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Toxicity of *Bacillus thuringiensis* var. *kurstaki* and Spinosad on three larval stages of beet armyworms *Spodoptera exigua* (Hübner) (Lep: Noctuidae)

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Abstract
The beet armyworm, *Spodoptera exigua* (Hübner), is a polyphagous pest that causes serious damages to various plants including beet, potato and cotton. In order to reduce application of chemical insecticides, two bioinsecticides, *Bacillus thuringiensis* and Spinosad, were evaluated for their efficacy in control of *S. exigua* in laboratory conditions. The LC_{25} and LC_{50} values of *B. thuringiensis* and Spinosad were estimated on three larval stages. Furthermore, LC_{50} values for *B. thuringiensis* treatments on 1st, 2nd and 3rd larval stages were 275. 305, 717.444 and 727.671 ppm and for Spinosad 276.715, 521.058 and 568. 914 ppm, respectively, and LC_{25} values of *B. thuringiensis* and Spinosad treatments on 1st, 2nd and 3rd larval stages were 104.105, 341.652 and 441.803 ppm and 92.812, 369.569 and 379.958 ppm, respectively. After determining the LC_{50} and LC_{25} values of both agents, to evaluate their combined effects, an experiment of completely randomized design was conducted with four treatments including LC_{50} (*Bt*), LC_{50} (Sp), LC_{25} (*Bt*) + LC_{25} (Sp) and control. Statistical analysis of the results showed that mortality percentage of two biological control combination on three larval stages 1st, 2nd and 3rd of *S. exigua* have significant differences ($P<0.05$) in comparition to control, Spinosad and *B.t* alone treatments. These results demonstrated that Spinosad was more effective in control of *S. exigua*, than *B. thuringiensis*. Thus our recommendation is the combination of two biological agents to control of beet armyworm.

Keywords: Spinosad, *Bacillus thuringiensis* (B.), *Spodoptera exigua* (H.), bioassay

1. Introduction

The beet armyworm, *Spodoptera exigua* (Hübner), is a widely distributed polyphagous pest of numerous cultivated crops and this pest has being treated with different insecticides, this has resulted in the development of resistance to a diverse array of chemical classesas well as environmental pollution and human health. Special emphasis is being placed on implementing environmentally safe strategies. Commercial formulates based on bacterium have been used for decades to control insect pests as an alternative to chemicals. Such formulates are environmentally friendly, harmless to humans and other vertebrates [9, 17, 23]. One alternative to chemicals is the application of bioinsecticides considered effective for the control of some lepidopteran pests. Among microbial entomopathogens, *Bacillus thuringiensis* (*B.t*) commercial products and Spinosad are used in recent years in worldwide. Spinosad may be good alternati0ves, as they have been used to control other insect pests successfully [12]. Spinosad is the first member of Dow AgroScience's naturally class of insecticides. It is comprised primarily of two macro cyclic lactones, Spinosyn A and Spinosyn D. These are secondary metabolites produced by the actinomycete, *Saccharopolyspora spinosa* Mertz and Yao, under natural fermentation conditions. The mode of action of Spinosad is twofold; the primary target site is the nicotinic acetylcholine receptor, but the GABA receptor is also affected to some degree**.** Routes of entry include both contact and oral. Symptoms of Spinosad poisoning include initial flaccid paralysis, followed by tremors and eventual death [20, 12]. Spinosad have rapid contact and ingestion activity in insects, causing excitation of the nervous system, leading to cessation of feeding and paralysis [11].

In present study, the effects of *B. thuringiensis* var. *kurstaki* and Spinosad on three larval stages beet armyworms *S. exigua* (Hübner) in laboratory conditions have evaluated.

2. Material and Methods

The present study was carried out in Entomology laboratory of the Plant Protection Department at the Faculty of Agriculture, Urmia University (Iran) during spring 2013 to summer 2015.

2.1 Insect rearing

The three larval stages of beet army worms were used in the present experiments. A colony of beet army worms was obtained from sugar beet farm near Urmia University. They were reared under laboratory conditions, on artificial diet containing 120 g mung bean powder, 10 g dried brewer yeast, 3 g methyl parahydroxy benzoate, 2 g sorbic acid, 2.5 g ascorbic acid, 12.5 g agar, 2 ml of 40 formalin, 30 ml of vitamin stock and 900 ml distilled water to make about 1080 ml diet) at 27 ± 3° C, with L: D 16: 8 and 55 ± 15% RH [6].

