Turkish Journal of Agriculture and Forestry

Volume 33 | Number 2 Article 12

1-1-2009

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Recommended Citation

KHASHAVEH, ADEL; ZIAEE, MASUMEH; SAFARALIZADEH, MOHAMMAD HASAN; and LORESTANI, FARZANEH ATTIGHI (2009) "Control of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) with Spinosad Dust Formulation in Different Oilseeds," *Turkish Journal of Agriculture and Forestry*: Vol. 33: No. 2, Article 12. https://doi.org/10.3906/tar-0806-38

Available at: https://journals.tubitak.gov.tr/agriculture/vol33/iss2/12

Turk J Agric For
33 (2009) 203-209
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doi:10.3906/tar-0806-38

Control of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) with Spinosad Dust Formulation in Different Oilseeds

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Received: 30.06.2008

Abstract: The insecticidal effect of spinosad dust, a formulation that contains 0.125% spinosad, was evaluated against adults of *Tribolium castaneum* (Herbst) on 4 commodities: safflower, sunflower, sesame, and canola. Fifty grams of each commodities were treated with 3 doses of the formulation: 100, 200, and 300 ppm, (corresponding to 0.125, 0.25, and 0.375 mg kg^{-1} a.i., respectively) at 27 °C and 60 ± 5% relative humidity. The mortality of *T. castaneum* adults was measured after 1, 2, 7, 14, and 21 days of exposure. After the mortality count on day 21, all surviving insects were removed while the samples were kept under the same conditions for a further 45 days to assess progeny production. The number of emerged individuals (adults and immatures) was then counted as progeny production. The increase of dose and exposure interval increased mortality. Significant differences were recorded among the 4 oilseed types and the mortality of *T. castaneum* on sesame was significantly lower compared to the other commodities. After 21 days of exposure, mortality reached 100% only on safflower at the highest dose (300 ppm). Progeny production was significantly lower on all 4 treated commodities in comparison with the untreated ones. Complete suppression in progeny production was achieved on safflower and sesame only at 300 ppm. In conclusion, our research revealed that application of spinosad dust formulation at higher rates and for longer exposure intervals could control *T. castaneum* in different oilseeds.

Key Words: *Tribolium castaneum*, oilseeds, progeny production

Introduction

Use of residual insecticides is becoming less desirable because of the development of resistance in major insect pests. Regulatory restrictions on the use of insecticides, awareness of environmental pollution, the increasing cost of storage, erratic supplies, worker safety, and consumer desire for a pesticide-free product have led to pest management specialists to evaluate alternative methods for the pest control of stored products (Arthur, 1996; Lorini and Galley, 1999; Athanassiou et al., 2003, 2004, 2007, 2008a; Kavallieratos et al., 2005, 2006). Spinosad is a bacterial insecticide with low mammalian toxicity and is very effective against a wide range of pest species (Sparks et al., 1995; Salgado, 1997; Bert et al., 1997; Fang et al., 2002b; Fang and Subramanyam, 2003; Subramanyam et al., 2003; Getchell and Subramanyam, 2007). Recently, spinosad was registered for use in stored products in the USA as an alternative to traditional grain protectants (Subramanyam et al., 2003).

Tribolium castaneum (Herbst) is an important world wide pest of stored products that is observed among several commodities. This pest may cause considerable economical losses if not adequately controlled because it has a very high rate of population increase (Hill, 1990). Although oilseeds are not the preferred hosts of *T. castaneum,* it was observed in some parts of Iran, such as

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Mazandaran and West Azarbaijan, where oilseeds were planted in a large area and stored in warehouses for further processing and the population of *T. castaneum* occasionally increased and caused quantitative and qualitative damage (unpublished data).

Previous works with spinosad on stored-products were performed using a liquid spinosad formulation (Fang and Subramanyam, 2003; Fang et al., 2002a, b; Huang et al., 2004; Athanassiou et al., 2008b) while there are many recent studies on the use of dust (dry) formulations (Mutambuki et al., 2003; Getchell, 2006; Getchell and Subramanyam, 2007; Nikpay, 2007; Athanassiou et al., 2008c; Chintzoglou et al., 2008). Dust formulation of spinosad may easily be removed from the grain surface and it may be more applicable compared to liquid formulations (Athanassiou et al., 2008c).

To the best of our best knowledge, no report on the efficacy of spinosad (liquid or dust formulation) on different oilseed commodities in suppressing major stored-grain insects has been published. The aim of the present study was to assess the efficacy of a dust formulation of spinosad on 4 different oilseed types.

