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SURVEY ON THE NEMATODES ISOLATED FROM AQUATIC SNAILS AND THEIR POTENTIAL AS BIO-CONTROL AGENTS OF SNAILS

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ABSTRACT

A survey of aquatic snails and their associated nematodes was carried out in the Nile, irrigation system and some rice fields in Cairo, Giza and Qualyobyia Governorates during 2002-2004. The survey revealed the existence of twenty species of molluscs including the snails intermediate hosts of *Schistosoma mansoni* Sambon, *Schistosoma heamatobium* Bilharz, *Fasciola gigantica* Cobbold, *F. hepatica* L., *Angiostrongylus cantonensis* Chen, and *Paramphistomum microbothrium* Carmyeriuis. Five species of snail parasitic nematodes were isolated from different snail species. Three parasitic species could infect all the mollusc species exposed to infection and *Bulinus truncatus* showed more suitability to the development of these nematodes than other snail species.

Key words: *bio-control agents, nematodes, screening, snails.*

1. INTRODUCTION

Aquatic snails infest rice plants causing damage in roots and small plants (Hassan and Kalliny, 1967; Lutfallah, 1974 and Azzam, 1995). Some of the snails serve as intermediate hosts of the human and animal parasitic diseases such as *Biomphalaria alexandrina* (Ehrenberg), the intermediate host of *Schistosoma mansoni* Sambon which infects man and wild rats (Mansour, 1978). *Bulinus truncatus* Audouin the intermediate host of *Schistosoma. heamatobium* Bilharz

which infects man and monkey (Malek, 1962) and rats (Mansour, 1978). Also this species acts with *Bulinus forskalii* (Ehrenberg) as intermediate hosts of *Schistosoma. bovis* (Sonsino) which infect human ,cattle and sheep (Malek,1962) and Ibrahim *et al*, (1999).

Meanwhile *Lymnaea collumella* L. and *L. cailliaudi* Bourguignat act as intermediate hosts of *Fasciola gigantica* Cobbold and *F. hepatica* L.(Azzam, 1995 and Ibrahim *et al*., 1999). These parasites infect human as well as farm animals (Abou Ragab, 1973). While *Lanistes carinatus* Olivier and *Bellamya unicolor* serve as intermediate hosts of *Angiostrongylus cantonensis* Chen which infects human and rats (Yousief and Ibrahim 1978, and Ibrahim *et al*., 1999).*Physa acuta* Draparnaud acts as intermediate host of *Echinostoma liei* Froelish that infects ducks ,geese and aquatic birds.Thus the control of these snails become very important. Therefore, the present study screened the nematodes associated with aquatic snails to search for new bio-control agents of these harmful snails in the Egyptian environment.

2. MATERIALS AND METHODS

Aquatic snails were collected from the Nile, irrigation system and rice fields in Qualyobyia Governorate, the Nile and irrigation system in Giza and Nile only in Cairo Governorate .

Snail collection was carried out by using a metallic strainer (22 cm in diameter) with an aluminum handle 70 cm which could be elongated to 200cm. The net was calmly pressed into the water, plant shacked . The net was then washed several times in the water to remove the mud and the collected samples were then evacuated into small plastic jars.

In the laboratory ,each species of mollusc after being identified was confined into an aquarium. Molluscs were screened for parasitization and suspected individuals were removed and kept individually using the same technique of isolation previously described by Azzam (1998). Some of the isolated nematodes were prepared for microscopic examination. For this purpose , specimens were directly taken from the mollusc cadavers, killed and fixed by hot T.A.F. then mounted in lactophenol or glycerol. All molluscs were identified according to Malek (1962) and Ibrahim *et al*., (1999). Nematodes were identified according to Chitwood and Chitwood (1950), Goodey 1963, Andrassy (1976,1978 and 1983, Pionar,(1977) and Soliman,(1996).

Isolated nematodes were tested with Koch's postulates to determine the pathogenic or parasitic species. The relative infectivity of these nematodes to different species of molluscs including the natural host, were tested on thirty lab-bred individuals from each species using the same technique previously described by Azzam, (1998) but using beakers (500ml)instead of Petri-dishes . Five individuals from one snail species were put in the beaker and 250 infective nematode were added to the beaker.

3. RESULTS AND DISCUSSION

Aquatic molluscs collected from different location and their medically and veterinary importantce, plants and nematodes associated with molluscs were recorded in (Table 1).

