Agricultural Science Research Journal Vol. 8(1): 1 – 5, January 2018
Available online at http://resjournals.com/journals/agricultural-science-research-journal.html
ISSN: 2026 –6073 ©2018 International Research Journals

Full Length Research Paper

Electrophoretic analyses of proteins and enzymes in the midguts of palm weevil, *Rhynchophorusphoenicis* F. (Coleoptera: Curculionidae)

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Abstract

Proteins and enzymes are very important in living organisms. Proteins play crucial roles in the repairs and growth of organisms while enzymes which are proteinous in nature, are important in food digestion. Electrophoretic profile of the midgut homogenate of the larvae and adults of *Rhynchophorusphoenicis* F. were carried out to determine their protein bands using sodium dodecyl-sulphate polyacrylamide gradient gel electrophoresis. The results of this work revealed eight distinct protein bands whose relative mobility Rm ranged from 0.06 – 0.75 in the larva and 0.08 – 0.89 in the adult. The molecular weights of the proteins in the larva are 17,780 Da, 25,120 Da, 31,620 Da, 50,120 Da, 63,100 Da, 79,430 Da, 100,000 Da and 199,500 Da while in the adult the molecular weights of the proteins are 11,220 Da, 22,390 Da, 25,120 Da, 31,620 Da, 50,120 Da, 95,500 Da, 177,800 Da and 190,500 Da. The midguts of *R. phoenicis* have so much proteins and enzymes that are responsible for the effective digestion of the fibrous tissues of the palm trees. The knowledge of the proteins and enzymes present in the midguts of the larva and adult palm weevils are essential information which could be used in crop engineering to formulate effective enzyme inhibitors in palm trees for the control of palm weevils. This method would adequately enhance a great reduction in palm weevils infestation of palm trees.

Key words: *Rhynchophorusphoenicis, e*lectrophoresis, buffer solution, enzymes, digestive tract, molecular weights, homogenate, proteinase inhibitors.

Introduction

The palm weevil, *Rhynchophorusphoenicis* F. is a key pest of all palm trees in the tropics. The main hosts of the adult beetles are coconut, oil and date, raphia and ornamental palms. The beetle eats into the wood of the palms and the gut microorganisms help in decomposing the plant materials, including dead standing palms, stumps and logs that are lying on the ground as well as saw dust. Palm weevils are prominent as pests of palm trees and they are notable edible insects that are highly relished by the people.

Palm weevils have inflicted more harm on palm trees in palm growing areas than any other pests. Insect pests are highly dangerous to farmers hence, their controls are necessary. Various methods have been adopted by authors for the control of crop pests. Amongst these are the use of medicinal botanicals, essential oil, chemicals and the use of proteinase inhibitors (Omotoso, 2014, Yazdani, 2014, Nasr, *et al.*2015, Sharma 2015). The importance of the use of proteinase inhibitors as a new effective way of controlling insect pests is gaining acceptance. The efficacy of proteinase inhibitors from host plants have been emphasized in the effective control of pests (Panchal and Karchole 2012). The use of proteinase inhibitors can only be possible if adequate knowledge of the proteins and the enzymes present in the guts of the insect is known.

Electrophoretic analyses of the gut systems of insect pests are needed to determine the proteins and enzymes profile in the gut of insects. Electrophoresis is a method used in biochemistry, molecular biology, genetics and clinical chemistry to separate macromolecules and are widely applied in DNA, RNA, proteins and enzymes analyses. Electrophoresis has also been employed by some workers to analyze the haemolymphs of insects (Thomas and Nair, 2011, Hyrsl *et al.* 2011). In electrophoresis, biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix and the biomolecules are separated by size in the agarose gel matrix (Sambrook and Russel, 2001). Thus, the gel electrophoresis is a technique used in the laboratory to separate charged molecules such as proteins, RNA and DNA. Gel is a size selective sieve

substance which separate proteins into bands during electrophoresis. As proteins move through a gel in response to an electric field, the smaller molecules travel more rapidly than larger proteins. As a result of dearth of information on the electrophoretic profile of the proteins and enzymes of *R. phoenicis,* this research work was conducted to provide information on the digestive physiology of the insect and to provide information that can be relied upon in the formulation of proteinase inhibitors for the effective control of palm weevils.

