Effects of some chemical and biological insecticides on beet armyworm (*Spodoptera exigua* Hubner (Lepidoptera: Noctuidae)) and natural enemies in sugar beet fields

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Beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae), is an important pest that feeds on various crops worldwide. It has many natural enemies including *Chrysoperla carnea* Stephens and *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae). The chemical control of this pest causes environmental pollution, destruction of natural enemies and threatens human health. Therefore, selection and use of non-destructive and safe pesticide is essential. In the present study, the efficacy of four pesticides i.e. spinosad with concentration 200 ml ha^{-1}, emamectin benzoate 200 g ha^{-1}, SeMNPV 5×10^{8} OB mL^{-1} and *Btk* 1 kg ha^{-1} in controlling larval stages of *S. exigua* at 1, 3, 7, 14 and 21 days after treatment in a sugar beet field were evaluated. The mortality of larval and adult stages of two predators, *C. undecimpunctata* and *C. carnea*, were also assessed. The highest efficacy in controlling the larval stage of *S. exigua* was observed in the emamectin treatment. The lowest efficacies were observed in the *Btk* and SeMNPV treatments, while spinosad had a moderate efficacy. The mortality of larvae and adults of both the predator species were highest in the emamectin treatment. *Btk* and SeMNPV had the least negative effects on the population of the predators, these two treatments were not significantly different. Spinosad caused a moderate mortality in population of the predators. In the damage assessment on plants, the highest and lowest percentages of damage were observed in the SeMNPV virus treatment and emamectin. Spinosad and emamectin, are recommended as the most effective compounds to control larvae of *S. exigua,* while SeMNPV and *Btk* are recommended for integrated pest management and are safe to the natural enemies.

Keywords: Beet armyworm, natural enemies, *Coccinella undecimpunctata* L., *Chrysoperla carnea* Stephens

Beet armyworm *Spodoptera exigua* is one of the three most important pest species of sugar beet in the world. It has a wide host range throughout tropical and subtropical regions (Darsouei et al. 2018). In most years, it causes considerable yield losses in many sugar beet growing regions. Chemical pesticides are the main method to control this pest (Jung and Kim 2006). A lot of research has been conducted on its chemical and biological control (Chidawanyika et al. 2012; Hu et al. 2016; Sukirno et al. 2017; Carlos et al. 2018). The effect of pesticides on non-target organisms has been a source of worldwide attention and concern for decades. Adverse effects of applied pesticides on non-target arthropods have been widely reported (Mahmood et al. 2016). The role of generalist predators as effective control agents is supported by both biocontrol theory and practice (De Castro et al. 2018). Unfortunately, natural insect enemies e.g., parasitoids and predators are most susceptible to insecticides and are severely affected (Fernandes et al. 2010). The destruction of natural enemies can exacerbate pest problems as they play an important role in regulating pest population levels. Usually, if natural enemies are absent, spraying additional insecticide is required to control the target pest (Fernandes et al. 2010). Pesticides often inflict severe mortality on both pests and natural enemies because of their basic physiological similarities, as they are both arthropods. However, much less is known about the effects of insecticides on natural enemies than on pests because the pest is usually the primary object of pesticide research (Che et al. 2013). Use of selective insecticides, could improve conservation of natural enemies and ensure success of Integrated Pest Management (IPM) programmes (Maroufpoor et al. 2010). Biological pesticides have attracted considerable interest in recent years (Ntalli et al. 2011; Ntalli and Caboni 2012; Martin et al. 2013). Reduced

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fecundity after exposure to abamectin has been demonstrated for natural enemies of spider mites such as *Galendromus occidentalis* (Bommarco et al. 2011). Galvan et al. (2005) reported that use of selective insecticides, such as spinosad and indoxacarb that are more toxic to lepidopteran pests than to *Harmonia axyridis* (Pallas), could facilitate conservation of this predator in sweet corn integrated pest management. Medina et al. (2003a) assessed the effect of three new pesticides, pyriproxyfen, spinosad and tebufenozide on survival and reproduction of adults of *Chrysoperla carnea* (Stephens). Thus, selective insecticides should effectively control the target pests but have minimal effects on the natural enemies present in the field during insecticide application (Varenhorst and O'Neal 2012). Nowadays biological control agents, such as *Bacillus thuringiensis* var. *kurstaki* (*Btk*) and *S. exigua* multiple nucleopolyhedrovirus (SeMNPV), are good environment friendly alternatives for chemical pesticides in controlling many pests including *S. exigua* (Zhang et al. 2009; Lasa et al. 2007). Spinosad, a mixture of spinosyns A and D, is derived from the naturally occurring actionomycete, *Saccharopolyspora spinosa*. Because of its unique mode of action, involving the postsynaptic nicotinic acetylcholine and Gamma-aminobutyric (GABA) receptors, spinosad has strong insecticidal activity against insects especially lepidopteran pests (Jones et al. 2005; Somers et al. 2015). Emamectin benzoate (Proclaim) is a novel semi-synthetic derivate of the natural product abamectin in the avermectin family; this insecticide has a high potency against a broad spectrum of lepidopteran pests with an efficacy about 1.5-fold more potent against certain armyworm species (Ishaay et al. 2002). *C. carnea* and *C. undecimpunctata* have been reported as two important generalist predators which can be found in many agricultural crops including sugar beet. Evaluation effects of bio pesticides on non-target insects (e.g. predators) is of high importance in IPM programmes (Ehler et al. 1997).

