

Efficacy of the Insecticidal Bacterium *Bacillus thuringiensis* (Berliner) (*Bt*) against the Cereal Leaf Miner *Syringopais temperatella* Led. (Lep., Scythrididae) under Laboratory Conditions

Firas A. Al-Zyoud¹, Nofal S. Al-Ameiri² and Ayed M. Alomary³

ABSTRACT

The cereal leaf miner, *Syringopais temperatella* Led. (Lep., Scythrididae) is one of the most economical pests on wheat in Jordan. The use of chemical insecticides is neither economic nor sustainable; therefore, biological pesticides (i.e. *Bacillus thuringiensis* (Berliner)) are becoming an important component in crop protection. The present study was conducted to evaluate the efficacy of *B. thuringiensis* against *S. temperatella*. Eight *B. thuringiensis* concentrations were used against *S. temperatella* L₃ on wheat and mortality was recorded at 1, 3, 5 and 7 days after spraying. The results indicated that *S. temperatella* mortality was significantly affected by *B. thuringiensis* concentration and time after bacterial application. After 1, 3, 5 and 7 days of spraying, 10⁸ concentration of *B. thuringiensis* had significantly caused the highest mortality to the pest with 63.3, 73.1, 78.3 and 80.0%, respectively. Then the mortality percent decreased significantly until it reached 3.3, 15.6, 17.4 and 45.0% at 10¹ concentration, respectively. In conclusion, the study showed that *B. thuringiensis* is effective in controlling *S. temperatella* under laboratory conditions.

Keywords: *Syringopais temperatella*, *Bacillus thuringiensis*, Cereal leaf miner, Control, Bio-pesticides.

INTRODUCTION

Wheat, *Triticum durum* L., is considered one of the most important cereal crops grown in Jordan. In 2005, Jordan produced a total of thirty four thousand tons of wheat from planted areas of about three hundred eighty

six thousand dunums with an average of 90 kg/dunum (Agricultural Statistics, 2006). However, the cereal leaf miner, *Syringopais temperatella* Led. (Lep., Scythrididae), is considered one of the most economic insect pests on wheat since 2001 in Jordan (Al-Zyoud, 2007, 2008; Al-Zyoud *et al.*, 2009). *S. temperatella* population increased over the years due to annual rainfall reduction and wrong agricultural practices applied by farmers (Al-Zyoud, 2007).

Intensive application of chemical insecticides has been used to suppress *S. temperatella* in the countries of our region (Fard, 2000; Vrieze, 2002; Jemsi and Rajabi, 2003). In Jordan, Al-Zyoud (2008) reported that diazinon, chlorpyrifos, imidacloprid and fenitrothion caused mortality higher than 90% to the pest under laboratory conditions. In Cyprus, diazinon, fenitrothion,

¹ Assist. Prof. of Biological Control and IPM, Dept. of Plant Protection and IPM, Faculty of Agriculture, Mu'tah University, Al-Karak, 61710 Jordan, P. O. Box: 7, Email: firaszud@yahoo.com. (Corresponding author).

² Assist. Prof. of Plant Pathology, Dept. of Plant Protection and IPM, Faculty of Agriculture, Mu'tah University, Al-Karak, 61710 Jordan, P. O. Box: 7, Email: Nofal00000@Yahoo.com

³ Associate Prof. of Forestry, Dept. of Plant Production, Faculty of Agriculture, Mu'tah University, Al-Karak, 61710 Jordan, P.O. Box: 7, Tel. (mobile): 00962- 795-292719, Tel. (office): 00962- 3- 2372380, Email: ayomary@mutah.edu.jo

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phosphamidon and trichlorphon caused mortalities greater than 90% to the pest (Melifronides, 1977). In addition, Kaya (1976) in Turkey and Jemsi and Rajabi (2003) in Iran reported that diazinon proved to be effective against *S. temperatella*.

