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Assessment of the efficacy of *Bacillus thuringiensis* and *Pseudomonas fluorescens* in the biological control of *Hypera postica* larvae and adults

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Abstract

The research aimed to assess the impact of two bacterial isolates, *Bacillus thuringiensis* and *Pseudomonas fluorescens*, on the larvae and adults of *Hypera postica* in a laboratory setting, utilising three concentrations (1×10^2, 1×10^4, and 1×10^6) CFU / ml for both bacterial kinds under investigation. The study's results indicated that the quantities employed varied in their efficacy in exterminating larvae and adults of the clover weevil *H. postica* in a laboratory setting. The findings indicated that the rates of mortality escalate with higher concentrations and prolonged feeding durations. The concentration of 1×10^6 CFU / ml of the *B. thuringiensis* exhibited the highest mortality rates after 96 hours of treatment, with rates of (75.8%, 73.9%, 69.7%, 68.2%, and 43.5%) for the first, second, third, fourth larval stages, and adults, respectively. In contrast, the lowest mortality rate was observed with the bacterium *P. fluorescens* at a concentration of 1×10^2 CFU / ml after 48 hours, yielding rates of (13.5%, 28.4%, 27.7%, 27.0%, and 6.6%) Subsequent to the aforementioned stages.

Keywords: Biological control, *Hypera postica*, *Bacillus thurngensis*, *Pseudomonas fluorescens*

Introduction

Hypera postica is the predominant beetle species prevalent in lucerne and clover fields globally, particularly in Iraq, and exerts a detrimental impact on crops. As diurnal insects, they commence eating on lucerne by nibbling the leaves, resulting in perforations that lead to an estimated 18% loss in the plant due to their fast reproductive rate and rapid feeding and growth (Maund and Hsiao, 2012).

Numerous strategies have been employed to mitigate the bug, with chemical pesticides being the primary approach for its control. Pesticides significantly diminish both the quantity and quality of human food, pose risks to human health and domestic animals, and disrupt the natural balance of biological pest enemies due to their toxic effects (Roh et al., 2007). A multitude of studies has concentrated on employing non-chemical solutions to address various insect pests that adversely affect plants economically, exemplified by the use of plant powders and biological control agents (Twigg et al., 2008). In this context, the utilisation of *B. thuringiensis* and *P. fluorescens* constitutes a critical

component of biological control (Mohan et al., 2008), as these bacteria possess various ways for eradicating insect pests. Numerous studies have demonstrated that these bacteria do not generate hazardous compounds for plants, do not adversely affect the environment, and lack parasitic capabilities in humans and animals (Feldhaar, 2011). The study sought to employ the bacterium *B. thuringiensis* and *P. fluorescens* for the biological control of the larval and adult stages of Hypera postica, which are commercially significant in Clover fields.

Materials and methods

Insect collection and breeding

Hypera postica insects (larvae and adults) were collected from some nearby fields in Samawah Governorate/southern Iraq planted with Medicago sativa. The insects were placed in plastic boxes 20 cm wide and 15 cm high at a rate of 3 boxes for each treatment, and some young leaves of the Clover plant were placed in them.

The pores of the boxes were covered with transparent cloth and tied with a rubber rope to prevent the moving insect stages from exiting them, and each stage of the insect was isolated separately with three replicates for each treatment, taking into account replacing the plant leaves whenever necessary in order to provide permanent food for the insect. The rearing boxes were transferred to the incubators at a constant temperature of 25±2°C and at the base were placed glass containers filled with water with a diameter of 19.5 cm and a height of 3.5 cm and 30 g of KH was dissolved in 100 ml of water to obtain a constant relative humidity (70±5%). The incubator was provided with a light source of 20 w with a timer to give a constant light period of 16 hours of light and 8 hours of darkness. The incubator was used under the same conditions in all subsequent laboratory experiments.

The insect eggs were collected from the adult breeding boxes and placed in transparent cylindrical plastic cups measuring 5 x 7 cm. Filter paper moistened with water was placed inside the cups to provide the necessary humidity for the eggs to hatch. The cups were covered with a mesh cloth and tied tightly to prevent the larvae from escape after hatching. The larvae were transferred to the containers designated for their rearing as previously indicated. When the adults emerged, each pair (male and female) of the newly adult insects were placed in the containers prepared for their rearing with three replicates and prepared for the study (Lu *et al*., 2013).

