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Evaluation of *Bacillus thuringiensis* (Berliner) (*Bt*) as an Alternative Control of the Cereal Leafminer on Barley under Laboratory and Field Conditions

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Abstract: The cereal leafminer, *Syringopais temperatella* Led. (Lep., Scythrididae) is one of the major insect pests that attack barely and causes significant damage to the crop. This study aimed to investigate the efficacy of different strains of *Bacillus thuringiensis* Berliner (*Bt*) against *S. temperatella* on barley. Experiments were conducted under Laboratory and field conditions using different strains of *Bt*. The *Bt* strains were *Bt* var. *israelensis* (*Bti*) and *Bt* var. *kurstaki* (*Btk*-China and *Btk*-USA), each with three different concentrations. Results showed that the lowest infestation was obtained at the high concentration of *Btk*-USA (60.63%) and *Bti* (40.15%) one and two weeks post *Bt* spraying, respectively. The highest mortality was significantly recorded at the higher concentration of *Btk*-China after 3 days (54.17%) and 5 days (71.43%) of *Bt* application, respectively. In conclusion, the present study showed that *Bt* is effective and may be used against *S. temperatella*.

Key words: *Syringopais temperatella* · *Bacillus thuringiensis* · Cereal leafminer · Control · Bio-pesticides · Barley

INTRODUCTION

The cereal leafminer, *Syringopais temperatella* Led. (Lep., Scythrididae) is one of the major insect pests that attack barely and causes significant damage to the crop [1]. The cereal leafminer is endemic to Jordan and has been reported in the country more than 50 years ago [2]. But, since 2001 the pest has been recognized as the most destructive insect pest limiting the production of barley in Jordan [3,4,5,6,7]. Field observations in Jordan indicate that *S. temperatella* larvae emerge from ground in early February and penetrate the leaf mesophyll cells of barley. The larvae live in the leaves for 2 months and make mines between the epidermal layers [3] leading to a sharp decline in barley production. The insect was reported in Jordan [3, 5], Iraq [8], Iran [9], Cyprus [10], Syria, Lebanon and Turkey [11] and Greek [12]. In addition to barley, *S. temperatella* also attacks wheat [3].

Intensive application of chemical insecticides was used to suppress *S. temperatella* in many countries of the region [4, 9, 12, 13]. In spite of the intensive use of insecticides to suppress *S. temperatella* in Jordan, the infested areas by the pest are continuously increasing [3]. The non-judicious use of chemicals has led to development of resistance to insecticides [14] and the use of chemicals has created problems of land degradation, pesticide residues in farm produces and environmental pollution. Furthermore, increased public concerns about the adverse effects of indiscriminate use of chemical insecticides prompted search of alternative methods for pest control. One of the promising alternatives was the use of biopesticides [15]. An important benefit of biopesticides is that they can replace, at least in part, some hazardous chemical pesticides.

The most widely used biopesticides is the bacterium, *Bacillus thuringiensis* (Berliner) (*Bt*) [16]. *Bt* is a rod-shaped, gram-positive bacterium that occurs naturally in soil, dead insects, water and grain dust [17]. During the sporulation process, these bacteria produce large crystal proteins that are toxic to many insect pests [18]. When orally ingested by insects, the crystal protein is solubilized in the midgut, forming proteins called

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delta-endotoxins, which are highly toxic to insect pests [19] and highly specific [17] without affecting beneficial insects [20, 21]. Therefore, *Bt* is ideally suited for incorporation into integrated pest management (IPM) programs [22].

The greatest success in biopesticides has come from the use of commercial preparations of *Bt*. These preparations were the most successful biological pest control products worldwide [23]. *Bt* was used to control a wide variety of insect pests [24] on food crops, ornamentals, forest trees and stored grains for more than 40 years [22]. In addition, *Bt* was exclusively used for the control of larvae of dozens of species of medically important and pestiferous black flies and mosquitoes around the world [25]. Strains of this bacterium are currently used to control larvae of several blood-sucking pests of domestic animals [26]. Furthermore, *Bt* is used against several species of lepidopteran, coleopteran and some dipteran pests in food and fiber crops [27]. For human health, *Bt* can be used for the effective control of populations of several dipteran disease vectors [21]. In 2001, 60% of the total area under brassicas and 40% of the area under tomato in the US were treated with *Bt* [28].

