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Effects of spinosad and spinetoram on larval mortality, adult emergence, progeny production and mating in *Cadra cautella* (Walk.) (Lepidoptera: Pyralidae)

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A B S T R A C T

The bacterial formulations, spinosad and spinetoram, were evaluated for their efficacy in suppressing development and mating success in *Cadra cautella* (Walk.) (Lepidoptera: Pyralidae), the almond moth. A dilution series of spinosad and spinetoram was sprayed on rice flour. Rice flour samples sprayed with water served as the control. Late instar *C. cautella* larvae were introduced onto spinosad-, spinetoram-, or water-treated rice flour. The first experiment tested the effects of spinosad and spinetoram on larval mortality, as well as emergence of adults and progeny at different insecticide concentrations. In the second experiment, the mating success of *C. cautella* adults that had emerged from larvae exposed to spinosad was tested inside a cubicle. Both spinosad and spinetoram increased larval mortality, whereas both compounds reduced adult emergence and progeny production. Natural mating was reduced in the presence of the synthetic sex pheromone (*Z,E*)-9,12-tetradecadienyl acetate. However, exposure of *C. cautella* larvae to spinosad did not alter mating in adult progeny. Spinosad was more effective than spinetoram at suppressing *C. cautella* development. The study concludes that both spinosad and spinetoram suppress the development of immatures of *C. cautella* to the adult stage as well as mating. Thus, the both compounds can be used to protect stored grains from infestation by *C. cautella*.

1. Introduction

The global population is estimated to reach 9.8 billion by 2050 and 11.2 billion by 2100 (Pimentel et al., 1994; United Nations, 2017). This will place increased demands on the food supply. Reduction of losses following harvest of food is thus of paramount of importance. Massive quantitative and qualitative losses to stored commodities are caused by insects and have been reported throughout the globe (Hagstrum and Subramanyam, 2006; Wijayaratne et al., 2018). *Cadra cautella* (Walk.) (Lepidoptera: Pyralidae), the almond moth, is a cosmopolitan insect species (Sinha and Watters, 1985), and is especially abundant in warm, humid climates (Soderstrom et al., 1987). This species is polyphagous on stored commodities, including raw cereal grains, processed flours, nuts, dried fruits and seeds (Cox, 1975; Sinha and Waters, 1985; Hill, 1990; Arbogast et al., 2005; Burks and Johnson, 2012), and assorted dried spices (Hagstrum and Subramanyam, 2009; Hagstrum et al., 2012). It is further found in grain elevators and mills (Good, 1937; Sinha and Watters, 1985); in animal feed (Larson et al., 2008) and barley (Imura, 1981); and in different types of packaged food (Edde et al., 2012). Female moths exhibit high fecundity, producing400–500 eggs over their lifespan (Bell, 1975), and populations are able increase in abundance by 50-foldover a month (Sinha and Waters, 1985). This indicates the importance of adopting control measures to manage moth populations.

The current management methods available for *C. cautella* and other stored-product insects are based on synthetic chemicals facing restrictions on their continued use, including fumigants, because of their negative impacts on the environment (Andersen et al., 2018). Resistance by *C. cautella* has been detected to malathion and phosphine (Sinha and Watters, 1985), complicating their use. In addition, chemical control measures may have non-target effects on other insect species, workers, and the environment (Fields, 1992; Arthur, 1996; Phillips and Throne, 2010; Wijayaratne et al., 2018).As a consequence, the use of reduced-risk tactics have play a key role in diversifying integrated pest management

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programs for stored-product protection (Arthur, 2007). For example, reduced-risk insecticides such as insect growth regulators (Shaaya and Pisarev, 1986; Kostyukovsky and Trostanetsky, 2006; Wijayaratne and Fields, 2010; Wijayaratne et al., 2012a,b; Scheff et al., 2019), diatomaceous earth (Korunić et al., 2020), zeolite (Lü et al., 2017), long-lasting insecticide netting (Morrison et al., 2018) and biorational fumigants (Morrison et al., 2019) have been assessed against stored-product insects, either alone or in combination with other factors.

