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Biochemical effects of bacterial symbionts of entomopathogenic nematodes on the Leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)

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Abstract

Tuta absoluta, a very fecund lepidopteran pest, seriously affects tomato production worldwide besides its growing insecticidal resistance; thus, seeking novel routes to combat it has become an exigency. Entomopathogenic nematodes (EPNs) and their symbiotic bacteria can be a choice as a new protection manner for tomato plants, at the same time nonhazardous to the environment.

The aim of this study is to investigate the biochemical effects of bacterial symbionts of entomopathogenic nematodes on the 3rd larval instar of the leaf miner, *Tuta absoluta*, under laboratory conditions. Biochemical changes were observed, including a marked decrease in total protein and an increase in protease contents in the pest. The data revealed that *P. luminescens* (EGAP2) had the most significant reduction in protein concentration (-66.62%), while *P. luminescens* (EGAP4) had the lowest (-23.59%) in treated insect proteins. Additionally, the highest increase in protease activity was detected in P. luminescens (EGAP2) (65.4±1.13) treated larvae.

Keywords: *Tuta absoluta, Photorhabdus luminescens, Xenorhabdus Nematophilus,* total protein, protease, electrophoresis

Introduction

The gelechiid moth, *Tuta absoluta* (Meyrick, 1917) [syn. *Phthorimaea absoluta*], represents a globally significant invasive species (Desneux *et al.*, 2022; Chang and Metz, 2021) [6, 4]. Originating in South America, with initial detection in Peru, this oligophagous pest of Solanaceae has subsequently established populations across diverse tomato-producing regions of China, resulting in substantial economic losses to the domestic industry. The rapid global dissemination of *T. absoluta* is attributed to anthropogenic activities, notably international trade, and the vectoring potential of infested plant material, including fruits, seedlings, and nursery stock, as well as the potential role of alternative host plants (Desneux *et al*., 2010) [5]. It is one of the most important pests of tomatoes in Egypt, which is posing a serious threat to tomato production. This pest is crossing borders rapidly and devastating tomato production substantially. Chemical control is not as easy as other insect pests because this leaf-mining insect lives inside leaves, fruits, and stems and requires continuous application of hazardous systemic insecticides. The rapid reproductive rate and short generation time of *T. absoluta* facilitate the accelerated evolution of resistance to commonly applied insecticides, necessitating dynamic adaptations in integrated pest management strategies. Entomopathogenic nematodes (EPNs) and their symbiotic bacteria, primarily belonging to the genera *Photorhabdus* and *Xenorhabdus*, represent a promising biocontrol strategy against various insect pests (Stock, 2015) [19].

Entomopathogenic nematodes (EPNs) initiate infection of an insect host via the release of their symbiotic bacteria, *Photorhabdus* or *Xenorhabdus*, into the hemocoel. This introduction triggers a pathogenic cascade culminating in host mortality and subsequent resource acquisition. The process is primarily mediated by a diverse suite of bacterial virulence factors, notably proteases, which catalyze the degradation of host tissues and facilitate nutrient mobilization. Proteolytic enzymes are fundamental to insect physiology, participating in both digestive catabolism and complex interactions with biotic agents. In insects, serine proteases, particularly trypsin and chymotrypsin, are dominant digestive enzymes, crucial for the hydrolysis of dietary proteins (Brabcová *et al.*, 2013) [2]. The adaptability of polyphagous insect pests, such as *Spodoptera frugiperda*, to diverse host plants is significantly influenced by the expression and activity of digestive protease enzymes and associated genes (Hafeez *et al*., 2021) [9]. Similarly, *Photorhabdus* and *Xenorhabdus* proteases exhibit broad substrate specificity, enabling the degradation of a wide range of host proteins, thereby supporting bacterial proliferation and nematode development.