Nevertheless, the continued damage to barley by *S. temperatella* is a daunting challenge and requires the use of novel technology. This has reflected an increased interest in the use of *Bt* as a part of IPM. Therefore, there is an urgent need of replacing agrochemicals with safer alternatives and adopting IPM practices to provide adequate crop protection for sustainable agriculture [29] and reduce the reliance of Jordanian farmers on chemical insecticides, that will be sustainable and minimize impact on environment and human health and increase barley production in the country, which will in turn increase farmers' incomes. However, up to date and to the best of our knowledge, no attention has been paid on the use of *Bt* against *S. temperatella* in Jordan and the world especially under field conditions. This study aimed to investigate the efficacy of different strains of *Bt* against *S. temperatella* on barley under Laboratory and field conditions. The final goal of this work is to encourage farmers to adopt such an alternative control measure in order to improve livelihoods and to help in investigation, development and promotion of effective plant protection measures, compatible with the environment and human health.

MATERIALS AND METHODS

Experimental Site and Growing Plants: The experiment was conducted in a barley field of Mu'tah variety in Faqqu Area (10 km north of the Faculty of Agriculture, Mutah University, Karak). The study was carried out in a field of two-dunum-area (dunum=1000 m^2) infested heavily by *S. temperatella* during 2011/2012 cropping season. The barley seeds were sown during December, 2011. The barley plants were under rain-fed conditions and no fertilizers were used.

Rearing of *S. temperatella* for Laboratory Experiments: The rearing of *S. temperatella* was initiated from newly emerged larvae collected from barley fields in Al-Qasr, Karak District and maintained on potted barley plants. The infested barley plants were kept in meshed cages of 50x50x80 cm under Laboratory conditions at 20±5°C temperature, 60±10% relative humidity and 12: 12h (L: D) photoperiod in a rearing room at the Faculty of Agriculture, Mu'tah University. The meshed cages were covered with gauze from their sides and tops to provide adequate ventilation. Barley plants of cultivar Mu'tah were used for rearing *S. temperatella* and conducting the Laboratory experiments. Barley plants were grown in an air-conditioned glasshouse in small pots of 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 instar (L_3) were used in the experiments and were randomly selected from the rearing cages and checked further under a Binocular microscope. The Laboratory experiment was conducted in 2012 under the above-mentioned controlled conditions.

Experimental Design and *Bacillus thuringiensis* Strains: Two different experiments were conducted. The first experiment was set up in the Laboratory and the second one was performed in the field. The experimental design was randomized complete block design (RCBD) with three replications each for the field experiment and complete randomized design (CRD) with three replications for the Laboratory experiment. Each experiment had two different treatments of *Bt* strains, in addition to a control treatment. Each *Bt* strain and concentration was replicated three times, in which each replicate (plot) consisted of an area of one meter square. In the field experiment, a distance of 10 m among the different treatments and 3 m among the plots was kept in order to prevent spray drift of the *Bt*. The *Bt* strains selected to conduct the experiments were *Bt* var. *israelensis* (*Bti*) and two isolates of *Bt* var. *kurstaki* (*Btk*-China and *Btk*-USA). *Bti* was obtained from a stock culture at the Department of Plant Protection, Faculty of Agriculture, Al-Balqa Applied University, while *Btk* strains (China and USA) were obtained from the National Center for Agricultural Research and Extension

(NCARE). *Btk* strains were used at three concentrations of 15, 20 and 25 g/L. *Bti* was 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 10,000 rpm for 15 min at 8°C. The pellet (3.5-4.0 g/L) was washed with distilled water to obtain 10^8 viable spores/ml for use in the experiments. Three *Bti* concentrations *viz.,* $1x10^8$, $3x10^8$ and $5x10^8$ viable spores/ml were prepared and they were counted by haemocytomer slide.

