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Effects of spinosad on the heat tolerance and cold tolerance of *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae)

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ABSTRACT

Sitophilus oryzae and *Rhyzopertha dominica* are serious insect pests of stored products in Sri Lanka. Currently pirimiphos methyl and phosphine fumigation are used as control measures but grain handlers seek for alternatives. Exposure to high or low temperature is popular in stored-product insect pest management but is expensive. Spinosad is effective against certain stored-product insects but has not yet been tested for its synergy with heat or cold. This experiment was conducted to evaluate the effect of spinosad on the heat tolerance and cold tolerance of *S. oryzae* and *R. dominica* adults. The experiment was a two-factor factorial, complete randomized design with four replicates. The spinosad concentration and exposure period were changed. Adults of *S. oryzae* and *R. dominica* were first exposed to a series of spinosad concentrations. Later they were held at higher (40 °C) or lower (6–11 °C) temperatures than room temperature for different durations.

Pre exposure of *S. oryzae* adults to Spinosad at 18 ppm or above synergized the adult mortality at high or low temperature showing a dose response. Pre-exposure of *R. dominica* adults to spinosad concentrations 12.5 ppm or higher synergized the mortality at high temperature whereas the spinosad synergized the mortality of *R. dominica* at low temperature when exposed to 6.25 ppm or higher concentrations; the effects followed a dose response. This study shows that heat and cold tolerance of *S. oryzae* and *R. dominica* adults are reduced by pre-exposure to spinosad. Therefore, spinosad is a potential grain protectant at high or low temperatures against these two insect species.

1. Introduction

Insects cause enormous quantitative and qualitative losses to stored food in Sri Lanka (Wijayaratne and Rajapakse, 2015). In developing countries, the magnitude of post-harvest losses reaches more than 20% of harvested yield (Rajapakse, 2001; Phillips and Throne, 2010); the loss is more prominent in tropical countries than in temperate climate (Wijayaratne et al., 2018). In Sri Lanka, annual post-harvest losses of cultivated crops reach approximately Rs. Million nine thousand. In grains, oil seeds and pulses, about 15% is lost during post-harvest operations: threshing, drying, transportation, storage, milling, etc. Storage loss of grains alone is 4–6%; insects are the most important factor as they cause 80% of this loss (Palipane, 2001; Wijayaratne and Rajapakse, 2015). Increased grain losses due to insects are frequently reported in Sri Lanka (Wijayaratne and Rajapakse, 2015).

Of the pest management methods available, synthetic insecticides have a quick lethal action on insects but they accompany negative impacts such as health risks (Arthur, 1996), effects on non-target organisms (Fields, 1992) and the environment (Phillips and Throne, 2010), and resistance development by insects (Wijayaratne et al., 2018). Accordingly, the pest management methods that use 'safer insecticides'/reduced-risk insecticides (Arthur, 2007) are considered to be better options as the treated commodities are later consumed. Besides, such attempts may prevent the negative effects accompanied by the synthetic insecticides on the nervous and/or respiratory systems of human (Phillips and Throne, 2010). Spinosad is a bacterial formulation (Subramanyam et al., 2007) derived from *Saccharopolyspora spinosa* (Bacteria: Actinobacteridae) (Mertz and Yao, 1990; Subramanyam

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et al., 2012). It is a reduced-risk insecticide due to its low mammalian toxicity (Thompson et al., 2000; Subramanyam et al., 2012), and safe on the environment (Cleveland et al., 2001). Spinosad effectively controls adults and immature stages of stored-product beetles, moths and psosids (Fang et al., 2002; Toews et al., 2003; Huang and Subramanyam, 2007; Athanassiou et al., 2008a,b,c; Chintzoglou et al., 2008a; Daglish et al., 2008; Hertlein et al., 2011; Athanassiou and Kavallieratos, 2014; Kavallieratos et al., 2016, 2017).

Survival of stored-product insects varies with the temperature (Fields, 1992). High (Dean, 1911) and low temperatures (Fields, 1992) have been used to control of stored-product insects but are expensive. Therefore, strategies to make these applications cost effective are timely needed. Combination of treatments has been successful as control measures against stored-product insects (Arthur and Dowdy, 2003; Wijayaratne and Fields, 2010). Spinosad has been tested in treatment combinations (Daglish, 2008; Daglish et al., 2008; Athanassiou et al., 2011; Athanassiou and Kavallieratos, 2014) but this has not been tested using high or low temperatures which facilitate heat treatments and cold treatments, respectively. Therefore, the objectives of this study were to evaluate effect of spinosad on the heat tolerance and cold tolerance of *Sitophilus oryzae* and *Rhyzopertha dominica*, major pests of stored grain and grain-based products in Sri Lanka.

2. Materials and methods

Experiment was laid out in a complete randomized design with two-factor factorial. Factor 1 was the different concentrations of Spinosad. Factor 2 was the different durations held at low or high temperature. A commercial preparation of Spinosad available at the local market was used as the insecticide. A concentration series (6.25, 12.5, 18 and 25 ppm) was prepared by diluting the commercial product 'Success' in distilled water. As the control, distilled water was used. For a given concentration and duration of exposure, there were four replicates; each replicate had twenty insects. In the preliminary experiments that tested the effect of ecdysteroid, the commercial product Mimic (containing Tebufenozide) was used whereas in the other experiment, the commercial product Rimon (Novaluron) was used as the chitin synthesis inhibitor.

