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SPINOSAD AFFECTS HEAT TOLERANCE AND HEAT ACCLIMATION OF *Tribolium castaneum* (HERBST) (COLEOPTERA:TENEBRIONIDAE) AND *Sitophilus oryzae (*L.) (COLEOPTERA:CURCULIONIDAE) ADULTS

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ABSTRACT

***Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) are serious pests of stored food. Exposure to high or low temperatures is effective in controlling these two species but accompany limitations. Spinosad, derived from bacterium *Saccharopolyspora spinosa,* is an effective insecticide but synergistic effect on heat- or cold-acclimated insects has not been reported. The objectives of this study were to determine if spinosad affects the mortality of *T. castaneum* and *S. oryzae* adults under temperature acclimated and unacclimated conditions. Adult insects were exposed to spinosad or water, acclimated at 35℃ and 40℃, and finally held at 45℃ for 0-30 h for *T. castaneum* and 0-12 h for *S. oryzae*. The mortality of adults at different durations was recorded and LT_{50} values were calculated. Exposure to spinosad before heat exposure reduced the heat tolerance of *T. castaneum* and *S. oryzae* adults. Acclimation at intermediate temperatures 35℃ and 40℃ increased the heat tolerance of both *S. oryzae* and *T. castaneum* adults. Further study is needed to determine the effect of spinosad on the heat tolerance and heat acclimation of other stored-product insect species.**

Keywords: Heat acclimation, Heat tolerance, LT_{50}, Spinosad, Stored-product insects

INTRODUCTION

The magnitude of losses caused by insects in the agricultural crop yield in storage varies geographically; 9% in developed countries and to 20% or more in developing countries (Phillips and Throne 2010; Wijayaratne *et al*., 2018). In Sri Lanka approximately 80% of the total loss in grains during storage occurs due to insect attack (Palipane 2001; Dissanayaka *et al*., 2018; Sajeewani *et al*., 2018). *Tribolium castaneum*, the red flour beetle, and *Sitophilus oryzae*, the rice weevil, are major pests of stored cereals, flour, nuts, spices, dried fruits and some pulses (Hill 1990; Hagstrum and Subramanyam 2006; Campbell *et al*., 2010). Current insect control methods in storage facilities mostly use synthetic contact insecticides (Arthur *et al*., 2019), or fumigants (Hwaidi *et al*., 2017). However, the factors such as phase out of effective fumigants (Andersen 2018), resistance in insects to the insecticides (Opit *et al*., 2012), and adverse impacts on the ecosystem (Phillips and Throne 2010) have emphasized the use of biorational alternatives in pest management. High temperature is used in the management of stored-product pests (Beckett *et al*., 2007). However, its expensiveness (Dosland *et al*., 2006) and possible damage to heat-sensitive equipment (Dowdy and Fields 2002) impose limitations for using this technology efficiently. Besides, the heat distribution inside a food processing facility is often uneven (Dowdy 1999) allowing insects to acclimate to high temperature and making them

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more difficult to be killed (Hagstrum and Subramanyam 2006).

Combination of treatments has been more effective in the management of stored-product insects than the use of a particular component alone (Banks 1987; Banks and Fields, 1995). Thus, heat increases the efficacy when used in combination with diatomaceous earth (Dowdy and Fields, 2002) and carbon dioxide + phosphine mixture (Mueller, 1994). Furthermore, Wijayaratne and Fields (2010) showed that Methoprene + heat combination reduces heat tolerance in *T. castaneum* enabling the protection of stored food infested with *T. castaneum* at a lower temperature which is economical. On the contrary, the said combination expands the use of methoprene formulation as a safe management tool for *T. castaneum*.

Spinosad derived from the bacterium *Saccharopolyspora spinosa* (Mertz and Yao 1990) has low mammalian toxicity (Thompson *et al*., 2000) and is environmentally benign (Cleveland *et al*., 2001). In general, spinosad has been tested against stored-product insects and its lethal effect has been reported (Athanassiou *et al*., 2011). Spinosad is effective in suppressing progeny emergence of *T. castaneum* (Dissanayaka *et al*., 2020). However, little is known how spinosad affects heat tolerance and heat acclimation of stored-product insects. Therefore, the objectives of this study were to determine if spinosad affects heat tolerance and heat acclimation of *T. castaneum* and *S. oryzae* adults.

MATERIALS AND METHODS

Test Insects

T. castaneum adults reared in wheat flour and *S. oryzae* reared on rice medium inside an incubator (FH-1200, Hipoint Laboratory, Taiwan) maintained at 30±0.5°C and 65±1% relative humidity. Adults about 30 days used for bioassay.

