African J. Biol. Sci., 17 (1): 221-231 (2021)
ISSN 1687-4870 **e- ISSN 2314-5501 (online)**
www.ajbs.journals.ekb.eg **E.mail: aasdjournal@yahoo.com**

Evaluation of the antimicrobial activity of purified *Spodoptera littoralis* hemolymph against some pathogenic bacteria

Hanan S. Amer[1], Doaa Soliman[2], Wael S. Abdel-Mageed[3], Shimaa A.Mo'men[2], Tamer Roshdy[3] and Nadia M. Lotfy[2]

1- Ain Shams Univ. Specialized Hospital, Cairo, Egypt
2- Entomology Department, Faculty of Science, Ain Shams Univ.
3- Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute, Sadat Univ., Menoufia, Egypt
4- Corresponding Author E-mail: Hantoosh27@yahoo.com

Received: May 12, 2021; Accepted: September 2, 2021; Available online: September 9, 2021

ABSTRACT

The effect of the Phenoloxidase (PO) on bacterial culture and its bioactivity is a big concerns nowadays because of the movement towards natural antibiotics instead of the chemical ones. In this study the prophenoloxidase (PPO) was activated to PO in *Spodoptera littoralis* by injection of series concentrations from *Bacillus thuringiensis kurstaki* (BT) (3200 IU/mg, AGERIN- wettable powder) $2X10^{10}$, $2X10^{20}$, $2X10^{30}$, $2X10^{40}$, $2X10^{50}$ and $2X\ 10^{60}$ cells/ml. 10 µl of each bacterial concentration was injected into groups from 10 larvae each, with a total 656 larvae. 519 of the injected larvae were live and 137 died. It was found that the stock concentration $2X10^{60}$ was the LC20 concentration. After the injection and inoculation for 24 hrs, the larvae had been disinfected and the haemolymph separated. Purification for PO from the haemolymph had been done using HiTrapTM CM FF 1ml column. After assuring the presence of PO by SDS gel, it was cultured against six types of bacteria, two gram +ve (*Staph. aureus*, *Enterococci*) and four gram –ve (*E. coli*, *Pseudomonas*, *Acinetobacter* and *Klebsiella*). It was effective against the gram positive bacteria and against *E. coli* only from the gram negative bacteria. Therefore, it could be concluded that the PO has bioactivity toward the gram positive bacteria more than the gram negative ones.

Keywords: *Spodoptera littoralis* , haemolymph, Phenoloxidase, *Bacillus thuringiensis*, gram +ve bacteria, gram –ve bacteria, bioactivity.

INTRODUCTION

Insects combat disease by mounting capable immune reactions that are interceded by hemocytes, the fat body, the midgut, the salivary organs and other tissues (Hillyer, 2016). They depend exclusively on a well-developed natural resistant framework to protect themselves against microbial diseases (Franssens, 2006). Insects need a procured immune system and depend exclusively on the intrinsic safe framework to combat microbial disease. Upon microbial infection, a course of action of small peptides and proteins are conveyed and released into the haemolymph (Prasad *et. al*. 2020). In insects, antimicrobial peptides/ polypeptides are synthesized mainly in a fat body (functional analogue of mammalian liver) and are released into haemolymph where they play a crucial role in innate immune systems and host defense mechanisms, and having a broad spectrum of activity against both Gram +ve and Gram

-ve bacteria and against fungi (Hoffmann, 1995; Hoffmann *et al*., 1996; Januszanis *et al.,* 2012). The era of Antimicrobial peptides (AMPs) is exceedingly inducible taking after a microbial contamination, the levels of AMPs modify from by and the large intangible in uninfected insects to micromolar concentrations in haemolymph of damaged ones. Expression of these AMPs comes essentially from fat body in show disdain toward of the reality that hemocytes as well contribute to their generation (Rosales, 2017). The components of AMP activity are thought of as an interaction with the bacterial cell membrane (Kumar *et al*., 2018). Mode of activity might relate to targeting metabolic forms within the bacteria including cell divider blend, nucleic corrosive or protein blend, which are crucial to the organism (Ebbensgaard *et. al.,* 2015).