Given the multifaceted roles of proteases in insect physiology, including digestion and pathogen interactions, their study is essential for understanding insect biology and developing effective pest management strategies. *Tuta absoluta*, a significant pest of solanaceous crops, demonstrates high susceptibility to EPNs and their bacterial symbionts (Kamou *et al*., 2024) [12]. Therefore, investigating the effects of *Photorhabdus luminescens* and *Xenorhabdus nematophilus* on total protein content and protease activity within *T. absoluta* larvae is crucial for optimizing biocontrol strategies.

Protein, a critical macromolecule for insect tissue synthesis, growth, and reproduction, serves as a key indicator of host nutritional status and physiological condition, which in turn influences susceptibility to pathogens and parasitoids. Fluctuations in total protein content following bacterial infection reflect both host metabolic responses and the enzymatic activity of bacterial proteases. Dietary protein availability and the insect's capacity for assimilation directly impact protein levels, which are intrinsically linked to various physiological processes, including the synthesis of immune molecules for pathogen defense (Ratcliffe *et al*., 2024) [17].

This study aims to quantify changes in total protein content and protease activity in *T. absoluta* larvae following exposure to *Photorhabdus luminescens* and *Xenorhabdus nematophilus*, thereby elucidating the mechanistic basis for the efficacy of these bacterial symbionts as biological control agents.

Materials and methods

The experiments were carried out under laboratory conditions to evaluate the biochemical effect of bacterial cell suspension and cell-free filtrate of EPNsB. This study builds upon previous findings published by Ahmed *et al*. (2018) [1], which demonstrated the high susceptibility of *T. absoluta* larvae to bacterial cell suspensions and cell-free culture filtrates of EPNsB. Specifically, in our previous study, we observed that larval mortality increased with higher cell suspension densities (up to 4×10^7 cells/ml) and undiluted cell-free supernatant (100%), achieving mortality rates up to 70%. The current research expands on these initial observations by examining the specific biochemical mechanisms underlying the observed toxicity of EPNsB in *T. absoluta*.

Microorganisms

Photorhabdus luminescens (EGAP1) MH368153, *Photorhabdus luminescens* (EGAP2) MH368154, *Photorhabdus luminescens* (EGAP3) MH368155, *Photorhabdus luminescens* (EGAP4) MH368156 and *Photorhabdus luminescens* (EGAP5) MH368157 and *P. luminescens subsp. laumondii* HP88 and *X. nematophilus* strains obtained from Pest Physiology Dept., Plant Protection Research Institute, Agricultural Research Centre, Egypt.

Insect

A laboratory colony of *Tuta absoluta* was established and maintained under controlled environmental conditions. Specifically, fresh apices of *Lycopersicon esculentum* Mill. were excised and placed in water-filled vessels within screened enclosures of 0.125 m^3 (0.5 m x 0.5 m x 0.5 m). These enclosures were maintained at a constant temperature of 25 ± 1°C. Adult male and female *T. absoluta* specimens were introduced into these enclosures to facilitate mating and subsequent oviposition on the provided *L. esculentum* foliage. Larval eclosion was observed to occur approximately five days post-oviposition. To ensure consistent nutritional resources and mitigate plant senescence, fresh *L. esculentum* apices were replenished at seven-day intervals.

Preparation of Samples for biochemical characteristics

Larval homogenates were prepared 48 hours post-treatment from larvae treated with 4×10^7 cells/mL of EPNsB strains or their undiluted cell-free filtrates. Specifically, treated larvae were subjected to mechanical disruption in ice-cold, sterile distilled water using a Teflon homogenizer operating at 500 revolutions per minute (rpm) for 180 seconds. The homogenizer was maintained at approximately 0°C via an external ice jacket. To mitigate enzymatic melanization, pre-chilled collection tubes were internally coated with phenylthiourea crystals. Homogenates were subsequently centrifuged at 6,000 x g for 10 minutes at 5°C. The resulting supernatant was fractionated into 0.5 mL aliquots and stored at -20°C for subsequent enzymatic activity assays and compositional analysis, following the methodology described by Salama et *al* (1985).

Determination of total protein content

Total proteins were determined by the method of Bradford *et al* (1976) using standard of Bovine serum albumin.

