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MORPHOLOGICAL AND PHAGOCYTIC PROPERTIES OF COELOMOCYTES OF THE EARTHWORM, *MEGASCOLEX TRAVANCORENSIS*

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The proportion of different types of coelomocytes and their morphological and cytological features and phagocytic properties in a native species of earthworm, *Megascolex travancorensis* have been studied. The three different classes of cells were noticed in the coelomic fluid of *M. trvancorensis* were amoebocytes (Type I and Type II), granulocytes (Group I and Group II) and eleocytes. Amoebocytes are the most representative cells in the coelomic fluid in *M. travancorensis*. All cells exhibited intense phagocytic activity during one hour of incubation. However, the amoebocytes were found to be more active in phagocytosis. The phagocytic activity of coelomocytes was found to increase as a function of time within observed time interval. Vacuolization in amoebocytes and formation of vesicular blebs on the membrane surface are signs of cellular aging.

Key words : Phagocytosis, coelomic fluid, amoebocytes, eleocytes, granulocytes

INTRODUCTION

Most earthworms eject coelomic fluid through the dorsal pores due to increased intra-coelomic pressure (Tuckova, 1996), seasonal variation (Morgan *et al.*, 2003) or in response to mechanical or chemical stimuli (Komiyama *et al.*, 2004) or when subjected to extremes of heat, cold or at times of stress. The consistency of the coelomic fluid differs between different species of earthworms, and also depends upon the humidity of the air in which the worms live. It is thicker and more gelatinous in worms in dry situations than those from wet habitats.

Coelomic fluid contains a heterogeneous population of coelomocytes having wide range of morphology, size, cytochemical properties and immune functions (Jamieson, 1992; Plytycz, 1997).

Kerala is known to be rich in earthworm species diversity (Nair *et al.*, 2007). Of the several species studied, only a few allow a common pattern of categorization. Worms of different species living under similar conditions and of comparable age have shown differences in the coelomocyte profile. Coelomocytes are formed from the epithelial lining of the coelomic cavity or from special structures associated with the epithelium (Valembois, 1971). The annual changes in the coelomocytes of four earthworm species have been examined by Kurek & Plytycz (2003). Several functions have been attributed to coelomocytes. These include the ability to recognize and eliminate foreign materials, primarily by phagocytosis and encapsulation (Englemann *et al.*, 2004) presumably driven by pattern recognition proteins (Nappi & Ottaviani, 2000) and to participate in clotting and wound healing (Cooper & Roch, 1992; Cikutovic *et al.*, 1999). The morphology and phagocytic properties of the earthworm, *Lumbricus terrestris* Linneus, 1758 have been studied by Stein *et al.*, (1977) and that of *Dendrobaena veneta* Rosa, 1886 by Admowicz & Wojtaszek (2001), and Kalac *et al.* (2002). Phagocytosis is of extreme importance in limiting the various microbial populations within the coelomic cavity and any inhibition to this function is a direct threat to the organism's ability to survive (Mc Donald, 2007).

The present attempt is to study the detailed morphology of the coelomocytes of *Megascolex travancorensis* Michaelsen, 1910 and throw some light on their functional significance by observing the phagocytic activity of different classes of cells.

MATERIALS AND METHODS

Preparation of beds : The worms belonging to the species *M. travancorensis* were collected from the field and were brought to the laboratory for rearing in artificial beds. The traditional methods using taxonomic keys were adopted for species identification (Blakemore, 2006). Large pots filled with alternating layers of cowdung and leaf litter to a depth of ½ to 1 ft were used for the purpose. The pot was covered with cotton cloth or plastic mesh, which allowed ventilation. After an initial period of restlessness, the worms seemed to get acclimated to the new surroundings. The adult clitellated worms were selected for coelomocyte retrieval.

Extrusion of coelomocytes : Earthworm coelomocytes are very convenient to work with, as they may be retrieved from coelomic cavity without killing the animals. The coelomic fluid was forcibly ejected by the application of 5v electric shock. Treatment of worms with 5% ethanol was also effective in releasing the coelomic fluid. However, the general vigour of animals is much better after electric stimulation than after ethanol treatment. The exudates containing coelomocytes were collected for total count and differential counts and for cytological observation.

Smears of coelomic fluid made on microscopic slides were air dried. Then they were stained with Methylene blue and Giemsa stain. Slides were then observed under the oil immersion objective of a research microscope for morphological features and then photomicrographs were taken. Differential counts of coelomocytes were made on twenty sample slides obtained from smears of twenty different worms belonging to *M. travancorensis*.