Antimicrobial resistance is rising to hazardously tall levels in all parts of the world. Unused resistance components are developing and spreading universally, threatening our capacity to treat common irresistible illnesses. A developing list of diseases such as pneumonia, tuberculosis, blood harming, gonorrhea, and foodborne infections are getting to be harder and now and then incomprehensible, to treat as antimicrobial gotten to be less successful (WHO, 2018). AMPs are conceivable candidates for the plan of unused antimicrobial agents since of their common antimicrobial properties and a low penchant for improvement of resistance by microorganisms. This composition surveys the current information of the fundamental science of AMPs and their applications in non-ruminant nourishment. Antimicrobial peptides not as it were have broad-spectrum movement against microscopic organisms, parasites, and infections but moreover have the capacity to bypass the common resistance instruments that are putting standard antimicrobials in risk (Wang *et al.,* 2016). The present study aimed at evaluating the resistant of different gram negative and gram positive bacteria against one of the AMP component, Phenoloxidase, in order to examine the antimicrobial activity as a primary step for new antibiotic era.

MATERIALS AND METHODS

Insect rearing and pathology assays

The adult leaf-worm *Spodoptera littoralis* was obtained from the Central Agribusiness Pesticides Investiagate Office (CAPL), Dokki, Giza, Egypt. Larvae were reared in insectarium on an artificial diet (Poitout *et al*., 1970) at 23±1˚C, with a photoperiod of 16 hrs light:8 hrs darkness and a relative humidity of 40± %.

Bacterial suspension

Pathogenicity experiments were performed by injecting a suspension of *Bacillus thuringiensis* kurstaki (BT) bacteria in the exponential growth phase (2 $X10^{10}$ cells/ml of LB broth) into fifth instar larvae (Bisch *et al*., 2015). Six independent pathogenicity assays were performed for *Bacillus thuringiensis* in the *S. littoralis* pathoassay to obtain the sublethal dose. 10 larvae were used for each concentration.

Haemolymph Collection:

The living infused and uninjected (control group) cotton leaf worms were expelled from the raising cages, and submerged in hot water shower at 60ºC for 2-5min, then permitted to dry on paper towel. The living larvae were severed at the rear coxa with fine scissors, the haemolymph was gotten with a fine-tipped calibrated glass capillary, which was kept at -20ºC for further use in detecting the antimicrobial peptides (Miranpuri and Khachatourians, 1993)

Antimicrobial peptide purification:

The refinement of the enzyme had been done utilizing HiTrap TM CM FF 1ml

column bought from GE Healthcare. CM Sephrose Fast Flow, is based on a vigorous, 6% highly cross-linked beaded agarose matrix with excellent stream properties and tall stacking capacities. Two groups of haemolymph (injected and control) had been centrifuged at 12,000 for 10 mins, the additives were washed out by filling the syringe with 5ml buffer (bis-tris PH 5). At that point washing with 5 ml of elution buffer (buffer with 1 M NaCl) (Jae-Joon and Woo-Yeon, 2013). Equilibrate with 5 ml start buffer. Apply the supernatant of the haemolymph to the column by the syringe, washed with start buffer with the same amount of connected haemolymph eluted with 5 ml solution buffer.

Haemolymph proteins were analyzed by SDS-polyacrylamide gel (Laemmli, 1970) method. Concentration of the protein in the injected and uninjected sample had been tested by NANODROP 2000c spectrophotometer from Thermo SCIENTIFIC.

Preparation of microbial cells A standardize bacterial suspension (0.5 McFarland) was obtained from microbiology unit, central lab, Ain Shams Specialized Hospital and prepared and measured by densitometer "BIOMERIEUX, DensiCHEK plus". Two gram positive bacterial suspensions had been prepared; *Staphylococcus aureus*, and *Entero cocci*, and four gram negative bacterial suspensions had been prepared; *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Klebsiella pneumonia*. All bacterial cultures were grown overnight at 37º C in rich nutrient media until reaching a stationary phase.

Antibacterial activity of collected haemolymph: Microbial growth inhibition was tested using agar well diffusion method (Magaldi *et al.,* 2004; Valgas *et al.,* 2007). In this procedure, agar plates were inoculated with a standardized inoculum of the test microorganism. Then, adding 10µ/drop from the purified haemolymph on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the tested microorganism and then the diameters of inhibition growth zones were measured.

RESULTS

Haemolymph purification:

Concentration of the protein in the injected and uninjected samples had been tested by "NANODROP 2000c spectrophotometer from Thermo SCIENTIFIC", 2µg/ml from the sample, the concentration of the injected sample was 106µg/ml and the protein concentration in uninjected sample was 190µg/ml.

