

## CONTACT AND GUSTATORY EFFECTS OF SPINOSAD ON THE SURVIVABILITY OF *SITOPHILUS ORYZAE* L. (COLEOPTERA: CURCULIONIDAE) IN WHEAT

Umme Habiba, W Islam\* and Selina Parween<sup>1</sup>

*Institute of Biological Sciences, University of Rajshahi,  
Rajshahi-6205, Bangladesh*

**Abstract:** The present study was planned to evaluate the effect of spinosad on the survivability and development of *Sitophilus oryzae* on four wheat varieties viz., BARI-26, BARI-28, Shatabdi-21 and Prodig-24. Three doses in three replications for spinosad were applied to four wheat varieties. Spinosad concentrations significantly increased the total developmental period compared to the control in a dose-dependent manner on four wheat varieties. The highest developmental period took  $41.67 \pm 0.33$  days to become adult was recorded in S-21 at  $0.0003 \mu\text{l/g}$  of spinosad in F<sub>1</sub>. All adults of F<sub>1</sub> did not reach in F<sub>2</sub> because surprisingly all adults died after emergence. So, no developmental period was found in S-21 ( $0.00 \pm 0.00$ ) and B-28 ( $0.00 \pm 0.00$ ) days at  $0.0003 \mu\text{l/g}$  spinosad in F<sub>2</sub>. On the other hand, five mated females were released on the treated wheat with different concentrations of spinosad for 10 - 15 days; then they were removed. Treated wheat was checked for up to 30 to 60 days and observed the progeny for two successive generations (1st and 2nd). Each combination of insect species, insecticide rate, and exposure duration were replicated three times. Among four wheat varieties, the lowest adult emergence was recorded as  $08.00 \pm 0.58$  in F<sub>1</sub> and totally controlled in F<sub>2</sub> generation in S-21 variety at  $0.0003 \mu\text{l/g}$ . Spinosad concentrations significantly increased the total developmental period compared to the control in a dose-dependent manner on four wheat varieties.

**Key words:** Contact and gustatory effects, spinosad, developmental period, *Sitophilus oryzae*

### INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important staple food used in many parts of the world and is a moderately salinity tolerance crop. Wheat grains are vulnerable to many insects in storage and insecticides are used as a most effective measure for protecting stored products from pest infestation. The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) has been reported as one of the severe pests of rice, sorghum, wheat, barley and other cereal grains and their products (Baloch 1992), is cosmopolitan in nature and causes intense losses in rice, maize, barley, wheat and other vegetation quantitatively and qualitatively throughout the world (Arannilewa *et al.* 2002, Tefera 2012). Both

\*Author for correspondence: <mwislam18@gmail.com>. <sup>1</sup>Department of Zoology, University of Rajshahi, Bangladesh.

the adults and larvae fed on the kernels leaving only the outer integument. The whole developmental stages of this pest passed in the grain (Banglapedia 2006, revised 2014). *S. oryzae* is an internal feeder. It is very harmful pest of stored wheat worldwide (Rees 2004). The larvae of *S. oryzae* can destroy 25 - 30% of the wheat kernel that reduces the market cost of wheat (Kadir et al. 2005). True proteins are definitely decreased as the insect feeds on both endosperm and embryo causing quantitative and qualitative damage (Prabhakumary and Sini 2008). Spinosad is an insecticide product from Dow Agro Sciences (Indianapolis, Indiana, USA) based on chemical compounds of a soil bacterium *Saccharopolyspora spinosa* was discovered in 1985 (Mertz and Yao 1990). This is aerobic, Gram-positive, nonacid-fast actinomycetes with fragmenting mycelium. Spinosad is a mixture of two spinosoid spinosyns A ( $C_{42}H_{67}NO_{16}$ ) is the major component and D ( $C_{41}H_{65}NO_{16}$ ) is the minor component, present in an approximately 85:15% ratio in the final product (Mertz and Yao 1990, Sparks et al. 1999). It is a naturally derived bio rational insecticide with an environmentally favorable toxicity profile (Bond et al. 2004). Spinosad degrades very quickly on soil surfaces by photolysis and below the soil surface by soil microorganisms (Saunders and Bret 1997, Thompson et al. 2000). It is classified as an environmentally and toxicologically reduced risk material by the U.S. Environmental Protection Agency (Cleveland et al. 2001). Its using rate is 1 mg (AI)/kg of grain, and the tolerance level was established at 1.5 mg (AI)/kg (Bruggink 2005).

