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Entomopathogenic bacteria *Photorhabdus luminescens* as natural enemy against the African migratory locust, *Locusta migratoria migratorioides* (Reiche & Fairmaire, 1849) (Orthoptera: Acrididae)

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Abstract

Background: The African Migratory Locust, *Locusta migratoria migratorioides* (Reiche & Fairmaire, 1849) (Orthoptera: Acrididae), is a major threat to agricultural crops and food security on a worldwide scale; hence, maintaining control over it is crucial. *Photorhabdus luminescens* bacteria can accomplish the efficient biocontrol agent criteria. As a result, the aim of this study was to assess the efficacy of the *P. luminescens* (EGAP3) strain and its cell-free filtrate against *L. migratoria migratorioides*, as well as to investigate changes in the activity of carbohydrates hydrolyzing enzymes, amylase, invertase, and trehalase in whole-body homogenates of the 5th nymphal instar under laboratory conditions and to investigate the histopathological changes in the midgut of the locust.

Results: The virulence of entomopathogenic bacteria was determined at different densities of 4×10^7, 4×10^6, 4×10^5, and 4×10^4 colony-forming units (CFU)/ml at different exposure times as well as different concentrations of its cell-free filtrate, undiluted cell-free filtrate (100, 50, 25, and 12.5%). The results indicated that higher-density cell suspension up to 4×10^7 cells.ml^{-1} and undiluted cell-free filtrate (100%) were the most effective insecticidal fluids, reaching up to 76.7 and 80%, respectively, after 7 days. The estimated LC_{50} value was 2.7×10^6 cells.ml^{-1} for bacteria, and the estimated LC_{50} value for cell-free filtrate was the 2nd dilution (50%).

Conclusions: The data clarified the toxicological and histopathological effects and carbohydrate hydrolyzing enzyme activities of the host insect *L. migratoria migratorioides,* following bacteria and cell-free filtrate infection; that provides an overview of the efficiency of bacteria and their cell-free filtrate on the host. In conclusion, *P. luminescens* (EGAP3) and its toxins can be an optimal option for bio-controlling of *L. migratoria migratorioides*.

Keywords: *Locusta migratoria migratorioides*, *Photorhabdus luminescens*, Histopathological effects, Enzymes activity, Biological control

Background

Although locusts are used as edible insects because of their beneficial proteins and minerals, their migration in terrible numbers is causing destruction to food crops, causing governments to find ways to combat it. The migratory locust, *Locusta migratoria migratorioides* (Fairmaire) (Orthoptera: Acrididae), is one of the most abundant agricultural pests which consume a large amount of grass and damage crops. Since the late 1990s, the number of individuals has increased and settled, especially in the south-west and west of Egypt. Outbreaks

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occurred in Sharq El-Owinat and Toshka from 2015 to 2019 (Moustafa 2019). It is characterized by its ability to exhibit solitary and gregarious phases (Uvarov 1921). Biocontrol is one of the most successful methods of specialized control because it does not expose the plant and does not harm bees. *Photorhabdus luminescens* (Thomas et Poinar) (Enterobacterales: Morganellaceae) is a non-spore-forming, motile, bioluminescent, gram-negative bacterium that belongs to the family Enterobacteriaceae (ffrench-Constant et al. 2007). This bacterium is highly pathogenic to many insect pests and has four toxin groups (Rodou et al. 2010). The *P. luminescens* genome comprises the highest number of toxin genes. Additionally, an analysis of the complete genome sequence of *P. luminescens* revealed numerous adhesins, proteases, and lipases, which may be expressed during the pathogenic phase of its complex life cycle (Duchaud et al. 2003). Many publications showed the efficacy of *P. luminescens* and its cell-free filtrate against pests. Oral application of *P. luminescens* on the third larvae and pupae of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) showed a significant toxicity with mortalities of up to 70–100%, 10 days after treatment, and its cell-free supernatant in the diet also doubled mortality rates of feeding larvae (Shawer et al. 2018). The mortality rates of *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) observed after diluting the fermentation broth of *P. luminescens* 50 times and dilution of the supernatant 5 times were 18.89 and 91.11%, respectively (Li-Hsin et al. 2020). In 65% of the eggs of the rice grain moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) exposed to *P. luminescens* cells alone or their toxins, the Corcyra egg shells became flaccid, and there was a significant reduction of up to 84% in the emergence of *Trichogramma* adults (Mohan and Sabir 2005). When *P. luminescens* cells and their toxins were applied to nymphs and adults of *Schistocerca gregaria* (Forsskål) (Orthoptera: Acrididae), either by injection into the abdomen or orally, mixed with brain or plant foliage, a lethal effect was observed (Mahran et al. 2004*).* Many "toxin complex" (tc) loci were also revealed on the chromosome of *P. luminescens* strains (ffrench-Constant et al. 2007), and all of these strains code for different high molecular weight insecticidal tc toxins. Some of the TC proteins may destroy the insect midgut, similar to *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) delta endotoxins. Through this research and many others, the use of these bacteria (*P. luminescens*) seems important and useful in the control of African locusts because of the types of toxins that have been proved to be effective in reducing locust numbers when attacking different agricultural crops as well as the fact that these bacteria can be introduced as part of integrated pest management (IPM).