2.2 Bioinsecticides

Spinosad and *B. thuringiensis* var. *kurstaki*, two bioinsecticides were evaluated for their efficiency against *S. exigua*. The commercial formulation based on *B. thuringiensis* used in our assay was Costar® (*B. thuringiensis* var. *kurstaki*, 90.4 MIU g^{-1}, WG) distributed in Spain by Syngenta Agro Science America (Madrid, Spain). This commercial formulation demonstrated high efficacy against *S. exigua* ([12]). The commercial formulation based on Spinosad used in our assay was Tracer (24% SC) distributed in Co Dow Agro Science America.

2.3 LC_{50} and LC_{25} values

The toxicity of the *B. thuringiensis* and Spinosad against different larval stages of *S. exigua* was tested. Range-finding studies were run to determine the appropriate testing concentrations. To estimate the LC_{50} and LC_{25} five concentrations from each biological agent after primary experiments with distilled water as control treatment in three replications used in leaf dipping method on 10 larvae *S. exigua*. The bioassays were performed in controlled conditions (27±3 °C and 55±15% RH). The mortality of different larval stages was recorded after 24, 48 and 72 hours.

2.4 The combination effects of *B. thuringiensis* and Spinosad

After calculating LC_{50} and LC_{25} values for *B. thuringiensis* and Spinosad on three larval stages, combination effects of *B. thuringiensis* and Spinosad on *S. exigua* larvae on the 1st, 2nd and 3rd by leaf dipping method in sugar beet plant were evaluated. All experiments in completely randomized block in 4 treatments include LC_{50} of *B. thuringiensis*, LC_{50} of Spinosad, LC_{25} *B. thuringiensis* plus LC_{25} Spinosad and distilled water as control in three replications on ten larvae were conducted. The mortality after 24, 48 and 72 hours were counted.

2.5 Statistical Analysis

The LC_{50} and LC_{25} values (with 95% confidence limits) were calculated by using Probit Analysis Statistical Method, mortality data of treatments subjected to analysis of variance (One Way ANOVA) and mean separation tests were conducted with Tukey's HSD with SPSS statistical analysis software (Version 19.0).

3. Results

3.1 LC_{50} and LC_{25} *B. thuringiensis* and Spinosad on 1st larval instars:

LC_{50} and LC_{25} of *B. thuringiensis* and Spinosad on 1st larval instars in three times are shown in Table 1.

Table 1: Lethal effect of *B. thuringiensis* and Spinosad on 1st larval instars

Bioinsecticide	Time	Slope±SE	Chi-square	Lethal concentration	
				LC_{25} (Upper and lower confidence limits)	LC_{50} (Upper and lower confidence limits)
B. t.	24	-4.173±1.280	3.111	396.119 (571.144-153.417)	1255.088 (7515.328-807.657)
	48	-2.989±1.173	0.262	156.292 (297.043-1.181)	681.139 (2600.419-420.719)
	72	-3.896±1.194	4.918	104.105 (189.624-15.698)	275.305 (384.586-121.871)
Spinosad	24	-3.601±1.183	1.183	183.902 (306.918-22.272)	611.643 (1130.931-408.778)
	48	-5.533±1.260	4.397	139.433 (207.752-58.067)	337.700 (500.825-104.720)
	72	-3.040±1.160	0.108	92.182 (199.346-17.780)	276.715 (365.331-175.138)

3.2 LC_{50} and LC_{25} *B. thuringiensis* and Spinosad on 2nd larval instars

LC_{50} and LC_{25} of *B. thuringiensis* and Spinosad on 2nd larval instars in three times are shown in Table 2.

Table 2: Lethal effect of *B. thuringiensis* and Spinosad on 2nd larval instars

Bioinsecticide	Time	Slope±SE	Chi-square	Lethal concentration	
				LC_{25} (Upper and lower confidence limits)	LC_{50} (Upper and lower confidence limits)
B. t.	24	-6.231±2.493	0.238	671.046 (871.858-96.746)	1478.619 (12065.964-1128.217)
	48	-6.714±2.413	0.870	465.707 (653.220-82.947)	924.922 (1201.260-666.993)
	72	-5.568±2.402	0.692	341.652 (560.423-3.352)	713.444 (974.776-248.050)
Spinosad	24	-6.692±2.415	0.052	457.718 (646.361-77.637)	909.483 (1167.378-641.087)
	48	-8.006±2.460	0.634	426.262 (587.889-138.232)	744.029 (886.669-493.040)
	72	-8.357±2.557	0.961	369.569 (526.884-110.671)	521.058 (757.710-353.506)

3.3 LC_{50} and LC_{25} *B. thuringiensis* and Spinosad on 3rd larval instars

LC_{50} and LC_{25} of *B. thuringiensis* and Spinosad on 3rd larval instars in three times are shown in Table 3.