Preparation of Spinosad Solutions

The commercial product of spinosad (25 g/L) was used in the experiment. A concentration series (1, 6.25, 12.5, 18 and 25 ppm) was prepared by diluting the commercial product in distilled water. As the control, distilled water was used. From each concentration four replicate solutions were prepared. The spraying was conducted immediately following the preparation of solutions.

Spaying and introduction of adults

Red raw rice medium (a mixture containing 60 % whole grains and 40 % cracked grains) was used for spraying. For each treatment, 250 g rice medium was laid out on an aluminum foil. Spraying of insecticides was done using artist's air brush (Paasche Airbrush Company, Chicago, USA). From each concentration, 3 mL was sprayed on rice using an artist's airbrush (VL-202s, Paasche Airbrush Company, Chicago) followed by shaking for 30 s (Arthur 2004) for uniform distribution of the insecticide in the grain sample. From the sprayed medium, 15g was placed in each plastic vial (50 mL). Each vial later received 20 adults of *T. castaneum* or *S. oryzae*. Vials were maintained under ambient environmental conditions (30 °C and 65 % relative humidity) for 36 h, before used in the heat treatment experiments.

Heat tolerance experiments

The vials having rice medium and insects were divided into two batches. One batch of vials (heat acclimated batch) were placed in an oven (Mermmert, Schwabach, Germany) maintained at 35 ℃ for 6 h, 40 ℃ for 3 h and finally held at 45 ℃ for different durations. The second batch of vials (unacclimated for high temperature) was placed in oven directly at 45℃. The temperature inside the oven was measured by data loggers (TM-305U, Neihu, Taiwan). The vials having *T. castaneum* adults were removed at 0, 6, 9, 12, 15, 18, 24 or 30 h after exposure at 45℃. The vials having *S. oryzae* were removed following 0, 2, 4, 6, 8, 10 or 12 h at 45℃. Following removed from the oven, the vials were maintained for 12 h under room temperature (30 ℃) and number of dead adults was counted.

Experimental design

Heat tolerance experiments for *T. castaneum* and *S. oryzae* adults were conducted as Completely Randomized Design (CRD) with four replicates. This was a three-factor factorial experiment; three factors were concentration of spinosad, duration of exposure and heat-acclimated/unacclimated situation.

Data analysis

Percentage values of adult mortality were transformed using the square root of the arcsine to accommodate the unequal variances associated with percentage data. Data were analyzed using ANOVA procedures of Statistical Analysis System (SAS Institute 2002-2008). Means were separated by Tukey's test at P= 0.05 level. The LT_{50} (confidence intervals) for particular spinosad concentration was calculated using PoloPlus LeOra software. For a given acclimation, LT_{50} for each spinosad concentration was compared with that of water control.

RESULTS AND DISCUSSION

In *T. castaneum* adults, increase in the exposure period at 45 °C increased mortality. Up to 9 h, there were no differences between the mortality of insects in the spinosad-treated and control (treated with water) rice samples under both unacclimated and heat-acclimated conditions (Tables 1 and 2). Furthermore, in both unacclimated and acclimated conditions, between 12 and 24 h, *T. castaneum* adult mortality in spinosad-treated samples were higher than the respective control treated with water. The LT_{50} values of spinosad-treated adults were lower than control (treated with water) in both unacclimated and acclimated adults. This indicates that pre-exposure to spinosad made *T. castaneum* adults more sensitive to heat. By acclimating adults at 35 ℃ for 6 h and 40 ℃ for 3 h, the heat tolerance of *T. castaneum* adults was increased; in the control LT_{50} (Confidence intervals) increased from 15.9 (14.8-17.1) h for unacclimated adults to 21.4 (20.4-22.5) h. In the adults exposed to spi-

Table 1: Percentage mortality (mean±SE) of *T. castaneum* adults treated with spinosad and exposed to 45°C without acclimation and held for different durations (n =4).

