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Electrophoretic (SDS-PAGE) studies on muscle albumin proteins of the male and female deep water mud shrimp *Solenocera melantho* (De Man, 1907)

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Abstract:

Activated muscle albumin proteins of males and females of Solenocera melantho have been resolved electrophoretically with water and with Tris- HCl buffer at five concentrations (0.1M to 0.5M) against five marker proteins - Phosphorylase, Bovine serum albumin, Ovalbumin, Carbonic anhydrase and Lactoglobulin (Molecular weight 18.4 to 97.4 kDa). Significant difference was observed between the sexes though there was some similarity in some of the band pattern. Males and females exhibited 15 and 10 albumin protein fractions with water, 12 and 13 albumin fractions with 0.1M, 15 and 12 fractions with 0.2M, 4 and 9 albumin fractions with 0.3M, 9 and 9 fractions with 0.4M and 10 fractions each with 0.5M Tris-HCl respectively. The albumin fractions resolved ranged from 12.58 kDa to 99.20 kDa.

Key words: *Solenocera melantho,* SDS-PAGE, Muscle albumins, Proteins, Deep water mud shrimp

Introduction

Protein constitutes about 20% of the muscle composition in shrimp and is subjected to change depending on the availability

of food, moult condition, spawning and migration (Viswanathan and Suseela Mathew, 2000). Albumins are the proteins of the fluid environment of the muscle *i.e.* within the myotomes and in between the myotomes. They constitutes about 16-22% of the total muscle proteins and can be extracted with water and salt solutions of low ionic strengths *i.e.,* < 0.5M. The albumins include the water soluble myoalbumins and water- insoluble myogens which are soluble in salt solutions of low ionic strength. The solubility of different albumin varies with different concentrations of salt solutions. The albumin proteins impart specific taste and flavour specific to the muscle of a particular species and ultimately different species possess different types of albumin proteins. In shrimps, protein is the major constituent of muscle and its composition varies with species, sex, size and season (Shaikhmuhmad and Magar, 1957).

Electrophoresis is the movement of charged particles under the influence of an electric field and is the main method for analysis of the biochemical systematics in various taxa (Avise, 1975). All proteins carry specific electric charge which is determined by their amino acid composition and the pH of the medium. Each species is identified by a specific number of proteins by means of high-resolution starch or polyacrylamide gel and isoelectic focusing which forms species-specific band patterns of different proteins. A protein is a translated phenotypic expression of a genetic code and the variations in the genotype usually result in change of the structure and function of proteins (Bye and Ponnaiah, 1983). These phenotypes show variation with species, sex and season both qualitatively and quantitatively. In an electric field, a protein moves towards the oppositely charged pole at a rate proportional to the magnitude of its charge. Since proteins have different charges, they may move at varying rates and directions in electrophoresis (Ferguson, 1980). Thus, the relative mobility of proteins at an electric field `depends on

their molecular weight, conformation and surface electric charge (Mc Laughlin *et al.,* 1982).

Most of the electrophoretic studies carried out so far are related to either total proteins or myofbrillar proteins or corneal proteins except those of Connell (1953) on low ionic strength proteins *i. e.,* 0.5M in fish, Chakravarty *et al* (2009) on Trachypenaeid shrimps- *T. curvirostris, T. sedili* and *T. pescadorensis* and Chakravarty *et al* (2013) on pomfrets *i.e.*, *Pampus argenteus*, *P.chinensis* and *Apolectis niger*. The present study aimed at the study of the electrophoretic mobility of water soluble albumin proteins and proteins at different concentrations of Tris- HCl up to 0.5M.

Material and methods

Specimens of the species *Solenocera melantho* were sampled from the catches of commercial fishing trawls operating from Visakhapatnam fishing harbour in the coastal waters of the Bay of Bengal. The specimens were kept on crushed ice in an insulated box and brought to the laboratory where they were separated as per sex. The abdominal muscles of male and female were separated. The water adhered to the material was blotted on a filter paper. The wet weight of the muscle of the individual specimen was taken on a sensitive electronic balance. The material was dried in hot air oven for about 48hrs at 55-65^{o}C and then the dry weight was taken. The dried tissue was finely powdered in a mortar and was used for electrophoretic studies following the method of Sambrook and Russell (1988). The albumin proteins of muscles were isolated with water and Tris-HCl buffer (0.1M, 0.2M, 0.3M, 0.4M and 0.5M). The marker proteins (MP) used were Phosphorylase, Bovine serum albumin, Ovalbumin, Carbonic anhydrase and Lactoglobulin with molecular weight ranging from (18.4 kDa to 97.4 kDa).