Using *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae) (Mertz and Yao, 1990; Subramanyam, 2006), the new biorational insecticide, spinosad, was developed (Subramanyam et al., 2007). The effects of spinosad on various stored-product insects has been elucidated to varying extents (Fang et al., 2002a,b; Toews et al., 2003; Huang and Subramanyam, 2007; Chintzoglou et al., 2008; Hertlein et al., 2011; Kavallieratos et al., 2017; Wijayaratne and Rajapakse, 2018; Dissanayaka et al., 2020). Spinetoram, a related compound, has also showed efficacy against several stored-product insects (Vassilakos et al., 2012; Isikber et al., 2013; Andrić et al., 2019). However, studies that have specifically evaluated effects of spinosad and spinetoram on *C. cautella* are lacking. Furthermore, effects of different concentrations of spinosad on mating success has not been investigated. Therefore, this study was conducted to determine if exposure of *C. cautella* larvae to spinosad and spinetoram affects larval mortality, adult emergence, and progeny production as well as mating in the F_1 (progeny) *C. cautella* adults. The study also tested if there is a dose response of spinosad and spinetoram on the above parameters.

2. Materials and methods

2.1. Experimental design

The experiment was conducted as a completely randomized design (CRD). The treatments consisted of different concentrations of spinosad and spinetoram to which *C. cautella* larvae were exposed. The effects of spinosad and spinetoram on the larval mortality, adult emergence and progeny production of *C. cautella* were tested. A second experiment tested how spinosad affects mating of *C. cautella* progeny.

2.2. Insect cultures

The laboratory population of *C. cautella* was initially collected from a rice mill in Puliyankulama, Anuradhapura, Sri Lanka. This population was maintained in the laboratory with regular culturing for nearly four years. The moths were reared inside an incubator (FH-1200, Hipoint Laboratory, Taiwan) maintained at 30 ± 1 °C, 65 ± 1% relative humidity (r.h.) using rice flour medium. Fifty adults from this population were introduced to 250 g of rice flour medium in a 5 L plastic bottle.

2.3. Insecticide application

The commercial products 'Success' (Spinosad 25 g/L SC,Hayleys Agriculture, Sri Lanka) and 'Radiant' (Spinetoram 25% WG, DOW AgroScience) were used in the current tests. A dilution series of spinosad (1, 6.25, 12.5, 18 and 25 ppm) and spinetoram (31.25 and 62.5 ppm) were prepared (Rumbos et al., 2018). Spinosad 25 ppm solution was prepared by diluting 100 μL of spinosad in 100 mL of distilled water. Using this stock solution, spinosad at 12.5 ppm was prepared by mixing 50 mL of 25 ppm spinosad solution in an equivalent amount of distilled water. Spinosad at 6.25 ppm was prepared by mixing 50 mL of 12.5 ppm spinosad solution in an equivalent amount of distilled water. The spinosad concentrations 18 ppm amd 1 ppm were prepared separately by diluting 72 μL and 4 μL of the commercial product in 100 mL distilled water. For spinetoram, a 62.5 ppm (label rate for field crops) solution was prepared by mixing 6.25 mg of spinetoram in 100 mL of distilled water. Using this solution, 31.25 ppm was prepared by mixing 50 mL of the 62.5 ppm spinetoram solution with an equivalent amount of distilled water. The dilutions of spinosad and spinetorm were prepared in volumetric flasks. Once the spinosad or spinetorm was added to water, they were mixed thoroughly 5 min by hand agitation. Rice flour was evenly spread into a layer with 1 mm thickness on aluminium foil. From each insecticide concentration, 3.75 mL was applied to 250 g of rice flour by using an artist's air brush (VL-202S, Paasche Airbrush Company, USA). After that, flour was put inside a zip lock bag and agitated by hand for 1 min.

There were n = 4 independent replicate solutions were prepared from each concentration of spinosad or spinetoram as described above. As the control, distilled water was used in place of insecticide solutions (Vayias et al., 2010), and rice flour was sprayed with the same amount of distilled water as did for spinosad or spinetoram.

