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Monitoring of four noctuidae species with pheromone traps and chemical control of *Helicoverpa armigera* (Hubner 1808) in Tunisian tomato open field crops

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RUNNING TITLE: SURVEY OF NOCTUIDAE SPECIES UNDER TUNISIA CONDITIONS

Abstract – The male flight activity of the most abundant noctuidae species (*Helicoverpa armigera*, *Spodoptera littoralis*, *S. exigua* and *Agrotis segetum*) was investigated using sex pheromone delta trap in tomato open field crops. This study was conducted in two Tunisian regions: Zaghouan (Northern half of Tunisia) and Takelsa (North-east of Tunisia) over a three years' period (2011, 2012 and 2014). The cotton bollworm *H. armigera*, is considered as the most harmful pest compared to the other species, which can cause several losses. So, development of an appropriate management program is a priority for tomato crop practitioners. This study showed that the four noctuidae species were able to realize up to 5 peaks in the two regions and the tomato of main season was found to be the best for the moth activity than the tomato of late season. For *H. armigera*, a summer pesticide trial (June, 2014) was conducted by the application of three insecticides in Zaghouan tomato open field plot. The tested insecticides cypermethrin, deltamethrin and *H. armigera* nucleopolyhedrovirus-based bio-insecticide were significantly effective in reducing the pest population. The bio-insecticide, which may be a new alternative to manage this pest, gave an encouraging result and confirmed the important effect of microbial control agents against lepidopteron species.

Keywords: insecticides, male flight activity, noctuidae species, open field tomato crop, population dynamics.

1. Introduction

Among the noctuidae species (Lepidoptera: Noctuidae) which are considered as polyphagous pests of cultivated crops (Brown and Dewhurss 1975), we noted *H. armigera* (Hubner 1808), *S. littoralis* (Boisduval 1833), *S. exigua* (Hubner 1808) and *A. segetum* (Denis and Schiffermüller 1775). These species can cause damage to different crops in Tunisia and in many countries around the word such as India (Singh 2005). In Tunisia the most harmful was *H. armigera* (Hubner). This insect pest is a polyphagous pest which feeds on diverse economically important crops, including soybeans, cotton, sorghum, corn, sunflower, peanuts, beans, peppers and tomatoes (Carneiro et al. 2014). *H. armigera* larvae are very dangerous and can cause severe damage particularly to reproductive organs (Sanja and Hrnčić 2012). This pest was reported in many countries around the word. Eggs are laid on plants when flowering (OEPP/EPPO 1981). This insect has several attributes (polyphagy, high mobility, high fecundity and facultative diapause) that allow its survival in adverse environments and its adaption to the most abrupt seasonal changes (Feng et al. 2005). Management strategies for this insect pest are based on monitoring, the use of chemical and biological control (Carneiro et al. 2014). For example, in Morocco, *H. armigera* was monitored on cotton cultivation by means of adult sex and light pitfall traps (Bourarrach et al. 1995). In Tunisia, several studies have proposed IPM strategies to control pests that attack tomato plants such as Lepidopteran species (Boukhris-Bouhachem et al. 2001, Lebdi-Grissa et al. 2010). To avoid damages caused by *H. armigera* and *T. absoluta*, farmers used uninfested plants and delta or water traps baited with sexual pheromones to survey these pests (Boukhris-Bouhachem et al. 2001, Lebdi-grissa et al. 2010). For *T. absoluta*, other means of control were used to manage this pest such as mass trapping with sexual pheromones and sprays of biological or chemicals insecticides (Abbes et al. 2014; Cherif and Lebdi-Grissa 2014; Hafsi et al. 2012). Among the active substances which are used by Tunisian farmers, we noted *Bacillus thuringiensis*, spinosad, indoxacarb and abamectin, which were proved effective on managing *T. absoluta* (Lebdi-grissa et al. 2010). Also, insecticides were frequently applied in Tunisian tomato plants to control *H. armigera*, by the use of some insecticides such as methomyl, endosulfan, *Bacillus thuringiensis* and *Melia azedarach* suspension (Boukhris-Bouhachem et al. 2001). Biological control was adopted also in Tunisia by the use of natural enemies such as *Trichogramma* spp. (for example *Trichogramma cacoeciae* and *T. evanescens*) which were found to be effective against *T. absoluta* and *H. armigera* (Boukhris-Bouhachem et al. 2002; Cherif and Lebdi-Grissa 2012). In tomato crops in many countries around the word, including Tunisia, chemical control is the most adopted method by farmers (Embrapa 2013) but the excessive use of chemical insecticides led this pest to develop resistance to many active ingredients (Ahmed et al. 1995; Alvi et al. 2012). Resistance of *H. armigera* has been reported not only to endosulfan, chlorpyrifos, and thiodicarb (Ahmed et al. 1995) but also to other conventional insecticides (such as pyrethroids and organophosphates) and to *Bacillus thuringiensis* Toxin Cry1Ac in Pakistan fields (Alvi et al. 2012). In order to avoid the problem of resistance to chemical insecticides, others control methods should be considered such as the use of new bioinsecticides and also consider eco-sustainable control methods which have no side effects on natural enemies in tomato crops.