Laboratory Experiment: The experiment was conducted in Petri-dishes of 5.5 cm in diameter and 1 cm in height that were partially filled with 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. Barley leaf discs of 10 cm^2 area cut from uninfected barley plants were placed in the Petri-dishes. *S. temperatella* L_3 instars were gently transferred using a Camel hairbrush into the Petri-dishes in groups of ten larvae/Petri-dish. Larvae in the control treatment (n=10) were sprayed with 1 ml of distilled water, while larvae in *Bt* treatments (n=10) were sprayed with 1 ml aqueous solution of the required *Bt* concentration and strain using a calibrated small sprayer. The Petri-dishes were kept under the fore-mentioned Laboratory conditions. Larval mortality was recorded at 1, 3 and 5 days post-spray. Larvae were considered dead if they did not move when gently prodded with forceps. The overall control mortality was 3.33, 20.0 and 30.0% at 1, 3 and 5 days after *Bt* application.

Field Experiment: In the field experiment, the larvae started infesting barley plants on 20th of March. The plants were sprayed once one week after the larval infestation. The experiment consisted of 30 plots, each had one meter square. Each three plots of barley were sprayed with the recommended concentration of each *Bt* strain using a calibrated little knapsack sprayer. The plots in the control treatment were sprayed with distilled water only. One week after *Bt* spraying on (4th of April), all the plants in the plots were harvested from the field and the plants of each plot were separately kept in a paper bag and brought to the Laboratory and data were recorded for the number of total plants/plot and number of infested plants/plot.

Statistical Analysis: The statistical analysis was performed using the proc GLM of the statistical package SigmaStat version 16.0 [30]. The mortality resulting from *Bt* treatments was adjusted for control mortality using Abbott's formula [31]. The data were analyzed using one way ANOVA to detect any differences in mortality of larvae caused by *Bt* [32] and means were compared using LSD at 0.05 probability level [33]. The *t-test* also was used for comparisons between only two means [34].

RESULTS

The results of *S. temperatella* infestation percent when barley plants were sprayed with different *Bt* strains in a direct spray test under field conditions after 1 and 2 weeks of spraying are shown in Fig. 1. Barley plants infestation by *S. temperatella* was significantly affected by *Bt* strain and their different concentrations. One week after *Bt* spraying, the highest infestation was significantly recorded in the control treatment with 98.04%, while the lowest infestation was observed at the high concentration of *Btk*-USA with 60.63%, followed by the high concentration of *Btk*-China with 65.10 (F=1.294; 9, 20 df; P=0.042). There were found non-significant differences between the control and the low and medium concentrations of the three *Bt* strains with infestation more than 74% (F=1.294; 9, 20 df; P=0.05) (Fig. 1). Two weeks after *Bt* application, the infestation was significantly the highest in the control treatment with 96.57% and the lowest was reported at the high concentration of *Bti* with only 40.15%, followed by the high concentration of *Btk*-USA with 56.08% and the high concentration of *Btk*-China with 57.25% (F=2.08; 9, 20 df; P=0.029). While non-significant differences were detected between the control and the low concentration of the three strains with infestation more than 73% (F=2.08; 9, 20 df; P=0.05). Within the same strain and concentration there were no significant differences between the one- and two-week treatments after application (t=0.317-2.748; 1, 4 df; P=0.05) (Fig. 1). The overall effect of the three concentrations (together) indicated after one week that the lowest infestation was significantly recorded for *Btk*-USA with 72.08%, while the highest was reported for *Bti* with 89.67% and control with 98.04% (F=2.759; 3, 26 df; P=0.044). In contrast, after two weeks the lowest infestation was achieved by the three strains (61.11-64.60%) and the highest for the control with 96.57% (F=2.817; 3, 26 df; P=0.005) (Fig. 1).

