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The relationship between the bioaccumulation of heavy metals in *Clarias gariepinus* tissues and endoparasitic helminths at Kafr El Sheikh Governorate, Egypt

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Abstract

A total number of 200 *Clarias gariepinus* were collected a live from two different ecosystem (100 *Clarias gariepinus* from each locality),where locality1 (Drainage Canal {Al-Gharbiya Drainage Canal}) and locality2 (River Nile Branch {Bahr Teara}) and examined for presence of endoparasitic helminths, *Clarias gariepinus* infected with cestodes (infestation rate was 19% and 4% in Drainage Canal and River Nile Branch, respectively) as: *Polyonchobothorium clarias* and *Monobothrium spp.* with an infestation rate 32.9% and 67.1%, respectively in Drainage Canal and 25% and 75%, respectively in River Nile Branch and nematodes (infestation rate was 41% and 23% in Drainage Canal and River Nile Branch, respectively) as: *Procamallanus laeviconchus* and *Paracamallanus cyathopharynx* with an infestation rate 56.3% and 43.7%, respectively in Drainage Canal and 55.2% and 44.8%, respectively in River Nile Branch hence, infestation rate of endoparasitic helminths in Drainage Canal was higher than River Nile Branch. The water of Drainage Canal was more polluted with heavy metals as (Fe, Cu, Hg, Pb, Ni, Mn and Cd) than the water of River Nile Branch while, Zn within Permissible limits. Heavy metal residues in the organs of *Clarias gariepinus* in River Nile Branch were higher than Drainage Canal. Heavy metals were bioaccumulated in *Clarias gariepinus* in order: liver> kidney> muscle in the two localities (River Nile Branch and Drainage Canal).

Keywords: Heavy metals, Prevalence, Endoparasitic helminths, *Clarias gariepinus*, Kafr El Sheikh Governorate.

Introduction

Fishes represent one of the best nutritious, relatively cheap food-protein, if compared with other animal protein source. Fishes like any other vertebrate are suffering from parasitic infestation either as final or intermediate hosts, with the possibility of transmission of these to human. Pollution of environmental water is a serious and growing problem all over the world. Fish bioaccumulate pollutants directly from contaminated water and indirectly by ingestion the contaminated feeding. Heavy metals are natural trace components of the aquatic environment but their levels have increased due to domestic, industrial, mining and agricultural activities **(Kalay and Canli, 2000)**. Discharge of heavy metals into river or any aquatic area can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behavior. Aquatic organisms such as fish and shell fishes accumulate metals to concentrations many times higher than present in water **(Olaifa et al., 2004)**.This investigation was carried out for studying the prevalence of endoparasitic helminths infect *Clarias gariepinus* and for studying the relationship between the collected endoparasitic helminths and the environmental pollutants (heavy metals) in the investigated areas.

Materials and methods

Fish samples:

A total of 200 *Clarias gariepinus* ranged between19 – 33cm in length and from 125 – 497gm in weight collected alive from two different ecosystem(100 *Clarias gariepinus* from each locality),where locality1 (Drainage Canal {Al-Gharbiya Drainage Canal}) and locality2 (River Nile Branch {Bahr Teara}).

Water samples:

4 water samples (from each locality), from the same source of fish collection (Drainage Canal and River Nile Branch) were taken. The flasks, one liter volume were rinsed several times with distilled water and sterilized in a hot air oven at 180̊ C / hour, then equipped with a cork stopper and open hand prides under water surface then equipped again and fixed drops with conc. nitric acid. Water samples were collected as replicates from various distances and depths along each locality and the collected water samples were labeled with the locality, date and time of collection and the average of their analysis were taken.

Parasitological examination:

The gastrointestinal tract as well as gall bladder was examined by naked eye for the presence of worms, encysted metacercaria and larval stages then the stomach and intestine were opened separately in petri dish which contains physiological saline. The mucosa of stomach and intestine was scraped. Cestodes were pressed between two slides and immersed in fixative (10% formal saline) and stained with acetic acid alum carmine, then dehydrated in ascending grades of ethyl alcohol, cleared in clove oil and mounted in Canada balsam. Nematodes fixed in glycerin alcohol, then cleared in lactophenol and mounted in glycerin-gelatin. The collected worms were identified according to **Yamaguti (1958, 1959 and 1961).**

Analytical procedures:

