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Efficacy of Spinetoram Against Red Flour Beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

This study, conducted at Department of Plant Protection, Faculty of Agriculture, Ankara University, aimed to evaluate the efficacy of Spinetoram (Radiant 120 SC) against the red flour beetle, *Tribolium castaneum*, across three temperatures (20, 25, and 30 °C) and at 65% relative humidity. Adults of *T. castaneum* were exposed to soft wheat treated with Spinetoram at concentrations of 0.01, 0.25, 0.50, 0.75, 1.00, 2.00, 5.00, and 10.00 ppm in PVC vials for of 1, 2, 3, 7, and 15 days. Bioassays at each dose rates were repeated 3 times with three replicates each. Adult mortality data were analyzed by repeated measures analysis of variance technique and the differences between the means were determined by Tukey's test. Mortality rates were found to be proportional to the dose, temperature, and exposure time. At 0.01, 0.25, 0.5, and 0.75 ppm doses, mortality rates were similar to those of the controls. At 1 ppm dose, mortality rates were 37.04 %, 52.59 %, and 72.59 % after 15 days of exposure at 20, 25 and 30 °C, respectively. The mortalities were 80.74 %, 68.52 % and 81.85 % at 2 ppm dose, 92.59 %, 98.15 %, 90.37 % at 5 ppm dose, and 98.89 %, 99.26 % and 99.63 % at 10 ppm dose, respectively. The development of F1 adults was significantly suppressed at 2 ppm, with the suppression rates of 80.0 %, 63.7 %, and 45.3 % at 20, 25, and 30 °C, respectively. In conclusion, Spinetoram can be considered as an alternative to chemical protectants in the control of *T. castaneum*.

Research Article

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1. Introduction

Wheat has been the most fundamental food source for humans throughout history. The history of collecting grains from nature dates back to 17.000 BCE (Tanno and Willcox, 2006). It is known that wheat was first domesticated in southeastern Turkey (Diamond, 2006). Today, it is known that there are many types of wheat and wheat is generally divided into three groups. These are defined as durum, bread and biscuit wheat (Kurt, 2012). Wheat has historically been one of the most internationally traded agricultural commodities. The main reason for this is that wheat is a staple food for many countries.

In recent years, the global population density has been steadily increasing, leading to a corresponding rise in the demand for food. Therefore, it is important to develop different methods to achieve high yields in agricultural production, which plays a crucial role in human nutrition, and to protect it from agricultural pests (Schöller et al., 1997; Azizoglu et al., 2011). From farm to fork, a range of factors can cause product losses at each stage. The causes of these losses may include climate events, diseases and pests, and agricultural infrastructure problems. Typically, losses caused by organisms of animal origin in stored products are estimated to be an annual average of 10 % (Donahaye and Messer, 1992). This damage rate can vary depending on the level of contamination. Studies have shown that losses caused by stored product pests can reach up to 100 % (Sallam, 2013).

The flour beetle, *Tribolium castaneum*, is a widespread pest of stored products, particularly those derived from wheat, such as flour. This species also inflicts damage on products like pasta, dried fruits, biscuits, and nuts, leading to significant losses (Sinha and Watters, 1985; Mills and Pedersen, 1990; Karunakaran et al., 2004). Additionally, this pest has a very high reproductive potential (Prakash et al., 1987).

Chemical control of these agricultural pests has been ongoing for a long time (Jackson and Jaronski, 2009). In recent years, studies have shown that the intensive use of chemicals has negative effects on human health, food safety, and the environment (Moore and Prior, 1993; Arthur, 1996; Zettler and Arthur, 2000; Ayvaz et al., 2008; Teng et al., 2013; Zhang et al., 2016; Tang et al., 2018; Perez-Parada et al., 2018; Al-Ghaim et al., 2019). The most common method used to combat stored product pests is chemical control, and it is reported that approximately 297 tons of pesticides are used for this purpose in our country (Emekçi and Ferizli, 1999).

Due to the successful results, synthetic pesticides are being heavily used in the fight against stored product pests (Tiryaki et al., 2010). These pesticides include crop protectants such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, as well as fumigants like methyl bromide and aluminum phosphide (Bond et al., 1984; Taylor, 1994). Recently, with the increasing environmental awareness and the negative effects of pesticides on human health, there has been a search for alternatives that are more rapidly and easily degradable compared to synthetic chemicals, and have minimal impact on non-target organisms and the environment (Arnason et al., 1989; Feng and Isman, 1995; Wewetzer, 1995).

