9.996	11.25	11.25
Level of significance	0.01	0.01	0.01	0.01
CV (%)	18.96	11.86	9.68	7.53

*HAIR=Hours after insect release, Different letters in a column differ significantly

Table 4: Mean mortality percentages of pulse beetle, *C. chinensis* treated with different doses of spinosad with different storage periods by indirect application method at different HAIR (Interaction of storage periods, doses and observation time)

Storage period	Dose (ppm)	Percent mortality			
		24 HAIR*	48 HAIR	72 HAIR	96 HAIR
1 Week	Control	13.33gh	16.67h	26.67j	40.00i
	100	20.00efg	30.00g	53.33fgh	83.33bcd
	500	33.33bc	53.33cd	76.67abc	93.33ab
	1000	43.33a	76.67a	86.67a	96.67a
2 Week	Control	10.00h	20.00h	30.00j	36.67i
	100	16.67fgh	33.33fg	53.33fhg	76.67def
	500	20.00efg	36.67fg	63.33def	83.33bcd
	1000	40.00ab	60.00bc	73.33bcd	96.67a
3 Week	Control	10.00h	16.67h	26.67j	43.33i
	100	16.67fgh	30.00g	43.33hi	63.33gh
	500	30.00cd	40.00ef	50.00gh	70.00efg
	1000	33.33bc	53.33cd	73.33bcd	90.00abc
4 Week	Control	10.00h	16.67h	26.67j	40.00i
	100	23.33def	30.00g	53.33fgh	56.67h
	500	30.00cd	46.67ef	66.67cde	66.67fgh

	1000	26.67cde	63.33cd	76.67abc	86.67abcd
5 Week	Control	10.00h	16.67h	26.67j	40.00j
	100	13.33gh	36.67fg	60.00efg	66.67fgh
	500	26.67cde	63.33b	73.33bcd	80.00cde
	1000	26.67cde	66.67b	80.00ab	86.67abcd
6 Week	Control	10.00h	20.00h	30.00j	40.00i
	100	10.00h	20.00h	33.33ij	60.00gh
	500	23.33def	40.00ef	43.33hi	63.33gh
	1000	20.00efg	36.67fg	46.67h	76.67def
LSD		8.941	9.996	11.25	11.25
Level of significance		0.01	0.01	0.01	0.01
CV (%)		18.96	11.86	9.68	7.53

*HAIR=Hours after insect release, Different letters in a column differ significantly

The interaction effects of storage period, dose and observation time on mortality were presented in table 7 varied significantly (P≤0.01). Mortality percentages at 1 and 2 weeks of storage periods indicated that the spinosad 1000 ppm possessed the highest residual effect on *C. chinensis* after 96 HAIR which remained statistically almost same up to 5 weeks of storage of chickpea and 76.67% was found after 6 weeks of storage which was statistically significant. The effect of 100 and 500 ppm of spinosad on mean mortality percentages of *C. chinensis* showed gradual decrease. For example, the mortality percentages of 100 ppm spinosad was 20.00, 16.67, 16.67, 23.33, 13.33, 10.00 for 1, 2, 3, 4, 5 and 6 weeks of storage periods, respectively (Table 4).

The above findings are supported by Hussain *et al.* (2009) [17], Huang *et al.* (2007) [16] and Khashaveh *et al*. (2011) [19] also observed and reported that at 2 days exposure interval; mortality was 61.66 and 100% at five days on chickpea. Chintzoglou *et al*. (2008) [5] stated that the difference of insect mortality in spinosad treated commodities may be due to the speed of pesticide degradation.

3.3 Residual effect of spinosad on oviposition rate of *C. chinensis*

The mean number of eggs laid by *C. chinensis* on the chickpea seeds in different treatments ranged from 17.97 to 160.56 per 250 seeds and differed significantly (P≤0.01). A decreasing trend in oviposition rate of *C. chinensis* with the increasing doses of spinosad was noticed. Among the treatments, the highest number of eggs was recorded in control (160.56) and the lowest (17.97) was found on the chickpea seeds treated with 1000 ppm spinosad. The inhibition on oviposition rate was increased from 66.01 to 88.81% comparing to control and maximum inhibition was recorded (88.81%) in 1000 ppm dose over control (Table 5). On the other hand, a gradual but steady increase in the oviposition rate of *C. chinensis* was observed with increasing storage periods and varied significantly (P≤0.01) (Table 6). The minimum (50.96) number of eggs was recorded when insect released after one week of storage which was statistically similar to that of two weeks after storage where 54.05 eggs were recorded. The maximum number of eggs (83.92) was found when insects were released after six weeks of storage which was statistically almost similar to eggs deposited at five weeks after storage (81.10) (Table 6). This indicated that the residual toxicity of spinosad was gradually decreased over time and hence the treated seeds of chickpea were preferred by the pulse beetle for oviposition.