Materials and Methods

Insect rearing

Adults of *T. castaneum* were reared on 95% wheat flour plus 5% brewers yeast at 28 °C and 65 ± 5% relative humidity in the dark. In the experiments, 1 to 7-day old mixed sex adults were used. Insects were obtained from cultures maintained in the laboratory for at least 5 years, with no history of exposure to insecticides.

Spinosad formulation

The spinosad dust formulation (Dow Agro Science) contained 0.125% a.i. of spinosad.

Oilseeds

Spring safflower, *Carthamus tinctorius* L. (Asteraceae) (var. IL-III), Sunflower, *Helianthus annus* L. (Asteraceae) (var. Armaveriski), winter canola, *Brassica napus* L. (Brassicaceae) (var. opera), and sesame, *Sesamum indicum* L. (Pedaliaceae) (white variety) obtained from the Oil Research Development Company of West Azerbaijan, Iran, were used for experimentation. The lipid content of safflower, sunflower, canola, and sesame recorded by the Oil Research Development Company was 28.46%, 42.37%, 45.28%, and 58.80%, respectively, that were measured with Soxhlet Extraction apparatus. The moisture content of the 4 commodities as determined by a Dickey–John moisture meter (Dickey–John Multigrain CAC II, Dickey-John Co, USA) ranged between 6.4% and 6.9%. These commodities were stored at -12 °C for a week prior to tests to kill any insect life stages that may have been present.

Bioassays

Spinosad formulation was applied at doses of 100 ppm (corresponding to 0.125 mg kg^{-1} a.i.), 200 ppm (corresponding to 0.25 mg kg^{-1} a.i.), and 300 ppm (corresponding to 0.375 mg kg^{-1} a.i.). Each dose was replicated 4 times. Fifty grams of each commodity were taken and placed in small glass vials separately. The samples were treated individually with respective quantities of spinosad dust; subsequently the vials were shaken for 2 min to obtain an even distribution of the dust on the seed sample. Thirty adults of *T. castaneum* were introduced into each glass vial covered with muslin cloth to provide sufficient aeration. The untreated commodities served as the control treatment with 4 replicates for each commodity. The vials were then placed in incubators set at 27 °C and 60 ± 5% relative humidity. The number of dead adults was counted after 1, 2, 7, 14, and 21 days of exposure. When no response of leg or antennal movements to hot needle was observed, insects were considered dead.

Progeny production counts

After the mortality count on day 21, all adults (dead and alive) were removed from the vials and the vials were left in the incubators at the same conditions for further 45 days to assess progeny production. The number of emerged individuals (adults and immatures) was then counted in controls and in the treated commodities and they were introduced to Aldryhim's (1990) formula for the calculation of percentage of reduction in progeny production.

Data analysis

The mortality counts were corrected using Abbott's (1925) formula. Percentage of reduction in progeny production was determined using Aldryhim's (1990) formula:

$$\left[\frac{\text{No. progeny in conrol - No. progeny in treatment}}{\text{No. progeny in control}} \times 100\right]$$

To equalize variances, mortality percentage of adults and percentage of reduction in progeny production were transformed using the square root of the arcsin. The data were analyzed using Analysis of Variance (SAS, 2000). Means were separated using the Tukey Multiple Range Test at P = 0.05. The concentration required to kill 50% of the insects (LC_{50}) was estimated using probit analysis (SPSS, 1999). For this purpose, we introduced 4 doses to the statistical software: 100, 200, 300, and 0 ppm as control treatment.

Results

Adults' mortality

All main effects as well as all associated interactions were significant at the $P < 0.0001$ level for dose (df = 2, 239; F = 190.28), exposure (df = 4, 239; F = 1024.57), commodity (df = 3, 239; F = 426.14), rate exposure (df = 8, 239; F = 16.9), exposure commodity (df = 12, 239; F = 32.729) with the exception of the rate commodity (df = 6, 239; F = 2.306), which was significant at P = 0.0360. The mortality within the control group 21 days after treatment was 3.33 ± 2.35, 4.16 ± 1.6, 5.83 ± 2.1, and 0.0% in safflower, sunflower, sesame, and canola, respectively. The mortality of *T. castaneum* exposed to spinosad in all the treated commodities was very low after 1 day of exposure (1 day) even at 300 ppm (Figures 1, 2, and 3). Also after 2 days of exposure, mortality was low and only 21.66% was recorded for safflower at 300 ppm (Figures 1, 2, and 3). However, after 7 days of exposure, the highest mortality (37.5%) was recorded on safflower treated with 300 ppm. In contrast, on sunflower, sesame, and canola, mortality did not exceed 17.49%. After 14 days of exposure, mortalities ranged between 9.9% (at 100 ppm on canola) and 77.96% (at 300 ppm on safflower). After 21 days of exposure, 100% mortality was noted only in safflower at 300 ppm. Of the 4 commodities, significantly lower mortalities, ranging from only 15.04% to 45.12% at 100 and 300 ppm, respectively, were recorded on sesame (Figures 1, 2, and 3).

