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Effect of Spinetoram on the Orientation of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Red Flour Beetle) Adults to Pheromone Traps

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ABSTRACT

Tribolium castaneum (Red Flour Beetle) is a cosmopolitan stored-product pest. Commercially available pheromone traps baited with its aggregation pheromone 4, 8-dimethyldecanal (4, 8 DMD) have a low trapping efficiency. Effects of biorational insecticides on orientation of *T. castaneum* have been reported. However, no such investigation has been carried out using spinetoram. Therefore, the objective of our study aimed to determine the effect of spinetoram on traps to catch of *T. castaneum* adults. The attraction of spinetoram pre-exposed to *T. castaneum* adults to pheromone traps were investigated under laboratory (experiment 1) and warehouse (experiment 2) conditions. Under laboratory conditions, the trapping of adults pre-exposed to spinetoram significantly differed from the respective control at each concentration. The highest trap catch (61.33%) was recorded on exposure to 31.25 ppm of spinetoram. The lowest trapping occurred at 0 ppm of spinetoram (water control). Under warehouse conditions, the highest trap catch was at 31.25 ppm and 46.875 ppm of spinetoram with no significant difference among them. The lowest trapping occurred at 0 ppm of spinetoram/ water control. The value obtained at 62.5 ppm was not significantly different from the value at 0 ppm. Our study showed that the pre-exposure to spinetoram enhanced the orientation of *T. castaneum* adults to pheromone traps baited with 4, 8 DMD, and the response was dose dependent. The observations made in this research may be useful in enhancing trapping efficiency of commercially available pheromone traps.

1. Introduction

Estimated losses by stored product insects during storage are 10% in temperate areas while 50% in tropical areas [1]. Out of this, 80% of storage grain losses in Sri Lanka are caused by insects [2]. Stored-product insects are common in warehouses, processing plants, flour mills and stores along the post-harvest distribution channels [3]. It is not easy to determine the actual pest population density in a given habitat. This complicates the stored-product pest management process. Semiochemicals such as pheromones and kairomones are used in monitoring of stored-product insects. Pheromones are used for intraspecific communication among the members of same species. Pheronomes attract individuals together for mating, to find oviposition sites, to face against predators or to overcome host resistance by mass attack. Therefore, they can be used to monitor, trap in large numbers and to disrupt mating. Aggregation pheromones and sex pheromones are widely used to monitor stored-product insects.

The red flour beetle (RFB) *Tribolium castaneum* (Coleoptera: Tenebrionidae) is a cosmopolitan stored-product insect that feeds on grains and grain-based products [4]. Both larvae and adults of *T. castaneum* cause serious damage to grains. *T. castaneum* secretes the aggregation pheromone 4, 8- dimethyldecanal (4, 8 DMD) that attracts both sexes [5]. Synthetic 4, 8- DMD is used in commercial traps to monitor *Tribolium* populations [6]. Dome traps are widely used to monitor populations of *T. castaneum* [6, 7]. However, the trapping efficiency of 4, 8 DMD used in dome traps is low as 25% [7, 8]. Thus, new methods are needed to improve the trapping efficiency.

Reduced-risk insecticides accompany advantages such as specificity on insects and low mammalian toxicity. Therefore, they are safer than the conventional neurotoxic insecticides. Spinosad and spinetoram belong to the spinosyns family of insecticides categorized as reduced risk insecticides. They are isolated from soil bacterium, *Saccharopolyspora spinosa.* Both spinosad and spinetoram disrupt nicotinic acetylcholine receptors and GABA receptors which affect impulse transmission [9].

