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RESEARCH

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Biocontrol efficacy of native protease-producing bacterial isolates against the olive leaf moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae)

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Abstract

Background The olive leaf moth (OLM), *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae), poses a significant threat to olive cultivation. This study focused on isolating protease-producing bacteria from moribund field strains of *P. unionalis* larvae and assessing their larvicidal efficacy against laboratory strains through estimated LC_{30}, LC_{50}, and LC_{90} values. Additionally, the long-lasting impacts of LC_{50} values on pupal and adult stages were evaluated.

Results Three entomopathogenic bacteria, *Bacillus amyloliquefaciens*, *Pseudomonas putida*, and *Bacillus subtilis*, were isolated. The larvicidal efficacies of these isolates varied, as *B. subtilis* exhibiting greater efficacy at LC_{30} and LC_{50} values, while *P. putida* showed the most potent effect at the LC_{90} value. Conversely, *B. amyloliquefaciens* demonstrated the lowest larvicidal potentialities at LC_{50} and LC_{90} values, with *P. putida* being the least effective at the LC_{30} value. The toxicity regression lines indicated increased larval lethality with each unit increase in concentration, as evidenced by the positive slope values. Chi-square (χ^2) values suggested a better fit and supported the reliability of the estimated lethal concentrations. Exposure to LC_{50} values s resulted in significant elongation of larval duration and reduction in weight, whereas pupal lifespan, adult emergence, and sex ratio showed non-significant differences compared to untreated controls.

Conclusions The study highlights the promising role of these biological candidates in pest control programs as eco-friendly alternatives, potentially enhancing safe plant production. The varied larvicidal efficacies of the bacterial isolates, along with their proteolytic capabilities, underscore their potential as biocontrol pathogens against OLM, contributing to sustainable agricultural practices.

Keywords *Palpita unionalis*, Bioinsecticides, Bacteria, *Bacillus amyloliquefaciens*, *Pseudomonas putida*, *Bacillus subtilis*, Cuticle, Plant defense, Microbial defense

Background

Economic losses due to agricultural pests are one of the main menaces facing the global agriculture economy (FAO 2021). Accordingly, the agriculture expansion aiming at bridging the food gap and offering foodstuffs for the ever-increasing population in conjunction with achievable pest management strategies has become a global demand. Out of the main biological stressors competing with humans for agricultural produce, insect

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pests are responsible for annual losses estimated to be one-fifth of the world's total production (Sallam 2013). Therefore, insect pest control is a top priority for maximizing the economic return of agricultural processes.

Jordan et al. (2021) reported that approximately 80% of insect diseases have been attributed to entomopathogens. Out of the entomopathogenic microbes, certain bacterial species, especially Gram-positive ones like *Bacillus* (Bacillales: Bacillaceae) and *Paenibacillus* (Bacillales: Paenibacillaceae) proved their field efficacies as highly sounded biocides in terms of adjusting the populations of a broad range of insect pests (Sindhu et al. 2017). This includes lepidopterans (Kaur et al. 2017), coleopterans (Ellis et al. 2002), and dipterans (Zhou et al. 2020), thereby reducing their induced infestation rates (Ongena and Jacques 2008). Other Gram-negative bacteria like *Pseudomonas* (Pseudomonadales: Pseudomonadaceae), *Chromobacterium* (Neisseriales: Neisseriaceae), and others have also drawn attention due to their insecticidal efficacy, inspiring both microbiologists and entomologists to unify their myriad efforts to assess their infectivity with the aim of adopting and accrediting new bacterial formulations (Chattopadhyay and Sen 2013). Although bacterial-based formulations have achieved much success in controlling insect pests (Stahly et al. 2006), repeated application has led to the emergence of resistant insect strains (Devi et al. 2022). The common pathogenic pathways of such entomopathogenic bacteria involving toxins that target specific receptors on the brush border membrane of the larval midgut epithelium besides other produced metabolites like antibiotics, bacteriocins, siderophores, enzymes (proteases and chitinases) and hydrogen cyanide (Sehrawat et al. 2022). AS such mechanisms have potential roles in insect pest control, the acquired resistance of pests became the case following repeated applications (Zeyu et al. 2023). Therefore, the need for finding new bacterial strains with novel modes of action is imperative.