Determination of Phagocytic Property : The coelomic fluid is collected in a vial. The yeast suspension of a concentration of 10^2 cells/ ml is prepared by using earthworm ringer (Diogeme *et al*., 1997). It is stained with saffranin dye. 0.05ml coelomic fluid is introduced into the yeast suspension on a clean glass slide. After incubation for 15 minutes, 30minutes, 45 minutes and 60 minutes, the number of cells in phagocytosis was counted. The percentage of cells involved in phagocytosis was calculated (Fornusek *et al*., 1985).

RESULTS

In the classification of coelomocytes, morphological as well as behavioural features such as phagocytic activity were taken into consideration.

Morphology of the cells

Three different classes of cells with distinct morphological characteristics were recognized. These were amoebocytes (Type I and Type II), granulocytes (G1 and G2) and eleocytes. The differential count of the coelomocytes was undertaken. The changes in

Table I : Phagocytic activity of coelomocytes of *M. travancorensis.*

Time of Exposure (min)	Phagocytic activity(% of cells in phagocytosis)		
	Amoebocyte	Granulocyte	Eleocyte
15	19.27	15.06	0
30	21.35	20.38	0
45	25.5	23.48	0
60	27.27	25.25	1.01
X(mean value)	23.35	21.04	1.01

the phagocytic activity as a function of time are shown (Table I).

Amoebocytes : These were amoeboid cells possessing slender cytoplasmic projections. The nucleus was moderate sized and oval or kidney shaped. The ability to form pseudopodia, the shape of the nucleus and the granular cytoplasm are characteristics of mature cells. Two types of amoebocytes have been identified. Those with numerous, long slender, finger like pseudopodia called filopodia were categorized as Type I amoebocyte (Fig. 1A). The other type with only one or two short, blunt pseudopodia called lobopodia was grouped as Type II amoebocyte (Fig. 1B). In the present observation, the Type I amoebocytes outnumbered the Type II amoebocyte. Vacuolated and nonvaculated amoebocyte were also noticed. These comprised 24±0.49% and 13±0.71%, respectively of total cell population. Nearly half of the amoebocytes were vacuolated. Many cells of these types were seen to be involved in phagocytosis (Figs.1C, D, E, F) at various stages of incubation with yeast. As many as 4 or 6 yeast cells could be seen to be engulfed by these coelomocytes.

Granulocytes : Granulocytes are spherical cells with centrally placed acidophilic nuclei. Their cytoplasm contains strongly eosinophilic granules, which stains pink. They comprised 14± 1.93% of the total cell population. Two types of granulocytes have been identified namely Group I and Group II. In Group I type, the granules are equally distributed (Fig. 2A). In Group II type the surface of the granulocyte cells have characteristic vesicular structures, so called blebs, having shapes varying from spherical to club like (Figs. 2B, C). Granulocytes show a slow, but progressive phagocytic activity upon incubation with yeast up to 60 minutes (Figs. 2D, E). The size of the blebs seems to progressively increase with maturation and aging of granulocytes.

Eleocytes : They have small, spherical eccentrically placed nuclei and show a high affinity for acid stains. They stain blue with Giemsa stain. The most important characterstic of the eleocyte is the presence of large membrane bound vesicles called chloragosomes in their peripheral cytoplasm (Fig. 3A). They had no pseudopodia. The cells tend to disintegrate upon contact with the substratum to which the granules adhere. The eleocytes are the largest cells among the coelomocytes. Some of these cells were found to be engulfed with various types of foreign bodies such as globules of stain (Fig. 3B). The eleocytes comprised 16.6± 0.051% of the total cell population. A slight phagocytic activity was observed during the 60 minutes of incubation (Fig. 4).