However, the use of chemical insecticides is neither economic nor sustainable and has a negative impact on the environment, natural enemies and farmers. Moreover, the use of chemicals has generally lagged due to cost constraints associated with wheat as a low-input crop (Debach and Rosen, 1991). Furthermore, the pest has developed resistance to chemical insecticides in Cyprus (Georghiou and Lagunes-Tejeda, 1991). Biological pesticides are becoming an important component in crop protection. They are natural disease causing microorganisms that infect specific pest groups (Carlton, 1988). *Bacillus thuringiensis* (Berliner) (*Bt*) is an ubiquitous bacterium that forms crystals containing insecticidal proteins, which are used to control lepidopteran, dipteran and coleopteran insects (Schnepf *et al.*, 1998). *B. thuringiensis* has been used in spray applications to reduce damage by agricultural and forest insect pests. This bacterium synthesizes crystalline inclusions that are toxic to the larvae of Lepidoptera and other orders of invertebrates. It exhibits high toxicity and specificity against insects, some parasitic nematodes, mites and protozoa (Schnepf and Crickmore, 1998; Al-Banna and Khyami-Horani, 2004). In Jordan, Abu-Dhaim *et al.* (2005) used six strains of *B. thuringiensis* and found that exposing the nematodes, *Meloidogyne javanica* and *Meloidogyne incognita* to 10^6 viable spores/ml resulted in complete inhibition of eggs' hatching and significantly increased the mortality of J_2S of both nematode species. The inclusion bodies produced during sporulation by *B. thuringiensis* are highly specific gut poisons causing the insect death within few hours of ingestion (Hofte and Whiteley, 1989; Gill *et al.*, 1992). Several formulated products based on *B. thuringiensis*

toxins are currently used as efficient tools for the control of agro-forestry insect pests (Cannon, 1996).

To the best of our knowledge, there are no studies on the use of *B. thuringiensis* against *S. temperatella* in Jordan, our region and the world as well. Therefore, in this study, the efficacy of *B. thuringiensis* against *S. temperatella* has been evaluated. The outcome of this study, will help in reducing the constraints to wheat production caused by *S. temperatella* in Jordan through development and application of appropriate low-cost and environmentally acceptable integrated pest management approaches.

MATERIALS AND METHODS

Rearing of *Syringopais temperatella* and Wheat Plants

The rearing of *S. temperatella* was initiated from newly emerged larvae collected from wheat fields in Al-Rabbeh, Al-Karak District and maintained on potted wheat plants. The infested wheat plants were kept in meshed cages of 50x50x80 cm under laboratory conditions of $20\pm 5^\circ\text{C}$ temperature, $50\pm 10\%$ relative humidity and 12:12 h (L: D) photoperiod at the Faculty of Agriculture, Mu'tah University. The meshed cages were covered with gauze from their sides and tops to provide adequate ventilation. Wheat plants of cultivar Horani 27 were used for rearing *S. temperatella* and conducting the experiments. Wheat plants were grown in an air-conditioned glasshouse in small pots, 12 cm in diameter and 12 cm in height and were frequently replaced whenever needed to maintain adequate host-plant supply. *S. temperatella* third larval instars (L_3) were used in the experiments and were randomly selected from the rearing cages and checked further under a binocular microscope. All experiments were conducted in the 2009 growing season at the Faculty of Agriculture, Mu'tah University under the previously mentioned controlled conditions.

Bacillus thuringiensis

Eight concentrations of *B. thuringiensis* var. *israelensis* were selected to conduct the experiments. *B. thuringiensis* was obtained from a stock culture at the Department of Biology, Faculty of Science, Mutah University. The bacteria were grown on nutrient broth (beef extract 10.0 g/L, peptone 10.0 g/L and sodium chloride 5.0 g/L) to aid sporulation. The culture was then incubated on a rotary shaker (300 rpm) at 28°C for four days to ensure sporulation and cell lyses. Spores and crystals were harvested by centrifugation at 1,000 rpm for 15 minutes at 8°C. The pellet (3.5-4 g/L) was washed with distilled water and diluted to obtain 10^1 - 10^8 viable spores/ml for use in the experiments. However, in order to prepare the different concentrations, *Bt* pellets were diluted in sterile distilled water to get 10^8 viable spores/ml and they were counted by haemocytometer slide. Hereafter, subsequent dilution to 10^7 until 10^1 was made from the 10^8 viable spores/ml.