SDS-PAGE Profiles:

Concerning the total cellular proteins of hemolymphs isolates, 14 bands of hemolymphs were fractionated in denaturing gel electrophoresis (SDSPAGE) MW 200, 150, 120, 100, 85, 70, 60, 50, 40, 30, 25, 20, 15 and 10 kDa (Fig. 1). Comparison of protein patterns from marker and purified hemolymph isolates indicated that there was 1 common band MW 65 kDa (Fig. 1).

Bacterial susceptibility against AMP'S:

The antibacterial activity of hemolymph of *S. litorallis* against different strains of Gram-positive and Gram-negative bacteria is indicated in Table (1). The Collected hemolymph was injected immediately after isolated. The inhibition zone with the gram positive bacteria *Enterococci* was (1, 1.1 and 0.9cm) and with *Staph aureus* was (2, 2.2 and 1.8cm) (Fig. 2). In case of gram negative the inhibition zone with *E.coli* was (1.5, 1.7 and 1.4cm). For other gram negative no inhibition zone was found (Fig. 3). In case of hemolymph tested after 60 minutes and

uninjected hemolymph there was no inhibition zone with gram positive or gram negative bacteria and there were no statistically significant difference between Gram positive and Gram negative Bacteria (Table 1).

Fig. 1. Protein bands of marker and purified haemolymph of *Spodoptera litorallis* adults determined from SDS- PAGE.