Other inclusions in the coelomic fluid included the breakdown products of the corpuscular bodies, protozoan and nematode parasites and bacteria. 'Brown bodies' were aggregated dark coloured masses or nodules usually found in the coelom at the posterior

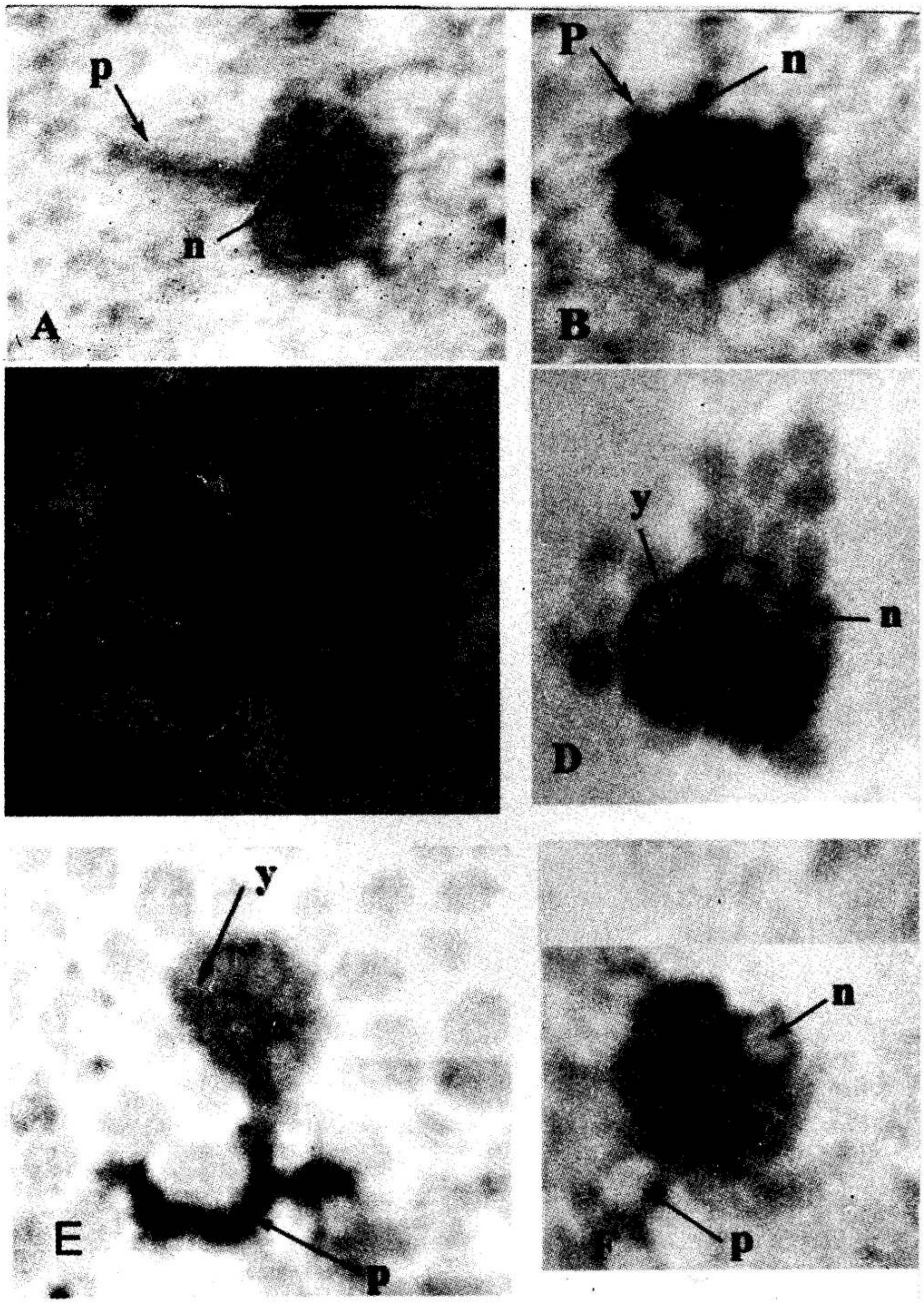

Fig. 1. Amoebocytes and their phagocytic activity. **A.** Amoebocyte with long pseudopodia (Type I); **B.** Amoebocyte with short pseudopodia (Type II); **C.** Phagocytosis of Type II amoebocytes in 15 minutes; D. Phagocytosis of Type II amoebocytes in 30 minutes; E. Phagocytosis of Type I amoebocytes in 45 minutes; **F.** Phagocytosis of Type II amoebocytes in 45 minutes. [Abbreviations : p, pseudopodia; n, nucleus; y, yeast. Scale bars: **A, E** -10 μm, **B, C, D, F** - 5 μm]

