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ROTIFERS AS A POSSIBLE BIOLOGICAL CONTROL AGENT AGAINST *BIOMPHALARIA ALEXANDRINA* EGGS

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ABSTRACT

The possibility of using the naturally occurring rotifers, present inadvertently in the snail aquaria, as biological control agent against snail eggs was tested. Two species of rotifers namely *Rotaria rotatoria* and *Philodina acuticornis* were identified, isolated and cultured intensively in controlled clean aquarium. Rotifers were fed on green algae of the genus *Scenedesmus*. Experiments were designed to test the ability of rotifers to destroy the snail egg masses coinciding with the abundance of rotifers in the media. The damage occurred to the snail egg masses by adult rotifers was monitored and assessed. The different stages leading to the egg masses destruction were identified. The results showed that the stage of development of embryos and the availability of rotifer's food were the two factors controlling this process.

INTRODUCTION

Schistosomiasis is among the most threatening diseases in most of the developing countries. Successful control of such disease should be based on an integrated approach which includes the control of the intermediate snail host as well as the parasite (WHO, 1993).

The application of molluscicides as a method of control has been the most common approach during the last decades. However, it may be successful in controlling snails, but it also has a number of major disadvantages, including the expenses of molluscicides and, very importantly the toxicity of the chemicals used, which may kill other non-target aquatic organisms such as fish. Also the possibility of development of resistance in schistosoma transmitting snails to commonly applied molluscicides is another important factor (Kloos & Mc-Cullough, 1982).

During the last decade, the search for new environmental friendly methods for combating schistosomiasis never stops. Among those is the use of biological agents which play a role in breaking the snail life cycle and / or compete with the free living stages of *Schistosoma* itself. For example, the ampullariid snails *Pila ovata*, *Lanistes carinatus* and *Marisa cornuarietis* can compete with *Biomphalaria* snails for food, and eat their eggs. The introducing of such snails into the infested habitat is considered in many countries (Hofkin *et al.*, 1991).

The Work of Chernin *et al.* (1957) demonstrated that the leech *Helobdella fusca* could be used as successful control tool for the intermediate host snail populations. It was also noticed that, in nature, juvenile and adult black carp feed exclusively on molluscs; thus, it has a great potential as a biological control agent against freshwater snails (Weinzettl & Jurberg, 1990).

Moreover growth of certain plants on the sides of waterways has been used to control snails. The berries of endod (*Phytolacca dodecandra)* have been shown to be naturally molluscicidal when they fall into the water (Lemma, 1970).

In the same direction, observations have long been made on a number of r5otifers that are found attached to shells of freshwater snails' especially *B. alexandrina.* The work of Stevens (1912) and Nekrassow (1928) proved that the two species of rotifers, *Proales gigantea* and *P. ovicola* were found attached to the egg masses of pulmonate snails. Their results suggested that rotifers can cause damage to eggs leading to destroy of the snail embryos. The same concept was tested by Stirewalt and Lewis (1981) in their monitoring of cercarial production by infected snails carrying rotifers.

The aim of the current study is to investigate the relationship between the presence of rotifers in the media surrounding the eggs of *B. alexandrina* on their development. The effect of rotifers was also evaluated as an attempt for using them as a bio-control agent.

MATERIAL AND METHODS

Materials:

Biomphalaria alexandrina snails were obtained from Giza drainage canals and reared in the animal house of the Zoology Department, Faculty of Science Al-Azhar University, Nasr City, Cairo. The egg-masses were collected on nylon sheets and divided into five stages of growth according to their embryonic development. These stages were one cell stage, gastrula stage, differentiation stage, pre-hatching stage and fully developed embryo (Plate 1, A,B,C and E) (Kamel, 1984).

The two tested species of rotifers; *Rotaria rotatoria* and *Philodina acuticornis* were obtained from a field strain aquarium containing *B. alexandrina* snails. The rotifers were isolated with their resting eggs using Pasteur pipette under a bi-nocular dissecting microscope, transferred to a clean aerated aquarium filled with dechlorinated tap water. The aquarium was provided with green algae that serve as a source of food for rotifers. This aquarium was maintained at 27 $\pm$ 2 °C and 12 hours light and dark cycle and remained as a permanent culture for these two species of rotifers and their resting eggs.

Eggs of rotifers were classified into normal and resting eggs according to the intensity of the outer sheath where the resting eggs have darker brown operculated capsule (Plate 2, E)

Experimental work :

Experiment 1: Rotifers invasion to snails eggs.

For determination of the rotifers invasion to snail egg masses, a number of egg-masses at different embryonic developmental stages were placed in rotifers intensive culture for nine days. The egg masses were examined at fixed intervals (every 12 hours) and photographed whenever any changes occur. The mechanism in which the egg mass was invaded was described in details. The experiment was repeated several times to ensure the same behavior from rotifers towards the egg masses.

Experiment 2: Rate of invasion of eggs by rotifers.