Spinosad efficacy was observed in layer treated wheat against five stored product pest including *S. oryzae* (Athanasziou et al. 2009). The effect of short exposures to spinosad-treated wheat or maize was also evaluated against adults of four stored-product insect species including *S. oryzae* and in spinosad-treated grain progeny production of *S. oryzae* and *R. dominica* is directly related to the speed of death of adults (Athanasziou et al. 2010). Getchell and Subramanyam (2008) found the instantaneous mortality of *S. oryzae* adults on wheat, maize, and sorghum treated with spinosad at various exposure intervals.

The spinosad's overall performance against stored insect pests and their offspring production relies upon on several factors like formulation, commodity, temperature, application rate and insect species (Athanasziou et al. 2008a, b, 2009, 2010, 2011, Vayias et al. 2010). Therefore, the present investigation characterizes the effectiveness of spinosad against *S. oryzae* adult mortality and the survivability of adult's emergence and its developmental time in economically important stored-product grain insect, *S. oryzae* in two successive generations on four wheat varieties.

## MATERIAL AND METHODS

In the present bioassay method spinosad were investigated against adults of *Sitophilus oryzae* by dietary exposure termed Treated Food Method (TEM) (Talukder and Howse 1994).

*S. oryzae* was obtained from the stock culture without any exposure to insecticides, maintained in the control temperature room, at Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh. Untreated and infestation free of wheat varieties viz., BARI-26, BARI-28, Prodip-24 and Shatabdi-21 were used.

Mass cultures were maintained in plastic containers (2.5 litre). The whole culture procedure was maintained in a control temperature room at  $30 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  RH. About 250 adults of *S. oryzae* were placed in the plastic container with 250 g of whole wheat grain for 10 - 15 days, then they were removed and placed on fresh grains to get a new progeny and avoid generation overlapping. The whole process was repeated several times to ensure 100% adult collection from the old culture medium to obtain homogenous generations throughout the experiment. Mouth of the container was covered with muslin cloth using a rubber band, to prevent the possible contamination and escape of insects (Mondal and Parween 1997).

*Preparation of food medium for mass-culture:* Whole wheat grains were used as the food for the weevils. Wheat grains were collected from the local market, Shaheb Bazar, Rajshahi. After washing in water, the wheat was sun-dried and finally sterilized in an oven at  $60^\circ\text{C}$  for 6 hrs. Sterilized wheat grains kept for 15 days to allow its moisture content (13.5%) to equilibrate with that of the environments. Sterilized wheat was used as food for mass-culture.

*Preparation of food medium for experiments:* Four kinds of wheat grains (B-26, B-28, P-24 and S-21) were collected from the Wheat Research Institute, Shampur, Rajshahi, Bangladesh. These grains were washed and cleaned by sieving through 500 micrometer aperture sieve and sterilized in an oven at  $60^\circ\text{C}$  for 6 hrs. Then grains were kept in plastic containers (3 liters) that were cleaned before and use throughout the experimental period for *S. oryzae*.

*Collection of weevils:* After tremble the container some of the grain was taken on the working table with the help of a medium sized spoon. The adults were collected using a camel hair brush and placed in treatment.

*Source of spinosad:* Spinosad is light grey to white in colour with slight odour stale water. About 500ml of spinosad (PRN- MAPP-12054, cfno 20012-019, Lot No-3068404) was obtained from Dow Agro Sciences, UK. Concentration of spinosad was 120g spinosad/litre. Spinosad 0.0018  $\mu\text{l}$  was obtained in a glass

vial by the help of micropipette and added 6 ml distilled water properly by using 2ml syringe and 0.0003, 0.00015 and 0.000075 µl/g were prepared by serial dilution.