Treatments

The experiments were conducted in Petri dishes, 5.5 cm in diameter and 1 cm in height, partially filled with a 0.5 cm thick layer of wetted cotton pad and the lid of each Petri dish had a hole closed with organdie fabric for ventilation. Wheat leaf discs of 10 cm² area cut from uninfected wheat plants were placed in the dishes. Third larval instars of *S. temperatella* were gently transferred using a Camel hairbrush into the Petri dishes in groups of ten larvae/Petri dish. Larvae in control groups (n=10) were sprayed with a 1 ml of distilled water, while larvae in treatment groups (n=10) were sprayed with a 1 ml aqueous solution of the required concentrations of *B. thuringiensis* by using a calibrated small sprayer. The Petri dishes were kept under the fore-mentioned laboratory conditions. For each concentration, three replicates were used and each Petri dish contained ten *S. temperatella* larvae. Larval mortality was recorded at 1,

3, 5 and 7 days post spraying. Larvae were considered dead if they did not move when lightly prodded with forceps. The overall control mortality was 0.00, 13.30, 23.33% and 33.30% at 1, 3, 5 and 7 days of the experiments.

Statistical Analysis

The statistical analysis was performed using the proc GLM of the statistical package SigmaStat version 16.0 (SPSS, 1997). The mortality resulting from *B. thuringiensis* treatment was adjusted for the control of mortality using Abbott's formula (Abbott, 1925). The data were analyzed by one way ANOVA to detect any differences in mortality of larvae caused by *B. thuringiensis* (Zar, 1999). When significant differences were detected, means were compared using LSD at 0.05 probability level (Abacus Concepts, 1991). Also, the correlation between the mortality and the progress of time after spraying as well as the mortality and increasing the concentration of *B. thuringiensis* was calculated by Spearman's correlation method (Zar, 1999)..

RESULTS

The results indicated that direct spraying of *S. temperatella* larvae by all *B. thuringiensis* concentrations tested exhibited a range of mortality after 1, 3, 5 and 7 days after spraying (Figure 1). *S. temperatella* larvae mortality was significantly affected by *B. thuringiensis* concentration and time after bacterial application. One day after spraying, 10^8 and 10^7 concentrations had significantly caused the highest mortality to the larvae with 63.3% and 56.7%, respectively. Then, the mortality percent decreased significantly until it reached 3.3% at 10^1 concentration ($F=17.68$; 7, 24 df; $P=0.000$). Three days after application, the mortality varied significantly among the different concentrations ($F=12.55$; 7, 24 df; $P=0.000$), in which it reached 73.1% at 10^8 concentration and started

to decrease with decreasing *B. thuringiensis* concentrations until it reached 15.6% at 10^1 concentration. Mortality levels were relatively higher at five days than at one day and three days post spraying, in which the mortality levels reached up to 78.3% at 10^8 concentration, whereas the least mortality was recorded at 10^1 concentration with 17.4% ($F=9.82$; 7, 24 df; $P=0.000$). Seven days after spraying, the percentage of mortality was the highest compared with 1, 3 and 5 days after application. However, there were significant differences in the mortality percent among the different concentrations of *B. thuringiensis*, where it was 80.0% at 10^8 concentration, while the least mortality of 45.0% was recorded at 10^1 concentration.

Further statistical analysis of results among the different days within the same concentration of *B. thuringiensis* indicated that with time after treatment, there was a significant increase in mortality for all *B. thuringiensis* concentrations (Figure 1). The percentage mortality of *S. temperatella* larvae was significantly higher after 7 days post spraying followed by 5 days and then by 3 days and the least mortality was recorded at 1 day after application for all concentrations ($F=4.60-24.23$; 3, 12 df; $P=0.000-0.037$).