Determination of protease activity

Using Ishaaya *et al* (1971)'s casein digestion method, the proteolytic enzyme activity was measured. 0.4 ml of 1.5% casein solution, 0.2 ml of homogenate sample, and 0.2 ml of glycine buffer (pH 10) made up the reaction mixture. After incubating for 60 minutes at 37°C and adding 1.2 milliliters of a 5% trichloroacetic acid solution, the enzyme activity was stopped. The supernatant was extracted for the assessment of enzyme activity after the reaction mixture was centrifuged for 15 minutes at 6000 rpm. The O.D. unit x 10^3 was used to measure the proteolytic activity at 280 nm.

Qualitative detection of protease activity of the bacterial cultures

Bacterial strains were propagated on nutrient agar slants (composition: 15 g/L agar, 3 g/L yeast extract, 5 g/L peptone, 5 g/L NaCl) and incubated at 28°C for 48 hours. For proteolytic activity assays, a skim milk agar medium was prepared by supplementing nutrient agar with 1% (w/v) skim milk. Aliquots of this medium were dispensed into Petri dishes and allowed to solidify for 30 minutes. Subsequently, 3 mm diameter plugs of crude culture from *Photorhabdus luminescens* strains EGAP1, EGAP2, EGAP3, EGAP4, and EGAP5, *Photorhabdus luminescens* subsp. *laumondii* HP88, and *Xenorhabdus nematophilus* strains were centrally inoculated onto the skim milk agar plates. The plates were then incubated at 28°C ± 2°C for 24 hours. Following incubation, the formation of clear zones, indicative of proteolytic activity, was observed and documented, consistent with the methodology described by Vijayaraghavan and Vincent (2013).

Fractionation of *Tuta absoluta* larvae homogenate protein

Sodium dodecyl sulphate polyacrlamide gel electrophoresis (SDS–PAGE) technique was used for fractionation the total protein in control and treated larvae. The method of Laemmli *et al* (1970) was adopted for mono dimensional electrophoresis.

Statistical analysis

Each test was conducted in triplicate using a fully randomized design. Analysis of variance (ANOVA) was used to establish the main effects' significance. Duncan's multiple range test was used to assess the significance of the different treatments (P<0.05). A software program called "Costat," produced by Berkeley, California-based Cohort Software Inc., was used for all analyses. Three replications were used to record all of the data.

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Results

Effect of EPNsB on the total soluble protein contentin *Tuta absoluta* larvae

Treatment of *T. absoluta* 3rd instar larvae with EPNsB caused a decrease in the total protein content of larval tissues compared with that of control insects. Table (1). Data revealed a significant reduction of protein concentration in *P. luminescens* (EGAP1) (-63.29%), *P. luminescens* (EGAP2) (-66.62%), and *P. luminescens* (EGAP1) (-63.37%) treatments. A significant decrease in insect proteins (-45.56, -43.74, and -37.72%) was detected in larvae treated with *P. luminescens subsp. laumondii* HP88, *X. nematophilus,* and P. luminescens (EGAP5), respectively. *P. luminescens* (EGAP4) produced a medium decrease (-23.59%) in proteins of treated insects. The treatments with EPNsB strains were significantly different (f = 143.10, df = 7, p = 0.0000) with respect to their total soluble protein content.

Table 1: Total protein contents (μgml^{-1}) of 3rd instars of *T. absoluta* as affected by different EPNsB strains

Sample	**μg.ml^{-1}** Mean ± SE
Control	881.6±10.13^{a}
Photorhabdusluminescens (EGAP1)	323.7±12^{e}
Photorhabdusluminescens (EGAP2)	294.3±14.5^{e}
Photorhabdusluminescens (EGAP3)	323±18.7^{e}
Photorhabdusluminescens (EGAP4)	673.7±22^{b}
Photorhabdusluminescens (EGAP5)	550±20.8^{c}
*Photorhabdus luminescenssubsp. laumondii*HP88	480±17.3^{d}
Xenorhabdus nematophilus	496±15.4^{d}

Means with different super scripts are significantly different (*p<0.01*)