Ten species of Pulmonate snails (Bassommatophora) were collected and one from Styllomatphora..The Basommatophorid represented by three families, *i.e* Family Lymnaeidae (*Lymnaea cailliaudi*) ,which was found at Cairo Governorate, *L. collumella* collected from Giza and Qualyobiya Governorates. The two Lymnaeid species serve as intermediate hosts of the liver fluke *Fasciola gigantica* Cobbold and *F. hepatica* which infect cattle, sheep and man (Abou Ragab,1973). Family Physidae represented by P*hysa acuta* Daparnaud was collected from Giza and Qualyobiya Governorates.

Seven species belong to the third family planorbidae. These species were *Biomphalaria alexandrina* Ehrenberg and *B.glabrata* the intermediate hosts of the blood worm, *Schistosoma mansoni* Sambon which infect man, gerbils and shrews(Malek,1962, Mansour,1978 and Ibrahim *et al.*,1999). *Bulinus forskalii* (Ehrenberg) the intermediate host of two parasites *Schistosoma bovis* (Sonsino) which infects sheep,goats, cattle, camel and man (Malek,1962 and Ibrahim *et al.*,1999) and *Paramphistomum microbothrium* Carmyeriuis which infects cattle and sheep. *Bulinus truncatus* (Audouin), the intermediate host of *Schistosoma haematobium* Bilharz and the last two parasites(Malek,1962 and Ibrahim *et al.*, 1999) .*Gyraulus ehrenbergi* Beck, *Planorbis planorbis*, and *Helisoma duryi* (Wetherby) were also recorded. The last species has been suggested as a potential biological control agent for *Biomphalaria* and *Bulinus* snail (Ibrahim *et al.*, 1999). It is worth mentioning that *Biomphalaria glabrata* Say is a major intermediate host of *Schistosoma mansoni* in U.S.A., Canada and South America (Ibrahium *et al.*, 1999). It has been collected for the first time in Egypt

in 1982 (Pfluger,1982) and confirmed later in 1995 from irrigation and drainage systems at Giza, Qualyobiya and Kafr El Sheikh Governorates (Yousif *et al.*, 1996).

This species was collected from irrigation and drainage systems at Giza Governorate with a hybrid of *B.alexandrina and B.glabrata.* Yousif *et al.*, (1998) recorded a hybrid of *B. glabrata* and *B. alexandina* in Fayoum and Dakahelya Governorates .

Studies of Yousif *et al.*, (1996) indicate that the density of cercariae (the infective stage of Schistosomiasis) liberated from one snail is 4-6 times that from *B. alexandriana* and hence is considered as a new threat for Schistosomiasis transmission in Egypt .

One species only from Pulmonate (Styllommatophora was found at Giza and Qualyobiya Governorates, this was *Succinea cleopatra* (Pallary).

On the other hand, six species from Prosobranchiate snails belonging to five families were reported *i.e. Gabbiella senaariensis* (Kuster) (Fam: Bithinyiidae) was reported at Qualyobiya Governorate only . While *Theodoxus niloticus* (Reeve) was recorded in both Cairo and Qualyobiya Governorates . *Lanistes carinatus* (Olivier) Fam: Pilidae was found in the three screened Governorates. Some individuals of this species which were collected from Kafre-Elo liberated larvae of the lung worm *Angiostrongylus cantonensis* which infect rats and human. Yousif and Ibrahim (1978) reported *L. carinatus* to harbour the infective larvae of the rat-lung nematode *Angiostrongylus cantonensis.* Two species belonging to family Thiaridae were recorded, the first was *Cleopatra bulimoides* which acts as intermediate host for the horse intestinal flucke *Gastrodiscus aegypticus* and *Prohemistomum vivax* which infect cats and dogs (Ibrahim *et al.*, 1999).The second species was *Melanoides tuberculata* . It serve as first intermediate host for *L.ecithodendrium pyramidum* which has the adult stage in bats (Ibrahim *et al.*, 1999). The last family of the Prososbranchiate snails was Viviparidae represented by *Bellamya unicolor* (Olivier) the second intermediate host of *E. liei* , it acts also as an intermediate host of *Angiostrongylus cantonensis* Chen.

Three families belonging to class Pelecypoda were also recorded in the Nile through the surveyed Governorates. These were Fam: Corbiculidae (*Corbicula consobrina)*, Fam:Mutilidae *(Mutela dubia nilotica*) and Fam:Unionidae (*Caelatura aegypitaca*).