The parameters of the probit analysis and LC_{50} values for *T. castaneum* adults in oilseeds treated with spinosad dust after 21 days of exposure are given in Table 1. Probit analysis showed that the lowest LC_{50} value was observed in safflower (63.64 ppm) and therefore adults of *T. castaneum* were more susceptible to spinosad dust formulation in safflower compared to other oilseeds after 21 days of exposure. The LC_{50} values were recorded by 97.86, 140.56 and 342.67 ppm for sunflower, canola, and sesame, respectively. No overlap was observed in 95% confidence limits of LC_{50} values in different oilseeds.

Progeny Production

All main effects and associated interaction were significant for dose (df = 2, 47; F = 21.66; P = 0.0001), commodity (df = 3, 47; F = 2.98; P = 0.0440), rate commodity (df = 6, 47; F = 0.815; P = 0.0001). Mean number of adult offspring produced (± SE)/vial in the control group was 5.75, 13.5, 8, and 5 for safflower, sunflower, canola, and sesame, respectively. The application of spinosad significantly reduced progeny production in the 4 commodities tested. In safflower, 100% control of progeny production achieved at 300 ppm. Although the mortality rate of *T. castaneum* in sesame at 300 ppm was significantly lower compared to

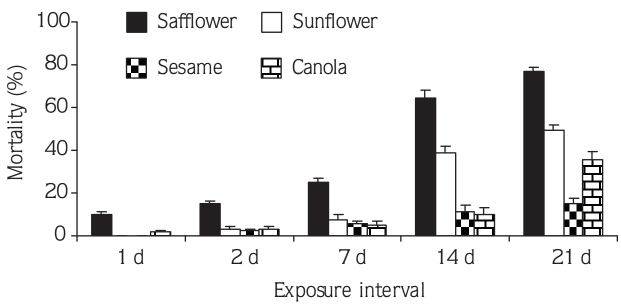

Figure 1. Mean mortality (% + SE) of *T. castaneum* adults in oilseeds treated with 100 ppm of spinosad dust after 1, 2, 7, 14, and 21 days of exposure.

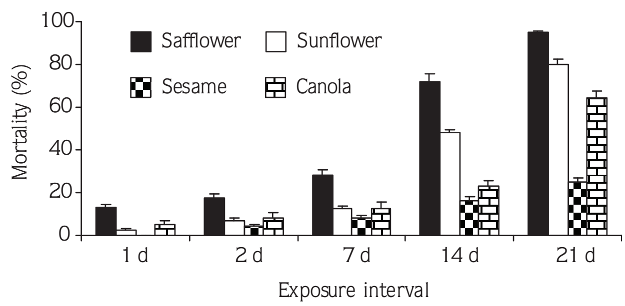

Figure 2. Mean mortality (% + SE) of *T. castaneum* adults in oilseeds treated with 200 ppm of spinosad dust after 1, 2, 7, 14, and 21 days of exposure.

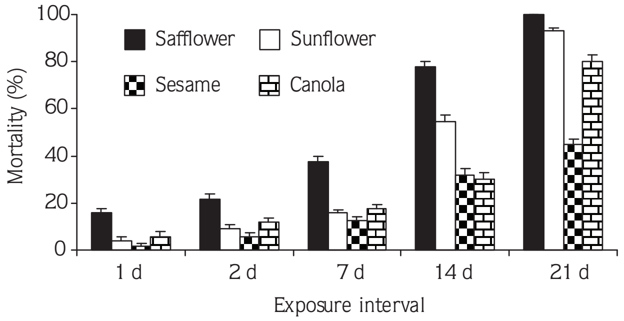

Figure 3. Mean mortality (% + SE) of *T. castaneum* adults in oilseeds treated with 300 ppm of spinosad dust after 1, 2, 7, 14 and 21 days of exposure.

the other commodities, for some unexplained reasons, complete suppression of progeny was recorded in this commodity (Table 2).

Discussion

The aim of our study was to investigate the efficacy of spinosad dust formulation against adults of *T. castaneum* in different oilseeds. Spinosad efficacy varied greatly among the type of oilseeds. The most significant result was that spinosad was effective against *T. castaneum* at the application dose of 300 ppm (0.375 mg kg^{-1}a.i.) in different oilseeds except sesame.