2.4. Effects of spinosad and spinetoram on larval mortality, adult emergence and progeny production

Late instar larvae of *C. cautella*, aged 15 days, were introduced into the flour with 100 larvae per bottle. A subset of 20 larvae exposed to each insecticide concentration in the flour were randomly selected and included in a separate 1 L plastic container with 250 g of flour to determine the effect on larval mortality. After 5 d, the mortality of larvae was determined visually and by prodding, and live larvae remaining were each introduced into an individual plastic bottle (3.6 cm diameter and 6.2 cm height) with the treated (or control) flour. They were sexed at the pupal stage by referring to the two nodes on the ventral side located close to the genital scar in the 8th segment of male pupa (Zhu et al., 1999). The differentiation of male and female pupae was done under a microscope (OPTIKA, Triace, Italy). The sexed pupae were maintained individually inside the same container until adult emergence. Adult emergence in each bottle was recorded. From each replicate, 20 bottles were used to determine adult emergence. Subsequently, one male and female from each replicate were paired in an individual bottle (3.6 × 6.2 cm D:H) containing 10 g of untreated rice flour, and maintained for two weeks afterwards at 33 ± 0.5 °C and 65 ± 1% r.h. inside an incubator (FH-1200, Hipoint Laboratory, Taiwan). The larval progeny that were produced in each replicate bottle were counted. For each replicate treatment combination, 20 males and females were paired with each other in individual bottles. As the control, male and female adults emerging from rice flour treated with water were used.

2.5. Effects of spinosad on mating

To assess the effect of spinosad on mating when mating disruption is simultaneously deployed, rice flour was treated separately with each of the four replicate solutions of a given spinosad concentration at the rate of 15 mL/kg of rice flour using the same manner as described previously. In each replicate, 10 g of treated rice flour was put into a plastic bottle (3.6 × 6.2 cm D:H), which later received one late instar larva aged 15 days. Sexing was done at the pupal stage and each pupa was maintained individually in a separate bottle at 33 ± 0.5 °C and 65 ± 1% r.h. in the incubator (FH-1200, Hipoint Laboratory, Taiwan) until adult emergence. Newly-emerged male and female adults (2–4 d old) from each treatment were used in the experiment. A pheromone lure loaded with 4.5 mg of (*Z*, *E*)- 9,12-tetradecadienyl acetate (ZETA; Trece Inc., Adair, OK, USA) was placed inside a monitoring trap (Storgard® kit insect monitoring system, Trece Inc., Adair, OK, USA) and hung at the center of a cubicle (1.5 × 1.5 × 1.5 m L:W:H dimensions) at least 3 h before the introduction of moths (Ryne et al., 2001). The top, bottom and two opposite vertical sides of cubicle were lined with transparent polythene (25 mm thickness). The other two vertical sides were lined with insect-proof netting material which facilitated air circulation. The polythene and netting material were attached to the frame of the cubicle by Velcro (Garment Accessories.lk, Rajagiriya, Sri Lanka). A total of 10 emerged males and 10 females were introduced into the cubicle individually. After 24 h, all the adults were collected and placed inside a freezer at −10 °C for 2 h, and then female moths were dissected under microscope, and evaluated for the presence of spermatophores, which are indicative of mating success. There were 4 replicates tested from each spinosad concentration using 4 separate cubicles. In addition, to determine trap shutdown, a key variable in mating disruption success (Cardé and Minks, 1995), male moths captured by the sticky trap were counted.

Two types of control experiments were conducted. The first control experiment was conducted to differentiate the effect of exposure or non-exposure to spinosad on mating. For this, *C. cautella* larvae were exposed to rice flour treated with distilled water (15 mL/kg) following the same procedure mentioned before. That flour treated with water was added to plastic bottles (each 3.6 × 6.2 cm D:H) as 10 g/bottle. One late-instar *C. cautella* larva aged 15-d was placed inside each plastic bottle and maintained inside the incubator at 33 ± 0.5 °C and 65 ± 1% r.h. Each larva was sexed at the pupal stage, placed back inside the same bottle individually and kept inside the incubator until adult emergence. Those emerged adults were used in the mating experiments at 2–4 d of age. The second control experiment was conducted to differentiate the effect of hexane and ZETA on mating. For this, female and male *C. cautella* adults emerged in individual bottles from larvae exposed either to different concentrations of spinosad or water (as described previously) were introduced into the cubicle having hexane only (solvent used for diluting pheromone) (e.g. lacking the pheromone lure).