In the present experiment, the mean number of eggs per 250 seeds at different storage periods varied significantly (P≤0.01) when combined with different doses of spinosad (Table 7). Number of eggs was zero at 1 week of storage period which was statistically almost similar with that of 2 weeks (7.87) of storage periods indicated that 1000 ppm spinosad possessed the highest toxicity effect on oviposition of *C. chinensis* followed by 3 (21.72), 4 (21.39), 5 (24.51) and 6 (32.33) weeks after storage. In 100 ppm and 500 ppm, the mean number of eggs of *C. chinensis* showed gradual increase with the advancement of storage periods. For example, mean number of eggs of 100 ppm spinosad was 28.22, 32.52, 50.78, 66.51, 72.67 and 76.78 per 250 seeds of chickpea for 1, 2, 3, 4, 5 and 6 week of storage periods, respectively (Table 7). The inhibition of oviposition rate was found 80.17% after 6 weeks of storage after treatment in 1000 ppm that was 100% at 1 week after storage (Table 7).

3.4 Residual effect of spinosad on adult emergence of *C. chinensis*

The mean number of adults emerged from different treatments ranged from 11.10 to 85.67 and those differed significantly (P≤0.01) (Table 5). The highest mean number of adults emerged (85.67) in control while the lowest number of adults emerged (11.10) in 1000 ppm of spinosad (Table 5). 34.95 adults were observed in 100 ppm. On the other hand, a gradual increase in mean adult number with the increasing storage periods similar to oviposition rate was observed (Table 6). The minimum number of adults (26.46) was counted after 1 week of storage which was statistically similar to adults emerged at 2 weeks (31.50). The maximum number of emerged adults (48.75) was found at 6 weeks after storage which was statistically almost similar to that of 5 (46.78) and 4 (44.54) weeks after storage (Table 6).

Again, the combined effects of storage periods and doses on mean number of adults emerged differed significantly (P≤0.01) and presented in table 7. Residual insecticidal effect was highest in 1000 ppm spinosad at 1 and 2 week of storage when adults obtained was 0.0 and 5.56 per 250 seeds, respectively. Statistically same number of adults was observed after 3, 4, 5 and 6 weeks of storage in case of 1000 ppm spinosad. Adults obtained in 100 and 500 ppm was statistically different and a gradual increase was observed. For example, in 100 ppm mean number of adults per 250 seeds after 1, 2, 3, 4, 5 and 6 week after storage was 15.70, 23.44, 38.73, 43.28, 44.11 and 44.43, respectively (Table 7). Inhibition of adult emergence was found 78.83% after 6 weeks of storage after treatment in 1000 ppm that was 100% at 1 week after storage (Table 7).

Table 5: Mean oviposition rate, adult emergence and inhibition rate (%) influenced by different doses of spinosad against pulse beetle, *C. chinensis* by indirect application method

Dose (ppm)	Mean no. of eggs / 250 seeds	Inhibition rate (%)	Mean no. of adults emerged	Inhibition rate (%)	Seed infestation rate (%)	Inhibition rate (%)	Seed weight loss at 45 DAIR* (%)	Inhibition rate (%)
Control	160.56a	-	85.67a	-	60.88a	-	4.35a	-
100	54.58b	66.01	34.95b	59.20	17.42b	71.39	1.15b	73.56
500	41.13c	74.38	27.41c	68.01	11.88c	80.49	0.74c	82.99
1000	17.97d	88.81	11.10d	87.04	5.09d	91.64	0.31d	92.87
LSD	8.493		6.897		2.594		0.1385	
Level of Significance	0.01		0.01		0.01		0.01	
CV (%)	5.66		7.92		4.97		3.77	

Different letters in a column differ significantly

It was found that mean number of eggs and adults emerged was increased with increasing storage periods after treatment but decreased with increasing doses of spinosad and residual toxicity remained up to 6 weeks of storage after treatment. These findings are supported by Fang *et al*. (2002a, b) [8, 9] who reported that spinosad at 1 mg/kg completely suppressed progeny production of *C. ferrugineus* and *R. dominica*. On the other hand, Flinn *et al*. (2004) [13] reported that the presence of low densities of *T. castaneum* adults in spinosad treated grain bins could be due to high immature mortality so that none of the eggs laid survived to become adults. Sanon *et al.* (2010) [25] found that the number of insects emerging from cowpea seeds was reduced 80% by coating seeds with spinosad after 6 months of storage.

