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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 10 (17): 2950-2954, 2007
ISSN 1028-8880

Susceptibility Status of Different Life Stages of *Tribolium castaneum* Herbst (Col: Tenebrionidae) to Spinosad

[1]Roghayeh Yousefnezhad-Irani and [2]Pourmirza Ali Asghar
[1]College of Agriculture, Urmia University, Urmia, Iran
[2]Department of Entomology, College of Agriculture, Urmia University, Urmia, Iran

Abstract: Red flour beetle, *Tribolium castaneum*, is one the most important and worldwide pest of stored-products. To evaluate the susceptibility of different life stages of this pest we used a commercial formulation of biorational insecticide, Spinosad, Tracer® against adults, young and old larvae and pupae using topical application bioassay method. Mortality was recorded after exclusion of pupae and 24, 48 and 72 h post-treatment for the other life stages. Spinosad did not cause pupal mortality. Comparison of LD_{50} values of different life stages of *T. castaneum* at 48 h exposure-time revealed that, young larvae and adults were more susceptible to spinosad than the old larvae. Overlapping of 95% CL revealed similarity of susceptibility of young larvae and adult insects to spinosad. Due to compatibility of spinosad with environment, this compound could be considered as a useful tool in the control of the pest in question.

Key words: Spinosad, susceptibility, life stage, bioassay, *Tribolium castaneum*

INTRODUCTION

Tribolium castaneum Herbst (Col: Tenebrionidae) is an important pest of stored-products worldwide. This insect is found in temperate areas, but will survive the winter in protected places, especially where there is central heat (Tripathi *et al*., 2001). Therefore, it's control is imperative

Contact insecticides application against stored-products insects is an important tool in grain industry and is very important in situations where storage structures are not sufficiently gas-tight for effective fumigation or controlled atmosphere storage (Daglish *et al*., 2003). Although insecticides provide quick control of pests but often require repeated applications to provide long term management. Repeated applications of insecticides can lead to the development of resistance in the target pest. Therefore, concerns about application of a biorational insecticide such as spinosad which is derived from natural sources and is efficacious against the target pest are warranted. This biopesticide is less toxic to non-target organisms and natural enemies (Schuster and Stansly, 2005).

Spinosad is a reduced risk insecticide based on metabolites of a soil bacterium, *Saccharopolyspora spinosa* Mertz and Yao (Mertz and Yao, 1990). It is a mixture of the two most active naturally occurring secondary metabolites from aerobe fermentation of *S. spinosa*: spinosyn A and D (Sparks *et al*., 2001). Spinosad is a commercial insecticide reported to be effective against insect pests in the orders Lepidopterane, Diptera and Thysanoptera and some species of Coleoptera (Cloyd and Sadof, 2000; Peck and McQuate, 2000). Spinosad acts as a stomach and contact poison (Cisneros *et al*., 2002) and degrades rapidly when exposed to sunlight (Liu *et al*., 1999). This biopesticide has a unique mode of action with a very low mammalian toxicity compared with other insecticides (Thompson *et al*., 2000). The primary action of spinosyns is activation of the nicotinic acetylcholine receptors, but there are also some effects on GABA receptor function (Salgado, 1998).

The objectives of this study were to determine the efficacy of spinosad on some life stages of *T. castaneum*, at different exposure intervals and elucidate the interaction profile between application rate and exposure time.

MATERIALS AND METHODS

Insects: Adults of *T. castaneum* were obtained from toxicology laboratory of agricultural faculty of Urmia University. Insect were reared on whole wheat flour in 1.5 L glass jars at 28±1°C, 65±5% RH and 10:14 L: D. Unsexed adults (2-week-old), 1-3-day-old pupae, old larvae (22-day-old) and young larvae (12-day-old) were used in the tests. The mean±SE (n = 20) weight of old larvae was 3.2±0.2 mg and that of young larvae was 1.1±0.1 mg.

Corresponding Author: Roghayeh Yousefnezhad-Irani, College of Agriculture, Urmia University, Urmia, Iran
Tel: +98-441-2779552 Fax +98-441-2779558

Insecticide: Spinosad, (Tracer 24, NAF-315; Batch TA13272015) was used in these experiments as a liquid mixture of spinosyn A and D, (Copping and Menn, 2002) with 22.8% purity.

Bioassay: This study was carried out at Toxicology Department of Agricultural Faculty of Urmia University, Iran at 2005-2006. Primary dosages that cause mortality between 25-75% (Robertson and Preisler, 1992), were calculated on the basis of initial tests for each life stage, separately. All experiments were carried under rearing conditions. Stock solutions were prepared using distilled water.

Adults: Seven to fourteen day old adults were used in this experiment. For each insect 1.5 μL of spinosad solution or distilled water was applied to the dorsal thorax using an Oxford microaplicator. Dosages used for this stage were: 385, 835, 1811, 3926 and 8511 mg L^{-1}. Four replicates were used for each dosage and control group was included. Twenty individuals were used in each replicate. Control group was treated with 1.5 μL of distilled water. After treatments insects were introduced into 8 cm diameter Petri dishes and each dish was covered with lid. Mortality was recorded 24, 48 and 72 h post-treatment. Insects were judged to be dead when probing the antenna with a hot needle failed to produce a response.