The corrected mortality percent of *S. temperatella* after different days of spray by different *Bt* strains in a direct spray test under Laboratory conditions are presented in Fig. 2. Mortality of *S. temperatella* larvae was significantly affected by *Bt* strain and concentration. One day after *Bt* spraying, the mortality of *S. temperatella*

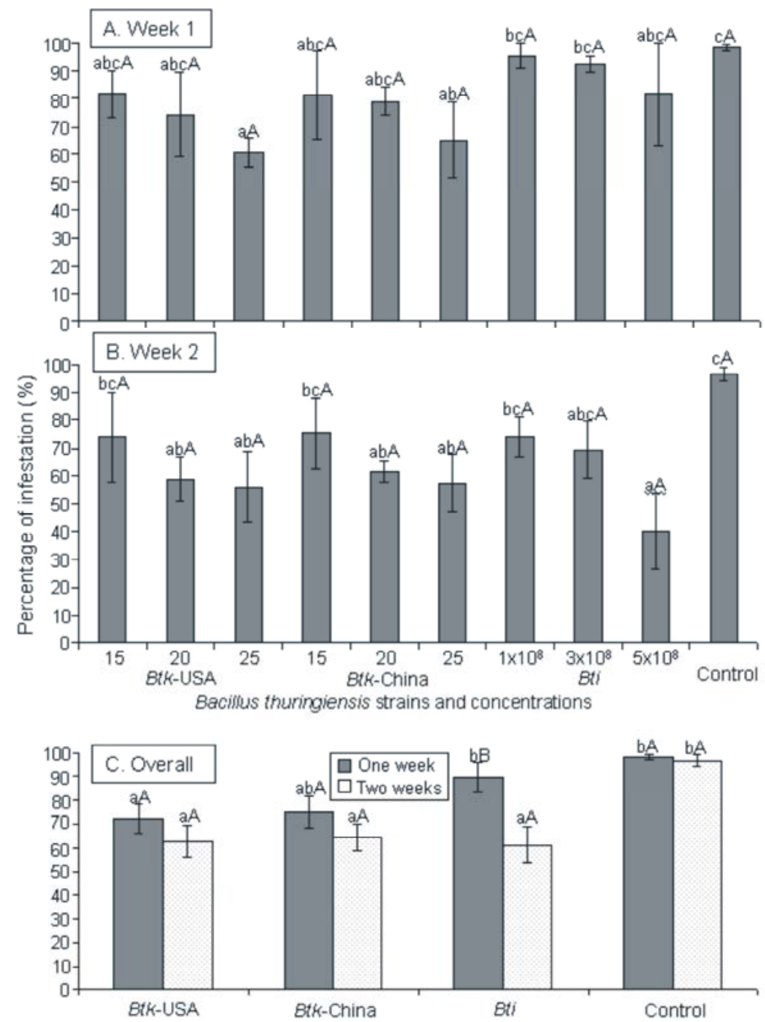

Fig. 1: *Syringopais temperatella* infestation percent (±*S.E.M.*) when barley plants were sprayed with different strains and concentrations of *Bacillus thuringiensis* in a direct spray test under field conditions. [Different small letters above bars indicate significant differences among the different strains and concentrations of *Bt* (A and B) within the same week and among the different strains (C), while capital letters above bars indicate significant differences among the different weeks within the same strain and concentration at $p<0.05$ (one-factor analysis of variance)].

ranged from 11.50 to 28.74% with non-significant differences among the three *Bt* strains (F=0.413; 8, 18 df; P=0.898). After 3 days of application, the highest mortality was significantly recorded for the medium and high concentrations of *Btk*-China with 54.17%, while the lowest mortality was recorded for the low concentration of both *Btk*-USA and *Bti* with 16.67% (F=1.112; 8, 18 df; P=0.05). Five days after *Bt* spraying, the highest mortality was significantly recorded at the high concentration of *Btk*-China with 71.43% and the lowest was observed at the low concentration of *Btk*-USA with 23.81% (F=1.491; 8, 18 df; P=0.049) (Fig. 2).