Red rice (variety Basmati, 30% bran removed) was used as the medium to spray Spinosad. For each replicate, 15 g rice was placed in a 90 mm (diameter) Petri dish (Thermo Fisher Scientific Inc., USA). From each Spinosad concentration 0.5 mL was sprayed onto rice medium inside one Petri dish, using a disposable 3 mL syringe (Changzhou Medical Appliances General Factory Co. Ltd., Jiangsu, China). Later, the Petri dish was covered with the lid, and was hand-shaken well on a table for 30 s to ensure uniform distribution of the sprayed concentration. Spraying was conducted from the lowest to the highest concentration. Before spraying spinosad concentrations, control Petri dishes were sprayed with water. For each replicate concentration sprayed, a fresh syringe was used.

The Petri dishes containing rice sprayed with insecticide or water were kept overnight under ambient environmental conditions until the insects were introduced. Four week old, twenty adults of *S. oryzae* or *R. dominica* were introduced using a brush (No. 3) (Modern Teaching Aids Pty Ltd., Brookvale, Australia) into each Petri dish containing rice sprayed with Spinosad or water. The Petri dishes were then held at room temperature (32±1 °C) for 36–48 h. Later, they were kept in an oven (OF-12GW, JEIO Tech Co. Ltd., Korea) maintained at 40 °C for 0–24 h. Another batch of adult insects exposed to Spinosad was exposed to low temperature in a refrigerator (GR-282 MVF, Lec Refrigeration Plc, Bognor Regis, U.K.) at 6 °C (*R. dominica*) or 11 °C (*S. oryzae*) for 0–12 days. Temperature inside the oven and the refrigerator was recorded by Hobo data loggers (Onset Computer Corporation, MA, U.S.A.). Mortality of adults was counted 12 h following the termination of the exposure at low or high temperature.

Survival of adults at a particular exposure period to high or low temperature was transformed using the square root of the arcsine value, and analyzed using ANOVA procedure of Statistical Analysis System (SAS Institute, 2002–2008). Means of mortality of a particular duration of exposure were separated by Tukey's test at P = 0.05 level. The LT_{50} (Confidence limits) for a particular spinosad concentration and LT_{50} ratio were calculated using PoloPlus LeOra software. Lethal time (LT_{50}) for each concentration was compared with the water control. The LT_{50} ratio for a given concentration was determined as LT_{50} for water/LT_{50} for a particular concentration.

3. Results

Survival of *S. oryzae* adults pre-exposed to spinosad and subsequently maintained at 40 °C ranged from 1.25 ± 1.25% to 97.5 ± 1.44% compared to that in the controls treated with water, from 91.25 ± 3.75 to 100% (Table 1). Increase in the exposure period at high temperature (40 °C) or low temperature (6 °C or 11 °C) gradually decreased the survival of *S. oryzae* and *R. dominica* adults; this is true in the control (0 ppm spinosad) as well as 6.25, 12.5, 18 and 25 ppm spinosad concentrations. There were occasions where the gradual decrease in the survival was not observed (in the *S. oryzae* heat tolerance experiment, 18 h at 6.25 ppm, 8 h at 12.5 ppm, 18 h at 18 ppm). This may be due to heat distribution problems in the oven. In the heat tolerance experiment, this decrease in the survival is comparatively narrow (100% at 0 h to 91.25 ± 3.75% at 24 h, thus a difference of 8.25%) in adults treated with water (0 ppm spinosad) (Table 1). Similarly, 6.25 ppm exhibited a difference of 28.75%. In contrast, survival of adults exposed to 18 or 25 ppm had a broader reduction in the survival over the 24 h exposure period; 66.25% and 82.5%, respectively when the exposure to high temperature increased from 0 to 24 h. Exposure to 12.5 ppm reduced the survival by an intermediate margin, 51.25%. Overall this indicates that higher concentrations of spinosad have additional effect on decreasing the survival of *S. oryzae* adults. This effect is also demonstrated by the LT_{50} values and LT_{50} ratio. LT_{50} of *S. oryzae* adults at 40 °C decreased when the spinosad concentration was increased.

For *S. oryzae*, with no involvement of heat (at room temperature), adults exposed to water (control) showed no difference than those pre-exposed to 6.25, 12.5 or 18 ppm spinosad concentrations tested. In contrast, at majority of the exposure periods (8, 12, 15, 18 and 24 h), spinosad at 12.5, 18 or 25 ppm decreased the survival than the untreated control (treated with water). For the above decreased survival levels of adults, there was a dose response of spinosad (Table 1). According to LT_{50} ratio, the survival of *S. oryzae* adults was significantly lower at spinosad concentrations 18 ppm and 25 ppm. This indicates that spinosad synergized the mortality of *S. oryzae* adults at high temperature 40 °C.

Similar to high temperature exposure, at no exposure to low temperature (maintained at the room temperature), survival of *S. oryzae* adults did not show significant differences between water (control) and 6.25, 18 or 25 ppm spinosad concentrations for 24 h. However, pre exposure to Spinosad synergistically reduced the adult survival when they were subsequently held at low temperatures. The survival of *S. oryzae* adults significantly decreased when they were pre exposed to spinosad and held at 11 °C for 1, 2, 3, 4, 6, 8 or 10 days (Table 2). On the whole, exposure of *S. oryzae* adults to low temperature without pre-exposed to spinosad reduced their survival from 100% to 55 ± 3.54% over 10-day period (a difference of 45%). In contrast, pre-exposure to spinosad reduced survivals to 3.75 ± 2.39%, 11.25 ± 2.39% and 5% at 12.5, 18 and 25 ppm,