Desert environments are among the promising agricultural habitats for the isolation of biocides. The scarcity of insecticidal spraying, harsh prevailing conditions, and the infrequency of exploration in desert agro-ecosystems increase the likelihood of successful isolation. Olive trees are a primary desert crop due to their resilience to harsh abiotic stresses. The jasmine moth, or OLM, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae), is one of the threatening insect pests to olive trees. The threat of this pest lies in its potential to devour both seedling and mature trees. Infested seedlings exhibit stunted growth due to larval attacks on young leaves and apical buds. In olive orchards, each terminal twigs, leaves, flower buds and fruits are targeted, and catastrophic infestation become the case in coinciding with pest population outbreaks (El-Salamouny et al. 2022).

Accordingly, the present study focuses on two aspects: firstly, to isolate protease-producing bacteria from moribund *P. unionalis* larvae showed bacterial infection symptoms in Matrouh governorate's, Egyptian olive orchards; and secondly, conducting laboratory trials to bioassay the larvicidal efficacy of the isolated bacterial strains against laboratory-reared OLM by calculating $LC_{30,}$ LC_{50} and LC_{90} values. The long-lasting impacts on the emerged pupal and adult stages were also considered.

Methods

Study site

Matrouh is a coastal governorate, located along the Mediterranean coast (31.352778° N 27.236111° E) in the Northwestern corner of Egypt. Rain-fed agriculture is the main cultivation profile in this coastal area. Fig, olive and vine orchards are the commonly cultivated crops constituting one of the main sources of income for a vast range of local communities. The experimental plots, selected for this study, were situated to cover olive assemblage alongside the coastal area, where the common farming practices got performed (minimal pruning practice and bit composting during winter season).

Experimental design

During 2021 growing season, four olive locations were extending along the coastal area. In each location, two olive farms with a history of *P. unionalis* infestation were selected as exploration sites. In each farm, twenty 15-year-old trees (represent a mature and productive trees) were monitored on a monthly basis for diseased larvae with bacterial infection symptoms. Once found, infected larvae were individually collected in sterilized 2-ml Eppendorf tubes to prevent cross-contamination, labeled with the date and location of collection, and transported under chilled conditions to the laboratory for further studies.

Isolation, purification, and preservation of bacteria

The proposed design for isolation, purification, and preservation was conducted according to the methodology adopted by Devi et al. (2022). Collected larvae were subjected to a surface sterilization process through exposure to 70% ethyl alcohol followed by two with distilled water. Thereafter, each larva was punctured using a sterile scalpel to collect the hemolymph. The obtained hemolymph from each larva was individually cultured on nutrient agar media in a culture plate. These plates were incubated at 30 °C for 48 h. Each initial grown colony was subsequently harvested and subjected to several subculturing processes until pure colonies were obtained. These pure

colonies were then inoculated in nutrient broth media for 16 h at 30 °C. For long-term storage, bacterial glycerol cultures were prepared.

Bacterial identification

Pure colonies were stained for scientific identification based on their distinctive morphological features (Murray et al. 1994). The identification of the isolates was confirmed by their 16S rRNA sequences. The gene was amplified by PCR using universal primers, forward primer 5′-GAGTTTGATCCTGGCTCAG-3′ as a forward primer, and 5′-AGAAAGGAGGTGATCCAG CC-3′ as a reverse one. The resulting sequences were then compared to those in the rRNA database (see Additional File 1 for the 16S rRNA gene partial sequences). Such a proposed procedure was conducted according to the methodology adopted by Suganthi et al. (2017). Obtained 16S rRNA gene sequence of *Bacillus amyloliquefaciens*, *Pseudomonas putida,* and *Bacillus subtilis* was deposited in the GenBank database with accession numbers ON493176, ON493173 and ON493164, respectively.

