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Field Efficacy of Azadirachtin, Chlorfenapyr, and *Bacillus thuringensis* against *Spodoptera exigua* (Lepidoptera: Noctuidae) on Sugar Beet Crop

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ABSTRACT

Spodoptera exigua (Hübner) is a highly dispersive and polyphagous species that can be a serious pest of vegetables such as sugar beet. Field experiments were conducted to evaluate the efficacy of azadirachtin, chlorfenapyr, and *Bacillus thuringensis* (Bt) against the pest population and its quantitative and qualitative damages on sugar beet during the cropping season 2014/2015 in Lorestan province, west of Iran. Samplings for estimation of *S. exiguae* densities were conducted 1 day before (DBT) treatment and 1, 3, 5, 7, and 10 days after treatment (DAT). Finally, total yield and sugar content of sugar beet in each treatment were recorded. Results showed that one day after treatment (DAT), chlorfenapyr significantly decreased the pest eggs and larvae densities, but azadirachtin and Bt did not significantly reduce the egg population. Similar performance of azadirachtin and Bt to reduce the larval population occurred with delayed effects at 5 and 7 DAT, respectively. Lastly, the result of yield and sugar value of sugar beet showed that azadirachtin was the most effective for controlling *S. exigua* population.

Key words: bio-insecticide, beet army worm, sugar beet, IPM.

INTRODUCTION

Sugar beet armyworms, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), is a pest that causes a significant amount of economic damage to a wide variety of crops that originate from Southeast Asia (Cook *et al*., 2004; Hill, 2008). In addition to its direct damage at reducing the photosynthetic area, its larval presence, feeding marks, and excrement residues, reduce the marketability of vegetables and ornamentals (Lesser *et al*., 1996; Capinera, 2001). The pest larvae are protected inside the leaves and are tolerant to many insecticides. Thus, inadequate insecticide deposition in the lower part of the plants seems to be one limiting factor in controlling this pest (Mink and Luttrell, 1989; Ali *et al*., 1990; Cook *et al*., 2004). In addition, insecticide resistance is a major problem in the management of this insect, possibly because it attacks crops are frequently treated with insecticides (Capinera, 2001).

Recent public concerns about the risks of chemical insecticides/pesticides on the environment have encouraged scientists to search for more effective and safe control agents (Ignoffo and Garcia, 1979). Efforts have been increased to discover more effective and safe biorational pesticides against hazardous insects in order to replace with the potentially hazardous chemical agents currently used (Ince *et al*., 2008). Based on their low ecotoxicological profile and short persistence in the environment, Bt and azadirachtin represent an important pest control option to integrated pest management (IPM). The bacterium *Bacillus thuringiensis* (Bt) produces delta-endotoxins that have toxic properties and can be used as biopesticides (Schunemann *et al*., 2014). Among the botanicals insecticides, azadirachtin isolated from the seeds of the neem tree, *Azadirachta indica* L., has been used to control various insect pest in different crops, particularly vegetables. It has many anti-insect properties, including anti-feedant activity, growth regulatory, and sterility effects, as well as influences more than 250 species of insect pests (Boadu *et al*., 2011). Chlorfenapyr is a pro-insecticide derived from halogenated pyrroles. Chlorfenapyr works by disrupting ATP production. This molecule has low mammalian toxicity and is classified as a slightly hazardous insecticide as per WHO criterion (Raghavendra *et al*., 2011).

In this regard, the present study was conducted and aimed to evaluate the field efficacy of azadirachtin, chlorfenapyr, and *Bacillus thuringensis* (Bt) to control *S. exigua* population and its quantitative and qualitative damages in sugar beet fields.

MATERIAL AND METHODS

Experimental design

This study took place during February 2014 to September 2015 growing season in Selseleh, north of Lurestan province, west of Iran. The trials were performed in a 4000 m^2 field, which was cultivated with the sugar beet var. Navaderow® (Maribo seed international co., USA) seeds (approximately 83,000-85,000 plants per hectare). The field was divided into 4 plots (250 m^2) and wide ridges (3 m) were made in each. Cultural practices were conducted according to practical advisements of the Lourestan province agricultural organization.

Treatments were arranged in a randomized complete block design with four replications (plots). The information about experimental treatments is presented in Table 1. The control was sprayed with water. Treatments were applied using a backpack sprayer (Matabi®, Taizhou Kaide Machinery *Co., Ltd*. *China*) in a broadcast application using the hollow cone, solid spray tip type of nozzle (TXVK-10). The equipment was set to deliver 1000L/ha, following the growers' usual practice. Sprayings were conducted after the first flight activity of *S. exiguae* moths. The male flight activity was monitored using sex pheromone lures (Russel IPM, U.K.) that was placed inside Delta sticky traps. The number of traps inside males caught was recorded weekly.

Sampling

Samplings for estimation of *S. exiguae* densities were conducted 1 day before treatment (DBT) and 1, 3, 5, 7, and 10 days after treatment (DAT). Number of sample unite were determined using Ruesink (1980) formula. At each sampling date, 6 randomly selected plants were checked by traveling in an X-shaped pattern through each plot and the numbers of live larvae and egg masses of the pest on the selected plant leaves were separately recorded.

