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Effect of Spinosad on Adults of *Tribolium castaneum* (Col: Tenebrionidae) and *Sitophilus oryzae* (Col: Curculionidae)

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Abstract: Toxicity of spinosad on adults of *Tribolium castaneum* Herbst (Col: Tenebrionidae) and *Sitophilus oryzae* L. (Col: Curculionidae) at different temperatures and exposure intervals was evaluated. A commercial formulation of spinosad, Tracer® was used, against 7-14-day-old adults of rice weevil and red flour beetle. Bioassays were conducted at 24, 28 and 32°C and 65±5% RH. Mortality was recorded at 24, 48 and 72 h post-treatment. Spinosad was diluted with water for making appropriate dosages. A control group (distilled water) was included in each bioassay. A dose-dependent response was observed in both species. Similar trend was detected between mortality and time intervals. In both species an inverse relationship between LD₅₀ values and temperature was detected. Based on LD₅₀ values and none overlapping of 95% CL, *S. oryzae* was more susceptible to spinosad than *T. castaneum*.

Key words: Spinosad, susceptibility, bioassay, stored-product insects, temperature

INTRODUCTION

Stored product pests are either beetles or moths (Koehler *et al.*, 2006). Several life stages of these insects may be present at the same time in infested products (Ogg, 2006). The two types of stored grain insects are internal-infesting and external-infesting insects. *Sitophilus oryzae* L. (Col: Curculionidae) is an internal insect that feed and develop internally in the kernels of cereal grains. *S. oryzae* larvae can destroy 25-30% of the wheat kernel that reduces the market value of wheat. *Tribolium castaneum* Herbst (Col: Tenebrionidae) is an external insect. This pest can cause a pungent, bad odor in the grain and contaminate the food with dead bodies and waste products (Kadir *et al.*, 2005). Therefore, the control of these pests is imperative.

Application of insecticides is an important tool to control stored product insects in grain industry (Daglish *et al.*, 2003). One chemical worthy of consideration is spinosad, which is produced from the metabolic by-products of a soil-dwelling bacterium and which is active against a range of insect species (Thompson *et al.*, 2000). Spinosad acts as a stomach and contact poison and degrades rapidly in the environment (Cisneros *et al.*, 2002) and has little toxicity to birds and mammals (Breslin *et al.*, 2000).

Temperature is one of the most important factors affecting biological processes in all living organisms and is also major factor affecting insecticide toxicity

(DeVries and Georghiou, 1979). The effect of temperature on efficacy can be either positive or negative. The response relationship between temperature and efficacy has been found to vary depending on the mode of action of an insecticide, target species, method of application and quantity of insecticide ingested or contacted (Johnson, 1990).

There are some reports about the more susceptibility of *S. oryzae* compared to *T. castaneum* exposed to spinosad by contact and oral toxicity. Therefore, the current study was carried out to examine direct toxicity (topical application) of spinosad against these insects.

MATERIALS AND METHODS

Insects: Adults of *T. castaneum* were collected from toxicology laboratory of agricultural faculty of Urmia University. *S. oryzae* was collected from infested store in Tonekabon. Insects were reared on 50% whole wheat and 50% rice (for rice weevil) and whole wheat flour (for red flour beetle). Insects were reared at 28±1°C, 65±5% RH and a photoperiod of 10:14 (L: D) h. In all experiments 7-14-day-old adults of two species were used.

Spinosad: Bacterial derived insecticide, spinosad (Tracer 24Sc; NAF-315; Batch TA13272015) was obtained from Substitute Dow AgroSciences, as a liquid mixture of Spinosyn A and D, Copping and Menn (2002) with 22.8% purity.

Bioassay: In all bioassays primary tests were conducted to determine the dosages that cause mortality between 25-75%, Robertson and Preisler (1992) for each species separately. The initial tests were carried out at 28±1°C, 65±5% RH and a 10:14 light: dark cycle. For all experiments, dosages of the insecticide were prepared using distilled water and a control group was included in each bioassay.

