Isolation of the Astacin-like metalloprotease coding gene (*astl*) and assessment of its insecticidal activity towards *Spodoptera littoralis* and *Sitophilus oryzae*

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Abstract

Astacin-like metalloprotease, possessing a zinc (II) ion in the catalytic center is one of the toxic proteases. It exists in a variety of organisms, including fish, frogs, birds and insects. The present investigation was conducted with the main goal of assessing the efficacy of astacin like metalloprotease toxin for pest control and to determine the probability of using it to produce a new biopesticide that is friendly to the environment. Therefore, the full length of *astl* cDNA was cloned from spider species, *Hasarius adansoni*. Sequencing of the cloned *astl* cDNA has proved that its full length includes 802 bp with 714bp open reading frame encoding 238 amino acids. 486bp of the catalytic domain was cloned and expressed by the yeast expression system *Pichia pastoris*, and its insecticidal activity was determined towards two species of agricultural insects from two different orders, *Spodoptera littoralis* (Lepidoptera:Noctuidae) and *Sitophilus oryzae* (Coleoptera:Curculionidae). Bioassay was performed using three concentrations (100,500 and 1000 μg/ml) for four days for *S. littoralis* and 14 days for *S. oryzae*. At the concentration 1000 μg/ml, the mortality ratio was 69.3%±2.51, 65%±2.6 and 64.0%±3.0 for first instar *S. littoralis*, second instar *S. littoralis and S. oryzae* adults respectively. Finally, the present study represents an evidence that the use of zinc metalloproteases derived from different spiders may play an effective role in insect control.

Keywords: Metalloprotease, *Spodoptera littoralis, Sitophilus oryzae*

1. Introduction

Spider venoms are a cocktail of several different peptide toxins, most of which are likely to have insecticidal activity towards different insect orders (King et al. 2002, Escoubas et al., 2006 and Windley et al., 2012). Many of the insecticidal peptide toxins have been isolated from spider venoms, and their activity has been examined. Some of spider toxins showed no adverse effects on economically important insects such as Hv1a that is harmless to the pollinating insect honey bee (Nakasu et al. 2014). Zinc metalloproteases (enhancins) expressed by some baculoviruses improve the baculoviral infection into the insect larvae by increasing the permeability of peritrophic matrix (PM) through digestion of the PM proteins (Derksen and Granados 1988; Wang et al. 1994 and Lepore et al., 1996). Therefore, zinc metalloproteases can be exploited in insect control field outside the baculoviruses context (Harrison and Bonning 2010). Astacin-like metalloprotease proteins are secreted as zymogens as low molcular mass proteases that are activated by the cleavage of the prosegment from the catalytic domain (Yiallouros et al. 2002 and Guevara et al. 2010). Stöcker et al. (1988) stated that astacin family consisted of six genes (*bmp1, tll1, tll2, mep1a, mep1b and*

astl). Astacin (*astl*) is a multi-domain metalloprotease that is distinguished by the presence of zinc binding motif (HEXXHXXGXXH) and methionine-turn (MXY) (Gomis-Rüth et al. 2012). The *astl* gene is expressed as the hatching enzyme in the oocyte and in the developing embryo (Gomis-Rüth et al. 2012). The hatching enzyme is responsible for degradation of embryonic envelopes in crustaceans, fish, frogs, and birds (Gomis-Rüth et al. 2012). It is also known as ovastacin in mammals (Quesada et al. 2004) and plays a role in egg-sperm interactions (Sachdev et al. 2012).

The agricultural and horticultural pest insects cause 40% loss of the crop yield worldwide, estimated by 17.7 billion dollars annually (Oerke et al. 1994 and Oliveira et al. 2014). *Spodoptera littoralis* and *Sitophilus oryzae* are two of the most dangerous agricultural insect pests that are able to cause a lot of harms to various important crops as cotton, maize, tomatoes and stored grains (Madrid et al. 1990 and Salama et al. 1970). These agriculture pests are controlled by different methods such as chemical insecticides which are extensively used in Egypt (Mansour 2004). Therefore, resistant strains of insect pests have appeared in the field (Sawicki 1986). An alternative control method used in the control strategy is biological agents such as natural enemies, nuclear polyhedrosis virus

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(Jones et al. 1994 and Atia et al. 2016), *Bacillus thuringiensis* and its derivatives (Navon et al. 1983 and Moussa et al. 2016) and fumigant toxicity of some plants as Uvaria afzelli (Olufemi-Salami et al. 2018). However, *S. littoralis* and *S. oryzae* developed resistance to biological control agents (Salama et al. 1989). New biopesticides are always required to be involved in control strategy. Many spider venoms toxins were exploited as bioinsecticides against various insect pests such as ω-hexatoxin, OAIP-1, brachyin and knottins (Fitches et al. 2012, Hardy et al. 2013, Pyati et al. 2014, Zhong et al. 2014 and Matsubara et al. 2017).

