ECOLOGIA BALKANICA
2015, Vol. 7, Issue 1 June 2015 pp. 39-44

Susceptibility of Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae) and Sitophilus oryzae (L.) (Coleoptera: Curculionidae) to Spinosad (Tracer®) as a Eco-friendly Biopesticide

Reza Sadeghi[1], Asgar Ebadollahi[2*]

1- Department of Entomology and Plant Pathology, College of Abouraihan, University of Tehran, Tehran, IRAN
2- Young Researchers and Elites Club, Ardabil Branch, Islamic Azad University, Ardabil, IRAN
*Corresponding author: asgar.ebadollahi@gmail.com

Abstract. *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) are internal feeder of various stored grains and introducted as major stored product insect pests. Due to the resistance by insect pests and negative effects of synthetic pesticides to the environment, it is necessary to use novel and suitable compounds in insect pest's management. Spinosad is a bio-insecticide that is derived from fermentation of a bacterium *Saccharopolyspora spinosa* Mertz and Yao. In the present study, the toxicity of Tracer® as a spinosad based insecticidise was evaluated against *R. dominica* and *S. oryzae*. Insect species were kept in stored-products insects rearing room in Agriculture Faculty of Tehran University at 27 ± 2ºC, 65 ± 5% relative humidity. Adult insects were exposed to different concentrations of Tracer® by oral trials for 10 and 20 days exposure periods. The mortality data were subjected to probit analysis using SPSS software to estimate LC (lethal concentration) values and their related information**.** Tracer® showed strong toxicity against the adults of *R. dominica* and *S. oryzae*. Maximum mortality was occurred in the concentration of 250 and 80 ppm after 20 days exposure for *R. dominica* and *S. oryzae*, respectively. Direct relationship between mortality of insects with concentration and exposure period was found. Probit analysis displayed *R. dominica* (10-days LC_{50} = 49.89 ppm) was more susceptible than *S. oryzae* (10-days LC_{50} = 50.75 ppm) to Tracer®. Results of present study stimulated the utilization of Tracer® as an eco-friendly and safe agent for insect pests' management.

Key words: Bioassay, *Rhyzopertha dominica*, *Sitophilus oryzae*, Toxicity, Tracer®.

Introduction

Cereal grains such as wheat and rice are the main sources of human diets. These grains are highly susceptible to infestation by stored product insects such as the lesser grain borer [*Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)] and the rice weevil [*Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)]. *R. dominica*, is a destructive insect pest of stored grains. Both larvae and adults of this insect feed on whole, sound grains and cause extensive damage (JOOD *et al.*, 1996; REES, 2007). *S. oryzae* is one of the most widespread and destructive insect pest of stored cereals. These pests are internal feeders and cause considerable loss to cereals affecting the quantity as well as quality of the grains (KUCEROVA *et al.*, 2003; REES, 2007).

Synthetic pesticides have been considered the most effective means to control insect pests of stored products.

Indiscriminate use of these chemicals have given increase to many serious problems, including resistance of pest species, toxic residues, and environmental and human health concerns (SANNA *et al.*, 2004; TAPONDJOU *et al.*, 2005; KOUL *et al.*, 2008). For example, the use of organophosphate was decreased, because of increasing resistance incidence in stored-product insects against these chemicals (FANG *et al.*, 2002). Fumigants such as phosphine and methyl bromide have been applied to control stored-products insects for a long time. Development of insect resistance to phosphine has been repoted recently (BENHALIMA *et al.*, 2004; COLLINS *et al.*, 2005) and application of methyl bromide has been largely scaled down because of its carcinogenicity and effect on the depleting ozone layer (MBTOC, 2002). There is, therefore, an urgent need to develop eco-friendly materials and methods with slight adverse effects on the environment and on consumers.