The aim of this work was a) to study the population dynamics of the four noctuidae species cited above in Tunisia open field tomato crops in Takelsa and Zaghouan regions and b) to determine under field conditions the effectiveness of three insecticides (two chemical and one bio-insecticide) in reducing population levels and losses caused by *H. armigera* larvae.

2. Materials and methods

2.1. Experimental sites

Monitoring of noctuidae species was studied using sex pheromone traps (PheroNorm®, Andermatt Biocontrol) at rate of 2 delta traps/ ha during a three-year study. Delta traps, which were setup one week before the beginning of the survey, were installed in tomato crops located in two different Tunisian regions: Takelsa (in Nabeul) and Bou slim (in Zaghouan). Pheromone capsules were inspected weekly, removed and replaced every 4 weeks. In the area of Takelsa, one typical crop covering an area of 1 ha was considered. However, in Bou slim region, one late season crop covering an area of 0.5 ha and two typical crops of 1 ha each were chosen. The specific characteristics of each studied crop are given in table 1. During this study and in order to determine the population dynamics of *H. armigera* on tomato plants, 40 tomato leaves and 20 fruits were weekly collected at random. The counting of eggs and larvae (all instars) in sampled tomato leaves and fruits were made in the laboratory under binocular microscope (Leica® Model MS5).

Table 1: Specific characteristics of each crop

Sampling sites	Number of surveyed fields	Description of the plots			Sampling dates
		Number of plants	Distance between rows (m)	Distance between plants (m)	
Takelsa (Nabeul) (Cap-Bon region, North-East of Tunisia)	1 open field (Typical crop)	30000 planted with three varieties (Ferrinz+Chebli+Chams) on 70 rows	1.5	0.5	06/04/2012 to 05/07/2012
Bou slim (Zaghouan) (northern half of Tunisia)	3 open fields	1 open field (late season) 6667 plants planted with Ferrinz on 30 rows	1.5	0.5	22/09/2011 to 31/12/2011
		2 open fields (Typical crops) The 1st and the 2nd plot contained, each, 33 000 plants on 63 rows. The 1st plot was planted with cxd while the 2nd plot was planted with Podium.	1.6	0.2	15/04/2014 to 05/08/2014

2.2. Management of H. armigera using different insecticides

The study was planned to test the efficacy of different insecticides in reducing the pest population and thus decreasing damage caused by this pest. For this purpose a trial was conducted in the Bou slim region (Governorate of Zaghouan) on June 20th, 2014, with three pesticides. The experimental design was a randomized complete block design with four treatments. Each experimental unit comprised 96 tomato plants and received one of the following treatments: one virus-based product, cypermethrin, deltamethrin and the untreated control (Table 2). Insecticides were sprayed in recommended doses when catches in traps reached 5 moths /day / trap as mentioned in a previous study realized in Gisborne processing tomato crops over two seasons (1992-1993 and 1993-1994) (Herman et al. 1994). For insecticide application, a hydraulic knapsack hand sprayer was used. Formulations and application doses are given in Table 2. Treatment efficacy against *H. armigera* was evaluated by taking 40 leaves and 20 fruits from each 32 tomato plants in each experimental unit. Three repetitions were considered for each treatment. Counting of *H. armigera* larvae was realized with a binocular microscope. Population was recorded after 3, 7, 14 and 21 days of the application of insecticides. Insecticide efficacy on *H. armigera* was evaluated with the Abbott's formula:

% efficacy = [(J0 – Jt / T0) x
100] where:
J0 (control) = Mean number of population in control tomato
plants Jt = Mean number of population in treated tomato plants

Table 2: Active ingredients and chemical groups used for the trial and their respective concentrations and doses

Active ingredient	Trade names	Conc. a.i.	Doses in l/ha or ml/hl
7.5 10^{12} cfu/l*Helicoverpa armigera*	Helicovex SC	> 7.5 × 10^{12} PIB/liter	0.2 l/ha
cypermethrin	Cypercal 250 EC	250g/l	50ml/hl
Deltamethrin	Decis Expert EC	100g/l	15ml/hl

a.i: active ingredient

2.3. Climatic data

The climatic data in the two typical crops (in Zaghouan and Nabeul) and in the late season crop (only in Zaghouan) were given by the Tunisian Meteorological Institute and shown in the table 3.

Table 3: Climatic conditions (Mean numbers of Temperature (T°c) and Relative Humidity (RH %)) in Zaghouan (2011 (a) and 2014 (c)) and Nabeul (2012 (b)).

(a) Months	**September**	**October**	**November**	**December**
T (°C)	25	18	15	11
Rh (%)	73	79	88	92

(b) Months	April	May	June	July	August
T (°C)	17	18	29	31	27
Rh (%)	83	77	72	70	72

(c) Months	April	May	June	July	August
T (°C)	18	20	23	28	28
Rh (%)	59	20	47	52	52

2.4. Statistical analysis

The statistical analyses were performed using the statistical software program SPSS 17. Data related to the effectiveness of insecticides treatment were subjected to one-way ANOVA. Duncan's multiple range test at *P*= 0.05 was used to separate Means of treatments (SPSS Inc, 2009).