Methods

Insect culture

The African migratory locust *L. migratoria migratorioides* was collected from the village of Abu Rawash in Giza governorate in Egypt. Insects are placed in wooden breeding cages covered with thin ventilation wires, cages exposure to sunlight throughout the day. Part of the cage was covered with a cloth to protect locusts during high heat (Hill and Taylor 1933). Nutrition was fresh leaves of Alfalfa, *Medicago sativa* (Linnaeus) (Fabales: Fabaceae) or Corn*, Zea mays* (Linnaeus) (Poales: Poaceae) as a season. Cages were cleaned daily.

Preparation of bacterial inoculum

Thirty µl of pure bacteria stock was placed into 5-ml nutrient broth tubes and cultured aerobically for 24 h. at 30 °C, then transferred to 50 ml of LB broth in a 250-ml Erlenmeyer flask, and incubated for 48 h at 150 rpm at 30 °C in a shaking incubator (Model: JSSI-2000). For 20 min, the bacterial suspension was centrifuged at 4200 rpm. The bacterium pellet was rinsed three times with a sterile saline solution before being centrifuged at the same pace until the saline solution was completely clear. Using a spectrophotometer set at 600 nm, cell densities in suspensions were calibrated to 4×10^7, 4×10^6, 4×10^5, and 4×10^4 cell.ml^{-1}.

Preparation of bacterial cell-free culture filtrates

To achieve a cell-free culture filtrate, the bacterial suspension was centrifuged at 4500 rpm for half an hour at 5 °C. The supernatant was filtered using a 0.2-m-pore filter syringe, with the resultant culture filtrates having a concentration of 100%. By adding the required distilled water aliquots, several dilutions of the filtrate, *i.e.,* 100, 50, 25, and 12.5% of the *P. luminescens* (EGAP3) strain, were generated.

Efficacy of bacterial cell suspension and bacterial cell-free culture filtrates

All of the experiments were done in a completely random order, with 8 treatments that included 4 different bacterial suspension densities, including 4×10^7, 4×10^6, 4×10^5, and 4×10^4 cell.ml^{-1}, 4 different concentrations of bacterial cell-free culture filtrates, including 12.5, 25, 50, and 100%, and a control consisting of the same volume of sterilized LB broth. Separate tests on bacterial suspensions and bacterial cell-free culture filtrates were conducted on nymphs in their 5th instar. The tests were repeated 3 times (3 replications), with 10 nymphs/replication. Individually, the nymphs were placed in a glass jar. Maize leaves dipped in the prepared culture suspensions containing a different density of bacteria and a different concentration of cell-free filtrates were applied topically

to the nymphs in each treatment, with a similar application in the control treatment according to Shepard (1958). The food was changed daily, and the nymphs were kept at 30 °C in the insect rearing room.

Assessment of mortality

Mortality of the nymphs was assessed daily, and the observations were recorded for 7 days. Accumulative mortality percentages of 5th instar were calculated and recorded using Abbott's formulation (Abbott 1925).

Histopathological studies

The LC_{50} of bacterial suspensions and the cell-free filtrates were used for a comparative study to clarify their effects on the histology of midgut of late 5th instar nymphs surviving treatment with the respective bacteria and its filtrate. Autopsy samples were taken from the nymphs in different groups and fixed in 10% formalin saline for 24 h. histopathological examination through the light microscope (Banchroft et al. 1996).

Preparation of homogenate samples of *L. migratoria migratorioides* for biochemical analysis

Nymphal samples were homogenized in distilled water and then centrifuged at 6000 rpm for 10 min at 5 °C using a Beckman GS-6R centrifuge. After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at −20 °C until analysis of the main components. Three replicates were carried out for each biochemical determination.