In the present study, effects of *Bacillus thuringiensis* var. *kurstaki (Btk*), *S. exigua* multiple nucleopolyhedrovirus (SeMNPV), spinosad and emamectin on *S. exigua* larvae and two natural enemies *C. carnea* and *C. undecimpunctata* were assessed on sugar beet in field conditions.

Materials and methods

Plot preparation and planting

The study was carried out in Noshin Shahr village, Urmia city, West Azerbaijan province, Iran, in the summer of 2019. The experiment was conducted in a randomised block design with three replicate blocks and five treatments consisting of four bio pesticides and a control (see below). The distances between the blocks was 10 m. Each block consisted of five plots (10 × 20 m) (one plot = one treatment) separated by 1 m bare ground from each other. Tillage, disking and other cultural practices were conducted. A variety of sugar beet (*Beta vulgaris* subsp. *vulgaris* var. *altissima*) was planted in rows 30 cm apart and 30 cm distance between plants (95,000–100,000 plants/ha after hand thinning).

Bio pesticides application

Spinosad (Tracer 240 SC, Dow Agro Sciences) at a rate of 200 ml ha^{-1}, emamectin benzoate (Proclaim®, Syngenta Co., Switzerland), 200 g ha^{-1}, *B. thuringiensis* var. *kurstaki* (Belthirul® WP, 32000 IU mg^{-1}, Probelte S. A. CTRA, Madrid, Spain) 1 kg ha^{-1}. SeMNPV with a concentration of 5×10^8 OB ml^{-1} (the calculated volume or amount of each product was obtained from the Iranian Research Institute of Plant Protection, Agricultural Research Education and Extension Organization, Tehran, Iran) were applied using a 16 L capacity sprayer (Hyundai HP1680 Chargeable Sprayer 16 liter, Korea) at a rate of 8 L per plot, equivalent to an application volume of 400 L ha^{-1}. Control plots were sprayed with water.

Sampling

The population densities of the pest and the predators were estimated by counting numbers of live larvae of *S. exigua* or larvae and adults of *C. undecimpunctata* and *C. carnea* per 10 plants in each plot (each treatment) before spray treatment and at 1, 3, 7, 14 and 21 days after treatment (DAT) (Karimzadeh et al. 2011). For this purpose, 30 points in each plot were randomly selected in a zigzag direction and all live pest larvae or adult and larvae of predators were estimated.

The Henderson-Tilton formula (Henderson and Tilton 1955) was used to calculate the efficacy of spinosad, emamectin, SeMNPV and *Btk* treatment on pest and predators:

$$\text{Treatment efficacy \%} = 1 - \left(\frac{\mathrm{Ta}\times\mathrm{Cb}}{\mathrm{Ca}\times\mathrm{Tb}}\right)\times 100$$

where, Ca and Cb = pest and predators rate in control plots after and before spraying; Ta and Tb = pest and predators rate in treated plots after and before spraying.

Damage assessment

Percentage pest damage on sugar beet plants was assessed 21 DAT with spinosad, emamectin, SeMNPV, *Btk* and water. In each plot 30 plants were randomly selected in a zigzag direction.

Statistical analysis

The calculated efficacy values of treatments or values of mortality effect of each treatment on pest and predators after assumption of the homogeneity of blocks (by Leven's test) were analysed by General Linear Model-univariate (GLM); mean separation tests were conducted with Tukey's HSD. SPSS statistical analysis software version 22.0 was used.

Results

Efficacy of treatments against larval stage of S. exigua

Homogeneity of the blocks was established by $F_{6, 2} = 1.016$, P = 0.417; $F_{6, 2} = 0.641$, P = 0.559; $F_{6, 2} = 2.897$, P = 0.132; $F_{6, 2} = 0.948$, P = 0.439 and $F_{6, 2} = 3.98$, P = 0.080 at 1, 3, 7, 14 and 21 DAT respectively.

