EFFECTIVENESS OF SPINOSAD AGAINST TRIBOLIUM CASTANEUM (HBST) (COLEOPTERA-ENEBRIONIDAE)

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ABSTRACT

Studies were conducted in the Grain Research Training and Storage Management Cell (GRTMC), Department of Agri- Entomology, University of Agriculture Faisalabad, to evaluate the effect of Spinosad (0.25%, 0.50%, 0.75% and 1.0%) at different doses with different exposure times against *Tribolium castaneum*. Spinosad at 1%, 0.75%, 0.50% and 0.25% concentrations killed 61.85%, 55.56%, 45.93% and 32.59% *Tribolium castaneum* adults respectively which were statistically different (P=0.05) than the percent kill of the test insect in the control. The toxicity of spinosad to the test insect was also dose dependent, the higher the concentration, the significantly more mortality of the insect. Exposure of the test insect to spinosad for different time intervals (24, 48 & 72 hours) resulted into different percent mortality of *T. castaneum*. The percent mortality of the insect after 72hours (51.33%) exposure was significantly different from the percent mortality after 48hours (40.89%) and 24hours (25.33%) exposure. The interaction effect of 1% spinosad and 72 hours exposure gave 75.55% kill, which was significantly (P=0.05) more than the percent kill of the other concentrations & exposure time except the interaction effect at 0.75% spinosad exposed for 72 hours. The population build-up of *T. castaneum* after exposure to Spinosad at different concentrations and different exposure time was significantly lower than that in the control. The negative effect of the chemical on the population build up of *T. castaneum* was dose and exposure time dependent.

Key words: Spinosad, *Tribolium castaneum*, Time interval, wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major food grain crop of Pakistan. Area and production of wheat for the year 2011-2012 was 8666 thousand hectares and 23517 thousand tons (Anonymous, 2011). In stores, wheat grains and grain products are severely damaged qualitatively and quantitatively by stored grain insect pests, especially in the warm climate, which favors rapid build-up of insect population. The losses by stored grain insect pests have been studied by several research workers. Jilani, (1981) observed that among various factors contributing losses in stored grain, insect pests are the most important one. Ahmad, (1986) estimated 10-15% losses to stored grains by insect pests. Similarly, Weifen *et al.*, (2003) in China and Singh and Yadav, (1995) observed 7 to 13% and 2.03%, respectively losses in stored wheat by stored grain insect pests.

Although, there are more than 40 insect species which attack stored grain and grain products, *Rhizopertha dominica* F., *Tribolium castaneum* Hbst., *Trogoderma granarium* Everts., *Sitophilus oryzae* L. and *Sitotroga cerealella* Oliv. are of great economic importance (Irshad and Talpur, 1993). Among the above mentioned insect pests, *Tribolium castaneum* is the most serious insect pest of stored grains and grain products world over. *Tribolium castaneum* adults are 3-4 mm in length and red brown in color. Female lays 327-956 eggs which hatch in 4-10 days. Larval stage remains for 22-25 day at $30C^0$.

Pupation takes place in flour and the stage lasts for 5-9 days. The life cycle is completed in 26-30 days in the summer but takes longer under unfavorable conditions.

The indiscriminate use of pesticides for the management of this notorious insect is not only hazardous to human beings but responsible for disturbing the eco-system as well. The use of these synthetic insecticide have also resulted resistance in *T. cateneum* against these insecticides. Shakoori *et al*. (1998) and Lessard *et al.* (1998) have also reported resistance in *T. castaneum* against synthetic pyrethroids, e.g., cypermetrin, deltamethrin, cyfluthrin, fenvalerate and some juvenile hormone analogues. The hazardous nature of fumigants has made it obligatory to test insecticides bearing novel modes of action, e.g., abamectin, spinosad, indoxacarb, azadirachtin and hydrocarbon against *T. castaeum* to minimize the risks and losses. Few research workers have tested these chemicals effectively against stored grain insect pests. Toews *et al*. (2003) found that *Tribolium spp*. was highly susceptible to spinosad on concrete (98-100% mortality). Similarly, Daglish and Nayak (2006) evaluated the persistence and efficacy of spinosad against *Rhyzopertha dominica* (F.) in wheat stored for 9 months. Spinosad applied at 0.5 or 1 mg kg^{-1} was effective for 9 months with 100% adult mortality after 14 days of exposure and no live F_1 adults produced. Mortality of *T. castaneum* adults increased with the increase in spinosad dose level (Fang *et al* 2002).