For determination of the infestation rate of rotifers to snail egg masses fifty clean egg-masses at different embryonic developmental stages were numbered on the back of the egg-mass nylon holding sheet . The numbered egg masses were placed in the rotifer culture tank, the number or rotifers and their normal and resting eggs were monitored daily on each sheet for nine days. Also, changes in the stage of development of snail eggs were recorded. The status of each egg-mass was recorded as fully hatched , partially hatched and dead egg-mass.

Experiment 3: Effect of food deficiency on the number of invading rotifers.

This experiment was designed to determine the effect of food deficiency on rotifers intensity on *B. alexandrina* egg masses. A group of fifty egg masses infested with rotifers were placed in dechlorinated tap water. Food for the rotifers was provided only once at the beginning of the experiment. Changes in number of rotifers and their resting eggs were monitored during three weeks period. The experiment was repeated three times with a new set of infested eggs every time and without adding any food.

RESULTS

A :The Rotifers Invasion :

Generally, the two tested rotifer species *Rotaria rotatoria* and *Philodina acuticornis* were found fixed to any substrate as snails shells, algae or debris suspended in water (Plate2, A-D). However, it was noticed that they prefer soft substrata than hard ones. They were found fixed on the ootheca of the snails egg mass by their star-shaped foot and begin to penetrate the egg mass tertiary membrane (Plate 1, D), leaving their upper half distinguishable by corona or the ciliary ring out in water (Plate3, A). When rotifers became well attached, their cilia draw a current of water carrying suspended particles to the mouth located between the two trochal disks.

When rotifers penetrate the egg mass they cause a series of mechanical damages; first they form pores due to their invasion by their star-shaped foot which never exceeds the outer layer. Second and as a result of pouching their bodies inside the rest of the egg mass layers, they form long tunnels (Plate 3, C) . The length of these tunnels may reach more than half of the rotifer length. The top part of these tunnels is somewhat swollen forming an area full of excreting materials (Plate 3, D).

With the increase in number of rotifers on the egg mass surface, the pores became obvious and with their migration from one point to another on the surface the egg mass it looked like a sieve (Plate 3, B). Through these pores, water protozoa and other micro-organisms can invade the egg, causing disturbance to the embryo which may lead to its death (Plate 4, A-D).

B :Rate of Rotifers Invasion :

The data representing changes in the number of rotifers recorded in the egg masses and their effect on the different stages of maturity is given in Table (1). In the second experiment, all the egg masses exposed to rotifers culture were found to contain a considerable number of rotifers regardless of their maturity stages. Among different stages of development, the only stage which survive rotifers invasion was the pre-hatching stage, while other stages always ended with the death of all individuals.

The results in table (1) showed that the number of rotifers showed a significant increase ($P>0.05$) after nine days of incubation. The highest number recorded was that in newly laid eggs (stage I&II) or those needing a longer period before hatching. The vast increase in number of rotifers per egg mass was reflected on the number of eggs being able to hatch. It was also noticed that, at the third stage of development where the embryo was able to move inside the egg capsule, those embryos in the center of the egg mass (about 5% of the total) were able to survive.

The egg masses introduced to the experiment in the pre-hatching stage were able to get out of the egg masses during the first three days prior to the rotifers heavy invasion. However , only 2% of these embryos were less developed, being affected by the presence of rotifers.

C: Effect of food deficiency on number of rotifers

The purpose of this experiment was to monitor the effect of food deficiency on the rotifer behavior towards the snail egg masses. A series of photographs demonstrating the results of this experiment is presented in plate 5 (A-F) and their data in Figure 1(a-b).

During the first week of experiment (Plate 5,A-B), where food was more available the mean number of rotifers on the egg masses was 11.16 ± 7.5 individual/egg mass. Meanwhile, the average number of rotifers resting eggs was 1.43 ± 1.9 per egg mass. At the end of the first week the mean number of rotifers increased significantly ($P>0.05$) on the outer surface of the egg masses, being 57.37 ± 26.0 individual/egg mass, regardless of the developmental stage of the eggs. However, the mean number of the resting eggs did not show any dramatic increase being 1.273 ± 1.2 egg/egg mass.

In the second week experiment (Plate5,B), and with a fresh group of egg masses the mean number of rotifers recorded on the egg mass surface was 9.49 ± 7.4 individual/egg mass. Also, the resting egg number was not changed from that recorded during the first week, being 1.44 ± 2.1 / egg mass in average. At the end of the second week and with decrease in food, the number of rotifers attached to new egg masses were lower than that recorded in the first week, being 33.57 ± 20.27 in average. On the other hand, the mean number of resting eggs showed an increase, being 10.56 ± 8.4 eggs/ egg mass.

During the third week experiment (Plate 5,D), and as a result of food deficiency, the number of rotifers found attached to the egg masses was reduced dramatically from 12.45 ± 7.38 to 2.2 ± 2.6 individual/egg mass with about 50 % of the egg masses found free of rotifers. The number of rotifers resting eggs continued to increase during this week, reaching 21.16 ± 10.5 eggs/egg mass. At the end of this experiments very few rotifers were seen attached to the egg masses and mostly what remained was the resting eggs.