Results

Water soluble albumins

Fifteen protein fractions in males and ten in females were recorded. The common protein bands present in both males and females ranged with a relative mobility of 0.06 to 0.91 and molecular weights from 12.58kDa to 94.76 kDa. The differed albumin bands in males and females were with relative mobilities of 0.04, 0.09, 0.68, 0.73 and 0.78 whose molecular weights were 99.20 kDa, 87.59 kDa, 28.32 kDa, 24.34 kDa and 20.37 kDa respectively (Table 1; Fig. 1a & 1b).

Males exhibited seven intense bands and eight medium bands while females showed five intense bands, four medium bands and one faint band (Table 2). The zonal-wise dispersion of protein bands in males showed seven slow, three intermediate, three fast and two very fast bands while in females they were five slow, three intermediate, one fast and one very fast (Table 3).

Albumins at 0.1M Tris-HCl

At this concentration, there were twelve activated proteins in males and thirteen in females. Of these, females differed from males in having two albumin protein fractions with molecular weights 99.20kDa and 83.36 kDa and in not having a protein with molecular weight 87.59 kDa. The common bands which are present in both the sexes were with relative mobilities of 0.06 to 0.91 and molecular weights of 12.58 kDa to 94.76 kDa (Table 1; Fig. 1a & 1b).

In males, four intense and eight medium bands were observed while in females, six intense bands and seven medium bands were present (Table 2). The zonal-wise dispersion showed five slow, three intermediate, two fast and two very fast in males whereas in females six slow, three intermediate, two fast and two very fast bands were observed (Table 3).

Albumins at 0.2M Tris-HCl

A total of fifteen bands were seen in males with relative mobilities ranging from 0.04 to 0.91 and the molecular weights from 12.58kDa to 99.20 kDa. In females, twelve bands were observed with relative mobilities ranging from 0.06 to 0.91 and the molecular weights from 12.58kDa to 94.76 kDa. The male differed from female at three additional protein whose relative mobilities/molecular weights were 0.04/99.20 kDa, 0.12/83.36 kDa and 0.23 /66.40 kDa (Table 1; Fig. 1a & 1b).

Males showed six intense, six medium and three faint bands while in females four intense, three medium and five faint bands were found (Table 2). In males seven slow, three intermediate, three fast and two very fast bands were observed. In females four slow, three intermediate, three fast and two very fast bands were observed (Table 3).

Albumins at 0.3M Tris-HCl

Four albumin fractions in males and nine bands in females were resolved. The common bands observed in both males and females were with relative mobilities of 0.04, 0.17, 0.54 and 0.91 and the molecular weights of 99.20 kDa, 76.58 kDa, 40.58 kDa and 12.58kDa respectively. The remaining bands found in females were with the relative mobilities ranging from 0.06 to 0.41 and the molecular weights from 51.56 kDa to 94.76 kDa (Table 1; Fig. 1a & 1b).

In males one intense, two medium and one faint band and in females four intense bands, two medium bands and three faint bands were found (Table 2). Two slow, one fast and one very fast band in males and in females five slow, two intermediate, one fast and one very fast band were observed in zonal-wise dispersion of bands (Table 3).

Albumins at 0.4M Tris-HCl

There was no difference between males and females. Nine activated proteins were noticed in both males and females. The

relative mobilities/ molecular weights of the protein fractions ranged from 0.04/99.20kDa, 0.06/94.76 kDa, 0.09/87.59 kDa, 0.12/83.36 kDa, 0.17/76.58 kDa, 0.33/58.97 kDa, 0.41/51.56 kDa, 0.57/37.56 kDa and 0.91/12.58 kDa (Table 1; Fig. 1a & 1b).

Both males and females showed three intense, four medium and two faint bands (Table 2). The zonal-wise dispersion of bands also exhibited similar pattern in both males and females with five slow, two intermediate, one fast and one very fast bands (Table 3).