In this article, the astacin like metalloprotease coding gene (*astl*) has been isolated from spider *Hasarius adansoni*, expressed in yeast and the toxicity of the astl protein was demonstrated towards *S. littoralis* and *S. oryzae*.

2. Material and methods

2.1. Spider collection

The spider samples were collected from human houses in Cairo, Egypt. The spider species used in the current study was identified as *Hasarius adansoni* using the DNA barcode technology by amplification of 889 bp of ribosaml RNA (*rRNA*) gene large subunit and 705bp of the 5'-end of mitochondrial cytochrome c oxidase subunit 1(*COI*) gene. The fragments of *rRNA* and *COI* were amplified using specific primer sets Sp28SN/Sp28SC (Starrett and Hedin, 2007), and SP-LCO 1490FJJ/SP-HCO2198RJJ (Astrin et al., 2016), respectively. The spider species has been confirmed by the complete homology of the two fragments with *H. adansoni* using "BLASTN" NCBI-BLAST alignments tool. The cephalothorax region was dissected in paraffin wax plate and isolated from the rest of the body.

2.2. Total RNA extraction

The total RNA was extracted from the cephalothorax region of the spider by Trizole® reagent (Invitrogen, USA, Cat.#15596-026). First strand cDNA was synthesized using SuperScript™II reverse transcriptase (Invitrogen Cat.# 18064014) according to the manufacturer's instructions.

2.3. Cloning of astl cDNA

A set of degenerate primers astlFD: CARTTCTCGGAGGTATTGAAGCWGG and astlRD:AATRAAAGGAAGCKAGCAGAA was designed to amplify a part of the nucleotides sequence of the astacin like metalloprotease cDNA using the first strand cDNA as a template. The 5' and 3' ends were amplified using First Choice ® RLM-RACE kit (Ambion, Austin, TX, USA). According to the kit instructions, two reverse primers, astlR1: CCGTCGAAGATGGAGCCGA and astlR2: CCTTGGGGATGTAGATGGACT, were used to amplify the 5' end. In a similar way, the 3' end was amplified using two forward primers;astlF1: TTCCCCTCCATCAACTGGCTC and astlF2: TTGCCTGTACGCAGCACTGGG TC.

2.4. Sequences and phylogenetic analysis

The alignment of *astl* cDNA sequence was performed using the "BLASTN" and "BLASTX" tools. The ExPASy translate tool (http://web.expasy.org/translate/) was utilized to deduce the amino acid sequences of astl cDNA clones. The phylogenetic tree was carried out by aligning astl amino acid sequence and other spiders and a scorpion sequences using the phylogeny.fr software (http://www.phylogeny.fr/index.cgi). Molecular weight and isoelectric point were predicted by ExPASy Proteomics website (http://web.expasy.org/cgi bin/compute_pi/pi_tool). The motifs were determined using PROSITE database (http://prosite.expasy.org). The glycosylation sites were scanned by NetNGLyc 1.0 software (http://www.cbs.dtu.dk/services/NetNGlyc/) and YinOYang program (http://www.cbs.dtu.dk/services/YinOYang/).

2.5. Cloning of astl, GNA catalytic regions and astl/GNA fused fragment into the yeast expression vector pPICZαA

The cloning strategy of astl catalytic domain in the expression vector pPICZαA was performed through the digestion by restriction enzymes EcoR1 and Xba1 then the ligation by T4 DNA ligase. The grown colonies were screened to select the positive clones.

2.6. Transformation of pPICZαA-astl, pPICZαA-GNA and pPICZαA-astl/GNA into yeast "Pichia pastoris" , KM71H strain.

pPICZαA-astl (3-5μg) was digested using *SacI* followed by purification and transformation into yeast cells, *Pichia pastoris* yeast competent cell strain "KM71H". The transformed cells were incubated at 28°C for 2-3 days on YPDS medium plates containing100mg/ml Zeocin. Then, the colonies were numbered and transferred to YPD plates containing100mg/ml Zeocin, and incubated for two more days and allowed to grow.

A number of grown yeast colonies were dissolved in 20mM NaOH then boiled for 45 min at 95°C. After centrifugation, 2μl supernatant was used as a template. The putative positive clones were identified using PCR screening.

2.7. Expression of Ha-astl protein

The secreted protein of pPICZαA-Ha-astl was collected through the induction of a single colony of KM71H harboring pPICZαA-Ha-astl by methanol for four days. The expression analysis was conducted on secreted proteins in the supernatant. SDS–PAGE, Western blot analysis and ELISA test were used to analyze the expressed proteins as explained in Salem et al. 2019.