Spinosad concentration (ppm)	Mortality±SE (%)[a]								LT_{50} (95% confidence intervals) (hrs)	LT_{50} ratio (95% confidence intervals)[b]
	Duration of exposure (hrs)									
	0	6	9	12	15	18	24	30		
0	0±0a	6.25 ±1.3 a	8.75± 2.4a	13.75 ±1.3b	38.75 ±1.3b	65± 2.9b	82.5± 1.4b	98.75 ±1.3a	15.90 (14.82-17.09)	1
25	2.5± 1.4a	8.75 ±1.3 a	13.75 ±2.4a	20±2a	46.25 ±1.3a	80±3. 5a	100±0 a	100±0 a	14.64 (13.75-15.44)	1.09 (1.08-1.11)

[a] For a given exposure, means followed by the same letter in a column are not significantly different at P = 0.05 according to Tukey's test following ANOVA

[b] LT 50 ratio = LT50 for control/LT50 for particular treatment. The LT50 values are not significantly different at p = 0.05, if the 95% confidence intervals for the ratio include 1.0.

nosad-treated rice, LT_{50} (Confidence intervals) increased from 14.6 (13.8-15.4) h in unacclimated adults to 18.6 (17.7-19.6) h in heat-acclimated adults. This shows that acclimation at intermediate temperatures increased the heat tolerance of *T. castaneum* adults in

Table 2: Percentage mortality (mean±SE) of *T. castaneum* adults treated with spinosad, acclimated at 35 ˚C for 6 hrs, 40 ˚C for 3 hrs, and held at 45 ˚C for different durations (n =4).

Spinosad concentration (ppm)	Mortality±SE (%)[a]								LT_{50} (95% confidence intervals) (hrs)	LT_{50} ratio (95% confidence intervals)[b]
	Duration of exposure (hrs)									
	0	6	9	12	15	18	24	30		
0	0±0a	0±0b	0±0b	3.75 ± 1.3b	16.25±1.3b	31.25±2.4b	55± 2b	90±2a	21.36 (20.37-22.50)	1
25	1.25 ±1.3 a	3.75 ± 1.3a	5±0a	10± 0a	30± 2a	47.5 ± 1.4a	72.5 ± 3.2a	93.75± 1.3a	18.611 (17.69-19.60)	1.14 (1.15 -1.16)

a For a given exposure, means followed by the same letter in a column are not significantly different at P = 0.05 according to Tukey's test following ANOVA

b LT 50 ratio = LT50 for control/LT50 for particular treatment. The LT50 values are not significantly different at p = 0.05, if the 95% confidence intervals for the ratio include 1.0.

Table 3: Percentage mortality (mean±SE) of *S. oryzae* adults treated with spinosad and exposed to 45 °C without acclimation and held for different durations (n =3).

Spinosad Concentration (ppm)	Mortality±SE (%)[a]							LT_{50} (Confidence intervals) hrs	LT_{50} ratio (95% confidence intervals)[b]
	Duration of exposure (hrs)								
	0	2	4	6	8	10	12		
0	0±0d	10±0d	15±2.8e	30±0d	43.3± 3.3e	71± 1.6e	100± 0a	6.91 (5.85-8.21)	1
1	6.6±1.6c	16± 1.6 cd	23± 1.6 de	36.6± 1.6 cd	58.3± 1.6d	76± 1.6 de	100± 0a	6.90 (5.99-7.62)	1 (0.98-1.08)
6.25	13.3± 1.6bc	21± 1.6c	30± 0cd	43.33 ±1.6c	68.3± 1.6 cd	80± 0cd	100± 0a	6.67 (5.91-7.27)	1.04 (0.99-1.13)
12.5	18.3± 1.6b	31± 1.6b	38± 1.6c	61.6± 1.6b	76.6± 1.6bc	83.3± 1.6bc	100± 0a	5.63 (4.58-6.35)	1.23 (1.28-1.29)
18	31.6± 1.6a	41.66 ±3.3b	51.66 ±3.3b	70±2.8b	83± 1.6b	86± 1.6b	100± 0a	5.38 (4.21-6.18)	1.28 (1.39-1.33)
25	43±4.4a	55±2.8a	66±1.6a	81±1.6a	91.6±1.6a	100±0 a	100± 0a	4.56 (3.17-5.34)	1.52 (1.85-1.54)

[a] For a given exposure, means followed by the same letter in a column are not significantly different at P = 0.05 according to Tukey's test following ANOVA

[b] LT 50 ratio = LT50 for control/ LT50 for particular treatment. The LT50 values are not significantly different at p = 0.05, if the 95% confidence intervals for the ratio include 1.0.

the water control as well as spinosad-treated batch. The non-overlapping nature of the confidence intervals of LT_{50} values between spinosad-treated and control batches in the heat acclimated *T. castaneum* proves that pre-exposure to spinosad decreased the heat acclimation in *T. castaneum* adults.