2.6. Data analysis

The percentage larval mortality and adult emergence at each spinosad or spinetoram concentration (or water control) were first transformed using the square root of the arcsine value to accommodate the heterogeneity of variances associated with percentage data (Zar, 1999). These data were then analyzed using ANOVA procedures of Statistical Analysis System (SAS) (SAS Institute, 2002–2008). The progeny production were directly analyzed using ANOVA procedures of Statistical Analysis system (SAS), because assumptions of normality and homogeneity of variance were fulfilled. In each case, the fixed explanatory variable was insecticide compound (spinosad and spinetoram) and concentration. The means were separated by Tukey's test, the significance was tested at $\alpha = 0.05$ significance level.

In the second experiment, which sought the effect of spinosad on mating, the percentages of mated females and males captures on sticky cards were square root- and arcsine-transformed before being analyzed using ANOVA procedures of Statistical Analysis system (SAS) (SAS Institute, 2002–2008). The means were separated by Tukey's test, the significance was tested at $\alpha = 0.05$ significance level.

3. Results

3.1. Effects of spinosad and spinetoram on larval mortality, adult emergence, and progeny production

The larval mortality was significantly different among the treatments ($F_{5,18} = 167.04$, $P < 0.01$). Larvae exposed to flour treated with the spinosad concentrations had higher larval mortality than those exposed to controls (Fig. 1). The larval mortality differed among different spinosad concentrations, and exhibited a dose-dependent response; mortality increased with increasing spinosad dose. The larval morality differed among spinosad concentrations in the following order: 25 ppm >18 ppm = 12.5 ppm > 6.25 ppm = 1 ppm (Fig. 1).

Adult emergence significantly differed among treatments as well ($F_{5,18} = 199.82$, $P < 0.01$). Emergence by larvae in spinosad-treated flour was lower than controls (Fig. 1). Furthermore, adult emergence varied among different spinosad concentrations, and

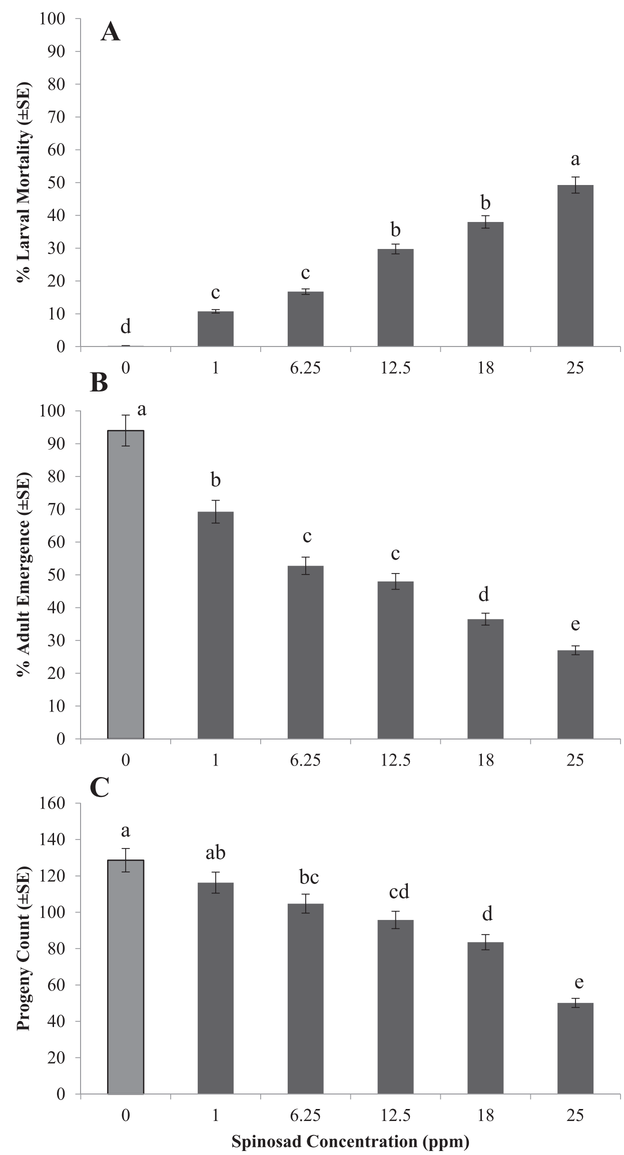

Fig. 1. Larval mortality (A), adult emergence (B) and progeny production (C) (mean±SE) of Cadra cautella following exposure to different concentrations of spinosad (n=20). Means followed by the same letter are not significantly different according to Tukey's test (a = 0.05).

also showed a dose-dependent response. Emergence increased among spinosad concentrations in the following order: 1 ppm > 6.25 ppm = 12.5 ppm > 18 ppm>25 ppm.

