

Contact and gustatory effects of spinosad on the larval and adult mortality of *Tribolium castaneum* L. (Herbst) in wheat varieties

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Abstract: Spinosad an eco-friendly bio rational insecticide found in the bacterial species *Saccharopolyspora spinosa* Mertz and Yao. It is treated as reduced-risk insecticide due to its low effective use rate and safety to the environment and mammals. This insecticide used more widely for the management of stored product insect pests. The contact and gustatory efficacy of spinosad was evaluated on four wheat varieties viz, Bari-26, Bari-28, Prodip-24 and Shstsbdi-21 against larva and adult of *Tribolium castaneum* L. (Herbst) were exposed to untreated wheat and wheat treated with spinosad at 0.98, 1.97 and 3.93 μ l/g concentrations after 24, 48 and 72 hour exposure. The 2nd instar larval mortality ranged from 15.00 \pm 2.89 to 80.00 \pm 2.89% and adult mortality ranged from 8.33 \pm 1.67 to 58.33 \pm 3.33% in treated wheat. The highest 2nd instar larval and adult mortality was recorded in variety Shatabdi-21 as 80.00% and 58.33% at 3.93 μ l/g of spinosad after 72 h exposure. Higher concentration of spinosad was extremely effective against 2nd instar larva of *T. castaneum* on all wheat varieties after higher exposure periods than control and other concentrations and exposures. Adult mortality exceeded >50% only in Shatabdi-21 wheat variety at 3.93 μ l/g of spinosad after 72 h exposure. The effect was positively related to concentrations of spinosad and exposure times of the beetle. The lowest larval and adult LC₅₀ value were 0.677 and 1.514 μ l/g recorded in S-21 after 72 h that indicated that spinosad is highly toxic against the 2nd instar larvae and moderate toxicity against the adult of *T. castaneum*. Our results suggest that spinosad to be an effective grain protectant for *T. castaneum* management in stored wheat.

Key words: Spinosad, *Tribolium castaneum*, wheat varieties

Introduction

Wheat (*Triticum aestivum* L.) is the maximum widely grown food crop, which ranks first in terms of region and production in the world (FAO, 1988). Wheat and cereal grains are the essential component of the human diet throughout the world. It is far stored within the government and public storages in Bangladesh and most of the countries of the world. However, large amounts of wheat as well as other grains are lost every year in storage due to both biotic and abiotic elements. The predominant biotic elements influencing wheat loss throughout storage are insects, molds, birds and rats (Baloch *et al.*, 1994).

The red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) an external-feeding pest or secondary pest attacks damaged grains in both larval and adult stages, and readily adapts to stored-grain environments for its excessive fecundity rates and relative longevity (Baldwin & Fasulo, 2004). *T. castaneum* causes considerable financial losses of stored grains throughout the world. Numerous synthetic insecticides have been used for control of this pest worldwide (Ali and Iman, 2015).

T. castaneum has a nicely-documented reputation for developing resistance to all classes of synthetic insecticides resulting in failure of management programs in different countries (Correa *et al.*, 2011, Ali & Iman 2015, Bajracharya *et al.*, 2016). Therefore, alternative pest control techniques are needed in post-harvest commodities to complement the existing more secure chemical grain protectants (Fang *et al.*, 2002a). One of such alternate insecticide of consideration is spinosad, through contact and ingestion which is highly toxic on a number of insect pests including *T. castaneum* (Copping & Duke, 2007).

