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Orijinal araştırma (Original article)

Residual toxicity of Spinetoram against to bean weevil, *Acanthocelides obtectus* Say. (Coleoptera: Bruchidae) on bean

Spinetoram'ın fasulye üzerinde fasulye tohum böceği, *Acanthocelides obtectus* Say. (Coleoptera: Bruchidae)'a karşı rezidüel toksisitesi

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Summary

In present study, residual contact toxicity of spinetoram suspension applied to bean against *Acanthocelides obtectus* Say. (Coleoptera: Bruchidae) adults was investigated under laboratory conditions. In laboratory bioassays, *A. obtectus* adults were exposed to bean sprayed with spinetoram suspension at 0.1, 0.25, 0.5 and 1 ppm (mg active ingredient/kg commodity) at 26±1 ºC temperature, 65±5 % relative humidity and completely dark condition. Paralysis and mortality of the adults were recorded after 1, 3, 5 and 7 day of exposure and 40 day later the bean was examined for progeny production. Based on the results obtained from the biological tests, concentration of spinetoram suspension and the exposure period had a significant effect on paralysis and mortality rate of *A. obtectus* adults on bean. Spinetoram treatments at all concentrations after 1 day of exposure resulted in low mortality of *A. obtectus* adults. Mortality of *A. obtectus* adults increased after 1 day of exposure period. Spinetoram treatments at low concentrations (0.1 and 0.25 ppm), resulted in low mortality of paralysis or mortality of *A. obtectus* adults at all exposure times. However, spinetoram treatment at higher concentrations (0.5 and 1 ppm) after 3 day of exposure resulted in almost 100 % paralysis or mortality of *A. obtectus* adults. These results indicated that 1 ppm concentration of spinetoram is enough to obtain the complete mortality of *A. obtectus* for 3 day of exposure. Spinetoram treatments at 0.25, 0.5 and 1 ppm completely hindered its progeny production. In conclusion, based on mortality and progeny production results spinetoram would be potential to be used for control of *A. obtectus* on stored beans as an alternative protectant to the conventional insecticides.

Keywords: Spinetoram, *Acantocelides obtectus*, residual action, toxicity, bean

Özet

Laboratuvar koşullarında yürütülen bu çalışmada fasulye tanelerine uygulanmış Spinetoram'ın, Fasulye tohum böceği, *Acanthocelides obtectus* Say. (Coleoptera: Bruchidae) erginlerine karşı reziduel kontak toksisitesi araştırılmıştır. Laboratuvar denemelerinde, *A. obtectus* erginleri 26±1 ºC sıcaklık, 65±5 % nem koşullarında ve tamamen karanlık ortamda 0.1, 0.25, 0.5 ve 1 ppm (mg aktif madde/kg ürün) konsantrasyonlarındaki Spinetoram solüsyonu uygulanmış fasulye ile muamele edilmiştir. Uygulamadan 1, 3, 5 ve 7 gün sonra felç ve ölü ergin bireyler sayılmış ve 40 gün sonra yeni nesil ergin çıkışları gözlemlenmiştir. Biyolojik testlerden elde edilen sonuçlara göre fasulye üzerine uygulanan Sipenetoram konsantrasyonları ve uygulama süreleri, *A. obtectus* erginlerinin felç ve ölüm oranları üzerine önemli derecede etkiye sahip olduğu bulunmuştur. Spinetoram'ın tüm konsantrasyonları, 1 gün uygulama süresinde *A. obtectus* erginlerin düşük ölümlerine neden olmuştur. Bir günden sonraki uygulama sürelerinde *A. obtectus*'un ölüm oranlarında önemli artış görülmüştür. Spinetoram'ın düşük konsantrasyonları (0.1 ve 0.25 ppm) tüm uygulama sürelerinde, *A. obtectus* erginlerin düşük felç ve ölümüne neden olmuştur. Ancak, yüksek konsantrasyonlarda (0.5 ve 1 ppm) 3 gün uygulama süresinde *A. obtectus* erginlerin hemen hemen % 100 felç ya da ölümü görülmüştür. Elde edilen bu sonuçlar *A. obtectus* erginlerinin tamamını öldürmek için 1 ppm uygulama konsantrasyonu ve 3 günlük uygulama süresinin yeterli olduğunu ortaya koymuştur. Spinetoram'ın 0.25, 0.5 ve 1 ppm uygulama konsantrasyonları yeni nesil ergin çıkışlarını tamamen engellemiştir. Ölüm ve yeni nesil ergin sonuçları, Spinetoram'ın konvensiyonel insektisitlere bir alternatif koruyucu insektisit olarak depolanmış fasulyelerde zararlı *A. obtectus* mücadelesinde kullanılabilme potansiyeline sahip olabileceğini göstermiştir.