Phylogenetic analysis

Sequences submitted to GenBank as its sequence dataset is the global accredited reference. Nucleotide sequences were analyzed using BLASTN and the National Center for Biotechnology Information database. A phylogenetic comparison of the 16S rDNA sequence with related spp. was performed. Then, a phylogenetic tree was constructed with MEGA11 software using the neighbor-joining method with bootstrap values based on 1000 replications according to Tamura et al. (2021). Pairwise deletion option was used to remove the ambiguous positions from the sequence pairs. The evolutionary distances were also estimated using Jukes–Cantor method (Jukes and Cantor 1969).

Qualitative determination of protease activity

Bacterial protease activity was determined following the method of Abdelmoteleb et al. (2017). Skimmed milk agar (SMA) was prepared by autoclaving 10% (w/v) skim milk powder in a 250-ml Erlenmeyer flask at 115 °C for 10 min. Agar solution was prepared and autoclaved at 121 °C for 20 min. While still heated, the two solutions were merged to prepare 1% of the final skim milk concentration and then poured into Petri dishes. For the qualitative determination of the bacterial protease activity, 50 µl of the adjusted bacterial cell suspensions (5×10^5 cells/ml) was poured into skim milk agar wells (5 mm in diameter) and the formed clear zone around the colony was measured after 48 h incubation at 30 °C. Inhibition zone diameter 12 mm or more surrounding the well of media indicated the proteolytic activity (Perez et al. 2009).

Preparation of concentrations

Following bacterial identification, laboratory preparation of the proposed concentrations was carried out as described by Kaur et al. (2017). Each bacterial isolate was inoculated in 100 ml of nutrient broth in 250-ml Erlenmeyer flask. Twenty-four h post-inoculation, each flask was subjected to a shaking incubation process, 180 revolutions per minute (rpm) at 30 °C. Then, bacterial culture broth was centrifugated at 10,000 rpm for 10 min and washed three times with phosphate buffer saline (pH 7.0) to separate the activated pellet cells. Finally, the activated pellets were suspended in sterilized distilled water to create a series of dilutions (0.25, 0.5 and 1.0 w/v) at 600 nm optical density that is equivalent to $1=8.6\times10^6$, 4.7×10^7 and 7.9×10^8 cells/ml, respectively.

Laboratory rearing of olive leaf moth

The insects were reared in the laboratory using the larvae collected from the olive orchards extended throughout the northwestern coast. Collected larvae were inspected carefully to ensure the absence of infection symptoms and then transferred to the insectariums of Desert Research Center to start up the laboratory rearing according to Mansour and Saber (2017). The larvae were kept in glass jars (1 l.) (Not more than 10 larvae per jar) and supplied with tender olive branches for nourishment. Daily maintenance included cleaning and olive branch replacement continued till reached pupation. Newly formed pupae were collected and transferred to separate jars until adult emergence. Newly emerged moths were sexed and transferred to glass chimney (2 males: 1 females/each chimney) and supplied with fresh tender olive branch for egg deposition and a piece of cotton wetted by 10% sugar solution for moth nourishment. The rearing conditions were maintained at 25 ± 2 °C and 65 ± 2% relative humidity.

Laboratory bio-assessment of the bacterial isolates

Bio-assessment was initiated from the 5th laboratory generation to ensure larval susceptibility. For each identified bacterial strain, ten 1st instar larvae were placed in one Petri dish and fed fresh olive leaves treated with the previously prepared concentrations (0.25, 0.5 and 1.0 cells/ml). Each larval group was exposed to one concentration, and each trial was repeated 3 times (30 larvae per concentration). Control groups were fed untreated leaves. Larval mortality was recorded over a 48-h period (for accurate assessment of immediate larvicidal efficacy), and average larval weight was measured two weeks post-treatment.

Statistical analysis

Probit analysis was employed to calculate the lethal concentrations ($LC_{30,}$ LC $_{50}$ and LC $_{90}$ values) with fiducial limits at 95%, using the SPSS Statistics program (version 26). The regression analysis estimated the slope, which represents the change in lethality per unit increase in the concentration of each bacterial isolate. This slope is crucial for understanding the rate at which lethality changes with varying concentrations of the bacterial isolates, with a steeper slope indicating a more pronounced effect. The standard error (SE) associated with the slope was also calculated, providing an estimate of the uncertainty in the slope estimate.