Spinetoram is a low-risk commercialized insecticide resulting from the chemical modification of spinosyns (spinosyn L and J) produced by the fermentation of the bacterium *Saccharopolyspora spinosa*, which belongs to the Actinomycete class (Mertz and Yao, 1990; DeAmicis et al., 2011). Compared to spinosad, spinetoram is a new spinosyn insecticide with faster action and higher potency (Dripps et al., 2008). When examining the mechanism of action of spinetoram, it is found to be effective on nicotinic acetylcholine and gamma-aminobutyric acid (GABA) receptors (Williams et al., 2003; Dripps et al., 2008). Spinetoram shares the same mechanism of action as spinosad, being effective through both contact and ingestion. When the correct dose rate is applied, it causes the pest to stop feeding within 24 hours, leading to death (Dripps et al., 2008).

Spinetoram is a broad-spectrum insecticide effective against harmful insects on numerous plants. It is effective against orders such as Lepidoptera, Thysanoptera, and Diptera. When applied at low rates, spinetoram shows low toxicity to predators belonging to the families Coccinellidae and Chrysopidae, as well as those in the order Hemiptera (DeAmicis et al., 1997; Copping and Menn, 2000; Williams et al., 2003; Gamal et al., 2007; Mahmoud and Osman, 2007).Spinosad is an insecticide with low toxicity to mammals, with an oral acute LD50 value of > 5000 mg kg-1 in rat trials (Thompson et al., 2000). Spinetoram was reported to be less toxic to the wasp *Bombus terrestris* (L.) (Hymenoptera: Apidae) than spinosad (Besard et al., 2011).

In this study, it was aimed to evaluate the lethal efficacy of Spinetoram, against *Tribolium castaneum* adults at different dose rates under 3 different temperatures (20 °C, 25 °C, and 30 °C) in the laboratory.

2. Materials and Methods

2.1. Test species

In this study, *Tribolium castaneum* (L.) reared in the stored product pests laboratory of the Plant Protection Department of the Faculty of Agriculture, Ankara University, was used.

2.2. Rearing of test species

Tribolium castaneum was maintained on a mixture of grounded soft wheat kernels and powdered dry yeast (5 %). The mixture was sterilized in a deep freezer for 72 hours, and then added to 1-liter glass jars in amounts of 250-300 grams. The mouths of the jars were covered by muslin cloths which were secured with rubber bands. The culture jars were kept in a temperature/humidity controlled room at 25 ± 1 °C and 60 ± 5 % relative humidity throughout the experiment.

2.3. Spinetoram

Spinetoram (Radiant 120 SC, Corteva Turkey Tarım A.Ş.) is used at the concentrations of 0.01, 0.25, 0.5, 0.75, 1, 2, 5, and 10 ppm

2.4. Bioassays

For each dose level, 500 grams of soft wheat was treated with 5 mL Spinetoram solution using a Badger Airbrush. The control group was treated with distilled water only.

The bioassays were conducted using 100 ml plastic vials, each contained 50 grams of wheat treated with the respective dose of Spinetoram and 30 *T. castaneum* adults of 7-21-day-old. Bioassays at each dose rates were repeated 3 times with three replicates each.

Test vials were placed in PVC boxes (28 x 40 x 16 cm, W:L:H) containing KOH solution for maintaining the humidity at 65 % (Solomon 1951), and they were kept in a temperature controlled incubator (Binder KB720, Germany) for 15 days. Adult mortalities were assessed 1, 2, 3, 7, and 15 days after the introduction of adults. In each counts till to last one, dead adults were removed while alive ones were returned to the same test vials. In the final count, all the adults, dead or alive, were removed, and the treated wheats were kept in the same vials for an additional 50 days to assess F1 adult development.

The study was conducted at 20, 25, and 30 °C and 65 % R.H., which were monitored continuously using Hobo® ProTemp/RH data loggers (Onset Comp. USA) placed in each PVC boxes.