Spinosad is a reduced-risk insecticide product from Dow Agro Sciences (Indianapolis, Indiana, USA) based on chemical compounds of a soil bacterium *Saccharopolyspora* was discovered in 1985 (Mertz & Yao, 1990). It is a naturally derived bio rational insecticide with an environmentally favorable toxicity profile (Bond *et al.*, 2004). Spinosad is known for its effective gustatory action against the external feeders of the stored-product commodities. Both dust (0.125%) and liquid formulations were effective against *T. castaneum* present in different commodities (Khashaveh *et al.*, 2009, Subramanyam *et al.*, 2012), but the liquid

formula was found to be more powerful than the dust one (Subramanyam *et al.*, 2012). It has proved to be very effective against an extensive range of stored-product pests and can retain its efficacy for a long time after application (Maier *et al.*, 2006, Daghli & Nayak, 2006, Subramanyam *et al.*, 2007, Vayias *et al.*, 2009, Athanassiou *et al.*, 2010, Hertlein *et al.*, 2011, Subramanyam *et al.*, 2012). It is a stomach poison with some contact activity and display some control on small beetle larvae (Thompson *et al.*, 2000). Ali & Iman (2015) showed that the spinosad had more effects against *T. castaneum*.

Among the major insect pests of stores *T. castaneum* often found damage to broken or cracked grains. Control of *T. castaneum* is quite difficult with the traditional chemical. Therefore, the present investigation was designed to evaluate the contact and gustatory effects of spinosad as a biological control agent against *T. castaneum* larvae and adults under laboratory conditions on four stored wheat varieties.

Materials and Methods:

Methods of the Bioassay: In the present study exposure of different life stages of *T. castaneum* and were exposed to Treated Food Method (TEM) (Talukder and Howse 1994) for evaluating the effects of spinosad against the larvae and adults of the beetle.

Collection of *T. castaneum*

T. castaneum beetles were obtained from the stock culture without any exposure to insecticides, maintained in the control temperature (CT) room, at Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh.

Preparation of standard food medium for mass culture of *T. castaneum*

The standard food medium, mixture of whole wheat flour with powdered Brewer's yeast (19:1) was used as the food medium throughout the mass culture of *T. castaneum* ((Park & Frank, 1948; Park, 1962). Both flour and yeast were previously passed through a 250µm sieve. The food medium was sterilized at 120°C for six hours in an oven and kept at least for 15 days before use, to equilibrate the moisture content with the environment (Khan, 1981; Mondal, 1984).

Source of Spinosad

About 500ml of liquid spinosad (PRN- MAPP-12054, cafno 20012-019, Lot No-3068404)

was obtained from Dow Agro Sciences, UK. The liquid is light grey to white in colour with slight odour stale water. Concentration of spinosad was 120g spinosad/Litre.

Preparation of spinosad concentrations

The spinosad was diluted in distilled water. In 50ml beaker 23.58µl spinosad was taken by using a micropipette, and 6ml distilled water were added properly in it by using 2ml syringe (3 times). The vial was shaken vigorously for equal mixing of spinosad and water. From this solution 1ml was taken off which contained 3.93µl spinosad, which was the stock concentration. The other concentrations of spinosad were prepared by serial dilution of stock solution and adding 2ml distilled water in each step. So, the desired concentrations of spinosad were obtained as 3.93, 1.97 and 0.98µl, which were used for toxicity study against *T. castaneum*.

Commodities used

Untreated and infestation free four wheat varieties viz, BARI-26, BARI-28, Prodip-24 and Shatabdi-21 were used in the experiments. The grains were washed with water and dried at room temperature before adjusting their moisture content to 13.5(%) by adding tap water. The grains were sieved through 500 micrometer aperture sieve and sterilized in an oven at 100°C for 8 h. After sterilization wheat grains were broken down by the hand blender and kept in clean plastic containers for using throughout the experimental period.

Bioassays

This cracked wheat was used as food medium for *T. castaneum* larvae and adults. One gram (1g) of wheat grain of a variety were placed in a petri-dish (6cm) and treated with freshly prepared aqueous spinosad solution of a definite concentration, using a 1ml syringe. Ten pairs of either 2nd or 5th instar larvae of *T. castaneum* were released in the treated wheat separately. Similarly, 10 pairs of untreated adults (5-6d old) were also released separately in the treated wheat. The petri-dish were covered with lid and kept in the CT room at 30±1°C and 75±0.5°C% RH. Mortality of the larval instars and adults of *T. castaneum* were recorded after 24, 48 and 72h exposure periods. For control batch wheat grains were treated with 1µl distilled water only. Three replications were taken for each of the spinosad concentrations, wheat varieties, each larval instar and adults of *T. castaneum*.