As shown in Table 1, efficacy of treatments in controlling larval stages of *S. exigua* were different at 1, 3, 7, 14 and 21 DAT. Emamectin had the highest efficacy at all sampling times. However, at 1 and 3 DAT SeMNPV had the lowest efficacy which was not significantly different to *Btk*, while spinosad had an intermediate efficacy. At 7 DAT, the efficacy of treatments was as follows: emamectin > spinosad > *Btk* > SeNPV. At 14 DAT, the lowest efficacy was observed in SeMNPV; *Btk* and spinosad were not significantly different and had an intermediate efficacy (Figure 1).

Table 1: Efficacy of selected bio-pesticide in controlling larval stages of *Spodopteraa exigua* at 1, 3, 7, 14 and 21 DAT under field conditions

Treatments	**Efficacy (mean ± SE) %**					**Mean**
	1 DAT*	3 DAT	7 DAT	14 DAT	21 DAT	
Emamectin	59.00 ± 3.98^{a}	71.00 ± 4.58^{a}	79.00 ± 4.52^{a}	91.67 ± 5.73^{a}	95.33 ± 6.49^{a}	79.20
Spinosad	43.00 ± 2.11^{b}	50.67 ± 4.11^{b}	60.00 ± 3.60^{b}	67.33 ± 5.50^{b}	64.00 ± 5.99^{b}	57.00
Btk	32.33 ± 1.68^{bc}	45.00 ± 3.50^{bc}	52.33 ± 3.02^{c}	53.67 ± 5.03^{c}	55.00 ± 4.33^{c}	48.27
SeMNPV	31.33 ± 1.12^{c}	39.67 ± 2.28^{c}	43.33 ± 3.21^{d}	46.67 ± 3.33^{c}	49.00 ± 2.41^{c}	42.00
P value	0.001	0.001	0.001	0.001	0.001	-
$F_{(3, 6)}$	30.03	139.82	214.93	184.05	452.80	-

Data are expressed as means ± standard error (SE) of three replicates of each bio-pesticide. *DAT: days after treatment. Means followed by the same superscript letter(s), within the same column are not significantly different ($P \leq 0.05$) according to Tukey HSD Test.

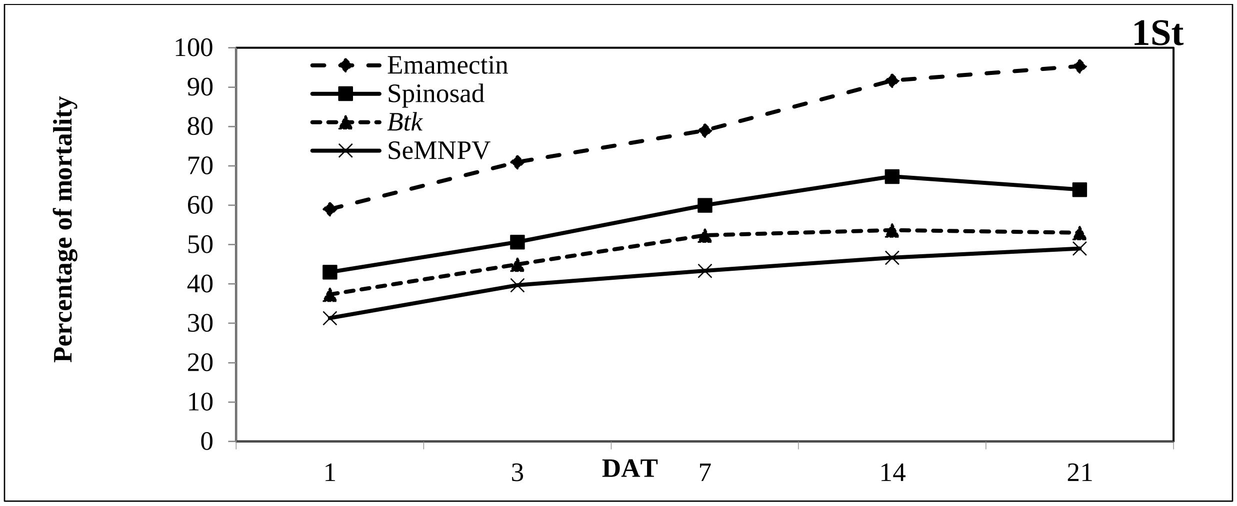

Figure 1: Trend of mortality in larval stage of *S. exigua* at 1, 3, 7, 14 and 21 days after treatment (DAT) by emamectin, spinosad, *Btk* and SeMNPV in a field trial.