Progeny production by *C. cautella* adults after exposure as larvae differed among the treatments ($F_{5,18} = 54.22$, $P < 0.01$). Except for spinosad at 1 ppm, all the other concentrations recorded lower progeny production than the controls. The progeny production differed from 6.25 to 25 ppm in a dose-dependent manner, with decreasing progeny as the dose increased (Fig. 1).

Exposure to spinetoram also resulted in similar effects on *C. cautella*. The larval mortality of *C. cautella* among treatments was significantly different ($F_{2,9} = 212.99$, $P < 0.01$). Spinetoram at 31.25 ppm and 62.5 ppm showed higher larval mortality than the control (Fig. 2). The three treatments (31.25, 62.5 and 0 ppm) also resulted in significantly different percentages of adults emerging ($F_{2,9} = 142.05$, $P < 0.01$). Adult emergence was significantly lower in spinetoram-treated rice flour than controls. Furthermore, adult emergence decreased when the spinetoram concentration was increased from 32.5 ppm to 62.5 ppm (Fig. 2). Exposure of *C. cautella* larvae to spinetoram-treated flour also reduced the progeny production by adults in the next generation ($F_{2,9} = 505.81$, $P < 0.01$). For example, the two spinetoram concentrations both showed lower progeny production than the controls, but the higher concentration reduced progeny even further than the lower concentration (Fig. 2).

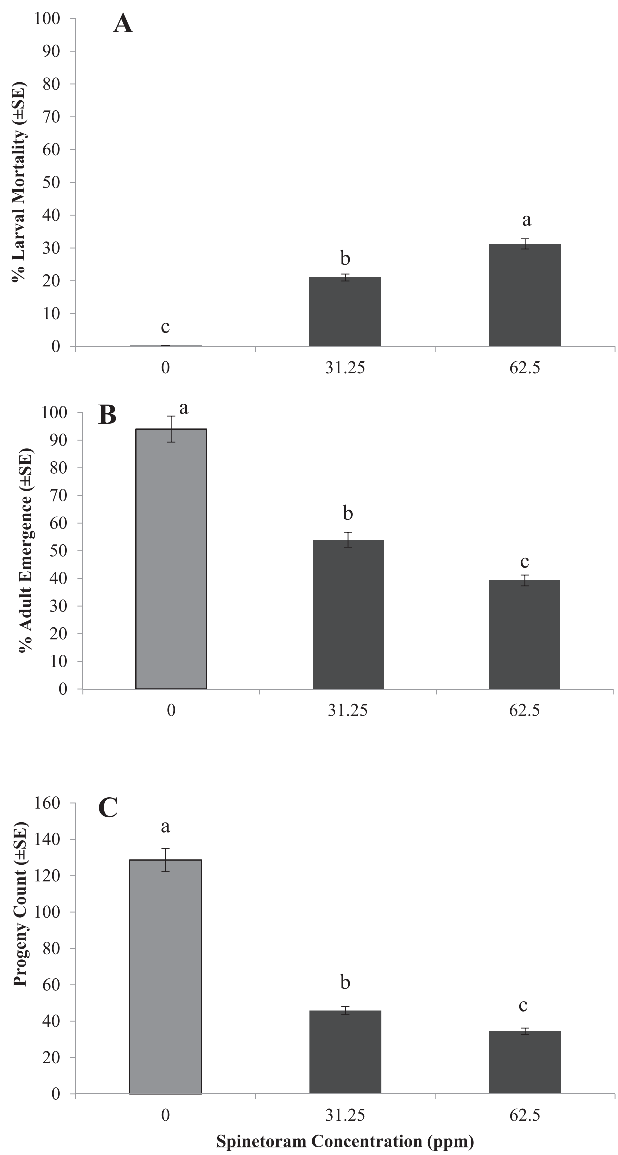

Fig. 2. Larval mortality (A), adult emergence (B) and progeny production (C) (mean±SE) of Cadra cautella following exposure to different concentrations of spinetoram (n=20). Means followed by the same letter are not significantly different according to Tukey's test (a = 0.05).