After filtration of water samples, they were examined chemically to estimate some heavy metals as (Cu, Fe, Pb, Zn, Hg, Cd, Ni and Mn) by using atomic absorption spectrophotometry model IL57 FAAS, according to **Chapman and Pratt (1978)**. Concerning detection of heavy metals residues in *Clarias gariepinus*, approximately 4gm of musculature, liver and kidney of *Clarias gariepinus* (10 *Clarias gariepinus* from each locality homogenized with each other) was dissected, washed with dis. water, dried with filter paper, weighed, packed in polyethylene bags and kept at -30°C until analysis. 0.5gm of these samples was digested using conc. H_2SO_4 giving deep brown color, shake and lift overnight, then put few drops of conc.H_2O_2 and lift it on a hot plate until the solution become colorless, the solution diluted to 50ml with dis. water using conical flask and shaken well and analyzed for (Cu, Fe, Pb, Zn, Hg, Cd, Ni and Mn) by using atomic absorption spectrophotometry model IL57 FAAS, according to **Cottenie (1980)**.

Statistical analysis:

A one way analysis of Varience (ANOVA) was under taken to analysis the result.

Results and Discussion

Clarias gariepinus infected with cesodes (with an infestation rate 19% and 4% in Drainage Canal and River Nile Branch, respectively) as: *Polyonchobothorium clarias* and *Monobothrium spp.* with an infestation rate 32.9% and 67.1%, respectively in Drainage Canal and 25% and 75%, respectively in River Nile Branch and nematodes (with an infestation rate 41% and 23% in Drainage Canal and River Nile Branch, respectively) as: *Procamallanus laeviconchus* and *Paracamallanus cyathopharynx* with an infestation rate 56.3% and 43.7%, respectively in Drainage Canal and 55.2% and 44.8%, respectively in River Nile Branch, as shown in table(1),table(2) and plate(1),plate(2).

Concerning occurrence of heavy metals in water the present results showed that, the water of Drainage Canal was more polluted with heavy metals as (Fe, Cu, Hg, Pb, Ni, Mn and Cd) than the water of River Nile Branch while, Zn within Permissible limits as shown in table (3) and table (4):

Concerning Heavy metals residues in different organs of *Clarias gariepinus* in the two examined localities, the current results showed that, heavy metal residues in the organs of *Clarias gariepinus* in River Nile Branch were higher than in the organs of *Clarias gariepinus* in Drainage Canal. Also, Heavy metals were accumulated in *Clarias gariepinus* tissues in order: liver> kidney> muscle in the two localities (River Nile Branch and Drainage Canal) as shown in table (5) and table (6).

In the present investigation, *Clarias gariepinus* was infected only with cesodes and nematodes with total infestation rate at two localities 32% and 11.5%, respectively. (Tissues of *Clarias gariepinus* were free from encysted metacercaria and larval stages)

Nearly similar results were observed by **Wanns (1977)** who recorded 11.6% and 22.5%, respectively. Also, **Noor El-Din (1981)** recorded 35% and 36.5%, respectively and lower than obtained by **Mohammed (2012)** recorded 65% and 34%, respectively. Also, **Chowdhury and Hossain (2015)** recorded cestodes, nematodes from fish *Channa punctata* from different water bodies at Jessore, Bangladesh with an infestation rate 41.1% and 29.7%, respectively.

Concerning *Polyonchobothrium clarias* with an infestation rate at two localities 31.8% which higher than obtained by **Oofintoye (2006)** recorded 5.3% and **Mwita (2014)** who recorded it from duodenum and gall bladder of *Clarias gariepinus* with an infestation rate 5.3% and 4.4%, respectively.

The infestation rate of *Clarias gariepinus* with *Monobothrium spp.* was 68.2% at two localities. This rate was higher than obtained by **Mahfouz (1991)**, **Ooniyte et al. (2004)** and **Mohammed (2012)** who recorded 1.5%, 13.33% and 14.5%, respectively.

The infestation rate of *Clarias gariepinus* with *Procamallanus laeviconchus* at two localities was 56%, this rate was higher than obtained by **Boomker (1982)** who recorded the prevalence 30.25% , also this result agree with **Aliyu and Solomon (2012)** who isolated *Procamallanus laeviconchus* from the intestine of *Clarias gariepinus*.