Data collection and statistical analysis

The data for percent kill of *T. castaneum* in all

used concentrations was recorded after 24, 48 and 72h exposure; the data were subjected to Analysis of Variance using SPSS-20 version. Means were compared by Turkey's tests ($P < 0.05$), and subjected to Probit Analysis using the Probit software for calculating average larval and adult mortality data and estimation of LC_{50} (lethal concentration) values. The regression lines were drawn using Microsoft Excel-2010 and Bio Stat-2009. 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:

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

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

$$P_t = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where, P_t = corrected mortality % P_0 = observed mortality % P_c = control mortality %

Results and Discussion

Toxicity of spinosad against 2nd instar larva: The mortal effect of spinosad on 2nd instar larvae of *T. castaneum* in wheat varieties at different concentrations and different exposure periods in different wheat varieties are shown in Table 1. The result showed that when the 2nd instar larvae were exposed to spinosad treated wheat at various concentrations (0.98, 1.97 and 3.93 $\mu\text{l/g}$) for different time interval (24, 48 and 72h) the toxic effects of the spinosad was differed with different exposure periods and wheat varieties. Spinosad at 0.98 $\mu\text{l/g}$ caused lowest mortality (15.00 \pm 2.89%) of 2nd instar larva of *T. castaneum* in P-24 variety after 24h, and highest mortality (80.00 \pm 2.89%) was observed at 3.93 $\mu\text{l/g}$ in S-21 variety after 72h exposure. The effect was positively related to concentration of spinosad and exposure time of the larvae.

The LC_{50} values, 95% confidence limits, regression equations, χ^2 for heterogeneity and regression lines of empirical probit mortality of

2nd instar larvae of *T. castaneum* are presented in Table 2. The LC_{50} values clearly show that spinosad toxicity was increased with the increase of exposure periods in four wheat varieties. The lowest LC_{50} value was 0.677 $\mu\text{l/g}$ in S-21 after 72h, which indicated that spinosad is highly toxic against the 2nd instar larvae of *T. castaneum*.

ANOVA results clearly indicated highly significant interaction effects were present between wheat varieties ($F = 57.60$, $df = 3$, $P < 0.001$), spinosad concentrations ($F = 1412.96$, $df = 3$, $P < 0.001$) and exposure time ($F = 419.25$, $df = 2$, $P < 0.001$) (Table 1).

Toxicity of spinosad in 5th instar larva: Results of the mortal effect of spinosad on 5th instar larvae of *T. castaneum* in different wheat varieties at different concentrations after different exposure periods are presented in Table 3. Among four varieties the highest mortality of 5th instar larvae was noted in S-21 as 75.00 \pm 2.89% at 3.93 $\mu\text{l/g}$ concentration and 72h exposure, and lowest mortality was 15.00 \pm 2.89% in B-26 after 24h exposure at the same concentration. Spinosad mortality against the 5th instar larvae of *T. castaneum* was positively related with both its concentrations and exposure periods, which were significantly different between the control and treatments; the percent mortality also significantly differed among the wheat varieties, S-21 being the most susceptible one.

The LC_{50} values, 95% confidence limits, regression equations, χ^2 for heterogeneity and regression lines of empirical probit mortality of 5th instar larvae are shown in Table 4. Toxic effect was maximum (LC_{50} 0.749) in S-21 wheat at 72h exposure. ANOVA results clearly expressed high significant interaction effects were found between varieties ($F = 36.36$, $df = 3$, $P < 0.001$), concentrations ($F = 595.61$, $df = 3$, $P < 0.001$) and exposure period ($F = 207.69$, $df = 2$, $P < 0.001$) (Table 3).