Previous studies have shown that 20% trapping of *T. castaneum* adults is possible up to 60 cm from 4,8 DMD [7] and pre-exposure to spinosad enhances the orientation of *T. castaneum* adults to pheromone traps. Not much research has been conducted to test the efficacy of insecticides on the trap catch of insects [10]. Previous studies have sought the simultaneous use of insecticides and pheromones on stored-product moth species [11]. However, the studies investigating the effect of spinetoram on stored-product beetle species are scarce. The ability of spinetoram to alter the orientation response is not yet known. Therefore, it is important to study the effective distance of the 4, 8 DMD when adults are treated with spinetoram. Such studies might provide insights on the improvement of trap catch efficiency. Our study investigated whether there is an effect of various concentrations of spinetoram on the orientation to pheromone traps by *T. castaneum* adults under laboratory and warehouse settings.

2. Material and Methods

2.1 Rearing and sub-culturing of adults

Pure laboratory cultures of red flour beetles were used for the research. Then parent adults were pre-sifted using metal 850 µm sieve (ASTME11, W.S. Tyler Industrial Group, USA) andthey were counted using the vacuum pump (Rocker 300, Rocker Scientific Co Ltd., New Taipei City). Counted adults were introduced into plastic containers containing whole meal (wheat) flour at the rate of 200 adults/ 250g of wheat flour/ container. The parent adults were removed 14 days following introduction.These insect cultures were maintained inside an incubator (FH-1200, Hipoint Laboratory, Taiwan) under ambient environmental conditions (30±0.5^{o}C, 65±0.5% RH). One month following the emergence, the progeny adults were used.

2.2 Insecticide treatments

A concentration series having 15.625, 31.25, 46.875 and 62.5 ppm spinetoram was prepared by diluting the commercial preparation of spinetoram (Radiant, SC, DOW Agrosciences) using the distilled water. For 0 ppm (control), distilled water was used.

Four replicate solutions were prepared from each concentration separately. From each spinetoram concentration, 3.75 mL was sprayed on to separate 250 g of rice flour medium [7] using an artist's airbrush (VL-202s, Paasche Airbrush Company, Chicago). Following spraying, each replicate rice flour sample was kept for 2 days under laboratory conditions (30±0.5 ^{o}C, 65±0.5% RH). Then one-month-old 1000 *T. castaneum* adults were introduced into each replicate of flour medium. These adults were kept for two weeks inside an incubator at 30 ^{o}C and 65% RH and the survived adults were used in the experiments.

2.3 Experiment 1: Determine if pre-exposure to spinetoram affects the orientation of *T. castaneum* adults to pheromone traps under laboratory conditions

This experiment was conducted using a transparent glass chamber (45 cm x 30 cm x 15 cm) with two holes in the bottom plate and one hole on the top plate. Two plastic vials (each 60 mL) were placed underneath each hole on the bottom plate. Two rubber septa containing commercial pheromone lure (0.5 µL) were placed inside one vial one hour before introducing adults. The remaining vial was kept empty (without pheromone to consider as control). For each spinetoram concentration has control vial (without pheromone) separately. Then fifty *T. castaneum* adults pre-exposed to a particular spinetoram concentration (15.625, 31.25, 46.875 or 62.5 ppm) were introduced gently into the glass chamber through the top cover using a funnel. Two hours following adult introduction, the number of adults trapped inside each vial (pheromone containing vial and empty vial) was counted. As for the 0 ppm of spinetoram, *T. castaneum* adults exposed to flour sprayed with distilled water was used. Above procedure was repeated for all three replicates of each concentration.

2.4 Experiment 2: Determine if pre-exposure to spinetoram affects the orientation of *T. castaneum* adults to pheromone traps under warehouse conditions

This experiment was conducted inside an empty room. Experimental arena was 3.5 m x 2 m area on the cement floor. The boundaries of experimental arena were marked using polytetrafluoroethylene (Teflon) (Sigma Aldrich, Saint Louis, USA) to prevent escape of insects. Six Dome traps, each having two rubber septa baited with 4,8 DMD (Trece Inc., Adair, USA) were placed inside the experimental arena two hours before releasing of adults. Two hundred healthy adults exposed to flour treated with each spinetoram concentration (15.625, 31.25, 46.875 or 62.5 ppm) were released at the middle of the experimental arena separately.