DISCUSSION

The greatest advantage of biological control is the possibility of interrupting the life cycle of a harmful organism, using another living organism without any substances or other factors that may damage the environment (Madsen,1990).Most studies carried out for biological control of schistosomiasis were mainly based on the introducing of snail predators or competitors (Pointier & Augustin, 1999 and Pointier& Jourdane, 2000) , crayfish (Loker *et al.*,1992 and Loker *et al.*,1993), fishes (Weinzettl and Jurberg, 1990) or leeches (Chernin *et al.*, 1957).

Garcia and Legner (1999) reviewed the developments in biological control of mainly freshwater snails specially those related to the transmission of trematode parasites. Their review pointed out some successful experiments of controlling snail vectors, including major concepts. These concepts include predation of snails by fishes and large invertebrates (Loker *et al.*, 1992 and Loker *et al.*, 1993); It also included introducing of competitor species of snails (Hofken *et al.*,1991). The results presented by Hassan *et al.* (1985) were mainly concerned with the effect of the rotifers presence in the media on the embryonic development of *B. alexandrina* snails. They suggested that rotifers have a growth inhibitory effect specially in young embryos till the blastula stage. The present results agree with their results except that their effect might continue throughout the different stages of development. However, the younger the stage of the egg , the more effective inhibition was recorded. The results also showed that even well developed moving embryos can be affected indirectly by the mechanical damage caused by rotifers to the egg ootheca permitting other micro-organisms to penetrate the egg mass.

The major effect of rotifers was explained by many authors (Stevens, 1912, ; Nekrassw, 1928; Stierwalt & Lowis, 1980 and Hassan *et al.*, 1985) as a result of chemicals that released from rotifers to the surrounding waters. They suggested that the water conditioned by rotifers may be the reason for less cercarial output of the snails. On the other hand, the test conducted by Hassan *et al.* (1985) showed not more than 10% mortality of eggs which occurs as a result of exposure to conditioned water. The mechanical effect presented in our study seems to have much greater effect on the snail eggs than the chemically conditioned water.

Concerning the rate of rotifers invasion to snail egg masses, it was found that rotifers tend to attach more to the newly laid eggs, where the ootheca covering the egg mass was still fresh and easy to penetrate. These results are shown in table (1), where the number of rotifers attached to egg masses of stages I and II were almost double those attached to eggs of stages III and IV. On the other hand, such increase in the number of rotifers causes an increase in number of their tunnels through the egg mass leading either to egg collapse or to invasion by other predatory species. These findings are in agreement with the brief review of Michelson (1957).

From the present results it is clear that the rotifers resting egg production was totally related to the inavailability of food indicated by their increase during the three weeks experiment, where no food was available.

It also could be concluded that under laboratory conditions rotifers can be very useful tool in controlling snail eggs in addition to their documented ability to control cercarial production. However, it is fair to say that in the field the situation may be different and in order to achieve similar results, certain conditions should be present. These conditions will include stagnant or slow moving water, large number of rotifers, plenty of phytoplanktonic algae for rotifers to feed. Furthermore, a field experiment is needed to investigate the effect of increasing number of rotifers under semi-field conditions.

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Table (1) Number of rotifers invaded the egg masses and their effect on the different stages of maturity after nine days of incubation.

Stage of Maturity	# Of Egg masses	Eggs		Rotifers		Egg
		Total	Mean ± SD	Total	Mean ± SD	Mortality%
I	15	218	14.53 ± 8.42	670	44.66 ± 8.34	100
II	15	278	18.53 ±10.6	770	51.30 ± 21.67	100
III	10	192	19.2 ± 5.49	460	46.0 ± 29.14	95
IV	10	231	23.1 ± 9.56	465	46.0 ± 38.46	2.0