3. Results

3.1. Male flight activity of *S. littoralis, S. exigua and A. segetum* in tomato open field crop in Zaghouan and Nabeul regions

In Zaghouan region, we noted the presence of four flight peaks for *S. littoralis* and *S. exigua* registered in September, October, November and December, 2011. For *A. segetum*, we noted the presence of 3 peaks recorded on 22/09/2011, 11/10/2011 and 06/12/2011 (Figure 1). This figure 1 showed that *S. exigua* and *A. segetum* were the most frequent at the beginning of the survey. In fact, the number of adults captured per delta trap ranged from 67 to 0 for *S. exigua* and from 48 to 0 for *A. segetum*. *S. littoralis* was present with an important number which reach 16 moths/ delta trap in the month of October (Figure 1). During the study period extending from early April, 2012 to late June, 2012, in Takelsa region, the figure 2 showed that all studied species were present with an important number and 3 to 5 flight peaks were recorded on April, May and June. Males of *S. littoralis* have been trapped from 06/04/2012 to 05/07/2012 with 4 male flight peaks recorded on 06/04/2012, 26/04/2012, 15/05/2012 and 07/06/2012. The highest trap counts (13males/trap/week) were registered in the second peak. *S. exigua* showed also 4 male flight peaks recorded on 06/04/2012, 10/05/2012, 07/06/2012 and 28/06/2012 with a highest trap noted on 10/05/2012. Finaly, for *A. segetum* the figure 2 showed that this pest was able to achieve 5 flight peaks. These peaks were recorded on 06/04/2012, 10/05/2012, 24/05/2012 and 28/06/2012 with a number of captured males of about 17, 13, 9, 13 and 3 respectively. According to the figure 1 and 2, we noted that the number of adults recorded in delta traps of main crop season in Nabeul was higher than that recorded in the tomato of late crop season located in Zaghouan region.

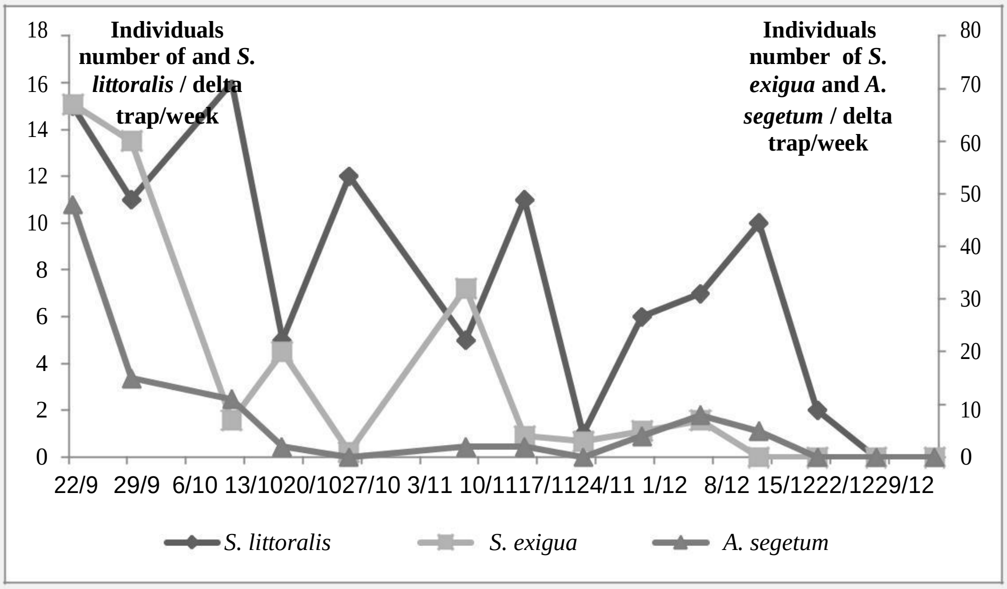

Figure 1: Male flight activity of *S. littoralis, S. exigua* and *A. segetum* captured in sex pheromone delta traps in tomato late crop season in Zaghouan region in 2011

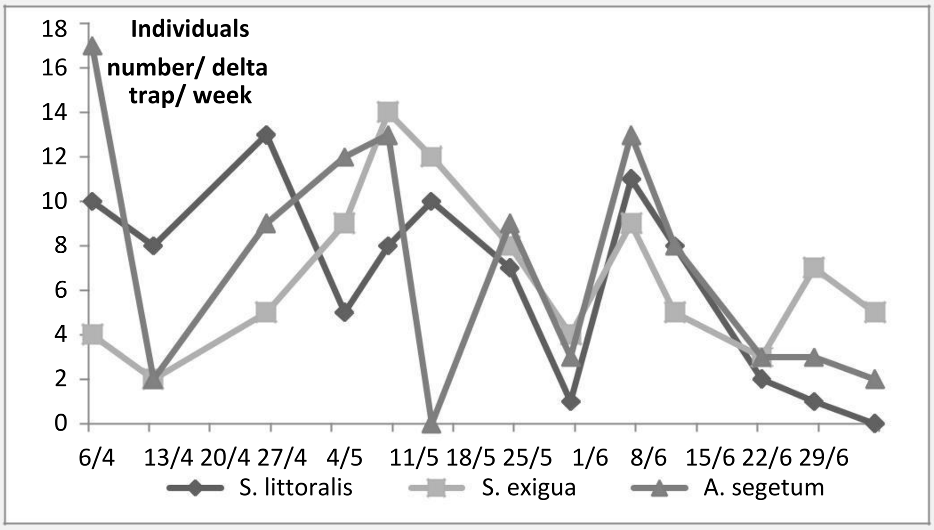

Figure 2: Weekly evolution of *S. littoralis, S. exigua* and *A. segetum* males caught in sex pheromone delta traps in Takelsa open field crop (tomato of main season) in 2012