The goodness of fit was determined using a χ^2 test with a significance threshold set at 5% was employed to determine the goodness of fit between the observed data and the expected values based on the concentration–response model. A high Chi-square value indicates great discrepancy between observed and expected values, while low value suggests the best fit. This test is integral in evaluating the reliability of the concentration–response model for each bacterial isolate's toxicity against the OLM. The latent effects on pupal and adult stages were evaluated by calculating pupal lifespan, adult emergence percentages, and sex ratio. These calculations were done when larvae were fed olive leaves treated with the LC_{50} value of each isolate, as well as untreated leaves serving as a control group. For the statistical analysis of these data, one-way ANOVA was conducted using SPSS, with mean differences determined at a 5% significance level through Tukey's test.

Results

Exploration and identification of bacterial isolates

The obtained findings of the present study targeted the exploration of olive groves looking for *P. unionalis* larvae exhibiting bacterial infection symptoms. Then, the scientific identification and the provision of the entomopathogenic properties of such bacterial isolates occurred. Out of the selected explorational sites, seven infected larvae had been collected from Zagarat 1 site, El-Negila locations. As per the taxonomic traits, the three isolates possessed the rod shape. The Ps-13 isolate was Gram-negative and developed under an aerobic condition. The other two isolates (Ps-5 and Ps-18) were Gram-positive and aerobically developed.

According to the phylogenetic outputs (Fig. 1), the first isolate (Ps-13) showed obvious similarity with *Pseudomonas putida*. It was deposited in the GenBank database under the accession number ON493173. The other two isolates had high convergence with the genus *Bacillus*. One of them (Ps-5) exhibited nucleotide similarity to *B. amyloliquefaciens*, and the other one witnessed nucleotide similarity to *B. subtilis*. Both *Bacillus* bacteria had been deposited in the GenBank database with accession numbers ON493176 and ON493164.1, respectively.

Enzymatic protease activity

The proteolytic capabilities of each bacterial isolate were estimated by measuring the diameter of the clear zones in a qualitative enzymatic assay. The Ps-5 isolate induced the largest clear zone with a diameter of 28 mm, while Ps-13 and Ps-18 produced inhibition zones of 23 and 17 mm, respectively. This indicated that the Ps-5 isolate had higher proteolytic potential than the other two isolates.

Bio-assessment of the bacterial isolates

The larvicidal efficacy of the examined bacterial concentrations was estimated against *P. unionalis* larvae by calculating LC values (LC_{30}, LC_{50}, and LC_{90}) (Table 1). The obtained outputs exhibited fluctuated larvicidal efficacies, where at the LC_{30} and LC_{50} levels, *B. subtilis* demonstrated higher larvicidal efficacy than the other two isolates (1.08×10^6 and 6.40×10^6, respectively), whereas the LC_{90} of *P. putida* was the most potent concentration compared to the LC_{90} for the other two *Bacillus* isolates. In contrast, as the LC_{50} and LC_{90} values of *B. amyloliquefaciens* bacterium exhibited the lowest larvicidal potential (1.93×10^7 and 3.53×10^9 at LC_{50} and LC_{90}, respectively), but *P. putida* had the weakest larvicidal effect at the LC_{30} value. As shown in Table 1, the slope of the toxicity regression lines for each bacterial isolate has been estimated. The pathogenicity regression lines' slopes were positive for all isolates, indicating an increase in bio-insecticidal properties with high concentrations. The steeper the slope, the more pronounced larval lethality per unit increases in the applied bacterial concentration. It implies the larvicidal efficacy of the bacterial isolates could be arranged in an ascending trend as *B. amyloliquefaciens*, *B. subtilis,* and *P. putida*. Chi-square (χ^2) values were another real guide to evaluate the fitness of concentration–response model (the lethal effects of bacterial isolates on the OLM). The lowest Chi-square value suggests a better fit and supports the reliability of the estimated lethal concentrations. Figure 2 provides a visual representation of *P. unionalis* larvae. These visual observations complement the larvicidal efficacy data, underscoring the response to bacterial exposure on larvae.