2.5. Statistical analyses

The mortality data were analyzed using Generalized Linear Models (GLM) with repeated measures ANOVA, and mean differences were assessed with Tukey's test at a significance level of 0.05. No correction was made in the adult mortalities due to low mortalities in the control groups.

3. Results and Discussion

3.1. The efficacy of Spinetoram against *Tribolium castaneum* adults

Statistical analyses revealed a significant interaction between exposure time, temperature, and dose rate (Table 1). Mortality rates at all the temperature levels increased with increasing dose and the exposure time In the control groups, mortality rates were observed to be low, only due to natural deaths. Mortality rates at the dose levels of 0.01, 0.25, 0.5, and 0.75 ppm were also observed to be low, similar to those of the control groups. At 1 ppm dose, mortality rates were 37.04 %, 52.59 %, and 72.59 % after 15 days of exposure at 20, 25 and 30 °C, respectively. The mortalities were 80.74 %, 68.52 % and 81.85 % at 2 ppm dose, 92.59 %, 98.15 %, 90.37 % at 5 ppm dose, and 98.89 %, 99.26 % and 99.63% at 10 ppm dose, respectively (Fig. 1, 2, and 3.).

Table 1. GLM repeated measures ANOVA test results

	SS	Degrees of freedom	MS	F	p
Intercept	878281	1	878280.7	3890.81	0.000000
Temperature	4018	2	2008.8	8.89	0.000193
Dose	1210675	8	151334.3	670.41	0.000000
Temperature*Dose	10331	16	645.7	2.86	0.000297
Error	48758	216	225.7		
Exposure time	73126	4	18281.4	1105.60	0.000000
Exposure time*Temperature	1119	8	139.9	8.46	0.000000
Exposure time*Dose	104447	32	3264.0	197.39	0.000000
Exposure time*Temperature*Dose	4581	64	71.6	4.33	0.000000
Error	14286	864	16.5		