Spinosad is a secondary metabolite from the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yoa. The parent strain was originally isolated from an abandoned rum still in the Caribbean by Elanco in 1982 and introduced commercially in 1997 by Dow AgroScience (KIRST *et al.*, 1992). This is a mixture of spinosyns A ($C_{41}H_{65}NO_{10}$) and D ($C_{42}H_{67}NO_{10}$) and through contact and ingestion is highly toxic on a number of insect pests (COPPING & DUKE, 2007). It affects on target insects through the activation of the nicotinic acetylcholine receptor, but at a different site than nicotine or the neonicotinoids. It also affects on GABA receptors, but their role in the overall activity is unclear. There is currently no known cross-resistance to other insecticide classes. The mode of action causes rapid death of target phytophagous insects. Its moderate activity reduces the possibility of the onset of resistance (THOMPSON *et al.*, 2000). Spinosad is sold as a water-based suspension concentrate (SC) formulation under the trade names Tracer®, Conserve, Success, SpinTor (Dow AgroSciences) and etc. Spinosad with high efficacy such as a broad insect pest spectrum, low mammalian toxicity, and minimal environmental profile is unique among existing products currently used for stored-grain protection (HERTLEIN *et al.*, 2011). Spinosad is considered to be practically non-toxic to birds but slightly to moderately toxic to fish. It is highly toxic to honey bees, with less than 1 mg/bee of technical material applied topically resulting in mortality. Once residues are dry, however, they are non-toxic (CISNROS *et al.*, 2002). Spinosad is rapidly degraded on soil surfaces by photolysis and, below the soil surface, by soil microorganisms (SAUNDERS & BRET, 1997; TOEWS *et al.*, 2003). Moreover, spinosad does not have carcinogenic, mutagenic and tumorigenic effects (SCHOONOVER & LARSON, 1995). Spinosad is classified by the U.S. Environmental Protection Agency as an environmentally and toxicologically reduced risk material (CLEVELAND *et al.*, 2002).

Therefore, the objective of this research was to investigate the toxicity of Tracer® as a biological control agent against two major stored-grain insects: *R. dominica* and *S. oryzae*.

Materials and Methods

Tracer®. The Tracer® (48% SC) used in this study was purchased from American Dow AgroSciences Company (Zionsville Road, Indianapolis, IN 46268). Cytowet oil as a 100% pure liquid was used to distribute the insecticides evently on glasses (5 cm diameter and 15 cm height). Distilled water was used for dilution of Tracer®.

Insect rearing. Both insect species were kept in stored-products insects rearing room in Agriculture Faculty of Tehran University at 27 ± 2ºC, 65 ± 5% relative humidity and 14 D: 10 L photoperiod. The rearing medium was wheat for *R. dominica* and soft wheat for rearing *S. oryzae*.

Bioassay. After preliminary tests with different concentrations and concentration fixing (ROBERTSON *et al.*, 2007), the main tests were performed using 5 concentrations of Tracer® - 60, 95, 150, 250 and 400 ppm for *R. dominica* and 15, 23, 36, 52 and 80 ppm for *S. oryzae*. The concentrations were prepared by diluting of insecticide in distilled water. A drop of cytowet oil which decreased the surface tension was used for even distribution of insecticide on the foodstuff surface (one drop of cytowet oil was used for a 50 milliliters of solution). All the surfaces of foodstuff (40 grams of wheat) were impregnated by 9 milliliters of a given concentration. After treatment of the wheat, their were dried under ambient conditions. Fifteen adult insects were introduced to each glass and glasses were covered with muslin cloth and kept under rearing conditions. All stages were done for control group without insecticide adding. Each concentration was replicated four times and for each concentration totally 60 adult insects were used.

Statistical analysis. The data were corrected using Abbott's formula (ABBOT, 1925) for the mortalities in the controls. Experiments were arranged in a completely randomized design and a two way analysis of variance was used to analyze the effect of varying concentrations and exposure periods on insect mortality. The means were separated by the Tukey test at the 5% level. The lethal concentration LC_{50}, chi-square, and 95% confidence intervals for each regression coefficient were calculated by using probit analysis (FINNEY, 1971). All statistical analyses were performed using the statistical software SPSS version 16.0 (SPSS, 2007).

Results and Discussion

Present study showed strong toxicity of Tracer® on the adults of *R. dominica* and *S. oryzae*. Results indicated that Tracer® with concentration of 250 ppm caused 100% mortality on *R. dominica* at 20 days' exposure time (Fig. 1). Figure 1 also displays the 88% mortality in *S. oryzae* adults with concentration of 80 ppm after 20 days. On the other hand, maximum mortality was occurred in the concentration of 250 and 80 ppm after 20 days exposure for *R. dominica* and *S. oryzae*, respectively. Compare means of mortalities revealed direct relationship between mortality of both insects with concentration and exposure times (Fig. 1). Concentration-mortality response lines for both insects exposed to different concentrations of Tracer® have shown in Fig. 2, too.