Toxicity of spinosad in unsexed adults: Results of adult mortality with different concentrations of spinosad after different exposure periods are presented in Table 5. The highest concentration of spinosad (3.93 $\mu\text{l/g}$) at 72h resulted in to >50% mortality in S-21 wheat variety which was significantly greater compared to the control and others concentrations and exposure periods. The highest mortality observed was in S-21 (58.33 \pm 3.33%). The lowest adult mortality was recorded as in P-24 wheat variety 8.33 \pm 1.67% at 0.98 μl of spinosad when exposed for 24h.

Table 1 Toxicity of different concentrations of spinosad against 2nd instar larvae *Tribolium castaneum* after 24, 48 and 72h of exposures.

Wheat varieties	Concentrations ($\mu\text{l/g}$)	Average Mortality %		
		Exposure period (h)		
		24	48	72
B-26	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	16.67 \pm 1.67c	28.33 \pm 1.67c	43.33 \pm 3.33c
	1.97	26.67 \pm 1.67b	40.00 \pm 2.89b	55.00 \pm 2.89b
	3.93	35.00 \pm 2.89a	50.00 \pm 2.89a	68.33 \pm 1.67a
B-28	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	21.67 \pm 1.67c	31.67 \pm 1.67c	48.33 \pm 1.67c
	1.97	33.33 \pm 1.67b	48.33 \pm 1.67b	61.67 \pm 1.67b
	3.93	45.00 \pm 2.89a	53.33 \pm 1.67a	75.00 \pm 2.89a
P-24	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	15.00 \pm 2.89c	23.33 \pm 3.33c	41.67 \pm 1.67c
	1.97	20.00 \pm 2.89b	38.33 \pm 1.67b	53.33 \pm 3.33b
	3.93	33.33 \pm 3.33a	48.33 \pm 3.33a	63.33 \pm 1.67a
S-21	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	25.00 \pm 2.89c	35.00 \pm 2.89c	56.67 \pm 3.33c
	1.97	36.67 \pm 4.41b	53.33 \pm 1.67b	70.00 \pm 2.89b
	3.93	48.33 \pm 2.89a	61.67 \pm 1.67a	80.00 \pm 2.89a
Source	F-value	DF		
Varieties	57.60***	3		
Concentrations	1412.96***	3		
Exposure Periods	419.25***	2		

In a column means with same letter do not significantly differed from each other within varieties at 0.05% level (Tukey's test). Note: ***= Significant at $P < 0.001$, *= Significant at $P < 0.05$, NS= Non-Significant.

Table 2 Representing χ^2 for heterogeneity, regression equations, LC₅₀ and 95% confidence limits of different concentrations of spinosad against 2nd instar larvae of *T. castaneum* after 24, 48 and 72h exposure period.

Wheat varieties	Exposure periods (h)	χ^2 for heterogeneity	Regression equation	LC ₅₀ ($\mu\text{l/g}$)	95% confidence limits	
					Lower	Upper
B-26	24	0.107	$Y = 3.143977 + 0.9294419X$	9.929	2.283	43.179
	48	0.020	$Y = 3.47904 + 0.959656X$	3.846	1.941	7.616
	72	0.028	$Y = 3.812216 + 1.023593X$	1.447	0.903	2.318
B-28	24	0.003	$Y = 3.107791 + 1.115524X$	4.969	2.370	10.417
	48	0.768	$Y = 3.683544 + 0.9026346X$	2.874	1.634	5.053
	72	0.005	$Y = 3.736731 + 1.209109X$	1.109	0.674	1.824
P-24	24	0.233	$Y = 2.961422 + 0.9731704X$	12.439	2.452	63.093
	48	0.183	$Y = 3.16654 + 1.129472X$	4.200	2.235	7.894
	72	0.002	$Y = 3.983191 + 0.8390487X$	1.629	0.956	2.774
S-21	24	0.01	$Y = 3.337842 + 1.009268X$	4.435	2.119	9.283
	48	0.389	$Y = 3.520077 + 1.138021X$	1.997	1.371	2.909
	72	0.005	$Y = 4.124046 + 1.054938X$	0.677	0.284	1.611

Table 3 Toxicity of different concentrations of spinosad against 5th instar larvae of *T. castaneum* after 24, 48 and 72 h of exposures.