3.2. Male flight activity and population dynamics of H. armigera in open field tomato crops

The cotton Bollworm *H. armigera* was more active in the typical crop than in the late crop season (Figs 3-8). In fact, the number of adults captured by the sex pheromone traps placed in Zaghouan tomato open field (late season crop in 2011) was very little (2 moths/ delta trap/week) (Figure 3a). On leaves, the number of eggs and larvae recorded was negligible (it did not exceed 2 eggs and 1 larva / 40 leaves) and no fruit was attacked (Figure 4a). Also, no pupae were present on leaves and fruits which confirm the idea that pupation occurs in the soil. The study of the male flight activity in the tomato typical crop revealed that *H. armigera* performed 3 peaks in Takelsa (From April to early July) and 6 peaks in Zaghouan (From April to early August) with maximum trap catches occurring in May in the two regions (19 adults/delta trap/week in Takelsa) and (25 moths/ delta trap/ week in the two plots in Zaghouan) (Figure 3 b, Figure 6). For the population dynamics, the figures 4, 5 and 7a showed that *H. armigera* eggs and larvae were more frequent again in the typical tomato crop. In fact, in Nabeul region, from April to July, 2012, eggs number ranged from 1 to 14 eggs/ 40 leaves and 3 peaks were recorded respectively on 10/05/2012, 13/05/2012 and 28/06/2012 (Figure 7a). Also, in Zaghouan region from April to August, 2014, the maximal number of eggs was 10 and 12 eggs/ 40 leaves respectively in the first and second plot (Figure 4 b, c). In fact, in the 1st plot of Zaghouan region, 4 peaks of eggs were recorded respectively on 13/06/2014, 24/06/2014, 11/07/2014 and 22/07/2014 while in the 2nd plot only 2 peaks were registered on 17/06/2014 and 11/07/2014. However, larvae were less frequent than eggs on tomato leaves. In fact, this number did not exceed 6 larvae/ 40 leaves in the two studied regions (Figure 4 and 7). The number of larvae in fruits did not exceed 9 larvae/ 20 fruits in the three experimental plots (Figure 5 and 7b). Pupae appeared only very rarely in fruits (no more than 1 pupa/ 20 fruits was found in all studied regions).

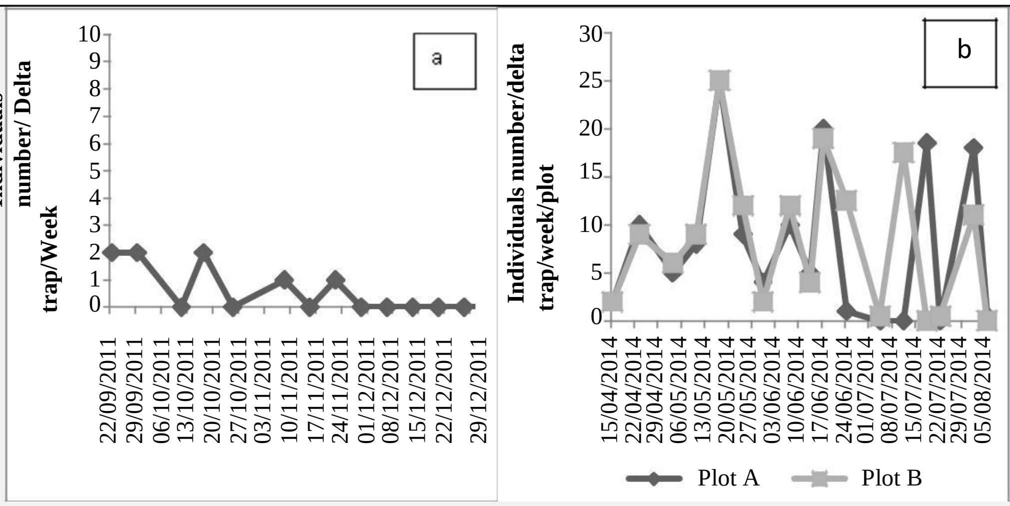

Figure 3: Weekly evolution of *H. armigera* males caught in sex pheromone delta traps in Zaghouan open field crops (a: late crop season in 2011; b: Typical crops in 2014: Plot A and Plot B)