Materials and methods

Collection and preparation of homogenate

The larvae (2.54±0.04-4.62±0.01 cm in length and 2.59±0.14-8.14±0.14 g in weight) and adult 5.30±0.01-6.3±0.12 cm in length and 5.50±0.11-8.51±0.12 g in weight) palm weevils, *R. phoenicis* used for this work were collected from Igbokoda (geographical coordinates 6°20' 59" North and 4° 48' 12" East) in Ondo State. A total of 100 larvae and 100 adult insects were collected and kept in separate plastic buckets containing raphia palm tree chippings (habitat of the weevils). The insect samples were transported to the laboratory and allowed to acclimatize for 24 h before being used for the work. The larvae and the adult stages were manually removed from the palm tree chippings into beakers and rinsed separately with distilled water. The insect samples were killed in a deep freezer maintained at -10º C for 30 minutes. The hard wings and the membranous wings of the adult insects were removed with dissecting set scissors and blade while insect pins were used to pin the insect in dissecting box with the dorsal side facing up. The insects were carefully dissected using dissecting set blade and scissors. The alimentary systems of the insects were carefully removed and put in separate beakers which were already placed inside bigger bowls containing ice-cubes so that the enzymes present would not be denatured. The midguts of the insects were carefully cut from the whole alimentary system and homogenized in a chilled glass homogenizer. The homogenate was prepared from 1g of midgut/ml of sample buffer (Tris-HCl pH 6.8). The homogenate was centrifuged at 5,000 rev/minute for 20 minutes at 4ºC in Beckman Optima Model LE-80K refrigerated centrifuge. The supernatant was boiled for 5 minutes and then used for electrophoresis. A modified method of Laemmli (1970) was used in preparing 4% acrylamide concentration and stock solution.

Preparation of solutions

Sample buffer

Sample buffer was prepared by adding 6 ml of 1M tris-HClph 6.8, 2 g of SDS, 10 ml of glycin, 7.013 ml of 2 M Mercaptoethanol and 0.005 g of Bromophenol blue and making everything to 50 ml with distilled water inside a measuring cylinder.

1.5 M Tris-HCl pH 8.8

For the preparation of 1.5 M Tris-HCl pH 8.8, 138.054 g of Tris-base and 56.88 g of Tris-acid were measured and poured in 1000 ml measuring cylinder and made up to 1 litre with distilled water. This gave 1.5 M Tris-HCl pH 8.8.

M Tris-HCl pH 6.8

To prepare 1.0 M Tris-HCl pH 6.8, 3.7057 g of Tris-base and 153.102 g of Tris-acid were measured and poured in 1000 ml measuring cylinder and made up to 1litre with distilled water. This gave 1.0 M Tris-HCl pH 6.8

Reservoir buffer

For reservoir buffer preparation, 3.028 g of tris-base, 14.42 g of Glycin 192 mM, 1 g of SDS 0.1% were measured and dissolved in a measuring cylinder and made up to 1 litre with distilled water.

Stock solution for polyacrylamide gel electrophoresis

All the following compounds were measured and added together. They include 3.72 ml of water, 0.65 ml of 1.0 M Tris-HCl pH 6.8, Acrylamide 30% 0.70 ml, 10% SDS 0.052 ml, TEMED 0.0052 ml and 10% APS (1000 mg/ml) 0.052 ml.

Fixing solution

Ten percent (10%) acetic acid was added to 40% methanol (v/v) and the gel was put inside and shook at intervals of 20 minutes for 1 h.

Staining solution

The following compounds were measured; 10 g Ammonium sulphate, 1 ml Phosphoric acid and 0.1 g Coomassie G250 and added together. Everything was made up to 100ml mark with distilled water in a measuring cylinder.

Electrophoretic assay

The gel components were mixed together and a glass slab was filled to mark with the gel solution using a pipette. Distilled water was carefully layered on the top of the gel solution to prevent the formation of air or gel meniscus. This ensured that a flat gel surface was obtained. The gel was left to polymerized after which it was ready for use. The sample mixture was applied on top of the individual gel with a micropipette after the layered distilled water had been discarded. Reservoir buffer was poured in the electrophoresis apparatus. Tracking dye was applied. The reference proteins used for the determination of molecular weights which included Myosin (200,000 Da), β-Galactosidase (119,000 Da), Phsphorylase b (97,400 Da), Serum albumin (66,000 Da), Ovalbumin (43,000 Da),