Wheat varieties	Concentrations ($\mu\text{g/g}$)	Average Mortality %		
		Exposure period (h)		
		24	48	72
B-26	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	15.00 \pm 2.89c	20.00 \pm 2.89c	33.33 \pm 4.41c
	1.97	23.33 \pm 4.41b	35.00 \pm 2.89b	48.33 \pm 3.33b
	3.93	28.33 \pm 1.67a	40.00 \pm 2.89a	65.00 \pm 5.00a
B-28	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	20.00 \pm 2.89c	31.67 \pm 4.41c	45.00 \pm 2.89c
	1.97	30.00 \pm 2.89b	40.00 \pm 2.89b	56.67 \pm 4.41b
	3.93	38.33 \pm 3.33a	45.00 \pm 5.00a	70.00 \pm 2.89a
P-24	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	16.67 \pm 1.67c	23.33 \pm 4.41c	35.00 \pm 2.89c
	1.97	20.00 \pm 2.89b	30.00 \pm 2.89b	43.33 \pm 3.33b
	3.93	26.67 \pm 4.41a	36.67 \pm 3.33a	63.33 \pm 4.41a
S-21	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	26.67 \pm 1.67c	36.67 \pm 3.33c	55.00 \pm 2.89c
	1.97	33.33 \pm 4.41b	45.00 \pm 2.89b	65.00 \pm 2.89b
	3.93	38.33 \pm 3.33a	53.33 \pm 3.33a	75.00 \pm 2.89a
Source	F-value	DF		
Varities	36.36***	3		
Concentrations	595.61***	3		
Exposure Periods	207.69***	2		

In a column means with same letter do not significantly differed from each other within varieties at 0.05% level (Tukey's test). Note: ***= Significant at $P < 0.001$, *= Significant at $P < 0.05$, NS= Non-Significant.

Table 4 Presenting χ^2 for heterogeneity, regression equations, LC_{50} and 95% confidence limits of different concentrations of spinosad against 5th instar larvae of *T. castaneum* after 24, 48 and 72h exposure period.

Wheat varieties	Exposure periods(h)	χ^2 for heterogeneity	Regression equation	LC_{50} ($\mu\text{g/g}$)	95% confidence limits	
					Lower	Upper
B-26	24	0.061	$Y = 3.26272 + 0.7361738X$	22.902	1.302	402.909
	48	0.471	$Y = 3.218521 + 0.9943705X$	6.188	2.317	16.527
	72	0.009	$Y = 3.179211 + 1.369976X$	2.133	1.554	2.928
B-28	24	0.054	$Y = 3.345081 + 0.8515831X$	8.777	2.027	37.998
	48	0.032	$Y = 3.915226 + 0.6187571X$	5.664	1.310	24.444
	72	0.025	$Y = 3.878657 + 1.009764X$	1.290	0.764	2.177
P-24	24	0.087	$Y = 3.418655 + 0.5987173X$	43.775	0.517	3704.895
	48	0.012	$Y = 3.593852 + 0.6725976X$	12.320	1.312	115.726
	72	0.688	$Y = 3.289111 + 1.262893X$	2.263	1.598	3.206
S-21	24	0.0004	$Y = 3.915482 + 0.4839118X$	17.424	0.461	658.653
	48	0.012	$Y = 3.983428 + 0.6891396X$	2.986	1.394	6.395
	72	0.014	$Y = 4.197139 + 0.9182762X$	0.749	0.300	1.870

Table 5 Toxicity of different concentrations of spinosad against adult (unsexed) *T. castaneum* after 24, 48 and 72h of exposure.