Albumins at 0.5M Tris-HCl

Ten protein fractions were observed with relative mobilities ranging from 0.04 to 0.91 and the molecular weights from 12.58kDa to 99.20kDa. Males differed from females in having a protein fraction with molecular weight of 58.97 kDa and in not having a protein with molecular weight 70.40 kDa (Table 1; Fig. 1a & 1b).

Three intense, four medium and three faint bands were found in males whereas in females three intense, five medium and two faint bands were observed (Table 2). The zonal-wise dispersion of bands showed six slow, two intermediate, one fast and one very fast in males while in females seven slow, and one intermediate, one fast and one very fast bands were noticed (Table 3).

Discussion

Muscle albumins are one of the most important of the fish proximate principles found in the aqueous system of the muscle *i.e.,* sarcoplasm and myoplasm extractable with water or dilute salt solutions (Lovell, 1989). Electrophoretic separation of muscle albumin proteins with water and salt solutions of low ionic strengths (0.1M to 0.5M Tris-HCl) reveals the difference between the sexes of the deep water mud shrimp *S. melantho* in terms of presence or absence of a particular protein fraction and

its relative mobility against marker proteins, staining intensity and molecular weights. Significant difference has been observed between the sexes though there is some similarity in some of the band patterns. In the present study males and females exhibited 15 and 10 albumin protein fractions in water soluble, 12 and 13 albumin fractions in 0.1M, 15 and 12 fractions in 0.2M, 4 and 9 albumin fractions in 0.3M, 9 and 9 fractions in 0.4M and 10 fractions each in 0.5M Tris-HCl respectively. The albumin fractions resolved have ranged from from 12.58 KDa to 99.20 KDa.

Connell (1953) has established the electrophoretic mobility of the codling, *Gadus callarias* skeletal muscle extracts- globulin X, myogen & myoalbumin at different ionic strengths such as 0.05I, 0.1I and 0.2I. Uglow (1969) has reported the electrophoretic resolution of hemolymph proteins of *Carcinus maenas* in which the proteins have resolved into three areas *viz.,* proximal (P), intermediate (I) and distal (D) fractions and the distal fraction again into two sub-fractions as "fast" and "slow" on the basis of their electrophoretic mobility confirming the earlier findings on the same species by Busselen(1970). Lim and Lee (1970) have found eight bands in the muscle myogens of *Metapenaeus stridulans* and *M. barbata.* According to them three bands were common in *M. nutatus*, *M. stridulans* and *M.barbata* and opined that this could suggest probable genetic relationships of the species of the two genera.

Kulkarni *et al* (1980) have observed a dense and fast moving fraction followed by a faint band in males of *Metapenaeus affinis* and a thick protein band in females. They have also found a total of five bands in males and females of *M. monoceros* of which males with three dense fractions followed by two faint bands whereas females with four dense bands and one faint fraction. In *Parapenaeopsis hardwickii* four fractions have been observed in both the sexes out of which males has two thick bands and two faint bands and females with three

faint bands and one thick band. In case of *P. stylifera* males and females have shown three protein fractions of which males have one dense fast moving followed by two light bands whereas females have two dense bands and one faint band. Lester (1985) has found a low level of genetic variation with little geographic differentiation within species among wild stocks of *Penaeus aztecus*, *P.setiferus*, *P. stylirostris* and *Litopenaeus vannamei* from American waters. Philip Samuel (1987) has observed 8, 9, 10 protein fractions in *Parapenaeopsis stylifera*, *P. sculptilis* and *P. hardwickii* respectively with three common fractions from the West coast of India. Chan *et al* (1988) have observed no changes in the major polypeptides but in case of less abundant polypeptides, one small (32kDa) and one high molecular weight (175kDa) polypeptide have shown variation in relative abundance in *L. vannamei* during the moulting cycle. Murthy (1991) has observed eight fractions in *Metapenaeus stridulans*, six fractions in *M. barbata* and the two species have two common fractions irrespective of the intensity of staining of the muscle extracts. Four calcium- binding proteins have been isolated from *Homarus americanus*, *Neptunus norvegicus*, *Carcinus maenas*, *Penaeu serratus* and *Acetes leptodactylus* by Arnold Sauter *et al* (1993). According to them the calmodulins of first four species contain trimethyl lysine (TML) and dimethyl lysine (DML) whereas those of *A. leptodactylus* have only TML.