*Bioassays:* Wheat grains 1 g of each variety of wheat was soaked in different concentrations of spinosad separately and then dried at room temperature for 24 hrs in a 6 cm glass Petri dish.

From mass culture five mated females were released on the treated wheat with different concentrations of spinosad for 10 - 15 days; then they were removed. The Petri dish was covered and the medium containing eggs and larvae were placed in the control room temperature until adult emergence. Treated wheat was checked for up to 30 - 60 days and observed the progeny for two successive generations (1st and 2nd). After every 10 days, newly treated fresh wheat was added with it. A similar set of experiment was carried out on wheat soaked with distilled water only, as a control batch. The room temperature was maintained at  $30 \pm 1^\circ\text{C}$  with 75% RH in the control temperature throughout the study period. As, *S. oryzae* is an internal feeder so, only adults production for this species was recorded for F<sub>1</sub> and F<sub>2</sub>. Adults unable to move when prodded gently with a hair brush were considered dead. The number of progeny for *S. oryzae* was based on all visible live adults found in wheat. Each combination of insect species, insecticide rate, and exposure duration was replicated three times, and each replicate was treated separately. Adult emergence and adult survivability of *S. oryzae* was determined to untreated and treated wheat with spinosad concentration for two successive generations.

*Data collection and statistical analysis:* Data were subjected to analysis of variance using SPSS-20 version. Means comparisons were performed by Turkey's tests ( $p < 0.05$ ). The percent reduction of adult emergence in treatments compared to control (PRC) was calculated by using the formula provided by Mian and Mulla (1982a) as follows:

$$\text{PRC} = 1 - \frac{\text{Average no. of adult emerged (treatment)}}{\text{Average no. of adult emerged (control)}} \times 100$$

The mortality data were corrected using Abbott's formula (Abbott 1925) as follows:

$$\frac{P_o - P_c}{100 - P_c} \times 100$$

## RESULTS AND DISCUSSION

*Effect on adult recovery/emergence:* Effect of spinosad on the adult emergence of *S. oryzae* on four wheat varieties in  $F_1$  and  $F_2$  are presented in Table 1. No mortality was found in the untreated (control) wheat. In control, the adult emergence ranged from  $56.33 \pm 1.86$  to  $70.00 \pm 2.89$  in  $F_1$  whereas  $145.00 \pm 3.00$  to  $165.33 \pm 2.91$  in  $F_2$ . In treated the adult emergence ranged from  $08.00 \pm 0.58$  to  $49.33 \pm 2.33$  in  $F_1$  whereas  $0.00 \pm 0.00$  to  $29.67 \pm 2.60$  in  $F_2$ . Among four wheat varieties, the lowest adult emergence was recorded as  $08.00 \pm 0.58$  in  $F_1$  generation and totally controlled in  $F_2$  in S-21 variety at  $0.0003 \mu\text{l/g}$  concentration. The highest PRC value was 85.80% noted in S-21 variety in  $F_1$ , 100% (extremely controlled) observed in S-21 and B-28 varieties treated with  $0.0003 \mu\text{l/g}$  concentration.

ANOVA showed that highly significant differences among wheat varieties in  $F_1$  ( $F = 40.88$ ,  $df = 3$ ,  $p < 0.001$ ) and in  $F_2$  ( $F = 23.26$ ,  $df = 3$ ,  $p < 0.001$ ) generation and concentrations in  $F_1$  ( $F=403.09$ ,  $df=3$ ,  $p<0.001$ ) and in  $F_2$  ( $F = 5746.87$ ,  $df = 3$ ,  $P < 0.001$ ). The relation between varieties and concentrations was not significant in  $F_1$  ( $F = 1.04$ ,  $df = 9$ ,  $p > 0.05$ ) and significant in  $F_2$  ( $F = 3.31$ ,  $df = 9$ ,  $p < 0.01$ ) generation (Table 1).