Worth mentioning that Bivalve molluscs can bio-accumulate, in their tissues, well known viruses that infect humans and higher animals (Elston, 1997). Meyers, (1984) mentioned that, bivalves are

widely known as bioaccumulators of viruses from human and other vertebrates, but such viruses do not infect and replicate in bivalves, rather the molluscs simply act, as a transient reservoir for entrained but infectious versions. Such entrained viruses are typically transferred to their vertebrate hosts upon ingestion of the bivalve or by contact after discharge into the water column.

Herpes-like viruses have been reported from three species of oysters (Farley *et al.,* 1972). Picorna-like virus and leukemia-like disease and HIV were reported in many bivalve species (Elston, 1997). The relationship of these viruses with viruses of higher vertebrate is an important evolutionary topic, but more definitive means of comparison are required.

Seven species of nematodes were isolated from different species of snails (Table1) *Rhabditis* $sp._1$ which isolated from *B. alexandrina* ,*B.. glabrata* , *H. duryi* and *M. tuberculata* . *Rhabditis* $sp._2$ isolated from *B. alexandrina, B. truncatus, L. carinatus, Melanoides tuberculata* , *Cleoptera bulimoides* and *Bellamya unicolor* . Mean while, *Phasmarhabditis* sp. isolated only from *Bulinus truncatus*.The predator nematode *Mononchus* sp. was found associated with *C. bulimoides* .

The infective stage (third stage) of the lung worm *Angiostrongylus catonensis* was isolated from *Lanistes carinatus* which was collected from Kafre – Elo at Cairo Governorate. Youssif and Ibrahim , (1978) recorded *Angiostrongylus catonensis* for the first time in Egypt as isolation from *Lanistes carinatus*. Godan (1983), reported *A. cotonensis* from *B. glabrata* , *Lymnaea stagnalis L.*, he also reported many species of aquatic nematodes as parasites of different fresh water snails. *i.e Daubaylia potomac* Chitwood & Chitwood, *Hexamermis albicans* (Siebold), *Leptodera flexilis*. (Dujardin) and *Mermis nigrescens* Dujardin on *Succinea putris* (L)

The snails *Onchomelania nosophora* (Robson) were highly infested in laboratory and field with the aquatic nematode *Rhabditis onchomelaniae* Jokko & Okabe (Okabe & Shiraishi,1971).

Infectivity of the isolated nematodes to the most important snail species was recorded in Table (2).

(Table :1): Aquatic molluses collected from three Governorates and their associated nematodes and plants with note on its medically and veterinary important in Egypt

Mollusks species.	Localities		Associated plants	Associated nematodes	Molluses medically and veterinary important	
	Gov.	Habitat			Parasites that molluscs serve as its intermediate hosts	Definitive host
Phylum Mollusca Class: Gatropoda SubClass: Pulmonata Order: Basommatophora						
Fam: Lymnaidae *Lymnaea cailliaudi* Bourgeuigant	Cairo	Nile	*Echornia crassipes* (Mort) *Cyperus articulatus*l. L.		*F.aesciola hepatica* L. *F. gigantica* Cobbold	Cattle , Sheep , and Man
Lymnaea collumella Say	Giza Qualyobiya	Nile& irrigation system Nile, rice field& irrigation system	*E.creassipes* *Lemna gibba L.* *Typha domingensis* Pers *Ceratophilum demersum* L. *Oryza sativa* L. *E. creassipes*. L. *gibba* *Phragmites communis*	Stronglid	*F.gigantica* *F.hepatica*	Cattle , Sheep , and Man
Fam:Physidae *Physa acuta* Draparnaud	Giza Qualyobiya	Nile &irrigation system Nile, rice fields and irrigation system	*E. creassipes* *T.domingensis* *L., gibba* *O. sativa*		*Echinostoma liei* (Froelich)	Ducks, geese and aquatic birds
Fam: Palnorbidae *Biomphalaria alexandrrina* (Ehemberg)	Cairo Giza Qulyobiya	Nile Nile & irrigation system Nile , irrigation system and rice fields	*E. creassipes* *Cyperus artilulatus* L. *E. creassipes* *T.domingensis* *E. creassipes* *T.domingensis* *O. sativa*	*Rhabditist* sp. ; *Rhabditis* sp. ; *Rhabditis* sp ;	*Schistosoma mansoni* Sambon	Man , rodents, gerbils and shrews
B. glabrata and hybrid of *B.. glabrata* and *B. alexandrrina*	Giza Giza	Irrigation system Irrigation system	*E. creassipes* *L. gibba* *E. creassipes* *L. gibba*	*Rhabditis* sp ; *Rhabditis* sp ;	*S. mansoni* *Angiostrongylus cantonensis*	Man , rodents, gerbils and shrews Man , Rodents.
BuBulinus forskalii Ehrenberg	Giza	Nile& irrigation system	*Ceratophyllum demersum* L.		*Schistosoma bovis* (Sansino) *Paramphistomum microbothrium* Carmyeriuics	Sheep, goats cattle, came& man Cattle and sheep