Concerning the infestation rate of *Clarias gariepinus* with *Paracamallanus cyathopharynx* at two localities was 44%. This result was lower than obtained by **Boomker (1982)** and **Atwa (2006)** who recorded the infestation rate 53.4% and 73.8%, respectively.

In the present study heavy metals in water was higher than those recorded by **Wehabe** and **Abo-Esa (2002)** who recorded that the mean concentration of heavy metals as lead, cadmium, mercury, copper and zinc in water samples in Qaroun Lake were 0.018 ± 0.0006 ppm, 0.052 ± 0.0007 ppm, 0.0007 ± 0.00003 ppm, 5.84 ± 0.13 ppm and 0.37 ± 0.008 ppm, respectively. and nearly similar to the results which reported by **Mohamed et al. (2008)** who recorded that the concentration of Cu, Fe, Zn and Mn in the water samples collected from Ismailia Canal and Abbassa fish farm ponds, were 0.41 ± 0.14 mg/l, 0.47 ± 0.33 mg/l, 0.33 ± 0.39 mg/l and 0.36 ± 0.15 mg/l, respectively. Also, The present results indicated that the concentration of heavy metals in Drainage Canal (Al-Gharbiya Drainage Canal) were higher than Rive Nile Branch (Bahr Teara), but out results disagreed With **Abou Zaid (2011)** who reported that concentration of heavy metals in River Nile Branch (Bahr Nashart) were higher than Drainage canal (Damroo Drainage Canal) this may be attributed to difference in the locality and quality of examined water. Also, **Mastan (2014)** reported that the values of heavy metals (Cd, Zn, Cu, Cr, Pb and Hg) concentration were within the maximum permissible levels for drinking water and fish.

Concerning the concentration of heavy metals in *Clarias gariepinus* organs in River Nile Branch (Bahr Teara) were higher than those in Drainage Canal (Al-Gharbiya Drainage Canal) this because high infestation rate of endoparasitic helminths in Drainage Canal and may be return to parasites accumulate heavy metals inside parasite by large quantities than their fish host. These results are in harmony with those of **Riggs et al. (1987)** who recorded elevation of selenium concentrations in the cestode *Bothriocephalus acheilognathi* in comparison to the tissues of its fish definitive host, **Sures et al. (1997a)** reported that mean concentrations of lead and cadmium in *Monobothrium wageneri* from the intestine of *Clarias gariepinus* were 75 and 40 times higher than in the musculature of the host, **Tenora et al. (2000)** found that *Philometra ovata* which parasitized fish from the genera Abramis, Rutilus and Vimba (Cyprinidae) accumulated high concentrations of heavy metals than the host fish. They also, investigated Cr, Pb and Cd concentrations in the *Ligula intestinalis* and found that, it showed a high capacity of bioaccumulation of heavy metals located in the intestines of their respective final host. Also our results agree with those of **Turaekova et al. (2002)** who reported that some tapeworms had demonstrated the greatest capacity to accumulate heavy metals than the host fish.

Heavy metals residues in *Clarias gariepinus* tissues in the present study illustrated that heavy metals accumulated in higher rate in liver followed by kidney then muscles because liver is considered the ware house for heavy metals. These results were relative to those of **Norrgren et al. (1985)** who reported that the gills were the primary organ of accumulation of cadmium in rainbow trout whereas, liver and kidney together came at first in stone loach and **Shereif and Moaty (1995)** who found that the heavy metals were significantly higher in fish viscera including liver tissue than in the edible muscles. Our results agree with those of **Mohamed et al. (2008)** who recorded that the highest heavy metals levels were accumulated in the liver followed by gills and then musculature of *Clarias gariepinus*.

The present study showed that the prevalence of endoparasitic helminths and heavy metals in locality 1 Drainage Canal (Al-Gharbiya Drainage Canal) were higher than in locality 2 River Nile Branch (Bahr Teara). This may be due to the presence of pollutants (as heavy metals) which affect the immune system of fish and reduce fish immunity to parasites causing the increase in the prevalence and the intensity of parasites and increase parasitic infection. These results agree with **Kabata (1985)** who reported that the unfavorable environmental conditions, contribute to stress which weakness immunity and open the pathway to the fish parasites. Also, **Sindermann (1990)** reported that parasitism may increase the host susceptibility to toxic pollutants and pollutants may result in an increase in the prevalence of certain parasites.