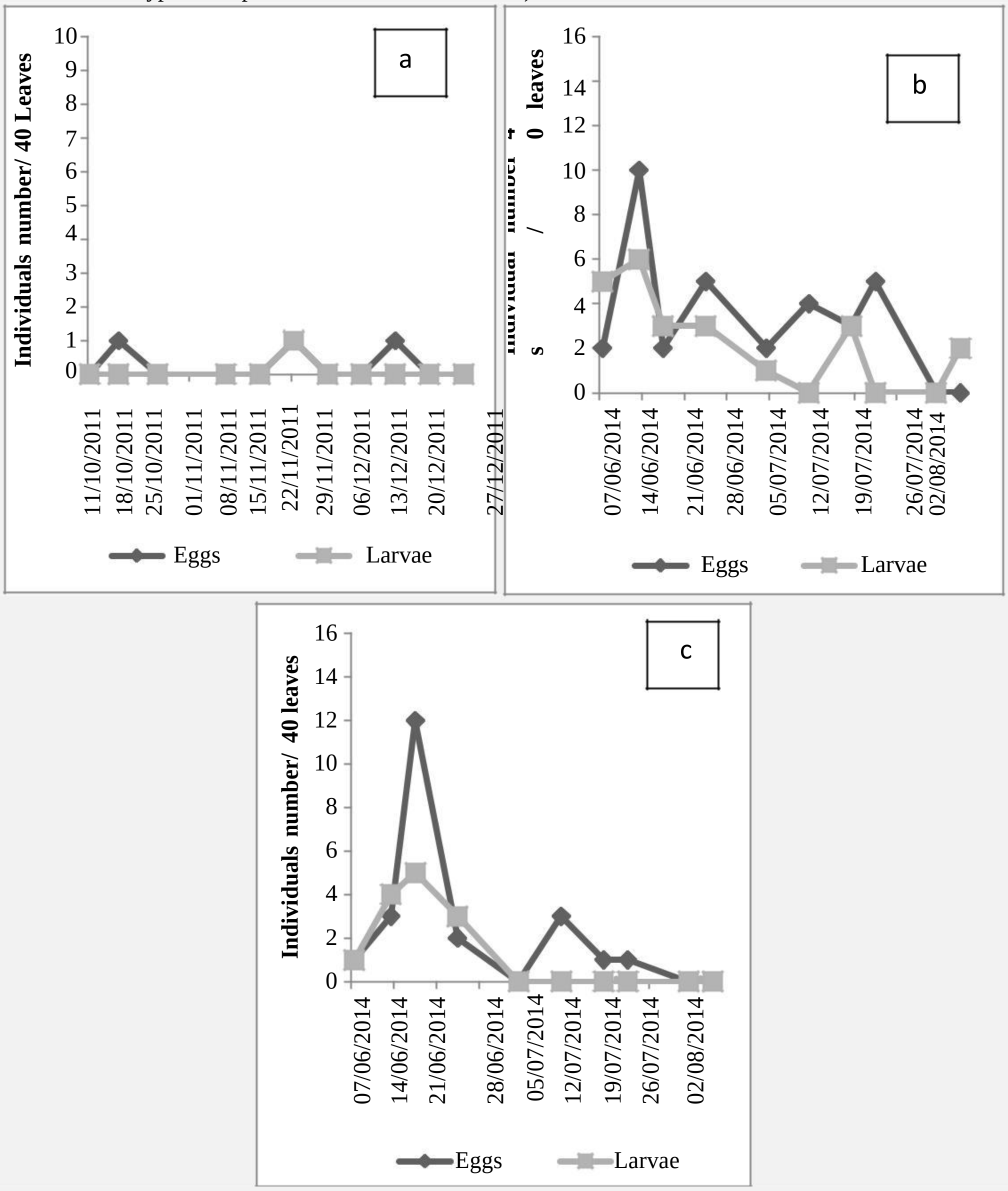

Figure 4: Population dynamics of *H. armigera* on leaves in Zaghouan open field crops (a: late crop season in 2011; b (Plot A) and c (Plot B): Typical crops in 2014)

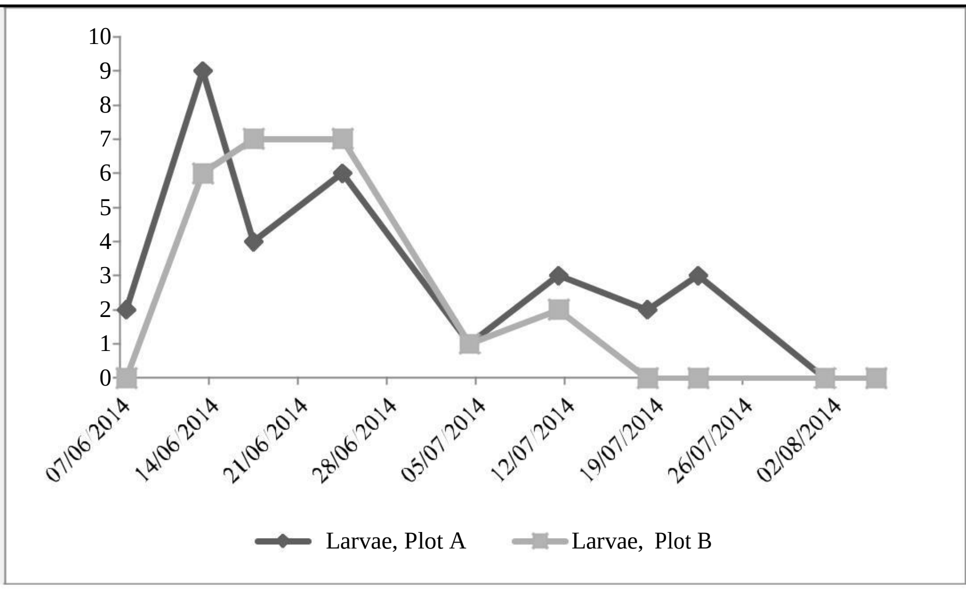

Figure 5: Population dynamics of *H. armigera* in fruits in Zaghouan typical crops in 2014: Plot A and Plot B