Determination of total proteins, lipids, and carbohydrates

The main components of *L. migratoria migratorioides* nymphal homogenates were determined (total proteins, total lipids, and total carbohydrates).

Determination of total protein content

Total proteins were measured using bovine serum albumin as a reference according to the method described by Bradford (1976).

Determination of total lipid content

Using phosphovanillin reagent and a standard curve, the total lipid content of *L. migratoria migratorioides* nymphal homogenate was calculated according to the method described by Knight (1972).

Determination of total carbohydrate content

Total carbohydrates were determined using anthron reagent according to the method described by Singh and Sinha (1977).

Determination of carbohydrate hydrolyzing enzymes activities (amylase, invertase, and trehalase)

The method was based on the digestion of trehalose, starch, and sucrose by trehalase, amylase, and invertase, respectively (Ishaaya and Swirski 1976). The free aldhydic group of glucose formed after trehalose, starch, and/or sucrose digestion was determined using 3, 5 dinitrosalicylic acid reagent, using the standard curve of glucose.

Fractionation of L. migratoria migratorioides nymph's homogenate protein

After toxicological and histopathological experiments, the sodium dodecyl sulfate polyacrlamide gel electrophoresis (SDS–PAGE) technique was used for fractionation of the total protein in control and treated nymphs. (Sambrook and Russell 2006). Chromatin prestained protein ladder (Vivantis, Malaysia, Cat. # PR0602) was used.

Data analysis

Analysis of variance was used to establish the significance of the major effects (ANOVA). Duncan's multiple range tests were used to assess the significance of various treatments (P 0.05). The software package "Costat," a product of Cohort Software Inc., Berkeley, California, was used for all analyses. Three replications were used to record all of the data.

Results

Efficacy of bacterial cell suspension and cell-free culture filtrates

Results presented in Table 1 and graphically illustrated in Figs. 1 and 2 show the effect of four different cell densities of the *P. luminescens* (EGAP3) strain and four concentrations of the cell-free filtrates on the survival of *L. migratoria migratorioides* nymphs during feeding technique. The cell suspension 4×10^7cell.ml^{-1} and the undiluted cell-free filtrate (100%) demonstrated the highest toxicity, resulting in 76.7% and 80% mortality, respectively, after 7 days post-infection. The low cell density (4×10^4 cell.ml^{-1}) and low concentration (12.5%) caused 16.6 and 20% mortality rates, respectively. The lethal susceptibility was different among the four densities and four concentrations. Statistical analysis revealed significant differences between bacterial suspensions and cell-free filtrates (Fig. 1; $df=1$, $F=8.47$, P 0.0044) as well as between different densities (Fig. 2) ($df=3$, $F=150.01$, P 0.0001) as well as between different days (Figs. 3, 5) ($df=6$, $F=142.2$, P 0.0000), ($df=6$, $F=82.9$, P 0.0000), and there were high significant differences for interactions between the densities and the days ($df=18$, $F=13.4$, P 0.0001). Also, significant differences ($df=3$, $F=78.6$, P 0.0000) were recorded for different concentrations

Table 1 Efficacy of bacterial cell suspension and cell-free culture filtrates on *Locusta migratoria migratorioides* during 7 days

Days	Density (cells/ml)	Mortality (%)	Concentration (%) (cell-free culture filtrates)	Mortality (%)
1st Day	4×10^4	0.0	12.5	0.0
	4×10^5	0.0	25	0.0
	4×10^6	0.0	50	0.0
	4×10^7	0.0	100	0.0
2nd Day	4×10^4	0.0	12.5	0.0
	4×10^5	0.0	25	3.3
	4×10^6	0.0	50	0.0
	4×10^7	0.0	100	10
3rd Day	4×10^4	10	12.5	10
	4×10^5	16.6	25	20
	4×10^6	26.7	50	26.7
	4×10^7	30	100	30
4th Day	4×10^4	10	12.5	13.3
	4×10^5	20	25	23.3
	4×10^6	30	50	33.3
	4×10^7	46.7	100	46.7
5th Day	4×10^4	13.3	12.5	13.3
	4×10^5	23.3	25	26.6
	4×10^6	33.3	50	36.7
	4×10^7	60	100	60
6th Day	4×10^4	16.7	12.5	20
	4×10^5	26.7	25	33.3
	4×10^6	36.7	50	50
	4×10^7	66.7	100	76.7
7th Day	4×10^4	16.7	12.5	20
	4×10^5	33.3	25	36.7
	4×10^6	46.7	50	50
	4×10^7	76.7	100	80