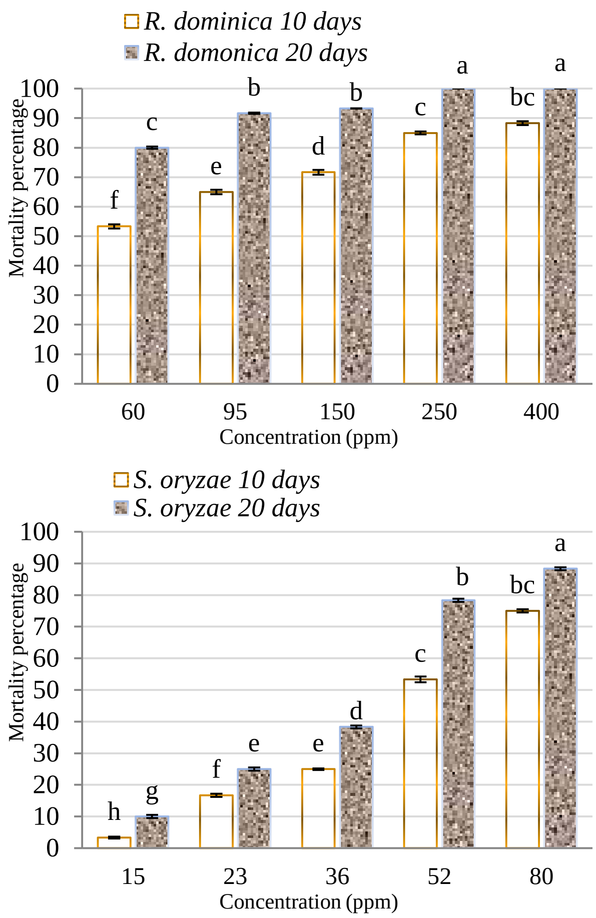

Fig. 1. Mean mortality (%) of *Rhyzopertha dominica* and *Sitophilus oryzae* adults exposed to different concentrations of Tracer® after 10 and 20 days of exposure times. Different letters on top of columns indicate significant differences according to Tukey's test at p = 0.05. Columns with the same letter are not significantly different.

Probit analysis demonstrated that *R. dominica* (10-days LC_{50} = 49.89 and 20-days LC_{50} = 31.39 ppm) was susceptible than *S. oryzae* (10-days LC_{50} = 50.75 and 20-days LC_{50} = 36.53 ppm) (Table 1). From the probit analyses, the calculated regression line equations of the first and second days' data were $Y = 1.99X - 2.37$ and $Y = 2.85X - 4.27$ for *R. dominica* and $Y = 3.31X – 5.65$ and $Y = 3.57X – 5.58$ for *S. oryzae*, respectively (Table 1). The concentrations were used as followed: 60, 95, 150, 250 and 400 ppm for *R. dominica* and 15, 23, 36, 52 and 80 ppm for *S. oryzae*. Number of insects for each time was 360 (15 insects × 4 replications × 6 concentration).

