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Contact and gustatory effects of Spinosad on inhibition of population build-up of *Cryptolestes pusillus* (Schon.) (Coleoptera: Cucujidae) after different storage periods in wheat

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Abstract

The adult population of *Cryptolestes pusillus* (Schon.) was significantly (P<0.001) reduced after 3-, 6- and 9-months storage periods at different concentrations of Spinosad than those of control medium. Spinosad at all concentrations reduced mean number of larval 5.33±0.72 to 52.00±0.94, 5.67±1.36 to 32.00±1.41 and 2.67±0.54 to 54.33±0.72 after 3, pupal 3.33±1.09 to 26.00±0.94, 3.33±0.72 to 26.00±1.25 and 2.33±0.29 to 16.33±0.27 after 6 and adult 5.33±1.19 to 62.00±0.47, 2.33±0.27 to 72.00±1.25 and 2.33±1.09 to 58.00±1.25 after 9 months storage period. The percent of repulsion (PRC) were always higher in higher concentrations but lower in lower concentrations in every cases. The total population of *C. pusillus* was 152.00±1.15 and 790.33±2.60 after 1st and 2.67±1.09 and 20.67±3.34 after 2nd generations. Spinosad effectively reduced the survivability and population of *C. pusillus* of the treated wheat.

Keywords: Population inhibition; Spinosad; *Cryptolestes pusillus*; Wheat

1. Introduction

Control of stored product insects is considered the best achieved through an integration of physical, chemical, and biological methods (Hagstrum *et al*. 1999, Phillips and Thorne 2010). In this context in storage facilities, where light is absent the bacterial insecticide Spinosad could be a potential agent; and was found to be remain stable for a long period, thus it can provide long-term protection for stored grains (Fang and Subramanyam 2003, Arthur *et al*. 2006, Hertlein *et al*. 2011). Spinosad, a reduced-risk commercial insecticide based on the fermentation products of an actinomycete bacterium, has been labeled for use on over 250 crops in more than 50 countries (Mertz and Yao, 1990; Thompson *et al*., 2000). Spinosad has low mammalian toxicity and degrades quickly when exposed to sunlight (Thompson *et al*., 2000), but it was relatively stable in stored-grain (Fang *et al*., 2002b; Flinn *et al*., 2004). Spinosad acts on the nicotinic acetylcholine receptors and this mode of action is unique among other known insecticides (Thompson *et al*., 2000). These benign properties make it an ideal product for use in stored grain. Laboratory and field tests on stored wheat showed that spinosad at 1 (a.i.) mg/ kg of grain was effective against several insect pests including the lesser grain borer *Rhyzopertha dominica* (F.), rusty grain beetles *Cryptolestes ferrugineus* (Stephens), and Indian meal moth *Plodia inter punctella* (Hübner) (Fang *et al*., 2002a, 2002b; Flinn *et al*., 2004; Huang *et al*., 2004).

A liquid formulation of spinosad (SpinTor® 2SC) containing 240 (a.i.) mg/ mL was obtained from Dow Agro Sciences (Indianapolis, Indiana, USA). Insecticide was diluted in distilled water to make solutions of different concentrations for grain treatment. In 2005, the United States Environmental Protection Agency registered spinosad at 1 mg/kg as a grain protectant on commodities including wheat, corn, rice, millets, oats, sorghum, and barley (Bruggink, 2005).

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Cryptolestes pusillus is a coleopteran pest which destroy stored grain. It is commonly known as flat grain beetle. The beetle is an external feeder and a serious cosmopolitan pest of stored product commodities especially cracked grain (Baker 1976). It multiplies rapidly and subsequently build up into a huge population within very short period of time (Rahman *et al*. 2009).

The present study was therefore, planned to study the efficacy of Spinosad on the population build -up of *C. pusillus* at different storage periods.