Table 3: Lethal effect of *B. thuringiensis* and Spinosadon 3rdlarval instars

Bioinsecticide	Time	Slope±SE	Chi-square	Lethal concentration	
				LC_{25} (Upper and lower confidence limits)	LC_{50} (Upper and lower confidence limits)
B. t.	24	-6.723±2.713	0.341	494.445 (664.325-202.309)	898.372 (1111.453-347.574)
	48	-7.955±2.840	0.079	454.084 (705.525-21.585)	776.787 (963.182-340.220)
	72	-11.503±3.257	1.796	441.803 (663.501-66.885)	727.671 (872.033-445.857)
Spinosad	24	-10.341±2.941	1.549	543.319 (720.241-227.675)	843.149 (989.376-566.210)
	48	-8.604±3.041	0.592	404.509 (617.582-62.964)	674.137 (860.568-256.624)
	72	-10.600±3.843	1.679	379.958 (580.466-56.655)	568.914 (750.546-175.288)

The first stages of pest were more susceptible to both biological agent controls. Results showed that percentage of mortality three larval stages of *S. exigua* in same concentration to both biological agents Spinosad was more lethal as compared to bacteria.

3.4. Combinate effects of *B. thuringiensis* and Spinosad on three larval stages

3.4.1. First instars

Effects of LC_{50} *B. thuringiensis*, LC_{50} Spinosad, combined effects of LC_{25} *B. thuringiensis* and Spinosad on first instar larvae was evaluated and counting the percentage mortality of larvae after 24, 48 and 72 hours (figure 1). The results showed that there was a significant difference between LC_{25}(*Bt*)+ LC_{25} (Sp) treatment with separate application treatments LC_{50} (*Bt*) and LC_{50} (Sp) with 95% confidence in three time (df= 3 & 8, $P< 0.05$, F= 200.000, Sig= 0.001 (df= 3 & 8, $P<0.05$, F= 108.889, Sig= 0.001 (df= 3 & 8, $P< 0.05$, F= 140.889, Sig= 0.001), respectively. The result showed that combined effects of treatments in three times were more lethality in compared other treatments.

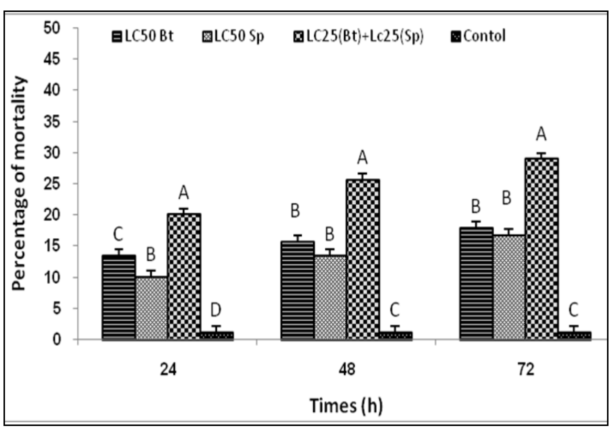

Fig 1: Mortality rate of different treatments on first instar larvae after 24, 48 and 72 hours. (Compare mean of treatments in three time independents from each other).

3.4.2 Second instars

Effects of treatments, LC_{50} *B. thuringiensis*, LC_{50} Spinosad, combined effects of treatments LC_{25} *B. thuringiensis* and Spinosad on second instar larvae was evaluated and counting the percentage mortality of larvae after 24, 48 and 72 hours (figure 2). The results showed that there was a significant difference between LC_{25} (*Bt*) + LC_{25} (Sp) treatment with separate application treatments LC_{50} (*Bt*) and LC_{50} (Sp) with 95% confidence in three time (df= 3 & 8, $P< 0.05$, F= 53.667, Sig= 0.001 (df= 3 & 8, $P<0.05$, F= 101.883, Sig= 0.001 (df= 3& 8, $P< 0.05$, F= 132.000, Sig= 0.001), respectively. According to result in three times showed that combined effects of treatments the highest mortality compared with other treatments.

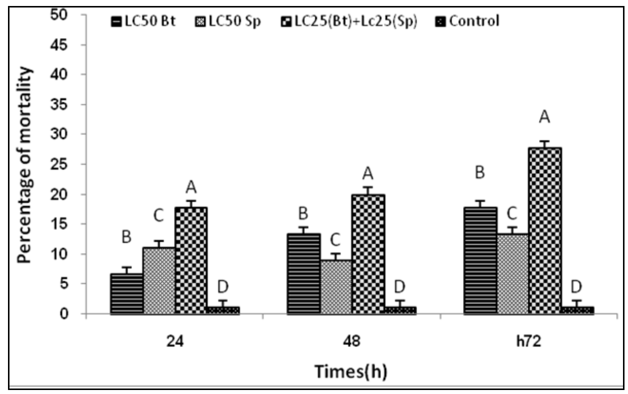

Fig 2: Mortality rate of different treatments on second instar larvae after 24, 48 and 72 hours (Compare mean of treatments in three time independents from each other).