Mortality effect of different treatments on adult of the predators

Homogeneity of the blocks was established by $F_{6, 2}$ = 3.759, P = 0.131; $F_{6, 2}$ = 3.971, P = 0.080; $F_{6, 2}$ = 4.285, P = 0.07; $F_{6, 2}$ = 3.426, P = 0.102 and $F_{6, 2}$ = 3.677, P = 0.091 at 1, 3, 7, 14, and 21 DAT respectively.

The mortality effect of different treatments on adults of *C. Uundecimpunctata and C. Carenage* was significantly different at 1, 3, 7, 14, and 21 DAT (Table 2). At 1 and 3 DAT, emamectin and spinosad caused the highest mortality in population of *C. undecimpunctata* (adults). Additionally, at 1 and 3 DAT, emamectin had the highest mortality effect on adults of *C. carnea* followed by spinosad. At 7, 14 and 21 DAT, emamectin had the highest mortality effect on adults of both predators followed by spinosad. However, *Btk* and SeMNPV had the minimum mortality effect on adults of both predators at all the sampling days (Figure 2).

Table 2: Mortality effect of different treatments on adults of *Coccinella undecimpunctata* and *Chrysoperla carnea* at 1, 3, 7, 14 and 21 DAT under field conditions

Predator	**Treatments**	**Efficacy (mean ± SE) %**					**Mean**
		1 DAT*	3 DAT	7 DAT	14 DAT	21 DAT	
Coccinella undecimpunctata	Emamectin	24.00±2.78^{a}	41.00±3.50^{a}	55.66±2.80^{a}	61.66±4.23^{a}	78.33±5.12^{a}	52.13
	Spinosad	18.66±1.78^{b}	29.33±2.10^{b}	33.00±1.50^{b}	47.66±2.16^{b}	47.66±4.89^{b}	35.26
	Btk	3.66±0.58^{c}	5.00±1.11^{c}	6.66±0.80^{c}	10.66±0.33^{c}	11.00±2.39^{c}	18.19
	SeMNPV	2.33±0.38^{c}	3.00±0.50 ^{c}	4.33±0.51^{c}	8.66±0.13^{c}	11.33±1.09^{c}	14.73
	P value	0.021	0.008	0.001	0.001	0.001	-
	$F_{(3, 6)}$	7.175	10.837	49.151	26.980	34.190	-
Chrysoperla carnea	Emamectin	35.00±3.22^{a}	57.33±4.57^{a}	78.66±4.90^{a}	82.66±5.94^{a}	91.00±5.45^{a}	68.93
	Spinosad	19.66±2.22^{b}	28.66±1.05^{b}	33.00±1.93^{b}	43.00±3.44^{b}	60.00±2.35^{b}	36.86
	Btk	4.00±1.32^{c}	5.66±0.51^{c}	8.33±0.90^{c}	12.00±1.31^{c}	13.33±1.25^{c}	11.66
	SeMNPV	3.66±0.21^{c}	4.00±0.25^{c}	6.00±0.50^{c}	11.66±0.34^{c}	12.00±0.42^{c}	12.46
	P value	0.001	0.001	0.001	0.001	0.001	-
	$F_{(3, 6)}$	33.501	76.938	113.520	576.587	651.684	-

Data are expressed as means ± standard error (SE) of three replicates of each bio-pesticide. *DAT: days after treatment. Means followed by the same superscript letter (s), within the same column are not significantly different ($P \leq 0.05$) according to Tukey HSD Test.