Table (1). Cont.

B. truncatus (Audouin)	Cairo Giza Qualyobiya	Nile Nile& irrigation system Nile , irrigation system and rice fields	*C.demersum* *C.demersum* *C.demersum* *O. sativa*	*Rhabditis* sp. *Phasmarhabditis* sp. *Rhabdihist* sp.	*P. microbothrium* *Schistosom haematobium* *S. bovi*	Cattle and sheep Man and monky Sheep, goats cattle, camel&man
Gyraulus ehrenbergi Beck	Giza Qualyobiya	Nile Nile	*C.demersum* *C.demersum*			
Helisoma jduryl (Wetherby)	Giza Qualyobiya	Nile &irrigation system Irrigation system	*C.demersum* *C.demersum*	Spirurid *Rhabditis* sp.		
Planorbis *Planorbis L.*	Giza Qualyobiya	Irrigation system Irrigation system and rice fields	*E. creassipes* *L. gibba* *L. gibba , Osativa*			
Sub Class: Prosobranchia Order Mesogastropoda Fam: Bithyniidae *Gabbiella senaariensis* (Kuster)	Qualyobiya	Nile, irrigation system and rice fields	*O .sativa* *P. communis*			
Fam: Neritidae *Theodoxus* *niloticus* (Reeve)	Cairo Qualyobiya	Nile Nile	*C. demersum ,* *E. Creassipes* *P. communis*			
Fam: Pilidae *Lanistes carinatus* (Olivier)	Cairo Giza Qualyobiya	Nile Nile& irrigation system Nile& irrigation system and rice fields	*C.articulatus* *E. creassipes* *E. creassipes* *E. creassipes* *O.sativa*	*Angiostrongylus cantonensis* Chen *Rhabditis* spp.	*Angiostrongylus cantonensis* Chen	Rats and man
Fam: Thiaridae *Cleopatra bulimoides* (Olivier)	Cairo Giza Qualyobiya	Nile Nile&irrigation system Nile, irrigation system and rice fields	*E. creassipes* *E. creassipes* *P. communis* *C. demersum* *E.creassipes* *O. sativa*	*Rhabditis* sp. *Rhabditis* sp. *Rhabditis* sp. *Mononchus* sp.	*Gastrodiscus engypticus* *Prohemistomum vivax*	Horses Cats and dogs

Table (1). Cont.

Melanoides tuberculata (Müller)	Cairo Giza Qualyobiya	Nile Nile&irrigation system Nile&irrigation system and rice fields	*C.articulatus* *E.creassipes* *C.demersum* *E. creassipes* *Potamogeton crispus* L. *C.demersum* *E. creassipes* *P. crispus*	*Rhabditis* sp_2 *Rhabditis* spp..	*Lecithodendrium pyramidium*	Bats
Fam: Viviparidae *Bellamya unicolor* (Olivier)	Cairo Giza Qualyobiya	Nile Nile& irrigation system Nile, irrigation system and rice fields	*C. demersum* *E. creassipes* *P. communis* *C. demersum* *E. creassipes* *P. communis* *O. sativa*	*Rhabditis* sp_2	*Angiostrongylus cantonensis* Chen	Rats and man
Class: Pelecypoda Order: Eulamellibranchia Fam: Corbiculidae Corbicula *consobrina* (Caillaud)	Cairo Giza Qualyobiya	Nile Nile Nile				
Fam: Mutilidae *Mutela dubia nilotica* (Caillaud)	Giza	Nile				
Fam: Unionidae *Caelatura aegyptiaca* (Caillaud)	Giza Qualyobiya	Nile Nile				

Table (2): Capability of different isolated nematodes to reinfect different snail species .