Following 5 hours of adult introduction, the adults trapped inside each trap were counted. As for the 0 ppm of spinetoram, *T. castaneum* adults exposed to flour sprayed with distilled water was used. Above procedure was repeated for all four replicates tested from each concentration.

2.5 Experimental design

Experiment 1 was set up as two factor- factorial, Complete Randomized Design with three replicates. The two factors were the treatment exposure (spinetoram or water) and the spinetoram concentration (0, 15.625, 31.25, 46.875 or 62.5 ppm). The Experiment 2 was set up as Complete Randomized Design with four replications.

In the experiment 1, the percentage of *T. castaneum* adults moved into the vial having pheromone septa was transformed into using square root of Arcsin and analyzed using ANOVA of SAS [12]. The main effects of treatment of exposure and concentration as well as their interaction were analyzed. Means were compared using Tukey's test at $P \leq 0.05$. In the experiment 2, the percentage of adults trapped inside each dome trap was transformed into using square root of Arcsin and analyzed using ANOVA of SAS. Means were compared using Tukey's test at P=0.05

3. Results and Discussion

3.1 Experiment 1: Determine if pre-exposure to spinetoram affects the orientation of *T. castaneum* adults to pheromone traps under laboratory conditions

The trapping response of *T. castaneum* adults varied on exposure to spinetoram ($F_{1,20}$=1167.46, P<0.0001) and with its concentration ($F_{4,20}$=151.93, P<0.0001). Furthermore, treatment*concentration was also significant ($F_{4,20}$=44.91, P<0.0001). The *T. castaneum* adults pre-exposed to spinetoram at 15.625, 31.25, 46.875 ppm showed higher trapping response than those either exposed to spinetoram at 62.5 ppm or not exposed to spinetoram (water control). The highest trap catch (61.33%) was recorded in beetles exposed to 31.25 ppm of spinetoram. The second highest trapping occurred at spinetoram exposure of 46.875 ppm. The third higher level of trapping occurred following exposure to 62.5 ppm and 15.625 ppm of spinetoram that were not significantly different. The lowest trapping percentage was observed in adults pre-exposed to water (0 ppm of spinetoram).

Table 1: Percentage (mean±SE) *Tribolium castaneum* adults trapped following exposure to different concentrations of spinetoram under laboratory condition

Spinetoram Concentration (ppm)	% adults trapped in the control (mean±SE)[a]	% adults trapped in pheromone grip(mean±SE)[a]
0	0 ± 0d	34.67 ± 1.33D
15.625	10.67 ± 1.33c	47.33 ± 1.76C
31.25	27.33 ± 0.67a	61.33 ± 2.40A
46.875	22.00 ± 1.15ab	48.67 ± 0.67B
62.5	20.00 ± 1.15b	37.33 ± 2.91C

[a]Means followed by the same uppercase or lowercase letters are not significantly different at P= 0.05 according to Tukey's test following ANOVA

3.2 Experiment 2: Determine if pre-exposure of spinetoram affects the orientation of *T. castaneum* adults to pheromone traps under warehouse conditions

The trapping percentage varied with the spinetoram concentration ($F_{4,15}$=15.97, P<0.0001). The trapping percentages were highest when adults pre-exposed to spinetoram at 31.25 ppm or 46.875 ppm with no differences among them (Table 2). The lowest trapping percentage was recorded at 0 ppm of spinetoram/ water control which showed no significant difference with 62.5 ppm. The trapping of adults exposed to 15.625 ppm spinetoram was lower than at 46.875 ppm and higher than 31.25 ppm.