The mortality of *T. castaneum* adults was significantly different in various commodities. As the moisture content of oilseeds was more or less the same, it cannot accounted for the difference. However, they were different in terms of lipid content and sesame had higher lipid content than the other 3 commodities. Therefore, the difference in lipid content may be the cause of difference in mortality. Although this result can not be a strong proof for our results, our observation indicated different mortality rates in different oilseeds at the same dose and with a high lipid content. Previously, some investigators explained that the amount of lipid in different commodities can determine the efficacy of some materials, such as diatomaceous earth, used as a dust against storage pests. Subramanyam (1995) explained the different mortality results observed between wheat and corn treated with diatomaceous earth as (1) greater retention of dusts on wheat than on corn and (2) loss of activity of dusts due to adsorption of lipids from corn surfaces. Corn has a much higher lipid content than wheat. Recently, Ziaee et al. (2007), when examining the effect of oilseed type on the efficacy of 5 diatomaceous earth (DE) formulations against *T. castaneum*, found considerable levels of variation in of susceptibility of adult *T. castaneum* in different oilseeds. The results indicated that the type of oilseed plays an even bigger role than the DE product employed in determining the level of control

Table 1. Probit analysis data for *T. castaneum* adults in oilseeds treated with spinosad dust after 21 days of exposure.

Oilseed	LC_{50} (ppm)	CL[a] (95%)	Intercept	Slope	P	χ^2
Safflower	63.64	41.91 - 78.06	-1.49 ± 1.37	3.59 ± 0.65	0.19	1.66
Sunflower	97.86	79.63 - 113.34	-1.91 ± 0.88	2.97 ± 0.4	0.61	0.262
Canola	140.56	118.96 - 159.86	-0.38 ± 0.8	2.5 ± 0.35	0.82	0.05
Sesame	342.67	269.2 - 570.88	0.82 ± 0.83	1.65 ± 0.36	0.34	0.89

[a] lower and upper confidence limit

Table 2. Mean percentage of reduction (± SE) in progeny production (F1) of *T. castaneum* in safflower, sunflower, canola, and sesame treated with spinosad dust

Sesame	Canola	Sunflower	Safflower	rate (ppm)
60 ± 8.25 cd	50 ± 8.83 d	55.55 ± 8 d	82.6 ± 7.1 ab	100
85 ± 5 ab	90.62 ± 5.98 ab	75.92 ± 4.65 bc	91.3 ± 5.02 ab	200
100 ± 0.0 a	93.75 ± 3.6 ab	94.44 ± 3.54 ab	100 ± 0.0 a	300

Means followed by the same letter are not significantly different (Tukey Multiple Range Test at P = 0.05).

of the infesting live adults. This factor is logical as the efficacy of spinosad dust formulation is a function of their sorptive capacity for cuticular waxes, and the adsorption of seed lipids should detract from their ability to adsorb insect wax.

Commodities differ in their physical and chemical properties. Pomeranz et al. (1988) found that kernel hardness was one of the most obvious differences between wheat classes and varieties. This property may have an influence on the level of infestation by insects and also on the adherence of different protectant dusts to the kernels. The reasons for the differences in performance of spinosad against adults of *T. castaneum* between different oilseeds could also be related to spinosad residue coverage, distribution, and retention on the kernel surfaces (Pomeranz et al., 1988; McCaughey et al., 1990). In comparison to sesame and canola, safflower and sunflower have a smaller total surface area for a certain amount of kernels that may result in a higher level of spinosad residue on safflower and sunflower compared to sesame and canola.

Several published reports are available on the insecticidal effect of spinosad dust against stored-grain insect pests, especially *T. castaneum*; however, there is not any references regarding oilseeds and different stored product insects to be compared with the results obtained in the present study. According to Fang et al. (2002a) the performance of liquid spinosad was different when evaluated on 4 classes of wheat (hard red winter, hard red spring, soft red winter, and durum wheat) against adults of the *R. dominica*, *Sitophilus oryzae* (L.), *O. surinamensis* (L.), and *T. castaneum*. On all wheat classes, spinosad at 0.1 and 1 mg kg^{-1} of kernel killed all exposed *R. dominica* adults within 7 days. Mortality of *O. surinamensis* and *T. castaneum* after 7 and 14 days of exposure at 1 mg kg^{-1} ranged from 46% to 76% only on durum wheat, while mortality of these species on the other wheat classes was <15%.