Preparation of bacterial concentrations

Bacterial isolates of *B. thuringiensis* and *P. fluorescens* were previously collected from the microbiology laboratories of the College of Science, University of Baghdad, Iraq. Three concentrations were prepared: 1×10^{2}, 1×10^{4}, and 1×10^{6} CFU/ml. A few drops of Tween 80 solution at a concentration of 0.10% were incorporated into each concentration as a moisture preservative and spreading agent. The CFU/ml of the initial solution was quantified using the methodology established by Goettel and Inglis (1997), which relies on the subsequent equation:

Volume for (A) cell = Mean no. × Conversion factor

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Pathogenicity of *B. thuringiensis* and *P. fluorescens* on larvae and adults of *H. postica*

All larval and adult stages were individually transplanted to Petri dishes. All dishes were stored in the refrigerator at 4°C for one hour to diminish insect activity and ease handling. The plates were removed, and the insects within their glass containers were treated with a suspension of varying concentrations of the specified bacterial species using a small hand sprayer. Three more dishes were treated alone with sterile distilled water for comparative purposes.

The treated insects were placed on filter paper for one hour, after which they were carefully transferred to glass test tubes measuring 20 cm in length and 3.5 cm in diameter. Each tube contained medical cotton saturated with sterile distilled water, along with several Medicago sativa leaves to provide continuous nourishment for the insects. The tubes were thereafter positioned in the electric incubator under the aforementioned parameters. Measurements were conducted after 48, 72, and 96 hours of therapy. The comparative efficacy of the specified bacterial concentrations was determined using the Abbot equation (1925), as outlined below:

$$P = \left(\frac{C - T}{C}\right) \times 100$$

The mortality percentages were converted to integer values for entry into the statistical analysis.

Results and Discussion

Effect of concentrations of *B. thurngensis* and *P. fluorescens* bacteria on the mortality of larval stages and adults of *H. postica* at different time periods (hour)

1- First larval stage

The results presented in Table (1) indicate that varying concentrations of *B. thuringiensis* bacteria over different time intervals significantly influenced the mortality rate of the first larval stage of *H. postica*. The highest mortality rate observed was 75.8% at a concentration of 1×10^6 spores/ml after 96 hours, while the lowest mortality rate was 39.3% at a concentration of 1×10^2 after 48 hours of exposure. *P. fluorescens* bacteria exhibited a marginally significant effect relative to the others, with the maximum insect mortality rate reaching 31.9% at a concentration of 1×10^6 spores/ml during a duration of 96 hours. The doses utilised in the investigation resulted in insect mortality at varying highly significant rates across different time intervals for both bacterial isolates examined, as well as their interactions.

Table (1): Effect of concentrations of B. thurngensis and P. fluorescens bacteria on the mortality of the first larval stage of H. postica at different time periods (hour)

Bacterial isolation	Bacteria concentration (cfu/ ml)	Insect death rate/ hour			The interference between focus and time
		48	72	96	
B. thurngensis	$10^2\times1$	39.3	57.8	68.8	55.3
	$10^4\times1$	54.6	64.7	72.9	64.0
	$10^6\times1$	58.0	67.7	75.8	67.1
	Average time effect	50.6	66.4	72.5	
P. fluorescens	$10^2\times1$	13.5	18.8	23.4	18.5
	$10^4\times1$	15.9	20.2	29.4	21.8

	$10^6 \times 1$	18.5	21.0	31.9	22.5
	Average time effect	15.9	20.0	28.2	
	L.S.D 0.01	0.441			1.178

2- The second larval stage

Variations in concentrations of *B. thuringiensis* bacteria and exposure durations significantly influenced the mortality rates of the second larval stage of the insect. The highest mortality rate was 73.9% at a concentration of 1×10^6 over 96 hours, followed by 69.0% at the same concentration over 72 hours. Conversely, the lowest mortality rate was 50.5% at a concentration of 1×10^2 over 48 hours (Table 2). All mortality rates diminished with the application of P. fluorescens bacteria. Less mortality rate reached 28.4 % at a concentration of 1×10^2 throughout a 48-hour exposure period. The interaction coefficients between the bacteria and the various time periods were all extremely significant for B. thuringiensis compared to P. fluorescens across all utilised time periods.

Table (2): Effect of concentrations of B. thurngensis and P. fluorescens bacteria on the mortality of the second larval stage of H. postica at different time periods (hour)

Bacterial isolation	Bacteria concentration (cfu/ ml)	Insect death rate/ hour			The interference between focus and time
		48	72	96	
B. thurngensis	$10^2 \times 1$	50.5	61.2	68.8	60.1
	$10^4 \times 1$	54.8	63.8	71.7	63.4
	$10^6 \times 1$	59.1	66.0	73.9	64.9
	Average time effect	54.8	63.6	71.4	
P. fluorescens	$10^2 \times 1$	28.4	34.9	42.2	35.1
	$10^4 \times 1$	30.1	38.6	45.9	38.2
	$10^6 \times 1$	32.5	40.2	48.3	40.3
	Average time effect	30.3	37.9	45.4	
	L.S.D 0.01	0.521			1.122