Snail species	Infectivity of the nematode			Rate% of snail mortality infected with the nematode			Ability of infected snail to recovered nematode			Rate of recovered individuals %		
	*R1	**R2	Ph	R1	R2	Ph	R1	R2	Ph	R1	R2	Ph
B. alexandrina	+	+	+	80	76.67	83.33	+	+	+	75	78.26	80
B. glabrata	+	+	+	76.67	76.67	76.67	+	+	+	73.91	73.91	73.91
B. truncataus	+	+	+	86.67	83.33	90	+	+	+	84.62	84	85.19
H. duryi	+	+	+	73.33	73.33	70	+	+	+	72.73	72.73	76.19
L. cailliaudi	+	+	+	60	60	63.33	+	+	+	77.78	72.22	78.95
L. carinatus	+	+	+	66.67	66.67	66.67	+	+	+	75	75	80
B. unicolor	+	+	+	70	70	73.33	+	+	+	71.43	76.19	77.27

Four species of isolated nematode were excluded from these tests the first was *Mononchus* sp. because it is a predator nematode, the second species was *A. cantonensis* because it spends some stage in the snail then its infective stage infects rats or man .Third and fourth were unidentified spirurid and stronglid.

R1 =*Rhabditis* sp $_1$ R2=*Rhabditis* sp $_2$ Ph = *Phasmarhabditis* sp

All the three parasitic species could infect all the molluscs species exposed to infection. The highest rate of mortality caused by *Rhabditis* sp. $_1$ (86.67%) was reported for *B. truncaus* snail, while the highest rates caused by *Rhabditis* sp.$_1$ and *Phasmarhabditis* sp. were 83.33% and 90% respectively and reported also for *Bulinus truncatus*. Meanwhile the lowest ratios recorded for the three species of nematodes were 60,60 and 63,33 respectively and reported for *L .carinatus* snail. This may be due to the bigger size of this snail that need higher number of nematodes to give higher mortality. Coupland (1995) reported that smaller snails were more susceptible to dauers of *Phasmarhabditis hermaphrodita* (Schneider) dying more quickly than larger snails. Charwat and Davies (1999) also reported that two isolates of cephalobid were more effective on *Cochlicella acuta* (Müller) (small size snail) than the big size snails *Cernuella virgata* (DaCosta) and *Theba pisana* (Müller) .

The highest ratios of recovered nematode individuals were reported also for *B . truncatus* (84.62-84 and85.19%) in the three nematodes species, respectively. This indicates more suitability of *B .truncatus* to the development of these nematodes than other snail species.

Azzam (1998) reported that the *Rhabdutis* sp. nematode isolated from terrestrial snails *Eobania vermiculata* (Müller) could infect *B.alexandriana* causing 100% mortality while the rate of individuals recovered nematodes was 93.33%. Azzam and Tawfik (2003) recorded 100% mortality of *B..alexandrina, B. truncates, L .carinatus, B. unicolor* and 80% of *C. bluimdoides* when infected with *Phasmarhabditis tawfiki* Azzam while recovery rate for the same species were 93,92,95,60 and 60 %, respectively.

From the above mentioned results , the parasitic nematodes may play a role in reducing the population of these harmful snails .Consequently the rate of infection with parasites transmitted by these snails will be reduced. Thus more investigation should be done in this aspect.

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حصر للنيماتودا المعزوله من قواقع مائيه و دورها كعامل مكافحه بيولوجيه للقواقع

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ملخـــــص

أجرى حصر للنيماتودا المرتبطه بالقواقع المائيه المنتشره فى النيل وقنوات ومصارف الرى وحقول الأرز فى محافظات القاهرة والجيزه والقليوبيه خلال أعوام ٢٠٠٢-٢٠٠٤ .

أسفر الحصر عن وجود عشرون نوعا من القواقع تتضمن الأنواع التى تعمل كعائل وسيط للبلهارسيا بنوعيها والدوده الكبديه والدوده الرئويه. أسفر الحصر أيضا عن وجود خمسة أنواع من النيماتودا تم تعريفها لمستوى الجنس ثلاثة أنواع منها متطفله على القواقع واثنين لم يتم التوصل لتحديد جنسيهما .

أثبتت الاختبارات المعمليه أن الثلاث أنواع من النيماتودا المتطفله لديها قدره على عدوىجميع أنواع القواقع التى عرضت للعدوى بها ،وأن القواقع المعديه لديها قابليه لانتاج الأطوار المعديه لتلك الأنواع النيماتوديه.

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