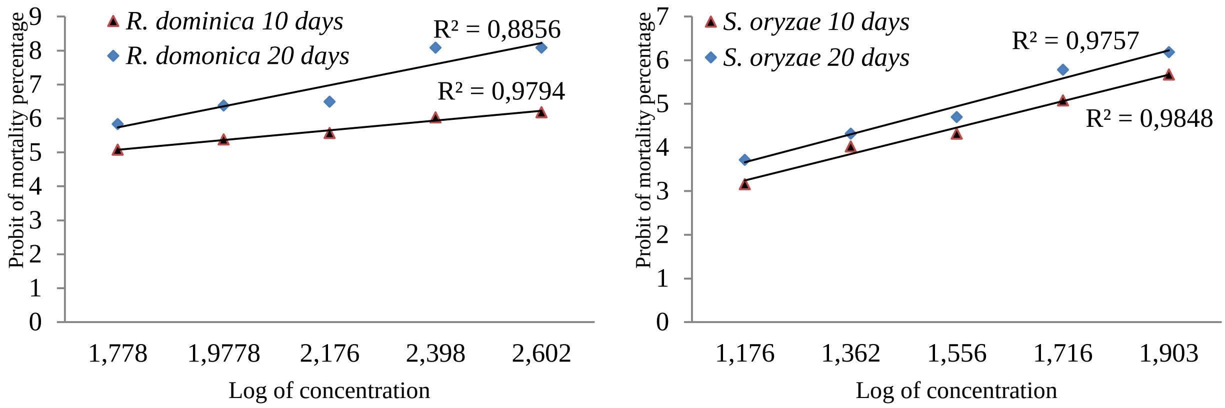

Fig. 2. Concentration-mortality response lines for adult of *Rhyzopertha dominica* and *Sitophilus oryzae* exposed to different concentrations of Tracer®.

Table 1. Probit analysis of diffirent treatments of Tracer® against two experimented insects in 10 and 20 days exposure times.

Insect	Time (day)	LC_{50} [a]	LC_{90} [a]	Intercept ± SE	Slope ± SE	χ^2 (df=3)	ρ [b]
R. dominica	10	49.89 (24.46 ± 70.87)	411.72 (275.59 ± 962.62)	-2.37 ± 0.61	1.99 ± 0.29	0.29	0.96
	20	31.39 (10.54 ± 45.63)	88.31 (71.20 ± 112.92)	-4.27 ± 1.45	2.85 ± 0.75	*0.84*	0.84
S. oryzae	10	50.75 (45.16 ± 58.30)	123.74 (86.62 ± 174.45)	-5.65 ± 0.62	3.31 ± 0.38	2.03	0.57
	20	36.53 (32.84 ± 40.71)	83.46 (70.27 ± 106.47)	-5.58 ± 0.57	3.57 ± 0.57	*4.72*	0.19

[a] 95% lower and upper fiducial limits are shown in parenthesis.
[b] Since goodness−of−fit Chi square is not significant (P > 0.15), no heterogeneity factor is used.

These results is similar to the results of FANG *et al.* (2002), MCLEOD *et al.* (2002) and TOEWS *et al.* (2003) that they have worked on some stored-products insect pests.

Spinosad is purposed for the control of a very wide range of important pests. For example, AHMED (2004 Cyro, Egypt, pers comm.) reported that the spinosad was the most effective compound against the newly hatched larvae of both pink and spiny bollworms after 12 days for laboratory and field strain, respectively. He added that spinosad contacts and affects the receptors of acetylcholine in different place contacts of acetylcholine, which caused hyper-activity in nervous system for a long time. SADAT & ASGHAR (2006) indicated that liquid formulation of spinosad (Tracer® 22.8%) had considerable contact toxicity against adults of *Callosobrochus maculatus* (F.). In that study, spinosad caused 75–100% mortality in 4 concentration rates: 400, 300, 185 and 150 ppm. In the study of SEMIZ *et al.* (2006), insecticidal effects of spinosad were expressed against the pine processionary moth, *Thaumatopoea wilkinsoni* Tams. In the other study, toxicity of Tracer® was evaluated to larve of common green lacewing, *Chrysoperla carnea* Stephens. In contact bioassay tests, a direct relationship was detected between the concentration of spinosad and mortality rate of first instar larvae so that the employing of 250 and 2500 ppm of Spinosad caused 33 and 67 percent mortality, respectively (MAROUFPOOR *et al.*, 2010). Toxicity of spinosad (Tracer® 24% SC) were evaluated against cotton leaf worm, *Spodoptera littoralis* (Boisd.) by KORRAT *et al.* (2012). Along with toxicity, pupation, fecundity, hatchability and sterility rates and adult emergence percentages of insect were affected by spinosad. Effectiveness of spinosad was evaluated against adults *C. maculatus* on four commodities: chickpea, split pea, cowpea and lentil by KHASHAVEH *et al.* (2011). Mortality of exposed individuals in all treated commodities was low at 1-day exposure even at 0.3 g/kg and did not exceed 20%. As expected, mortality increased with the increase of exposure interval and dose rates. After 10 days of exposure, mortality reached 100% in all commodities except for split pea. The application of spinosad significantly reduced progeny production in four commodities tested in comparison with the untreated ones. Recently PIRI *et al.* (2014) displayed sublethal effects of spinosad including reduction in glutathione S-transferase activity, percentage of larval pupation and female fecundity against lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae).

