STUDIES ON THE CONTACT EFFECT OF SPINOSAD ON ADULT OF *TRIBOLIUM CASTANEUM* (TENEBRIONIDAE), *SITOPHILUS ORYZAE* (CURCULIONIDAE) AND *ORYZAEPHILUS SURINAMENSIS* (SILVANIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT: Due to economical importance of stored-products insects and resistance to conventional insecticides, it is necessary to use novel and suitable compounds in control programs. Therefore, we evaluated the toxicity of spinosad to three important storage insects viz, Red flour beetle, *Tribolium castaneum* (Herbst), Rice weevil, *Sitophilus oryzae* (L.), and Sawtoothed grain beetle *Oryzaephilus surinamensis* (L.). One-day old adult insects were exposed to different concentrations of spinosad. The exposure times were 24, 48 and 72 hours in all experiments. Experiments were performed in complete randomized block design with 4 replications. After treatment the samples were held under constant conditions in rearing room at 27±2°C, 65±5 R.H and 14 D: 10 L photoperiod. In contact tests for *T. castaneum* the maximum mortality rate was achieved with 500 ppm of spinosad after 72 h exposure. Application of 400 ppm of spinosad after 72 h caused maximum mortality in *S. oryzae*. For contact treatment the complete control of *O. surinamensis* was obtained with 500 ppm spinosad at 72 h exposure time. It is possible to recommend 500 ppm of spinosad for complete control of the tested insects through contact application. Estimated LC_{50} values of spinosad in 72 h after exposure for adults of *T. Castaneum*, *S. oryzae* and *O. surinamensis* were 1287, 279 and 53.17 ppm, respectively. Results showed that *O. surinamensis* is most susceptible insect to spinosad in these experiments.

KEY WORDS: Spinosad, Bioassay, *Tribolium Castaneum*, *Sitophilus oryzae*, *Oryzaephilus surinamensis*.

To protect the agricultural products against insect pests in warehouses, chemical insecticides have been the most effective tool for a long time (Sadeghi & Pourmirza, 2009). To preserve the quantity and quality of stored-product foodstuff particularly cereals it is necessary to decrease the population of the insect pests (Pourmirza & Tajbakhsh, 2001). It is well documented that the number of contact insecticides with lower toxicity to human and environment are very limited (Arthur, 1999; Leesch, 1995). Studies show that the most important and conventionally used contact insecticide to protect wheat against stored-products insects has been malathion (Daglish, 1998), but since 1999 its application in stores is no longer approved (Arthur, 1999). The use of organophosphate was decreased, because of increasing resistance incidence in stored-product insects against these chemicals (Fang et al., 2002). Fumigants have been applied to control stored-products insects for a long time but in recent years the number of usable fumigants has been decreased considerably (Leesch, 1995). Methyl bromide is used as a fumigant to control insects pests on stored cereals (United Nations Environmental Programme, 2001). Application of methyl bromide has been largely scaled down because of its carcinogenicity and its effect on depleting ozone layer (Johnson et al., 1998; United Nations Environmental Programme, 2001). Therefore this fumigant will be phase-out in immediate future and could be replaced by new compound such as spinosad (Cisnros et al., 2002). Spinosad is less persistent with formidable insecticidly properties owing low level of toxicity against mammals and birds (Carson & Trumble, 1997; Cisnros et al., 2002; Toews et al., 2003). Moreover, spinosad does not have carcinogenic, mutagenic and tumorigenic effects (Schoonover & Larson, 1995). This compound is also compatible with other integrated pest management program measures (Thompson et al., 1999) and it could be a useful tool in insect control programs (Bessin, 2001).

MATERIALS AND METHODS

Spinosad is a secondary metabolite from aerobic fermentation of *Saccharopolyspora spinosa* (Mertz & Yao) which is formed on foodstuffs. During this process, spinosad is extracted as a concentrated substance (Anonymous, 1996). By stimulating the nervous system of insects spinosad causes involuntary muscle contraction, insect knock down and severe shakings; the insect is then paralyzed and dies after 4-5 days. These effects are caused by nicotine receptors of acetylcholine. Spinosad also affects GABA (gamma- amino butyric acid) receptors (Salgado, 1998; Salgado et al., 1998; Salgado, 1997). The spinosad used in this study was purchased from American Dow Agro Science Company 48 % SC.