Activity of proteases in *T. absoluta* as affected by EPNsB strains

The obtained data show that the protease activity was higher in *T. absoluta* 3rd instar larvae treated with EPNsB (Table 2) than in control untreated larvae**.** The highest increase in the enzyme activity was detected in *P. luminescens* (EGAP2)-treated larvae. A significant increase was recorded in *P. luminescens* (EGAP1) and *X. nematophilus* treated larvae, respectively. Treatments with *P. luminescens* (EGAP3), *P. luminescens* (EGAP4), *P. luminescens* (EGAP5), and *Photorhabdus luminescens subsp. laumondii* HP88 produced significant elevation of larval enzyme. The differences according to treatments with examined EPNsB strains are significantly different (f = 15.74, df = 7, p = 0.0000) with respect to their proteolytic activities.

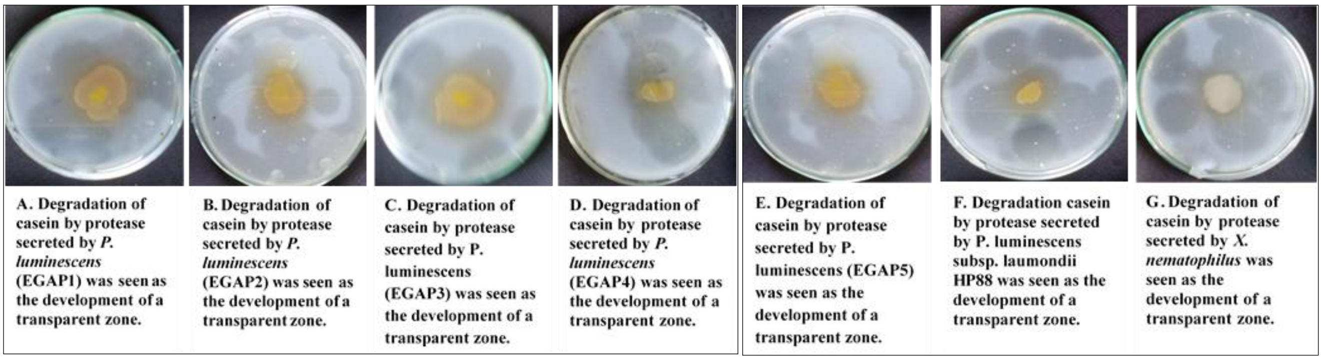
Table 2: Protease activity (μg.ml^{-1}) in *T. absoluta* larvae 3rd treated with different EPNsB strains

Sample	**μg.ml^{-1}** Mean±SE
Photorhabdus luminescens (EGAP1)	54.3±1.62^{b}
Photorhabdus luminescens (EGAP2)	65.4±1.13^{a}
Photorhabdus luminescens (EGAP3)	44.8±1.14^{c}
Photorhabdus luminescens (EGAP4)	49.2±2.28^{bc}
Photorhabdus luminescens (EGAP5)	45.2±2.25^{c}
Photorhabdus luminescens sub *sp. Laumondii* HP88	44±2.09^{c}
Xenorhabdus nematophilus	54.9±2.53^{b}
Control	33.1±1.28^{d}

Means bearing different super scripts are significantly different at (p<0.01, ANOVA, Duncan's multiple range test

Qualitative determination of protease activity of EPNsB on skimmed milk agar

The symbiotic bacteria, *P. luminescens* (EGAP1), *P. luminescens* (EGAP2), *P. luminescens* (EGAP3), *P. luminescens* (EGAP4), *P. luminescens* (EGAP5), *P. luminescens* subsp. laumondii HP88 and *X. nematophilus* were examined for their ability to produce proteases on skimmed milk agar plates. Clear zones were observed on the skimmed milk agar plates (Fig. (1): A - G) as a result of the proteolytic activities caused by all the symbiotic bacteria.



A. Degradation of casein by protease secreted by *P. luminescens* (EGAP1) was seen as the development of a transparent zone.

B. Degradation of casein by protease secreted by *P. luminescens* (EGAP2) was seen as the development of a transparent zone.