Anahtar sözcükler: Spinetoram, *Acantocelides obtectus*, rezidüel etki, toksisite, fasülye

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Introduction

Worldwide, the dry bean (*Phaseolus vulgaris* L.) (Fabaceae) is the most economically and nutritionally important legume for human consumption (Jones, 1999). Bean is one of the most commonly used vegetables in human nutrition particularly in developing countries (Jones et al., 2011; Lopes et al., 2015) and the common bean is estimated as the third-largest source of calories and the second-largest source of dietary protein (Hillocks et al., 2006). However, attack by bruchids (Coleoptera: Bruchidae) during storage compromises the quality and commercial value of beans. The bruchid, *Acanthoscelides obtectus* (Say) is one of the major insect pests affecting the common bean (Hagstrum & Subramanyam, 2009; Mutungi et al., 2015). Larvae developing within the grain cause the largest damage (Swella & Mushobozy, 2007), causing a reduction of dry matter and, hence, grain mass (Padin et al., 2002). Thus, besides the reduction in grain weight, the insects destroy the embryo while feeding, reducing the germination ability of the beans (Padin et al., 2002; Caneppele et al., 2003). Given the destructive power of bruchids, many farmers sell their entire crop of beans immediately after harvest when the prices in the market are still low, and they do not store seeds for the next sowing season or for their own consumption (Schmale et al., 2006; Lopes et al., 2015).

The control of *A. obtectus* relies mainly on the application of fumigation and synthetic insecticides on stored beans. Currently, phosphine (PH3) gas has been used for fumigation of stored beans infested by the insect pests. But resistance problems of phosphine has already reported in a number of countries, with very high levels of resistance in some parts of Asia and Africa (Mills, 1983; Taylor & Halliday, 1986; Taylor, 1989; Zettler, 1997; Sayaboc et al., 1998; Rajendran, 1999), Australia (Collins et al., 2001; Nayak et al., 2010; Emery et al., 2011) more recently in America (Opit et al., 2012; Saglam et al., 2015). The synthetic insecticides used against stored bean insects are primarily organophosphorus and pyrethroid compounds, and the residues from a single application can often prevent insects from establishing in stored beans. However, use of residual insecticides is becoming less desirable because of the resistance in major insects (Pimentel et al., 2007), regulatory restrictions on use of insecticides, awareness of environmental pollution, the increasing cost of storage insecticides, erratic supplies, worker safety and consumer desire for a pesticide-free product. All the above issues raise the need for the development of new active ingredients that pose fewer concerns for both humans and the environment and are more compatible with Integrated Pest Management (IPM) approaches in stored-grain protection.

Spinosyns group insecticides exhibit low mammalian toxicity and are considered harmless for the environment since they degrade to simpler fragments containing only carbon, oxygen, nitrogen, and hydrogen (Dripps et al., 2011). Spinosad is a naturally occurring mixture of spinosyns A (primary component) and D (minor component) (Sparks et al., 1999; Saldago & Sparks, 2005), Spinosad acts on the insect nervous system at a unique site on the nicotinic acetylcholine receptor, and is active through contact or ingestion (Dripps et al., 2011). Spinosad can be used effectively for organophosphate and pyrethroid resistant strains of several stored product insects (Daglish, 2008). Spinosad possesses a unique mode of action in insects and controls insect strains resistant to other grain protectants (Hertlein et al., 2011). Also, in comparison with OPs and pyrethroids, Pozidi-Metaxa & Athanassiou (2013), reported that spinosad was more effective than chlorpyriphos-methyl and equally effective as deltamethrin and pirimiphos-methyl against the larger grain borer, *Prostephanus truncates* (Horn) (Coleoptera: Bostrychidae).