The overall corrected mortality percent of third larval instars of *S. temperatella* in all days as a result of spraying with different concentrations of *B. thuringiensis* is shown in Figure 2 (A). The 10^8 concentration resulted in the highest mortality with 73.6% and it was significantly higher than that caused by any of the other *B. thuringiensis* concentrations, while the lowest mortality was recorded for 10^1 concentration with only 19.5% ($F=11.84$; 7, 96 df; $P=0.000$). There was also a positive correlation of 0.688, significant (0.000) at 0.01 probability level. This means that with increasing the concentration of *B. thuringiensis* up to 10^8 there was an increase in the mortality of *S. temperatella*.

The overall corrected mortality percent of *S. temperatella* in all concentrations of *B. thuringiensis* on the different days after spraying is illustrated in Figure 2 (B). The results indicated that the mortality was significantly increased with time and was 28.8%, 46.7%, 54.4% and 63.8% after 1, 3, 5 and 7 post spraying, respectively ($F=13.22$; 3, 96 df; $P=0.000$). Also, there was a positive correlation of 0.538, significant (0.000) at 0.01 probability level.

DISCUSSION

Hundreds of insects have been described on wheat worldwide and many of them have their origins in West and Central Asia and along the Mediterranean rim (Harlan, 1992), where Jordan is located. While most of these insects cause insignificant damage, others annually cause serious yield and forage reduction. *S. temperatella* is one of the major insect pests on wheat in the Mediterranean region and the Middle East (Jemsi *et al.*, 2002; Al-Zyoud, 2007, 2008; Al-Zyoud *et al.*, 2009), and it causes quantitative and qualitative damages to the crop (Jemsi and Rajabi, 2003).

Because of the rapid knock-down, high target mortality and ease of application, the use of chemical pesticides soon became the predominant method of controlling pests and overshadowed programs of alternative methods of control (Osteen and Szmedra, 1989). Chemical insecticides were widely and effectively tested against *S. temperatella* in some countries (Fard, 2000; Jemsi and Rajabi, 2003; Al-Zyoud, 2008). But, it is well known that continuous use of chemical insecticides is neither economic nor sustainable and has a negative impact on the environment, natural enemies and farmers (Debach and Rosen, 1991). Moreover, *S. temperatella* has developed resistance to some chemical insecticides (Georghiou and Lagunes-Tejeda, 1991). Therefore, efforts are needed to develop integrated pest

management strategies for the management of this pest

through the use of biological insecticides.