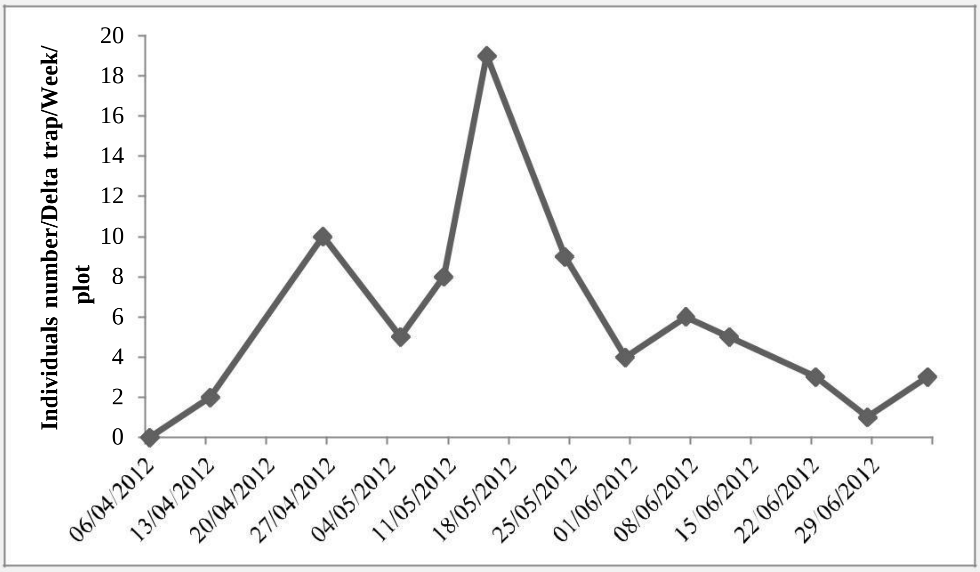

Figure 6: Weekly evolution of *H. armigera* males caught in sex pheromone delta traps in Nabeul open field crop (Typical crop) in 2012

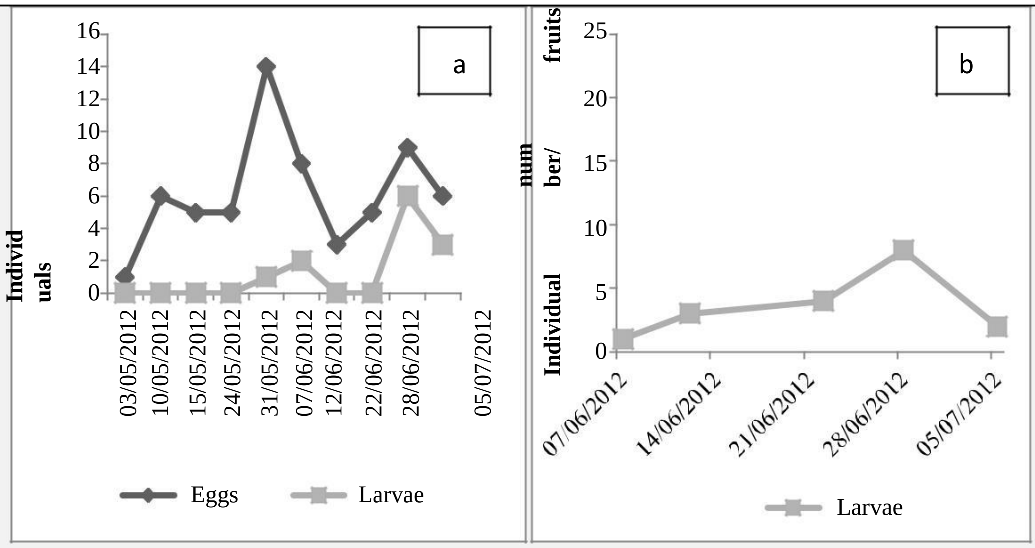

Figure 7: Population dynamics of *H. armigera* in Nabeul open field crop (Typical crop), on leaves (a) and in fruits (b), in 2012

3.3. Effect of tested insecticides on *H. armigera* larvae

This study aimed assess the efficacy of three insecticides (cypermethrin, deltamethrin and the bio-insecticide) tested, on *H. armigera* larvae. The results obtained are presented in figure 8 and tables 4 and **5** below. The number of larvae decreased significantly following spraying ($F=3, 49$, dfs (16, 3) and P=0.04) in comparison to the control and in the three treated blocks (figure 8). Three days after treatment (DAT), cypermethrin and the virus based product induced a decrease of the pest population of about 83.33%. However, deltamethrin generated a 69.28% efficacy in decreasing the infestation caused by the pest (Table 4). The three tested insecticides caused a 100% mortality 7 DAT (figure 8).