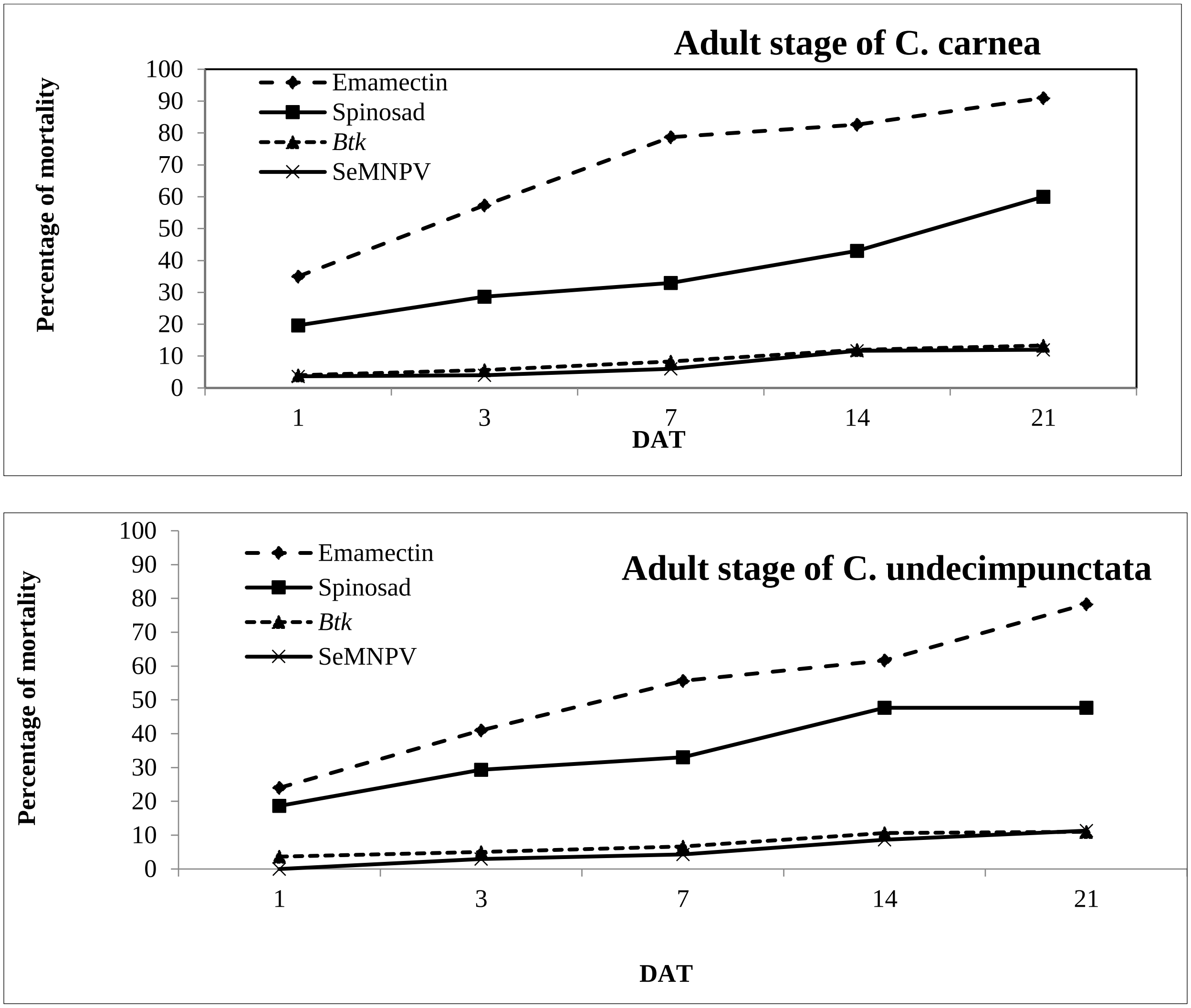

Figure 2: Trend of mortality in larval stage of *Coccinella undecimpunctata* and *Chrysoperla carnea* at 1, 3, 7, 14 and 21 days after treatment (DAT) by *Btk*, SeMNPV, emamectin and spinosad in a field trial.

Mortality effect of different treatments on larval stages of the predators

Homogeneity of the blocks was established by $F_{6,2} = 3.103$, $P = 0.119$; $F_{6,2} = 0.303$, $P = 0.749$; $F_{6,2} = 0.135$, $P = 0.877$; $F_{6,2} = 0.495$, $P = 0.633$ and $F_{6,2} = 2.706$, $P = 0.145$ at 1, 3, 7, 14, and 21 DAT, respectively.

The mortality effect of different treatments on larval stages of the predators was significantly different at 1, 3, 7, 14, and 21 DAT (Table 3). At 1, 3, 7 and 14 DAT, emamectin and spinosad moderately reduced larval density of *C. undecimpunctata* while *Btk* and SeMNPV slightly reduced the density of this predator. At 21 DAT, the highest mortality in larval stage of *C. undecimpunctata* was caused by emamectin followed by spinosad while the lowest mortality was caused by *Btk* and SeMNPV. The mortality in larval stage of *C. carnea,* which caused by emamectin at 1 DAT, was equal to that caused by spinosad. At 3, 7, 14 and 21 DAT, the mortality effect of the treatments on larval stage of *C. carnea* had the order: emamectin > spinosad > *Btk* and SeMNPV (Figure 3).