3.4.3 Third instars

Effects of treatments, LC_{50} *B. thuringiensis*, LC_{50} Spinosad, combined effects of treatments LC_{25} *B. thuringiensis* and Spinosad on third instar larvae was evaluated and counting the percentage mortality of larvae after 24, 48 and 72 hours (figure 3). The results showed that there was a significant difference between LC_{25} (*Bt*)+ LC_{25} (Sp) treatment with separate application treatments LC_{50} (*Bt*) and LC_{50} (Sp) with 95% confidence in three time (df= 3 & 8, $P< 0.05$, F= 46.333, Sig= 0.001 (df= 3 & 8, $P<0.05$, F= 81.222, Sig= 0.001 (df= 3 & 8, $P< 0.05$, F= 162.667, Sig= 0.001), respectively. According to result in three times showed that combined effects of treatments the highest mortality compared with other treatments.

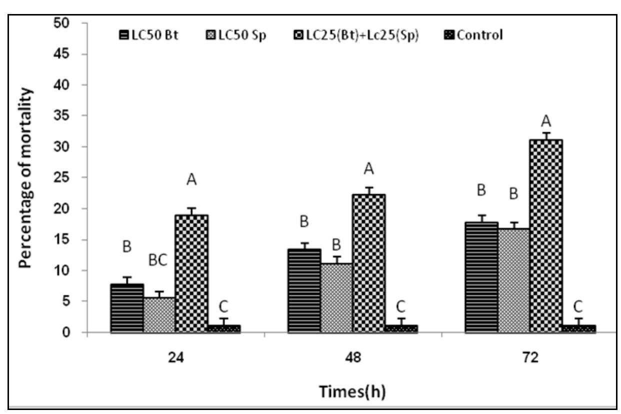

Fig 3: Mortality rate of different treatments on third instar larvae after 24, 48 and 72 hours (Compare mean of treatments in three time independents from each other).

4. Discussion

The practical application of different insecticides extensively has resulted in several problems such as development of resistance in field population of insects [10, 1]. Due to this reason, many researchers have concentrated on alternative control methods. Biologically active substances from plants and microbes affect the growth and development of insects and provide protection against herbivores including Lepidoptera pests [21, 19, 14, 4, 5]. El-Sheikh *et al*. (2012) [8] reported that use of Spinosad and *Bacillus thuringiensis* var. *kurstaki* in controlling *Spodoptera littoralis* Boisduval are better than synthetic pyrethroid cypermethrin. Kausar *et al*. (2012) [15] concluded that *Bacillus thuringiensis* can be made by combination of biopesticides to control many pests. This will be environment safe having capacity to kill the target organism more rapidly with no side effect on non target organisms. Also Ayden and Gurkan (2006) [2] applied Spinosad against *S. littoralis* larvae and suggested that Spinosad is potentially important in the control of *S. littoralis*.

In this study, two bioinsecticides, *Bacillus thuringiensis* and Spinosad, were evaluated for their efficacy in the control of *S. exigua*, in laboratory conditions. These results demonstrated that Spinosad was more effective in control of *S. exigua* in compare with *B. thuringiensis.* The combination of *B. thuringiensis* and Spinosad has additive effect when applied individually. In experiment control larval stages of Helicoverpa *armigera* (Hubner) with different agents, Spinosad with 0.009% showed 92.52% mortality after 3 days that was the most effective treatment in pest control [3]. The highest reduction percentage in the number of larvae was occurred in cause of using entomopathogenic bacteria, *B. thuringiensis* combined with botanical extract, Neem [16]. Dong *et al.* (2013) [7] suggested that the combination of lethal and sub lethal effects of Spinosad might affect *S. exigua* population dynamics significantly by decreasing its survival and reproduction by delaying its development. Ranjbari *et al.* (2012) [18] bioassays were carried out to evaluate the insecticidal activities of Spinosad alone against the various larval instars of *Plutella xylostella* (L.) under laboratory conditions and showed that the highest mortality rate for 1st, 2nd, 3rd and 4th larval instars in 600, 700, 800 and 900 ppm of Spinosad caused 100, 95, 98.3 and 93.3% mortality after 72 h, respectively. The combination of *B. thuringiensis* and Spinosad were most effective on mortality of *Tuta absoluta* than their separate application [13]. Thus our study supports previous research and demonstrated the excellent effect of two natural friendly biopesticides, Spinosad and *B. thuringiensis*. Thus our recommendation is the combination of these biological agents to control beet armyworm.

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