Recently, spinetoram that is a mixture of two synthetically modified spinosyns (spinosyn J and spinosyn L), which are metabolites of the bacterium *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae), was introduced as a new spinosyn insecticide with greater potency and faster speed of action in comparison with spinosad (Dripps et al., 2008; Sparks et al., 2008). Recently, spinetoram has been tested and found to be effective for the control of several stored grain beetle species (Vassilakos et al., 2012; Isikber et al., 2013) while its efficacy was practically not affected by temperature and relative humidity (RH) (Vassilakos & Athanassiou, 2013). Spinetoram has some surface treatment studies against all life stages of *Tribolium confusum* du Val. (Saglam et al., 2013). Vassiliakos & Athanassiou (2012) suggested that spinetoram is very effective against *R. dominica*, moderately effective against *S. oryzae*, and not very effective against *T. confusum.* Spinetoram is considered more active and more persistent than spinosad (Dripps et al., 2011).

In spite of the fact that there are several published studies for the efficacy and toxicity of Spinetoram against some stored grain insects (Vassilakos et al., 2012; Vassiliakos & Athanassiou, 2012; Vassiliakos & Athanassiou, 2013). However, to our knowledge, the efficacy of spinetoram against stored bean insects has not been tested so far. In the present work, residual toxicity of spinetoram against bean weevil, *A. obtectus* on beans was tested under the laboratory conditions.

Material and Methods

Test insect

The *A. obtectus* strain used in this study was obtained from laboratory culture that originated from bean seeds collected around Mersin Province, Turkey in October 2010. *A. obtectus* was reared on uninfested bean (*Phaseolus vulgaris*) at 1 l glass jars (8.6 cm in diameter and 17.5 cm in height) in incubators set at 65±5 % RH and 27±1 °C, under continuous darkness. New subcultures were established weekly, by removing approximately 100 beetles from each of the two oldest jars, the oldest then being discarded.

Commodity

Uninfested and untreated bean (*Phoselus vulgaris* L. var. Elbistan) with 8±0.5 % of moisture content was used for the bioassays and insect rearing. The bean was placed a freezer at -18 °C for one week to destroy any remaining insects before the seed was used for insect rearing and laboratory trials. Moisture content of the bean was measured by using by using KETT-Pm-600 moisture meter (Kett Electric Laboratory, Japan).

Insecticide and insecticide treatment

A water dispersible granule (WG) formulation of spinetoram (Delegate 250 WG) that contained 250 g of active ingredient (AI) per liter and was supplied by Dow AgroSciences, UK was used for bioassays. One kg of bean was sprayed with spinetoram to create four concentration levels: 0 (control), 0.1, 0.25, 0.5 and 1 ppm (mg AI/kg of bean). The spinetoram WG formulation was suspended with distilled water to prepare each concentration and 1 ml of the appropriate suspension was sprayed in each lot. The insecticide application was made by using HSENG Airbrush AS18 model (Ningbo Haosheng Pnömatik Machinery Co., Zhejiong, China). To achieve even distribution of the insecticide, the bean was spread into a plastic tray (48 x 33 x 8 cm) providing a thin mono layer as a spraying surface. Then, the sprayed bean lots in the plastic tray were shaken manually for approx. 1 min to enhance the insecticide distribution. Additional lots of 1 kg of beans were sprayed with distilled water as a control treatment. After application, commodities were left one day for drying under laboratory conditions

Bioassays

Cylindrical glass vials with 450 ml of capacity were used as the experimental units for bioassays. For each spinetoram concentration, five samples, each of 200 g, were taken from each jar of treated bean and placed in vials. Then, 25 one to two-day old and mixed sex adults of *A. obtectus* were introduced into each vial (separate vials for each concentration). All these vials were placed in incubators set at 25±1 °C, 65±5 % RH and continuous darkness. Dead (no motion) and paralysis (only moving antenna and legs) of the exposed individuals were recorded after 1, 3, 5 and 7 day of exposure in the treated and untreated substrate. After the 7 day of exposure, all adults (dead or alive) were removed and the jars returned at the experiment conditions. Forty days later (Rees, 2004), adult progeny emergences (F_1) were counted in the vials.