Table 3: Mortality effect of different treatments on larval stages of *Coccinella undecimpunctata* and *Chrysoperla carnea* at 1, 3, 7, 14 and 21 DAT under field conditions

Larval stage	**Treatments**	**Efficacy (Mean ± SE) %**					**Mean**
		1 DAT*	3 DAT	7 DAT	14 DAT	21 DAT	
C. undecimpunctata	Emamectin	35.00 ± 4.17^{a}	42.00 ± 3.91^{a}	57.00 ± 4.55^{a}	73.00 ± 5.31^{a}	86.33 ± 5.15^{a}	58.66
	Spinosad	20.667 ± 2.47^{b}	31.00 ± 2.20^{b}	50.00 ± 4.11^{b}	60.30 ± 3.91^{b}	68.33 ± 3.11^{b}	45.85
	Btk	3.33 ± 0.17^{c}	4.66 ± 0.41^{c}	5.00 ± 0.65^{c}	8.66 ± 1.21^{c}	15.00 ± 2.16^{c}	22.33
	SeMNPV	1.667 ± 0.07^{c}	2.33 ± 0.11^{c}	4.667 ± 0.25^{c}	7.00 ± 1.02^{c}	10.00 ± 1.65^{c}	14.73
	P value	0.021	0.008	0.001	0.001	0.001	-
	$F_{(3, 6)}$	7.175	10.837	49.151	26.980	34.190	-
C. carnea	Emamectin	38.33 ± 3.46^{a}	67.00 ± 4.42^{a}	70.00 ± 4.74^{a}	80.33 ± 5.63^{a}	87.00 ± 5.55^{a}	68.53
	Spinosad	28.00 ± 2.14^{b}	30.00 ± 3.42^{b}	37.00 ± 3.87^{b}	43.33 ± 4.16^{b}	55.33 ± 4.81^{b}	38.73
	Btk	4.66 ± 0.26^{c}	5.66 ± 0.72^{c}	7.33 ± 1.71^{c}	10.66 ± 1.64^{c}	13.00 ± 1.89^{c}	14.46
	SeMNPV	2.66 ± 0.06^{c}	3.00 ± 0.41^{c}	5.33 ± 0.77^{c}	8.33 ± 0.96^{c}	9.00 ± 1.85^{c}	16.66
	P value	0.001	0.001	0.001	0.001	0.001	-
	$F_{(3, 6)}$	33.501	76.938	113.520	576.587	651.684	-

Data are expressed as means ± stander error (SE) of three replicates at each bio-pesticide. *DAT: days after treatment. Means followed by the same superscript letter (s), within the same column are significantly different ($P \leq 0.05$) according to Tukey HSD Test.