2. Material and methods

2.1. Insects

Ten adults of *C. pusillus* were taken from the stock culture maintained five years in Control Temperature Room (CT Room) and kept in 500 ml beakers. Wheat was soaked with different concentrations (1.25, 0.63, 0.32, 0.16 and 0.08µg/ml) of Spinosad separately and dried at room temperature for 24h. fifty gram of wheat grains were taken and soaked with one of the Spinosad concentration from each beaker. A separate batch of control was maintained. Thirty (30) unsexed adult beetles of 10-15 days old were introduced into beaker and covered with muslin cloth by the help of rubber bands. Control batch was simultaneously running. Every after 30 days additional 10 g uninfested wheat was added to each beaker. The experiment was conducted at CT room. Three replications were used for each Spinosad concentrations. Beetles were observed after 3-, 6- and 9-months exposure and the number of larva, pupa and adult were counted separately and only the dead ones were discarded.

All data were analyzed by Factorial ANOVA to compare mortality percentage as the respose variable and concentrations, life stages, exposure periods main effects. For the comparison of means the Tukey's test (1953) was used. The percent reduction of population to control (PRC) was calculated according to Mian and Mulla (1982a) by the following formula:

Percentage reduction in population

$$\text{PRC}=1\frac{Average\ no.of\ adult\ emergence\ (treatment)}{Average\ no.of\ adult\ emergence\ (Control)}\times 100$$

Where,
C= No. of population in control
T= No. of population in treated media

2.2. Preparation of concentration

The formulation of liquid spinosad was diluted in distilled water. By using a micropipette (maximum range 10 µl) 20.79µg spinosad was taken in 50 ml beaker. 6ml distilled water by using 2ml syringe (3 times) were added properly in it. For equal mixing of spinosad and water the vial was shaken vigorously. After that 1ml was taken off from the solution containing each 1 ml 3.47µg spinosad. This was the first concentration. The other spinosad concentrations were prepared by serial dilution by taking 1ml of solution and in each step 2ml distilled water was added. Then, the desired Spinosad concentrations as 3.47, 1.74 and 0.87µg were selected for toxicity as a mother dose of *C. pusillus* . Spinosad stock solutions for wheat and rice treatment were made in distilled water. This solution was preserved in refrigerator carefully.

Then prepared expected doses by serial dilution, by taking one ml of Spinosad in each step and adding 2 ml distilled water. Selected Doses were 1.25, 0.63, 0.32, 0.16 and 0.08µg/ ml.

All experiments were conducted under laboratory conditions at CT room.

3. Results and discussion

Different gustatory action of Spinosad concentrations drastically decreased the number of larvae of *C. pusillus* after 3 months of storage periods (Table 1). It was noticed that larval population was significantly reduced in all concentrations of Spinosad. Mean number of larval populations ranged from 5.33±0.72 to 52.00±0.94 after 3 months of storage but in control in was 60.00±0.47. It was due to problem of space accumulation for increased number of larvae after 3 months storage. The PRC values were noted 13.33 to 91.11. Significant effect of mean larval population was noted ($F= 225.236$ $df= 5$, $P<0.001$).

The number of pupae decreased according to dose dependent. In higher doses it was recorded 3.33±1.09 in higher doses (1.25µg/ml) but 26.00±0.94 in lower doses (0.08µg/ml. The PRC values were 33.90 to 91.53. Significant differences were noticed among concentrations (F= 105.209, df=5, P<0.001.

Table 1 Effect of Spinosad on PRC value of different concentrations on different life stages of *C. pusillus* after 3 months of storage condition (N=30)

Concentrations (µg/ml)	Average no. of life stages					
	Larvae	**PRC**	**Pupae**	**PRC**	**Adults**	**PRC**
Control	180 (60.00±0.47)a	-	118 (39.33±0.72)a	-	348 (116.00±1.70)a	-
0.08	156 (52.00±0.94)b	13.33	78 (26.00±0.94)b	33.90	186 (62.00±0.47)b	46.55
0.16	120(40.00±1.25)c	33.33	60 (20.00±0.82)bc	49.15	102 (34.00±0.82)c	70.69
0.32	61(20.33±1.96)d	66.11	43 (14.33±1.09)c	63.56	78 (26.00±0.47)d	77.59
0.63	42 (14.00±1.25)e	76.67	23 (7.67±1.44)d	80.51	38 (12.67±0.54)e	89.08
1.25	16 (5.33±0.72)f	91.11	10 (3.33±1.09)d	91.53	16 (5.33±1.19)f	95.40

In a column means with same letter do not significantly differ from each other within doses at 0.05% level (Tuke's test).

Similarly, the adult population was increased in higher doses and decreased in lower doses.