Table 4: Efficacy rates (%) of the different insecticides used against *H. armigera* larvae per 32 tomato plants in Zaghouan open field plot

Treatments	**Efficacy rates (%)**			
	DAT			
	3	**7**	**14**	**21**
Control	–	–	–	–
Cypermethrin	83	100	100	100
Deltamethrin	69	100	100	100
7.5 10^{12} cfu/l*Helicoverpa armigera*	83	100	100	100

**** DAT: Days After Treatments**

Table 5: Effect of insecticide treatment on *H. armigera* larvae on tomato plants

Treatments	**Mean number of larvae/20 Fruits/32 tomato plants**
Control	3.99 a
Cypermethrin	1.06 b
Deltamethrin	1.13 b
7.5 10^{12} cfu/l*Helicoverpa armigera*	1.06 b

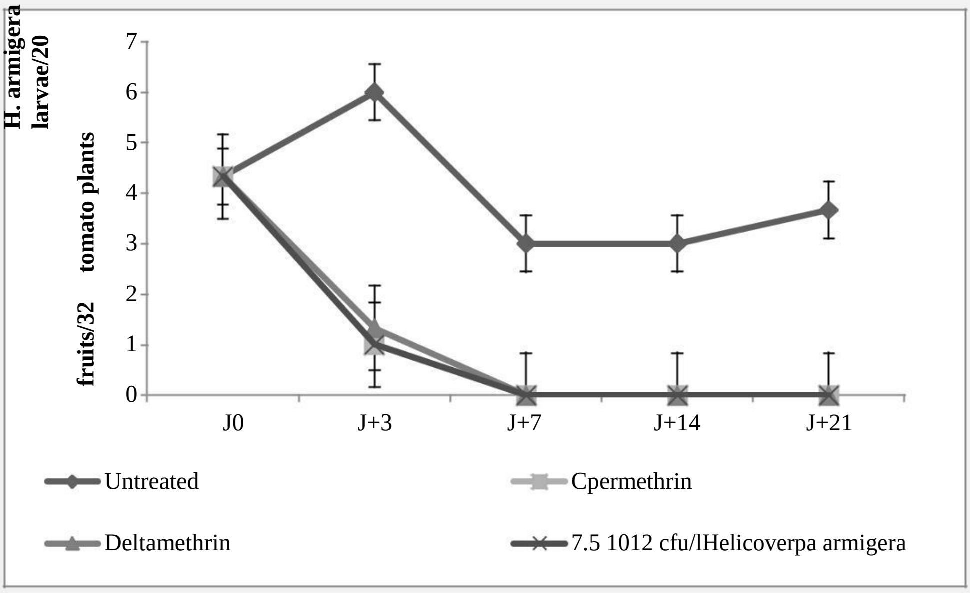

Figure 8: Evolution of Helicoverpa larvae after the spray of the different insecticides in Zaghouan tomato open field plot in 2014

Statistical analysis
Effect of tested insecticides on *H. armigera* larvae

ANOVA
NUMBER

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31,770	3	10,590	3,496	,040
Within Groups	48,469	16	3,029		
Total	80,239	19			

NUMBER
Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
2,00	5	1,0660	
4,00	5	1,0660	
3,00	5	1,1320	
1,00	5		3,9980
Sig.		,956	1,000

Means for groups in homogeneous subsets are displayed. a.

Uses Harmonic Mean Sample Size = 5,000.

4. Discussion

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In the present study, results of male flight activity of the four noctuidae species showed that, these pests may realize up to 5 generations. Also, we noted that these pests were more active in the tomato of main crop season than in the tomato of late crop season. Many studies were carried out in Tunisia and other countries around the world in order to study the male flight activity of these pests. In fact, the male flight activity data showed that these noctuidae were more active in the typical crops (from April to August) than in the tomato of late season (September to December). Ahmad (1988) studied the seasonal flight of *S. littoralis* using pheromone trap during two years (1983 and 1985) in vegetable crops in central Iraq. The author indicated the presence of many adult flight peaks during the study period and showed that more males are attracted in trap baited with septa that had been field-aged for 2 weeks compared to other traps baited with septa up to 4-weeks old and fresh septa (Ahmad 1988). For *S. exigua*, in a previous study, the male flight activity was measured by the aid of flight actograph Saito (2000). This author demonstrated that 2 days after adult emergent, males showed far higher activities than females, being several times higher at the time of maximum flight activity. Also, in this study, Saito (2000) showed that *S. exuiga* being regarded as long-distance migratory insects. In a previous study realized in 2004 in two regions located in Slovenia, Zalokar (2006) studied the male flight activity of *A. segetum* in a suggarbeet crop (*Beta vulgaris* var. altissima Döll.) by using pheromone traps. Zalokar (2006) ascertained that, under favorable climatic conditions when the air temperature rises above 12°C, the turnip moth realized two generations and its bionomics did not be influenced by a high rainfall doses. In another study, Srebernjak (2009) confirmed the occurrence of three generations of *A. segetum* on the corn field. The cotton bollworm, *H. armigera* has been studied by many authors in different countries on tomato and on other crops. For example, in a previous study realized in tomato open field crops in two different regions in Tunisia (Korba and Manouba), Boukhris-Bouhachem et al. (2007) demonstrated that *H. armigera* was active from late May to early November and the maximum trap catches occurred in July. Also, these authors showed that the greatest number of eggs and larvae were observed in July which coincides with the maximum moth activity) (Boukhris-Bouhachem et al. 2007). Bourarrach et al. (1995), demonstrated in a study done in cotton cultivation in Morroco that the activity period of *H. armigera* was short compared to other lepidopteron species. The authors showed that this pest spreads from June to October (Bourarrach et al. 1995). Bues et al. (1988) noted that in the south of France where *H. armigera* causes serious damage to tomato principally, three flight periods can be recorded (mid may-mid June, July-August, Sebtember-October). Tripathi and Singh (1991) mentioned that the study of the population dynamics of *H. armigera* (Hubner) in different cultures including chickpea, okra, maize and tomato indicated that the insect passed through five overlapping generations in a year. These authors noted that the growth of the population of *H. armigera* and its generation survival were maximum in second generation followed by fourth, third, fifth and first generations (Tripathi and Singh 1991). In another study**,** Kumar-Sharma et al. (2012) demonstrated under chickpea crop field that the maximum number of males trapped through pheromone traps were 105.66/trap/week, while during 12th standard week, the maximum number of larvae recorded was 30.0/10 plants.