Wheat varieties	Concentrations (μg)	Average Mortality %		
		Exposure period (h)		
		24	48	72
B-26	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	11.67 \pm 1.67c	23.33 \pm 1.67c	33.33 \pm 1.67c
	1.97	23.33 \pm 1.67b	36.67 \pm 1.67b	41.67 \pm 1.67b
	3.93	26.67 \pm 1.67a	40.00 \pm 2.89a	45.00 \pm 2.89a
B-28	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	16.67 \pm 1.67c	20.00 \pm 2.89c	36.67 \pm 1.67c
	1.97	23.33 \pm 1.67b	35.00 \pm 2.89b	43.33 \pm 3.33b
	3.93	33.33 \pm 1.67a	41.67 \pm 1.67a	48.33 \pm 1.67a
P-24	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	8.33 \pm 1.67c	18.33 \pm 3.33c	25.00 \pm 5.00c
	1.97	16.67 \pm 1.67b	26.67 \pm 1.67b	38.33 \pm 1.67b
	3.93	21.67 \pm 1.67a	36.67 \pm 1.67a	41.67 \pm 1.67a
S-21	Control	0.00 \pm 0.00d	0.00 \pm 0.00d	0.00 \pm 0.00d
	0.98	20.00 \pm 2.89c	28.33 \pm 1.67c	46.67 \pm 1.67c
	1.97	26.67 \pm 1.67b	41.67 \pm 1.67b	51.67 \pm 1.67b
	3.93	35.00 \pm 2.89a	45.00 \pm 2.89a	58.33 \pm 3.33a
Source	F-value	DF		
Varities	52.66***	3		
Concentrations	937.99***	3		
Exposure Periods	245.43***	2		

In a column means with same letter do not significantly differed from each other within varieties at 0.05% level (Tukey's test). Note: ***= Significant at $P < 0.001$, *= Significant at $P < 0.05$, NS= Non-Significant.

Table 6 Presenting χ^2 for heterogeneity, regression equations, LC_{50} and 95% confidence limits of different concentrations of spinosad against adult (unsexed) *T. castaneum* after 24, 48 and 72h exposure periods.

Wheat varieties	Exposure periods (h)	χ^2 for heterogeneity	Regression equation	LC_{50} (μg)	95% confidence limits	
					Lower	Upper
B-26	24	0.576	$Y=3.001223+0.9017737X$	16.462	2.089	129.742
	48	0.465	$Y=3.517256+0.8069621X$	6.878	1.880	25.167
	72	0.134	$Y=4.003893+0.5709572X$	5.554	1.165	26.473
B-28	24	0.032	$Y=3.182795+0.8464992X$	14.019	1.977	99.397
	48	0.315	$Y=3.12011+1.083684X$	5.429	2.397	12.294
	72	0.035	$Y=4.211056+0.4649989X$	4.973	0.865	28.582
P-24	24	0.372	$Y=2.683409+0.9878488X$	22.135	2.231	219.595
	48	0.012	$Y=3.136717+0.9591558X$	8.763	2.333	32.905
	72	0.306	$Y=3.589646+0.7892432X$	6.123	1.804	20.780
S-21	24	0.0006	$Y=3.457781+0.7205223X$	13.817	1.479	129.079
	48	0.541	$Y=3.751808+0.7280239X$	5.182	1.625	16.527
	72	0.0006	$Y=4.462005+0.4559123X$	1.514	0.546	4.195

The LC₅₀ values, 95% confidence limits, regression equations, χ^2 for heterogeneity and regression lines of empirical probit mortality of *T. castaneum* adults are presented in Table 6. The lowest LC₅₀ value (1.514 μ l/g) was recorded in S-21 after 72h indicates that spinosad is toxic against the adult *T. castaneum*. ANOVA expressed high significant effects were found between varieties (F=52.66, df=3, P<0.001), concentrations (F=937.99, df=3, P<0.001) and exposure period (F=245.43, df=2, P<0.001) (Table 5).