The obtained data showed an elongation pattern of the larval periods following bacterial treatments with LC_{50} value than those in the control group (Table 2). This implies, as the lifespan of treated larvae exceeded 20 days, the untreated ones survived ~17.12 ± 0.42 days on average. Additionally, the effect of bacterial treatments was extended to cause an obvious reduction in

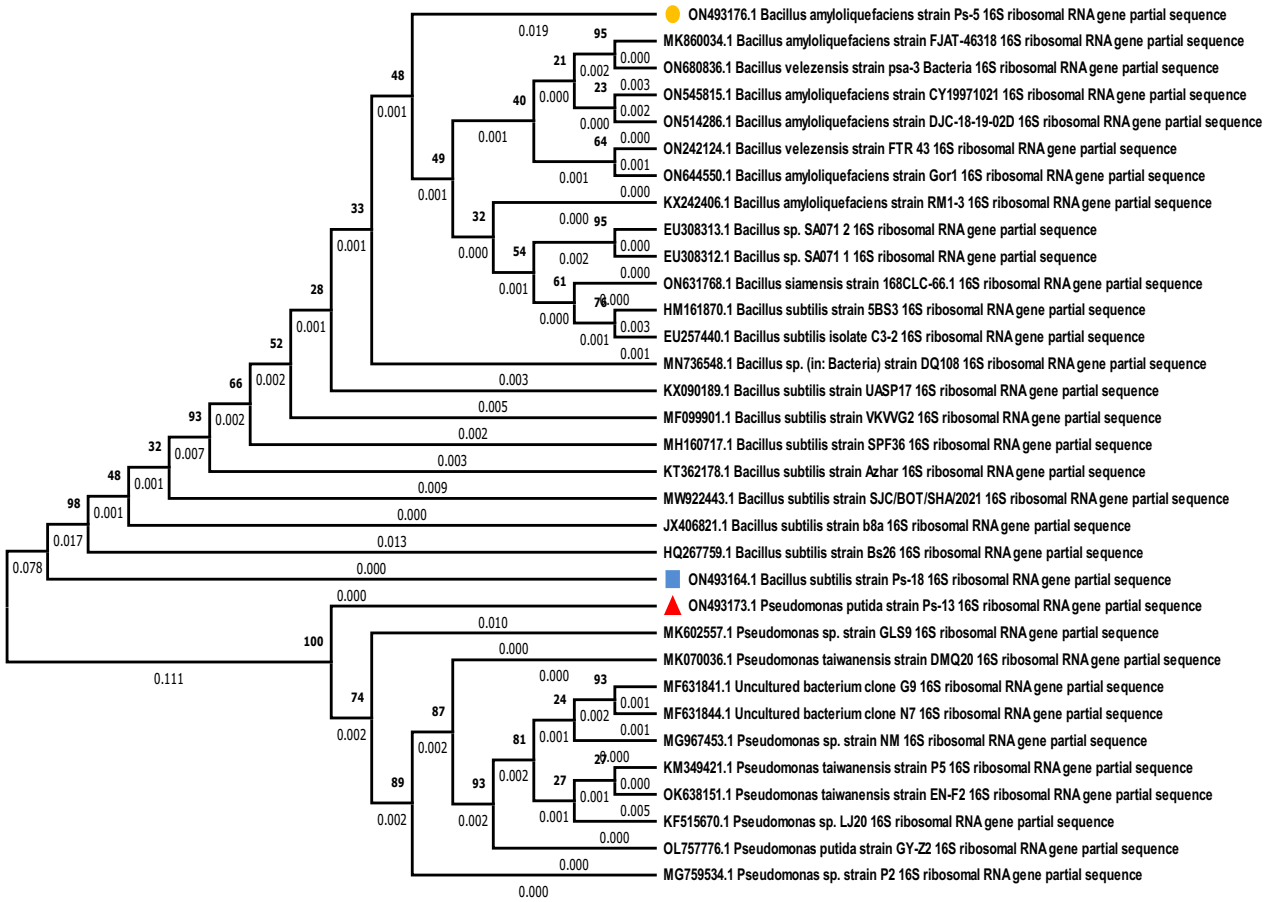

Fig. 1 Phylogenetic analysis of *Bacillus amyloliquefaciens*, *Pseudomonas putida* and *Bacillus subtilis* based on sequencing of the 16S ribosomal RNA gene. Our strains have been marked and colored