The concentration of protein was determined at protein A280 using Nanodrop 2000/2000C spectrophotometer, (ThermoScientific, USA).

2.8. Insect cultures

The *S. littoralis* colony was reared in the insectory of the Agriculture Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC) under standard conditions. The larvae were reared on cleaned castor (*Ricinus communis*) leaves at 25±2°C and 16 h /8 h light-dark period .

S.oryzae was kindly supplied by the Plant Protection Research Institute (PPRI), ARC. The adults of the same age were reared on wheat seeds in glass jars under standard conditions and then used to assess the insecticidal activity.

2.9. Bioassay

The insecticidal activity of astl was assessed by feeding the *S. littoralis* larvae and the *S. oryzae* adults on three different concentrations (100, 500 and 1000 µg/ml) of the protein. The castor leaves were immersed in solution containing the assayed amount of protein with agitation for half an hour. The leaves were then picked up from the expressed culture supernatant and allowed to air dry. Twenty five and twenty of first and second stadium of *S. littoralis* larvae, respectively, were added to treated castor leaves which were replaced daily with fresh treated leaves for four days. Each treatment was repeated three times. The larval mortality was recorded daily during experiment period. The sensitivity of *S.oryzae* adults to astl was assayed for 14 days period. Ten g of wheat seeds were immersed in protein solution for 30 minutes. The treated wheat seeds were then filtered and allowed to dry. Twenty-five adults were added to each 10 g of treated wheat seeds in glass jar with three replicas for each concentration. The jars were incubated at 27°C for 16h/8h light/dark period. The adult mortalities were recorded on day 14. For all bioassays, both larvae and adults were treated with the control "expressed culture supernatant of native pPICZαA"

2.10. Statistical analysis

the significance of mortality results was calculated by Student's t-test in the Excel program using the calculations (Total, Percentage and STDEV) of the three replicates for each experiment .

3. Results

3.1. Amplification of the full length of Ha-astl cDNA sequence

The first strand cDNA of the *H. adansoni* cephalothorax, was used as a template to amplify Astacin-like metalloprotease coding gene (*astl*). One degenerate primer set was designed for astacin-like metalloprotease coding gene (*astl*) from the conserved regions of the same gene in the spider *Latrodectus hesperus* and the scorpion *Tityus serrulatus*. A 119 bp fragment was firstly amplified using the degenerate primers. The 5' and 3' ends were amplified using two PCR rounds of RACE based on the sequence of the first amplified fragments; it was deduced that the full length of astacin like metalloprotease of *H. adansoni* (*Ha-astl*) cDNA is 802 nucleotides (accession no. MN453831). The open reading frame consists of 714 nts which encodes 238 amino acids and with catalytic domain between N46 and C238. The calculated molecular weight of encoded protein is 27.33 kDa with an isoelectric point (pI) of 8.29. The analysis of Ha-astl protein reveals the presence of two disulfide bridges between cysteine residues at positions 87-238 and 108-128. In additions, three zinc binding sites at histidine residues were found at positions 136,140 and 146 using the site (http://prosite.expasy.org). Also, a putative N-glycosylation site was predicted at aa 149, and 0-β-glycosylation sites were predicted at T56, S70 and T94 (Fig 1a).