The corrected mortality percent of *S. temperatella* as a result of spray with three concentrations (together) of *Bt* according to strains and days under Laboratory conditions is shown in Fig. 3A and B. For the three concentrations (together), the mortality after 5 days was significantly higher than after 1 and 3 days, in which it was 36.51% for *Btk*-USA (F=3.875; 2, 24 df; P=0.035), 65.08% for *Btk*-China (F=7.289; 2, 24 df; P=0.003) and 50.79% for *Bti* (F=5.739; 2, 24 df; P=0.009) (Fig. 3A). Within the same day, the mortality was not significantly different among the three different *Bt* strains after one day (F=0.997; 2, 24 df; P=0.384), while after 3 and 5 days,

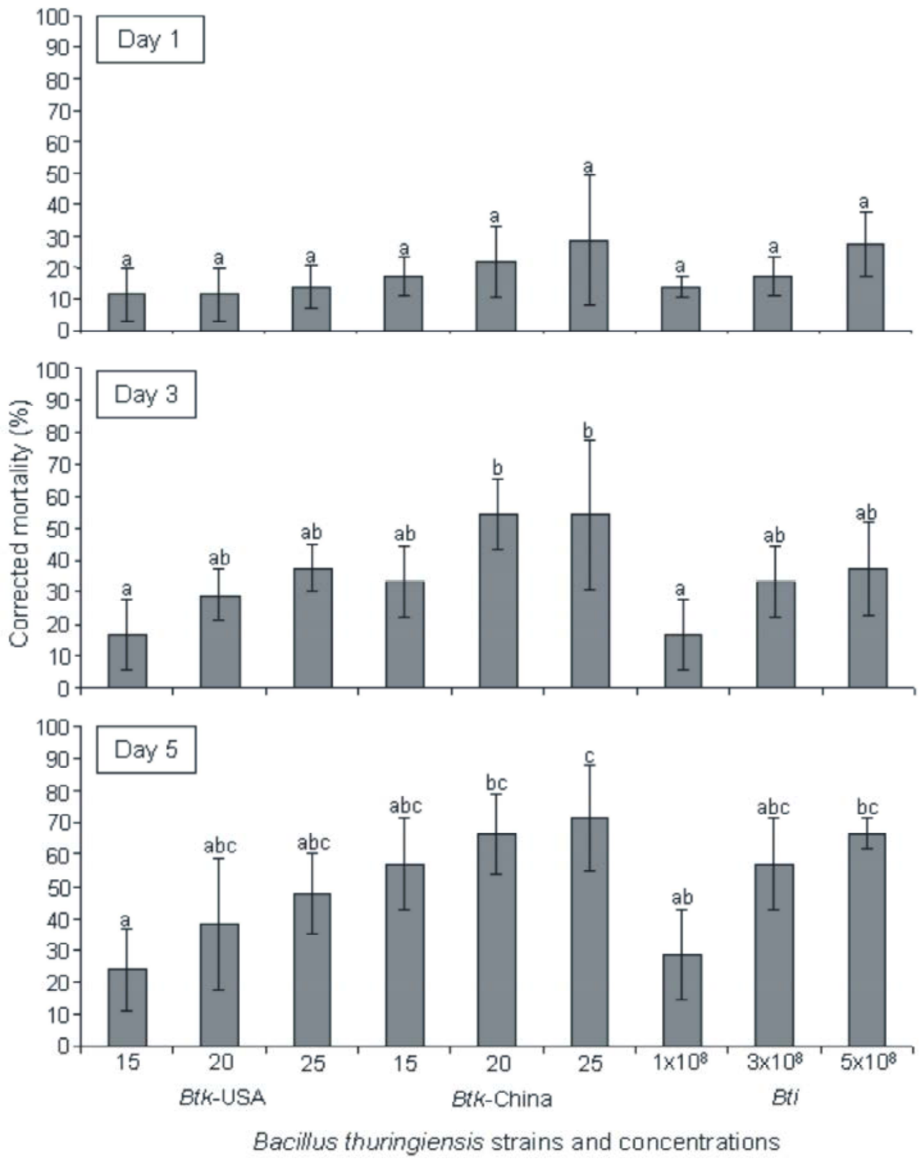

Fig. 2: Corrected mortality percent (±*S.E.M.*) of *Syringopais temperatella* (L_3) after different days of spraying by different strains and concentrations of *Bacillus thuringiensis* in a direct spray test under laboratory conditions. [Different small letters above bars indicate significant differences among the different strains and concentrations of *Bt* within the same day at $p<0.05$ (one-factor analysis of variance)].

the mortality in the *Btk*-China was significantly higher than *Btk*-USA and *Bti* (*F*=2.288, 3.056; 2, 24 df; *P*=0.05, 0.021) (Fig. 3B).