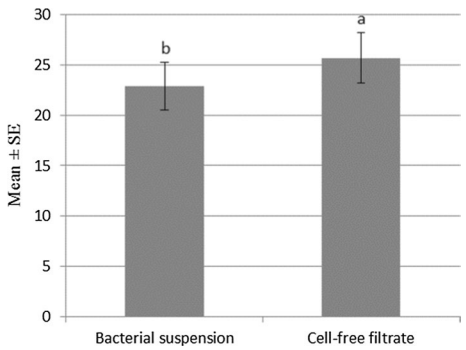

Fig. 1 Mortality of *Locusta migratoria migratorioides* after treatment with bacterial suspension of *P. luminescens* (EGAP3) and its cell-free filtrate. Bars (mean ± SE in the same and between time intervals with the same letter(s) are not significantly different ($P < 0.005$)

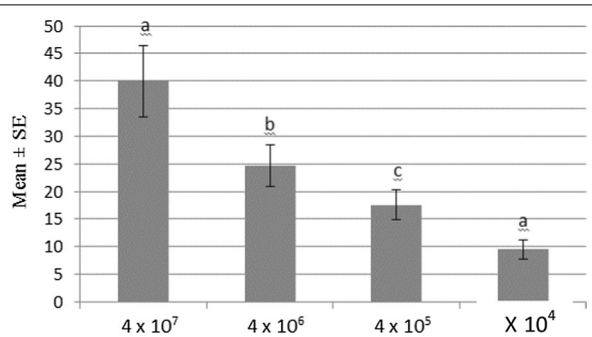

Fig. 2 Mortality of *Locusta migratoria migratorioides* after treatment with 4 different densities of *Photorhabdus luminescens* (EGAP3). Bars (mean ± SE in the same and between time intervals with the same letter(s) are not significantly different ($P < 0.005$)

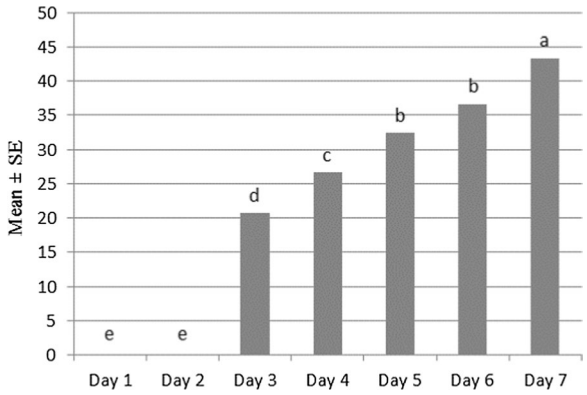

Fig. 3 Mortality of *Locusta migratoria migratorioides* during 7 Days after treatment with *Photorhabdus luminescens* (EGAP3). Bars (mean ± SE in the same and between time intervals with the same letter(s) are not significantly different ($P < 0.005$)

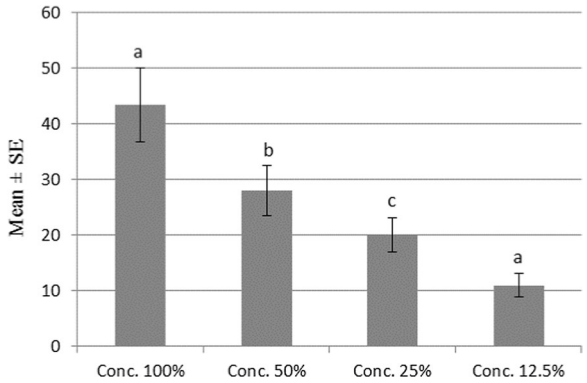

Fig. 4 Mortality of *Locusta migratoria migratorioides* after treatment with 4 concentrations of cell-free filtrate of *Photorhabdus luminescens* (EGAP3). Bars (mean ± SE in the same and between time intervals with the same letter(s) are not significantly different ($P < 0.005$)

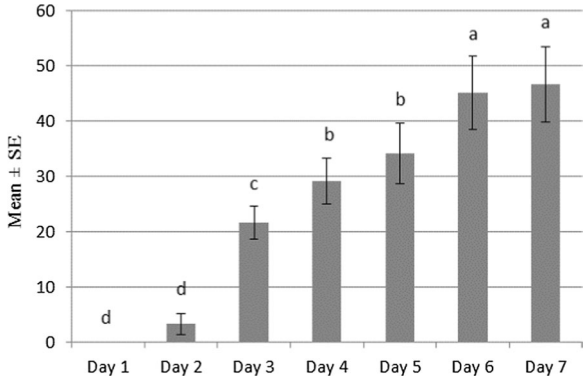

Fig. 5 Mortality of *Locusta migratoria migratorioides* during 7 Days after treatment with cell-free filtrate of *Photorhabdus luminescens* (EGAP3). Bars (mean ± SE in the same and between time intervals with the same letter(s) are not significantly different ($P < 0.005$)

(Fig. 4) and high significant differences were recorded for interactions between all different concentrations and days ($df = 18$, $F = 6.2$, P 0.0000). This might imply that the treatment's effect was not constant, but rather changed with time (Fig. 5).