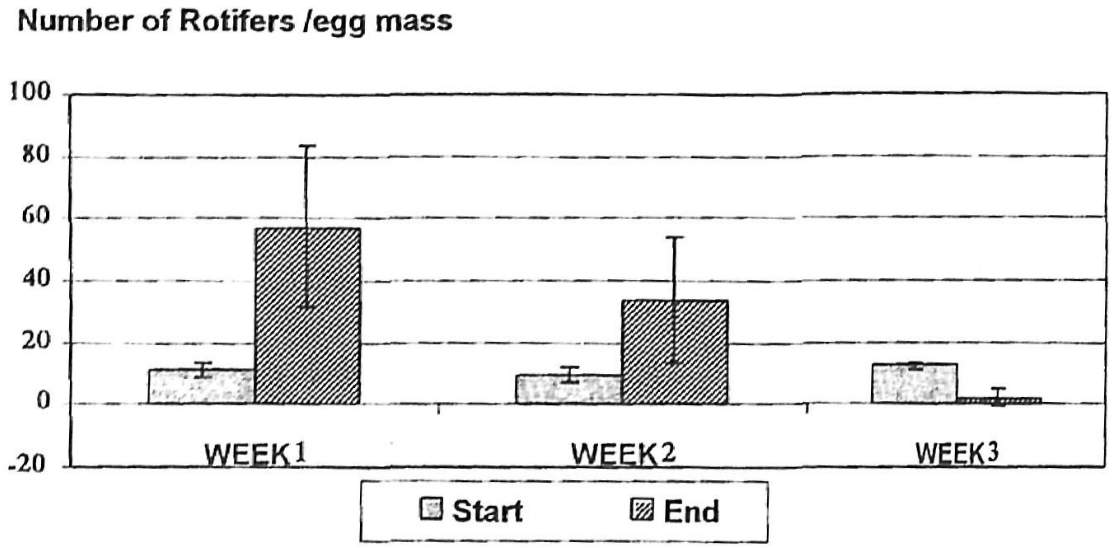


Figure 1,a: the changes in number of rotifers during experiment III.

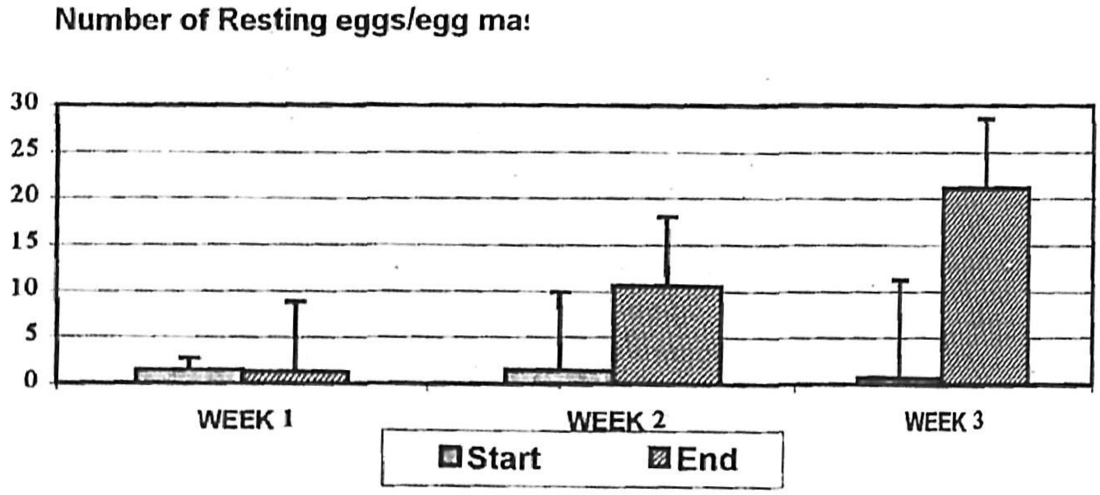
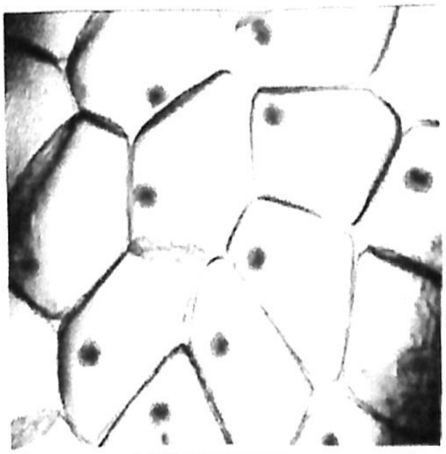
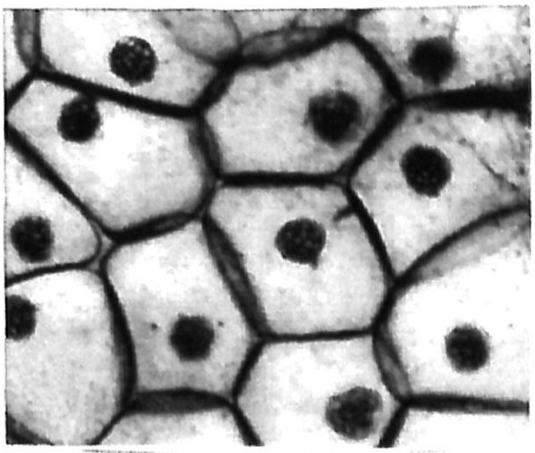


Figure 1,b: the changes in number of rotifers resting eggs during experiment III.

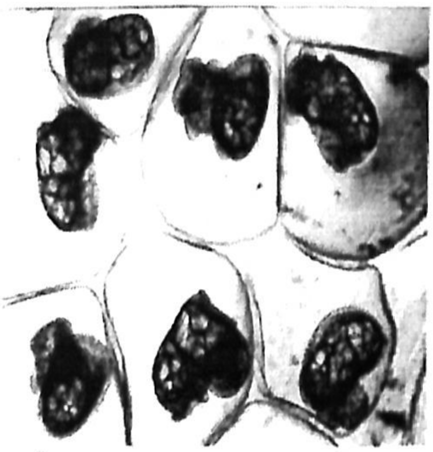
PLATE I



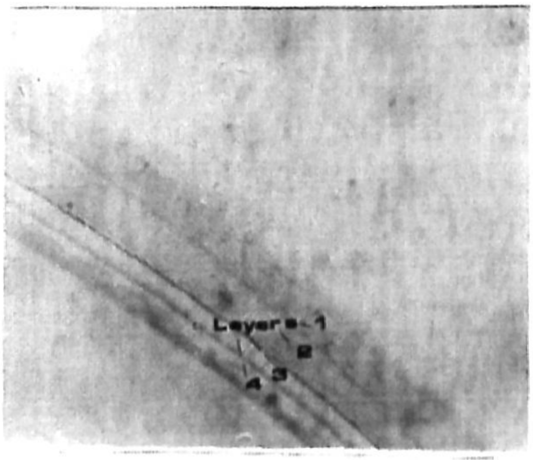
A (single cell stage)