Keeping in view the hazards of conventional insecticides and resistance development in insects against these chemicals, the present studies were under taken to evaluate the insecticidal activities of spinosad for the management of *T. castaneum* and to study the residual activity of spinosad in term of post treatment population build-up of *T. castaneum.*

MATERIALS AND METHODS

Research trials were carried out in the Grain Research Training and Storage Management Cell (GRTSMC), Department of Agricultural Entomology, University of Agriculture, Faisalabad to study the response of *Tribolium castaneum* (Hbst.) to spinosad.

Adults of *Tribolium castaneum* were collected from local godowns of the Punjab Food Department and cultured at $30\pm2^{o}C$ and 60 ± 5 R.H on whole wheat grains mixed with flour in 800 gm capacity jars sterilized at $60C^{o}$ for 60-90 minutes. Each jar was filled with 200 gm wheat grains admixed with 20 gm flour and about 100 beetles were added to each jar assuming 50 males & 50 females. The jar were then covered with muslin cloth and tied with rubber bands to avoid the escape of beetles and the entry of ants or other insects. Beetles were allowed for 3 days to oviposite in the medium. After 3days, the insects were removed with the help of sieves using camel hair brushes from the media and were added to another set of sterilized jars filled with 200 gm wheat grains + 20 gm flour for further infestation for the purpose of fresh culture development. The flour containing eggs of the test insect was placed in other jar to get different larval stages of the test insects and the fresh adults. It takes approximately 28 days for reaching to 6^{th} instars larvea and 39 days to become adult.

The Spinosad (Tracer 24%) was purchased from the Bayer Crop Sciences of Pakistan and diluted in ethanol to obtain 0.25%, 0.50%, 0.75% and 1.0% solution to be

tested against the test insect. From the spinosad 0.25%, 0.50%, 0.75% and 1.0% concentrated were prepare for experimental purpose by talking 0.25ml, 0.50 ml, 0.75 ml and 1.0 ml spinosad added with 99.75, 99.50, 99.25 and 99 ml ethanol, respectively.

To determine the response of *Tribolium castaneum* (Hbst.) to spinosad at different doses for different time intervals Circular plastic petri dishes (150 X 25 mm) were used as testing arena. A filter paper (Fisher Sci., Pittsburg, Penn.) was placed in the bottom of each Petri dish. Ethanol solution of 0.25%, 0.5%, 0.75% and 1.0% Spinosad was applied separately to the filter papers in the dishes through micropipette. The Petri dishes were left open to air for an hour or so to completely evaporate the solvent. Thirty adults of *T. castaneum* were released in each treatment including control for up to 72hours. The Petri dishes were covered with lid to avoid the escape of the test insect. All the treatments including control were replicated thrice. Though, the mortality of the test insect in each concentration was recorded after 24, 48 and 72 hours, an overall mean percent mortality in each concentration in the mentioned three time intervals were analyzed with Analysis of Variance (ANOVA) and means for each treatment were separated with LSD (α= 0.05).

In other experiment the same method for testing spinosad against *T. castaneum* was tested and the data for percent kill of the test insect in all used concentration was recorded after 24, 48 and 72 hours. The mean percent mortality of the insect in each time interval was analyzed with analysis of variance (ANOVA) and means separated with LSD ($\alpha = 0.05$).

To see the post treatment population build-up of the test insect, the survived insects in each treatment of both trials were released separately in glass jars with 800 gm capacity containing 200 gm whole wheat grains + 20gm of wheat flour. The mouth of the jars were covered with muslin cloth and secured by rubber bands to prevent the entry of any foreign insect. The jars were placed in the controlled environment. The rearing temperature of the controlled environment was $30\pm2\ ^{0}$C and 60 ± 5 R.H with 12h light. The numbers of *T. castaneum* adults in each treatment were recorded for post treatment population build up and finally the data for each aspect was analyzed statistically and means separated with LSD for significant differences.

RESULTS

The result of Table-I showed that spinosad at all levels of concentration proved to be toxic to *Tribolium castaneum*; as 61.85%, 55.565, 45.93% and 32.59% mortality of the test insect at 1%, 0.75%, 0.50% and 0.25% concentration, respectively was significantly more than the percent kill in the control**.** Results also indicated that the percent kill of the test insect by spinosad was dose dependent. The higher the concentration of the spinosad, the more the percent kill of the test insect. The percent kill of the test insect in all treatments was significantly (P=0.05) different from each other.

Table-I. Mean percent mortality of *Tribolium castaneum* at different levels of

concentration of spinosad up to 72 hours.