The deduced amino acid sequence was aligned using the BLASTp tool in the NCBI website. The covered score was 88-99%, and the identity ranged between 74-84% in spiders such as *Trichonephila clavipes*, *Latrodectus hesperus*, *Stegodyp husmimosarum* , *Parasteatodate pidariorum* and scorpion as *Tityus serrulatus* with accession no. PRD25795.1, ADV40108.1, KFM63176.1, XP_015909675.1 and CDJ26716.1, respectively. The alignment results of the deduced amino acids sequence with other published astacin like metalloprotease toxin are shown in Fig (1b). The phylogenetic tree performed by the phylogeny.fr software is shown in Fig (1c). These results demonstrated that the Ha-astl aa sequence is closely related to that of the spider *T. clavipes* and the scorpion *T. serrulatus*.
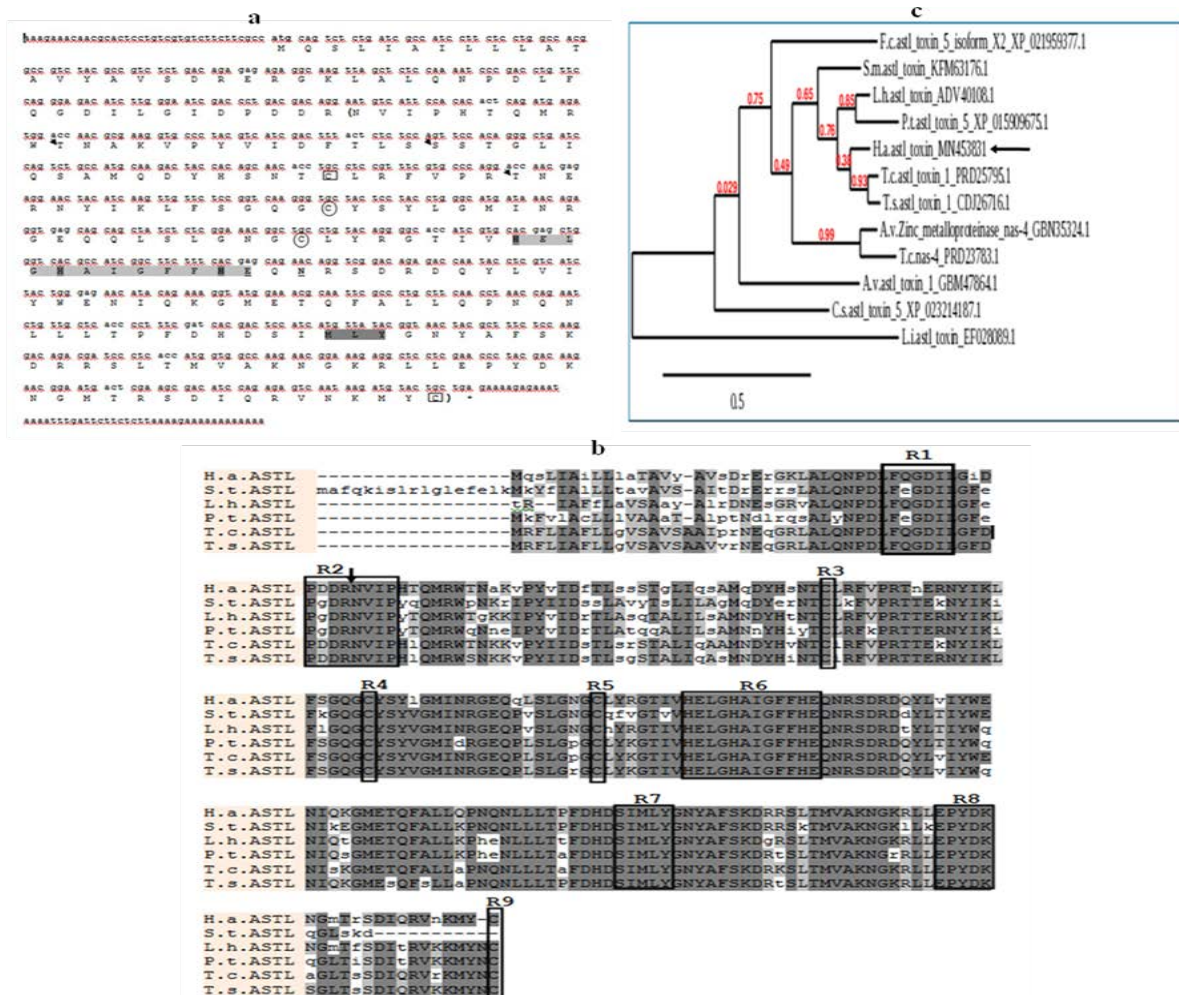
Figure 1. (a) Nucleotides and deduced amino acid sequence of *Hasarius adansoni* Astacin-like metalloprotease. The brackets mark to the active domain that start with asparagine N46 and ended at C238. The zinc motif is grey highlighted containing the three metal sites (Zinc) (H: 136,140 and 146) highlighted by dark grey. The astacin hallmark (E 137) is underlined The methionine (MXY) turn is highlighted by grey. The first disulphide bridge is between the two squares (C:87 and C: 238). The second disulphide bridge is between the two ovals (C:108 and C: 128). Amino acid that predicted to be N-glycosylated was underlined (N: 149). Predicted 0-β-glycosylation sites are noted by arrows (T:56, S70 and T:94). The stop codon is referred by asterisk. (b) amino acid alignment of the astacin like metalloprotease toxin in *Hasarius adansoni* (H.a.) (MN453831) with other spiders *Stegodyphus mimosarum* (S.t.), *Trichonephila clavipes* (T.c.) (PRD25795.1), *Latrodectus hesperus* (L.h.)(ADV40108.1) and *Parasteatoda tepidariorum* (P.t)(XP_015909675.1) and a scorpion *Tityus serrulatus* (T.s.)(CDJ26725.1). The identical aminoacids and the conservative substitution are highlighted in dark and light grey respectively. The rectangles (R) marked the regions containing the conserved aspartic residue "D" within the prosegment (R1), the activation site containing the first amino acid in the mature protein asparagines"N" (R2) ,the four conserved cysteine residue (R3,R4,R5 and R9), astacin signature sequence containing the hallmark glutamate"E" (R6), the methionine turn (R7) and the segment mainly engaged in shaping of subsite S_1 (R8) respectively. The black arrow referred to the cleavage point between the prosegment and the catalytic segment. (c) Phylogenetic analysis of amino acids of *Hasarius adansoni* (H.a.)Astacin-like metalloprotease (astl) toxin (referred by black arrow) with other correspondings in other spiders *Folsomia candida* (F.c.), *Stegodyphus mimosarum* (S.t.), *Latrodectus hesperus* (L.h.), *Parasteatoda tepidariorum* (P.t)., *Trichonephila clavipes* (T.c.), Araneus ventricosus (A.v.) and Loxosceles intermedia (L.i.) and scorpions *Tityus serrulatus* (T.s.). , Centruroides sculpturatus (Cs.).