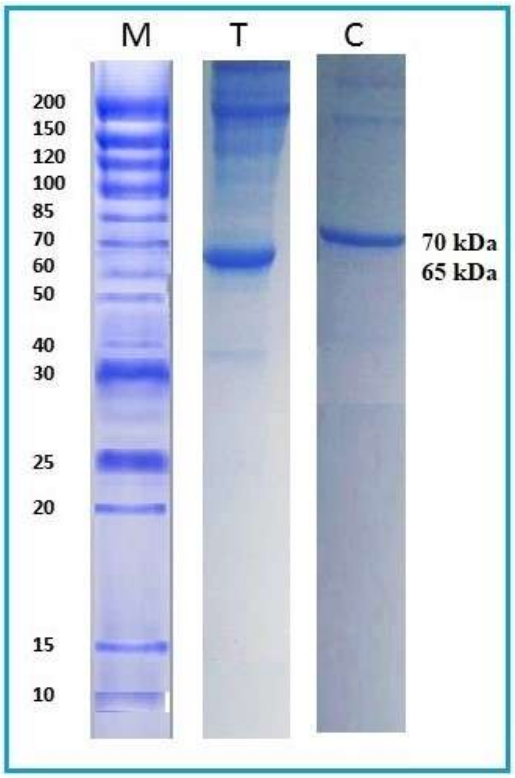

Fig. 2. inhibition zone with gram positive bacteria

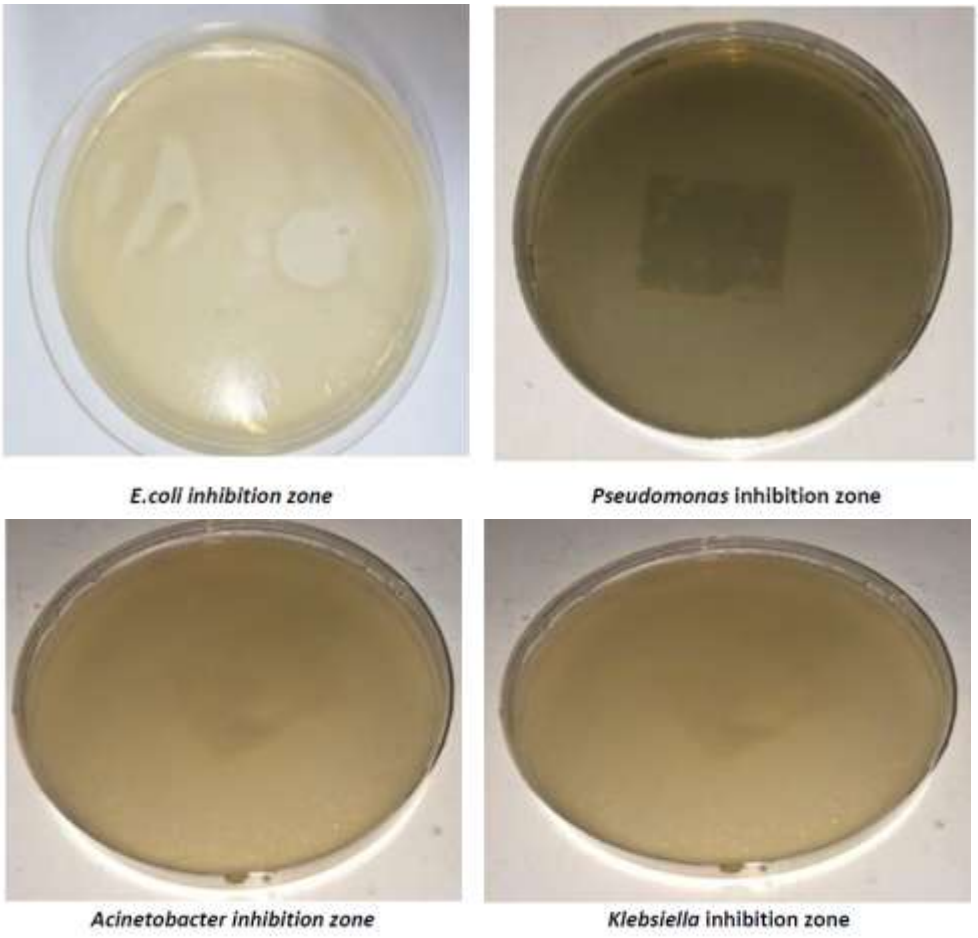

Fig (3): Inhibition zone with gram negative bacteria

Table (1): Antibacterial activity of **the haemolymph of *S. litorallis*** against different strains of Gram-positive, Gram-negative bacteria

Collected hemolymph	**Inhibition zone (cm) (mean ± SE)**		**t-test**	**p-value**
	Gram +ve Bacteria	**Gram -ve Bacteria**		
Immediately isolated	1.53±0.09	1.50±0.07	0.374	0.714
After 60 min	0±0	0±0	---	---
Uninjected sample	0±0	0±0	---	---

Using: Independent Sample t-test; p-value >0.05 NS

DISCUSSION

Multidrug resistant bacteria are a global threat to the human health and abusing of antibiotics is the main cause of this problem. Research for new natural antibiotic is the new era those days. Insects resist the bacterial infection by mounting powerful immune responses that are intervened by hemocytes, the fat body, the midgut, the salivary glands and other tissues (Hillyer, 2016). Phenoloxidase is the authoritative of invaders' PAMPs on

PRPs actuates the mix of antimicrobial proteins or starts the proteolytic incitation of phenoloxidase cascade (Yu XQ *et al*., 2002; Marmaras and Lampropoulou, 2009; Tsakas and Marmaras, 2010). *Spodoptera littoralis* was the insect chosen in this study, since it had demonstrated a great success in this kind of immune-logical thinks (Paterson *et al*., 1987; Seufi *et al*., 2011; Basiouny *et al*., 2016). This comes from the reality that they can be easily reared with cheap media and materials, can be kept up in huge numbers, easily identified, have a quick lifecycle and they have huge blood volume. According to Gholami *et al.*, (2013) , the Phenoloxidase (PO) microbial activity may decrease by 50% after mins. This is why three samples had been tested and it has been supported by this study as the sensitivity showed in the immediate phenoloxidase had been disappeared after 1hr. The antimicrobial impact of responsive intermediates deliverd in the phenol oxidase-catalyzed responses. After being treated with *Manduca sexta* phenoloxidase and dopamine, tiny examination appeared melanin testim ony on cell surface, accumulation of bacteria, and misfortune of cell versatility. Viability tests uncovered the major diminishes within the bacterial colony counts and since the diminish remained noteworthy after scattering of the cell clumps, the receptive compounds were derived to have amassed and killed *E. coli* and *B. subtilis* cells. Beneath the exploratory conditions, 60–94% of the Gram-negative microscopic organisms (*E.coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) and 52–99% of the Gram-positive microscopic organisms (*Bacillus cereus, B. subtilis, Micrococcus luteus,* and *Staphylococcus aureus*) were killed. wWithin the nearness of phenol oxidase (Zhao *et al.*, 2007). While in this study the effect of PO on gram positive bacteria (*Staph. aureus* and *Enterococci*) was greater than the effect on gram negative bacteria, as long as on the gram negative it affect only the growth of the *E.coli* and this may be due to the weakness of the *E. coli* as a strain, while on the remaining strong pathogenic gram negative tested strain (*Pseudomonas, Acinetobacter* and *Klebsiella*) it showed no effect on their growth. The results mentioned in this study indicated that the PO has an antibiotic activity toward the bacteria, specially the gram positive and may have effect on the weak gram negative bacteria.

