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Influence of spinosad on the reproductive potential of *Tribolium castaneum* (Herbst), (Coleoptera: Tenebrionidae) infesting wheat

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ABSTRACT: Spinodad of different concentrations were screened against *Tribolium castaneum* (Herbst) reared on four local wheat varieties to observed the effects on reproductive potentials for two successive generations.The lowest number of eggs laid was 119 (23.80±0.97) observed in Shatabdi-21 (S-21) in 45 d in F_1 and 15 (3.00±0.71) in 15 d in F_2 generation when treated at 0.12µl/g of spinosad. Spinosad at all concentration totally inhibited egg laying oroviposition rate on day 45 in all wheat varieties except P-24 variety in F_1 generation.The lowest fertility was found in Shatabdi-21 variety as 12.61percent in F_1 and 6.67 percent in F_2 generation at 0.12µl/g.The latent effect of spinosad on number of eggs, larva, pupa and adult obtained in Shatabdi-21 as 2.00±0.32,0.80±0.37, 0.40±0.40 and 0.20±0.20 at 0.12µl/g of spinosad in F_2 generation.There was no significant difference between the number of males and females in F_2 generation wheat varieties. Spinosad ultimately reduced the fecundity, fertility and decreased the egg to adult survivability in four wheat varieties compared to control and F_1 and F_2 generations. Results of the research revealed that comparatively higher concentrations of spinosad that used in this study would potentially control development and reproductive potentiality of *T. castaneum* in wheat varieties.

INTRODUCTION

In a recent post-harvest compendium *T. castaneum* is reported as most common secondary pest of all plant commodities in store throughout the world (Babarinde and Adeyemo, 2010; Stejskal *et al*., 2014). This external feeder pest makes serious damage on flour and over whelms cereal particularly at larval and adult stages (Baldwin and Fasulo, 2004) making it unfit for human intake.This insect causes substantial loss in storage because of its high reproductive potential (Campbell and Runnion, 2003). Diet type and initial population density may also have an immediate or indirect impact on the reproduction, development rate, number of progeny and body weight of *T. castaneum* (Longstaff, 1995; Assie *et al*., 2008). Shukla and Upadhyay (2011) noticed that the progeny production rate of *T. castaneum* was very excessive and the fourth instar larvae were enormously energetic in rainy season causing very excessive infestation where in adults could live for 2 years or greater in the course of duration the female producing nearly 1000 eggs.

Spinosad is an insecticide product from Dow Agro Sciences (Indianapolis, Indiana, USA) based on

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chemical compounds of a soil bacterium *Saccharopolyspora* was discovered in 1985 (Mertz and Yao, 1990). Hertlein *et al*. (2011) stated that spinosad has an aggregation of high potency, minimum mammalian toxicity, and secure environmental profile which is unique among existing products recently used for stored-grain protection.The mode of action of spinosad is characterized by involving the disruption of nicotinic acetylcholine receptors and γ-amino butiric acid (GABA)-gated ion channels of insect nervous systems (Salgado and Sparks, 2005; Kirst, 2010; Sparks *et al*., 2012). Spinosad must be sprayed directly into the eggs, but larvae and adults can be efficaciously dosed through contact butit is most effective when ingested (Cleveland *et al*., 2001). Spinosad is more toxic through ingestion than via contact (Athanassiou and Kavallieratos, 2014).

Wheat is the major and second staple food/ worldwide and Bangladesh respectively. Different wheat varieties were developed in different countries, among which the factors like pest resistant and insecticide susceptible varieties gaining importance from the point of view of reducing infection stress during post-harvest storage. In this research four local varieties of wheat were used to screen out the variety that is susceptible to spinosad treatment without leaving any hazardous effect on human health. The objective of this experiment was to evaluate the effect of different concentrations of spinosad on the reproductive potentials of *T. castaneum* for two successive generations as a result of contact action to eggs and contact and gustatory effects on the adults of *T. castaneum* in four wheat varieties local of Bangladesh.