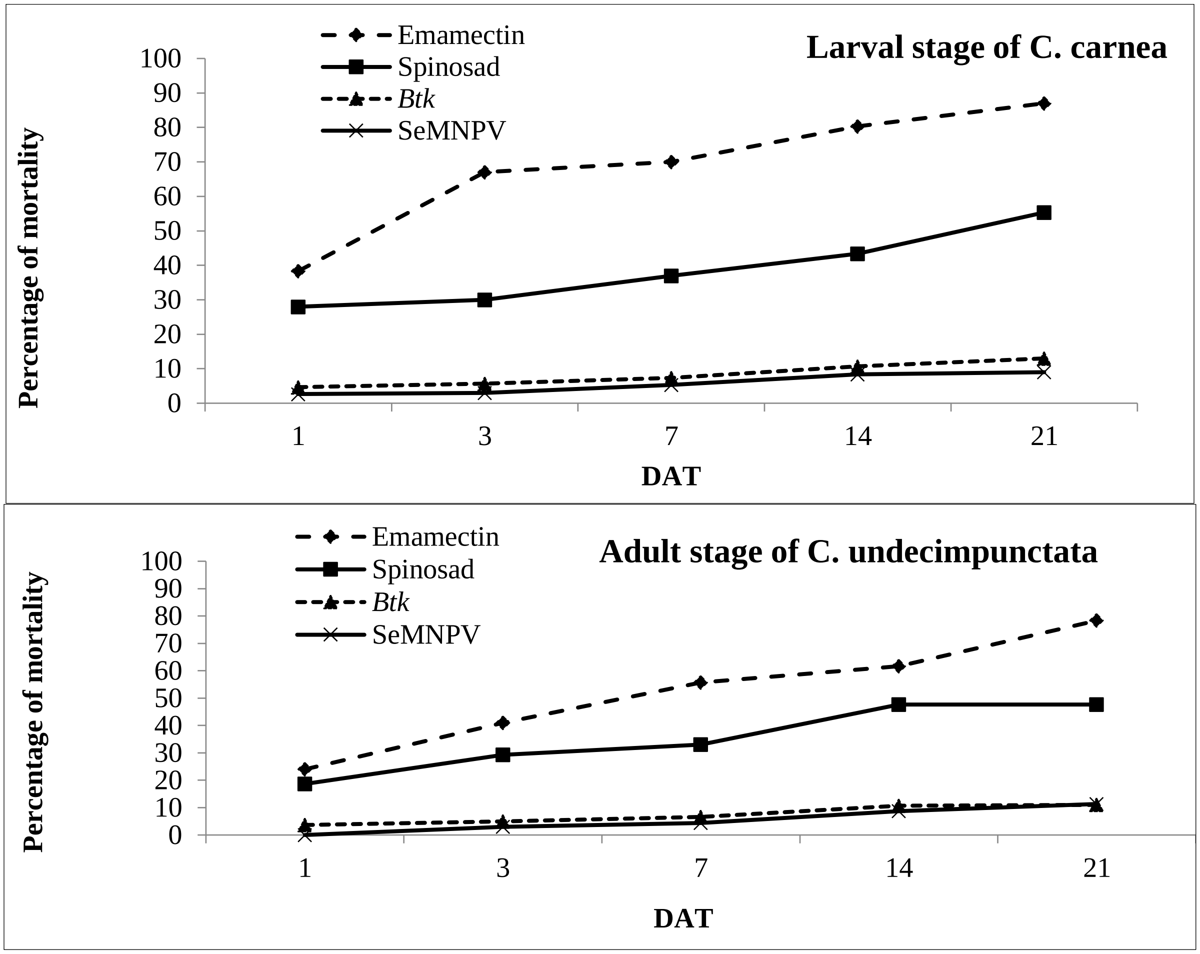

Figure 3: Trend of mortality in larvae of *Coccinella undecimpunctata* and *Chrysoperla carnea* at 1, 3, 7, 14 and 21 days after treatment (DAT) by *Btk*, SeMNPV, emamectin and spinosad in a field trial.

Damage assessment

Analysis of variance of bush damage to the sugar beet after 21 days by pests on plants treated with spinosad, emamectin, SeMNPV, *Btk* and water as control showed a significant difference between treatments ($F_{4,10} = 95.550$, $P = 0.001$) (Figure 4).

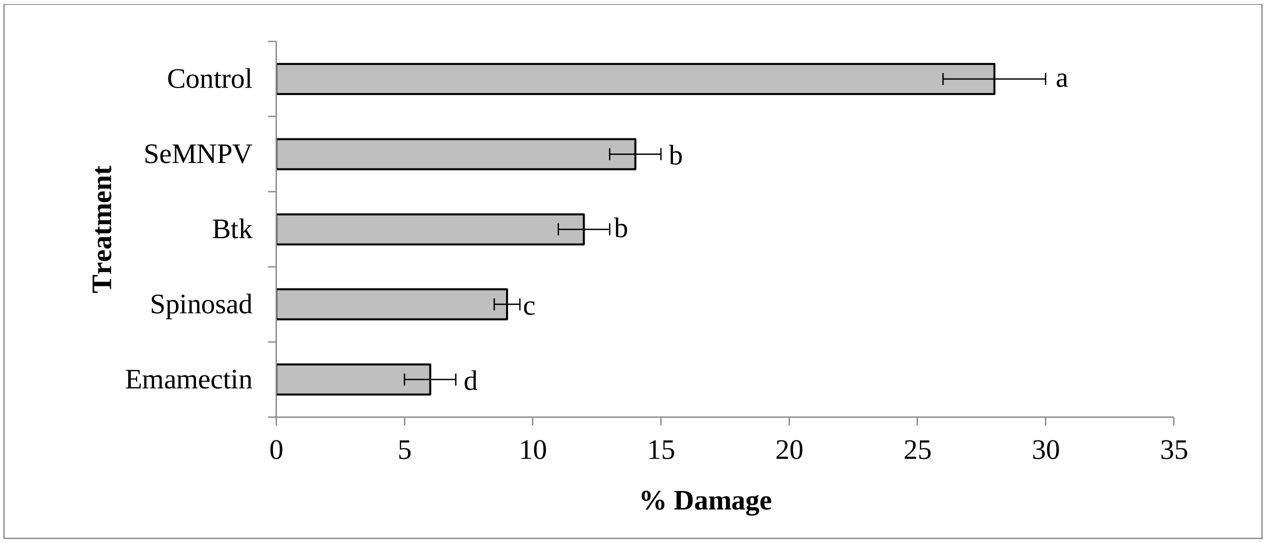

Figure 4: Comparison of sugar beet plant damage 21 days after treatment at 95% probability level with Tukey test. The columns with the same letters are not significantly different. The bars represent standard errors.

According to the comparison of the mean of treatments, the highest damage was due to control treatment and the lowest damage was reported for emamectin.