Referring to Gonzalez-Santoyo and Cordoba-Aguilar (2011) PO are expressed as dormant zymogens (proPOs) in all insects and are changed over to active PO when required. ProPOs are polypeptides with a total weight of 50–60 and 70–80 kDa in their active and inactive frame.

In this study, PO was purified with different purification protocols. The molecular weight of PO was estimated on SDSPAGE with a single band of approximately 70 kDa. The molecular mass of PO from other markers has been reported as follows: protein markers were separated into 14 bands with molecular weights (MW) 200, 150, 120, 100, 85, 70, 60, 50, 40, 30, 25, 20, 15 and 10 kDa. These marker proteins used as a reference for the apparent separated protein bands. Schnepf *et al.* (1998) and De Maagd *et al.* (2001) reported that Delta-endotoxins from the spore-forming bacterium, *Bacillus thuringiensis,* are toxic to a variety of insect species with a very high specificity. Susceptibility of pests to these toxins is influenced by the accomplishment of many steps such as crystal solubilization, protoxin activation by midgut proteases (Lightwood *et al*., 2000; Rausell *et al*., 2004), and binding of the toxin to the receptors located on the brush

border membrane vesicles (BBMV) (Schnepf *et al*., 1998). An alteration in one of these steps may be a cause of larvae sensitivity modification or resistance emergence (Ferré and Van Rie, 2002). Abdelkefi *et al. (*2011) reported that *B. thuringiensis* toxin has a higher LC50 when tested against *S. littoralis* larvae. The authors demonstrated that B. thuringiensis was active against first instar larvae of the polyphagous S. littoralis with an LC50 of about 305 ng/cm^2.

In the present study the susceptibility tests of *S. litorallis* adult indicated that the mortality percentages were recorded after 24 hr for *B. thuringiensis* post-injection. The estimated LC50 value, at 95% probability, was 2 x 10^7 cells/ml. The estimated LC20 was 2x10^6 cells/ml. This concentration was found to stimulate the immune response of larvae and at the same time did not cause high mortality rate. Therefore, this concentration 2x10^6 cells/ml was used as sublethal concentration to investigate the subsequent experiments.

The Hemolymph PO has been implicated in resistance to a range of pathogens, including nucleopolyhedro viruses (NPVs), fungi, nematodes and parasitoids (Rowley *et al*, 1990; Ourth and Renis, 1993; Hagen *et al*., 1994; Hung and Boucias, 1996; Washburn *et al*., 1996; Bidochka and Hajek, 1998; Reeson *et al*., 1998). However, PO in other parts of the body may also play an important role in immunity. NPVs enter the body via the midgut and proceed by infecting the associated tracheal cells (Washburn *et al*., 1996). The authors showed that in refractory Helicoverpa zea, these infected cells were encapsulated and melanized, halting the spread of the virus. This suggests a possible role for midgut PO in viral resistance. In *Anopheles gambiae*, *Plasmodium cynomolgi* ookinetes are encapsulated between the midgut epithelial cells and the midgut basal lamina. It has been shown that refractory individuals have higher midgut PO levels than susceptibles after an infective blood meal. This suggests that their refractoriness may, in part, be due to phenoloxidase activity (Paskewitz *et al*., 1989). PO has also been used as an indicator of immune function, for example, Reeson *et al.* (1998) showed that larvae of the African armyworm, *Spodoptera exempta*, that had been reared at high densities had significantly higher haemolymph PO levels and higher NPV resistance than those reared solitarily. However, a direct link between PO activity and intra-specific variation in parasite resistance has yet to be conclusively demonstrated and it is important to examine the association between PO and pathogen resistance, and to determine any associated costs of pathogen resistance (Wilson *et al*., 2001).