Concentrations	Percent Mortality
0.25%	32.59 d
0.50%	45.93 c
0.75%	55.56 b
1.0%	61.85 a
Control	0.000 e
LSD Value	**4.170**

Means followed by the same letters are not significantly different from each other at $\alpha = 0.05$

The results of Table-II showed that when the test insect was exposed to spinosad treated media at various concentrations for different time intervals, the toxic effect of the chemical was different at different exposure period. The 72 hour exposure time of the insect at various doses resulted in 51.33% kill, which was significantly more than the 40.89% and 25.33% mortality of the test insect after 48 and 24 hour exposure, respectively. Results also indicated that the longer the insect was exposed to treated media, the higher was the percent kill of the test insect.

Table-II. Mean percent mortality of *Tribolium castaneum* with Spinosad at different exposure times.

Exposure Time (Hours)	Percent Mortality
24	25.33 c
48	40.89 b
72	51.33 a
LSD Value	**3.230**

Means followed by the same letters are not significantly different from each other at $\alpha = 0.05$

The Table-III showed the interaction effect of spinosad at various concentration and exposure times. The 75.55% mortality of the test insect occurred in the media treated with spinosad at 1.00% concentration exposed for 72 hour was significantly more than any other treatment and time interval in the experiment except the interaction effect at 0.75% spinosad and 72 hour time interval. Results clearly indicated that there was significant interaction effect among the spinosad concentration and exposure times. The 36.67% mortality with 0.75% for 24 hour exposure was statistically similar to the 42.22% mortality at 1.00% treatment for 24 hour exposure which was significantly less than the 60.00% kill at 0.75% for 48 hours exposure and 67.78% mortality of the insect at 1.00% for 48 hour exposure. Spinosad at 1% concentration resulted in 42.22% mortality when the test insect was exposed for 24 hour and it was statistically similar to 44.44% kill at 0.5% when exposed for 48 hour and 47.78% kill at 0.25% when exposed for 72 hour. The percent mortality of the insect with 0.75% after 24 hour

exposure, 0.5% after 24 hour of exposure and 0.25% after 48 hours exposure time were statistically at par to each other. Spinosad at higher concentrations after72hour exposure time resulted into higher percent mortalities as compared to that in the control as well as the rest of the treatment except the kill of the insect at 0.75% spinosad exposed for 72 hour.

Table-III: Interaction Effect of Spinosad doses and exposure time on the mean percent mortality of *Tribolium castaneum.*

Treatment	Mean Percent Mortality Exposure Times		
	24Hour	**48Hour**	**72Hour**
0.25%	17.78 g	32.22 f	47.78 d
0.50%	30.00 f	44.44 d	63.33 bc
0.75%	36.67 ef	60.00 c	70.00 ab
1.0%	42.22 de	67.78 b	75.55 a
Control	0.000 h	0.000 h	0.000 h
LSD Value		**7.22**	

Means followed by the same letters are not significantly different from each other at α = 0.05

The *T. castenum* adults which survived after feeding on media treated with spinosad at different concentration for different time intervals were allowed to develop its F1 progeny on untreated medium. The Table-IV showed that the population developed at 1.0% concentration of spinosad was 574.8, which was significantly lower than the population build-up of *T. castaneum* in all other treatments. The post treatment population build-up of the test insect at 0.25% and 0.50% concentrations of spinosad was 657.8 and 652.5 adults of the test insect which were statistically at par with regard to the post treatment population build-up of the test insect. The maximum population was recovered from the control.

Table-IV. Post treatment population build-up of *Tribolium castaneum* at different dose rates of Spinosad up to 72 hours.

Concentrations	***Mean Population Buildup***
0.25%	657.8 b
0.50%	652.5 b
0.75%	626.1 c
1.0%	574.8 d
Control	795.2 a
LSD Value	**5.913**

Means followed by the same letters are not significantly different from each other at α = 0.05.

The Table-V showed that when the survived *T. castaneum* fed on spniosad treated media at different concentrations for 24 hour, 48 hour and 72 hour were allowed to develop its F1 progeny on untreated medium, the highest mean population (677.8) was found for 24 hour exposure of the test insects which was significantly more than the 670.5 and 635.5 individuals obtained after 48 hour and 72 hour exposure, respectively. It indicates that when more the exposure time the less will be the population build-up of
T. castaneum.

Table-V. Post treatment population build-up of *Tribolium castaneum* after exposure to different doses of spinosad treated media at different time interval.