*Effect on total developmental period:* Dietary treatment of *S. oryzae* with spinosad concentrations significantly increased the total developmental period compared to the control (Table 2) in a dose-dependent manner on four wheat varieties. In  $F_1$ , developmental period ranged from  $29.67 \pm 0.33$  to  $31.33 \pm 0.33$  days in control and  $31.00 \pm 0.58$  to  $41.67 \pm 0.33$  days in treatment whereas, the range of developmental period was from  $29.33 \pm 0.67$  to  $31.67 \pm 0.33$  days in control and  $0.00 \pm 0.00$  to  $45.67 \pm 0.33$  days in treatment in  $F_2$ . The highest developmental period was  $41.67 \pm 0.33$  days to become adult was recorded in S-21 at  $0.0003 \mu\text{l/g}$  of spinosad compared with the control and rest of other concentrations in  $F_1$ . All adults of  $F_1$  did not reach in a  $F_2$  because surprisingly they died after emerge. So, no developmental period was recorded in S-21 ( $0.00 \pm 0.00$ ) and B-28 ( $0.00 \pm 0.00$ ) days at  $0.0003 \mu\text{l/g}$  spinosad in  $F_2$ .

Table 2 showed significant effect among varieties in  $F_1$  ( $F = 22.04$ ,  $df = 3$ ,  $p < 0.001$ ) and in  $F_2$  ( $F = 534.67$ ,  $df = 3$ ,  $p < 0.001$ ) generation and concentrations ( $F = 311.84$ ,  $df = 3$ ,  $p < 0.001$ ) and ( $F = 1108.78$ ,  $df = 3$ ,  $p < 0.001$ ) in  $F_2$ . But, interactions between varieties and concentrations was not significant in  $F_1$  ( $F = 1.23$ ,  $df = 9$ ,  $p > 0.05$ ) and highly significant in  $F_2$  ( $F = 769.88$ ,  $df = 9$ ,  $p < 0.001$ ) generations.

*Effect on total developmental period:* Dietary treatment of *S. oryzae* with spinosad concentrations significantly increased the total developmental period compared to the control (Table 2) in a dose-dependent manner on four wheat varieties. In F<sub>1</sub>, developmental period ranged from 29.67 ± 0.33 to 31.33 ± 0.33 days in control and 31.00 ± 0.58 to 41.67 ± 0.33 days in treatment whereas, the range of developmental period was from 29.33 ± 0.67 to 31.67 ± 0.33 days in control and 0.00 ± 0.00 to 45.67 ± 0.33 days in treatment in F<sub>2</sub>. The highest developmental period 41.67 ± 0.33 days to become adult was recorded in S-21 at 0.0003 µl/g of spinosad compared with the control and rest of other concentrations in F<sub>1</sub>. All adults of F<sub>1</sub> did not reach in a F<sub>2</sub> because surprisingly all adults were died after emerge. So, no developmental period was found in S-21 (0.00 ± 0.00) and B-28 (0.00 ± 0.00) days at 0.0003 µl/g spinosad in F<sub>2</sub>.

**Table 1. Effect of spinosad on survivability of adult emergence of *S. oryzae* in F<sub>1</sub> and F<sub>2</sub> generations**