Hassan *et al*. (2012) reported that the present findings showed that, larval hemolymph of untreated and treated samples of *S. littoralis* had 18 bands of proteins. The protein patterns had Rf ranged from 0.02 to 0.74. Also, there were differences in the protein patterns between treated and untreated larvae. The treatment with novaluron and pyrialyl caused disappearance of normal bands and /or appearance of abnormal bands as compared to the control samples.

Ashida and Brey (1997) reported that PO are expressed as inactive zymogens (proPOs) in all insects and are converted to active PO when required. ProPOs are polypeptides that contain two copper atoms per protein molecule, with a total weight of 50–60 and 70–80 kDa in their active and inactive forms, respectively.

In this study the larvae of *S. littoralis* were more susceptible to gram positive bacterial than gram negative bacterial. This

difference in pathogenicity came from the presence of an outer membrane in the cell wall of G -ve bacteria, made up of LPS and acted as an endotoxin, which was absent in gram positive bacterial (Sewify *et. al*. 2017).

The normal insects exhibited a very weak antibacterial activity towards virulent bacteria without receiving any antigenic challenge, because of naturally occurring antimicrobial substances found in the food. Indeed, a weak antibacterial response to water challenge (control) was observed and may be due to either a lower sensitivity or a higher induction-specificity of the larval immune system. The bacterial virulence factor (SEA) induced the strongest antimicrobial activity in larval hemolymph against all the studied bacterial species.

Hemolymph of bacteria-injected *S. littoralis* larvae recorded drastic changes in both the total protein content and the protein banding patterns following injections.

The PO antimicrobial activity decreased significantly after 1 h post-injection. This can be attributed to the intensive consumption of plasma proteins during multiplication and growth of bacteria. Also, some hemolymph sticky and soluble proteins may be involved in the attachment of the injected pathogens to the hemocytes or some native proteins might be converted into glycoproteins or lipoproteins after injection (Gholami *et. al* 2013).

Demir *et al*. (2002) reported that *Klebsiella* spp. and *Enterobacter* spp. are closely associated with many insect species and species belonging to these genera and they are not generally insect pathogens. They probably play roles in the digesting processes in the insect gut and in the physiological developments of *S. littoralis* larvae (Ademolu and Idowu, 2011). *Klebsiella* species (SL2 and SL5) and 2 *Enterobacter* (SL3 and SL4) strains were isolated from *S. littoralis*. The *Enterobacter* isolates did not show good activity against *S. littoralis*, while the *Klebsiella* species caused significant mortalities in *S. littoralis* larvae. (Demir *et al.,* 2002; Ademolu and Idowu, 2011).

Staphylococcus species have been isolated from different insect species (İnce *et al.*, 2008), although Bucher (1981) indicated that *Staphylococcus* species are rarely associated with insects. In the current study *Staphylococcus* sp. was isolated from SL9 *S. littoralis* larvae, but this isolate did not show significant mortality in the pest. Ravensberg (2011) indicated that all larval stages of *S. littoralis* were susceptible to the isolate Bacillus thuringiensis subsp. Kurstaki (MnD) at the same rate, except for the second-instar larvae. The second-instar larvae were also infected with MnD, but they were found to be more resistant than the other development stages. In most cases, all larval stages of insect pests are susceptible to pathogens; therefore, often the larval stage is the preferred stage in field studies (Ravensberg, 2011).

REFERENCES

Basiouny, A.; Ghoneim, K.; Tanani, M.; Hamadah, K.H. and Waheeb, H. (2016). Disturbed protein content in Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) by some novel chitin synthesis inhibitors. Int. J. Adv. Res. Biolog. Sci., 3(3):, 1-12.

Ben, M.S. and Schmid-Hempel, P. (2006). Insect immunity shows specificity in protection upon secondary pathogen exposure. *Current biology : CB*: 16(12):1206-1210.

Broderick, N.A.; Robinson, C.J.; McMahon, M.D. (2009). Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera . BMC Biol., **7:** 11

Carlos, R. (2017). Cellular and molecular mechanisms of insect immunity. Insect Physiology and Ecology, published by INTECH open science, 179-212. DOI: 10.5772/67107.

Campbell,JJR. and Konowalchuk, J. (1948). Counts of Bacteria in Raws Milk. Canad. J. Res., 26e(6): 295-298

De Maagd, R.; Bravo, A. and Crickmore, N. (2001). How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends in genetics (TIG), 17: 193-9. DOI:10.1016/S0168-9525(01)02237-5.