MATERIALS AND METHODS

Culturing of *T. castaneum*: *T. castaneum* beetles were obtained from the stock culture maintained in the control temperature (CT) room, at Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh. The standard food medium, mixture of whole wheat flour with powdered Brewer's Yeast (19:1) (Park and Frank, 1948; Park, 1962) was used as a food medium throughout the mass culture of *T. castaneum*. Both flour and yeast were previously passed through a 250μm sieve sterilized at 60°C for six hours in an oven, and kept at least for 15 days before use, to equilibrate the moisture content with the environment (Khan, 1981; Mondal, 1984).

Mass cultures were maintained in plastic containers (2.5 liters) in a Control Temperature (CT) room at 30±1°C and 70±5% RH providing 250g of wheat flour /container. About 500 adults of *T. castaneum* were introduced into each container. The cultures were checked at regular intervals and eggs and larvae were separated to avoid cannibalism. A crumpled filter paper was placed inside each container for easy movement of the beetles. Mouth of the container was covered with muslin cloth using a rubber band, to prevent the possible contamination and escape of insects (Mondal and Parween, 1997). A series of stock culture were maintained for the constant supply of these insects to conduct different experiments.

Preparation of spinosad concentrations:

Spinosad is light grey to white in colour with slight odour stale water. About 500ml of spinosad (PRN-MAPP-12054, cafno 20012-019, Lot No-3068404) was obtained from Dow Agro Sciences, UK. Concentration of spinosad was 120g a.i./l.

Spinosad measuring 0.72μl was pippetted in a glass vial and added 6ml distilled water properly by using 2ml syringe. The vial was shaken vigorously for thorough mixing of spinosad and water serving as stock having 0.12μl spinosad/ml of other desired concentrations of spinosad (0.06 and 0.03μl/g) were prepared by serial dilution by taking 1 ml of solution and adding 2 ml distilled water in each step.

Wheat varieties: Infestation free four wheat varieties viz., BARI-26, BARI-28, Prodip-24 and Shatabdi-21 (B-26, B-28, P-24 and S-21) were used for the experiment. The grains were washed with water and dried at room temperature before adjusting their moisture content to 13.5 by adding tap water, cleaned by sieving through 500 micrometer aperture sieve and sterilized in an oven at 60°C for 8 h. After sterilization wheat grains were kept in separate plastic containers according to variety.Grains were partially broken down by the hand blender before further use.

Bioassay: The efficacy of spinosad against reproductive potentiality of *T. canstaneum* was investigated by dietary exposure termed Treated Food Method (TEM) (Talukder and Howse, 1994). Newly emerged 50 pupae from culture were sexed by the electric microscopic examination of the exo-genital process. In female the structure of exo-genital lobes are larger than male. The genital papillae of females are pointy but male's genital papillae are stubby, conjoined. Female papillae resemble fingers like 2 combined thumbs (Halstead 1963). After adult emergence, 20 male and females were paired up to 15 days and collected about 350 numbers of freshly eggs with the help of microscope.Two grams of each wheat variety was soaked with different concentrations of spinosad as treated wheat and 2gms of each wheat variety was soaked with distilled water as for control or untreated wheat. About 20 eggs were placed on each petri-dish and provided with 2gms treated wheat and untreated wheat of each variety which was changed after every three days. The larvae were checked regularly until they emerged as pupae. Pupae from each treated concentration were paired by microscopic examination of the exo-genital process. After 48 h emerged adults were paired and 5 pairs of beetles from each concentration were placed in separate petri-dishes and provided with 2gms of treated wheat of each variety of different concentrations for egg collection.

Eggs from each pair were collected after three days up to a 45 day. Total number of eggs laid by each pair up to 45 daywas recorded and daily egg production/female was determined. Fertility of the eggs was assessed by the how many larvae were recovered from the total number of eggs up to 45 days of 5 pairs adults into 100 was recorded. The ultimate effects of the spinosad were assessed by recording the total number of larvae produced, recoveries of total pupae and adults up to 45 d. About fifty pupae were sexed and the males and females were kept separately again.