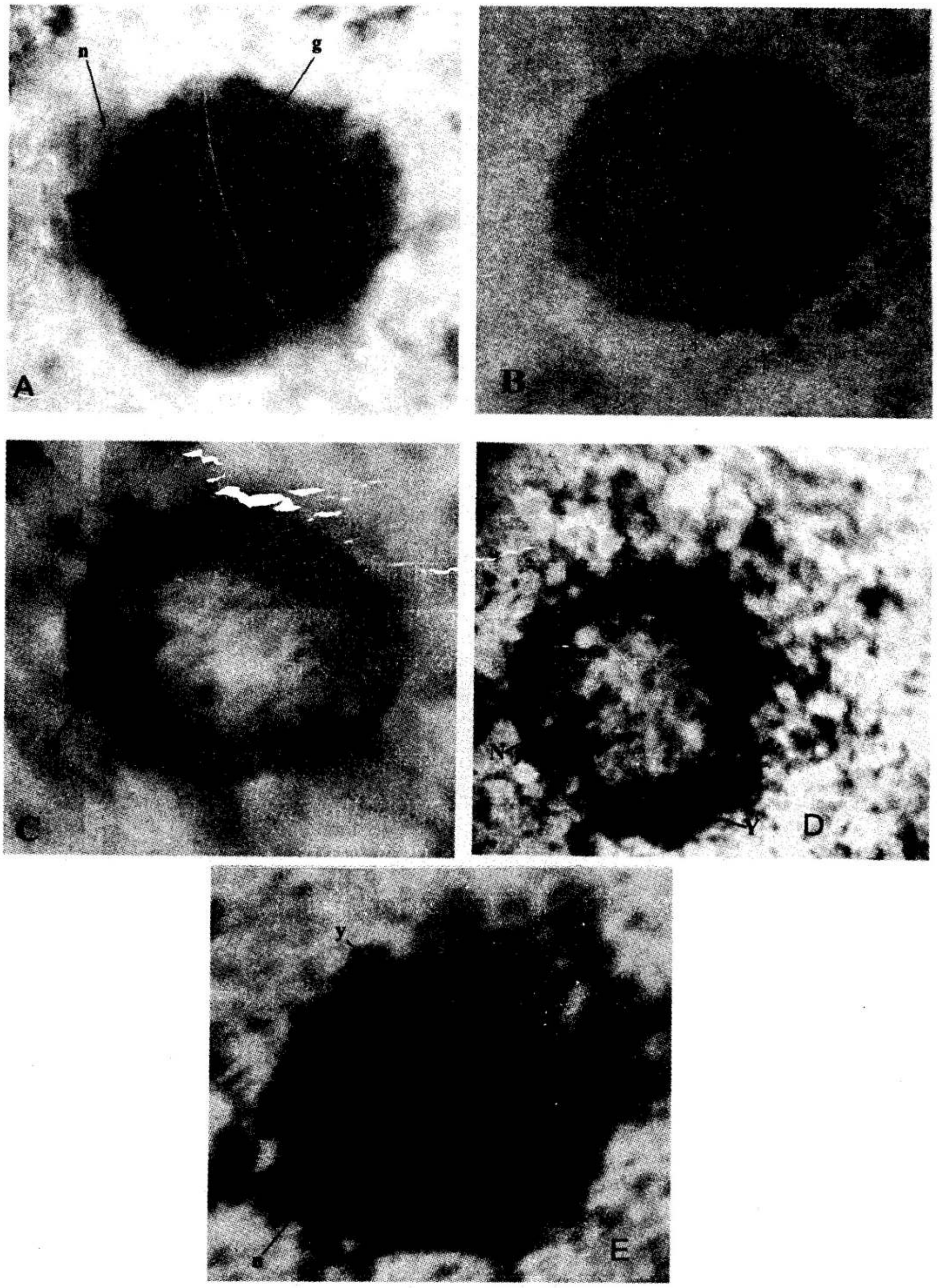

Fig. 2. Granulocytes and their phagocytic ability. **A.** Group I type with equally distributed granules; **B.** Group II type with characteristic vesicular structures, so called blebs; **C.** Group II type with spherical to club like granules; **D.** Phagocytosis of granulocyte in 15 minutes; **E.** Phagocytosis of granulocytes in 60 minutes. [Abbreviations : N, nucleus; Y, yeast; g, granules. Scale bars: **A, E** - 3 μm, **B** - 10 μm, **C, D** - 5 μm].