Table 2: Percentage (mean±SE) *Tribolium castaneum* adult trapped following exposure to different concentrations of spinetoram under warehouse condition

Spinetoram Concentration (ppm)	% adults trapped (mean ± SE)[a]
0	31.63 ± 3.52C
15.625	44.63 ± 2.18B
31.25	55.38 ± 1.93A
46.875	46.25 ± 1.16AB
62.5	38.13 ± 1.52C

[a]Means followed by the same letters are not significantly different at P= 0.05 according to Tukey's test following ANOVA.

According to the current study, trapping percentage was increased when *T. castaneum* adults were exposed to spinetoram 0 ppm (no spinosad exposure/ water control) to 31.25 ppm.

The highest trap catch was shown by *T. castaneum* adults pre-exposed to 31.25 ppm spinetoram under laboratory condition. But the trapping percentage was higher at 31.25 ppm or 46.875 ppm under warehouse condition. When the concentration of spinetoram was increased further, trapping percentage of beetles was decreased. Amount of aggregation pheromone produced by male adults of *T. castaneum* may vary due to different reasons. In crowded conditions, amount of aggregation pheromone production is lower than normal condition. The composition of isomers/ enantiomers of aggregation pheromone may also vary in different *T. castaneum* populations. Due to two chiral centres situated at C-4 and C-8, four conceivable stereoisomers can be synthesized (optical purity ≈100%) [13]. Then pheromone perception and their response to the synthetic pheromone may also be varied with the stereoisomers of 4,8- DMD [14].In addition to 4, 8- DMD, they produce some volatile compounds which serve as defensive/ repellent compounds such as 2-methyl-1, 4- benzoquinone [15, 16, 17]. During high concentration of insecticide exposure, they may produce such defensive compounds in high amounts, thus may reduce their pheromone perception. However, this needs to be investigated in a future research. Insecticides may interfere with pheromonal systems and affect sexual communication, which may disrupt mate choice and decrease mating success [18, 19, 20]. Latest findings show that pre-exposure to spinosad enhances the orientation of *T. castaneum* adults to pheromone traps which agrees with current study. According to the current study, higher trapping efficiency of *T. castaneum* was observed in laboratory experiment under limited/ controlled condition compared with warehouse. In the laboratory, space and air flow was limited. But in the current study, the warehouse experiment was conducted in 7 m^2 area. Distance from the pheromone or kairomone affects the movement of insects [21] and *T. castaneum* adults can effectively catch up to 60 cm from the pheromone source [7]. In the current warehouse study, distance between pheromone and insect releasing point was 87.5 cm. In addition to that, cracks and holes present in the warehouse gather more insects and sometimes they can't move towards the pheromone or away from the pheromone. Other than that, some insects tend to aggregate along the walls of the warehouse [6], thus may lead reduction of trapping them into dome traps maintained away from corners of the room. However, under real warehouse condition, these results may be altered due to the availability of live and/ or dead insect populations of in that environment [22]. This is due to the presence of a residual alarm pheromone produced by insects before death. This areas need to be further investigated in future research.

Previous studies have shown the direct effect of spinetoram on stored-product insects [23]. The current study reveals the possibility of using the same to facilitate trapping of *T. castaneum*. Thus, this study highlights the extended use of spinosad as a grain protectant.

The increased post-harvest losses of crop yield across the world amounting to one-third of the total food production or 1.3 billion tons annually is reported. This is a great challenge for the food security [24]. Furthermore, the unique role of insects on post-harvest losses has also been shown [25]. The findings of this study demonstrate new avenues for the protection of stored grains from devastating insect infestation and thus ensure food security.

4. Conclusion

Pre-exposure to spinetoram enhances the orientation to pheromone traps in *T. castaneum* adults in both laboratory and warehouse conditions. Therefore, the refugees following initial treatment of spinetoram in a storage facility can be further eliminated by the deployment of traps containing 4, 8- DMD. The improved trap catch through our method may resolve low trapping efficiency reported in commercially available pheromone traps having 4, 8 DMD, the aggregation pheromone of *T. castaneum*.

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