3.2. Effects of spinosad on mating

Overall, the percentage *C. cautella* females that were mated differed across the treatments ($F_{6,\ 21} = 55.32$, $P < 0.01$). This difference, however, was observed primarily between adult moths exposed to hexane and those exposed to the synthetic pheromone ZETA. None of the spinosad or water control treatments differed in percentage of mating success (Fig. 3). Male attraction was not significantly different between any of the treatments ($F_{6,\ 21} = 0.5$, $P = 0.80$). As a result, regardless of treatment *C. cautella* males were captured in equal abundance (Fig. 3).

4. Discussion

Fang et al. (2002a) reported that in *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), the Indian meal moth, spinosad at 1 ppm causes >97% mortality of larvae and suppresses adult emergence from eggs by at least 93%. However, in the current study, only 11% mortality of *C. cautella* larvae was observed after exposure to 1 ppm spinosad on rice flour. Nonetheless, prior work has documented that increased concentrations of spinosad and spinetoram result in higher *C. cautella* larval mortality (Rashed et al.,

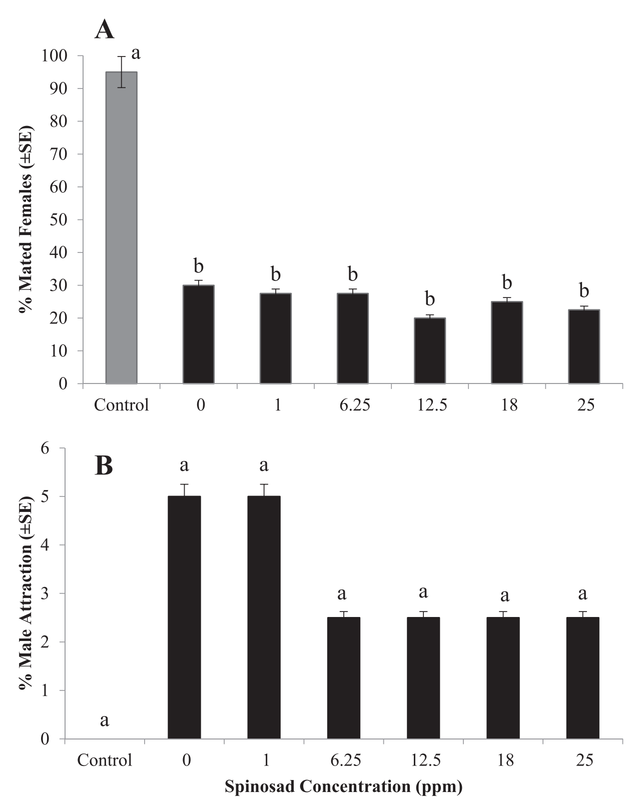

Fig. 3. Percentage (mean±SE) of successfully mated Cadra cautella females (A) and male Cadra cautella captured on a pheromone-baited sticky card (B) following exposure to different concentrations of spinosad (n=4) in a wind tunnel. Means followed by the same letter are not significantly different according to Tukey's test (a = 0.05).

2018). Our work supports this finding. Previously, spinosad or spinetoram at 0.4 mL/L has been documented causing >85% mortality of *C. cautella* larvae on treated dried dates within one day (Rashed et al., 2018). However, here we found that the highest mortality of *C. cautella* larvae was 49% at 25 ppm spinosad and 31% at 62.5 ppm spinetoram following a 5-d exposure. In food facilities, suppression of the subsequent generation is a good characteristic of a grain protectant (Arthur, 1996). Previous research has indicated that spinosad has the ability to provide long-term protection with higher efficacy than chlorpyrifos-methyl for *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), the lesser grain borer, and *P. interpunctella* (Fang et al., 2002b). We have found here that spinosad and spinetoram suppressed the progeny production of *C. cautella*.