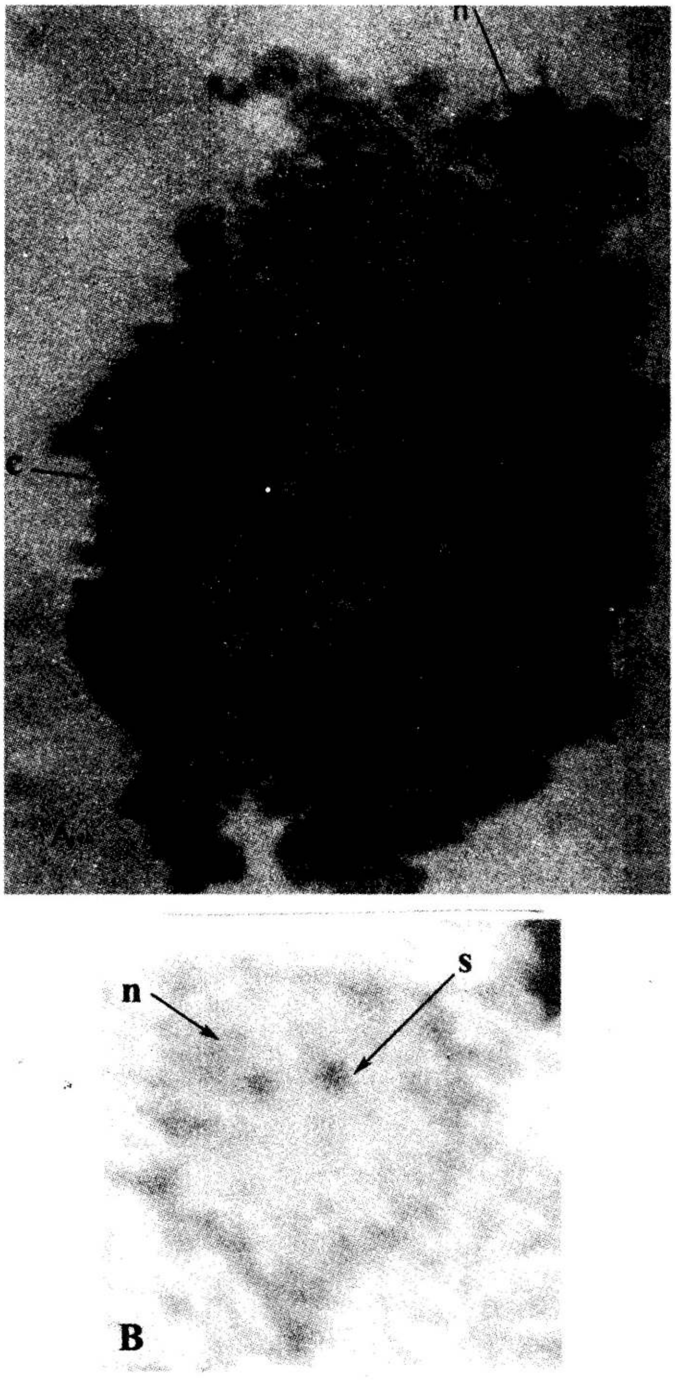

Fig. 3 : Elecoytes and their phagocytic ability. **A.** Chloragosomes with spherical eccentrically placed nuclei; **B.** Phagocytosis of stain particles by eleocytes; [Abbreviations : p, pseudopodia; n, nucleus; c, chloragosomes; s, phagocytosed stain particle. Scale bar: A - 3 μm, B - 10 μm].

end of the body. They consisted of disintegrated solid debris, the remains of amoebocytes and also cysts of nematodes and protists.

DISCUSSION

There is a surge of research relating to immune function and processes in invertebrates. As coelomocytes are immune cells, the investigation on them will contribute to our understanding of the invertebrate cellular immunity. The present study has revealed the proportion of different types of coelomocytes and their morphological, cytological features and phagocytic properties in a native species of earthworm, *M. travancorensis*.

Three different classes of cells noticed in the coelomic fluid of *M. trvancorensis* were amoebocytes (with or without pseudopodia - Type I and Type II), granulocytes (Group I and Group II) and eleocytes.