Results of the present and mentioned studies adequately expressed the toxicity of spinosad against a wide variety of insect pest along with stored product insect pests. With retrospect, it could be concluded that spinosad and Tracer® as one of the spinosad based traditional formulation is merit to be considered as a potential compound in controlling the insects in question.

Acknowledgements

The authors thank the Aburihan campus, University of Tehran, for providing facilities for this research.

References

ABBOTT W. 1925. A method for computing the effectiveness of an insecticide. - *Journal of Economic Entomology*, 18: 265-267.

BENHALIMA H., M. Q. CHAUDHRY, K. A. MILLS, N. R. PRICE. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. - *Journal of Stored Products Research*, 40(3): 241-249. DOI:10.1016/S0022-474X(03)00012-2.

CISNROS J., D. GOULSON, C. L. DEWENT, I. D. PENAGOS, O. HERNEZ, T. WILLIAMS. 2002. Toxic effects of spinosad on predatory insects. - *Biological Control*, 23(2): 156-163. DOI:10.1006/bcon.2001.1000.

CLEVELAND C. B., M. A. MAYES, S. A. CRYER. 2002. An ecological risk assessment for spinosad use on cotton. - *Pest Management Science*, 58(1): 70-84. DOI:10.1002/ps.424.

COLLINS P. J., G. J. DAGLISH, H. PAVIC, R. A. KOPITTKE. 2005. Response of mixed age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentrations and exposure periods. - *Journal of Stored Products Research*, 41(4): 373-385. DOI:10.1016/j.jspr.2004.05.002.

COPPING L. G., S. O. DUKE. 2007. Natural products that have been used commercially as crop protection agents. - *Pest Management Science*, 63(6): 524–554. DOI:10.1002/ps.1378.

HERTLEIN M.B., G.D. THOMPSON, B SUBRAMANYAM., C.G. ATHANASSIOU. 2011. Spinosad: a new natural product for stored grain protection. - *Journal of Stored Products Research*, 47(3): 131-146. DOI:10.1016/j.jspr.2011.01.004.

JOOD S., A. C. KAPOOR, R. SINGH. 1996. Effect of infestation and storage on lipids of cereals. - *Journal of Agricultural and Food Chemistry*, 44(6): 1502-1506. DOI:10.1021/jf950270e.

KIRST H. A., K. H. MICHEL, J. S. MYNDERSE, E. H. CHIO, R. C. YAO, W. M. NAKASUKASA. 1992. Discovery, isolation, and structure elucidation of a family of structurally unique, fermentationderived tetracyclic macrolides. – In: Baker D., J. Fenyes, J. Steffens (Eds.), *Synthesis and Chemistry of Agrochemicals III*, Ch. 20, American Chemical Society, Washington.

KHASHAVEH A., M. ZIAEE, M. H. SAFARALIZADEH. 2011. Control of pulse beetle, *Callosubruchus maculatus* (F.) (Coleoptera: Bruchidae) in different cereals using spinosad dust in storage conditions. - *Journal of Plant Protection Research*, 51: 77-81. DOI:10.2478/v10045-011-0014-z.

KOUL O., S. WALIA, G. S. DHALIWAL. 2008. Essential oils as green pesticides: potential and constraints. - *Biopesticides International*, 4(1): 63-84.

KORRAT E., A. ABDELMONEM, A. HELALIA., H. KHALIFA. 2012. Toxicological study of some conventional and nonconventional insecticides and their mixtures against cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noectudae). - *Annual Agricultural Sciences*, 57(2): 145-152. DOI:10.1016/j.aoas.2012.08.008.

KUCEROVA Z., R. AULICKY, V. STEJSKAL. 2003. Accumulation of pest-arthropods in grain residues found in an empty store. - *Journal of Plant Disease and Protection*, 110: 499-504.