Data analysis

For each count, mortality rate, paralysis rate and morality + paralysis rate of *A. obtectus* adults were calculated. Control mortality was generally low, so no correction was considered necessary. Adult mortality rate, paralysis rate and morality + paralysis rate were analyzed separately for each species using the MANOVA Fit Repeated Measures Procedure with Wilk's lambda estimate of JMP software (Sall et al., 2001), with dose rate as main effects, and time as the repeated variable. Arcsine transformation was applied to mortality and paralysis data that were subjected by one-way ANOVA (Factor: concentration). For progeny production, one-way ANOVA was performed, by using the same software, with number of progeny as the response variable, and concentration as main effect. In this case, the number of progeny in the control vials was also included in the analysis. The means were separated using Duncan test at the 5% level (Proc CM: One-way ANOVA, SPSS Statics 18, 2009).

Results

Regarding to mortality counts, all main effects and their interactions were significant as repeated measures MANOVA parameters (Table 1). Generally, mortality of *A. obtectus* adults increased with increasing of concentration at each exposure time, apart from first day. In all exposure times, except first day, spinetoram treatments at concentration of 1 ppm resulted in significantly higher mortality than those at the other concentrations. The lowest mortalities were achieved at 0.1 ppm concentration at each exposure time. After 7 day of exposure, 48.8%, 66.4%, 93.6% and 100% of mortality of *A. obtectus* were obtained at 0.1, 0.25, 0.5 and 1 ppm concentration of spinetoram respectively. Thus, the complete mortality was achieved only at 1 ppm for 5 and 7 day of exposure (Fig. 1).

Table 1. Repeated measures MANOVA parameters for mortality, paralysis and mortality + paralysis counts of the bean weevil (in all cases, error df=20)

	df	Mortality	Paralysis	Mortality + Paralysis	*P*
		F	F	F	
All between	4	106.4055	80.6058	190.5743	<0.0001
Intercept	1	1178.7295	948.0423	1959.0035	<0.0001
Dose	4	106.4055	80.6058	190.5743	<0.0001
All within	12	9.9648	13.7511	9.1045	<0.0001
Time	3	401.5952	44.5557	181.5643	<0.0001
Time*Dose	12	9.9648	13.7511	9.1045	<0.0001

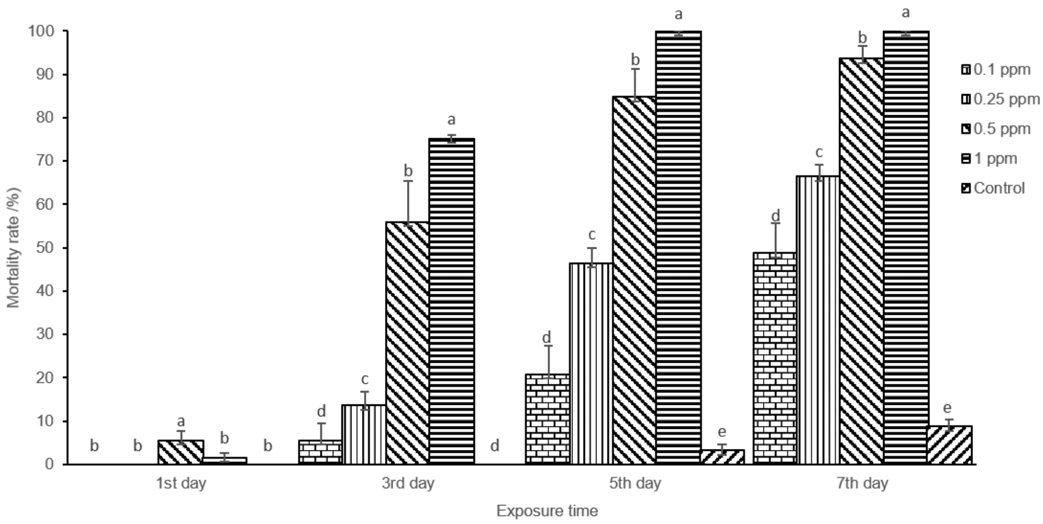

Fig. 1. Mean mortality (%±SE) of *Acanthocelides obtectus* on beans treated by spinetoram at the different concentrations for 1, 3, 5 and 7 day of exposure time (Means followed by the same lower case letter at each concentration are not significantly different; Duncan test at 0.05; Errors bars on the graph indicate the standard error of mean mortality of each treatment).