3.2. Expression of pPICZαA-Ha-astl into yeast "Pichia pastoris" "KM71H strain"

Expressing target protein, pPICZαA-Ha-astl bound to His tag, was transformed into the yeast *P. pastoris* "KM71H" competent cells. The cell cultures were induced by methanol to express pPICZαA-- Ha-astl protein. The expressed protein was demonstrated on SDS-PAGE gel.

Expressed Ha-astl showed faint band on the SDS-PAGE at expected molecular mass (Fig. 2a); the extracellular expressed colonies were detected by Western blot analysis (Fig. 2b) in addition to the sandwich ELISA test that detect the expressed proteins targeted His Tag with His-specific antibody with different reading values at OD 450 (Fig 2c).
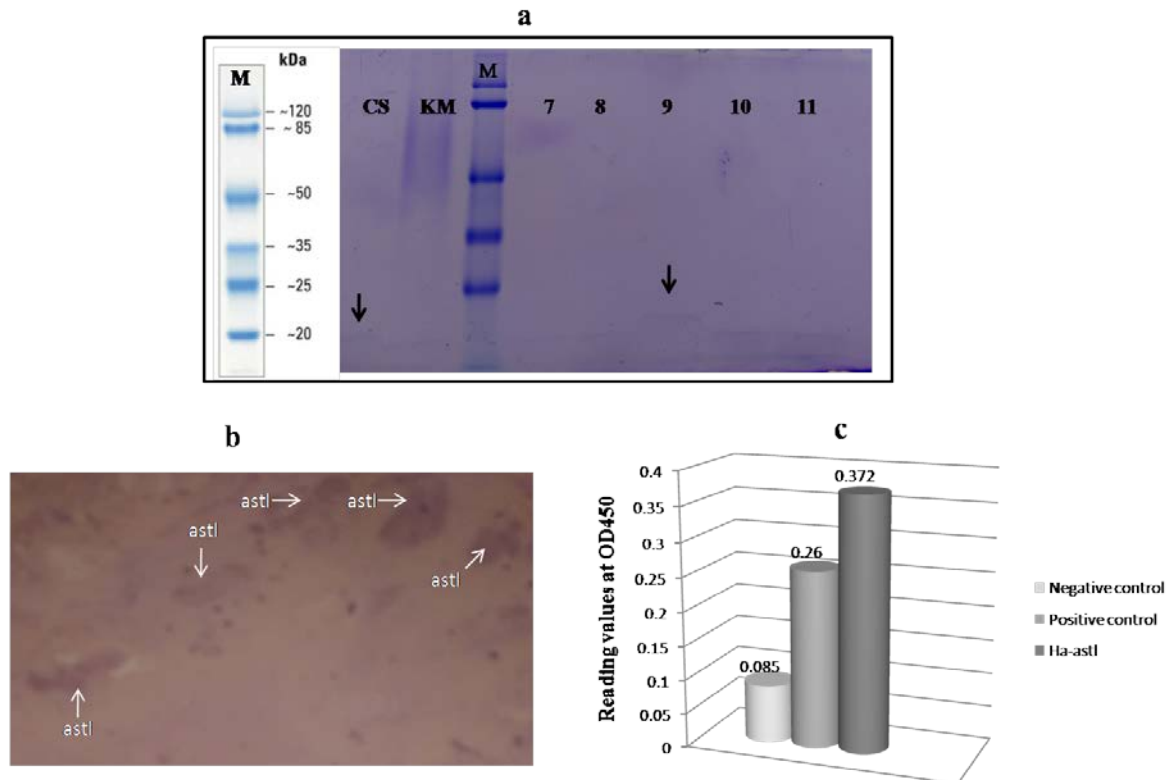
Figure 2. (a) Extracellularly expressing colonies (pPICZαA-Ha-astl). The black arrow refers to the positive clone that revealed the expected expression. KM: empty KM71H strain and CS: secretion control afforded by invitrogen. M. prestained protein molecular weight marker (Thermoscientific, Cat no. 26612) (b) Western blot analysis for the expressed yeast colonies containing the recombinant vectors pPICZαA-Ha-astl (c) Sandwich ELISA test showing the average values of negative control, positive control, pPICZαA-Ha-astl , at OD 450.