After emergence, adults were paired again (5 pairs) and kept separately. Similarly, the total number of eggs laid by each pair up to 15 days, fertility of these eggs, the total number of larvae produced, and recoveries of pupae and adults were recorded. The second generation (larvae to adults) was developed on untreated wheat. A similar set of experiment was conducted with same number of beetles in untreated wheat, as the control batch. All the experiments were conducted at CT room (30±1°C) and 75% RH and replicated thrice. Data obtained from the experiments were analyzed using the Analysis of variance (Factorial) using SPSS version 20. Means were compared by Tukey's test, accepting significant differences at $P<0.05$. Sex ratios were determined by χ^2 test ($P>0.05$).

RESULTS AND DISCUSSION

i) Effect on fecundity in two successive generations

The total number of egg laid by *T. castaneum* ranged from 656 to 1125 in control and 119 to 761 in treatments in F_1 and 163 to 384 in control and 15 to 145 in treatments in F_2 generation.The lowest number of eggs laid was 119 (23.80±0.97) observed in S-21 up to 45 days in F_1 and 15 (3.00±0.71) up to 15 days in F_2 generation at 0.12µl/g and the highest number of egg was found in P-24 as 761 (152.20±2.60) in F_1 and 145 (29.00±1.30) in F_2 generation at 0.03µl/g concentration (Tables 1 and 2).

ii) Effect on fertility of the eggs in two successive generations

Spinosad at all concentrations reduced fertility of the laid eggs in a concentration- dependent manner in F_1 and F_2 generations in all wheat varieties (Figure 1). The percentage of fertility of eggs ranged from 74.70 to 89.67 percent in untreated wheat and 12.61 to 64.91 percent in treatments in F_1 generation. The lowest fertility was noted in S-21 variety as 12.61 percent at 0.12µl/g in F_1 generation. In F_2 generation, the percentage of fertility ranged from 69.94 to 85.16 percent in untreated wheat and 6.67 to 45.52 percent in treatments. The same trend was also observed in F_2 generation where the lowest fertility was recorded in S-21 variety as 6.67 percent.

iii) Latent effect of spinosad on the life stages produced in second generations

The latent effect of different concentrations of spinosad at different life stages and sex ratio of *T. castaneum* deviated from 1:1 in F_2 generation; the effects were concentration-dependent and in all wheat varieties (Table 2).The mean number of eggs production ranged from 32.60±1.47 to 76.80±1.39 in control and 3.00±0.71 to 29.00±1.30 in treatments. Out of four wheat varieties, the lowest number of eggs was produced in S-21 as 3.00±0.71 at 0.12µl/g of spinosad compared with control in F_2 generation (Table 2).

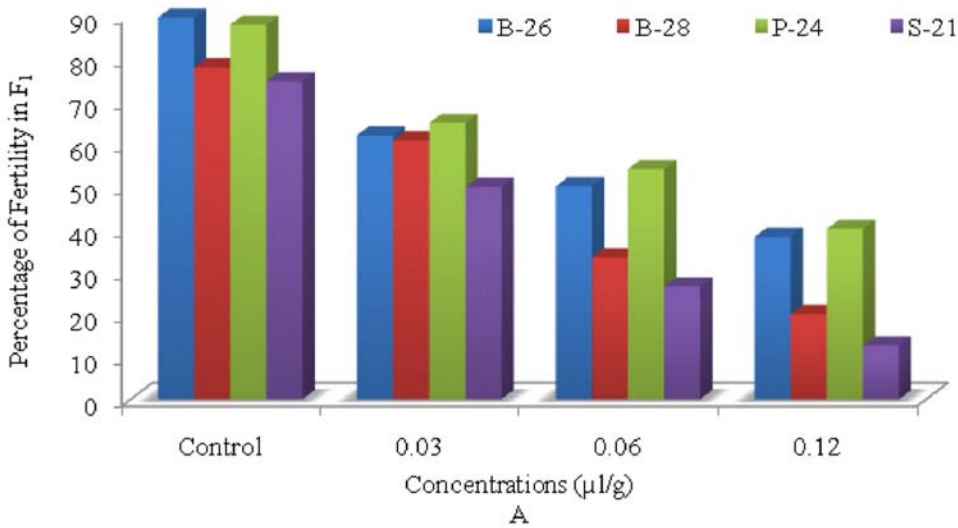
Larval mortality was found to be very high especially in spinosad at 0.12µl/g concentration. Few larvae became black and shriveled, failed to shed the old cuticle and died while hatching after 2-3 days. Larval survival ranged from 22.80±1.02 to 65.40±0.86 nos in control and 0.20±0.20 to 13.20±0.86 nos in treatments. The lowest number of larva was recorded in S-21 as 0.20±0.20 at 0.12µl/g compared with the control and rest of other concentrations (Table 2). The mean number of pupal survival ranged from 20.80±1.02 to 50.20±1.80 nos in control and 0.00±0.00 to 9.00±0.711 nos in treatments. The lowest number of pupa was recorded in S-21 as 0.00±0.00 at 0.12µl/g compared with the control and other concentrations (Table 2).