Table 1 Pathogenicity and lethal effects of bacterial isolates against 1st instar *Palpita unionalis* larvae

Bacterial isolates	*N*	Slope ± SE	χ^2	Lethal concentrations (cells/ml) (95% CL)*		
				LC_{30}	LC_{50}	LC_{90}
Bacillus amyloliquefaciens	207	0.57 ± 0.10	6.54	2.30×10^{6} (3.24×10^{5}–6.72×10^{6})	1.93×10^{7} (6.57×10^{6}–4.32×10^{7})	3.53×10^{9} (9.53×10^{8}–4.39×10^{10})
Pseudomonas putida	202	1.14 ± 0.20	4.24	2.37×10^{6} (7.80×10^{5}–4.44×10^{6})	6.83×10^{6} (3.44×10^{6}–1.12×10^{7})	9.04×10^{7} (4.68×10^{7}–2.99×10^{8})
Bacillus subtilis	204	0.68 ± 0.12	5.42	1.08×10^{6} (1.54×10^{5}–3.14×10^{6})	6.40×10^{6} (1.94×10^{6}–1.39×10^{7})	4.91×10^{8} (1.89×10^{8}–2.66×10^{9})

*Lethal concentrations and 95% confidence limits (CL) were estimated using Probit analysis (SPSS)

the larval weight gain, where the larvae exposed to LC_{50} value of *B. amyloliquefaciens*, *P. putida* and *B. subtilis* weighted ~ 0.038, 0.038 and 0.036 g, respectively, compared to 0.083 g for the control. Statistical analysis showed significant differences in both larval duration and weight between treated and control groups. The long-lasting impact was noticed on pupal and adult stages. Although treated pupae showed shorter lifespan than those emerged from control larvae (8.12 ± 1.12, 10.50 ± 1.5 and 10.50 ± 2.0 days at *B. amyloliquefaciens*, *P. putida* and *B. subtilis*, respectively, than about 12 ± 2.3 days for the control), statistical analysis categorized such variation as a non-significant difference. On the same statistical trend, both adult emergence percentages and sex ratio showed non-significant difference compared to the control.

Discussion

In the present study, the entomopathogenic efficacy of the lethal concentrations was assessed. The larvicidal efficacy of three bacterial isolates showed an ascending