The overall corrected mortality percent of *S. temperatella* as a result of spray with different concentrations and strains of *Bt* in all days is presented in Fig. 4. The results indicated that the high concentration of *Btk*-China with 51.44% caused significantly the highest mortality, followed by the medium concentration of *Btk*-China with 47.56% and the high concentration of *Bti* with 42.92%, while the lowest mortality was recorded at the low concentration of *Btk*-USA with only 17.32% and at the low concentration of *Bti* with 19.68% (*F*=2.183; 8, 72 df; *P*=0.039) (Fig. 4A). In regards to concentration and strain, the mortality increased gradually with the progress of the time, in which the mortality after 5 days with 50.79% was significantly higher than after 3 days with 34.72% and after 1 day with 18.14% (*F*=2.78; 2, 78 df; *P*=0.000) (Fig. 4B).

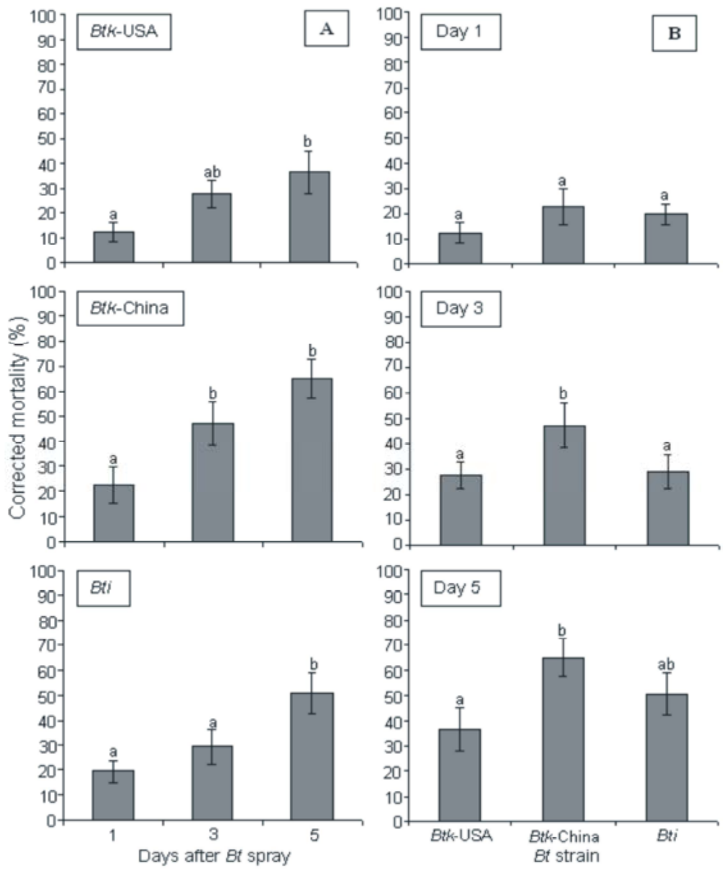

Fig. 3: Corrected mortality percent (±*S.E.M.*) of *Syringopais temperatella* (L_3) as a result of spraying with three concentrations (together) of *Bacillus thuringiensis* according to strains (A) and days (B) under laboratory conditions. [Different small letters above bars indicate significant differences among the different days (A) and strains (B) at $p<0.05$ (one-factor analysis of variance)].