Wheat varieties	Concentrations (µl/g)	Adult emergence (Mean ± SE)			
		1st generation	PRC	2nd generation	PRC
B-26	Control	64.67 ± 2.91a	-	156.67 ± 2.03a	-
	0.000075	44.33 ± 2.33b	31.44	29.67 ± 2.60b	81.06
	0.00015	30.67 ± 2.03c	52.58	7.00 ± 0.58c	95.53
	0.0003	17.00 ± 0.58d	73.71	2.67 ± 0.33d	98.30
B-28	Control	57.33 ± 1.86a	-	150.67 ± 3.53a	-
	0.000075	39.67 ± 1.45b	30.81	25.67 ± 2.73b	82.96
	0.00015	28.00 ± 1.15c	51.16	4.33 ± 0.33c	97.12
	0.0003	15.67 ± 0.88d	72.67	0.00 ± 0.00d	100.00
P-24	Control	70.00 ± 2.89a	-	165.33 ± 2.91a	-
	0.000075	49.33 ± 2.33b	29.52	29.33 ± 2.33b	82.26
	0.00015	39.33 ± 1.76c	43.81	8.67 ± 1.20c	94.76
	0.0003	20.00 ± 1.15d	71.43	3.00 ± 0.58d	98.19
S-21	Control	56.33 ± 1.86a	-	145.00 ± 3.00a	-
	0.000075	34.67 ± 3.28b	38.46	17.00 ± 1.53b	88.28
	0.00015	19.67 ± 2.03c	65.09	2.00 ± 0.58c	98.62
	0.0003	08.00 ± 0.58d	85.80	0.00 ± 0.00d	100.00
Source	DF	1 <sup>st</sup> generation F value		2 <sup>nd</sup> generation F value	
Vareties	3	40.88***		23.26***	
Concentrations	3	403.09***		5746.87***	
Vareties * Concentrations	9	1.04 <sup>NS</sup>		3.31**	

Column means with same letter do not differ significantly from each other within varieties at 0.05% level (Tukey's test). \*\*\*Significant at  $p < 0.001$ , \*\*Significant at  $p < 0.01$ , NS = Non significant.

Table 2 showed that highly significant effect found among varieties ( $F = 22.04$ ,  $df = 3$ ,  $p < 0.001$ ) in F<sub>1</sub> and ( $F = 534.67$ ,  $df = 3$ ,  $p < 0.001$ ) in F<sub>2</sub> and concentrations ( $F = 311.84$ ,  $df = 3$ ,  $p < 0.001$ ) in F<sub>1</sub> and ( $F = 1108.78$ ,  $df = 3$ ,  $p < 0.001$ ) in F<sub>2</sub>. But, interactions between varieties and concentrations was non-

significant ( $F = 1.23$ ,  $df = 9$ ,  $p > 0.05$ ) in  $F_1$  and highly significant ( $F = 769.88$ ,  $df = 9$ ,  $p < 0.001$ ) in  $F_2$ .

**Table 2. Effect of spinosad on developmental period of *S. oryzae* in  $F_1$  and  $F_2$  generations**

Wheat varieties	Concentrations ( $\mu\text{l/g}$ )	Developmental period (Mean $\pm$ SE)	
		1st generation	2nd generation
B-26	Control	30.00 $\pm$ 0.58d	30.33 $\pm$ 0.33c
	0.000075	31.33 $\pm$ 0.67c	33.67 $\pm$ 0.67b
	0.00015	35.67 $\pm$ 0.33b	41.33 $\pm$ 0.67a
	0.0003	39.33 $\pm$ 0.67a	45.67 $\pm$ 0.33d
B-28	Control	30.33 $\pm$ 0.33d	30.67 $\pm$ 0.33c
	0.000075	32.67 $\pm$ 0.33c	34.67 $\pm$ 0.33b
	0.00015	36.33 $\pm$ 0.33b	42.67 $\pm$ 0.33a
	0.0003	40.33 $\pm$ 0.33a	0.00 $\pm$ 0.00d
P-24	Control	29.67 $\pm$ 0.33d	29.33 $\pm$ 0.67c
	0.000075	31.00 $\pm$ 0.58c	33.33 $\pm$ 0.33b
	0.00015	33.33 $\pm$ 0.33b	39.33 $\pm$ 0.67a
	0.0003	38.67 $\pm$ 0.67a	43.67 $\pm$ 0.88d
S-21 I	Control	31.33 $\pm$ 0.33d	31.67 $\pm$ 0.33c
	0.000075	33.00 $\pm$ 0.58c	36.00 $\pm$ 0.58b
	0.00015	37.33 $\pm$ 0.67b	43.67 $\pm$ 0.33a
	0.0003	41.67 $\pm$ 0.33a	0.00 $\pm$ 0.00d
Source	DF	1st generation F value	2nd generation F value
Wheat varieties	3	22.04***	534.67***
Concentrations	3	311.84***	1108.78***
Wheat varieties *Concentrations	9	1.23 <sup>NS</sup>	769.88***

In a column means with same letter do not vary significantly within varieties at  $p < 0.05$  level (Tukey's test). \*\*\*Significant at  $p < 0.001$ , NS = Non Significant.