MBTOC. 2002. *Report of the Methyl Bromide Technical Options Committee (MBTOC) 2002 Assessment*. UNEP, Nairobi, Kenya, 437 pp.

FANG L., B. SUBRAMAYAN, H. F. ARTHUR. 2002. Effectiveness of spinosad on four classes of wheat against five stored insects. - *Journal of Economic Entomology,* 95(3): 640- 650. DOI:10.1603/0022-0493-95.3.640.

FINNEY D. J. 1971. *Probit Analysis*, 3rd edn. Cambridge University Press, London.

MAROUFPOOR M., M. H. SAFARALIZADEH, A. A. POURMIRZA, S. ALLAHVAISY, S. GHASEMZADEH. 2010. Lethal effects of Spinosad on *Chrysoperla carnea* larvae (Neuroptera: Chrisopidae) under laboratory conditions. - *Journal of Plant Protection Research*, 50(2): 179-183. DOI:10.2478/v10045-010-0030-4.

MCLEOD P., F. J. DIAZ, D. T. JOHNSON. 2002. Toxicity persistence and efficacy of spinosad, chlorfenaptro and thamethoxam on eggplant when applied against the eggplant flea beetle (Coleoptera: Chrysomelidae). - *Journal of Economic Entomology*, 95(2): 331-335. DOI:10.1603/0022-0493-95.2.331.

PIRI F., A. SAHRAGARD, M. GHADAMYARI. 2014. Sublethal effects of spinosad on some biochemical and biological parameters of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). - *Plant Protection Science*, 50: 135-144.

ROBERTSON J., R. RUSSELL, H. PREISLER, N. SAVIN. 2007. *Bioassay with Arthropods*. CRC Press, England.

REES D. 2007. I*nsects of stored grain: a pocket reference*. Australia. CSIRO Publishing.

SADAT K., P. ASGHAR. 2006. The influence of post-exposure temperature on the toxicity of Spinosad against adults of *Callosobruchus maculatus* Fabricius (Coleóptera: Bruchidae). In: *Proc. 9th Int. Work. Conf. Stored-Prod. Protect.* Campinas, Sauo Paulo, Brazil.

SANNA G., E. BAZZONI, M. MORETTI. 2004. Microencapsulated essential oils active against Indian meal moth. - *Boletín de Sanidad Vegetal Plagas*, 30: 125-132.

SAUNDERS D.G., B.L. BRET. 1997. Fate of spinosad in the environment. - *Down Earth*, 52: 21-28.

SCHOONOVER J.R., L.L. LARSON. 1995. Laboratory activity of spinosad on non-target beneficial arthropods. - *Arthropod Management Tests*, 20: 357.

SEMIZ G., H. CETIN, K. ISIK, A. YANIKOGLU. 2006. Effectiveness of naturally derived insecticide, spinosad, against the pine processionary moth, *Thaumatopoea wilkinsoni* Tams. (Lepidoptera: Thaumatopoeidae) under laboratory conditions. - *Pest Managment Science*, 62(5): 452-455. DOI:10.1002/ps.1181.

SPSS. 2007. *SPSS Version 16.0.SPSS Inc,* 233 S. Wacker Drive, Chicago, Illinois. [ibm.com/software/analytics/spss/]

TAPONDJOU A. L., C. ADLER, D. A. FONTEM, H. BOUDA, C. REICHMUTH. 2005. Bioactivities of cymol and essential oils of Cupressus sempervirens and Eucalyptus saligna against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. - *Journal of Stored Products Research*, 41(1): 91-102. DOI:10.1016/j.jspr.2004.01.004.

THOMPSON G. D., R. DUTTON, T. C. SPARKS. 2000. Spinosad – a case study: an example from a natural products discovery programme. - *Pest Managment Science*, 56(8): 696-702. DOI:10.1002/1526-4998(200008)56:8<696::AID-PS182>3.0.CO;2-5.

TOEWS M. D., B. SUBRAMAYAN, J. M. ROWAN. 2003. Knockdown and mortality of adults of eight species stored-products beetle exposed to four surfaces treated with spinosad. - *Journal of Economic Entomology,* 96: 1967-1973. DOI:10.1603/0022-0493-96.6.1967.

Received: 23.01.2015
Accepted: 12.03.2015

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House