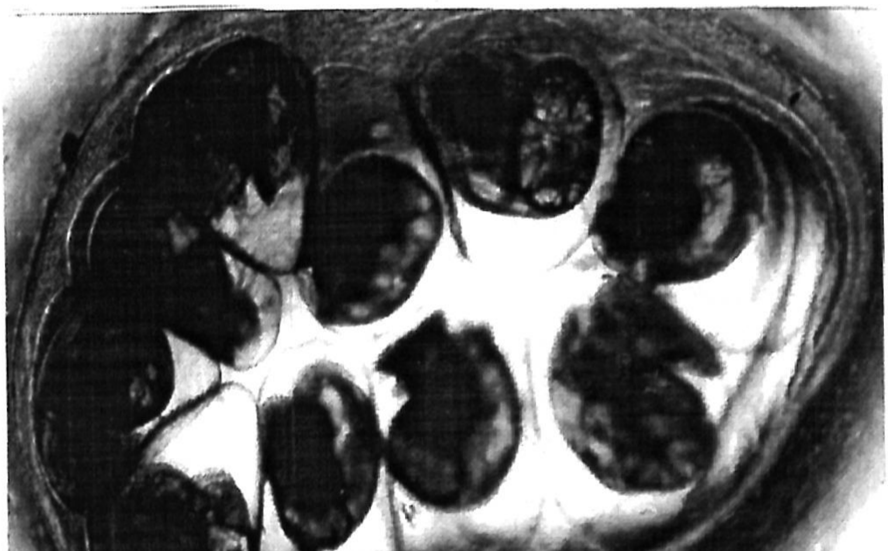
B (Blastula stage)



C (Pre-hatching stage)

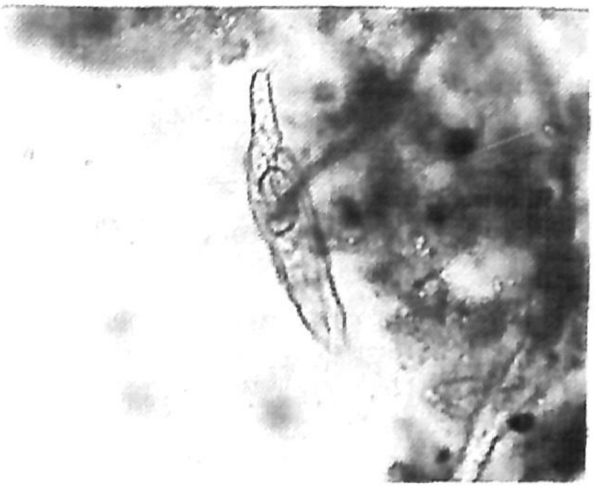


D (Egg capsule wall)



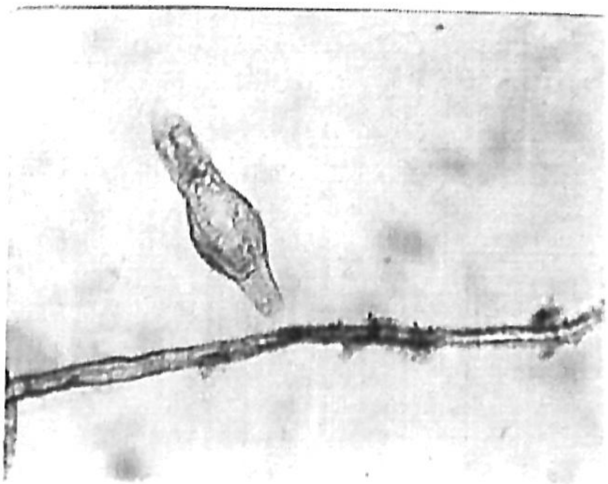
E (Fully developed embryos)

PLATE II

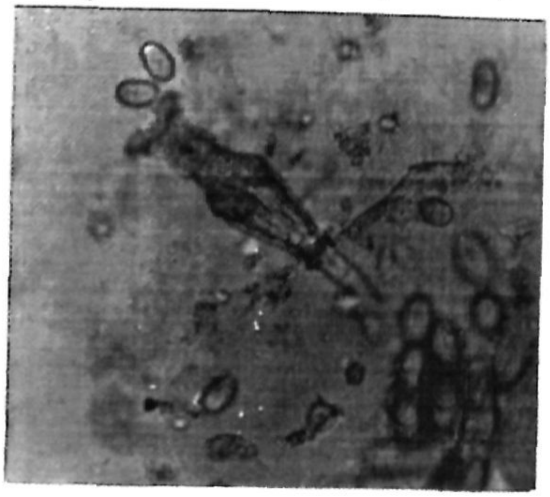


A (Free swimming rotifer)