Table 2 Effect of Spinosad on PRC vale of different concentrations on different life stages of *C. pusillus* after 6 months of storages condition (N=30)

Concentrations (µg/ml)	Average no. of life stages					
	Larvae	**PRC**	**Pupae**	**PRC**	**Adults**	**PRC**
Control	342 (114.00±0.47)a	-	150 (50.00±1.63)a	-	533 (177.67±1.44)a	-
0.08	96(32.00±1.41)b	71.93	78 (26.00±1.25)b	48.00	216 (72.00±1.25)b	59.47
0.16	90 (30.00±0.94)b	73.68	42 (14.00±0.94)c	72.00	132(44.00±0.47)c	75.28
0.32	42 (14.00±0.82)c	87.72	25 (8.33±0.72)cd	83.33	37 (12.33±0.98)d	93.06
0.63	25 (8.33±1.52)cd	92.69	17 (5.67±0.54)d	88.67	22(7.33±0.72)de	95.87
1.25	17 (5.67±1.36)d	95.03	10 (3.33±0.72)d	93.33	7(2.33±0.27)e	98.69

In a column means with same letter do not significantly differed from each other within doses at 0.05% level (Tuke's test).

Like 3 months record, the population of *C. pusillus* at larval, pupal and adult stages were effective on different concentrations after 6 months storage periods. The mean number of larvae was 5.67±1.36 to 32.00±1.41 in five doses. In higher doses lower number larvae was recorded but in lower doses higher number of larvae was counted. The PRC were 71.93 to 95.03. Significant effect of larval population was recorded (F=194.225 df= 5, P< 0.001).

The pupal population was also decreased in higher doses (1.25 µg/ ml) and increased in lower doses 0.08 µg/ ml). The PRC values were 48.00 to 93.33 in different doses. Pupal population was also significant (F=3.298, df =5, P<0.001).

The mean number of adult populations were found 2.33±0.27 to 72.00±1.25 in different concentrations. The PRC values were 59.47 to 98.69 in different storage periods. Significant results were noticed between concentration and storage periods.

Table 3 Effect of Spinosad on PRC value of different concentrations on different life stages of *C. pusillus* after 9 months of storages condition (N=30)

Concentrations (µg/ ml)	**Average no. of life stages**					
	Larvae	**PRC**	**Pupae**	**PRC**	**Adults**	**PRC**
Control	458 (152.67±1.44)a	-	216 (72.00±163)a	-	645 (215.00±1.41)a	-
0.08	163 (54.33±0.72)b	64.41	49 (16.33±0.27)b	77.31	174 (58.00±1.25)b	73.02
0.16	80 (26.67±0.72)c	82.53	24 (8.00±1.70)c	88.89	121 (40.33±0.72)c	81.24
0.32	37 (12.33±0.54)d	91.92	17 (5.67±0.27)c	92.13	37 (12.33±0.27)d	94.26
0.63	17 (5.67±0.27)e	96.29	9 (3.00±0.47)c	95.83	14 (4.67±0.54)e	97.83
1.25	8(2.67±0.54)e	98.25	7 (2.33±0.29)c	96.76	7 (2.33±1.09)e	98.91

In a column means with same letter do not significantly differed from each other within doses at 0.05% level (Tuke's test).

The Larval, pupal and adult populations of *C. pusillus* was effectively suppressed in different concentrations of Spinosad in 9 months storage periods (Table 3). In lower doses, highest mean number of larvae of *C. pussilus* (54.33±0.72) and in higher doses lowest number (2.67±0.54) was recorded. The PRC vales were 64.41 to 98.25. Significant effect of concentrations and storage periods were recorded.

The pupae were 2.33±0.29 to 16.33±0.27in higher and lower doses. The PRC values were 77.31 to 96.76. Pupae was effective in different doses as well as different storage periods.

Mean number of adults were recorded after 9 months storage period and tabulated in Table 3. The PRC values were 73.02 to 98.91.

Spinosad resulted in suppression of total population in *C. pusillus* after different storage periods are presented in Table 4. The number was positively related to storage periods. The mean total population in control was 1161.00±2.65 to 43.00±5.56 in first and second generation. In the treatment it was 152.00±1.15 to 790.33±2.60 in the 1st generation and to 2.67±1.09 to 20.67±3.34) in the 2nd generation. Analysis of variance revealed that highly significant effects of Spinosad on total population of *C. pusillus* was noted.