Table 6: Effect of different storage period of chickpea treated with spinosad by indirect application method on oviposition and adult emergence of pulse beetle, *C. chinensis*

Storage period	Mean no. of eggs/250 seeds	Mean no. of adults emerged	Seed infestation rate (%)	Seed weight loss at 45 DAIR (%)
1 Week	50.96d	26.46c	16.10 e	1.23 e
2 Week	54.05d	31.50c	18.87 d	1.32 e
3 Week	65.96c	40.65b	22.34 c	1.51 d
4 Week	75.36b	44.54ab	26.70 b	1.70 c
5 Week	81.10ab	46.78ab	26.56 b	1.93 b
6 Week	83.92a	48.75a	32.34 a	2.14 a
LSD value	8.493	6.897	2.594	0.1385
Level of Significance	0.01	0.01	0.01	0.01
CV (%)	5.66	7.92	4.97	3.77

Different letters in a column differ significantly

Table 7: Mean no. of eggs and adult emergence of pulse beetle, *C. chinensis* treated with different doses of spinosad with different storage periods by indirect application method

Storage period	Dose (ppm)	Mean no. of eggs /250 seeds	Inhibition rate (%)	Mean no. of adults emerged	Inhibition rate (%)	Seed infestation rate (%)	Inhibition rate (%)	Seed weight loss at 45 DAIR (%)	Inhibition rate (%)
1 Week	Control	166.70a	-	85.00a	-	55.09d	-	4.35a	-
	100	28.22ghi	83.07	15.70g	81.53	7.06kl	87.18	0.42gh	90.34
	500	8.97j	94.62	5.16h	93.93	2.24m	95.93	0.13i	97.01
	1000	0.00k	100	0.00h	100	0.00m	100	0.00i	100
2 Week	Control	155.0b	-	82.33a	-	60.17c	-	4.34a	-
	100	32.52gh	79.02	23.44ef	71.53	8.13k	86.49	0.53g	87.79
	500	20.82i	86.57	14.66g	82.19	5.21l	91.35	0.32h	92.63
	1000	7.87jk	94.92	5.56h	93.25	1.97m	96.73	0.07i	98.39
3 Week	Control	158.0b	-	85.33a	-	62.90b	-	4.34a	-
	100	50.78f	67.86	38.73bc	54.61	12.70ij	79.81	0.80ef	81.57
	500	33.33g	78.91	25.83de	69.73	8.33k	86.76	0.53g	87.79
	1000	21.72i	86.25	12.71g	85.10	5.43l	91.37	0.35h	91.94
4 Week	Control	162.0ab	-	87.33a	-	65.81a	-	4.34a	-
	100	66.51de	58.94	43.28b	50.44	21.34g	67.57	1.27d	70.74
	500	51.54f	68.19	32.53cd	62.75	14.31i	78.26	0.86e	80.18
	1000	21.39i	86.80	15.02g	82.80	5.35l	91.87	0.33h	92.40
5 Week	Control	158.7ab	-	87.33a	-	56.16d	-	4.29a	-
	100	72.67cd	54.21	44.11b	49.49	25.24f	55.06	1.77c	58.74
	500	68.54cde	56.81	40.71b	53.38	18.73h	66.65	1.23d	71.33
	1000	24.51hi	84.56	14.97g	82.86	6.13kl	89.08	0.43gh	89.98
6 Week	Control	163.0ab	-	86.67a	-	65.18ab	-	4.41a	-
	100	76.78c	52.90	44.43b	48.74	30.07e	53.87	2.13b	51.70
	500	63.55e	61.01	45.57b	47.42	22.44g	65.57	1.33d	69.84
	1000	32.33gh	80.17	18.35fg	78.83	11.67j	82.10	0.70f	84.13
LSD value		8.493		6.897		2.594		0.13085	
Level of Significance		0.01		0.01		0.01		0.01	
CV (%)		5.66		7.92		4.97		3.77	

Different letters in a column differ significantly

3.5 Residual effect of spinosad on seed infestation rate (%) caused by *C. chinensis*

The percentage of damaged seeds differed significantly (P≤0.01) among the treatments and varied from 5.09 to 60.88%. The highest seed infestation was recorded in control (60.88%), which differed significantly from other treatments and the lowest seed infestation was recorded when seeds were treated with 1000 ppm spinosad (5.09%). The percent infestation in 500 ppm was recorded 11.88% followed by 100 ppm (17.42%) of spinosad. Spinosad treatment inhibited the seed infestation according to its concentration and inhibition was found increased from 71.39 to 91.64% (Table 5).