In *S. oryzae*, increase in the exposure of adults to 45°C increased their mortality than at 0 h exposure in both unacclimated and heat -acclimated batches. In the unacclimated insects, LT_{50} of spinosad-treated adults decreased than untreated controls at three higher spinosad concentrations tested 12.5, 18 and 25 ppm. In the heat-acclimated batch, the LT_{50} was lower than untreated control when the insects were pre-exposed to spinosad 1, 6.25, 18 and 25 ppm (Tables 3 and 4). This shows that pre-exposure to spinosad reduced heat tolerance in *S. oryzae* adults. As the confidence intervals of LT_{50} values between spinosad-treated and control batches do not overlap in the heat acclimated batch, spinosad has decreased the heat acclimation in *S. oryzae* adults.

The death of stored-product insects at increased temperatures occurs due to the changes occurred in the lipoprotein layers, balance of biochemical reaction rates, ionic activities or due to desiccation (Fields, 1992; Denlinger and Yocum, 1998). Synthesis of heat shock proteins (Lurie and Jang, 2007); increased levels of insect blood sugar trehalose (Singer and Lindquist, 1998), glycerol or sorbitol content

Table 4: Percentage mortality (mean±SE) of adult *S. oryzae* treated with spinosad, acclimated at 35˚C for 6 hrs and 40˚C for 3 hrs, and held at 45˚C for different durations (n =3).

Spinosad Concentration (ppm)	Mortality ± SE (%)[a] Duration of exposure (hrs)							LT_{50} (Confidence intervals) hrs	LT_{50} ratio (95% confidence intervals)[b]
	0	2	4	6	8	10	12		
0	0±0d	5 ±2.8b	10 ±2.8b	13.3 ±1.6d	26 ±1.6d	63 ±1.6b	83± 1.6cd	8.92 (7.45-11.44)	1
1	3.3 ±1.6cd	6.6 ±1.6b	15 ±2.8ab	15 ±0d	36± 1.6cd	63.3 ±1.6b	90± 2.8bc	8.63 (7.06 -11.34)	1.03 (1.05-1.01)
6.25	6.6 ±2.8bc	8.3 ± 1.6b	10 ±2.8b	18.33 ±1.6cd	46 ±1.6c	63 ±1.6b	81.6 ±1.6d	8.83 (8.16 -9.48)	1.01 (1.91-1.21)
12.5	10± 2.8abc	11.66 ±1.6ab	15 ±5b	25 ±0bc	33.3 ±1.6d	70 ±2.8b	90 ± 0bc	8.85 (8.18 -9.40)	1.01 (0.91-1.28)
18	13.3 ±1.6ab	13.33 ±4.4ab	21.6± 4.4 ab	31.6 ±1.6b	65± 2.8b	73.3±1.6b	95± 0b	7.63 (6.91 -8.22)	1.17 (1.08-1.39)
25	21.66 ±1.6a	28.3 ±1.6a	35 ±0a	45 ±2.8a	81.6± 3.3a	93±1.6 a	100± 0a	6.67 (5.74 -7.18)	1.34 (1.30-1.59)

a For a given exposure, means followed by the same letter in a column are not significantly different at P = 0.05 according to Tukey's test following ANOVA

b LT50 ratio = LT50 for control/LT50 for particular treatment. The LT50 values are not significantly different at p = 0.05, if the 95% confidence intervals for the ratio include 1.0.

(Denlinger and Yocum 1998; Wolfe *et al*., 1998), amino acids (Malmendal *et al*., 2006) or dopamine (Rauschenbach *et al*., 1993) are some physiological changes that insects undergo in response to heat stress to overcome adverse effects. One or more of these protective activities may have been adversely affected by spinosad making the insects more susceptible to increased temperature and hence reducing their survival following exposure to high temperature. Insects produce heat shock proteins when their body temperature rises above the optimal temperature for growth (Lurie and Jang 2007). Another study specified that heat acclimation enhances the survival of *T. castaneum* and *S. zeamais* adults subsequently exposed to high temperatures. Particularly, acclimation to high temperature significantly increases the heat tolerance of *S. zeamais* adults (Lu and Zang 2016).

This study highlights the success of using heat combined with the bacterium-derived insecticide spinosad for the management of *T. castaneum* and *S. oryzae* under laboratory settings. Further studies need to test this technology under practical situations such as inside food processing facilities to determine the possible drawbacks during implementation and suggest alternatives to overcome the same. Future tests also need to test the response of other stored-product insects to the combined use of heat and spinosad.

CONCLUSION

Pre-exposure to spinosad reduces heat tolerance of *T. castaneum* and *S. oryzae* adults. The mortality of both species is reduced by acclimation at 35^0C and then 40^0C before the exposure at 45^0C. Pre-exposure to spinosad also reduces heat acclimation of both species. Therefore, pre-exposure to spinosad increases the sensitivity of *S. oryzae* and *T. castaneum* adults to high temperatures.

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