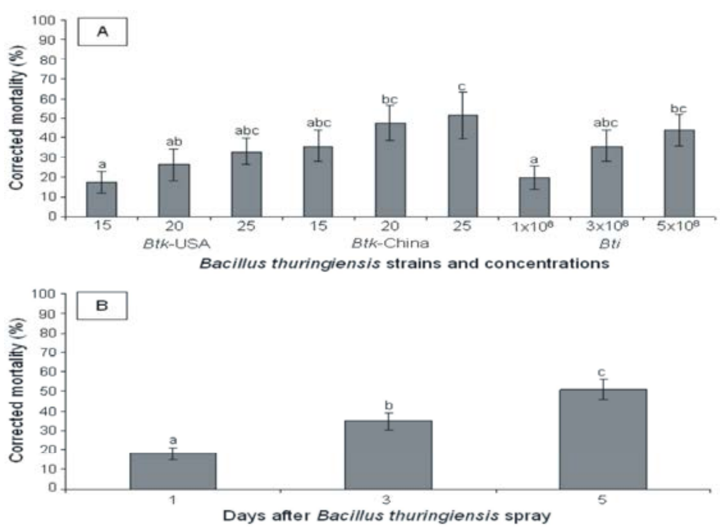

Fig. 4: Overall corrected mortality percent (±*S.E.M.*) of *Syringopais temperatella* (L_3) as a result of spraying with different concentrations and strains of *Bacillus thuringiensis* in all days (A) and in all concentrations and strains (B) under laboratory conditions. [Different small letters above bars indicate significant differences among the different *Bt* strains and concentrations (A) and days (B) at $p<0.05$ (one-factor analysis of variance)].

DISCUSSION

Increased public concerns about the adverse environmental effects about the use of chemical insecticides prompted search of alternative methods for insect-pest control. One of the promising alternatives has been the use of biopesticides and entomopathogenic microorganisms [15]. Microbial control agents can be effective and serve as alternatives to broad-spectrum chemical insecticides [16]. *Bt* has been known to be reservoir of several insecticidal proteins that efficiently utilized to safely and effectively control a wide variety of insect pests [24]. Compared to chemical pesticides, *Bt* is highly toxic to insects and yet highly specific. Therefore, *Bt* is ideally suited for incorporation into IPM programs [22].

Results of the field experiment indicated that barley plants infestation by *S. temperatella* was significantly affected by *Bt* strain and concentration. One and two weeks after *Bt* spraying, the lowest infestation was significantly recorded for the high concentration of both *Btk*-USA (60.63%) and *Bti* (40.15%), respectively. The overall effect of the three concentrations (together) indicated after one week that the lowest infestation was significantly recorded for *Btk*-USA with 72.08%. In contrast, results of Laboratory experiment indicated after 3 and 5 days of application that the highest mortality was significantly recorded for the high concentration of *Btk*-China with 54.17 and 71.43%, respectively on barley. In this regard, after 1, 3, 5 and 7 days of spraying, 10^8 concentration of *Bti* caused significantly the highest mortality to the pest with 63.3, 73.1, 78.3 and 80.0%, respectively in Laboratory experiment on wheat [7]. Comparing the results of this study with another conducted by Al-Zyoud [4] on chemical control under the same Laboratory conditions, *Btk*-China (71.43%) caused higher mortality to *S. temperatella* larvae than Methomyl (66%), Lamda cyhalothrin (63%) and Cypermethrin (48%). Ammouneh *et al*. [35] reported that a single-dose showed that all obtained *Bt* isolates were very toxic (100% mortality) to *Ephestia kuehniella* Zeller, *Phthorimaea operculella* Zeller and *Cydia pomonella* L. (Lepidoptera) larvae. Similarly, Iriarte *et al*. [36] reported that most of the *Bt* tested isolates showed insecticidal activity (above 25% mortality) against some lepidopteran species. One of the most important factors affecting differences in toxicity among the different studies is the *cry* gene content of the *Bt* strains used, in which the type of *cry* genes present in a strain correlates to some extent with its insecticidal activity [35]. Furthermore, *Bt* has been used to control lepidopteran larvae attacking stored products [37]. *Bti* is used exclusively or in combination with other interventions for the control of larvae of dozens of species of medically important and pestiferous black flies and mosquitoes around the world [25]. Bt has been used to control other lepidopterans such as *Plodia interpunctella* (Hubner) [38]. Furthermore, *Bt* showed activity on the western corn rootworm, *Diabrotica virgifera* (LeConte) [39].