Old larvae: Old larvae were exposed to dosages 11668, 30269, 78162, 201836 and 521194 mg L^{-1} of spinosad. The larvae were dosed topically with 1.5 μL of spinosad solution or distilled water as a control group. In each dose twenty larvae were used. Insects were introduced into 8 cm diameter Petri dishes. Mortality was recorded after 24, 48 and 72 h post-treatment. Each test was replicated four times on four different days and results were pooled. Those insects that did not move when probed or shaken in the light and mild heat considered dead.

Young larvae: Young larvae were exposed to 1006, 3548, 12502, 44055 and 155238 mg L^{-1} of spinosad. The larvae were dosed topically with 1 μL of spinosad solution. In each test, the control group was treated identically except that no spinosad was used. In each dose twenty larvae were used. After treatments insects were introduced into 8 cm diameter Petri dishes and each dish was covered with lid. Larval mortality was scored after 24, 48 and 72 h after treatment. Each test was replicated four times on four different days and results were pooled. The criterion for dead was similar to those described for the old larvae.

Pupae: Dosages of 32000, 64000, 128000, 256000 and 512000 mg L^{-1} were used for initial experiment on 1-3-day-old pupae. One milliliter of spinosad solution or distilled water was applied to the dorsal thorax using an Oxford microaplicator. Control group were treated with 1 μL of distilled water. Mortality was recorded after exclusion. Pupae were considered dead if adults did not emerged and deformed adults were considered as live.

Data analysis: Mortality percentage of insects was transformed using arcsine to stabilize treatment variances for statistical analysis. All data were subjected to one-way analysis of variance (ANOVA) to compare mortality percentage ($p>0.05$). Treatment means were separated using Duncan's Multiple Range Test ($p<0.05$). Lethal dosages and the associated 95% CL were calculated by probit analysis with SPSS software (SPSS, 1993). Significant difference at LD_{50} values were compared by constructing 95% CL around the means. Means with no overlapping 95% CL were considered significantly different ($p<0.05$). Data were analyzed separately for each life stages.

RESULTS

Adults: Main effects application rate ($F = 104.75^{**}$; df = 5, 71) and exposure interval ($F = 43.52^{**}$; df = 2, 71) and the exposure×rate interaction ($F = 2.74^{**}$; df = 10, 71) were all significant at 99% confidence level ($p<0.01$). The amounts of intercept were positive for all three intervals and it was the lowest after 24 h post-treatment interval. The amounts of slope were positive for three intervals and it's highest amount was at 24 h after treatment. LD_{50} and LD_{95} values were decreased with increasing the time (Table 1).

Old larvae: Effects of application rate ($F = 92.203^{**}$; df = 5, 51) and exposure-time ($F = 101.444^{**}$; df = 2, 51) were significant at 99% confidence level ($p<0.01$). Effect rate×exposure interaction ($F = 1.263$; df = 10, 51) was not significant for mortality ($p>0.05$). It means that this interaction did not affect each other for mortality of old larvae. The amounts of intercept and slope were positive in all three intervals. An inverse relationship between LD_{50} and LD_{95} values and exposure times was observed (Table 2).

Young larvae: Main effects application rate ($F = 125.276^{**}$; df = 5, 51) and exposure interval ($F = 113.038^{**}$; df = 2, 51), plus interaction: rate×exposure ($F = 2.775^{**}$; df = 10, 51) were all significant at 99% confidence level ($p<0.01$). Slope and intercept were positive for all time intervals and the lowest amount of intercept was at 24h post-treatment interval. LD_{50} and LD_{95} values were decreased with increasing of the time (Table 3).

Table 1: Probit regression estimates (Mean±SE) for adults treated with spinosad

Post treatment intervals	Total No. insects	Intercept (±SE)	Slop (±SE)	LD_{50} (95%CL) mg L^{-1}	LD_{95} (95%CL) mg L^{-1}	χ^2 (df)	p-value
24h	480	0.46±0.51	1.26±0.15	3836(2976-5285)	76516(37394-239620)	4.08 (3)	0.25
48h	480	2.60±0.45	0.80±0.13	973(581-1409)	109223(36301-973048)	0.20 (3)	0.97
72h	480	1.77±0.50	1.18±0.15	528(334-724)	12819(7602-30060)	2.11(3)	0.55

Since goodness-of-fit of the probit model to dose/response data is not significant (p>0.05), no heterogeneity factor is used in the calculation of confidence limits

Table 2: Probit regression estimates (mean±SE) for old larvae treated with spinosad

Post treatment intervals	Total No. insects	Intercept (±SE)	Slop (±SE)	LD_{50} (95%CL) mg L^{-1}	LD_{95} (95%CL) mg L^{-1}	χ^2 (df)	p-value
24h	480	1.2(±00.59)	0.65(±0.12)	607174 (320966-1964603)	195354573 (25366209-13726252246)	6.06(3)	0.1
48h	480	0.74(±0.67)	0.81(±0.13)	161244 (107085-271495)	16524529 (4544992-192860999)	0.73(3)	0.86
72h	480	2.58(±0.61)	0.56(±0.12)	18240 (5130-34768)	14051029 (2690284-797881088)	1.38(3)	0.7