In our study, for *H. armigera*, the largest number of eggs and larvae was observed on leaves in late May- Early June and on fruits from June to mid-July. Pupae were totally absent on leaves and present with very little number on fruits. This result, which is similar to that obtained by OEPP/CABI (1996), led us to conclude that pupation occurred in the soil. In fact, these authors suggested that once growth is achieved larvae leave the plant and pupate in the soil (3-15cm depth) and in some cases pupate at the end of a corn cob in Zimbabwe region (OEPP/CABI 1996).

The difference of male moth catches and larval population of noctuidae species in the two types of tomato crops (typical crops and late season crop) may be explained by two factors. Firstly, it may be linked to abiotic factors like temperature and relative humidity. In fact, during the survey of these pesst in the tomato late season crop in Zaghouan, the temperature and relative humidity values ranged from 32.40 to 7.30° C and from 53.60 to 97.30% respectively. While, in the typical crops temperature and relative humidity values are higher. Our findings are similar to those mentioned by Kumar-Sharma et al. (2012), who demonstrated that abiotic factors like maximum and minimum temperature had positive correlation with moth catches and larval population of *H. armigera*, contrarily to the relative humidity which had a negative correlation. Secondly, biotic factors may operate on noctuidae species populations. In fact, in a previous study for *H. armigera*, Tripathi and Singh (1991) demonstrated that the key mortality factor operating between generations was the variation in natality (log of maximum potential natality and actual eggs). Concerning the results related to the efficacy of the tested insecticides on reducing *Helicoverpa* larvae, this study showed that cypermethrin, deltamethrin and 7.5 10^{12} cfu/l*Helicoverpa armigera* were significantly effective in reducing *H. armigera* populations. Also in this study, we demonstrated that the treatment by the virus-based product (biopesticide) appeared to be effective against *H. armigera* larvae and its efficacy was comparable to synthetic pesticides. These findings were promising and as important as those obtained by Boukhris-Bouhachem et al. (2007) who got effective control of *H. armigera* after application of two synthetic (methomyl and endosulfan) and bio- (*Bacillus thuringiensis* and *Melia azedarach*) insecticides in Tunisian tomato open-fields. In this study, the authors demonstrated that applications of the two bio-insecticides reduced significantly pest populations and were comparable to methomyl (Boukhris-Bouhachem et al. 2007). Also, in other previous study, Razaq et al. (2005), demonstrated that cypermethrin when applied to reduce *H. armigera* pest population in a cotton plot with a dose of 625 ml/ha, gave an important larval mortality (near of 60%) 3 days after treatments. Kraaz and Zingg (2012), showed that the efficacy of Helicovex® has been assessed when applied at rates of 100 and 200 ml/ha in numerous field trials from 2006 to 2011. The efficacy ranged between 68 and 89% in protected tomato and pepper crops and between 51 and 100% in others open field crops (Kraaz and Zingg 2012).

5. Conclusion

The typical crop season was considered the best for noctuidae species activity than the tomato late crop season which was characterized by fewer adults catches per traps and less attacks in leaves and fruits. The study of *H. armigera* population dynamics, which is considered the most harmful pest to tomato crops in Tunisia helps not only to better know the pest but also to improve the efficiency of insecticides tested. The population dynamics of the other studied noctuidae pests must be studied, in order to estimate their damages on tomatoes and to establish a control program. An integrated pest management based mainly on the monitoring of the moth and the application of synthetic and biological insecticides is necessary to protect tomato crops. Results of chemical treatment on *H. armigera* are promising. To better manage this insect, other chemical or biological insecticides could be tested in Tunisian tomato crops to avoid resistance of this pest to some active ingredients. Results of the *H. armigera* nucleopolyhedrovirus-based bio-insecticide were very interesting due to its high efficacy and selectivity towards non-target organisms. This product, which leaves nil residues on the crop, can not only solve the problem of insect resistance already established in many countries but also offers new and effective solutions for integrated plant protection strategies.

Acknowledgements

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6. References

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