Histopathological effect of bacterial cell suspension and cell-free culture filtrates

The results revealed that when nymphs were exposed to LC_{50} of *P. Luminescens* (EGAP3) suspension or its cell-free filtrate, they died. Histopathological investigations of the effects of *P. Luminescens* and cell-free filtrate on orthopteran nymph *L. migratoria migratorioides* were undertaken at the end of the 5th instar. These effects were displayed and are described in Fig. 6A–C: Fig. 6A depicts the histological anatomy of the midgut of untreated nymphs. The hemolymph site has two layers of muscle fibers: longitudinal and circular (musculosa). The musculosa is quite close to the epithelial cells' basement membrane. The food material within the lumen is surrounded by a thin peritrophic membrane. The epithelium is made up of a single layer of various cell types. Columnar, calyciform (goblet), and regenerative (interstitial) cells are the three main types. The columnar cells are cylindrical, with a big, coarsely formed nucleus in the middle of each cell. The edge of these cells is striated or brush-like (microvilli). The goblet cells have a calyx-like structure and are plentiful between the columnar cells.

Treatment of *L. migratoria migratorioides* with the LC_{50} of *P. lumenescens* (EGAP3) cell suspension (Fig. 6B) showed exfoliation of the midgut epithelium from the underlying circular muscle fibers, leaving a large vacuole or space. Vacuolization of the midgut epithelium disrupted the peritrophic membrane. Some of the degenerated columnar cells were fused with the disrupted peritrophic membrane.

On the other hand, treatment of *L. migratoria migratorioides* with LC_{50} of cell-free filtrate of *P. Luminescens* (EGAP3) (Fig. 6C) showed that the lumen of the gut was collapsed and globular bodies and cytoplasmic fragments were observed pinching off from the tips of some of the epithelial cells within the lumen vicinal to the deteriorated peritrophic membrane. The muscles have lost their compact appearance.

Protein profile of nymph homogenate

To study the impact of the examined bacterial bioagents on the protein constituents of *L. migratoria migratorioides* nymph homogenates, the total protein contents in untreated and treated 5th instar nymphs of *L. migratoria migratorioides* with the LC_{50} of *P. luminescens* suspension and cell-free filtrate were fractionated on SDS polyacrylamide gel electrophoresis (Table 2) and (Fig. 7). All

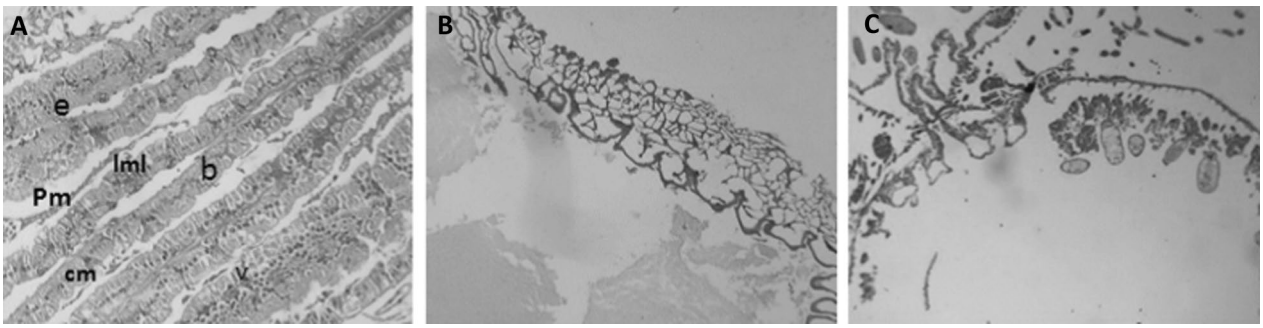

Fig. 6 Midgut region of untreated 5th nymph instar of *Locusta migratoria migratorioides* (**A**), Exposure to *Photorhabdu luminescens* (EGAP3) (**B**), and exposure to cell- free filtrate (**C**) (X 200). lml = longitudinal muscle layer, cml = circular muscle layer, b: basement membrane, v: vacuole, e: epithelium and Pm: preitrophic membrane