Regarding to paralysis levels, all main effects and their interactions were significant (Table 1). After 7 day of exposure, 0%, 12.8%, 60.8% and 95.2% of mortality of *A. obtectus* were obtained at 0.1, 0.25, 0.5 and 1 ppm respectively. Spinetoram treatments at 0.5 and 1 ppm after 1 day of exposure had significantly higher paralysis levels of *A. obtectus* than those at 0.1 and 0.25 ppm. However, very low paralysis levels were obtained at same concentrations after 5 and 7 day of exposure. Paralysis data indicated that adults of *A. obtectus* exposed on beans exposed spinetoram at high concentrations (0.5 and 1 ppm) were highly paralyzed just after 1 day of exposure (Fig. 2).

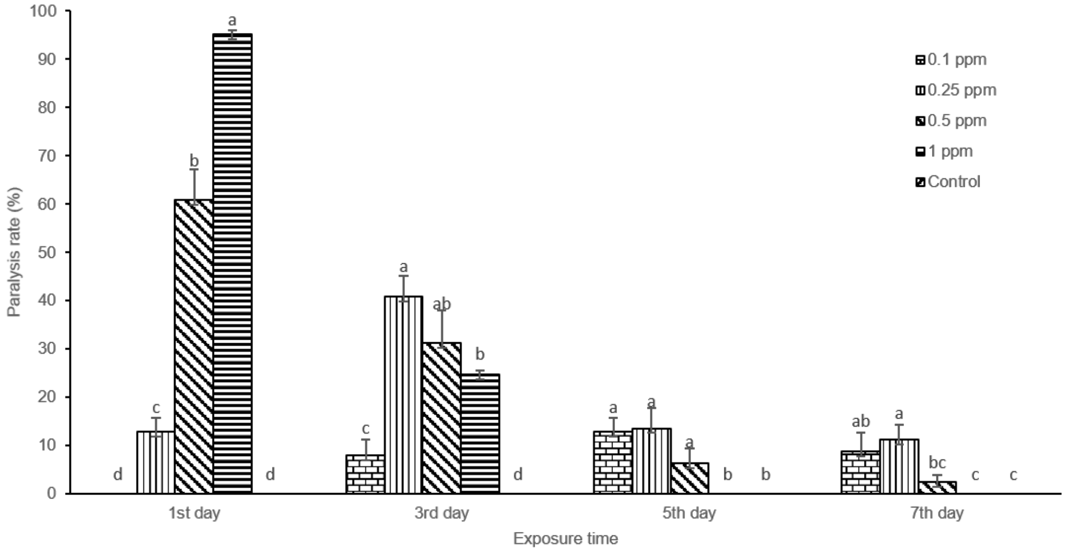

Fig. 2. Mean paralysis levels (%±SE) of *Acanthocelides obtectus* adults on beans treated with spinetoram at the different concentrations for 1, 3, 5 and 7 day of exposure (Means followed by the same lower case letter at each concentration are not significantly different; Duncan test at 0.05; Errors bars on the graph indicate the standard error of mean mortality of each treatment).

Analysis of mortality+paralysis data indicated that all main effects and their interactions were also significant (Table 1). Generally, mortality+paralysis level of *A. obtectus* adults increased with increasing of concentration at all exposure times, apart from 7-day of exposure. Mortality+paralysis levels of *A. obtectus* adults at 1 ppm were higher than those at the other concentrations for 1, 3 and 5 day of exposure. However, after 7 day of exposure, mortality+paralysis levels at 0.5 and 1 ppm were statistically similar whilst they were higher than those at 0.1 and 0.25 ppm. 100% or nearly 100% mortality+paralysis level of *A. obtectus* adults was achieved at 1 ppm after 1, 3 and 5 day of exposure, whilst it was obtained at 0.5 and 1 ppm after 7 day of exposure.