The duration of storage periods had influence in seed infestation rate which ranged from 16.10% after 1 week of storage to 32.34% after 6 weeks of storage (Table 6). Infestation rate after 2, 3, 4 and 5 weeks of storage was found 18.87, 22.34, 26.70 and 26.56%, respectively. It was observed that seed infestation (%) increased gradually with progressive storage periods (Table 6). On the other hand, the interaction effect of storage periods and different doses on seed infestation was found significantly different (P≤0.01). The results indicates that residual effect was highest in 1000 ppm after 1 week of storage which was statistically similar with 2 weeks of storage when infestation was recorded 0.0 and 1.97%, respectively (Table 7). There was a gradual increase in seed infestation with the increase in storage periods and it was 11.67% after 6 weeks of storage. Seed infestation was higher in 100 ppm than 1000 and 500 ppm which also followed similar trend. For example, in 100 ppm spinosad seed infestation was recorded 7.06, 8.129, 12.70, 21.34, 25.24 and 30.07% after 1, 2, 3, 4, 5 and 6 weeks of storage, respectively. Inhibition of seed infestation was found 82.10% after 6 weeks of storage after treatment in 1000 ppm that was 100% at 1 week after storage (Table 7).

3.6 Residual effect of spinosad on seed weight loss (%) caused by *C. chinensis*

The highest seed weight loss was recorded in control (4.35%), which differed significantly (P≤0.01) from other treatments and the lowest seed infestation was recorded when seeds were treated with 1000 ppm spinosad (0.31%). The seeds treated with 100 and 500 ppm showed 1.15% and 0.74% weight losses, respectively which were statistically significant. Inhibition in seed weight loss was observed increasing with the increasing doses. The inhibition of seed weight loss was increased from 73.56 to 92.87% and maximum inhibition was registered 92.87% in 1000 ppm (Table 5).

On the other hand, weight loss due to insect infestation at different storage periods after treatment showed a gradual increasing trend (Table 6). The weight loss of seed when insect released after different storage periods was found significant and it ranged from 1.23 to 2.14%. The highest seed weight loss (2.14%) was recorded when insect were released at 6 weeks after storage which differed significantly from other storage periods and the lowest weight loss (1.23%) was recorded when insect were released after 1 week of storage with spinosad which was statistically similar to 2 weeks (1.32%) (Table 6).

The interaction effect of storage periods after treatment, different doses on seed weight loss (%) presented in table 14 and varied significantly (P≤0.01). Here, the highest residual toxicity was found in 1000 ppm at 1 week after storage which remained statistically similar to 2 weeks after storage. Weight loss in 1000 ppm spinosad when insect released at 1, 2, 3, 4, 5 and 6 weeks after storage was recorded 0.00, 0.07, 0.35, 0.33, 0.43 and 0.70%, respectively. Weight gradient was found too low with increasing storage period in 1000 ppm treatment. In 100 ppm treated seed, weight loss when insect released after 1, 2, 3, 4, 5 and 6 weeks of storage was observed 0.42, 0.53, 0.80, 1.27, 1.77 and 2.13%, respectively. The inhibition rate of seed weight loss was found 84.13% after 6 weeks of storage in 1000 ppm that was 100% at 1 week after storage (Table 7).

From the present study, it was found that with the increase of spinosad concentration seed infestation and seed weight loss were decreased due to the residual toxicity of spinosad.

Similar trend also observed by Vishwamitra *et al*. (2014) [36] who reported 0.7% damaged grain and 1.24% seed weight loss when treated with spinosad 45SC at 0.2ml/kg seed. Khashaveh *et al*. (2011) [19] also stated that spinosad controlled the emerged adults and recorded less damaged grains (0.71%), the lowest weight loss (1.24%). The present report indicates the toxicity of spinosad to pulse beetle, *C. chinensis* and also supported by the reports of Subramanyam *et al*. (2002, 2003) [30, 31], Sadat and Asghar (2006) [24], Vayias *et al*. (2009) [35] and Hertlein *et al*. (2011) [15] who reported the toxicity of spinosad to coleopteran stored-grain pests.

4. Conclusion

The highest residual activity of spinosad was observed at maximum doses (1000 ppm). A gradual decrease in mortality was noticed with the progressive storage periods of chickpea. Again, the mortality percentages at 1 and 2 weeks of storage periods indicated that the 1000 ppm spinosad possessed the highest residual effect on *C. chinensis* at 96 HAIR (96.67% mortality) which remained statistically almost same up to 5 weeks and 76.67% was found after 6 weeks of storage of chickpea which was statistically significant while minimum was recorded in untreated seeds. Residual toxicity tests against *C. chinensis* also revealed that minimum number of eggs, adults, seed infestation (%) and seed weight loss (%) was found in 1000 ppm. From the results of the present research, it could be concluded that biotoxicity of spinosad was increased significantly with the increasing doses and duration of exposure. Again, residual toxicity decreased with the increase of interval between biopesticide mixing and insect release after different storage periods. But even at 6 weeks of storage after treatment the biopesticide gave good result in all the spheres of investigation. Therefore, spinosad might be an alternative, safe and eco-friendly tactic for the management of pulse beetle.

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