Effects on *T. castaneum*: Red flour beetle adults of 7-14-day-old were treated topically with 1.5 µL of spinosad solution or distilled water as a control group using an Oxford microapplicator. Primary dosages that used for this species were: 385, 835, 1811, 3926 and 8511 mg L⁻¹. Four replications were used for each dosage and twenty insects were treated for each replicate. Control specimens were treated with distilled water alone. After treatment, insects were introduced into 8 cm diameter Petri dishes and each dish was covered with lid. The experiments were carried out at 24, 28 and 32°C, 65±5% RH and a photoperiod of 10:14 (L: D) h separately. Mortality was recorded at 24, 48 and 72 h post-treatment. Insects were considered dead; if no movement was observed after probing the antenna with a hot needle.

Effects on *S. oryzae*: One microlitre of either the spinosad solution or distilled water was applied to the thorax of each 7-14-day-old adult using a microapplicator. Dosages used for this species were: 282, 461, 753, 1230 and 2009 mg L⁻¹. Control group was treated identically with distilled water. Fifty insects were treated for each dosage and control group. After treatment insects were introduced into 8 cm diameter Petri dishes and each dish was covered with lid. The experiments were conducted at three different temperatures viz., 24, 28 and 32°C and 65±5% RH and a photoperiod of 10:14 (L: D) h. Mortality was counted at 24, 48 and 72 h post treatment. The criterion for mortality was similar to those described earlier. Each bioassay was replicated four times on four different days.

Data analysis: Mortality percentage of insects was transformed to arcsine \sqrt{x} to normalize treatment variances. Means were compared using Duncan's Multiple Range Test (p<0.05). LD₅₀ values and the associated 95% confidence limits were calculated using probit analysis with SPSS software (SPSS, 1993). Failure of 95% CL to overlap was used as criterion for significant difference at LD₅₀ level.

RESULTS AND DISCUSSION

Adults of *T. castaneum*: Main effects application rate (F = 267.31**, df = 5, 159), exposure interval (F = 163.13**, df = 2, 159) and temperature (F = 172.73**, df = 2, 159) were significant for mortality at 99% confidence levels (p<0.01). Interactions viz., exposure × spinosad rate (F = 4.5**, df = 10, 159), temperature × spinosad rate (F = 7.08**, df = 10, 159) (p<0.01) and temperature × exposure (F = 2.55*, df = 4, 159) (0.01 < p < 0.05) were significant at 99% (p<0.01) and 95% (p<0.05) confidence levels. The overall interaction among temperature, exposure and spinosad rate was not significant (F = 0.88^{ns}, df = 20, 159, p>0.05). Based on the results it is concluded that temperature is the most important factor on mortality and could affect other factors.

Comparison of LD₅₀ values at three different temperatures and exposure time revealed that an inverse relationship between LD₅₀ values and temperatures does exist. A similar trend was observed between LD₅₀ values and exposure intervals (Table 1).

Adults of *S. oryzae*: Effects of application rate (F = 268.01**, df = 5, 159), time interval (F = 413.14**, df = 2, 159) and temperature (F = 952.32**, df = 2, 159), plus interactions viz., exposure × spinosad rate (F = 11.14**, df = 10, 159), temperature × spinosad rate (F = 21.84**, df = 10, 159) and exposure × temperature (F = 18.87**, df = 4, 159) and the overall interaction of temperature, spinosad rate and exposure (F = 3.391**,

Table 1: Probit regression estimates for adults *T. castaneum* exposed for 24, 48 and 72 h to spinosad at three temperatures

Temp. °C	Post treatment intervals															
	24 h						48 h						72 h			
	Intercept (±SE)	Slop (±SE)	LD ₅₀ (95% CL) (mg L ⁻¹)	χ ² (df)	p-value	Intercept (±SE)	Slop (±SE)	LD ₅₀ (95% CL) (mg L ⁻¹)	χ ² (df)	p-value	Intercept (±SE)	Slop (±SE)	LD ₅₀ (95% CL) (mg L ⁻¹)	χ ² (df)	p-value	
24	1.84 (0.59)	0.61 (0.17)	140363 (30161-26714745)	0.6 (3)	0.8 (9)	3.11 (0.46)	0.42 (0.13)	27811 (8569-3763959)	0.36 (3)	0.95 (0.44)	3.5 (0.5)	0.41 (0.15)	4613 (2270-33164)	0.07 (3)	0.99 (3)	
28	0.46 (0.51)	1.26 (0.15)	3836 (2976-5285)	4.08 (3)	0.2 (5)	2.6 (0.45)	0.8 (0.13)	973 (581-1409)	0.02 (3)	0.97 (0.5)	1.77 (0.15)	1.18 (0.15)	528 (334-724)	2.11 (3)	0.55 (3)	
32	0.99 (0.48)	1.2 (0.14)	2061 (1628-2721)	1.65 (3)	0.6 (4)	2.5 (0.48)	0.87 (0.14)	762 (427-1116)	0.68 (3)	0.87 (0.6)	2.66 (0.19)	1.06 (0.19)	162 (48-299)	0.89 (3)	0.82 (3)	