Spinosad at 0.08, 0.16, 0.32, 0.62 and 1.25µg/ ml concentrations possess higher insecticidal effect on larval, pupal and adult population of *C. pusillus* at 3-9 months storage. In all concentrations larval population was significantly reduced in all treatments compared to control. Hertlein *et al*. (2011) reported that Spinosad residues are extraordinarily stable on grains stored in bins, offering protection ranging 6 months to 2 years. Spinosad formulations were found to be extremely powerful against *Plodia interpunctella* and *Rhyzopertha dominica* allowing negligible larval survivability and adult emergence on wheat and maize (Subramanyam *et al*. 2012). These consequences are consistent with greater susceptibility of *P. interpunctella* eggs and larvae to a commercial Spinosad formulation as reported by Huang *et al*. (2004). Yousefnezhad-Irani and Asghar (2007) reported that younger larvae and adults of *Trobolium castaneum* were more susceptible to spinosad than the older larvae; adult being less prone and the first instars are incredibly susceptible (Towes and Subramanaym 2003). Daglish and Nayak (2006) reported the persistence and efficacy of Spinosad against *R. dominica* in wheat stored for 9 months. Their result support the results of the present investigation. Spinosad is an effective grain protectant and also have splendid impact on progeny reduction of stored grain insect pests. The present work almost similar.

Subramanyam *et al*. (2006) reported that Spinosad at 1 mg/kg provided fantastic control of adults of the *R. dominica*, *T. castaneum*, *C. ferrugineus*, *S. zeamais* and *P. interpunctella* for the duration of the six months to one year with the exception of *T. castaneum*, which was less prone; progeny production of all of the mentioned species was greatly suppressed. Fang et al. (@002a) revealed that *O. surnamensis* and *T. castaneum* adults were sensitive to Spinosad, exhibited excessive mortality on durum wheat. Sanon *et al*. (2010) stated that a dry Spinosad formulation implemented at 0.94 ppm to cowpeas provided up to six months of non-stop protection while these treated seeds were bagged in plastic and stored under traditional warehouse conditions in Burkina Faso. Control of *Callosobruchus maculatus* by Spinosad was better than that supplied by the commercial widespread pyrethroid, deltamethrin.

Nayak *et al*. (2005) pronounced that Spinosad was effective in controlling *Liposcelis. entomophila* and a psocid species, on wheat at 1ppm, but did not give successful result against the other related species.

Table 4 Effect of spinosad on the total population of *C. pusillus* in F1 and F2 generation in wheat

Food medium	Concentrations (µg/ml)	1st generation (42d period)			2nd generation (15d period)		
		Total no. of eggs laid Mean±SE	No. of eggs/day/ female	PRC	Total no. of eggs laid Mean±SE	No. of eggs/day/ female	PRC
Wheat	Control	3483 (1161.00±2.65)a	27.64	-	129 (43.00±5.56)a	2.87	-
	0.08	2371 (790.33±2.60)b	18.82	31.93	62(20.67±3.34)b	1.38	51.94
	0.16	1962 (654.00±2.31)c	15.57	43.67	44 (14.67±2.60)ab	0.98	65.89
	0.32	1258 (419.33±1.86)d	9.98	63.88	29(9.67±2.60)ab	0.64	77.52
	0.63	773(257.67±1.45)e	6.13	77.81	14 (4.67±0.72)ab	0.31	89.15
	1.25	456 (152.00±1.15)f	3.62	86.91	8 (2.67±1.09)c	0.18	93.80

In a column means with same letter do not significantly differ from each other within doses at 0.05% level (Tuke's test).

Overall, the present findings the investigation revealed that Spinosad has good potential properties against *C. pusillus* as target species of insect pest control programs.

4. Conclusion

The mentioned research concluded that, a reduced-risk bacterial insecticide was very effective in the suppression of *C. pusillus* in different storage periods. At concentration >1.25µl/ g resulted in 100% control of *C. pusillus* on wheat after 9 months storage periods. Therefore, in order to maximize the negative effects of the synthetic chemicals on the environment and natural enemies in the pest management programme, Spinosad could be integrated in IPM programme.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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