Results of the experiments indicate that low concentrations spinosad is a potential gustatory toxic agent causing significant mortal effect larva and adult stages of *T. castaneum* at 24-72h exposure. However, the efficacy of spinosad varied according to the concentrations, the exposure times and as well as the wheat commodities. The larvae irrespective of their age became intoxicated by spinosad and so the unsexed adults. The larvae were more susceptible to spinosad compared to the adults in all the wheat varieties. Mortality (%) was increased with the concentrations of spinosad and exposure time. The mortal effect was 80.00% mortality in 2nd instar larvae at 3.93 μ l/g concentration in S-21 variety at an exposure of 72h.

Published literature showed that larval *T. confusum* was much susceptible to spinosad than their adults (Vayias *et al.*, 2010). Fang *et al.*, (2002a), Huang *et al.*, (2007), Huang & Subramanyam (2007) reported that susceptibility of *P. interpunctella* larvae showed dose dependent mortal effect in spinosad treated medium. Toews & Subramanyam (2003) evaluated that mortality of *T. castaneum* in spinosad treatment and the effect was related to concentrations of spinosad. These results are found to be similar to the present results.

Athanassiou *et al.*, (2008a) found that 3rd to 4th instar larvae of six *T. confusum* strains of different locations of Europe were susceptible to spinosad than their adults. In the present results *T. castaneum* adults were found to be less susceptible to spinosad even at higher rate than its young and old larvae. Similar trend of spinosad mortality in wheat were shown by Fang *et al.*, (2002a, b), Subramanyam *et al.*, (2007). Susceptibility of *T. castaneum* adults to spinosad at the labeled rate (1mg/kg) varied with the wheat class (Sehgal *et al.*, 2013).

Toews & Subramanyam (2003) revealed that contact toxicity of spinosad resulted in 12-48% mortal effect in adult *T. castaneum*, when

exposed to 0.001-0.79mg/cm² of spinosad for 24h; and 0, 0.0016 and 0.016mg/cm² at 48h exposure. The authors also noted that *T. castaneum* was more tolerant to spinosad than *R. dominica* and *S. oryzae*. Similar results have also been reported by other researchers like Huang *et al.* (2004), Nayak *et al.*, (2005), Getchell (2006). Andrić *et al.*, (2011) reported that *T. castaneum* was less susceptible to spinosad than *S. oryzae*. The adults of *O. surinamensis* and *T. castaneum* are moderately susceptible to spinosad (Nayak *et al.*, 2005, Sehgal *et al.*, 2013). Nikpay (2007) observed the 65% mortality of *T. castaneum* in treated spinosad at 1mg/kg of wheat; and Athanassiou *et al.* (2010), observed a maximum 10% adult on wheat and maize.

The larger grain borer, *Prostephanus truncatus* and *R. dominica* are very susceptible to spinetoram (product of secondary metabolites spinosyn J and L (Herbert, 2010) to protect grain while *Tribolium confusum* and *Oryzaephilus surinamensis* are less susceptibility to spinetoram (secondary metabolites spinosyn J and L) (Vassilakos *et al.*, 2012). Spinetoram was effective against *T. confusum* adults and young larvae but ineffective against *T. confusum* on wheat (Vassilakos & Athanassiou 2013). Babarinde *et al.*, (2018) noted that a synergistic effect of spinosad with *Piper guineense* and *Eugenia aromatica* powders causing the death of *T. castaneum* at 3-7 DAT (days after treatment). Vassilakos *et al.*, (2014) recorded 72.4% mortality against *R. dominica*. Hameed *et al.*, (2012) evaluated the mortality up to 50% against *T. castaneum* for spinosad and two extracts, neem (*A. indica*) and *Nerium oleander* L. Rehman *et al.*, (2019) recorded that highest toxicity of *T. castaneum* was 79.8% for spinetoram at higher concentration along with longer exposure periods and it can be an effective alternative to synthetic insecticides for eco-friendly management of stored commodity insect pests.

The present results revealed that spinosad, a reduced-risk bacterial insecticide, can potentially control against the life stages of *T. castaneum* through its contact and gustatory effects. To obtain toxicity concentrations <1 μ l/g of spinosad is enough to produce >50% mortality in larval and adult *T. castaneum* in 3 days.

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