Sriraman *et al* (1995) have studied the muscle proteins of eleven species of shrimps belonging to six genera from Porto Novo by polyacrylamide disc gel electrophoresis and found a total of 12 protein fractions of which, the fractions 6,7,8 and 11 are common in four species of the genus *Penaeus i.e Fenneropenaeus indicus, P. monodon, P. merguiensis* and *P. semisulcatus* and the fractions 5,7,10 and 12 are common in *Metapenaeus monoceros* and *M. dobsoni* showing their genetic affinity, whereas the fractions 6,7and 11 are common to *P. stylifera* and *P. maxillepedo* referring to their affinity. The

presence of fraction 7 in both *Solenocera crassicornis* and *Metapenaeus stridulans* establishes their relationship with other species of the genera *Penaeus, Metapenaeus* and *Parapenaeopsis*. Absence of fractions 1 to 5 in *Macrobrachium idella* shows the specificity of the family Palaemonidae and the presence of fractions 6 to 12 indicates some relation between the species of the family penaeidae. Lubzens *et al* (1997) have isolated the high density lipoproteins (HDL) in male and female hemolymph of *Penaeus semisulcatus*. They found that the male HDL containing lipoprotein I composed of a peptide with 110 kDa whereas the female HDL contains two proteins- the lipoprotein I which is identical to male LPI(110 kDa) and vitellogenin with molecular weights of 200kDa, 120kDa and 80kDa.

Yehezkel *et al* (2000) have isolated the high density lipoproteins LPI and LPII from the hemolymph of *Cherax quadricarinatus* and found that both contained a carotenoid moiety and LPI is made up of a single polypeptide with a mol. wt. of 96kDa and LPII is of two native components LPIIa and LPIIb, having molecular weight of 80kDa and 177kDa. According to them, LPII serves as a marker indicating the onset of secondary vitellogenesis in females. Avarre *et al* (2003) have isolated two protein bands of 74kDa and 199kDa in hemolymph and four bands of 72kDa, 79kDa, 100kDa and 207kDa by SDS-PAGE in *Penaeus semisulcatus* in ovary. Laxmilatha and Laxminarayana (2003) have reported 16 bands in male and female by SDS- PAGE in the haemolymph of white shrimp, *Fenneropenaeus indicus*. Oliveira *et al* (2006) have found high levels of actin and myosin in post moult, reaching a plateau in intermoult and a decrease in pre moult by SDS-PAGE in *Litopenaeus vannamei* and have suggested muscle fiber-rearrangement in pre- and post- moult stages and the abdominal muscle build- up mostly during the intermoult stage. Kruevaisayawan *et al* (2007) have isolated and characterized the cortical rods in *Penaeus monodon* consisting of a number of

major protein bands ranging from 35-230kDa. Kazuo shiomi *et al* (2008) have purified a new crustacean allergin with 20kDa which is a sarcoplasmic calcium binding protein (SCP) from the muscles of the black tiger shrimp *Penaeus monodon*.

Yedery and Reddy (2009) have reported the identification and characterization of a 11kDa antimicrobial protein (SSAP) from granular haemocytes of the mangrove crab, *Scylla serrata*. Chakravarty *et al* (2009) have found at 0.1M, a total of 14 fractions in three species, at 0.2M 10 fractions in *T. curvirostris*, 8 in *T .sedili* and 12 in *T. pescadorensis* and 11,10,9 fractions in *T.curvirostris*, *T.sedili* and *T. pescadorensis* are observed respectively at 0.3M. At 0.4M and 0.5M, numbers of protein fractions have been reduced. Chakravarty *et al* (2013) have reported that a maximum number of seven and four albumin protein fractions with water, three in males and six in females at 0.1M Tris-HCl in black pomfret *Apolectis niger*. In case of males and females of white ponfret *Parastromateus argenteus* and Chinese pomfret *P. chinensis* the albumins observed are at 0.1M, 0.2M and 0.3M Tris-HCl are 5 & 3,4 & 5; 5 & 6, 4 & 5 and 8 & 9, 10 & 8 respectively. At 0.4M and 0.5M Tris-HCl, the band pattern observed is low.