Discussion

Use of natural enemies to control pests has become an important tactic for the management of insect pests in many agricultural crops. Biological control has been increasingly used in crop protection over the past 30 years, with the production of biological control agents also increasing, with more than 130 species of predators and parasitoids on the market (van Lenteren 2012). Therefore, it is necessary to use effective compounds on pests with the least effect on natural enemies. In the present study, emamectin and spinosad effectively controlled the larval stages of *S. exigue*. Therefore, these two insecticides are suitable candidates for suppressing outbreaks of *S. exigua*, but emamectin and spinosad negatively affected the populations of *C. carnea* and *C. undecimpunctata*. Zhang et al. (2014) reported that spinosad and emamectin could effectively control larvae of *S. exigua*. In our study, *Btk* and SeMNPV had minimum adverse effect on population of these predators with acceptable control on *S. exigua*. Venkateswari et al. (2008) evaluated the effects of emamectin and abamectin on the fourth larval stage of *S. littoralis* (Boisduval), the LC_{50} value of emamectin was 19.1µg ml^{-1}. Altaf Sabri et al. (2016) showed that order of mortality of *S. exigua* caused by four insecticides was: methoxyfenozide > spinosad > indoxacarb > emamectin > lufenuron. Moadeli et al. (2014) reported that the LC_{50} value of spinosad after 48 hours on the first larval stage of *S. exigua* was calculated as 0.096 mg L^{-1}. In a study carried out by Rehan and Shoaib (2014), spinosad was reported as the most effective insecticide against *S. litoralis* among other insecticides: emamectin, methoxyphenozide, fipronil, indoxacarb, prophenphos, lufenuron and deltamethrin; they reported values of 1.23 and 0.0305 mg L^{-1} for LC_{50} of spinosad and emamectin, respectively. Ishtiaq et al. (2012) reported that the most toxic insecticide against *S. exigua* among four conventional and six new insecticides, were emamectin and spinosad. Stanley et al. (2017) reported that *S. litura* is highly susceptible to emamectin and spinosad, which is similar to our results. Kumari and Singh (2009) and Ali et al. (2011) reported that SlNPV effectively controlled the *S. litura* larval population.

Biological control agents are a good alternative to chemical insecticides in pest management programmes. However, natural enemies may not always provide adequate control of insect pests and mites (Kuris et al. 2005). Therefore, the use of biopesticides compatible with natural enemies is essential in pest management programmes. Medina et al. (2003b) reported that spinosad, tebufenozide and azadirachtin are not toxic to eggs and pupae of *C. carnea* and spinosad at the highest concentrations caused a slight significant reduction in adult life span and fecundity. However in our study spinosad caused lower mortality in comparison with emmamectin on the larval and adult stages of *C. carnea* and *C. undecimpunctata.* Studies have demonstrated that spinosad has no direct or indirect negative effects on green lacewing (*Chrysoperla carnea*) (Medina et al. 2003a); this is not in agreement with our results. De Castro et al. (2018) evaluated the effects of synthetic and organic insecticides on *S. exigua* and its predator *Podisus maculiventris* (Say); the results showed that spinosad had a toxic effect on the pest and its natural enemies. Numerous studies have investigated the effects of *Bt* on insect pests and their natural enemies (Sanchis 2011). Jiang et al. (2006) studied the effects of triazophos, shachongshuang, abamectin, and *Bt* + imidacloprid on the insect pest-natural enemy community in early rice fields. The results showed that all of the test insecticides had significant effects on pest and natural enemy populations, but *Bt* had least effect on natural enemies. Salama et al. (2009) investigated the effect of *B. thuringiensis* on *S. littoralis* and its natural enemies including *C. undecimpunctata* and *C. carnea*; the result showed that *Bt* had effect on the pest, but negligible effects on the parasites and predators. Kumar et al. (2011) investigated the efficacy of polyhedrosis virus in controlling lepidopteran pests. The result showed that most lepidopteran larvae were susceptible to the polyhedrosis virus. Similar to our results, Studebaker and Kring (2003) showed that emamectin is harmful to adults of *C. undecimpunctata* and *C. carnea*. Studebaker and Kring (2003) also reported that the predatory bug *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) was very sensitive to emamectin under laboratory conditions.

Conclusion

The repeated use of synthetic pesticides against major pests could be the main reason of pest resurgence, and reduction of natural enemies. The results of this study provide a useful insight to build a framework for future investigations and to reduce the use of toxic chemicals for the control of insect pests of major crops and conservation of the natural enemies. According to our results *Btk* and SeMNPV can be recommended to manage the larval stage of *S. exigua* with least hazard effect on the natural enemies in sugar beet fields. Hopefully, a more rational approach will be gradually adopted towards microbial pesticides such as *Btk* and SeMNPV in the near future and short-term profits from chemical pesticides will not determine the fate of natural enemy.

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