3- The third larval stage

The study's results (Table 3) indicated that varying concentrations of *B. thuringiensis* bacteria over different time intervals resulted in a significant increase in third larval stage mortality, with the highest mortality rate reaching 71.4% at a concentration of 1×10^6 after 96 hours. Conversely, the lowest mortality rate of 45.1% occurred at a concentration of 1×10^2 over 48 hours. Additionally, the mortality rate was lowest throughout the experiment when *P. fluorescens* bacteria were used at a concentration of 1×10^2 for 48 hours, yielding a mortality rate of 27.7%. Statistical analysis revealed highly significant differences in the interaction rates between the various bacterial concentrations and time periods.

Table (3): Effect of concentrations of *B. thurngensis* and *P. fluorescens* bacteria on the mortality of the third larval stage of *H. postica* at different time periods (hour)

Bacterial isolation	Bacteria concentration (cfu/ ml)	Insect death rate/ hour			The interference between focus and time
		48	72	96	
B. thurngensis	$10^2 \times 1$	45.1	60.9	67.9	57.9
	$10^4 \times 1$	52.4	63.7	69.8	61.9
	$10^6 \times 1$	59.3	67.1	71.4	65.9
	Average time effect	52.2	63.9	69.7	
P. fluorescens	$10^2 \times 1$	27.7	31.4	39.6	32.9
	$10^4 \times 1$	29.8	33.1	45.1	36.0
	$10^6 \times 1$	30.5	36.6	47.8	38.3
	Average time effect	29.3	33.6	44.0	
	L.S.D 0.01	0.430			0.859

4- The fourth larval stage

The data in Table (4) showed that the use of different concentrations of *B. thurngensis* bacteria and different time periods led to significant differences in the percentages of death of the fourth larval stage of the insect. The peak mortality rate was 68.2% at a concentration of 1×10^6 over 96 hours, while the minimum mortality rate was 27.0% at a concentration of 1×10^2 employing *P. fluorescens* bacteria over 48 hours. Significant changes in insect fatality percentages were seen in relation to the concentration of bacteria and the duration of exposure, peaking at 60.4% with *B. thuringiensis* at a concentration of 1×10^6 over 96 hours.

Table (4): Effect of concentrations of *B. thurngensis* and *P. fluorescens* bacteria on the mortality of the fourth **larval stage of *H. postica* at different time periods (hour)**

Bacterial isolation	Bacteria concentration (cfu/ ml)	Insect death rate/ hour			The interference between focus and time
		48	72	96	
B. thurngensis	$10^2 \times 1$	43.5	54.6	62.2	53.4
	$10^4 \times 1$	48.7	57.5	64.7	56.9
	$10^6 \times 1$	52.1	61.1	68.2	60.4
	Average time effect	48.1	57.9	65.3	
P. fluorescens	$10^2 \times 1$	27.0	32.0	37.2	32.0
	$10^4 \times 1$	28.4	33.1	43.1	34.8
	$10^6 \times 1$	30.9	35.4	45.6	37.3

	Average time effect	28.7	33.5	41.9	
	L.S.D 0.01	0.554			1.108

5- Adult stage

The data in Table (6) show that use of different concentrations of *B. thurngensis* bacteria had little effect on the percentage of adult death at different time periods compared to the four larval stages of the insect. The peak adult death rate of the examined insect was 43.5% at a concentration of 1×10^6 over a duration of 96 hours, succeeded by a rate of 39.7% at the same concentration of bacteria but at 1×10^4 during the identical time frame. Nonetheless, varying doses of *P. fluorescens* bacteria resulted in a reduction of adult mortality to a minimum of 6.6% at a concentration of 1×10^2 for a duration of 48 hours. The results of the identical table similarly revealed substantial disparities in the percentage of adult mortality at the same concentration across all evaluated time intervals.

Table (5): Effect of concentrations of *B. thurngensis* and *P. fluorescens* bacteria on the mortality of the adult stage of *H. postica* at different time periods (hour)

Bacterial isolation	Bacteria concentration (cfu/ ml)	Insect death rate/ hour			The interference between focus and time
		48	72	96	
B. thurngensis	$10^2\times1$	22.0	30.3	36.6	29.6
	$10^4\times1$	24.1	32.4	39.7	33.0
	$10^6\times1$	27.5	34.7	43.5	35.2
	Average time effect	24.5	32.4	39.9	
P. fluorescens	$10^2\times1$	6.6	9.9	12.5	9.6
	$10^4\times1$	8.2	10.9	16.2	11.7
	$10^6\times1$	9.1	11.5	19.3	13.3
	Average time effect	7.6	10.7	16.0	
	L.S.D 0.01	0.695			1.390