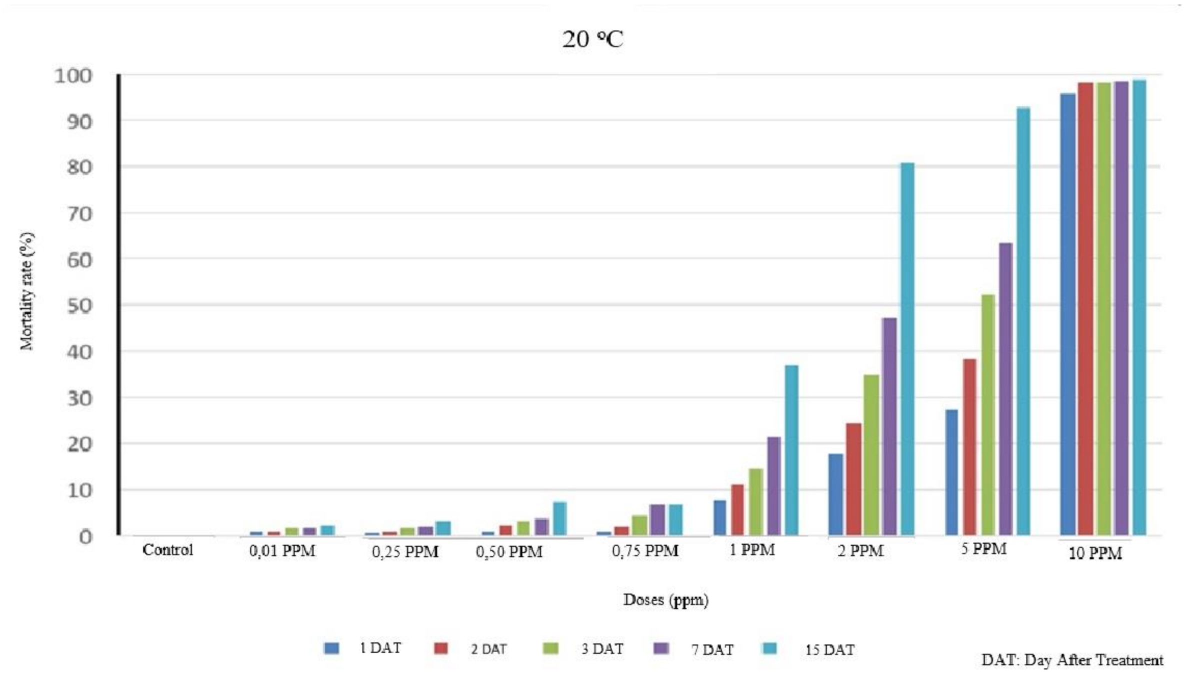

Figure 1. Adult mortality in *Tribolium castaneum* -at 20 °C

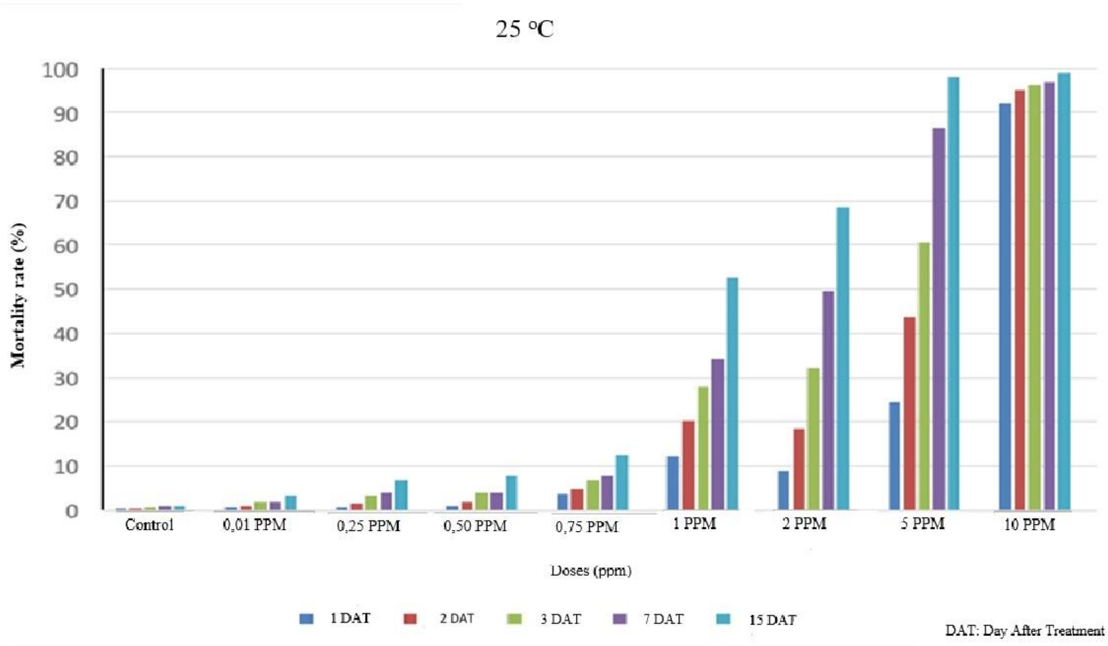

Figure 2. Adult mortality in *Tribolium castaneum* -at 25 °C

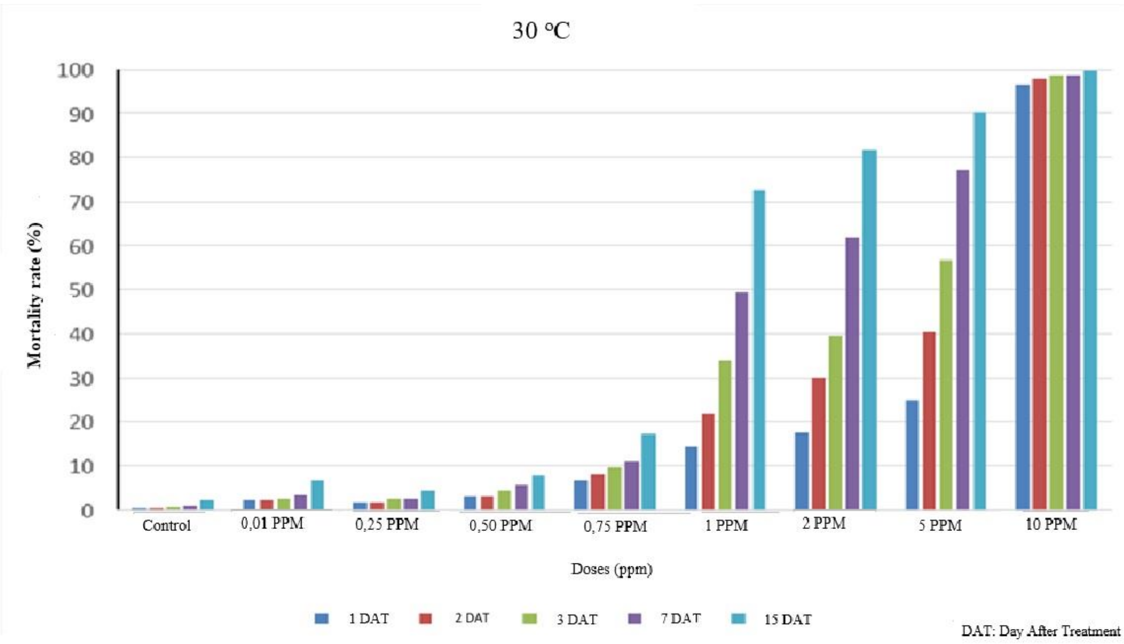

Figure 3. Adult mortality in *Tribolium castaneum* -at 30 °C

3.2. The effect of spinetoram on F1 adult development of *Tribolium castaneum*

Development of F1 adults of *Tribolium castaneum* has been suppressed in a dose dependent manner. It was observed that Spinetoram applied at doses of 0.75 ppm and lower did not suppress the population growth at all three temperature levels (20, 25, and 30 °C). The development of F1 adults was suppressed by 56.3 %, 57.0 %, and 51.3 % at 1 ppm dose at 20, 25, and 30 °C, respectively, and by 80.0 %, 63.7 %, and 45.3 % at 2 ppm dose, respectively. At 5 and 10 ppm, almost the entire populations were suppressed at all three temperature levels (Figure 4).

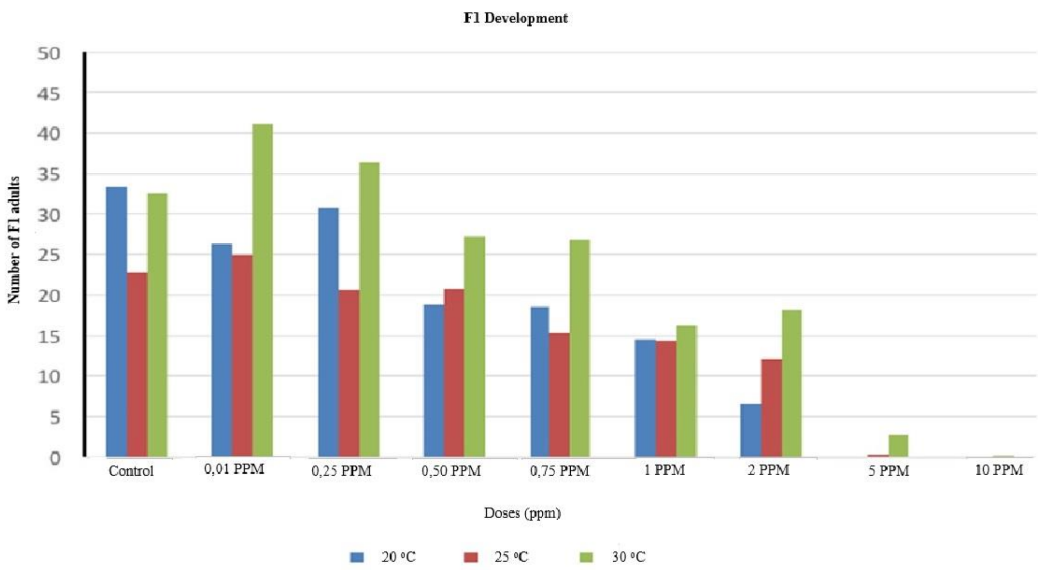

Figure 4. The effect of Spinetoram at different temperatures and doses on the F1 development of *Tribolium castaneum*

Tribolium castaneum is one of the most resistant species to insecticides among stored product pests. For example, in a study conducted by Arthur et al. (2004), *Sitophilus zeamais*, *Oryzaephilus surinamensis*, and *Tribolium castaneum* adults were exposed to maize treated with thiametoxham at the doses of 0, 0.5, 1.0, 2.0, and 4.0 ppm at 22-27 °C and 32 °C for 1-6 days. For *S. zeamais*, a mortality rate of 58-90 % was observed at a 0.5 ppm dose, and a mortality rate of 95-100 % occurred at a 4 ppm dose. *O. surinamensis* showed more tolerance compared to *S. zeamais*, with a mortality rate of 18-80 % at a 5 ppm dose. In the experiment with *T. castaneum*, the mortality rate did not exceed 40 % on the 1st, 2nd, and 3rd days for all doses. At 27 °C, the mortality rate did not exceed 20 % on the 1st and 2nd days. Complete mortality could only achieved at 32 °C by the 6th day.