Dillon, R.J. and Dillon, V.M. (2004). The gut bacteria of insects, Nonpathogenic Interactions. Ann. Rev. Entomol., 49:71-92.

Ebbensgaard, A.; Mordhorst, H.; Overgaard, M.T.; Nielsen, C.G.; Aarestrup, F.M. and Hansen, E.B. (2015). Comparative evaluation of the antimicrobial activity of different antimicrobial peptides against a range of pathogenic bacteria. PLoS ONE, 10(12): e0144611. doi:10.1371/journal.pone.0144611

Franssens, V. (2006). Study of two types of immune responses in insects: nodulation in the flesh fly, *Neobellieria bullata*, and prophenoloxidase activation in the desert locust, *Schistocerca gregaria*. MEI press.

González-Santoyo, I. and Córdoba-Aguilar, A.(2011). Phenoloxidase: a key component of the insect immune system. J. Entomol Experimentalis et Applicata. DOI: 10.1111/j.1570-7458.2011.01187.x

Gholami, T.; Ghadamyari, M.; Oliaee, A. O. and Ajamhasani, M. (2013). Effects of inhibitors on haemolymph phenoloxidase from rosaceous branch borer, *Ospheranteria coerulescens* (Coleoptera: Cerambycidae). J. Plant Prot. Res., 53(4), 324-332. At https://doi.org/10.2478/jppr-2013-0049

Heimpel, A.M. and Angus, T.A. (1960). Bacterial insecticides. Bacteriol. Rev., 24: 266–288.

Hillyer, J.F. (2016). Insect immunology and hematopoiesis. Dev. Comp. Immunol., (58): 102–118.

Kamal, M. (1951). The biological control of the cotton leafworm *Prodenia litura* F. in Egypt. Bull. Soc. Ent. Egypt, 35: 221-270.

Kim, J.J. and Kim, W.Y. (2013). Purification and characterization of polyphenol oxidase from fresh ginseng. J. Ginseng Res., 37(1): 117–123. https://doi.org/10.5142/jgr.2013.37.117

Kumar, P.; Kizhakkedathu, J.N. and Straus S.K. (2018). Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. Biomolecules, 8(1): 4.

Marmaras VJ, Lampropoulou M. Regulators and signalling in insect haemocyte immunity. Cell Signal. 21: 186-95, 2009.

Miranpuri, G.S. and Khachatourians, G.G. (1993). Role of bioinsectictdes in integrated pest management (IPM) and insect resistant management (IRM) practices. J. Insect Sci., (6): 161-172.

Moore, I. and Navon, A. (1973). Studies of the susceptibility of the cotton leafworm, *Spodoptera littoralis* (boisduval), to various strains of *Bacillus thuringiensis* Phytoparasitica, 1: 23–32.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680-685.

Paterson, R.R.M.; Simmonds, M.S.J. and Blaney, W.M. (1987). Mycopesticidal effects of characterized extracts of Penicillium isolates and purified secondary metabolites (including mycotoxins) on *Drosophila melanogaster* and *Spodoptora littoralis.* J. Invert. Pathol., 50(2): 124-133.

Prasad, S.V.; Fiedoruk, K.; Daniluk, K.; Piktel, E. and Bucki, R. (2020). Expression and Function of Host Defense Peptides at Inflammation Sites. Int. J. Mol. Sci., 21(1): 104. doi.org/10.3390/ijms21010104

Schnepf, E.; Crickmore, N.; Van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J. and Zeigler, D.R. (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Reviews, 62(3): 775-806. DOI: 10.1128/MMBR.62.3.775-806.1998

Seufi, A.M.; Hafez, E.E. and Galal, F.H. (2011). Identification, phylogenetic analysis and expression profile of an anionic insect defensin gene, with antibacterial activity, from bacterial-challenged cotton leaf-worm, *Spodoptera littoralis*. BMC Molecular Biology, 12(1): 47.

Sewify GH., Hamada HM., Alhadrami HA.(2017). In Vitro Evaluation of Antimicrobial Activity of Alimentary Canal Extracts from the Red Palm Weevil, Rhynchophorus ferrugineus Olivier Larvae. BioMed Res. Int. Vol 2017, Article ID:8564601. doi.org/10.1155/2017/8564601

Tsakas, S. and Marmaras, V. (2010). Insect immunity and its signaling: an overview. Invert. Surv. J. (ISJ), 7:228-238.