Table 2 Relative frequency values and molecular weights of SDS protein bands detected in the whole-body homogenates of *Locusta migratoria migratorioides* 5th instar nymph treated with LC_{50} of bacterial suspension and its Cell-free filtrate

Band no	Control		Bacterial suspension		Cell-free filtrate	
	Rf	MW	Rf	MW	Rf	MW
1	0.455	77.9	0.635	43.6	0.630	44.1
2	0.559	53	0.719	35.2	0.683	38.9
3	0.619	46.8	0.789	27.9	0.797	27.2
4	0.921	20	0.924	20	0.832	24.4
5	–	–	–	–	0.963	20

treatments were separated into different bands according to their relative frequency (RF) values and molecular weight (MW) on the gel.

Table 2 and Fig. 7 reveal that several distinct protein bands were observed in non-treated larvae but not in treated larvae, and vice versa.

Effects on the total soluble protein, carbohydrate, and lipid contents

Proteins, lipids, and carbohydrates are the main nutrients which provide insects with energy needed for movement and flight; they are also essential for building important insect tissues. In this study, the effects of LC_{50} (as shown in Table 3) of the tested compounds on total proteins, carbohydrates, and lipids in the 5th instar nymphs were also examined.

Data in Table 3 show that total protein levels decreased significantly in the *L. migratoria migratorioides* treated with LC_{50} of the cell suspension of *Photorhabdu luminescens*, recording 74.25 mg/ml compared to 103.33 mg/ml of body weight in the whole-body homogenate of the untreated nymphs, which was slightly higher than the LC_{50} of cell-free filtrate effect (71.24 mg/ml) ($df = 2$, $F = 9.44$, P 0.0140). After 7 days of treatment.

Data in Table 3 indicate that total lipid in *L. migratoria migratorioides* 5th instar nymphs was significantly decreased in the treated nymphs with LC_{50} of *Photorhabdu luminescens* (93.72) mg/ml compared to 132.72 mg/ml for the untreated nymphs. However, both levels were significantly higher than that in cell-free filtrate effect treated, which recorded 78.69 mg/ml ($df = 2$, $F = 114.21$, P 0.0000).

Data in Table 3 indicate that total carbohydrate levels in *L. migratoria migratorioides* 5th instar nymphs were not significantly different between treated with LC_{50} of *Photorhabdu luminescens* bacterial suspension (54.75) mg/ml and the untreated nymphs (56.71 mg/ml). However, LC_{50} of cell-free filtrate caused a significant decrease in total carbohydrate levels, recording 49.66 mg/ml ($df = 2$, $F = 12.04$, P 0.0079).

Determination of carbohydrates hydrolyzing enzymes activities (amylase, invertase and trehalase)

Three digestive enzymes, amylase, invertase, and trehalase, were determined in 5th nymphal instar of *L. migratoria migratorioides* which are treated with LC_{50} of the tested *Photorhabdu luminescens* (EGAP3) bacterial suspension and its cell-free filtrate. Data in Table 4 showed that decreased the amylase activity which was

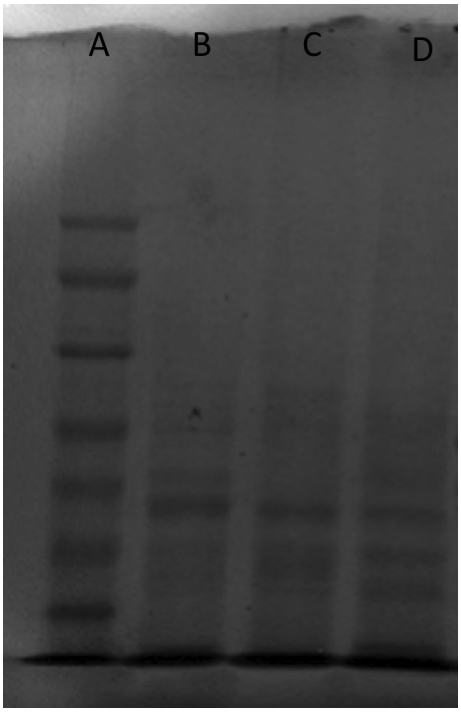

Fig.7 SDS protein bands detected in the whole-body homogenates of *Locusta migratoria migratorioides* 5th instar nymph treated with LC_{50} of bacterial suspension and its Cell-free filtrate. (**A**) Marker, (**B**) Control, (**C**) Bacterial suspension, (**D**) (filtrate)

1942.99, 1462.48 (UI/ml) with *P. luminescens*(EGAP3) bacterial suspension, cell-free filtrate, respectively, while it was 2476.08 with control ($df=2$, $F=48.71$, P 0.0002).