The mean number of adult survival ranged from 17.80±0.86 to 41.60±1.50 nos in control and 0.00±0.00 to 5.60±0.40 nos in treatments. The lowest number of adult was observed in S-21 as 0.00±0.00 at 0.12µl/g of spinosad compared with the control and other concentrations. Few adults became black and shriveled (Table 2). The sex ratios for the treatment variables did not differ significantly from each other in any generation as judged by χ^2 test (P>0.05) (Table 3).

Results of the present experiments have demonstrated that spinosad affected egg to adult survivability and reproductive potentiality of *T. castaneum* in F_1 (45 d) and F_2 (15 d) generations. There was significant decrease in egg number of *T. castaneum* laid on different wheat varieties. The lowest number of eggs laid (119) in 45 d was observed in S-21 in F_1 generation; and that was 15 laid in 15 d in F_2 generation; at highest concentration 0.12µl/g. Egg laying was decreased in all wheat varieties and in F_1 generation females laid small number of eggs after 33 d; and at highest concentration was totally failed in the later part of oviposition period.

Spinosad at 0.12µl/g concentration totally inhibited egg laying on day 45 in F_1 and day 15 in F_2 generation in all wheat varieties. Absence of adequate published literature on the spinosad toxicity on the fecundity and oviposition rate of stored product insects, make the present result quite difficult to compare. Similarly fertility rate lowest in S-21 variety as 12.61 percent in F_1 and 6.67 percent in F_2 generation at 0.12µl/g concentration. Accordingly, lowest number of larva, pupa and adult was recorded in S-21 at 0.12µl/g of spinosad in F_2 generation.

Bajracharya *et al.* (2013) reported that spinosad caused high mortality and complete progeny suppression of *Rhizopertha dominica* at five storage periods (2, 84, 168, 252, and 336 d) which was in agreement with Bonjour *et al*. (2006) who demonstrated that stored wheat treated with 1 ppm of spinosad completely controlled *R. dominica* adults and progeny production for all the post treatment storage periods (28, 84, 182, 252, 336, and 672 d). Subramanyam *et al*. (2012) reported that complete suppression in *R. dominica* (0.0±0.0) and near complete suppression of adult progeny in *T. castaneum* (1.2±1.0 and 0.6±0.4) achieved on spinosad-treated wheat (spinosad I and spinosad II) after 42 days.The author additionally reported that no *Plodia interpunctella* adults have been found on liquid spinosad treated wheat after 42 days. These findings are consistent with Huang *et al.* (2004) who stated that spinosad was highly toxic against *P. interpunctella* inhibiting larval survivability and adult emergence on wheat and maize. Adult mortality of *Cryptolestes ferrugineus*, *R. dominica*, *Oryzaephilus surinamensis*, *S. oryzae*, and *S. zeamais* was found as >98% at 1 and 2 mg of spinosad/kg on corn after 12 days. Spinosad absolutely suppressed egg-to-larval