Wang, S.; Zeng, X.; Yang, Q. and Qiao, S. (2016). Antimicrobial Peptides as Potential Alternatives to Antibiotics in Food Animal Industry. Int. J. Mol. Sci., 17(5): 603.

Wang, Q.; Ren, M.; Liu, X.; Xia, H. and Chen, K. (2019). Peptidoglycan recognition proteins in insect immunity. J. Mol. Immunol., 106:69-76.

WHO (2018). Antibiotic Resistance -Fact Sheets. At https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

Yu, X.Q.; Zhu, Y.F.; Ma, C.; Fabrick, J.A.; Kanost, M.R. (2002). Pattern recognition proteins in *Manduca sexta* plasma. Insect Biochem. Mol. Biol., 32:1287-1293.

Zhao, P.; Li, J.; Wang, Y. and Jiang, H. (2007). Broad-spectrum anti-microbial activity of the reactive compounds generated in vitro by *Manduca sexta* phenoloxidase. Ins. Bio. Chem. and Mol. Biol., (9):952-959.

Evaluation of the antimicrobial activity of purified *Spodoptera littoralis* hemolymph against some pathogenic bacteria

تقييم النشاط المضاد الحيوى للهيموليمف المنقى من حشرة دودة القطن سبودوبترا ليتوراليس ضد بعض البكتيريا الممرضة

حنان سيد عامر[1] , دعاء سليمان[2] ,وائل سعد عبد المجيد[3] , شيماء احمد مؤمن[2] ,تامر رشدى[3] ,نادية محمد لطفى[2]

1 - مستشفى عين شمس التخصصى , القاهرة, مصر.

2 - قسم الحشرات-كلية العلوم جامعة عين شمس, القاهرة,مصر.

3-قسم البيولوجيا الجزيئية,معهد ابحاث الهندسة الوراثيا والبايوتكنولوجى , جامعة السادات, المنوفية,مصر.

المستخلص

تاثير وفاعلية المضاد الحيوى للفينول اوكسيدز هو محل اهتمام كبير هذه الايام بسبب الاتجاه للبحث عن مضادات حيوية طبيعية بدلا من المضادات الحيوية الكيميائية . فى هذخ الدراسة تم تفعيل البروفينول اوكسيدز الى فينول اوكسيدز داخل حشرة دودة القطن سبودوبترا ليتوراليس عن طريق حقن سلسة من التركيزات المختلفة من بكتيريا الباسيلس (3200 ميكروجرام –بودرة) $2X10^{50}$, $2X10^{40}$, $2X10^{30}$, $2X10^{20}$, $2X10^{10}$و $2X\ 10^{60}$ خلية/مل. تم حقن 10 ميكرلتر من كل تركيز داخل مجموعات من يرقات حشرة دودة القطن (10 يرقات فى المجموعه) بمجموع 656 يرقة تم حقنهم. تم بقاء 519 على قيد الحياة و ماتت 137 يرقة. وجد ان تركيز $2\ X\ 10^{60}$ هو تركيز قتل 20% , بعد الحقن والتحضين ب 24 ساعة تم تطهير اليرقات الحية وتم سحب و فصل الهيموليمف . تنقية الفينول اوكسيدز من الهيموليمف تمت عن طريق عمود فصل هاى تراب عمود 1مل, بعد التاكد من وجود الفينول اوكسيدز عن طريق جل الس دي اس , تم زرعه مع 6 انواع من البكتيريا الممرضة, نوعين من موجبين الصبغة (ستاف اوريس, انتيروكوكاى) و اربعة أنواع من سالبة الصبغة (اى كولاى, سودوموناس, اسينتوباكتر,كليبسيلا). ولقد كانت النتائج ايجابية ضد البكتيريا الموجبة للصبغة وفقط مع بكتيريا الاى كولاى (من البكتيريا السالبة للصبغة) , ولذلك يمكن استنتاج ان فاعلية المضاد الحيوي الفينول اوكسيدز تؤثر على البكتيريا الموجبة للصبغة اكثر من تاثيره على البكتيريا السلبية للصبغة.