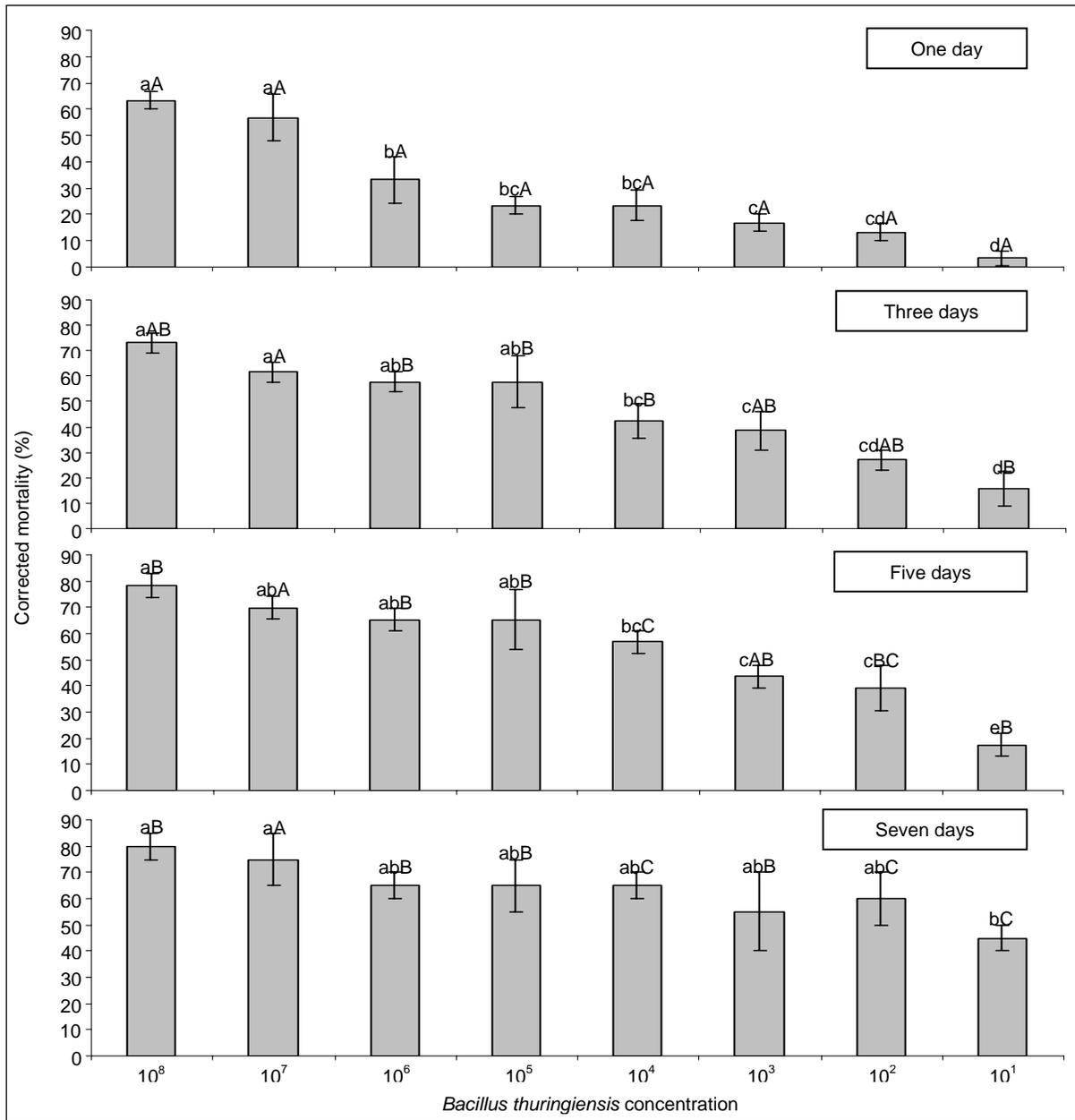


Figure 1. Corrected mortality percent of third larval instars of *Syringopais temperatella* after one, three, five and seven days of spraying of *Bacillus thuringiensis* in direct spray test. [Different small letters above bars indicate significant differences among the different *B. thuringiensis* concentrations within the same day, while capital letters above bars indicate significant differences among the different days with the same *B. thuringiensis* concentration at $p < 0.05$ (one-factor analysis of variance)].