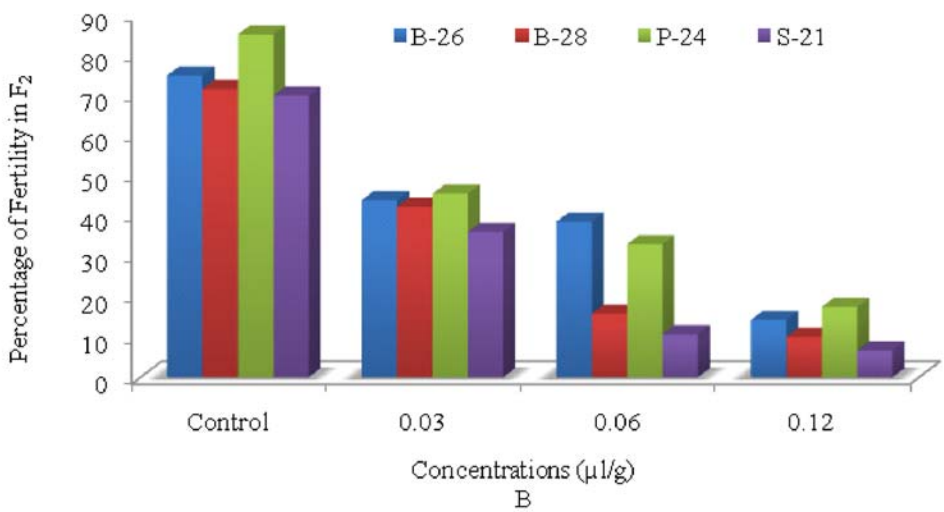


Fig..1 Effect of spinosad on the fertility in F_1 (A) and F_2 (B) generations of *T. castaneum* in wheat varieties.

survival at ≥0.5 mg/kg after 21 days and egg-to-adult emergence of *P. interpunctella* after 49 days, whereas 16 percent adults of *T. castaneum* survived at 1 mg of spinosad/kg of flour after 12 days. Spinosad at 1 or 2 mg/kg provided complete or close to entire suppression of progeny production and kernel damage of all species after 49 days (Huang and Subramanyam, 2007).The present findings are more or less consistent with above results. There was no significant deviation was found in sex ratios at any concentrations of spinosad, wheat varieties and F generations.

Spinosad (Tracer®) showed powerful toxicity against the adults of *R. dominica* and *S. oryzae* and maximum mortality occurred at 250 and 80 ppm

Table 1. Latent effects of spinosad on the life stages of F_1 generation of *T. castaneum* in different wheat varieties (N=5 pairs).

Wheat varieties	Concentrations (µl/g)	Total no. and Mean ± SE			
		Eggs	Larvae	Pupae	Adults
B-26	Control	1075 215.00±1.30a	964 192.80±1.93a	839 167.80±3.32a	655 131.00±3.32a
	0.03	579 115.80±1.69b	359 71.80±2.31b	314 62.80±2.24b	205 41.00±1.18b
	0.06	352 70.40±1.17c	176 35.20±2.31c	111 22.20±1.83c	65 13.00±1.10c
	0.12	265 53.00±1.22d	101 20.20±2.15d	62 12.40±1.44d	43 8.60±1.17d
B-28	Control	761 152.20±2.01a	593 118.60±1.81a	424 84.80±3.57a	328 65.60±2.38a
	0.03	380 76.00±1.41b	23 146.20±2.35b	14 929.80±2.84b	76 15.20±1.83b
	0.06	174 34.80±2.18c	58 11.60±1.33c	39 7.80±1.16c	17 3.40±0.68c
	0.12	130 26.00±0.89d	26 5.20±0.58d	14 2.80±0.37d	3 0.60±0.24d
P-24	Control	1125 225.00±2.21a	990 198.00±1.76a	735 147.00±3.74a	553 110.60±3.59a
	0.03	761 152.20±2.60b	494 98.80±4.67b	337 67.40±6.19b	220 44.00±6.96b
	0.06	405 81.00±0.45c	219 43.80±1.39c	141 28.20±2.11c	80 16.00±1.87c
	0.12	300 60.00±1.58d	120 24.00±1.30d	69 13.80±0.73d	52 10.40±0.81d
S-21	Control	656 131.20±3.92a	490 98.00±1.30a	368 73.60±3.39a	271 54.20±3.22a
	0.03	331 66.20±2.15b	165 33.00±1.70b	98 19.60±1.72b	44 8.80±1.07b
	0.06	185 37.00±2.10c	49 9.80±0.37c	25 5.00±0.55c	12 2.40±0.60c
	0.12	119 23.80±0.97d	15 3.00±0.32d	10 2.00±0.32d	2 0.40±0.24d