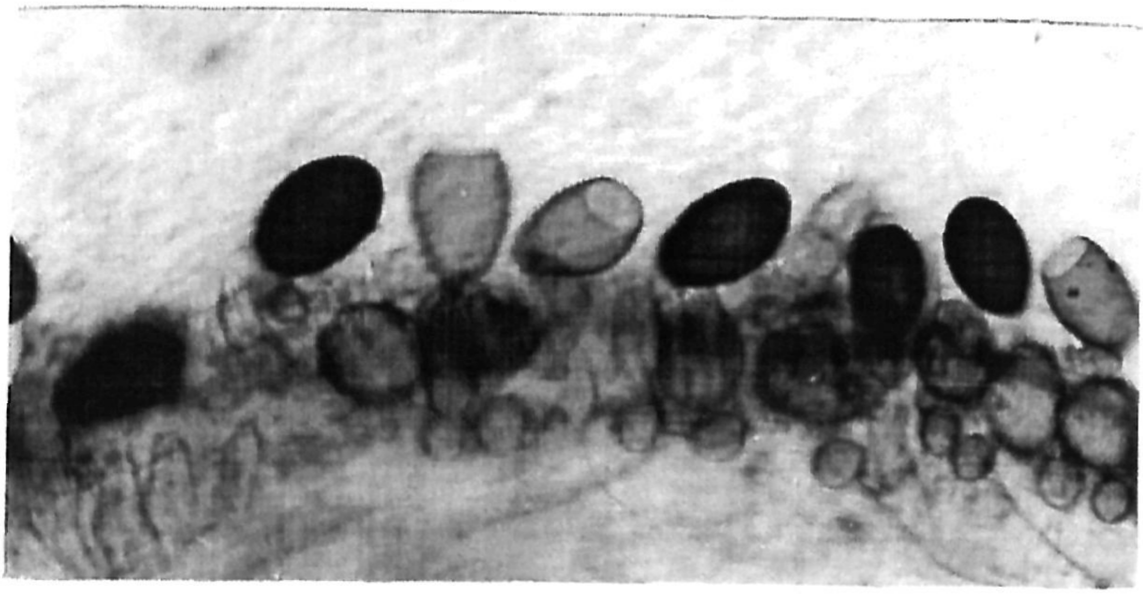
B (Rotifer attached to plant leaf)



C (Rotifer attached to Algae)