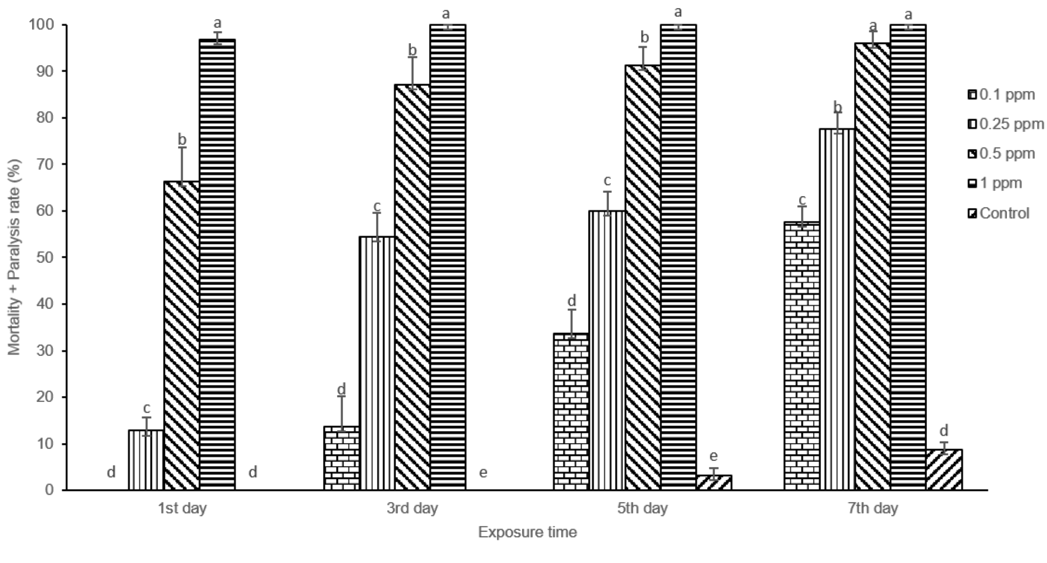

Fig. 3. Mean mortality + paralysis levels (%±SE) of *Acanthocelides obtectus* on beans treated with spinetoram at the different concentrations for 1, 3, 5 and 7 day of exposure (Means followed by the same lower case letter at each concentration are not significantly different; Duncan test at 0.05; Errors bars on the graph indicate the standard error of mean mortality of each treatment).

Progeny production (F_1)

There were significant differences in progeny production between spinetoram concentrations and control treatment ($F_{3,20}$=433.9, P<0.0001). At 0.25, 0.5 and 1 ppm, no progeny of *A. obtectus* was produced, whilst progeny production was observed at the lowest concentration (0.1 ppm). However, progeny production at 0.1 ppm was significantly lower than that at control treatment. These results indicated that spinetoram treatment at 0.25, 0.5 and 1 ppm would completely suppress the progeny production of *A. obtectus* (Fig. 4).

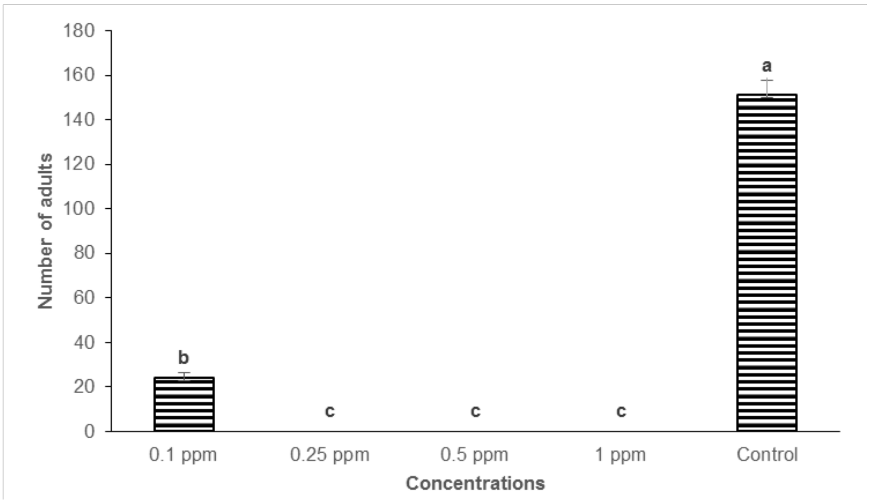

Fig.4. Mean number of adult progeny of *Acanthocelides obtectus* on beans treated by spinetoram at the different concentrations (Means followed by the same upper case letter at each concentration are not significantly different; Duncan test at 0.05; Error bars on the graph indicate the standard error of mean number of adult progeny of each treatment).