3.3. *The insecticidal impact of Astacin-like metalloprotease (Ha-astl), protein on S. littoralis*

The toxic efficacy of Ha-astl was evaluated on the first and second instars of *S.littoralis* larvae *per os* using three different concentrations, i.e. 100, 500, 1000 µg/ml of the protein. The oral activity was performed on treated castor leaves which were replaced daily for four days. While the control larvae were fed on treated castor leaves with the supernatant of expressed native pPICZαA for the same period. The mortality was counted daily throughout the experiment period. The larval mortality ratio showed daily increment during the four-day experiment. For the first instar of the *S.littoralis*, the mortality percentages were 42.6%±2.5, 46.6%±3.05 and   69.3%±2.51 for the three concentrations, i.e. 100, 500, 1000 µg/ml of the protein, respectively. While for the second instar of *S.littoralis* the mortality percentages became 25%±1.0, 58.3%±2.0 and 65%±2.6 for the same three concentrations , respectively. The statistical analysis of the mortality ratio showed significant (b) and highly significant (c) effects of the Ha-astl protein against control larvae at (p>0.05) and (p>0.01) respectively.

Larval mortality of *S. littoralis* and their significance are shown in Fig. (3a) and (3b).

The results of the feeding experiment also demonstrated that the larvae survived in the different treatments showed a significant retardation in their growth (Fig 4a). Some of them retarded to pupate, failed to pupate or developed to malformed pupae compared to the control (Fig 4b). After a recovery period when larvae were transferred onto untreated castor leaves and allowed to grow for 10 to 15 days, the larvae did not restore normal weight. The difference of consumed castor leaves by control and treated larvae was clearly notable as shown in Fig (4c). The average larval weight of the control and treated larvae was 0.05 and 0.013g after 10 days and 0.08 and 0.0085 g after 15 days, respectively.
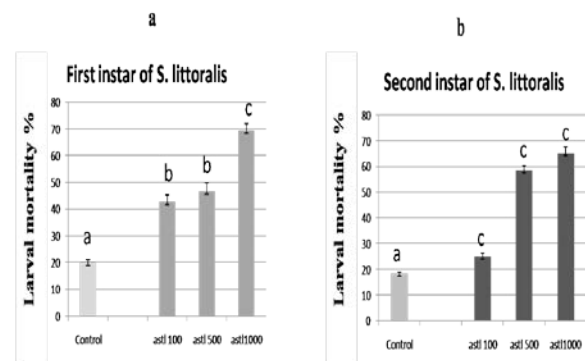

Figure 3. The mortality ratio of the first instar (a) and   for the second instar (b) of *Spodoptera littoralis* after 96 hours using three concentrations (100, 500, 1000 µg/ml) of Ha-astl protein comparing with the control (expressed native pPICZαA protein). Error bars illustrate ±SE. Within the same protein concentration different letters indicate significantly (b) at P < 0.05. and highly significant (c) at P>0.01.
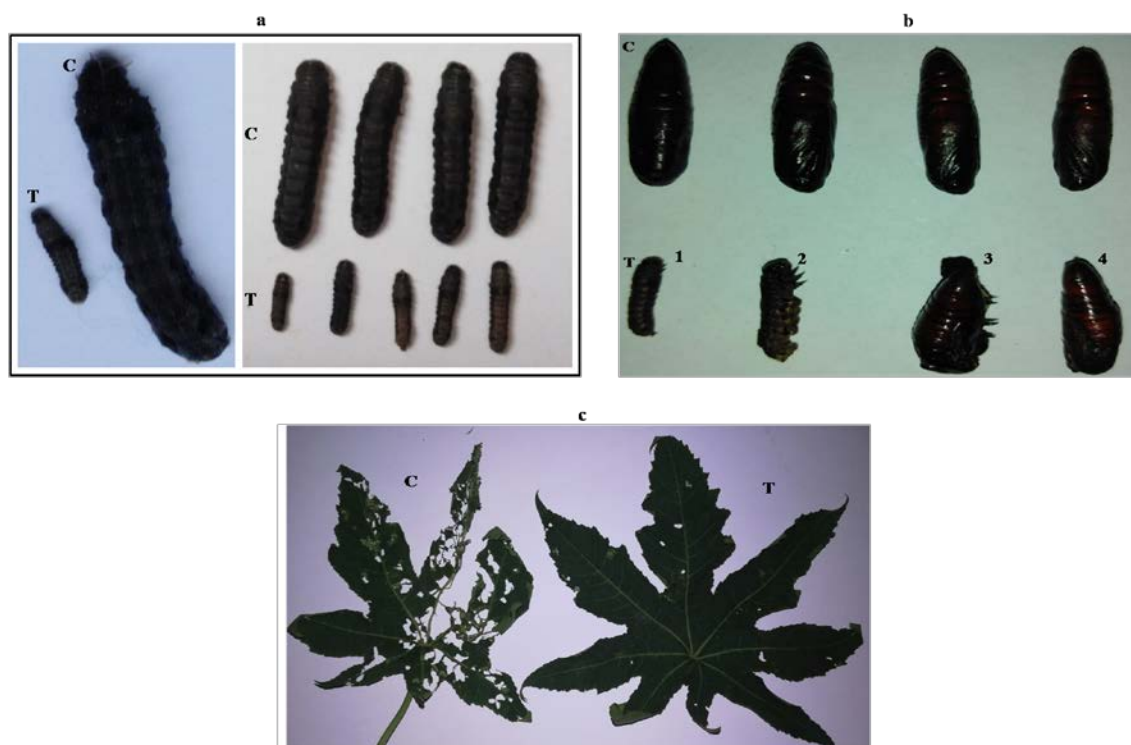
Figure 4. (a) Retardation in growth for the treated larvae using Ha-astl protein (T) against the control (C) in the same age. (b) The development of treated larvae (T) to retarded to pupate (1), failed to pupate (2) or malformed pupae (3 and 4) compared to the control (C). (c) The fed castor leaves by the control (C) and the treated larvae (T).