Since Goodness-of-fit of the probit model to concentration/response data is not significant (p>0.05), no heterogeneity factor is used in the calculation of confidence limits

Table 2: Probit regression estimates for adults *S. oryzae* exposed for 24, 48 and 72 h to spinosad at three temperatures

Post treatment intervals															
		24 h				48 h				72 h					
Temp. °C	Intercept (±SE)	Slop (±SE)	LD ₅₀ (95% CL) (mg L ⁻¹)	χ ² (df)	p-value	Intercept (±SE)	Slop (±SE)	LD ₅₀ (95% CL) (mg L ⁻¹)	χ ² (df)	p-value	Intercept (±SE)	Slop (±SE)	LD ₅₀ (95% CL) (mg L ⁻¹)	χ ² (df)	p-value
24	-	-	-	-	-	0.58 (1)	1.11 (0.33)	9449 (3783-284188)	0.36 (3)	0.94 (4)	-0.3 (0.89)	1.53 (0.29)	2932 (1988-6439)	1.8 (3)	0.61
28	0.64 (0.76)	1.37 (0.26)	1493 (1129-2404)	0.61 (3)	0.89	3.01 (0.71)	0.78 (0.24)	333 (80-53)	0.45 (2)	0.9 (0.9)	0.44 (0.32)	1.88 (0.32)	265 (161-352)	0.8 (3)	0.84
32	-0.8 (0.92)	1.85 (0.31)	1342 (1080-1835)	1.2 (3)	0.75	1.29 (0.92)	1.63 (0.33)	187 (81-278)	0.6 (3)	0.89 (9)	1.01 (2.1)	2.1 (0.8)	72 (0-158)	0.62 (3)	0.89

Since Goodness-of-fit of the probit model to concentration/response data is not significant (p>0.05), no heterogeneity factor is used in the calculation of confidence limits. Spinosad did not affect the adults of *S. oryzae* at 24°C and 24 h after treatment

Table 3: Mean mortality% (±SE) *T. castaneum* adults treated with different dosages of spinosad at different time intervals and temperatures

Temp. °C	Time interval (h)	Dosage of spinosad (mg L ⁻¹)					
		0	385	835	1811	3926	8511
24	24	0.63±0	12.61±4.20	15.06±1.38	16.02±5.46	25.53±1.72	28.28±1.00
	48	0.63±0	28.28±1.00	29.94±1.36	32.15±3.28	37.72±1.50	39.91±2.74
	72	0.63±0	35.44±1.94	37.75±0.84	40.61±2.80	44.28±1.36	47.92±3.54
28	24	0.63±0	13.70±4.80	22.46±7.68	40.64±2.78	45.74±3.00	52.52±4.38
	48	0.63±0	36.18±6.36	44.21±3.84	50.22±4.08	56.99±2.88	60.92±1.60
	72	0.63±0	42.08±4.30	51.57±2.50	56.66±4.26	71.07±6.54	79.34±5.80
32	24	0.63±0	22.65±1.66	36.05±3.38	45.00±1.16	52.29±1.92	60.06±1.36
	48	11.23±3.76	38.94±5.24	49.39±3.04	54.68±3.00	60.2±2.440	64.75±3.12
	72	17.06±1.38	58.95±6.78	61.22±3.60	72.45±6.70	77.67±4.52	78.75±3.74

Each mean is based on four replicates

df = 20, 159) were all significant for mortality at 99% confidence level (p<0.01). It means that different levels of spinosad rates, exposures and temperatures have different effect on mortality and all interactions affect each other significantly.