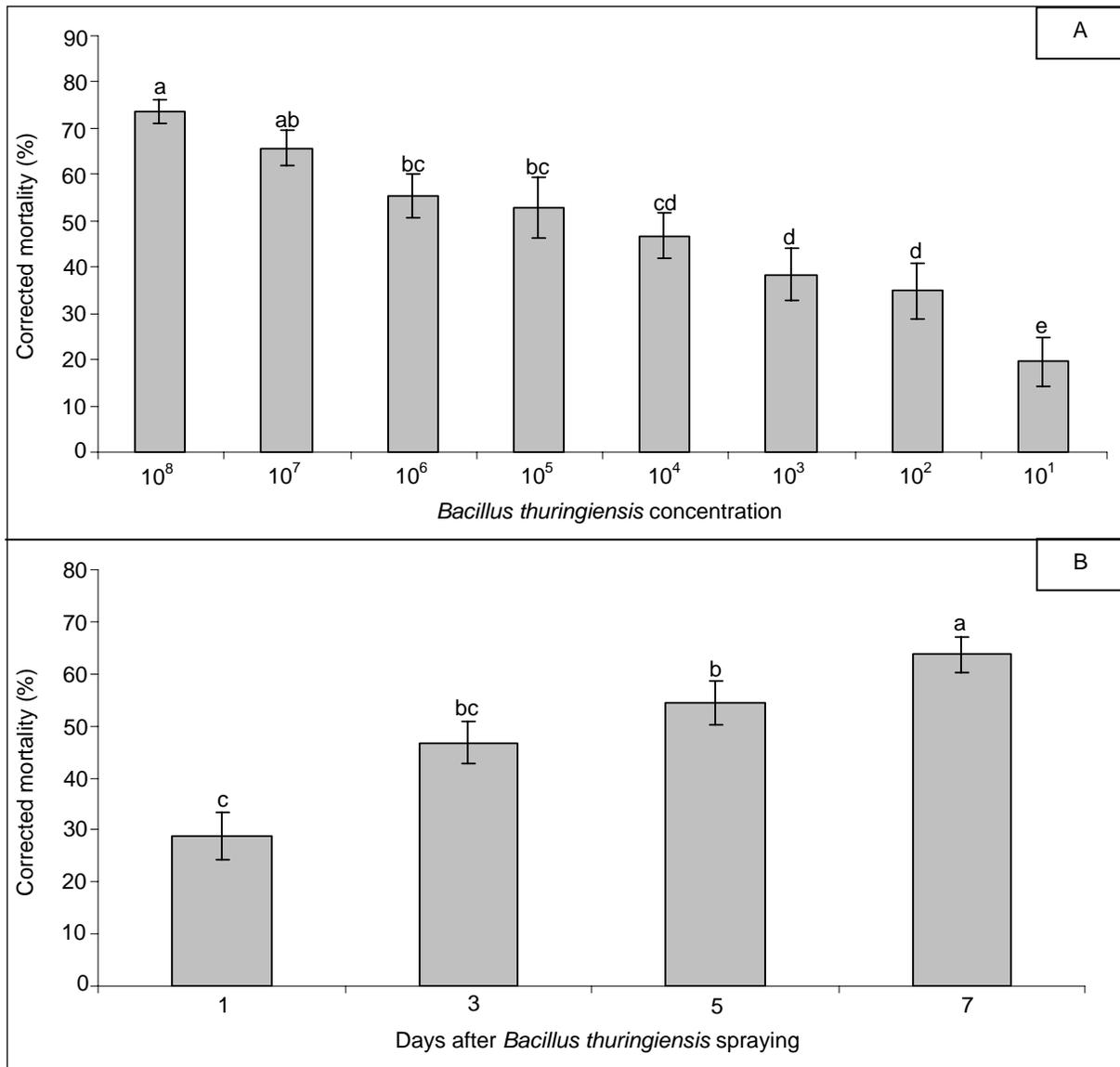


Figure 2. Overall corrected mortality percent of third larval instars of *Syringopais temperatella* on all days as a result of spraying with different concentrations of *B. thuringiensis* (A) and in all concentrations of *B. thuringiensis* on the different days after spraying (B). [Different small letters above bars indicate significant differences among the different *B. thuringiensis* concentrations (A) and among the different days (B) at $p < 0.05$ (one-factor analysis of variance)].

Results of the present study demonstrated that *S. temperatella* larval mortality was significantly affected by *B. thuringiensis* concentration and time after bacterial application. After 1, 3, 5 and 7 days of spraying, 10^8

concentration of *B. thuringiensis* had significantly caused the highest mortality to the pest with 63.3, 73.1, 78.3 and 80.0%, respectively. No studies on the efficacy of *B. thuringiensis* against *S. temperatella* have been

reported so far. Comparing the results of this study with another study conducted by Al-Zyoud (2008) on chemical control under the same laboratory conditions, *B. thuringiensis* (80.0%) caused higher mortality to *S. temperatella* larvae than Abamectin (74%), Methomyl (66%), Cypermethrin (48%) and Lamda cyhalothrin (63%). *B. thuringiensis* has been used to control other Lepidopterous pests such as *Plodia interpunctella* (Hubner) (Shelton *et al.*, 1993). Dubois and Dean (1995) found that *B. thuringiensis* spores have received much less attention than toxins but are known to increase the toxicity of *B. thuringiensis* proteins to the target insects.