The experimental results demonstrate that a concentration of 1×10^6 cfu/ml of *B. thuringiensis* bacteria is more effective in causing mortality in the four larval stages of *H. postica* than other concentrations of *P. fluorescens* bacteria, The concentration of bacteria and the extension of the feeding period significantly contribute to the rise in insect mortality rates. A direct correlation exists among concentration, illumination, and mortality rates; as bacterial concentration, lighting intensity, and feeding durations increase, so too does the proportion of insect mortality, This may result from the discontinuation of feeding by insect larvae when exposed to bacterial isolates, coupled with a progressive rise in mortality rates as the duration of treatment extends. The findings align with Luca Ruiu's (2015) assertion that the consumption of certain bacterial isolates of *Bacillus* by scaly and

coleopteran insects leads to mortality within 3-4 days for younger larvae, extending to a duration of up to 4 days as the larvae mature.

The mortality of insects may result from ingesting food infected with bacteria and containing the bacteria's crystalline proteins, as it was noted that the adult insects ceased feeding, became entirely paralysed, and then died, Vilcinskas (2010) asserted that toxins generated by crystal bodies and protein degradation within the insect gastric membrane bind to specific receptor sites on the epithelial cell membranes of the midgut, resulting in the formation of pores that disrupt ionic transport systems across the midgut wall. Furthermore, the application of high doses of bacteria leads to the disintegration of midgut epithelial cells, culminating in the rapid mortality of insects (Reddy et al., 2016). Furthermore, damage to the gastric cells is adequate to inhibit normal secretions in the stomach, thereby diminishing the acidity of the wall and facilitating the germination and infiltration of bacteria into the green cells, leading to proliferation in the insect's hemolymph, which results in harm to the blood cells and ultimately death. These effects manifest over a protracted duration relative to the elevated concentrations of bacteria employed, corroborating the findings of Bajwa et al. (2014).

References

[1] Reddy .GVP, Antwi FB, Shrestha G, Kuriwada T (2016). Evaluation of toxicity of biorational insecticides against larvae of the alfalfa weevil. Toxicology Reports 3: 473-480. doi: 10.1016/j. toxrep.2016.05.003.

[2] Feldhaar, H.(2011). Bacterial symbionts as mediators of ecologically important traits of insect hosts. Ecol. Entomol. 36, 533–543.

[3] Lu F, Kang X, Jiang C, Lou B, Jiang M et al. (2013). Isolation and characterization of bacteria from midgut of the rice water weevil (Coleoptera: Curculionidae). Environmental Entomology 42 (5): 874-881. doi: 10.1603/en13111.

[4] Abbott. W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of economic entomology, 18: 265- 267.

[5] Maund CM and TH Hsiao. 2012. Differential encapsulation of two *Bathyplectes* parasitoids among alfalfa weevil strains, *Hypera postica* (Gyllenhal). The Canadian Entomologist123(1):197-203.

[6] Goettel, M. S. and Inglis, D. G. 1997. Fungi: Hyphomycetes. *In* "Manual of Techniques in Insect Pathology", (L. Lacey, Ed.), pp 213–249. Academic Press, London.

[7] Tawij, Nabil Salim Saeed and Mohammed Mohsen Al-Rafi'i and Waqdad Abdul-Ilah. (2008). Study of the possibility of integrating the biological preparation manufactured from Beauveria bassiana fungus vaccine with some chemical pesticides to control the green peach aphid Myzus persicae. Journal of the University of Kufa. Proceedings of the First Scientific Conference for Pure and Applied Sciences. 143-153 pages.

[8] Bajwa, W. ; M. O'Connor ; S. Slavinski and E. Butts. (2014). Comprehensive Mosquito Surveillance and Control. New York City Department of Health and Mental Hygiene, New York, NY. p. 40.

[9] Vilcinskas, A.(2010). Coevolution between pathogen-derived proteinases and proteinase inhibitors of host insects. Virulence. 1, 206–214.

[10] Luca Ruiu. (2015). Insect Pathogenic Bacteria in Integrated Pest Management. Insects REV 6, 352-367.

[11] Roh, J.Y.; Choi, J.Y., Li,MS; Jin, B.R. Je, YH. 2007. Bacillus thuringiensis as aspecific, safe, and effective toolfor insect pest control. Journal of microbiology and biotechnology 17,pp:547-559.

[12] Mohan, M., S. N. Sushil, J. C. Bhatt, G. T. Gujar, and H.S. Gupta. 2008. Synergistic interaction between sublethal doses of Bacillus thuringiensis and Campoletis chlorideae in managing Helicoverpa armigera. Biocontrol 53, 375-386.