To distribute the insecticides evenly on Petri-dish with the diameter of 5 and height of 1cm surface, cytowet oil was used. Cytowet oil consisted of emulsifier was applied as a 100% pure liquid. Distilled water was used for dilution of spinosad and had the pH value of 6.7. All three insect species were kept in stored-products insects rearing room in Agriculture Faculty of Urmia University at 27±2°C, 65±5% R.H and 14 D: 10 L photoperiod. The rearing medium for *T. castaneum* was wheat flour mixed with 5% of yeast (Lale & Yusuf, 2001). Soft Wheat was used for rearing *S.* oryzae (Padin et al., 2002) and oat used as rearing medium for *O. surinamensus* (Tunçbilek, 1997).

Contact bioassay with one-day old insects

After preliminary tests with different concentrations and dose fixing the main tests were performed using 5 concentrations of spinosad and a control group. 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 Petri-dish surface (one drop of cytowet oil was used for a 50 milliliter of solution). All the internal surfaces of Petri-dish were impregnated by 2 milliliter of a given concentration. After all surfaces were impregnated, the surplus liquid was removed and Petri-dishes were left to one hour under ambient conditions. Fifteen one-day old adult insects were introduced to each Petri-dish. The dishes were kept at rearing conditions. Mortality was recorded after 24, 48 and 72 hours and each experiment was replicated four times. After treatment of the Petri-dishes these were dried under ambient conditions. Fifteen one-day old adult insects were introduced to each Petri- dish and Petri- dishes were covered muslin cloth and kept under rearing conditions. Each concentration was replicated four times and for each concentration totally 60 one- day old adult insects of each species were used.

Basic Design and Data Analysis

The completely randomized block design through factorial tests was used. Data transformation were performed as Arcsin $\sqrt{x}$ and $\sqrt{x}$ whenever needed and MSTATC program was used for variance analysis. Mortality data from all bioassays were analyzed with SPSS software (SPSS Inc, 1993).

RESULTS AND DISCUSSION

Results of table 1 based on the LC_{50} values showed that after 24 hours of contact exposure, adults of *T. castaneum* are more tolerant than two other insects and adults of *O. surinamensis* are more susceptible than *T. castaneum* and *S. oryzae* (*T. castaneum*> *S. oryzae* > *O. surinamensis*). Amounts of RR_{50} values prove this claim. Results of F values showed that there is no significant difference between the concentrations in the case of *T. castaneum*, but a significant difference was observed between concentrations that used on *S. oryzae* in $\rho<0.05$ and *O. surinamensis* in $\rho< 0.01$.

Data from table 2 showed that, after 48 hours since the application of contact exposure, adults of *S. oryzae* were more tolerant than two other insects and adults of *O. surinamensis* were more susceptible than *T. castaneum and S. oryzae*. (*S. oryzae* > *T. castaneum* > *O. surinamensis*). RR_{50} values prove this claim. The results showed that there is a significant difference between concentrations which were applied against these three species.

Results showed that there is no significant difference between concentrations in the case of *T. castaneum*, but a significant difference was observed between concentrations which were used against *S. oryzae* and *O. surinamensis*. RR_{50} values in 72h exposure times showed that adults of *T. castaneum* are more tolerant than two other insects and adults of *O. surinamensis* are more susceptible than *T. castaneum* and *S. oryzae* (*T. castaneum*> *S. oryzae* > *O. surinamensis*) (Table3).

Data from table 4 showed that, F values of time, concentrations and the interaction between concentrations and time periods (Time×Concentration) are significant on the three experimented insects.