Carbonic anhydrase (29,000 Da), Trypsin (20,000 Da), Lysozyme (14,500 Da) and Aprotinin (6,500 Da) together with all other reagents were obtained from the Department of Biochemistry, Obafemi University, Ile-Ife, Nigeria (Sigma). Electrophoresis of the samples and the protein standards were run together by applying a current of 10 mA per gel. Electrophoresis power pack, Vokam Model SAE 2761 was used to pass current through the gels. Electrophoresis was stopped when the tracking dye was 6/7th down the gel. The gel was removed from the slab by squirting water from a syringe between the gel and the glass wall. The gel was fixed for 1 h and stained with Coomassie brilliant blue G250 for 8 h. The gel was de-stained in 500 ml of distilled water every 24 h until the bands became clearer. The de-stained gel was photographed. A graph of relative mobility was plotted against the log of molecular weight using the formula:

$$\text{Relative mobility} = \frac{\text{Distance of protein migration}}{\text{Distance of dye migration}}$$

The molecular weights of the proteins were determined from the standard curve obtained and the comparative relative mobility values were determined.

Results

The result of the electrophoretic profile of the larval and adult stages of palm weevil, *R. phoenicis*is shown in plate 1. The larvae and adults have eight types of proteins each in their midguts (Table 1.). The relative mobility value of the first protein band in the larva was 0.06 while it was 0.08 in the adult. The Rm value of the second protein band was 0.25 in the larva while it was 0.1 in the adult. The Rm value of the third protein band was 0.31 in the larva while it was 0.28 in the adult. The Rm values of the fourth, fifth, sixth and seventh protein bands in the larva were 0.39, 0.42, 0.58 and 0.64 respectively while in the adult the Rm values were 0.42, 0.58, 0.64 and 0.69 respectively. Protein bands with Rm values of 0.42, 0.58 and 0.64 were present in both the larva and adult stages. The Rm value of the eighth protein band was 0.75 in the larva while it was 0.89 in the adult

The molecular weights of the proteins in the larva ranged from 17,780 Da to 199,500 Da while in the adult it ranged from 11,220 Da to 190,500 Da. The proteins of molecular weights 25,120 Da, 31,620 Da and 50,120 Da were found in both the larval and adult stages. Proteins with molecular weights of 199,500 Da, 100,00 Da, 79,430 Da, 63,100 Da and 17,780 Da were specific to the larval stage while those with molecular weights of 190,500 Da, 177,800 Da, 95,500 Da, 22,390 Da and 11,220 Da were specific to the adult stage.

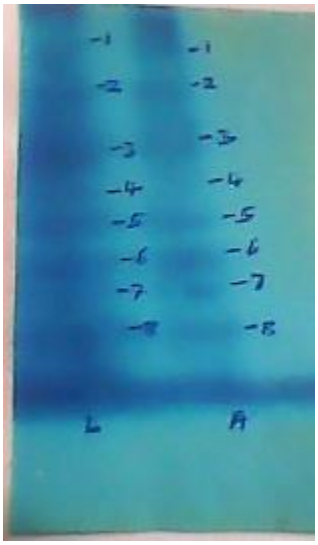

Plate 1: SDS-PAGE of the midgut of palm weevil, *R. phoenicis* . The samples were loaded onto a 4% gel. **Lane L:** Larva of *R. phoenicis,* **lane A:** Adult of *R. phoenicis.*

Table 1: Molecular weights (Da) of the proteins of larva and adult palm weevil, *R. phoenicis*

Standard protein		Larva		Adult	
Name	(Mol. Weight (Da)	Rm	(Mol. Weight (Da)	Rm	(Mol. Weight (Da)
Myosin	200,000	0.06	199,500	0.08	190,500
β-Galactosidase	119,000	0.25	100,000	0.10	177,800
Phosphorylase b	97,400	0.31	79,430	0.28	95,500
Serum albumin	66,000	0.39	63,100	-	
		0.42	50,120	0.42	50,120
Ovalbumin	43,000	0.58	31,620	0.58	31,620
Carbonic anhydrase	29,000	0.64	25,120	0.64	25,120
Trypsin	20,000	-		0.69	22,390
		0.75	17,780	-	
Lysozyme	14,500	-		-	
		-		0.89	11,220
Aprotinin	6,500	-		-	