Conclusion

This study was carried out for the relationship between the endoparasitic helminths of *Clarias gariepinus* in certain sites at Kafr El-Sheikh Governorate and heavy metals pollution.

Fish samples were collected from two different localities as follow:

- 100 *Clarias gariepinus* were collected from Drainage Canal (Al-Gharbiya Drainage Canal) which receives agriculture effluents, dead animals, human and animal wastes.
- 100 *Clarias gariepinus* were collected from River Nile Branch (Bahr Teara) which receives agriculture wastewater.

Clarias gariepinus infected with cestodes (infestation rate was 19% and 4% in Drainage Canal and River Nile Branch, respectively) as: *Polyonchobothorium clarias* and *Monobothrium spp.* with an infestation rate 32.9% and 67.1%, respectively in Drainage Canal and 25% and 75%, respectively in River Nile Branch and nematodes (infestation rate was 41% and 23% in Drainage Canal and River Nile Branch, respectively) as: *Procamallanus laeviconchus* and *Paracamallanus cyathopharynx* with an infestation rate 56.3% and 43.7%, respectively in Drainage Canal and 55.2% and 44.8%, respectively in River Nile Branch**.**

For studying the pollution degree, water samples from the two different localities were examined chemically to measure the degree of heavy metals pollution; the present study showed that the water of Drainage Canal was more polluted with heavy metals as (Fe, Cu, Hg, Pb, Ni, Mn and Cd) than the water of River Nile Branch while, Zn within Permissible limits

Heavy metal residues in the organs of *Clarias gariepinus* in River Nile Branch were higher than in the organs of *Clarias gariepinus* in Drainage Canal.

Amounts of heavy metal in the water, the prevalence and the intensity of endoparasitic helminths in locality1 (Drainage canal) were higher than locality2 (River Nile Branch).

Table (1): Prevalence of helminth parasites in examined *Clarias gariepinus* in the two localities:

Locality / Parasite	Locality1 (Drainage Canal)		Locality2 (River Nile Branch)	
	Clarias gariepinus No.= 100		*Clarias gariepinus* No.= 100	
	No. of infested fish	Percentage of infestation%	No. of infested fish	Percentage of infestation%
Cestode	19	19%	4	4%
Nematode	41	41%	23	23%

Table (2): Incidence of helminth parasites in examined *Clarias gariepinus* in the two localities:

Locality / Parasite	Locality1 (Drainage Canal) Fish species	Locality2 (River Nile Branch) Fish species
	Clarias gariepinus	*Clarias gariepinus*
Polyonchobothrium clarias	32.9% (24/73)	25% (3/12)
Monobothrium spp.	67.1% (49/73)	75% (9/12)
Procamallanus laeviconchus	56.3% (98/174)	55.2% (32/58)
Paracamallanus cyathopharynx.	43.7% (76/174)	44.8% (26/58)

Table (3): Heavy metals in water from the two examined localities (Mean value ± SE):

Water source	Heavy metals detected in PPM							
	Zinc	Iron	Manganese	Copper	Mercury	Lead	Nickel	Cadmium
Locality1 (Drainage Canal)	0.9± 0.2	1.3± 0.7	0.4± 0.05	2.1± 0.4	1.8± 0.8	0.4± 0.1	1.3± 0.4	0.2± 0.08
Locality2 (River Nile Branch)	0.3± 0.1	0.9± 0.4	0.3± 0.11	0.7± 0.1	0.36± 0.32	0.2± 0.1	0.6±0.4	0.05±0.007

Table (4): Permissible limits of trace elements in water detected in PPM according to Egyptian Law 1589/2005:

Zinc	Lead	Manganese	Copper	Nickel	Cadmium	Mercury	Iron
3	0.05	0.1	2	0.02	0.01	0.001	0.3

Table (5): Heavy metals residues in different organs of *Clarias gariepinus* in locality1 (Drainage Canal) (Mean value ± SE):

Locality / Organ	Locality1 (Drainage Canal)							
	Zn	Fe	Mn	Cu	Hg	Pb	Ni	Cd
Muscle	1.36± 0.3	0.82± 0.3	0.1± 0.06	0.20± 0.03	0.05± 0.002	0.82± 0.1	0.42± 0.008	0.008± 0.0002
Liver	1.98± 0.2	1.9± 0.1	0.18± 0.03	0.59± 0.02	0.19± 0.04	1.2± 0.01	0.72± 0.003	0.06± 0.0001
Kidney	1.61± 0.3	0.9± 0.2	0.13± 0.04	0.32± 0.02	0.08± 0.002	1.1± 0.1	0.6± 0.006	0.009± 0.0002