Exposure Time (Hours)	***Mean Population Buildup***
24	677.8 a
48	670.5 b
72	635.5 c
LSD Value	**4.580**

Means followed by the same letters are not significantly different from each other at $\alpha = 0.05$

The interaction effect of spinosad at various concentrations and different exposure time on the population build-up is evident from the results in Table-VI. The 500.0 individuals with spinosad at 1.0% concentration for 72 hours was significantly lower than any other interaction in the experiment followed by 607.8 and 617.4 individuals in the treatment for 48 hour and 24 hour, respectively exposures at the same concentration of spinosad. Spinosad at 0.75% concentration for 24 hour exposure resulted into 649.4 population build-up which was statistically similar to 639.3 individuals of *T. castaneum* exposed for 48 hour at the same concentration and was also statistically similar to 652.5 insect at 0.50% concentration at 48 hour exposure as well as to 659.1 and 642.6 individuals at 0.25% for 48 hour and 72 hour, respectively exposure of the test insect. The post treatment population build-up (668.3) at 0.50% concentration of spinosad for 24 hour exposure was statistically identical to 659.1 individuals at 0.25% concentration for 48hour exposure. Maximum population build-up was observed in the control at 72 hour exposure which was 808.9% followed by 48 hour and 24 hour which were 794.4 and 782.2 insects, respectively.

Table-VI. Post treatment population build-up of *Tribolium castaneum* at different doses of spinosad with various exposure times.

Treatment	**Mean Percent Population Build-up Exposure Times**		
	24 Hours	**48 Hours**	**72Hours**
0.25%	671.6 d	659.1 ef	642.6 ghi
0.50%	668.3 de	652.5 fg	636.6 i

0.75%	649.4 fgh	639.3 hi	589.6 l
1.0%	617.4 j	607.0 k	500.0 m
Control	782.2 c	794.4 b	808.9 a
LSD Value		**10.24**	

Means followed by the same letters are not significantly different from each other at $\alpha = 0.05$

DISCUSSIONS

Adults of Tribolium castaneum responded differently to spinosad at different levels of concentration in different time intervals. All the tested concentrations of spinosad significantly killed more *T. castaneum* and adversely affected the post treatment population build-up of the insect. Spinosad showed 61.85% kill at 1.0% concentration up to 72hours. Maximum mortality (51.33%) of the test insect was found when it was exposed to spinosad with all tested concentrations for 72hours. As compared to the 795.2 post treatment population build-up in the control, the 574.8 individuals obtained at 1.0% concentration was the lowest population build-up in all treatments in the trails. Spinosad has also been tested against stored grain insect pests by other research workers. The research workers in their studies almost obtained identical results to the present studies. Little deviation in the results of few workers may be because they used different concentrations of spinosad and different stored grain insect species. Toews and Subramanyam (2003) evaluated the contact toxicity of spinosad to adults *Tribolium castaneum*. Mortality of *T. castaneum* ranged 12 to 48% when adults were exposed to 0.001–0.79 mg cm^{-2} deposits of spinosad in 24-h tests and 0, 0.0016 and 0.016mg cm^{-2} deposits in 48-h tests. Nipkay (2007) found that mortality of *T. castaneum* was 65% when treated with spinosad at the rate of 1 mg (a.i.)/kg.of wheat. Progeny production was significantly lower in comparison with the untreated ones. In other studies, Yousefnezhad-Irani and Asghar (2007) reported that young larvae and adults of *T. castaneum* were more susceptible to spinosad than the old larvae. Daglish *et al*. (2003) reported that an application of 1 mg/kg of spinosad in wheat may be suitable for controlling *R. dominica* strains that are resistant to organophosphates, pyrethroids or the insect growth regulator methoprene. Fang and Subramanyam (2003) reported that all adults of *Rhyzopertha dominica* were killed when treated with spinosad at the rate of 0.1 and 1 mg/kg of wheat. Spinosad with 100% adult mortality and progeny reduction after 4d exposure at 1 mg [a.i.]/kg was the most effective chemical against *R. dominica* (F.) (Nayak *et al.* 2005). In laboratory tests, Huang and subramanyam (2007) found that on spinosad-treated corn, adult mortality of *Cryptolestes ferrugineus*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, *Sitophilus oryzae*, and *S. zeamais* was > 98% at 1 and 2 mg/kg after 12 days and almost complete suppression of progeny production and kernel damage of all species after 49 days.

Subramanyam *et al.* (2007) found that a single application of spinosad at 1 mg/kg is effective for managing common stored-grain insects, including *R. dominica*, for at least 6 months. According to the above

said authors spinosad is an effective grain protectant and also have great effect on progeny reduction of stored grain insect pests.

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