Also, the results in Table 4 indicate that invertase activity in *L. migratoria migratorioides* 5th instar nymphs was significantly different treated with LC_{50} of *P. luminescens* (EGAP3) bacterial suspension (822.8 UI/ml) than the untreated nymphs (889.6 UI/ml). Furthermore, the LC_{50} of cell-free filtrate caused a significant decrease in invertase activity, recording 616 UI/ml ($df=2$, $F=47$, P 0.0002).

Furthermore, data in Table 4 indicate that trehalase activity in *L. migratoria migratorioides* 5th instar nymphs was significantly different treated with LC_{50} of *P. luminescens* (EGAP3) bacterial suspension (2339.9) UI/ml than the untreated nymphs (2271.6 UI/ml). Furthermore, LC_{50} of cell-free filtrate caused a significant increase in trehalase activity, recording 1408.6 UI/ml ($df=2$, $F=190.9$, P 0.0000).

Discussion

Efficacy of bacterial cell suspension and cell-free culture filtrates

The symbiotic bacteria of the genus *Photorhabdus* cause superior mortality of *L. migratoria migratorioides*. The obtained results manifest the pathogenicity of *P. luminescens* (EGAP3) strains against the pest nymphs. Therefore, *P. luminescens* (EGAP3) strain demonstrated an alternative bacterial agent for effective biocontrol of *L. migratoria migratorioides,* which causes severe losses in yield. The findings are consistent with previous findings of the desert locust *S. gregaria*, infected with infective juveniles (IJs) of the entomopathogenic nematode *Heterorhabditis*

Table 3 Effect on total proteins, lipids and carbohydrates of *Locusta migratoria migratorioides*

Treats	The main components content of *L. migratoria migratorioides* mg/g body weight		
	Total Proteins (Mean ± SE)	Total lipids (Mean ± SE)	Total carbohydrates (Mean ± SE)
Bacterial suspension	74.25 ± 2.945	93.72 ± 0.684	54.75 ± 0.651
Cell-free filtrate	71.24 ± 7.966	78.69 ± 2.945	49.66 ± 0.524
Control	103.33 ± 3.620	142.72 ± 3.470	56.71 ± 0.912

Table 4 Effect on amylase, invertase, and trehalase enzymatic activities of *Locusta migratoria migratorioides*

Treats	Enzymatic activities of *Locusta migratoria migratorioides* µg glucose released/ minute/ gram body weight.)		
	Amylase (Mean ± SE)	Invertase (Mean ± SE)	Trehalase (Mean ± SE)
Control	2476.08 ± 57.7	889.6 ± 20.5	2771.6 ± 70.6
Bacterial Suspension	1942.99 ± 73.3	822.8 ± 12.5	2339.9 ± 24.4
Cell-free filtrate	1462.48 ± 84.3	616 ± 26.9	1408.6 ± 45.4

bacteriophora Poinar (HP88) with its mutualistic bacterium *P. luminescens* (Souad et al. 2015).

In addition, the obtained results refer to the possible use of the examined *P. luminescens* (EGAP3) strain with the significance of potential insecticide that allows different species of insect pests to be effectively controlled by a single natural product rather than multiple chemical products (Elizabeth et al. 2015). Bacteria secrete entomopathogenic factors directly into the growth medium. Interestingly, these bacteria or their toxic factors are insecticidal when they are ingested. A few previous reports reported insecticidal activities of cell-free filtrates of various strains of *Photorhabdus* spp. The bacteria from the genus *Photorhabdus* are proving to be a genomic gold mine, encoding a multitude of insecticidal toxins. In vitro *cultures of Photorhabdus* produce many secondary metabolites molecules, including genistine (afuran derivative), anthraquinone derivatives, stilbene derivatives, and a phenol derivative, which have been identified. The study showed that they had insecticidal activities (Shi et al. 2017).