**<u>SE</u> (Standard Error). **<u>PPM</u> (Part Per Million).

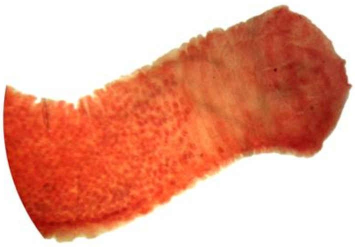
Table (6): Heavy metals residues in different organs of *Clarias gariepinus* in locality2 (River Nile Branch) (Mean value ± SE):

Locality / Organ	Locality2 (River Nile Branch)							
	Zn	Fe	Mn	Cu	Hg	Pb	Ni	Cd
Muscle	1.60 ± 0.3	1.1± 0.2	0.21± 0.04	0.34± 0.2	0.16± 0.03	1.3± 0.6	0.63± 0.09	0.071 ± 0.002
Liver	2.2± 0.2	3.2± 0.1	0.41± 0.01	0.84± 0.1	0.24± 0.02	1.6± 0.2	0.83± 0.04	0.28± 0.002
Kidney	1.89 ± 0.3	1.8± 0.1	0.32± 0.02	0.54± 0.2	0.17± 0.02	1.5± 0.4	0.74± 0.09	0.12± 0.008

Table (7): Permissible international levels of heavy metals in fishes:

Metal	Permissible limits in fishes	References
Lead (Pb)	0.3 mg/Kg	EOSQC (2007).
	0.5 ppm	FAO/WHO (1992).
Cadmium (Cd)	0.1 mg/Kg	EOSQC (1993).
	0.05 ppm	FAO/WHO (1992).
Mercury (Hg)	0.5 mg/Kg	EOSQC (1993) as methyl mercury.
	0.5 ppm	FAO/WHO (1992).
Copper (Cu)	20.0 ppm	Food stuffs, Cosmetics and Disinfectants (1972).
	20.0 µg/Kg	Boletin Official De Estando (1991) in Schulhmacher and Domingo (1996).
Zinc (Zn)	50.0 ppm	Food stuffs, Cosmetics and Disinfectants (1972).

**** EOSQC** (Egyptian Organization for Standardization and Quality Control).
**** WHO** (World Health Organization).
**** FAO** (Food and Agriculture Organization).



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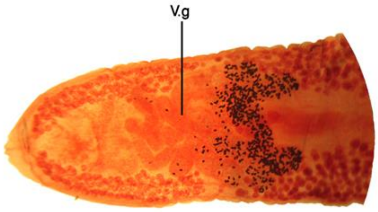

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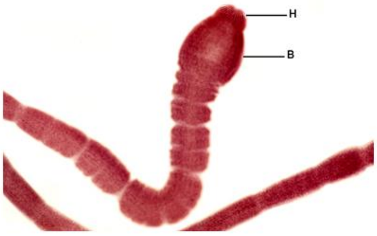

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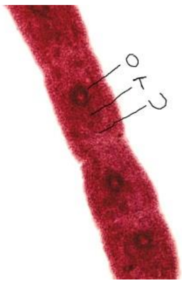