Harvesting was started late August and continued until early September. After harvesting, the total yield of each experimental treatment was separately determined. In addition, sucrose content in sugar beets of each treatment was measured according to the Sachs-Le Docte process method (Janshekar and Mor, 1977) by quality check laboratory of Lourestan sugar factory.

Table1. Information about the experimental treatments.

No.	Treatment	Trade name	Formulation	Mode of action	Applied rate per hectare
1	Chlorfenapyr [1]	Box®	24%EC	Disrupting the production of adenosine triphosphate	400 ml
2	*Bacillus thuringiensis Kurstaki*[2] (Bt)	Liponex plus®	32000 spore/gr WP	Its delta-endotoxin act as digestive toxin	1 kg
3	Azadirachtin[3](Az)	Neem Azal F. ®	5% EC	Insect growth regulators	1 lit

1-Shanghai Chemical Pharm-Intermediate Tech.Co., China. 2-Interchem, Italy. 3-Trifolio, Germany.

Data analysis

The data were statistically analyzed by ANOVA. Duncan’s Multiple Range Test was used for means separation using SPSS (version 16).

RESULTS AND DISCUSSION

Chlorfenapyr significantly reduced sugar beet armyworm egg masses compared to the other treatments at 1-10 DAT (Table 2). Plots treated with Bt and azadirachtin had no significant effect on numbers of the pest egg masses at all sampling dates. This indicated that chlorfenapyr affect *S. exigua* oviposition, but Bt and azadirachtin have no effects on the pest oviposition. The results of the present study is similar to Liu *et al.,* 2002 in which chlorfenapyr provided an excellent control of cabbage looper *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae) egg on cabbage. Our finding is also in agreement with the results of Donovan *et al*., 2001 who reported that Bt does not have any effect on *S. exigua* eggs. A similar result was obtained by Liu and Zhang, 1997 for *Plutella xylostella* L., *Spodoptera litura* Fabricus, and *Pieris rapae* L. In this regard, Groeters *et al.*, 1992 showed that the oviposition preference of *P. xylostella* is not affected by Bt toxins.

In contrast to our finding, Polanczyk and Alves, 2005 demonstrated that some Bt isolates affected the adult physiology of *Spodoptera frugiperda* Smith in a such way that was reflected in the quality and amount of eggs laid. Similar results were reported by Abdul-Sattar and Watson, 1982 who reported that Bt affected the mating behavior and oviposition of *Helicoverpa virescens* F., with the number of eggs hatching close to zero. This different result may be due to the different moth sensitivity of these species with *S. exiguae*.

Table 2. Mean ± SE of S.exigua eggs in chlorfenapyer, Bt and azadirachtin treatments at 1, 3, 5, 7 and 10 days after treatment.

Days after treatment					
Pesticide	1	3	5	7	10
Chlorfenapyer	2.270.193 a	2.070.179 a	1.60.21 a	1.280.141 a	0.90.11 a
Bt	2.550/19 b	2.520/185 b	2.350.17 b	2.220.186 b	2.120.18 b
Azadirachtin	2.420.17 b	2.300.187 b	2.20.18 b	2.150.14 b	1.980.13 b
Control	2.550.20 b	2.500.19 b	2.470.12 b	2.300.191 b	2.220.18 b
Df	15,3	15,3	15,3	15,3	15,3
ANOVA (P>F)	0.01	0.01	0.001	0.002	0.002

Means in the column followed by a same letter are not significantly different (Duncan, P <0/05).

In a previous study, deterrent effects of neem based insecticides on *S. exigua*, under laboratory conditions, were shown by Greenberg *et al.,* 2005. The same results were documented for *Mamestra brassicae* L. (Lep., Noctuidae) (Seljansen and Meadow 2006; Ikeura *et al*., 2013) and *Spodoptera littoralis* Boisduval (Pineda *et al*., 2009). Differences in the laboratory and field conditions are probably the main reason for the different results. Differences in compounds of various neem based insecticides may be another reason for the different results. There are two types of harvestable neem materials: 1- azadirachtin, which is extracted from the neem oils of neem seeds, concentrated, and purified, and 2-neem seed kernel oil cake (Ikeura *et al*., 2013). It has been documented that the feeding repellent effect of field spray of azadirachtin on *M. brassicae* is less than seed kernel oil cake (Ikeura *et al*., 2013). Laboratory and glasshouse trials conducted by Charleston *et al*., 2005 showed that when female adults of *P. xyllostella* were exposed to the botanical insecticide derived from neem tree, the moths did not discriminate between control plants and treated plants. Whereas, *Melia azedarach* L. extracts appear to have a suitable repellent effect for the moth.