In a laboratory study conducted by Sağlam et al. (2016), adults of the bean weevil, *Acanthocelides obtectus* Say. (Coleoptera: Chrysomelidae) were exposed to bean treated with spinetoram at 0.1, 0.25, 0.5, and 1 ppm dose rates at 26±1 ºC, and 65±5 % R.H. for 1, 3, 5, and 7 days. Significant increases in mortality rates of *A. obtectus* were observed with exposure times longer than one day. Low concentrations of Spinetoram (0.1 and 0.25 ppm) caused low paralysis and mortality in adults at all exposure times. However, at higher concentrations (0.5 and 1 ppm), nearly 100% paralysis or mortality was observed in *A. obtectus* adults after 3 day of exposure. These results showed that an application at 1 ppm for 3 days was sufficient to kill all *A. obtectus* adults. Application rates of 0.25, 0.5, and 1 ppm spinetoram completely inhibited F1 adults development.

Similarly in our curent study, we observed low mortality rates in *T. castaneum* adults after 1 day of exposure at 0.1 ppm and 0.25 ppm. Spinetoram applied at 0.5 ppm and 1 ppm also resulted in low mortality rates, and complete paralysis or mortality in *T. castaneum* adults were only achieved at 5 ppm or above.

The efficacy of Spinetoram varies according to species, dose rate, and exposure time. Previous studies have shown that *Tribolium castaneum* is more resistant to higher application rates compared to many other species. Although mortality rates increase with longer exposure durations, the required exposure time for mortality in other species is shorter compared to *T. castaneum*. For example, Vassilakos and Athanassiou (2012), reported that *Prostephanus truncatus* and *Rhyzopertha dominica* was found to be very susceptible to spinetoram. At 0.1 ppm Spinetoram, the mortality rate in wheat and corn was reported to be nearly 100 % after 7 days of exposure. *Tribolium confusum*, however, was found to be less susceptible showing 95 % mortality at 10 ppm after 14 days of exposure. Mortality in *Oryzaephilus surinamensis* adults was reported to be 95 % at 5 ppm after 14 days of exosure. Similarly, in our study, at the lowest dose rate of 0.25 ppm, adult mortality after 7 days o exposure was very low across three different temperature degrees. At 20 °C, only 1 dead individual was found in 9 replications, and no dead individuals were found in 9 replications at 25 °C and 30 °C. At 10 ppm, the mortality rate for *Tribolium castaneum* was calculated to be 100 % at the end of 15th day of exposure. These studies demonstrate that the efficacy of Spinetoram varies among species, and lower dose rates and shorter exposure times were reported for susceptible species.

4. Conclusions

In this study, the efficacy of Spinetoram against *Tribolium castaneum*, an important pest of stored grains, was determined at different dose rates at different temperatures for different exposure times. The high insecticidal efficcay of Spinetoram in *Tribolium castaneum* adults was observed at the dose rates of 5 ppm and above. The development of F1 adults varied depending on the dose and temperature. At a dose of 1 ppm, it was observed that more than half of the population was suppressed across all the temperatures. In higher doses, suppression was higher and reached to 100 % at 5 and 10 ppm.

In this study, it was determined that Spinetoram is more effective when applied at higher dose rates compared to other insecticides, and that a minimum of 5 ppm is required for a successful application.

In conclusion, More than 500 pest species have been found to develop resistance to one or more insecticides (Plapp, 1984). Many organophosphorus insecticides are also considered unsafe for humans and the environment. Synthetic pyrethroids are considered more suitable due to their low toxicity to mammals. However, resistance to synthetic pyrethroids has also been detected. Therefore, new-generation insecticides such as Spinosad and Spinetoram may be considered as an alternative to residual pesticides for suppressing pests in stored products.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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