Major differences were observed in the morphology of amoebocytes and the eleocytes. Amoebocytes are the most representative cells in the coelomic fluid in *M. travancorensis*. In *L. terrestris*, amoebocytes are termed as basophils and in electron microscope they are thought to correspond to lymphocytic coelomocytes of type I and type II, because of some structural similarity to immature young lymphocytes of lower vertebrates and some mammals (Linthicum *et al.*, 1977; Stein & Cooper, 1981). Their centrally or peripherally located nuclei vary in shape from oval to kidney shaped and show strong affinity to acid stains, while their cytoplasm contains basophilic granules. The most characteristic feature observed in eleocytes is the presence of large distinct membrane bound granules (chloragosomes). The present observations agree with the previous findings of Affar *et al.* (1998), Peeters-Joris (2000) and Adamowicz & Wojtazek (2005). These cells resemble the liver cells of invertebrates with regard to certain functions like storage and transport of nutrients through the coelomic fluid (Hamed *et al.*, 2002). Similar to our results, the eleocytes of *L. terrestris*, the cytoplasm is not diversified, while chloragosomes are regularly distributed in the entire cell (Linthicum *et al.*, 1977; Stein & Cooper, 1981). The two types of granulocytic coelomocytes observed in *M. travancorensis* are similar to those reported by Adamowicz & Wojtaszek (2001) and Linthicum *et al.* (2004).

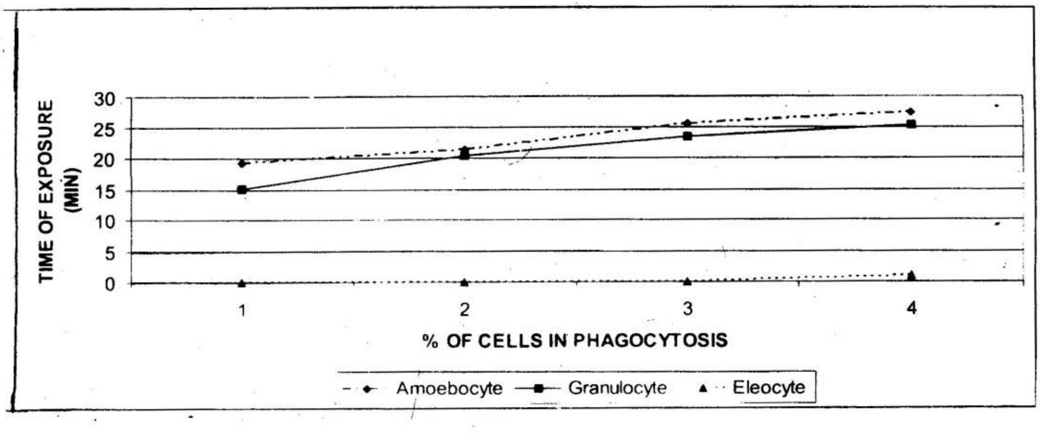

Fig. 4 : Phagocytic activity of coelomocytes.

In most of the worms studied, nearly half the amoebocytes were vacuolated. The increased number of vacuolocytes observed in the worms may be correlated with the aging process, since all the worms studied were fully mature or aging ones. According to Lavia & Hill (1972) the vacuolization and nuclear pycnosis are signs of cell death.

The differential count shows that the amoebocytes were the most represented coelomocytes. Kale & Krishnamoorthy (1979) have reported species-specific variation in the total counts and differential counts of coelomocytes in the five species of worms.

Among the three types of coelomocytes in *M. travancorensis*, the amoebocytes were found to be more active in phagocytosis. Ameobocytes are extremely efficient in removing foreign particles from the coelomic cavity, by phagocytosis (Millar & Ratcliffe, 1994). All cells exhibited phagocytic activity. Amoebocytes and granulocytes are capable of producing pseudopodia (Stein *et al.*, 1977; Linthicum *et al.*, 1977; Dhainaut & Scaps, 2001). The phagocytic activity of coelomocytes was found to increase as a function of time within an observed time interval. This is in agreement with the studies of Adamowicz & Wojtaszek (2001) in *D. venta*. Many granulocytes were found to be loaded with one or two yeast cells by 15 minutes of incubation. The peak phagocytic activity measured by the number of cells in phagocytosis is observed after 30 minutes. This is in correlation with the reports of Stein & Cooper (1981) in *L. terrestris*. In Type I amoebocyte and granulocytes, the phagocytic activity continued to increase after 45 minutes. A small number of yeast cells were phagocytosed by eleocytes during the 60 minutes of incubation. A similar phagocytic activity was observed by Stein and Cooper (1981); Adamowicz & Wojtaszek (2001), while Ratcliffe & Rowley (1981) showed that eleocytes are not capable of phagocytic activity. But in *M. travancorensis* a peak phagocytic activity was observed in all the cells after at 60 minutes of incubation. Similar to our observation Kalac *et al.* (2002) reported that the optimum period for the bacterial phagocytosis of coelomocytes of *D. veneta* is at 60 minutes.

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