Conclusion

Significant difference was observed between the sexes though there was some similarity in some of the band pattern. Males and females exhibited 15 and 10 albumin protein fractions with water soluble, 12 and 13 albumin fractions with 0.1M, 15 and 12 fractions with 0.2M, 4 and 9 albumin fractions with 0.3M, 9 and 9 fractions with 0.4M and 10 fractions each with 0.5M Tris-HCl respectively. The albumin fractions resolved ranged from 12.58 kDa to 99.20 kDa.

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Table 1 Relative mobility and molecular weights (kDa) of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M & 0.5M Tris- HCl in relation to marker proteins of males and females of *S. melantho*

Marker proteins	Molecular Weights (KDa)	Relative mobility's of marker proteins
Phosphorylase b	97.4	0.05
Bovine serum albumin	66	0.23
Ovalbumin	43	0.48
Carbonic anhydrase	29.0	0.66
Lactoglobulin	18.4	0.86

S.No.	Rm values	Molecular weight(kDa)	Water soluble		0.1M		0.2M		0.3M		0.4M		0.5M	
			Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0.04	99.20	+	-	-	+	+	-	+	+	+	+	+	+
2	0.06	94.76	+	+	+	+	+	+	-	+	+	+	+	+
3	0.09	87.59	+	-	+	-	+	+	-	+	+	+	+	+
4	0.12	83.36	+	+	-	+	+	-	-	+	+	+	+	+
5	0.17	76.58	+	+	+	+	+	+	+	+	+	+	+	+
6	0.20	70.40	+	+	+	+	+	+	-	-	-	-	-	+
7	0.23	66.40	+	+	+	+	+	-	-	-	-	-	+	+
8	0.28	64.15	+	+	+	+	+	+	-	-	-	-	-	-
9	0.33	58.97	+	+	+	+	+	+	-	+	+	+	+	-
10	0.41	51.56	+	+	+	+	+	+	-	+	+	+	+	+
11	0.54	40.58	+	+	+	+	+	+	+	+	-	-	-	-
12	0.57	37.56	-	-	-	-	-	-	-	-	+	+	+	+
13	0.68	28.32	+	-	-	-	+	+	-	-	-	-	-	-
14	0.73	24.34	+	-	+	+	+	+	-	-	-	-	-	-
15	0.78	20.37	+	-	+	+	+	+	-	-	-	-	-	-
16	0.91	12.58	+	+	+	+	+	+	+	+	+	+	+	+
Total No. of Bands			15	10	12	13	15	12	4	9	9	9	10	10

+ Presence **- Absence**

Table 2 Relative mobility, molecular weights (kDa) and band intensity of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M & 0.5M Tris- HCl of males and females of *S. melantho*.

S.No.	Rm values	Molecular weight(kDa)	Band Intensity											
			Water soluble		0.1M		0.2M		0.3M		0.4M		0.5M	
			Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0.04	99.20	I	-	-	I	I	-	I	I	I	I	I	I
2	0.06	94.76	M	I	I	M	M	I	-	F	M	M	M	M
3	0.09	87.59	M	-	M	-	M	F	-	F	F	F	F	F
4	0.12	83.36	M	F	-	M	M	-	-	F	F	F	F	F
5	0.17	76.58	I	I	I	I	I	I	M	I	I	I	I	I
6	0.20	70.40	I	M	M	M	M	M	-	-	-	-	-	M
7	0.23	66.40	M	M	M	M	M	-	-	-	-	-	F	M
8	0.28	64.15	M	I	M	M	M	F	-	-	-	-	-	-
9	0.33	58.97	I	M	M	I	I	M	-	M	M	M	M	-
10	0.41	51.56	I	M	M	I	I	M	-	M	M	M	M	M
11	0.54	40.58	I	I	I	I	I	I	M	I	-	-	-	-
12	0.57	37.56	-	-	-	-	-	-	-	-	I	I	I	I
13	0.68	28.32	M	-	-	-	F	F	-	-	-	-	-	-
14	0.73	24.34	M	-	M	M	F	F	-	-	-	-	-	-
15	0.78	20.37	M	-	M	M	F	F	-	-	-	-	-	-
16	0.91	12.58	I	I	I	I	I	I	F	I	M	M	M	M
Total No. of Bands			15	10	12	13	15	12	4	9	9	9	10	10

I = Intense **M = Medium** **F = Faint**

Table 3 Zonal - wise protein band dispersion of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M & 0.5M Tris- HCl of males and females of *S. melantho*.