Histopathological effect

Our findings are similar to the histopathological effects of *B. thuringiensis* on the midgut of the Mediterranean locust *Dociostaurus maroccanus* (Thunberg) (Orthoptera: Acrididae) (Quesada-Moraga and Santiago-Alvarez 2001). In addition, the obtained results are consistent with the histopathological alterations in the midgut epithelial cells of adult *L. migratoria manilensis*, according to the findings (Wu et al. 2011). Interestingly, despite various presumed mechanisms of action and, in the instance of protein toxin from *P. luminescens*, variable means of delivery, orally active toxins elicit similar ranges of histopathological effects on the midgut of *L. migratoria migratorioides*. Although many insect toxins appear to target the insect midgut, it is apparent that bacteria like *Photorhabdus, Xenorhabdus,* and *B. thuringiensis*, as well as their toxins, can harm other insect tissues.

Protein profile of nymph homogenate

On SDS-PAGE, fractionation of protein content throughout the whole homogenate of *L. migratoria migratorioides* nymphs demonstrated that protein depletion was related with the absence of several proteins and a reduction in band intensity in treated nymphs' comparison to untreated nymphs. The findings suggest that the studied bacterial solution and its cell-free filtrate may have an effect on the protein profile of the nymph homogenate. The protein depletion was considered as a parameter to study the influence of the entomopathogenic bacteria *P. luminescens* and its cell-free filtrate in this study.

Effects on the total soluble protein, carbohydrate, and lipid contents

The observed decrease in total protein content in *L. migratoria migratorioides* post-bacterial suspension infection may be attributed to the action of bacteria. Bacteria may cause a complete elimination of some hemolymph enzymes and proteins which may be involved in attachment of the infected bacteria to the hemocytes or some native proteins which may be converted into lipoproteins or glycoproteins after injection. The same explanation was reported for the desert locust *S. gregaria* infected with *B. thuringiensis* (Barakat and Meshrif 2007),

The protein reduction seen could possibly be an indirect result of changes in the insect fat body produced by the nematode *Mermis nigrescens* (Dujardin) (Mermithida: Mermithidae), which depletes both fat body protein and amino acids in the desert locust *S. gregaria* (Gordon and Webster 1971). Bennett and Shotwell (1972) proposed that infected larvae secrete enzymes which use lipids to eliminate the invading organisms. They also postulated that pathogens may break down hemolymph lipids into simpler moieties that can be used as a carbon source for growth and sporulation as infection continued. These findings are consistent with those of the present study.

Carbohydrates are rich and sustained energy sources, which play a crucial role in insect physiology. Obtained results showed that all treatments cause significant decreases in total carbohydrate content that are in agreement with those found by Naglaa et al. (2014). Bacteria utilize carbohydrates as a carbon source for energy and building new cells. This may decrease the available carbohydrates in treated insects, especially glucose, which plays an important role in energy supply, adult maturation, and building up a new chitin (El-sheikh et al. 2013).

Determination of carbohydrates hydrolyzing enzymes activities (amylase, invertase and trehalase)

Metabolism of carbohydrate hydrolyzing enzymes, which are essential for insect digestion and carbohydrate use (Wyatt 1967), is controlled mainly by amylase, invertase, and trehalase enzymes. An indicator of toxic effects is a violation of the intensity of the digestive processes. Toxicosis is usually accompanied by a decrease in the activity of digestive enzymes. Amylases play an important role in the hydrolysis of starch in insects (Darvishzadeh et al. 2014). Also, it was revealed a significant decrease in the activity of amylases when *L. migratoria migratorioides* 5th instar nymphs were exposed to *P. luminescens*

(EGAP3) bacterial suspension and their cell-free filtrate, which indicated a violation of homeostasis in the gut and the destruction of cells responsible for the secretion of enzymes during bacterial toxicosis. The invertase enzyme, which has been found in the digestive tracts of various insects, is thought to be vital for sucrose digestion and utilization (Wigglesworth 1953). In the case of the invertase enzyme, all treatments resulted in a decrease in activity in *L. migratoria migratorioides* when compared to the control. The reduction in enzyme synthesis, according to Kurappasamy et al. (2001), is attributable to the direct influence of toxicants on the synthesis. Trehalose is a major storage carbohydrate found in practically all forms of life except mammals. In addition, trehalase played a significant role in the supply of energy to the insect, and the activity of trehalase might serve as an indicator of energy reserves resulting from the availability of carbohydrate nutrients (Wyatt 1967).

Conclusions

It was concluded that *P. Luminescens* (EGAP3) had a high virulence against *L. migratoria migratorioides.* The present study highlights the importance of entomopathogenic nematode bacterial symbionts as promising sources of natural bioactive compounds that could be used in biological control strategies.

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