The efficacy of *B. thuringiensis* was decreased with decreasing its concentration and was 3.3, 15.6, 17.4 and 45.0% at 10^1 concentration after 1, 3, 5 and 7 days after spraying, respectively. This might be due to the fact that *B. thuringiensis* product proteinase activity was lower in its effect to *S. temperatella*. These results are in agreement with results obtained by Abu-Dhaim *et al.* (2005), who found that low concentration of *B. thuringiensis* caused low mortality to *Meloidogyne* spp.

The present results indicated that mortality significantly increased with time. It was 28.8, 46.7, 54.4 and 63.8% at 1, 3, 5 and 7 days after spraying, respectively. *B. thuringiensis* occurs naturally in the environment and isolated from the soil (Schnepf *et al.*, 1998) and since the pest diapauses as larvae in the soil (Jemsi and Rajabi, 2003), it might increase the success of using this bacterium in controlling the pest. There are many factors affecting larval survival; i.e., predation, parasitism, rainfall, ploughing, sowing date and varieties (Fard, 2000). Therefore, *B. thuringiensis* could be used with other control methods to suppress the pest. Al-Zyoud (2007) reported that in Jordan, parasitism by the

parasitoid, *Anilastus* sp. Förster (Hym., Ichneumonidae), reached 49%, high enough to make a sufficient reduction in the pest population. Therefore, using of *B. thuringiensis* to control the pest will have no side effects on the parasitoid (Worthing, 1983) and both of them could be used in an IPM program to suppress the pest in the future.

In conclusion, the present study provides basic information on the efficacy of *B. thuringiensis* against *S. temperatella*. It showed that it is toxic to the pest and should be able to reduce its population if it is used at high concentration up to 10^8 . The efficacy of *B. thuringiensis* against *S. temperatella* should also be tested under field conditions and if the results are similar to those obtained under laboratory conditions, then the use of the bacterium should be in preference to chemical insecticides. Future studies should focus on economic injury and economic threshold levels of the pest, studying the dynamics of the insect population in comparison with the phenological development of host plants to optimize planting and harvesting dates, conducting economic feasibility studies on the replacement of wheat with alternative crops such as chickpeas and monitoring natural enemies to increase their impact on the pest populations. Conducting economic feasibility studies on the replacement of wheat with alternative crops should also be carried out. Such future studies, together with the current results and those of Al-Zyoud (2007, 2008) on the biology and ecology of the pest and the efficacy of insecticides under laboratory conditions as well as Al-Zyoud *et al.* (2009) on the susceptibility of wheat and barley varieties to the pest are expected to form the foundation of an IPM program for *S. temperatella* in Jordan.