C. Degradation of casein by protease secreted by P. luminescens (EGAP3) was seen as the development of a transparent zone.

D. Degradation of casein by protease secreted by *P. luminescens* (EGAP4) was seen as the development of a transparent zone.

E. Degradation of casein by protease secreted by P. luminescens (EGAP5) was seen as the development of a transparent zone.

F. Degradation casein by protease secreted by P. luminescens subsp. laumondii HP88 was seen as the development of a transparent zone.

G. Degradation of casein by protease secreted by *X. nematophilus* was seen as the development of a transparent zone.

Fig 1: Clear zones were observed on the skimmed milk agar plates (A - G) as a result of the proteolytic activities caused by all the symbiotic bacteria

Protein profile of *T. absoluta* larvae homogenate

The total protein contents of untreated and treated third-instar larvae of *T. absoluta* were separated on SDS polyacrylamide gel electrophoresis in order to investigate the effects of the investigated bacterial bioagents on the protein constituents of *T. absoluta* larvae homogenates (Table 3 and 4). Based on their molecular weight (MW) and relative frequency (RF) values on the gel, each treatment was divided into 25 distinct bands.The total numbers of protein bands were 14, 10, 8, 10, 8, 8, 9, and 10, which appeared in the whole body homogenate of control (lane 1a), *Photorhabdus luminescens* (EGAP1) (lane 2a), *Photorhabdus luminescens* (EGAP2) (lane 3a), *Photorhabdus luminescens* (EGAP3) (lane 4a), *Photorhabdus luminescens* (EGAP4) (lane 5a), *Photorhabdus luminescens* (EGAP5) (lane 6a), *Photorhabdus luminescens subsp. laumondii* Hp88 (lane 7a), and *Xenorhabdus nematophilus* (lane 8a) treated larvae (Table 3). The total numbers of protein bands of treated larvae with their cell-free filtrates were 11 (lane 2b), 9 (lane 3b), 11 (lane 4b), 9 (lane 5b), 10 (lane 6b), 11 (lane 7b), and 12 (lane 8b), respectively (Table 4). The obtained results point out a possible effect of the examined EPNsB strains on the protein profile of the larvae homogenate. This can be simply extrapolated by comparing profiles from larvae treated with broth cultures of the examined strains or their cell-free supernatant. Several specific protein bands can be detected in the non-treated larvae but not in the treated ones, as shown in Tables 3 and 4.

Table 3: Relative frequency values and molecular weights of SDS protein bands detected in the wholebody homogenates of *Tuta absoluta* 3rd instar larvae treated with seven bacterial cell suspensions

			Control	**EGAP1**	**EGAP2**	**EGAP3**	**EGAP4**	**EGAP5**	**Hp88**	**BA2**
Band No.	**Rf**	**MW**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**
			1(a)	**2(a)**	**3(a)**	**4(a)**	**5(a)**	**6(a)**	**7(a)**	**8(a)**
1	0.091	207.50	+	-	-	-	-	-	-	-
2	0.126	155.00	+	-	-	+	-	-	-	+
3	0.235	69.93	+	+	-	+	-	-	-	-
4	0.247	67.47	-	+	-	-	+	+	-	+
5	0.301	56.81	+	-	-	-	-	-	-	-
6	0.306	55.78	-	-	+	-	-	-	+	+
7	0.384	41.75	+	+	-	+	+	+	-	-
8	0.416	34.00	-	+	+	-	-	-	+	+
9	0.429	31.67	+	-	+	+	+	-	+	-
10	0.486	24.58	-	+	-	+	-	+	-	-
11	0.508	21.65	+	-	+	-	+	-	+	+
12	0.553	16.20	+	-	-	-	-	+	-	+
13	0.585	13.94	-	+	+	-	+	-	+	-
14	0.605	13.15	+	-	-	-	-	-	-	-
15	0.661	12.41	+	-	-	-	+	-	-	+
16	0.675	12.32	-	+	-	+	-	+	+	-
17	0.692	12.15	-	-	+	-	-	-	-	-
18	0.721	11.49	+	-	+	-	+	-	-	+
19	0.752	10.29	+	-	-	+	-	-	-	-
20	0.772	9.28	-	+	-	-	-	+	+	-
21	0.816	6.64	-	+	+	-	-	+	+	+
22	0.872	3.47	+	-	-	-	-	-	-	-
23	0.874	3.37	-	+	-	+	-	-	-	-
24	0.898	2.21	-	-	-	-	-	-	+	+
25	0.924	0.89	+	-	-	+	+	+	-	-
Total No. of bands			14	10	8	10	8	8	9	10