Little information has been available on the effect of spinosad on *C. cautella.* In general, as mentioned below, the susceptibility of lepidopterans to spinosad is high. A reduction of more than 95% of the F_1 progeny production by *P. interpunctella* and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), the Angoumois grain moth, is reported when maize seeds were treated with spinosad at 1 ppm (Szabela, 2005; Huang and Subramanyam, 2007). In *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), the rice moth, immatures were completely killed by 0.5–2 mg/kg of spinosad (Sharma and Michaelraj, 2006), while larval mortality was >93% at 0.5–1 mg/kg of spinosad when sprayed on maize and sunflower seeds (Huang and Subramanyam, 2003). Similarly, we found a reduction in the progeny production and increase in the larval mortality for *C. cautella*, which is in alignment with prior work for other stored-product moth species.

The current study revealed that the progeny production by *C. cautella* declined with the increased spinosad concentration. By contrast, the mating percentage of *C. cautella* was not varied with the concentration of spinosad. This is comparable with a previous study by Wijayaratne et al. (2012a) where the reduced progeny production by *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle adults previously exposed to methoprene as larvae was not varied with the concentration. Future studies should investigate the factors underlying these outcomes. Furthermore, as performed by Wijayaratne et al. (2012a), exposing only one of the sexes to insecticide (spinosad/spinetoram) followed by pairing with untreated opposite sex would enhance understanding on the mode of action of these insecticides on progeny production.

Previous studies evaluating spinetoram efficacy on stored-product Lepidoptera in food facilities is very rare. However, there has been some work evaluating spinetoram on stored-product Coleoptera. For example, the larval mortality of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), the confused flour beetle, following 21-d exposure to spinetoram was low (<15%) with no differences between two doses (Rumbos et al., 2018). Larval mortality achieved 21–31% in 5-d exposures at 31.25 and 62.5 ppm of spinetoram in our study. By contrast, both young and old larvae of *T. castaneum* suffered 100% mortality after 14-d of exposure to spinetoram at 0.05 and 0.1 ppm (Saglam et al., 2013). We found adult emergence in *C. cautella* varied with spinetoram concentration, but only with a maximum 31% larval mortality observed at a much higher 62.5 ppm of spinetoram following 5-day of exposure.

Reduced progeny production has been reported in the granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *T. castaneum* and *T. confusum* after exposure to spinetoram at 0.25 and 0.5 ppm (Rumbos et al., 2018), while the same has been reported for *Sitophilus oryzae, S. granarius, R. dominica, Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) the larger grain borer and *T. confusum* after spinetoram exposure at 0.01–10 ppm (Vassilakos et al., 2012; Vassilakos and Athanassiou, 2013). Rumbos et al. (2018) observed no differences in progeny production between the two doses of spinetoram at 0.25 and 0.5 ppm. By contrast, we used much higher spinetoram doses in this study comparatively, with *C. cautella* progeny production reduced at both concentrations, but not proportionally to the increase in concentration. Spinetoram sprayed on rice does not suppress progeny in *S. oryzae* but does so in wheat (Athanassiou et al., 2008). However, *R. dominica* progeny declined on both rice and wheat sprayed with spinetoram (Vassilakos and Athanassiou, 2012). Because the commodity used in prior work affected the efficacy of spinetoram (Vassilakos and Athanassiou, 2012; Rumbos et al., 2018), future research should evaluate how effective spinetoram is against *C. cautella* on a variety of other grains types given its polyphagous nature. *C. cautella* undergoes larval diapause (Hagstrum and Sharp, 1975; Bell et al., 1983), which may affect susceptibility to spinosad and spinetoram. Other lepidopteran species such as *P. interpunctella* also undergoes larval diapause (Bell and Savvidou, 1991; Wijayaratne and Fields, 2012), and exhibits tolerance to insecticides such as methyl bromide during this phase in their life cycle (Bell and Savvidou, 1991). As such, it may be worthwhile to investigate if the differential susceptibility to spinosad and spinetoram observed in *C. cautella* is linked with the diapause. Overall, the current study shows that both spinosad and spinetoram reduce larval survival, adult emergence and progeny production in *C. cautella*; spinosad is more effective than spinetoram. Mating is reduced by spinosad but its success not altered by different concentrations of spinosad.

CRediT authorship contribution statement

A.M.P. Sammani: Conceptualization, Investigation, Data curation, Formal analysis, Writing - original draft. **D.M.S.K. Dissanayaka:** Investigation. **L.K.W. Wijayaratne:** Conceptualization, Methodology, Validation, Supervision, Writing - review & editing, Resources, Funding acquisition, Project administration. **W.R. Morrison:** Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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