The results of the present study indicate that there was a significant impact of spinosad on the adult emergence and total developmental period of *S. oryzae* in wheat varieties. Since spinosad acts as a contact insecticide, the present research assumes that at control room temperature the increased spinosad resulting in increased mortality. From a practical point of view, 0.000075 and 0.00015  $\mu\text{l/g}$  of spinosad gave decreasing adult emergence levels. Consequently, spinosad at 0.0003  $\mu\text{l/g}$  was higher concentration and can satisfactorily control the adult emergence of *S. oryzae* in successive two generations.

It was showed that insecticidal efficacy often vary depending upon the particular insecticide and the commodity that is treated. Spinetoram (group of spinosyn) efficacy against *S. oryzae* was notably increasing from the size of the treated layer, and elevated length drastically increased mortality and reduced progeny production. Mortality was high only on totally treated wheat for layer

treated wheat ranged between 32 and 72%. Mortality became substantially lower on rice than wheat which suggests that spinetoram became much less effective on rice than on wheat (Vassilakos and Athanassiou 2012). The present investigation revealed that spinosad at 0.0003  $\mu\text{l/g}$  concentration significantly gave the lowest percentage ( $08.00 \pm 0.58\%$ ) of adult emergence of *S. oryzae* in S-21 in  $F_1$  and absolutely controlled the adult emergence in S-21 and B-28 wheat varieties in  $F_2$  compared with the control and rest of other concentrations.

The efficacy of spinosad in the present study was more or less similar with the findings of Huang *et al.* (2007) who revealed that spinosad at 1 mg (AI)/kg provided 100% reduction of egg-to-adult emergence of *Sitotroga cerealella*. Minimal presence of spinosad on wheat had some lethal effect on parental *S. oryzae*. On the other hand, the lower percentage of treated kernels significantly increased progeny production of *S. oryzae* for wheat. The complete control of adult *S. zeama* and progeny production on maize with two liquid formulations is in agreement with Huang and Subramanyam (2007), who mentioned similar consequences with a commercial liquid formulation of spinosad used on field crops (SpinTor 2SC). The present finding is in agreement with the above results where complete control of adult emergence of *S. oryzae* was observed in varieties S-21 and B-28 in  $F_2$ . Andrić *et al.* (2019) reported that all doses of spinetoram achieved high mortality (96 - 100%) of *S. granarius* on both wheat varieties, *viz*, variety with high (HVWG) and another with low (LVWG) endosperm vitreousness. While high mortality of *S. oryzae* (97 - 100%) and both populations of *S. zeama* is (93 - 100%) was achieved using 1 - 2 mg doses on the HVWG and 2 mg dose on the LVWG variety after 14 days.

In the present work, there was significant increase in time taken for development at all concentration of spinosad in comparison with the control. Developmental periods was increased in all wheat varieties at 0.0003  $\mu\text{l/g}$  of spinosad and no developmental period was observed in S-21 and B-28 wheat varieties in  $F_2$  at 0.0003  $\mu\text{l/g}$ .

The present investigation revealed that spinosad at 0.0003  $\mu\text{l/g}$  concentration significantly gave the lowest percentage ( $08.00 \pm 0.58\%$ ) of adult emergence of *S. oryzae* in S-21 in  $F_1$  and absolutely controlled the adult emergence in S-21 and B-28 wheat varieties in  $F_2$  compared with the control and rest of other concentrations.

The results of the present study indicate that there was a significant impact of spinosad on the adult emergence and total developmental period of *S. oryzae* in wheat varieties. From a practical point of view, 0.000075 and 0.00015  $\mu\text{l/g}$  of spinosad gave decreasing adult emergence levels. Consequently, spinosad at



0.0003 µl/g was higher concentration and can satisfactorily control of adult emergence of *S. oryzae* in successive two generations.

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