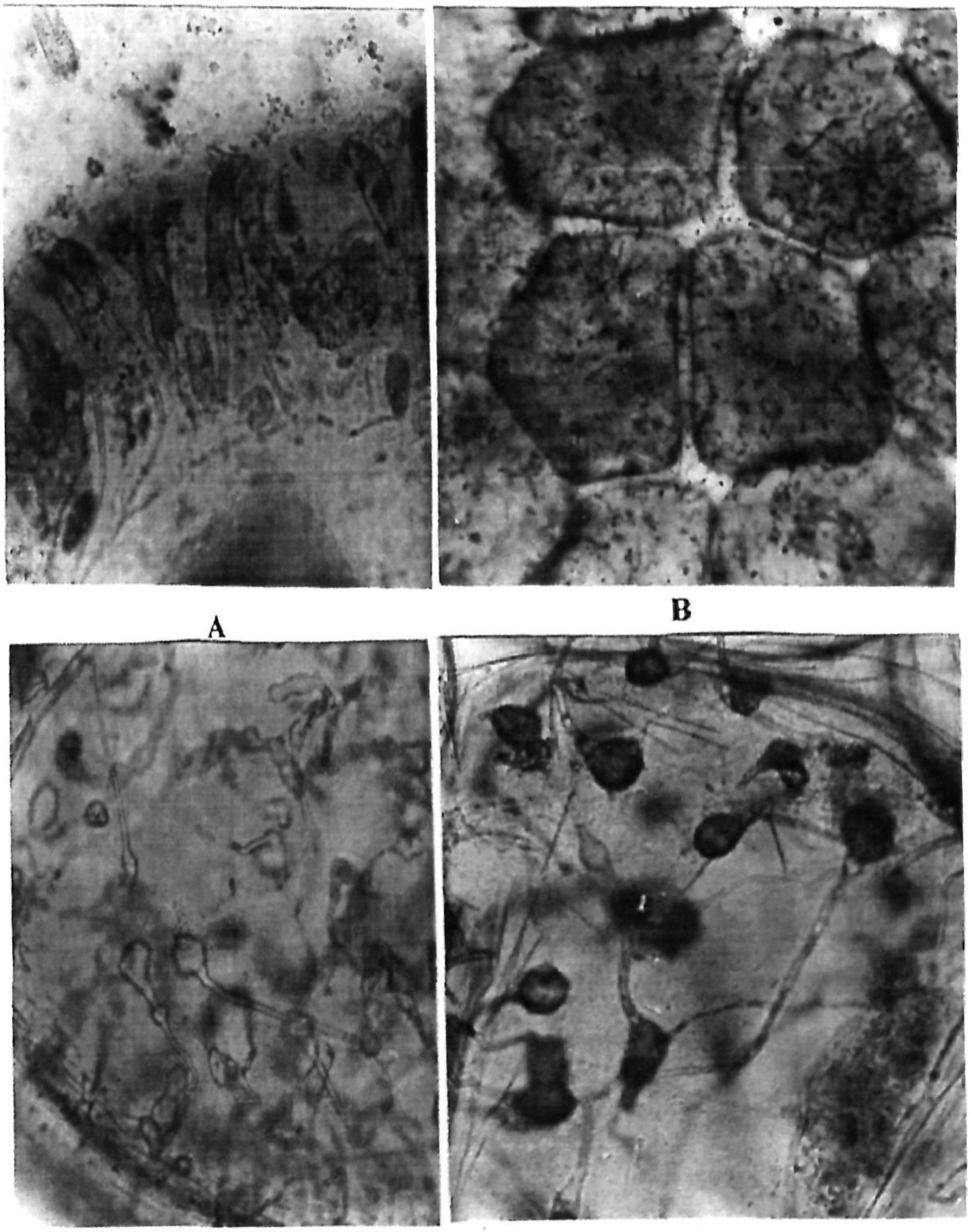


D (Rotifer attached to debris)



E : The normal (light) and resting eggs (dark) of rotifers

PLATE III



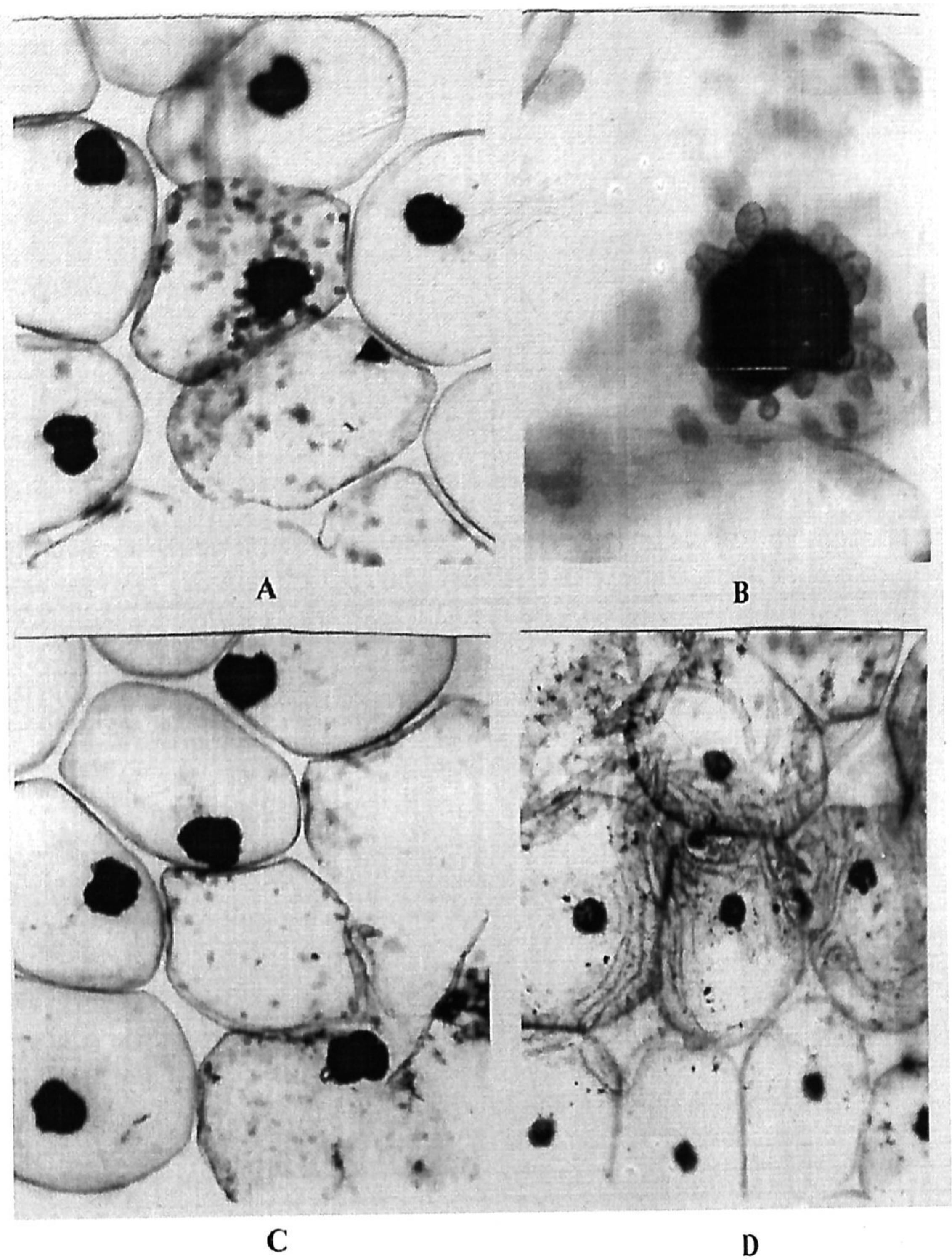
A : Large number of rotifers attached to the outer layers of the egg mass X400.