3.4. The insecticidal activity of Astacin-like metalloprotease (Ha-astl) protein on S.oryzae

The toxicity of the astacin-like metalloprotease was evaluated on the adult of *S. oryzae* by *per os* alongside the control (native expressed pPICZαA). Three concentrations were used, i.e.: 100, 500, 1000 µg/ml of the protein with three replicates each and 25 adults per replica. The adult mortality was recorded two weeks post-treatment. The mortality ratio for Ha-astl protein was 46.6%±0.577, 48%±1.7 and 64%±3 for the three concentrations 100, 500, 1000 µg/ml, respectively. The statistical analysis showed that the mortality ratio was highly significant (c) at (p>0.01) for all the concentrations of the Ha-astl protein. (Fig 5).
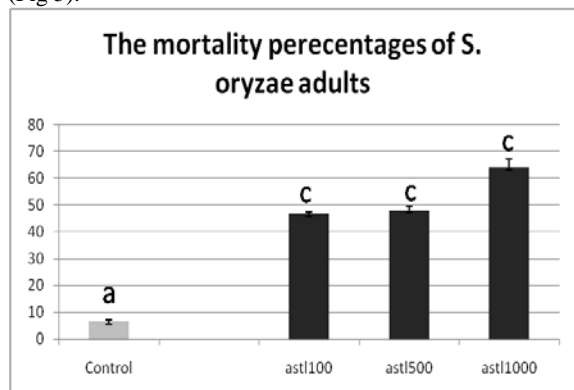
Figure 5. The mortality ratio of the adults of *Sitophilus oryzae* after fourteen days using three concentrations (100, 500, 1000 µg/ml) of Ha-astl protein comparing with the control (expressed native pPICZαA   protein). Error bars illustrate ±SE. Within the same protein concentration different letters indicate highly significant (c) at P>0.01.

4. Discussion

Bioinsecticides are exploited as potential alternatives to pesticides. The sources of biopesticides are natural organisms, or their metabolic products including insecticidal toxins derived from parasitoids   and insect predators as spiders and (Hajek 2004, Chandler et al. 2011, Lacey et al.2015, Silva et al. 2018 and King 2019). This study examined the susceptibility of agricultural insect pests, *S. lettoralis* and *S. oryzae*, (belonging to two insect orders lepidoptera and coleoptera towards expressed metallopeptidase peptide, astacin, derived from the Adanson's house jumper spider, *H. adansoni*. The *Ha-astl* sequence was identified in the total RNA content of *H. adansoni* spider venom. The presence of metalloproteases as components of spider venom was previously detected in *Loxosceles* spider species (Feitosa et al. 1998, Young and Pincus 2001, Da Silveira et al. 2002 and 2007, Zanetti 2002 and Barbaro et al. 2005). Moreover, nine possible isoforms of astacin-like metalloproteases were identified from the Peruvian, *L. laeta* venom (Medina-Santos et al., 2019). Presence of metalloproteases in the spider venom provides evidence of its significant biological activity and its conserved feature in the venom of spider species (Da Silveira et al., 2002; Zanetti, 2002 and Barbaro et al., 2005).