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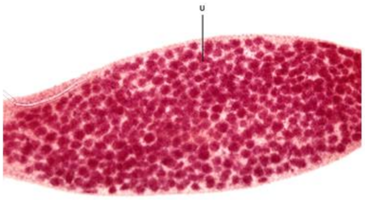
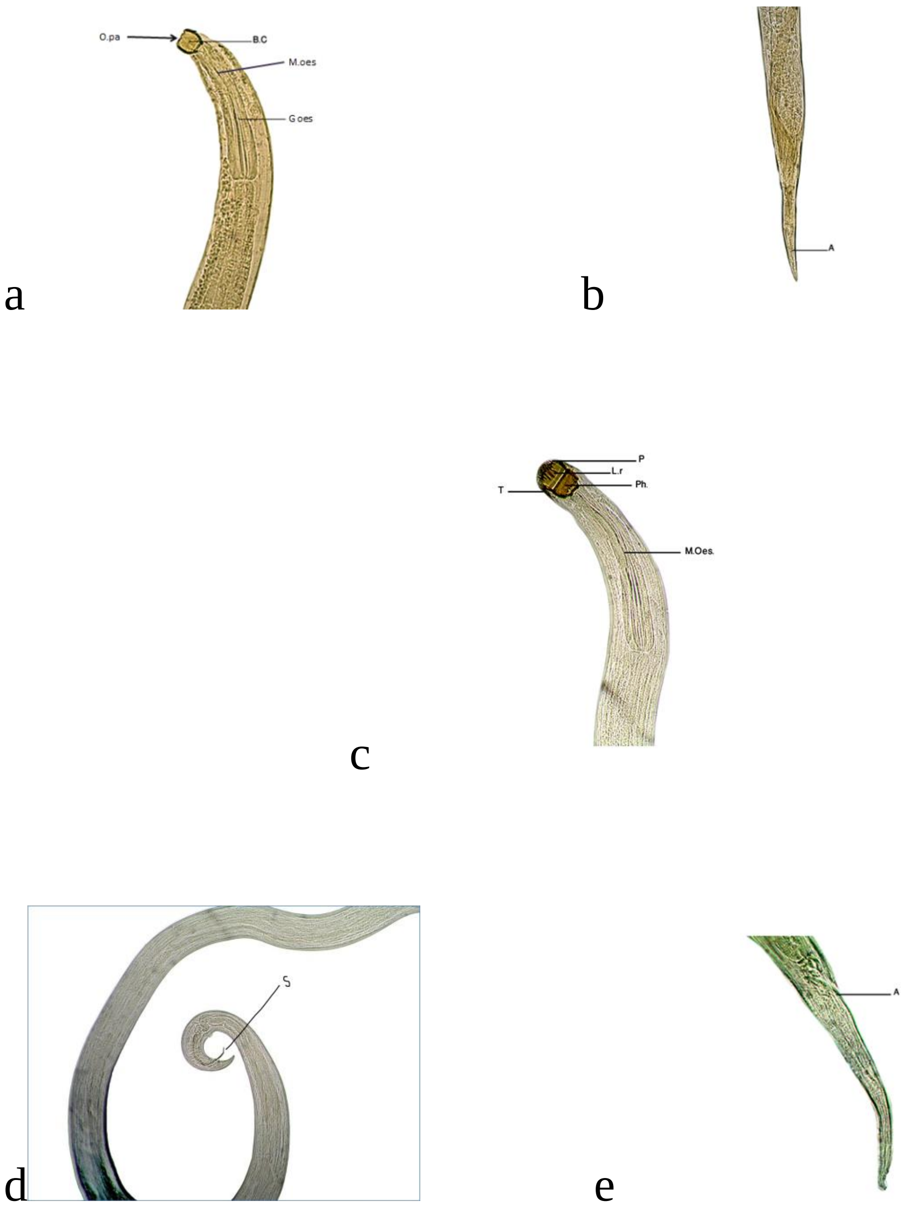


e

<u>Plate (1): Cestodes.</u>

a-Anterior part of *Monobothrium spp.***(X30)**.
b-Posterior part of *Monobothrium spp.* Where V.g (Vitelline gland) **(X30)**.
c- Scolex of *Polyonchobothrium clarias.* Where H (Hook) and B (Bothrium)**(X100)**.
d- Mature segments of *Polyonchobothrium clarias.*Where O (Ovary), T (Testis) and U(Uterus) **(X200)**.
e- Gravid segment of *Polyonchobothrium clarias.* Where U (Uterus) **(X200)**.



<u>Plate (2): Nematodes.</u>

a- Anterior part of female *Procamallanus laeviconchus.* Where O.p (Oral papillae),B.C (Buccal Capsule), M.oes (Muscular oesophagus) and G.oes (Glandular oesophagus) **(X100)**.

b- Posterior part of female *Procamallanus laeviconchus.* Where A (Anus) **(X100)**.

c- Anterior part of *Paracamallanus cyathopharynx.* Where P (Plate), L.r (Longitudinal rib), Ph. (Pharynx), M.oes (Muscular oesophagus) and T (Trident) **(X100)**.

d- Posterior part of male *Paracamallanus cyathopharynx.* Where S (Spicule) **(X100)**.

e- Posterior part of female *Paracamallanus cyathopharynx.* Where A (Anus)**(X200)**.

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