REFERENCES

- Abacus Concepts. 1991. SuperAnova user's manual. Version 1.11, Berkeley, CA.
- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265-267.
- Abu-Dhaim, E., Al-Banna, L. and Khyami-Horani, H. 2005. Evaluation of some Jordanian *Bt* strains against two root-knot nematodes. *Jordan Journal of Agricultural Sciences*, 1(1): 49-57.
- Agricultural Statistics. 2006. Department of Statistics, Amman, the Hashemite Kingdom of Jordan. 132 pp.
- Al-Banna, L. and Khyami-Horani, H. 2004. Nematocidal activity of two Jordanian strains of *Bacillus thuringiensis* on root-knot nematodes. *Nematol. Medit.*, 32: 41-45.
- Al-Zyoud, F. 2007. Investigations on certain biological and ecological parameters of the cereal leaf miner *Syringopais temperatella* Led. (Lepidoptera: Scythrididae). *Bull. Fac. Agric., Cairo Univ.*, 58(2): 164-172.
- Al-Zyoud, F. 2008. Effects of direct spray and residual exposure of different insecticides on the cereal leaf miner *Syringopais temperatella* Led. (Lep., Scythrididae) under laboratory conditions. *Jordan Journal of Agricultural Sciences*, 4(1): 1-11.
- Al-Zyoud, F.A., Salameh, N.M., Ghabeish, I. and Saleh, A. 2009. Susceptibility of different varieties of wheat and barley to cereal leaf miner *Syringopais temperatella* Led. (Lep., Scythrididae) under laboratory conditions. *International Journal of Food, Agriculture and Environment-JFAE*, 6(3 and 4): 235-238.
- Cannon, R.J.C. 1996. *Bacillus thuringiensis* use in agriculture: a molecular perspective. *Biol. Rev.*, 71: 561-636.
- Carlton, B. 1988. Development of genetically improved strains of *Bacillus thuringiensis*. In: Hedin, P., Menn, J. and Hollingworth, R. (Eds.), *Biotechnology for Crop Protection*, American Chemical Society, Washington D. C., 260-279.
- Debach, P. and Rosen, D. 1991. *Biological control by natural enemies*. Cambridge University Press, Cambridge, UK, 440 pp.
- Dubois, N.R. and Dean, D.H. 1995. Synergism between Cry1A insecticidal crystal proteins and spores of *Bacillus thuringiensis*, other bacterial spores and vegetative cells against *Lymantria dispar* (Lepidoptera: Lymantridae) larvae. *Environ. Entomol.*, 24: 1741-1747.
- Fard, P.A. 2000. Loss evaluation of wheat leaf miner *Syringopais temperatella* in Khuzestan Province. International Congress of Entomology, Brazil, 674.
- Georghiou, G.P. and Lagunes-Tejeda, A. 1991. The occurrence of resistance to pesticides in arthropods: an index of cases reported through 1989, FAO, Rome, Italy, 11-22.
- Gill, S.S., Cowles, E.A. and Pietrantonio, P.V. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. *Ann. Rev. Ent.*, 37: 615-636.
- Harlan, J.R. 1992. *Crops and man*. 2nd edition, American Society of Agronomy, Madison, Wisconsin, USA, 284 pp.
- Hofte, H. and Whiteley, H.R. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.*, 53: 242-255.
- Jemsi, G. and Rajabi, G. 2003. Study on harvesting agronomic measures and effect of chemical application in controlling the cereal leaf miner *Syringopais temperatella* Led. (Lep., Scythrididae) in Khuzestan province. *Appl. Entom. Phytopath.*, 70: 13-15.
- Jemsi, G., Shojai, M., Radjani, G. and Ostovan, H. 2002. Study on economic population dynamic, biology, host range and economic threshold of cereal leaf miner in Khuzestan. *J. Agric. Sci.*, 8(3): 1-21.
- Kaya, O. 1976. The cereal leaf-miner, *Syringopais temperatella*. Report, CENTO Scientific Programme: recd. 1978, 49-50.
- Melifronides, I.D. 1977. The cereal leaf miner *Syringopais temperatella* and its control in Cyprus. *PANS*, 23(3): 268-271.
- Osteen, C.D. and Szmedra, P.I. 1989. Agricultural pesticide use trends and policy issues. Agricultural Economic Report No. 622, Washington DC, USDA, USA.
- Schnepf, E. and Crickmore, N. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.*, 62: 775-806.
- Schnepf, E., Crickmore, N., van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. and Dean, D.H. 1998. *Bacillus*

- thuringiensis* and its pesticidal crystal proteins. **Microbiol. Molec. Biol. Rev.**, 62: 775-806.
- Shelton, A.M., Robertson, J.L., Tang, J.D., Perez, C., Eigenbrode, S.D., Preisler, H.K., Wilsey, W.T. and Cooley, R.J. 1993. Resistance of diamondback moth (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* subspecies in the field. **J. Econ. Entomol.**, 86: 697-705.
- SPSS, Statistical Product and Service Solutions, INC. 1997. SIGMASTAT 2.03: Sigmastat statistical software user's manual, Chicago, United States.
- Vrieze, M.De. 2002. Interessante Wahrnehmungen von *Lepidoptera* in Griechenland. **Phegea**, 30: 41-47.
- Worthing, C.R. 1983. The pesticide manual: a world compendium. Croydon, England: The British Crop Protection Council.
- Zar, J. 1999. Bio-statistical analysis. Prentice Hall, Upper Saddle River, NJ, 663 pp.

		<i>Bacillus thuringiensis</i> (Berliner)									
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% 45 17.4 15.6 3.3		10 ¹									
		<i>Bacillus thuringiensis</i>				<i>Syringopais temperatella</i> :					

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