Athanassiou et al. (2008d) and Chintzoglou et al. (2008) found that the effectiveness of spinosad dust was greater on wheat than on barley, rice, and maize whereas in the case of *R. dominica,* spinosad dust was less effective on maize compared to other commodities. These results confirmed our results that the type of commodity affects the efficacy of spinosad. Laboratory experiments were carried out by Nayak et al. (2005) on relevant resistant strains of 4 beetle species with the aim of determining the potential of spinosad as a new grain protectant. Adults of each strain were exposed to wheat treated with spinosad at 0.1, 0.5 and 1 mg [a.i.] kg^{-1} of grain. Among beetles, spinosad was most effective against *R. dominica*, with 100% adult mortality and progeny reduction after 14 days of exposure at 1 mg [a.i.] kg^{-1}. Efficacy of spinosad was less against *S. oryzae*, and least against *T. castaneum* and *O. surinamensis*. They reported that among *R. dominica*, *S. oryzae*, *T. castaneum*, and *O. surinamensis*, spinosad was most effective in reducing the progeny production of *R. dominica*. Spinosad at 1 mg [a.i.] kg^{-1} prevented the production of progeny in *R. dominica* but failed to achieve the complete reduction of progeny in the other species.

Huang et al. (2004) studied the susceptibility of laboratory and field strains of 4 stored-product insect species to spinosad. Adults of *T. castaneum* were relatively less susceptible to spinosad compared with the other species tested. Getchell and Subramanyam (2007) indicated that dry formulation of spinosad even at 10 mg (a.i) kg^{-1} could not control *T. castaneum*. According to Athanassiou et al. (2008b) *R. dominica* and *Prostephanus truncatus* were very susceptible to liquid spinosad, followed by *S. oryzae* whereas *T. confusum* was the least susceptible. Mutambuki et al. (2003) tested the effect of a dust formulation against adults of *T. castaneum*, *S. zeamais*, *R. dominica*, and *P. truncates*. In that study all species were highly susceptible to spinosad, even 0.35 mg kg^{-1} a.i. caused >95% mortality. On the other hand, for *T. castaneum*, mortality was low (only 50%) even at 1.4 mg kg^{-1}. In our research, we recorded that *T. castaneum* is relatively susceptible to spinosad dust formulation at the dose of 300 ppm. Other investigators, as mentioned above, noted that this species is not susceptible to different formulations of spinosad contrary to our results. We have not any strong proof for our results but it is possible that the used strain of this species is the cause of this difference. Athanassiou et al. (2008c) investigated the susceptibility of 6 strains of *Tribolium confusum* to spinosad dust formulation. For this purpose, adults and larvae of *T. confusum* were exposed on wheat treated with 2 doses of the dust formulation, 0.05 and 0.15 g kg^{-1}. The increase of dose and exposure interval increased mortality and significant differences were noted among populations. This investigation upholds our results that increase of dose and exposure interval could affect efficacy of spinosad against storage pests. These results also mean that different strains of one species have different susceptibility and this factor may help us to understand of difference of our results with other investigations regarding the susceptibility of *T. castaneum* to spinosad.

Huang and Subramanyam (2007) evaluated the effectiveness of liquid spinosad against 7 major stored grain insects on corn. They demonstrated that spinosad was extremely effective in suppressing progeny production. For *T. castaneum*, progeny production was 28.0 ± 4.2 per container on control treatment after 49 days. This number was reduced by 91% at 0.5 mg kg^{-1} and >96% at 1 and 2 mg kg^{-1}. These results certify our results that spinosad is effective in suppressing progeny production and there is an inverse relationship between progeny production and spinosad rate.

Insecticides formulated as dusts can be advantageous to stored-grain managers because they are easy to handle and apply. Unlike liquid formulations, dust formulations do not require water for dilution and power supply for the sprayer, and they can be directly admixed with grain. They are relatively easy to use by producers, as sprayer calibration is not necessary. Dust formulations can be applied to grain in the truck prior to unloading, to grain stream as it is augered into a bin, or to the grain surface after bin-loading (Ware, 1991; Getchell, 2006).

In conclusion, our research revealed that spinosad dust formulation could control *T. castaneum* in different oilseeds but longer exposure intervals and higher doses are needed to achieve satisfactory control of T. *castaneum*.

Acknowledgements

We would like to express our special thanks to Mr. Baradari and Mr. Nazari (Dow Agrosciences, Tehran office) for providing the spinosad dust for experimentation. We are grateful to Ali Khorsandi, Ashkan Zarei, and Mehran Omran for their great assistance.

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