Zone	Number of bands present in											
	Water soluble		0.1M		0.2M		0.3M		0.4M		0.5M	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Slow (0.10 – 0.25)	7	5	5	6	7	4	2	5	5	5	6	7
Intermediate (0.26 – 0.50)	3	3	3	3	3	3	-	2	2	2	2	1
Fast (0.51 – 0.75)	3	1	2	2	3	3	1	1	1	1	1	1
Very fast (0.76 – 1.0)	2	1	2	2	2	2	1	1	1	1	1	1
Total No. of Bands	15	10	12	13	15	12	4	9	9	9	10	10

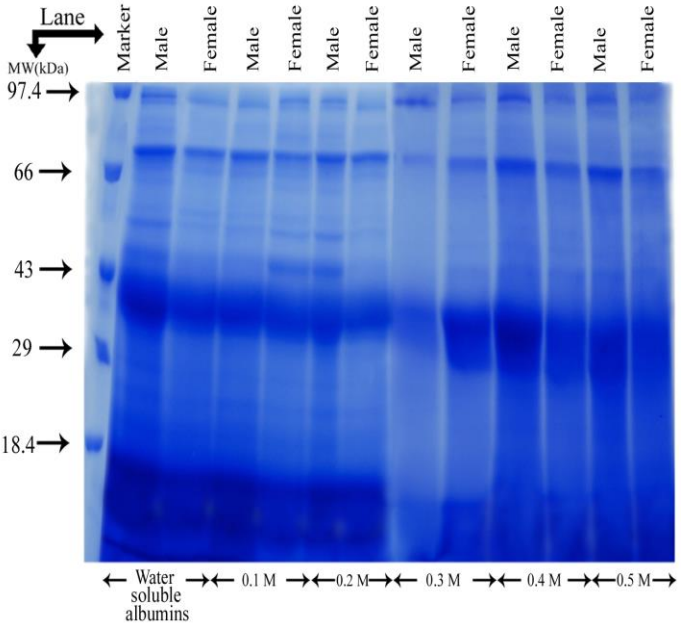

Fig. 1 (a) Electropherogram of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M and 0.5M Tris- HCl in males and females of *S. melantho*.

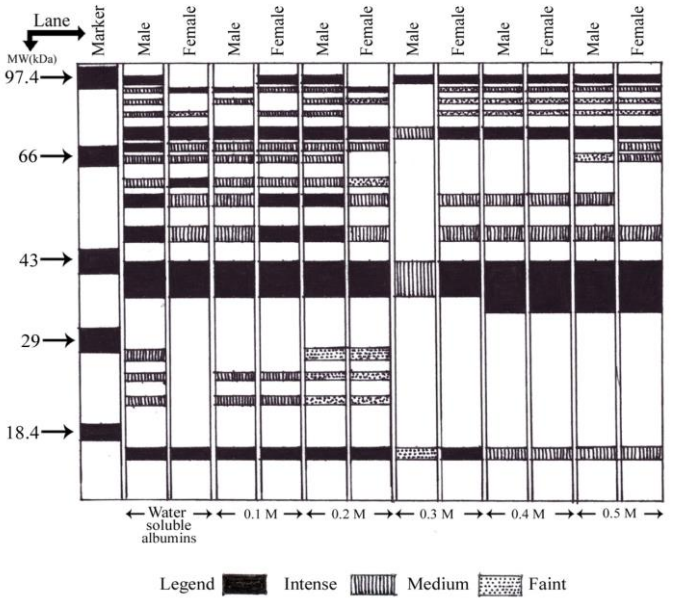

Fig. 1(b) Digrammatic representation of electropherogram of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M and 0.5M Tris- HCl in males and females of *S. melantho*.