Our results indicated that the efficacy of *Bt* has decreased with decreasing its concentration in the two strains tested. This is in complete agreement with the results obtained by Al-Zyoud *et al*. [7] on *S. temperatella* infesting wheat and Abu-Dhaim *et al*. [40] on *Meloidogyne* spp, who found that low concentration of *Bt* causes low mortality. It might be due to a fact that *Bt* product proteinase activity was lower in its effect to *S. temperatella*.

The current results showed that the mortality increased gradually with the progress of the time after *Bt* application, in which the mortality after 5 days was significantly higher than those obtained after 1 and 3 days of *Bt* applications. These results are in strong agreement with those of Al-Zyoud *et al*. [7], who reported an increased mortality of 28.8, 46.7, 54.4 and 63.8% at 1, 3, 5 and 7 days post spraying, respectively.

In order to determine the correct time of *Bt* application; it is important to know the phenology of *S. temperatella* in the field. In Jordan, Al-Zyoud [3] reported that the larvae start feeding on the plants early February until early April. Therefore, the time of control should take place from mid to late February, because the larvae within this period are very active, small in size and sensitive. There are also many factors affecting larval survival, i.e., predation, parasitism, rainfall, ploughing, sowing date and varieties Therefore, *Bt* could be used [13] with other control methods to suppress the pest. In this regard, Al-Zyoud [3] reported in Jordan that the parasitism by the parasitoid, *Anilastus* sp. Förster (Hym., Ichneumonidae) reached up to 49%, which is high enough to make a sufficient reduction in the pest population. Therefore, using of *Bt* to control the pest will have no side effect on the parasitoid and both of them could be used in an IPM program to suppress the pest in the future.

In conclusion, the current study provides basic information on the efficacy of Bt against *S. temperatella* under Laboratory and field conditions. The used strains exhibit toxic potential and, therefore, could be adopted for future applications to control the pest. Application of *Bt* plays a central role in protecting crop plants; thereby it will drastically reduce and replace, at least in part, some of the most dangerous chemical insecticides currently used against the pest in Jordan making it an ideal component of IPM. Better pest control strategy can improve quality of life of farmers by reducing inputs on insecticides and sprayers thereby increasing their incomes and offering time savings. However, future studies should focus on economic injury and economic threshold levels of the pest, studying of the dynamics of the insect population in comparison with the phonological development of host plants to optimize planting and harvesting dates, conducting economic feasibility studies on monitoring natural enemies to increase their impact on the pest populations. Such future studies, together with the current results and those of Al-Zyoud [3-5] on the pest biology and ecology, efficacy of insecticides and effect of crop rotation on the pest, as well as Al-Zyoud *et al*. [6, 7] on the susceptibility of wheat and barley varieties and accessions to the pest are expected to form the foundation of IPM program for *S. temperatella* in Jordan.

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