Source	DF	F	Sig.*	F	Sig.*	F	Sig.*	F	Sig.*
Varieties	3	164.58***	0.00	187.22***	0.00	146.68***	0.00	65.72***	0.00
Concentrations	3	1278.25***	0.00	1790.61***	0.00	2120.40***	0.00	1117.38***	0.00
Varieties * Concentrations	9	50.95***	0.00	96.15***	0.00	72.95***	0.00	39.84***	0.00

In a column means with same letter do not significantly differed from each other within varieties at 0.05% level (Tukey's test). Note: *Significant value, *** 0.001 = P < 0.001.

Table 2. Latent effects of spinosad on the life stages of F_2 generation of *T. castaneum* in different wheat varieties (N=5 pairs).

Wheat varieties	Total no. and Mean±SE				
	Concentrations (µl/g)	Eggs	Larvae	Pupae	Adults
B-26	Control	343 68.60±0.93a	257 51.40±1.63a	207 41.40±0.81a	175 35.00±1.00a
	0.03	123 24.60±2.01b	54 10.80±0.80b	35 7.00±0.55b	21 4.20±0.37b
	0.06	70 14.00±1.22c	27 5.40±0.40c	13 2.60±0.24c	6 1.20±0.37c
	0.12	28 5.60±0.51d	4 0.80±0.20d	3 0.60±0.24d	2 0.40±0.24c
B-28	Control	211 42.20±1.77a	151 30.20±1.43a	138 27.60±1.17a	109 21.80±2.44a
	0.03	123 24.60±2.24b	52 10.40±1.17b	30 6.00±0.55b	18 3.60±0.51b
	0.06	38 7.60±0.74c	6 1.20±0.20c	5 1.00±0.32c	1 0.20±0.20c
	0.12	20 4.00±0.83d	2 0.40±1.75d	0 0.00±0.00d	0 0.00±0.00c
P-24	Control	384 76.80±1.39a	327 65.40±0.86a	251 50.20±1.80a	208 41.60±1.50a
	0.03	145 29.00±1.30b	66 13.20±0.86b	45 9.00±0.71b	28 5.60±0.40b
	0.06	88 17.60±0.81c	29 5.80±0.66c	16 3.20±0.37c	8 1.60±0.24c
	0.12	40 8.00±0.84d	7 1.40±0.40d	3 0.60±0.24d	2 0.40±0.24c
S-21	Control	163 32.60±1.47a	114 22.80±1.02a	104 20.80±1.02a	89 17.80±0.86a
	0.03	100 20.00±0.95b	36 7.20±0.86b	18 3.60±0.40b	12 2.40±0.24b
	0.06	285.60±0.40c	30.60±0.24c	10.20±0.45c	00.00±0.00c
	0.12	15 3.00±0.71d	1 0.20±0.20d	0 0.00±0.00d	0 0.00±0.00c

Source	DF	F	Sig.*	F	Sig.*	F	Sig.*	F	Sig.*
Varieties	3	164.58***	0.00	187.22***	0.00	146.68***	0.00	65.72***	0.00
Concentrations	3	1278.25***	0.00	1790.61***	0.00	2120.40***	0.00	1117.38***	0.00
Varieties * Concentrations	9	50.95***	0.00	96.15***	0.00	72.95***	0.00	39.84***	0.00

In a column means with same letter do not significantly differed from each other within varieties at 0.05% level (Tukey's test).Note: *Significant value, *** 0.001 = P < 0.001.

Table 3. Effect of spinosad on the sex ratio of the 2nd generation of *T. castaneum* in different wheat varieties (N=5 pairs).