At 1 DAT, chlorfenapyr significantly reduced beet armyworm larval densities compared to those observed in the Bt, azadirachtin, and control treatment (Table 3). In addition, larval densities in Bt was significantly lower than azadirachtin and control at 3 DAT. At 5 and 7 DAT, all insecticide treatments significantly reduced larval densities compared to control. No larvae were collected in plots treated with all tested insecticides at 10 DAT. Delayed effects of azadirachtinon (Scmuttere, 1988) and Bt (Saleh *et al*., 1990) in comparison with chemical insecticides, particularly neurotoxin, on target pests has been previously reported. Azadirachtin has specific anti-feedant and deterrent activity, suppressing and stopping of insect feeding, reduction of molting and deformations in pupae and in the imago, and can decrease fecundity of the females (Isman, 2006). Despite the sensitivity of insects of most orders to azadirachtin, neem based insecticides are selective as do not harm important natural enemies of the pests. In addition, they are non-toxic to warm-blooded animals. Therefore, neem-seed extracts have considerable potential for integrated pest control measures, not only in developing countries but also in industrialized countries (Schmuttere, 1988).

Table 3. Mean ± SE of S.exigua larvae in chlorfenapyer, Bt and azadirachtin treatments at 1, 3, 5, 7 and 10 days after treatment.

	Days after treatment				
Pesticide	1	3	5	7	10
Chlorfenapyr	0.820.04 a	0.370.02 a	0.250.015 a	0.150.01 a	00a
Bt	40.062 b	1.850042 b	0.950.03 b	0.60.024 ab	00b
Azadirachtin	3.970.07 b	2.720.05 c	1.370.03 b	0.770.025 b	00b
Control	3.550.06 b	3.1250.055 c	2.620.05 c	2.150.046 c	1.30.03 b
Df	36,3	36,3	36,3	36,3	36,3
F value	34.477	17.061	13.228	9.553	5.505
ANOVA (P>F)	0	0	0	0	0

Means in the column followed by a same letter are not significantly different (Duncan, P <0.05).

These results for azadirachtin are similar to those reported by Tanzubil and Mc Caffery, 1990 in which the effect of azadirachtin was dilatory and caused 100% mortality to *Spodoptera exepta* Walk after seven days. The present study demonstrated that chlorfenapyr persisted throughout the time period evaluated. Our results are in agreement with those found by Huai-Hengl *et al.*, 2011 who reported that chlorfenapyr had a short and long-term effect on *S. litura*.

ANOVA showed that there is a significant difference for average weight of harvested sugar beet (df=3,5; F=31.04; P <0.0001) (Table 4) and sugar content (df=3, 19; F=7.54; P=0.002) (Table 5) in different treatments. Yields from plots treated with azadirachtin were significantly higher than chlorfenapyr, Bt, and control (Table 4). Crop yield increased significantly in treated plots over control plots. Despite higher larval density of *S. exiguae* with azadiractin treatment compared to chlorphenapyr, the highest sugar beet yield (Table 3) was observed in this treatment. This result may be due to the anti-feedant property of the insecticide residues. The anti-feedant effect of azadiractin on *S. littoralis* was reported by Martinez and van Emden (1999).

Table 4. Mean yield±SE (tone/hectare) of sugar beet in the different experimental treatments.

Treatment	Azadirachtin	Bt	Chlorfenapyr	Control
The average weight per treatment	1125±59.5 a*	975± 14.4 b	837.5± 12.5 c	600±50 d

The average weight per treatment otherwise the same letters in the row indicates significant differences in the level of 5% (Duncan).

The promising effect of azadirachtin against *S. exigua,* which in turn increased the yield in the present investigation, is concordant with those by Mudathir and Basedow, 2003 and El Shafie and Abdelraheem, 2012. Their findings showed that neem formulations significantly reduced pest attack on tomato and increased yield. These findings are in agreement with Elshafie, 2001 who reported that the average yield of potato treated with NeemAzal ®, a formulation of azadirachtin, was increased in comparison to control. The repellency, anti-feedant, deterrence activities (Mochiah *et al*., 2011), and safety to the beneficial insects make neem a sufficient pesticide to control *S. exigua*.

Table 5. Mean ± SE sugar levels (gr/ Kg) measured in experimental treatments.

Treatment	Azadirachtin	Bt	Chlorfenapyr	Control
Sugar levels	1.31±20 a*	0.67 ±18.4 b	0.66 ±17.8 bc	0.24 ±16.5 c

* Same letters in the row represents no significant difference in the level of 5% (Duncan test).

In conclusion, our field trials suggest the effectiveness of the tested compounds (azadirachtin, chlorfenapyr, and Bt) on the pest larvae. Although chlorfenapyr tended to be a powerful suppressor of *S. exigua,* the yield and sugar level of sugar beet were lower with this treatment than tested bio-insecticides. Since azadirachtin and Bt could suppress the pest one week after spraying, it is recommended that the pest population can be further reduced by other management methods, such as chlorphenapyr application, during this period. The botanical pesticides degrade in the environment within hours or days. In addition, it has been shown that the use of natural plant products provide a low risk when compared to chemical insecticides (Sengottayan, 2013). Thus, biopesticides that are currently under investigation could be effectively used as a different pest management option in the production of organic tomato in order to reduce the pest populations below economic threshold and increase yield.

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