With retrospect results of these experiments show that maximum mortality of red flour beetle occurred in the concentration of 500 ppm after 72 h exposure which agree with the results of Toews et al. (2003) and Mcleod et al. (2002) working with some stored-products pest insects. Considering the results from this study the highest mortality of rice weevil adults was obtained in the concentration of 400 ppm after 72 hours which is in agreement with the results of Mcleod et al. (2002) and Fang et al. (2002) which reported a direct relationship between mortality rate of *R. dominica* and exposure time. The present study showed that 500 ppm concentration of spinosad over 72 hours exposure caused the highest mortality rate of sawtoothed grain beetle. This finding is similiar to the results of Fang et al. (2002) which reported that mortality percentage of *R. dominica* was increased by increasing the concentration of spinosad. With retrospect it could be concluded that spinosad is merit to be considered as a potential compound in controling the insects in question.

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Table 1. Toxicity of spinosad to *T.castaneum*, *S.oryzae* and *O.surinamensis* in 24h exposure times.

Toxicity value	*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Lethal concentration	17668	127995	15625	36826	12751	94986
Slope (b)	0.33		0.69		0.42	
χ^2	2.37		0.129		0.584	
ρ	0.499		0.988		0.90	
F	$1.16^{n.s}$		4.13^{*}		15.78^{**}	
$RR_{50}{}^{a}$	1.38		1.22			

The concentrations were used as followed: 0, 100, 150, 220, 340 and 500 ppm for *T.castaneum*.
The concentrations were used as followed: 0, 150, 190, 250, 310 and 400 ppm for *S.oryzae*.
The concentrations were used as followed: 0, 40, 75, 145, 270 and 500 ppm for *O.surinamensis*.
[a]Resistance Ration(RR) is equal to LC_{50} each species/LC_{50} of the most susceptible species(*O.surinamensis*).

Table 2. Toxicity of spinosad to *T.castaneum*, *S.oryzae* and *O.surinamensis* in 48h exposure times.

Toxicity value	*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Lethal concentration	2405	32842	3701	42910	217	2544
Slope (b)	1.44		0.796		1.54	
χ^2	1.52		1.63		7.55	
ρ	0.67		0.65		0.05	
F	14.00^{**}		5.06^{*}		158.03^{**}	
$RR_{50}{}^{a}$	11.08		17.05			

The concentrations were used as followed: 0, 100, 150, 220, 340 and 500 ppm for *T.castaneum*.
The concentrations were used as followed: 0, 150, 190, 250, 310 and 400 ppm for *S.oryzae*.
The concentrations were used as followed: 0, 40, 75, 145, 270 and 500 ppm for *O.surinamensis*.
[a]Resistance Ration(RR) is equal to LC_{50} each species/LC_{50} of the most susceptible species(*O.surinamensis*).

Table 3. Toxicity of spinosad to *T.castaneum*, *S.oryzae* and *O.surinamensis* in 72h exposure times.

Toxicity value	*T. castaneum*		*S. oryzae*		*O. surinamensis*	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Lethal concentration	1287	21584	279	1515	53.17	530.2
Slope (b)	1.34		2.23		1.64	
χ^2	1.26		0.99		10.21	
ρ	0.73		0.80		0.01	
F	$2.06^{n.s}$		25.79^{**}		122.71^{**}	
$RR_{50}{}^{a}$	24.20		5.24			

The concentrations were used as followed: 0, 100, 150, 220, 340 and 500 ppm for *T.castaneum*.
The concentrations were used as followed: 0, 150, 190, 250, 310 and 400 ppm for *S.oryzae*.
The concentrations were used as followed: 0, 40, 75, 145, 270 and 500 ppm for *O.surinamensis*.
[a]Resistance Ration(RR) is equal to LC_{50} each species/LC_{50} of the most susceptible species(*O.surinamensis*).

Table 4. Variance analysis of diffirent treatments of three experimented insects mortality in 24, 48 and 72h exposure times.

S. V	*T. castaneum*			*S. oryzae*			*O. surienamensis*		
	df	Mean square	F	df	Mean square	F	df	Mean square	F
Time	2	2421.45	276.24^{**}	2	2804.14	152.46^{**}	2	7599.92	295.56^{**}
Concentration	5	226.364	25.82^{**}	5	1244.83	67.68^{**}	5	4214.64	163.90^{**}
Time×Concentration	10	150.81	17.20^{**}	10	126.39	6.87^{**}	10	305.84	11.89^{**}
Total	17			17			17		

** *p* is significant at 0.01 level.