L=lane, RF=relative frequency, Mw=Molecular weight, (+) = detected protein, (-) = non-detected protein

Table 4: Relative frequency values and molecular weights of SDS protein bands detected in the homogenates of *Tuta absoluta* larvae treated or not with the seven EPNsB cell-free supernatant

			Control	**EGAP1**	**EGAP2**	**EGAP3**	**EGAP4**	**EGAP5**	**Hp88**	**BA2**
Band	**Rf MW**		**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**
No.			**1(b)**	**2(b)**	**3(b)**	**4(b)**	**5(b)**	**6(b)**	**7(b)**	**8(b)**
1	0.091	207.50	+	-	-	-	-	-	-	-
2	0.126	155.00	+	-	-	-	+	-	-	+
3	0.235	69.97	+	+	-	+	-	+	+	-
4	0.268	63.43	-	-	+	-	-	-	+	-
5	0.296	57.88	-	+	-	+	-	+	-	+
6	0.301	56.81	+	-	-	-	-	-	-	-
7	0.340	50.35	-	+	+	-	-	+	-	+
8	0.384	41.75	+	+	-	+	+	+	+	-
9	0.407	36.14	-	+	-	-	-	-	+	+
10	0.429	31.67	+	-	-	+	+	-	+	-
11	0.451	28.64	-	+	-	+		+	+	+
12	0.483	24.97	-	+	-	-	-	+	-	+

13	0.508	21.65	+	-	+	-	+	+	-	+
14	0.533	18.388	-	+	-	+	-	-	+	-
15	0.553	16.198	+	-	+	+	-	+	-	+
16	0.573	14.600	-	+	-	-	+	-	-	+
17	0.605	13.151	+	-	-	+	-	+	+	-
18	0.661	12.408	+	+	+	-	+	-	-	-
19	0.721	11.491	+	-	+	-	-	-	+	+
20	0.752	10.290	+	-	-	+	-	-	-	-
21	0.767	9.561	-	-	+	-	+	-	-	+
22	0.825	6.098	-	-	+	-	-	-	-	+
23	0.872	3.474	+	+	-	+	-	-	+	-
24	0.898	2.211	-	-	+	-	+	+	-	+
25	0.924	0.895	+	-	-	+	+	-	+	-
Total No. of bands			14	10	8	10	8	8	9	10

L=lane RF=relative frequency, Mw=Molecular weight, (+) = detected protein, (-) = non-detected protein

Discussion

Our study demonstrates that treatment of *Tuta absoluta* 3rd instar larvae with various strains of *Photorhabdus luminescens* and *Xenorhabdus nematophilus* significantly impacts both total soluble protein content and protease activity. Specifically, *P. luminescens* strains EGAP1, EGAP2, and EGAP3 elicited the most substantial decrease in protein levels, indicating potent proteolytic activity or a rapid disruption of host protein synthesis. This reduction in protein content aligns with previous observations that EPNsB-mediated infection disrupts host metabolic processes, leading to the degradation of host tissues and a decline in overall protein levels (Muhammad *et al*, 2022) [15].