Discussion

Several grain protectants can provide long-term protection against a wide range of stored-product beetle species. However, despite the fact that persistence is a desirable characteristic of a given grain protectant, the use of an insecticide that is toxic to mammals in conjunction with high residues on the products, is not permitted in stored product protection. Therefore, the use of an insecticide of very low mammalian toxicity, such as spinetoram (Rat oral LD_{50} > 5000 mg/kg of body weight) can be considered as a safe solution in this respect.

Based on the results obtained from the biological tests, the concentration of spinetoram suspension and the exposure period had a significant effect on paralysis and mortality rate of *A. obtectus* adults on beans. The mortality of *A. obtectus* significantly increased with increasing the concentration of spinetoram and exposure time. Spinetoram treatments at all concentrations after 1 day of exposure resulted in low mortality of *A. obtectus* adults. Mortality of *A. obtectus* adults increased after 1 day of exposure period. Spinetoram treatments at low concentrations (0.1 and 0.25 ppm), resulted in low mortality of paralysis or mortality of *A. obtectus* adults at all exposure times. However, spinetoram treatment at higher concentrations (0.5 and 1 ppm) after 3 day of exposure resulted in almost 100 % paralysis or mortality of *A. obtectus* adults. These results indicated that 1 ppm concentration of spinetoram is enough to obtain the complete mortality of *A. obtectus* for 3 day of exposure. At 0.25, 0.5 and 1 ppm, no progeny of *A. obtectus* was produced, whilst progeny production was observed at the lowest concentration (0.1 ppm). These results indicated that spinetoram treatments at 0.25, 0.5 and 1 ppm completely hindered its progeny production.

Currently, no studies for efficacy of spinetoram against *A. obtectus* on beans have been published in literature. However, there are some studies published about efficacy of spinetoram against several stored grain insects. Vassilakos et al. (2012) found that spinetoram was effective against *Tribolium confusum* du Val. (Coleoptera: Tenebrionidae) only in the high doses of 5 and 10 ppm (mg of AI/kg of grain) and ineffective at 2 ppm after 21 days of exposure in treated wheat. *Tribolium confusum* young larvae are susceptible to both spinosad and spinetoram (Vayias et al., 2009; Saglam et al., 2013). *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) was the most susceptible among the species, while concentrations of 0.5 and 1 ppm were needed to control *Sitophilus granarius* L. (Coleoptera: Curculionidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) adults, respectively. These findings for the concentration of spinetoram required to obtain the complete mortality of *R. dominica, S. granarius* and *S. oryzae* on wheat stand in accordance with the results obtained in present study for *A. obtectus* on beans. However, compared with the findings for *T. confusum* on wheat, reported by Vassilakos et al. (2012), the concentration of spinetoram required to obtain the complete mortality of *A. obtectus* in present study is much lower than that for *T. confusum.* This discrepancy can be due to the difference in insect species and commodity tested. Likewise, previous studies document that the insecticidal efficacy of spinosad and spinetoram is affected by several biotic or abiotic factors, such as the target species, the type of commodity, the exposure interval and the type of surface that spinosad is applied to (Fang et al., 2002; Subramanyam et al., 2003; Toews & Subramanyam 2003; Toews et al., 2003; Nayak et al., 2005; Daglish & Nayak, 2006; Subramanyam, 2006; Subramanyam et al., 2007; Vassilakos et al., 2015).

In this study, spinetoram proved to be effective against *A. obtectus* on bean. Based on mortality and progeny production results, at 1 ppm, spinetoram was found to result in the complete mortality of *A. obtectus* and completely prevent its progeny production. In conclusion, present study indicated that spinetoram would be potential to be used for control of *A. obtectus* on bean as an alternative protectant to the conventional insecticides. However, further research is needed to obtain data on its persistence on beans, its toxicity for other stored bean insects under laboratory and field conditions.

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