The full length of *Ha-astl* cDNA sequence is a total of 802 nucleotides encoding 238 amino acids peptide. Ha-astl protein shows the same general features of metalloproteases family members (Dumermuth et al. 1991; Bond and Beynon 1995 and Mohrlen et al. 2004). Ha-astl primary structure includes a prosegment region (M1:R45), a catalytic domain (N46:C238) and a conserved methionine- turn MXY (Met192 and Tyr194). The

catalytic domain contains the consensus signature sequence responsible for binding of the catalytic zinc ion, HEXXHXXGXXHE (His136 and Glu147). Approximately 90% of spider-venom toxins possess between one to seven disulfide bridges, however, about 60% of spider toxins have three bridges only (Windley et al. 2012). Two disulfide bridges are potentially present in Ha-astl between Cys108-Cys128 and Cys87-Cys238. The calculated molecular mass and pI of the deduced amino acid of *Hasarius adansoni* (27.3 kDa/8.29) are closely related to other astacin-like metalloproteases in spiders, *Trichonephila clavipes* (27.3 kDa /8.97) , *Latrodectus hesperus* (27.1 kDa /8.81) and a scorpion *Tityus serrulatus* (27.3 kDa /8.9).

The biological significance of native astacin-like venom toxin is not clearly known. However, the toxin is assumed to play a role in the predation of the insect prey as well as a defensive role against predators (Da Silveira et al. 2007). The metalloproteases proteases facilitate the diffusion of other venom toxins through prey bodies by increasing the permeability of prey tissues, and then act in complement with other active toxins (Da Silveira et al. 2007).

Astacin may play role, in synergism with other venom toxins, in the deleterious effects of the prey after envenomation (Futrell 1992) as well as in the activation of peptide derived toxins after proteolysis. However, the toxic efficacy of solely astacin-like metalloprotease on insects is questionable. Could these proteases present the venom cocktail be used in insect control? To answer this question, the insecticidal activity of Ha-astl was assayed *per os* on cotton leaf worm and rice weevil. The N-terminal pro-segments of astacin-like metalloproteases inhibit the catalytic zinc domain and its removal is necessary to uncover a deep active-site cleft that contains aspartate residues in the specificity pocket (Gomis-Rüth et al. 2012). Hence, the catalytic region was used for expression as active astl. To maintain functional configuration of the expressed recombinant protein, an expression system performs post translation modifications was required. Thus, *Pichia pastoris* was used to express Ha-astl and secrete the recombinant protein into culture supernatant. The expressed recombinant proteins were exploited for sensitivity assays. A moderate toxic effect (mortality) of Ha-astl was demonstrated in both insect species. 1000 µg/ml of astacin peptide causes 69.3%±2.51 and 65%±2.64 for first and second instars of *Spodoptera* larvae, respectively. On the other hand, 64% ±3 % of *S. oryzae* adults were killed by the same concentration of Ha-astl protein.. The similar level of lethality was demonstrated by spider neurotoxins on pest species. The insect-selective neurotoxin μ-agatoxin-Aa1 from the venom of the Western grass spider, *Agelenopsis aperta*, cause convulsive paralysis in insects and show variable toxicity against different insect orders. It is very potent to dipterans, moderate to orthopterans, while it shows weak activity towards lepidopterans (Adams et al. 1989). The ω-Hexatoxin-Hv1a toxins from the venom of Australian funnel-web spiders have low ED50 values in Orthoptera, Hemiptera, Dictyoptera, Diptera, Coleoptera, Acarina and Lepidoptera (Atkinson et al. 1996, Fletcher et al. 1997 and Bloomquist 2003). However, proper manipulation of these toxins revealed the prospect use of spider toxins in insect pest control. The

transgenic tobacco expressing ω-HXTX-Hv1a shows tolerance levels against *Helicoverpa armigera* and *S. littoralis* larvae (Khan et al. 2006). The topical application of recombinant thioredoxin-ω-HXTX-Hv1a has also been shown to be lethal to these caterpillar species (Khan et al. 2006).

5. Conclusion

The mortality resulting from the feeding of the astl protein in *Spodoptera littoralis* and in *Sitophilus oryzae* , Also, the retardation in growth of the larvae in *Spodoptera littoralis* proved that the astl protein may play an effective role in insect control as oral bioinsecticides. The actual effect of the astl protein will appear when it is used under different field conditions, and this will determine whether it can be used lonely in insect control or maybe introduced as a new biopesticide agent in the integrated pest management programs. Finally, the using of astl protein is considerd the starting point for the exploiting zinc metalloproteases derived from different spiders in insect control fields, particularly in the view of the decreasing numbers of bioinsecticides available for use.

Acknowledgments

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