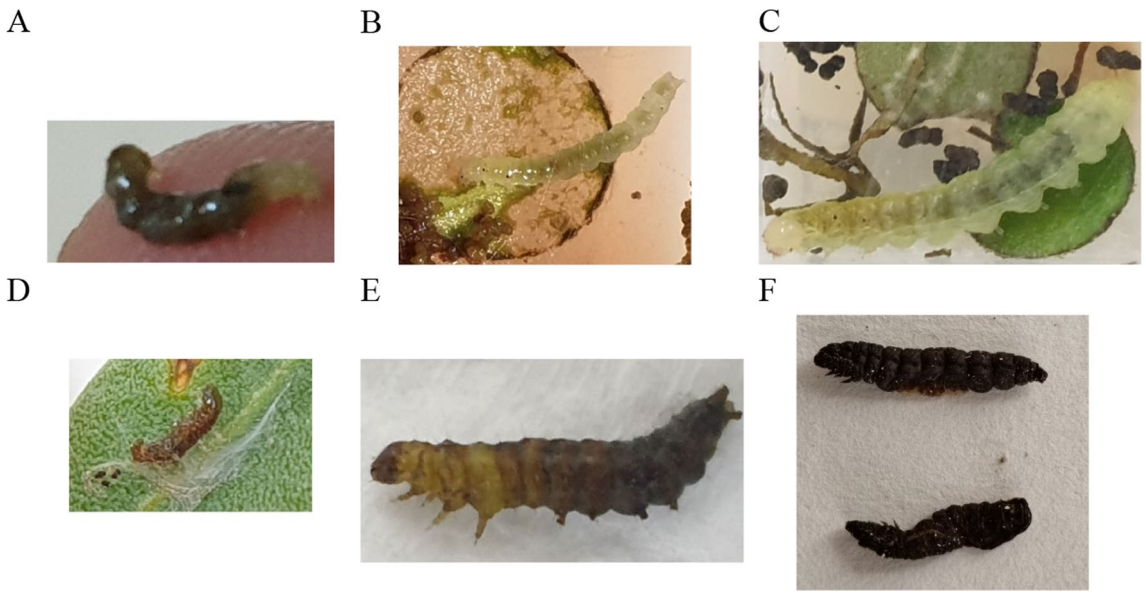

Fig. 2 Various developmental stages and health conditions of *Palpita unionalis* larvae; **A** An infected larva collected from the field, exhibiting signs of infection, **B** A healthy, young larva exemplifying normal development, **C** A mature larva, displaying characteristic features of a healthy state, **D** A young larva showing infection symptoms, **E** A mature larva showing infection symptoms, **F** A larva in the late stages of infection

Table 2 Larvicidal impacts of LC_{50} values and the long-lasting effects on the biological traits of pupal and adult stages of olive leaf moth, *Palpita unionalis*

Treatment	Larval period (days)	Larval weight (gms)	Pupal period (days)	Adult emergence (%)	Sex ratio
Control	17.12 ± 0.42^{a}	0.08 ± 0.04^{a}	12 ± 2.3	1 ± 0.01	1 ± 0.03
Bacillus amyloliquefaciens	22.28 ± 2.03^{ab}	0.04 ± 0.01^{b}	8.12 ± 1.12	0.68 ± 0.23	0.67 ± 0.33
Pseudomonas putida	21.33 ± 2.18^{ab}	0.04 ± 0.01^{b}	10.50 ± 1.5	1 ± 0.01	0.16 ± 0.16
Bacillus subtilis	23.07 ± 1.11^{b}	0.04 ± 0.02^{b}	10.50 ± 2.0	1 ± 0.03	0.41 ± 0.08
F value	2.81	29.94	0.80	0.86	1.02

Means (± standard error) followed by different letters within a column are significantly different at $P = 0.05$ according to Tukey's test

trend with the applied concentrations. The proportional relation between larval mortality and the applied LC levels has been stated by Sarkhandia et al. (2023). The applied LC_{90} value of *Pseudomonas* sp. was succeeded in fulfilling higher percentage of *S. litura* larval death than those of LC_{50} value. Another worthy output was observed upon comparing the larvicidal capabilities of the tested LC concentrations. It implies the present results showed an obvious variation in the insecticidal ability of the three bacterial isolates at the $LC_{30,}$ $LC_{50,}$ and LC_{90} values.

The next part of the present study targeted the proteolytic capability of protease enzyme, secreted by our bacterial isolates, and the insecticidal role of such process. Regarding the proteolytic activity, the highest the induced clear zone as in case of Ps-5 isolate, the highest the potentiality in enzymatic proteolysis was. Such findings were in line with Tiwari et al. (2015).