Wheat varieties	Concentrations (µl/g)	Male No. (%)	Female No. (%)	Sex ratio (Male: Female)	÷^{2} value
B-26	Control	91(43.96)	116(56.04)	1:1.27	1.81^{NS}
	0.03	14(40.00)	21(60.00)	1:1.50	1.76^{NS}
	0.06	9(69.23)	4(30.77)	1:0.44	3.61^{NS}
	0.12	3(100.00)	0(0.0)	0:0.00	0.00^{NS}
B-28	Control	79(57.25)	59(42.75)	1:0.75	2.42^{NS}
	0.03	14(46.67)	16(53.33)	1:1.14	2.11^{NS}
	0.06	4(80.00)	1(20.00)	1:0.25	0.00^{NS}
	0.12	0(0.0)	0(0.0)	0:0.00	0.00^{NS}
P-24	Control	116(46.22)	135(53.78)	1:1.16	3.72^{NS}
	0.03	25(55.56)	20(44.44)	1:0.80	1.80^{NS}
	0.06	10(62.50)	6(37.50)	1:0.60	2.84^{NS}
	0.12	2(66.67)	1(33.33)	1:0.50	0.00^{NS}
S-21	Control	53(50.96)	51(49.04)	1:0.96	1.45^{NS}
	0.03	9(50.00)	9 (50.00)	1:1.00	2.20^{NS}
	0.06	1(100.00)	0(0.0)	1:0.00	0.00^{NS}
	0.12	0(0.0)	0(0.0)	0:0.00	0.00^{NS}

Note: NS= Not significant at 5% level.

spinosad after 20 days exposure for *R. dominica* and *S. oryzae*. These results inspired the usage of Tracer® as an safe agent for insect pests' management (Sadeghi and Ebadollahi, 2015). Huang and Subramanyam (2007) reported that progeny production was 28.0±4.2 in control treatment after 49 days and decreased by 91 percent at 0.5 mg/kg and >96 percent at 1 and 2 mg/kg for *T. castaneum*. These consequences certify our results that spinosad is powerful in suppressing progeny production and there is an inverse relationship between progeny production and spinosad concentrations.

The present result revealed that higher concentrations of spinosad were highly effective against progeny production of *T. castaneum* in all wheat varieties in both generations. Among the wheat varieties used S-21 was found to give best result. So, it is revealed that spinosad inhibits the potentiality of *T. castaneum* at 0.12µl/g, and the latent effect transmitted to the next generation. It ultimately reduced the fecundity, fertility and decreased the egg to adult survivability in four wheat varieties in F_2 generations. So, it can be said that effect of spinosad at higher concentrations could effectively control development and reproductive potentiality of *T. castaneum* in stored wheat.

REFERENCES

Assie L. K., Brostaux Y. and Haubruge E. (2008) Density-dependent reproductive success in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Product Research 44: 285-289.

Athanassiou C.G. and Kavallieratos N. G. (2014) Evaluation of spinetoram and spinosad for control of *Prostephanus truncatus, Rhyzopertha dominica, Sitophilus oryzae*, and *Tribolium confusum* on stored grains under laboratory tests. Journal of Pest Sciences 87: 469-483.

Babarinde S.A. and Adeyemo Y.A. (2010) Toxic and repellent properties of *Xylopiaaethiopica* (Dunal) A. Richard on *Tribolium castaneum* Herbst infesting stored millets, *Pennisetum glaucum* (L.) R. Br. Archives of Phytopathology and Plant Protection 43: 810-816.

Bajracharya N.S., Opit G.P., Talley J. and Jones C.L. (2013) Efficacies of Spinosad and a Combination of Chlorpyrifos-Methyl and Deltamethrin against Phosphine-Resistant *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) on Wheat. Journal of Economic Entomology106: 2208-2215.

Baldwin R. and Fasulo T.R. (2004) Red and confused flour beetles-*Tribolium* sp. http:// creatures.ifas.ufledu /urban/beetles/red- flour-beetle.html.