Since goodness-of-fit of the probit model to dose/response data is not significant (p>0.05), no heterogeneity factor is used in the calculation of confidence limits

Table 3: Probit regression estimates (mean±SE) for young larvae treated with spinosad

Post treatment intervals	Total No. insects	Intercept (±SE)	Slop (±SE)	LD_{50} (95%CL) mg L^{-1}	LD_{95} (95 %CL) mg L^{-1}	χ^2 (df)	p-value
24h	480	2.09(±0.41)	0.59(±0.1)	69657(38595-170484)	38450192 (5709544-1444061575)	0.94(3)	0.81
48h	480	3.63(±0.37)	0.45(±0.09)	1069(181-2615)	4795829 (748573-327809465)	0.55(3)	0.9
72h	480	3.7(±0.42)	0.54(±00.1)	237(22-727)	249170(81301-2586861)	1.1(3)	0.77

Since goodness-of-fit of the probit model to dose/response data is not significant (p>0.05), no heterogeneity factor is used in the calculation of confidence limits

Pupae: Results of the initial test (a = -7.348, b = 1.98, χ^2 = 1.12(3), p = 0.77, total mean = 4% showed that spinosad did not affect pupal mortality of the *T. castaneum.*

DISCUSSION

Adults: Positive amounts of intercept showed there was natural mortality in all three intervals and its lowest amount was at 24 h post-treatment interval. According to positive amounts of slope in all three intervals a direct relationship between mortality rate and dosage was detected (Table 1). Results were similar to those reported by Eger *et al.* (1998) in which mortality of *Frankliniella* spp showed a dose dependent response and Toews and Subramanyam (2003) working with *T. castaneum* reported similar results. A biochemical reason for this similarity is imposing of the same enzymes with spinosad in these experiments. Mortality of adults showed a time dependent response and 72 h after treatment caused the most mortality, similar to those reported by Ludwing and Oetting (2001) for *Frankliniella occidentalis* Pergands. The interaction effect of rate×exposure imposed positive effect on efficacy of spinosad. Fang *et al.* (2002) reported that mortality of *T. castaneum* was increased with increasing the interaction effect of rate with time on Durum, Hard red spring and Hard red winter, similar to those obtained in this experiment.

Old larvae: Positive amounts of intercept showed natural mortality in all three intervals. According to positive amounts of slope in all three intervals a direct dose dependent response was concluded (Table 2) and this is similar to those reported by Aydin and Gürkan (2006) in which mortality of third instar larvae of *Spodoptera littoralis* Biosduval increased as dose increased. Exposure interval and rate×exposure interaction affected mortality of old larvae positively.

Young larvae: The amounts of intercept were positive in all three intervals that show the natural mortality for these intervals and its lowest amount was at 24 h post-treatment interval. Positive amounts of slope indicated that mortality was increased with increasing the rate of spinosad, which was similar to those reported by Brunner and Doerr (1996) in which neonate larvae of *Pandemis pyrusana* Kearfortt and *Choristoneura rosacena* showed a direct dose dependent response to spinosad. Mortality of young larvae after 72 h post-treatment was higher than 24 h and 48h after treatment. Other results showed that toxic effect of spinosad on 2nd instar of *Lymantria dispar* L. was consistent 9 day after treatment Wanner *et al.* (2000) which differs with our results. One explanation to this disparity could be differing the insects of two experiments and experimental conditions. Rate×exposure interaction positively affected the mortality.

Pupae: Pupae of *T. castaneum* were not affected by topical application of spinosad, possibly due to low penetration of this compound. Based on our findings similar results reported by Pineda *et al.* (2004) on *S. littoralis* pupae earlier. Merdina *et al.* (2001) also have

been reported previously lack of spinosad effect to *Chrysoperla Carnea* Stephens pupae.

LD_{50} values for adults, young and old larvae after 48 h post-treatment were 973, 1069 and 161244 mg L^{-1}, respectively. Therefore, adults and young larvae are more susceptible to spinosad in comparison with old larvae. Toews and Subramanyam (2004) reported that young larvae were susceptible to spinosad. LD_{50} value of adults was less than young larvae but due to overlapping of 95% CL of LD_{50} values for adults and young larvae, no significant difference between their susceptibility to spinosad was detected.

The current study indicated that *T. castaneum* adults and larvae are controllable using spinosad at dose levels within the range of those tested here. The dosages required for effective control can be lowered if provision can be made to extend exposure periods. By speculation similar species may also be controlled by such dosages at extended exposure time, but this would need conformation by toxicity studies. Also the high effective dosages can be due to using the Tracer® formulation and it's inert ingredients because we can not obtain pure active ingredient for using in this study.

ACKNOWLEDGMENT

We thank the Plant Protection Department of Urmia University for providing facilities of this study. We are grateful to Mr. Nazari for providing the technical spinosad used in this study.

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