Based on Table 2 spinosad did not affect the adults of *S. oryzae* at 24°C and 24 h after treatment. Comparison of LD₅₀ values at three different temperatures and time intervals revealed that LD₅₀ values were decreased with increasing the temperature and exposure interval. The minimum value was of LD₅₀ (72 mg L⁻¹) was observed at 32°C and 72 h after treatment.

Adults of *T. castaneum*: Comparison of means (Table 3) showed that dosage of 8511 mg L⁻¹ caused the highest mortality rate of *T. castaneum* and mortality was increased with increasing the dosages in all temperatures. Results were similar to those reported by Eger *et al.* (1998) in which mortality of *Frankliniella* sp. showed a concentration dependent response and Toews and Subramanyam (2003) that reported mortality of *T. castaneum* was increased with increasing the dosage of insecticide. A biochemical reason for this similarity is imposing of the same enzymes with spinosad in these experiments. Mortality of adults in all dosages is dependent to time and 72 h after treatment caused the most mortality in all temperatures. Similar results have

been reported by Ludwing and Oetting (2001) for *Frankliniella occidentalis* Pergand. The interaction of rate×temperature affected mortality of adults positively. This result was different from that reported by Musser and Shelton (2005) for *Ostrinia nubilalis* Hübner. One explanation for unsimilarity of the results could be due to difference of the insects, species, enzymes that were imposed by spinosad in two species and bioassays conditions.

Adults of *S. oryzae*: Table 4 shows that dosage of 2009 mg L⁻¹ caused the most mortality rate of rice weevil and a direct relationship was observed between mortality and dosages. Similar trend was detected between mortality and temperature. Lewis and Neese (2005) reported the similar results for *Agrilus planipennis* Fabrimaire. Mortality of *S. oryzae* was increased with increasing the time and 72 h post-treatment caused the highest mortality similar to those reported by (Kristensen and Jespersen, 2004). These authors reported LD₅₀ and LD₉₅ values of spinosad on *Musca domestica* L. was decreased as time intervals increased. Temperature affected efficacy of spinosad for control of *S. oryzae* adults positively. Spinosad rate×temperature, rate×exposure and temperature×exposure interactions and the overall interaction rate×exposure×temperature affected mortality of rice weevil positively.

Table 4: Mean mortality% (\pm SE) *S. oryzae* adults treated with different dosages of spinosad at different time intervals and temperatures

Temp. °C	Time interval (h)	Dosage of spinosad (mg L ⁻¹)					
		0	282	461	753	1230	2009
24	24	0.65±0	0.65±0.00	0.65±0.00	0.65±0.00	0.65±0.00	0.65±0.00
	48	0.65±0	7.77±4.10	16.52±1.62	17.00±6.38	23.98±1.48	24.51±8.00
	72	0.65±0	14.25±5.30	18.15±1.88	21.06±2.38	33.17±1.20	39.96±5.18
28	24	0.65±0	23.90±1.54	29.86±2.08	35.20±1.66	39.90±2.08	50.78±1.58
	48	0.65±0	44.99±2.48	46.90±1.56	49.80±0.96	54.77±1.66	60.12±2.10
	72	14.89±0	49.80±0.96	54.77±1.66	63.44±0.02	71.78±1.86	82.10±4.10
32	24	11.33±3.56	23.98±1.48	29.70±2.94	33.90±4.46	43.04±3.52	55.82±1.94
	48	14.89±0	54.71±0.00	59.28±3.64	68.98±7.32	77.60±6.78	84.04±5.16
	72	16.52±1.62	73.39±1.60	82.76±6.44	85.65±3.54	89.20±0.01	89.20±0.01

Each mean is based on four replicates

At LD₅₀ value levels none overlapping of 95% CL was observed. It is concluded that adults of *S. oryzae* are more susceptible to spinosad than *T. castaneum*. The trend of species susceptibility to spinosad was similar to those reported by (Subramanyam *et al.*, 2004; Huang *et al.*, 2004 and Flinn *et al.*, 2004). One line of explanation for this similarity could be similarly of target enzyme. Comparison of regression lines revealed that the lines are parallel and only negligible differences were observed in intercept values. This could be due to differences between rearing conditions.

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