Bonjour E.L., Phillips T.W. and Pitts J.T. (2006) Spinosad provides long-term protection for stored wheat. In: Lorini I, Bacaltchuk B, Beckel H, Deckers D, Sundfeld E, dos Santos J P, Biagi J D, Celaro J C, Faroni L R D A, Bortolini de O F L, Sartori M R, Elias M C, Guedes R N C, da Fonseca R G and Scussel V M (Eds.), Proceedings of the 9th International Working Conference for Stored-Product Protection, 15-18 October 2006. ABRAPOS, Campinas, Säo Paulo, Brazil. pp. 1189-1193.

Campbell J.F. and Runnion C. (2003) Patch Exploitation by female Red flour beetles, *Tribolium castaneum*. Journal of Insect Science 3: 20.

Cleveland C. B., Mayes M.A. and Cryer S. A. (2001) An ecological risk assessment for spinosad use on cotton. Pest Management Science 58: 70-84.

Halstead D.G.H. (1963) External sex differences in stored-products Coleoptera. Bulletin Entomological Research 54: 119-134.

Hertlein M.B., Thompson G.D., Subramanyam B. and Athanassiou C.G. (2011) Spinosad: A new natural product for stored grain protection. Journal of Stored Product Research 47: 131-146.

Huang F. and Subramanyam B. (2007) Effectiveness of spinosad against seven major stored-grain insects on corn. Insect Science 14: 225-230. Journal of Compilation © Institute of Zoology, Chinese Academy of Sciences.

Huang F., Subramanyam B. and Toews M.D. (2004) Susceptibility of laboratory and field strains of four stored-product insect species to spinosad. Journal of Economic Entomology 97: 2154-2159.

Khan A.R. (1981) The combined action of organophosphorus insecticides and microsporidians on *Tribolium castaneum*. Unpublished Ph D Thesis, University of Newcastle upon Tyne, UK. 162 pp.

Kirst H. A. (2010) The spinosyn family of insecticides: realizing the potential of natural products research. Journal of Antibiotics 63: 101-111.

Longstaff B. (1995) An experimental study of the influence of food quality and population density on the demographic performance of *Tribolium castaneum* (Herbst). Journal of Stored Product Research 31: 123-129.

Mertz F.P. and Yao R.C. (1990) *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar mill rum still. International Journal of Systematic Bacteriology 40: 34-39.

Mondal K.A.M.S.H. (1984) A method of determining the larval instars of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Laboratory Practice 33: 120-121.

Mondal K.A.M.S.H. and Parween S. (1997) Laboratory culturing of flour beetles, *Tribolium* species. Tribolium Information Bulletin 37: 153-162.

Park T. and Frank M. B. (1948) The fecundity and development of the flour beetles, *Tribolium confusum* and *Tribolium castaneum* at three constant temperatures. Ecology 29: 368-375.

Park T. (1962). Beetles competition and population Science 138: 1369-1375.

Sadeghi R. and Ebadollahi A. (2015) Susceptibility of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to Spinosad (Tracer®) as a eco-friendly Biopesticide. Ecologia Balkanica 7: 39-44.

Salgado V. L. and Sparks T. C. (2005) The spinosyns: chemistry, biochemistry, mode of action and resistance. In: Gilbert L. I., Iatrou K. and Gill S. (Eds.), Comparative Insect Molecular Science 6: 137-173.

Shukla G. S. and Upadhyay V. B. (2011) Economic Zoology. Rastogi. Publs. 487pp.

Sparks T. C., Dripps J. E., Watson G. B. and Paroonagian D. (2012) Resistance and cross-resistance to the spinosyns –A review and analysis. Pesticidal Biochemistry Physiology 102: 1-10.

Stejskal V., Aulicky R. and Kucerova Z. (2014) Pest control strategies and damage potentials of seed-infesting pests in the Czech stores-A review. Plant Protection Science 50: 165-173.

Subramanyam B., Hartzer M. and Boina D. R. (2012) Performance of pre-commercial release formulations of spinosad against five storedproduct insect species on four stored commodities. Journal of Pest Science 85: 331-339.

Talukder F. A. and Howse P. E. (1994) Efficacy of pithraj (*Aphanamixis polystachya*) seed extracts against stored-product pests. Proceedings of 6th International Working Conference on stored-product Protection 2: 848-852, Canberra, Australia.

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