Rm = Relative mobility
Mol. Weight = Molecular weight

Discussion

It is known that insects are good sources of proteins and as pests; they have enormous quantities of enzymes which help them in degrading their foods. The results of sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis showed that both the larva and adult palm weevils contained several proteins and enzymes with wide ranging molecular weights in their midguts (Plate 1.). The proteins obtained in the midgut of the larva of sweet potato weevil, *Cylasformicariuselegantulus* by Baker *et al.* (1984) are similar to those found in the larva and adult of the insect in this study. Proteins whose molecular weight compared favourably with that of myosin occurred in both the larval and adult stages of this insect. Myosin and actin molecules are very effective in effecting muscle contraction thus, causing movement of organism. The presence of this protein in the larva and adult insects must be responsible for the active lifestyle (burrowing and flight) they exhibited. β-galactosidases were observed in both the larva and adult midguts. These are powerful enzymes that act on carbohydrase in the midgut of the insect. The presence of α- and β- galactosidases have been observed in palm weevil, *R. ferrugineus* (Riseh, *et al.* 2012). Other enzymes obtained in the insect include α- and β-Glucosidases (Riseh, *et al.* 2012). Baker *et al.* (1984) observed that carbohydrases with relative mobility (Rm) values abound in *C. formicarius.* Similar carbohydrases were also observed in the insect in this study. Proteins with the same Rm were detected in both the larva and adult insects. The detection of proteins with the same Rm in the larva and adult weevils connotes that the proteins are essential for their development. This finding agrees with the observation of Nunamaker and Mckinnon (1989). These authors detected identical protein components in the egg, larva, pupa and adult stages of *Culicoidesvariipennis.* They reported that the proteins were necessary for developmental changes; hence they were present in all the life stages and that under the right sets of metabolic conditions, the same proteins could be used to construct the necessary tissues and mediate the appropriate metabolic processes unique to the particular life stage.

Proteins with Rm of 0.58 were discovered in both the larva and adult palm weevil in this work. This finding agreed with the work of Thomas and Nair (2011) who had earlier reported protein bands with the same Rm value of 0.58 in the adult haemolymph of *Rhynchophorusferrugineus* Olivier. A total of 15 protein bands were reported in the haemolymph of *R. ferrugineus* by Thomas and Nair (2011). Protein bands with Rm values of 0.24, 0.40, 0.66, 0.71 and 0.88 reported in the haemolymph of the developmental stages of *R. ferrugineus* by Thomas and Nair (2011) were also observed in this work (i.e. larva and adult stages). The greater number of enzymes found in the midguts of both the larva and adult palm weevils could have been responsible for the faster digestion and enormous destruction of palm trees by the larval and adult stages of the insect.

The presence of enzymes with the same molecular weights in both the larva and adult in this work were expected since both stages feed on the same type of food. Amylase of molecular weight of 60,000 Da was reported in *P. americana*by Due *et al.* (2008). The enzyme of molecular weight 63,100 Da found in the larva of palm weevil was comparable to those reported for the α-amylases of *Thermusfiliformis* and *Haloferaxmediterranei* by Egas *et al.* (1998) and Perez-Pomares, *et al.,* (2003) respectively.

Plants have developed various methods of defences against pests. Among the methods adopted by plants are biochemical and molecular defence mechanisms, production of toxins and proteinase inhibitors that alter biological processes in pests (Mithofer and Boland, 2012, War, *et al.* 2012). The knowledge of the proteins and enzymes profile in the midgut of the larva and adult stages of palm weevils in this study are essential information which can be utilized in crop engineering to incorporate proteinase inhibitors which will hinder palm trees digestion by palm weevils. The incorporation of proteinase inhibitors has been noted to cause appreciable increase in the resistance of crops to pests (Alahmadi, *et al.* 2012,

Sharma 2015). Protease inhibitors have been effective in controlling insects (Alahmadi, *et al.* 2012). In order to record efficient management of pests, it is highly necessary to know the types of enzymes present in the insect pests (Bandani, *et al.* 2010, Macedo and Freire, 2011).

Conclusion

All the developmental stages of palm weevil, *R. phoenicis*are dangerous to palm trees in palm growing regions of the world thereby lowering the yield or output of the farmer. Efforts have been made to cut down or minimize the effects of this pest in palm plantations but some of the methods used have not been able to deliver this crop from the pest. However, the new method of incorporating proteinase inhibitors into crops, which have worked for other crops can be extended to palm trees. The method can only be used now that adequate knowledge of the proteins and enzymes profile of palm weevils are known. The knowledge of this can be utilized in crop engineering to formulate effective enzyme inhibitors in palm trees for the control of palm weevils. This method will adequately enhance a great reduction in palm weevils infestation of palm trees.

Acknowledgement

The author is very grateful to Dr. Adewale, I.O. of the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife for his assistance with the electrophoretic pack and for the unrestricted access to his laboratory.

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