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Electrophoretic protein pattern of male and female haemolymph of *Rhynchophorus ferrugineus* Olivier

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ABSTRACT

The protein pattern in the haemolymph of male and female *Rhynchophorus ferrugineus* has been characterized. Native polyacrylamide gel electrophoresis (PAGE) analysis revealed eleven protein bands in adult males, ten in adult females and nine in the young female. Male haemolymph showed an additional protein band when compared with that of adult female haemolymph, whereas, in adult as well as young female a female specific protein band was present. The protein metabolism of an insect could be studied by observing the haemolymph protein pattern.

Keywords: *R ferrugineus*, coconut pest, haemolymph, protein, PAGE, Rm value

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Insecta: Coleoptera: Curculionidae) is a widely distributed serious pest of the young coconut palms. This pest is known to cause serious damage to the crop since mid fifties (Nirula 1956). The occurrence of *R ferrugineus* on various oil palms in India has been reported by many workers (Abraham et al 1989, Dhileepan 1991, Rajan and Nair 1997). The present study was undertaken to investigate the protein pattern in the haemolymph of male and female *R ferrugineus*. The biochemical composition of haemolymph is highly variable among the insect species at different developmental stages (Florkin and Jeuniaux 1974). Since metabolic activities have been reported to be different at different developmental stages in an insect, the fluctuation in the metabolites could indicate rhythmic changes that occur in the biochemistry of insect haemolymph. This could be related to the rhythm of insecticide susceptibility occurring in insects and hence may be of importance in the study of the mode of action of insecticides on the mechanism of resistance to development.

Insect haemolymph contains many different proteins with a variety of functions.

The total quantity of protein in the blood varies in the course of development. These proteins are usually classified as storage proteins, lipid transport proteins, vitellogenins, enzymes, proteinase inhibitors, chromo proteins, and a range of different proteins that are involved in various immune responses in insects. Most proteins, though not all, in an insect body, have been reported to be synthesised in the fat body released into the haemolymph and at least some of them sequestered by the ovaries during vitellogenesis. A correlation, therefore, should exist between the protein pattern of these tissues not only in the number of protein fractions present in each tissue (haemolymph) but also in the timings of their appearance and disappearance.

Insects have been found to survive microbial and parasitic infections by humoral and cellular defence mechanisms. In response to invading microbes, insect defence is composed of this two-stage strategy, haemocytic to be followed by humoral. The haemocytic defence is especially by phagocytosis, encapsulation, and nodulation of foreign matter by phagocytosis mainly by plasmatocytes and granulocytes. The humoral response that follows the cellular one takes time, in view of de novo synthesis of antibacterial proteins in the haemolymph. A number of antibacterial proteins have been isolated and characterised from many insects, which include lysosymes, cercopins, attacins, haemolins, diptericins, coleoptericins etc (Gudderra et al 2002, El-Sadawy and Abdelshafy 2007). Insects are known to lack lymphocytes and immunoglobulins and, therefore, have developed a relatively simple sequence of inducible antimicrobial peptides that are distinctively different from those of mammalian origin (Steiner et al 1981, Van Hofsten et al 1985, Williams 2007). Acidic polyacrylamide gel electrophoresis of the haemolymph of the American bollworm, *Helicoverpa armigera* showed the presence of at least ten major proteins with molecular weights ranging from 14 to 77.5k Da, in response to bacterial inoculation (Subramanian and Gujar 2000). Similar induction of lysozyme activity was also reported by Hughes et al (1983), Anderson and Cook (1979) and Rowley et al (2005).

Female specific protein, vitellogenin, has been studied in many insects, which was found to be the precursor protein of egg yolk. This protein was synthesised under the influence of juvenile hormone (Chen and Wyatt 1981, Scharff et al 2005) in the fat body and then released into the female haemolymph during vitellogenesis and subsequently taken up by the oocyte (Nordin et al 1990).

In the present study, protein in the haemolymph of adult male and female and young female of *R ferrugineus* was characterised. The haemolymph native proteins were separated by Non-sodium dodecyl sulphate-polyacrylamide gel electrophoresis. General proteins in the haemolymph of the different stages were

stained by specific staining methods. The relative mobility of these proteins was also calculated.

MATERIAL AND METHODS

The native protein of the haemolymph of red palm weevil was analysed by 10 per cent polyacrylamide gel electrophoris. The haemolymph protein pattern of both adult male and female and young female were compared. Determination of relative mobility was also done electrophoretically. The polyacrylamide gel electrophoresis (PAGE) method of Laemmli (1970) was followed with necessary modifications.

Relative mobility (Rm) value

The total length of the separating gel, the distance travelled by the marker dye in the separating gel and the various distances migrated by the different protein fractions were measured. The relative mobility/relative fraction values of each band was calculated as follows:

$$\text{Relative Mobility (Rm)} = \frac{\text{Distance travelled by the protein fraction}}{\text{Distance travelled by the marker dye}}$$

RESULTS

Native PAGE analysis of the haemolymph of *R ferrugineus* of adult male and female and young female were conducted to study the qualitative changes in the protein pattern.