B : Invaded egg mass showing the number of pores in the outer surface X100.

C : The tunnels left by rotifers in the egg mass surface X400.

D: The swollen part of the tunnels filled with excretions of rotifers X400.

PLATE IV



A: The protozoan invasion of the eggs through the rotifers tunnels X100.

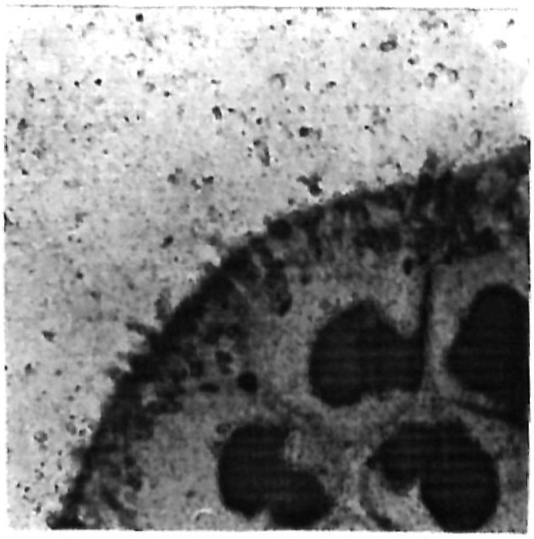
B: The protozoan animals surrounding the embryo in the early stages X400.

C: The empty egg capsules after invasion X100.

D: Oligocheates feeding on what's left of the egg materials X100.

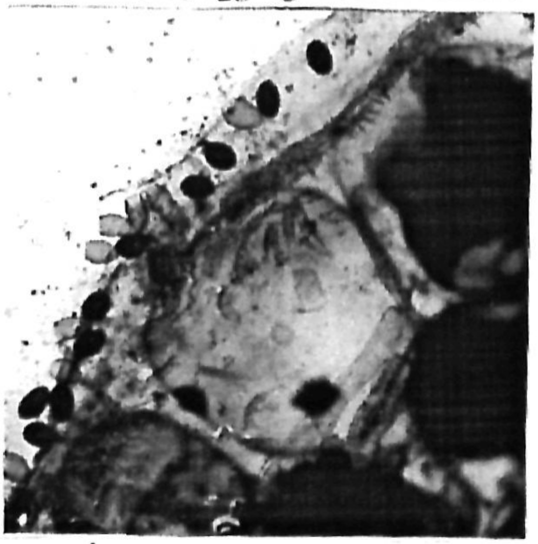
PLATE V

A: 1st week, large number of Rotifers

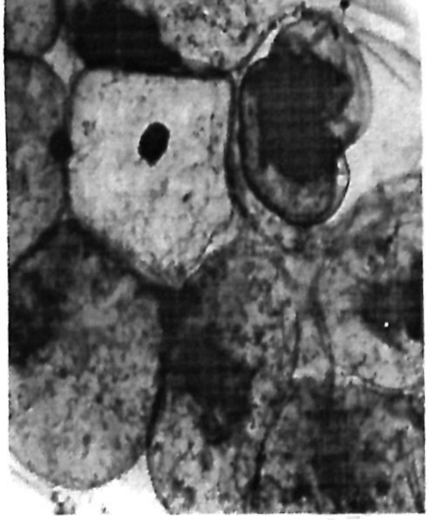


B: 1st week, aggregation of rotifers

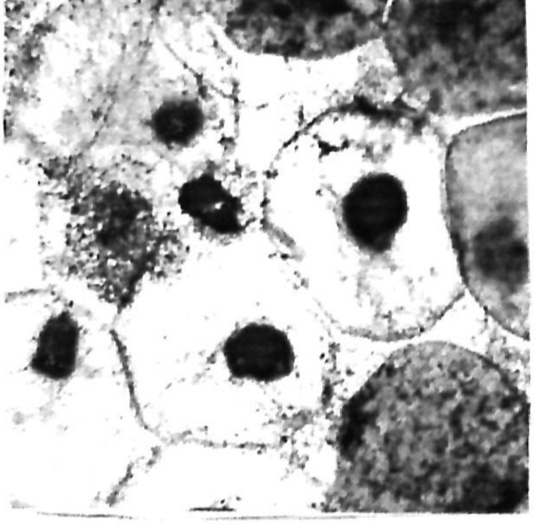
C: 2nd week increase in egg laying



D: 3rd week , resting eggs increase



E: Damaged egg mass



F: Dead embryos after 9 days