The promising entomopathogenic capabilities of the isolated bacterial pathogens matched a vast range of similar studies. *Pseudomonas paralactis* fulfilled promising larvicidal potentiality against *Spodoptera litura* (He et al. 2019). Recent reports documented the acquired resistance of the targeted pests following massive entomopathogenic treatments or exposure (Suganthi et al. 2017). Therefore, the need for exploration and exploitation of the non-traditional insecticidal capabilities of entomopathogens became imperative (Sarkhandia et al. 2023). In this regard, Harrison and Bonning (2010) highlighted the effectiveness of protease-based interventions in causing larval pathogenicity through the possible enzyme action on insect midgut, hemocoel, and cuticle. Harrison and Bonning (2010) suggested that protease's insecticidal action stems from its ability to break down proteins essential for the insect's peritrophic membrane, cuticle, and basement membrane. Fang et al. (2009) identified protease as a microbial factor with larvicidal effects. A lot of previous studies noted that pathogens can access the host's hemocoel via lesions, vectors, or

ingestion, enabling proteases to exert their pathogenic functions. They also demonstrated that *Bacillus* bacteria's bel (*Bacillus* enhancin-like) encoded protease disrupts the midgut membrane and mucin in *Helicoverpa armigera* and *Trichoplusia ni* larvae, causing significant mortality (Fang et al. 2009). Sarkhandia et al. (2023) found that proteases from *Pseudomonas* bacteria were responsible for about 74% larval mortality in *S. litura*. Similarly, Andrejko et al. (2014) reported that larval death in *Galleria mellonella* was due to bacterial metalloprotease degrading gut proteins, observable 48 h after treatment.

Apart from the larval mortality, the induced disorders of *P. unionalis* biological stages in the present study could be returned to such an enzymatic role. *Pseudomonas* sp. is succeeded in extending the developmental periods of *S. litura* where inducing histopathological and morphological deformities in both larvae and emerging moths (Sarkhandia et al. 2023). In addition, the significant weight loss of treated larvae could be attributed to the induced disruption of midgut epithelium that could lead to the deficient performance of the digestive enzymes (Nathan et al. 2006). Similar impacts of peritrophic membrane damage, midgut disruption, and imbalance of digestive enzymes on caterpillars have been stated due to the infection with entomopathogenic *Bacillus* and *Pseudomonas* bacteria (Loper et al. 2016). Harrison and Bonning (2010) summarized such discussion upon they pointed two vital remarks; the pathogenicity of microbial proteases toward insect pests and the role of such enzymes as components of predator's venoms. In line with this finding, Harrison and Bonning (2010) mentioned the defensive role of plant-secreted protease against herbivores.

Conclusions

The current successful isolation and identification of entomopathogenic bacteria from *P. unionalis* larvae in olive groves mark a significant advancement in biocontrol research. Notably, *B. amyloliquefaciens* strain (Ps-5) demonstrated the highest larvicidal and proteolytic activities, indicating its potential as a potent biopesticide. The correlation between the concentration and effectiveness of the bacterial isolates, especially *B. subtilis* and *P. putida*, highlights their potential in high concentrations. The notable proteolytic capability of these isolates underscores the critical role of enzymatic action in microbial pathogenicity, offering new insights into biological pest control mechanisms. These findings pave the way for the development of environmentally sustainable pest management strategies, emphasizing the importance of diverse microhabitats like those around olive trees for discovering novel biocontrol agents (Additional file 1).

Abbreviations

OLM	Olive leaf moth
LC_{50}	Lethal concentration 50
PCR	Polymerase chain reaction
rRNA	Ribosomal RNA
SMA	Skimmed milk agar
rpm	Revolutions per minute
SE	Standard error
χ^2	Chi-square
ANOVA	Analysis of variance
RH	Relative humidity

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-024-00783-7.

Additional file 1. DNA Sequencing of Bacteria Isolates: This file contains the 16S ribosomal RNA gene partial sequences for *Bacillus amyloliquefaciens* strain Ps-5, *Pseudomonas putida* strain Ps-13, and *Bacillus subtilis* strain Ps-18. These sequences were instrumental in confirming the identity of the bacterial isolates used in our study for their larvicidal efficacy against *Palpita unionalis larvae*.

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Author contributions
AAK, AII, and ANM were instrumental in conceiving the idea and designing the methodology for this study. AAK provided the necessary research materials. All three, AAK, AII, and ANM, actively participated in field visits. ANM was responsible for maintaining the laboratory colony, while AAK and all conducted the experiments and collected data. Data analysis was carried out by ANM, and the manuscript was written by AII. The manuscript underwent review and editing by AAK and ANM. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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