Native PAGE analysis revealed eleven protein bands in adult males, ten in adult females and nine in young females. Compared with the haemolymph protein pattern of the female, the haemolymph protein pattern of male was in excess of one band (Plate 1). The relative mobility value of the first protein band in all these stages was 0.14. The second protein band was present only in adult male and young female, with Rm value of 0.15 each. The third protein band which was a diffused one was present only in young female with Rm value of 0.19.The next two bands with Rm values 0.22 and 0.24 were present in both adult male and adult female, but were absent in young female. The sixth band, a darker one, was present in all the three stages with Rm value of 0.40.The seventh protein band was present only in adult female with Rm value of 0.46.The eighth protein band which was present in the adult male and young female had Rm value of 0.51. The ninth band was present only in adult male but absent in adult and young female. This band had Rm value of 0.58. The tenth protein band, which was a darker one, was present in adult and young female with Rm

values of 0.60 each. The next two protein bands were very feeble and diffused and were present in all the three stages with Rm values 0.66 and 0.71, respectively. The next protein band having Rm value of 0.78, was present only in adult male. Similarly the fourteenth band with Rm value of 0.84 was characteristic of adult female, but was absent in both adult male and young female. The protein band with Rm value of 0.88 was present in all the three stages. (Table 1).

DISCUSSION

In the present study, striking similarities were noticed between the protein components of male and female haemolymph of *R ferrugineus*. Male haemolymph showed an additional protein band when compared with adult female haemolymph, whereas, adult and young females had a protein band with Rm value of 0.60 each, which was absent in males. This may be regarded as a female specific

Table 1. Rm values of haemolymph proteins of *R ferrugineus* in adult male and female and young female

Protein band	Rm value		
	Adult male	Adult female	Young female
1	0.14	0.14	0.14
2	0.15		0.15
3			019
4	0.22	0.22	
5	0.24	0.24	
6	0.40	0.40	0.40
7		0.46	
8	0.51		0.51
9	0.58		
10		0.60	0.60
11	0.66	0.66	0.66
12	0.71	0.71	0.71
13	0.78		
14		0.84	
15	0.88	0.88	0.88

protein band in *R ferrugineus*. Female specific protein bands have been observed in many insects like *Manduca sexta* (Ryan et al 1985) in *Periplaneta americana* (Kim and Lee 1994) and in *Oxya hyla hyla* (Ghosh and Chel 2000). Studies made in *Locusta migratoria* have shown the presence of female specific protein band on native PAGE (Chinzei et al 1981). Native PAGE analysis of the haemolymph of the American cockroach has shown the presence of five major protein bands, of which some were involved in mediating immune responses (Laura et al 1991). The protein bands in various species are stage dependant and species specific.

In the present study, the adult female red palm weevil showed the presence of an additional protein band in the haemolymph compared to the protein pattern of young female. This extra protein band might be associated with egg maturation in the adults of *R ferrugineus*. This is supported by the view of Bodnaryk and Morrision (1966) suggesting that the concentration of haemolymph protein is low at emergence, increases as growth proceeds and fluctuates during egg maturation in response to ovarian demand.

As the haemolymph composition of insects reflects the nature and degree of metabolism of the tissues bathed in this fluid, changes in protein of haemolymph may show the level of modification in the organism. Therefore, by studying the haemolymph protein pattern, it is possible to have a clear picture of the protein metabolism of the insect. However, the variation of protein fractions during the different developmental stages of *R ferrugineus* in the present investigations apparently indicates both the synthesis and breakdown of specific proteins.

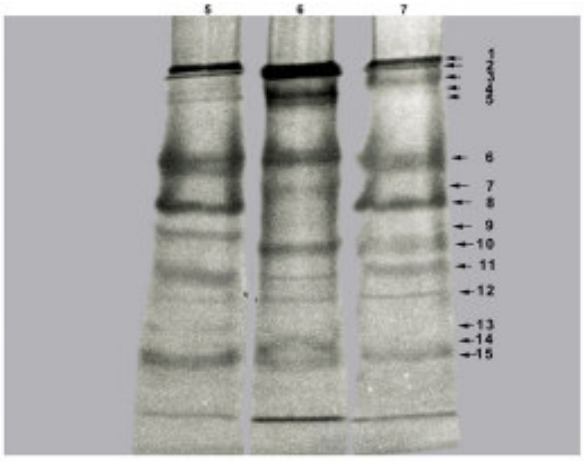

Plate 1. Electrophoretic separation of haemolymph proteins in male and female *Rhynchophorus ferrugineus,* (Lane 5- Male haemolymph, Lane 6- Female haemolymph, Lane 7- Young female haemolymph)

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