Interestingly, the degree of protein reduction varied among the different EPNB strains, suggesting variations in virulence or protease activity. *P. luminescens* EGAP4 exhibited a moderate decrease in protein content, indicating a potentially lower virulence or a different mode of action compared to the other strains. These variations underscore the importance of strain-specific characteristics in determining the efficacy of EPNB as biocontrol agents. The observed alterations in protein align with previous findings by Fathy *et al*. (2023) [8], who demonstrated that sublethal exposure (LC_{50}) of the desert locust *Schistocerca gregaria* to *Photorhabdus luminescens* resulted in the amounts of protein in the treated locusts being drastically reduced.

The observed decrease in total protein content can be attributed to several factors. Firstly, the secretion of bacterial proteases into the hemocoel facilitates the breakdown of host proteins, providing nutrients for bacterial proliferation and nematode development. This proteolytic activity is consistent with the broad substrate specificity of *Photorhabdus* and *Xenorhabdus* proteases, which enables them to degrade a wide range of host proteins (Massaoud *et al*. 2010) [14]. Secondly, the infection process may induce a systemic immune response in the host, leading to the mobilization of host proteins for defense mechanisms, which are subsequently depleted (Ratcliffe *et al*., 2024) [17]. However, the significant protein decrease observed in this study, indicates that the bacterial proteases where more impactful than any potential host immune response. Differential protease activity was observed across *Photorhabdus luminescens* strains. Specifically, *P. luminescens* EGAP2 demonstrated the highest proteolytic activity, which coincided with the most substantial reduction in total protein content. This correlation strongly suggests that the observed protein depletion is primarily attributed to bacterial protease activity. Conversely, *P. luminescens* subsp. *laumondii* HP88, *P. luminescens* EGAP5, and *P. luminescens* EGAP3 exhibited significantly lower, though still elevated compared to the control, protease activities. These lower levels were also associated with corresponding reductions in protein content. As anticipated, the control group displayed the lowest proteolytic activity. The variations in protease activity observed among the different strains may reflect differences in the expression or secretion of specific proteases, as well as variations in their catalytic efficiency.

As the complete protein contents of *T. absoluta* larvae's body homogenate were fractionated using SDS-PAGE, it was discovered that a decrease in protein concentration was associated with the loss of several proteins and a decrease in the strength of the bands in treated larvae as compared to control ones. These results are consistent with those of Eman R. and Hanan S. (2017) [7], who discovered that when *T. absoluta* was treated with phenolphthalein, imidacloprid, and dinotefuran, many protein bands with varying intensities of difference in fractionated proteins of insect tissues disappeared, appeared, and varied in degree when compared to the control. The observed alterations in protein profiles align with previous findings by Muhammad *et al*. (2022) [15], who demonstrated that sub lethal exposure (LC_{50}) of *Locusta migratoria migratorioides* to *Photorhabdus luminescens* resulted in significant qualitative and quantitative variations in fractionated tissue proteins compared to untreated controls. These findings are in accordance with results obtained by Radwan and El- Malla (2008) [16] who reported that treatment of the med fly, *Ceratitis capitata* with LC_{50} from Vertimec, sumi-gold and tracer caused disappearance, appearance and degrees of difference in fractionated proteins of insect tissues compared with control treatment of the spiny bollworm, *Earias Insulana* larvae with LC_{50} of abamectin and esfenvalerate caused disappearance and appearance of numerous protein bands with different intensity. Also Hassan and Abdel Hafez (2009) [10] reported that the fractionation proteins of 6th larval instar of *S. littoralis* with LC_{50} of spintor and Spintoram caused the appearance of some bands and disappearance of others.

Conclusion

In conclusion, this research elucidates that infection by entomopathogenic nematode-associated bacteria (EPNB) induces a significant decrease in total soluble protein content, alterations in protein profiles, and a concurrent increase in protease activity within *Tuta absoluta* larvae. These results underscore the potent pathogenic effects of

Photorhabdus and *Xenorhabdus* symbionts, emphasizing their potential as effective biocontrol agents. Future investigations should prioritize the identification and characterization of specific proteases responsible for host tissue degradation. Furthermore, elucidating the molecular mechanisms governing the observed variations in virulence across different EPNB strains is crucial. These studies will facilitate the development of optimized biocontrol strategies for the sustainable management of T. absoluta populations.

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