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Use of *Artemisia cina* Against *Gyrodactylus rysavyi* Infecting *Cyprinus carpio* in Comparison with Praziquntel

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ABSTRACT

The present study analyzed recent trends of approved drugs derived from previously untapped species. on effective agents derived from natural sources against *Gyrodactylus rysavyi* infections in *Cyprinus carpio*. Praziquntel (4g/kg diet) was screened for their anthelmintic efficacy. *Artemisia cina* showed efficacy against infections by the monogenean in vitro and feeding trials. For challenge trials, natural agent (2.5g/kg diet) was effective in laboratory trials were also given to the fish in feed. Praziquntel prevented horizontal infection, furthermore, the survival of groups treated were significantly higher than the negative control(diet) and other groups additionally, the number of matured parasites on the branchial cavity, which occurred from the beginning of the challenge trials,decreased in the groups treated with Praziquantel. *Artemisia cina* was effective in the dislodgement and mortality of monogenean parasites, *G. rysavyi* of juvenile *C. carpio* at concentrations ranging from 50 to 200 mg/l. There were positive correlations between the number of parasites dislodged/killed concentration of *A. cina* extract, and the duration of exposure of affected fish to the substances. This led to the conclusion that *A. cina* contains substances that are effective against *G. rysavyi* and provide a knowledge can be explored in the aquaculture industry to eliminate the use of conventional synthetic organic drugs that may be detrimental to consumers of aquaculture products.

Keywords:

INTRODUCTION

Monogeneans are generally found as parasites on or in cold-blooded vertebrates, mainly the elasmobranches, bony fish and in some amphibians and reptiles. In fish, the majority of monogenean is parasitic on the gills or skin. These parasites are

site- and host-specific, generally occurring in relatively low numbers (El-Naggar and Serag, 1987). However, monogeneans parasites can easily multiply and disperse in confined areas, in a very high intensity so, the establishment of a heavy infection, particularly under unfavorable culture conditions, may give rise to mass epizootics with severe economic loss (Ramadan, 2000 and El-Abbassy (2001). Skin and gills of affected host are damaged by the attachment hooks resulting in secondary infection by bacteria or fungi. They cause severe economic losses among fish farms (Mohamed *et al*., 2010).

Antiparasihc drugs for fish are lately being more widely studied due to the need to keep farmed fish free of infestations supported by intensive farming. Praziquantel have been tested on monogeneans other than Gyrodactylus. The genus *Artemisia* belongs to the large family of Asteraceae, encompassing more than 300 species. *Artemisia annua* L. (Asterales: Asteracaea), commonly known as sweet wormwood or annual wormwood, grows widely in Europe and America and is planted to a large extent in China, Turkey, Vietnam, Afghanistan, and Australia (Alawa *et al*., 2003 and Abdel-Hadi *et al*., 2008). They equally affect the early stage of gametocyte development, which reduces further retransmission of the parasites from humans to mosquitoes in areas of low transmission. Many other bioactive compounds isolated from *A. annua* have equally displayed unique pharmacological activities against a wide range of bacteria (Bone and Morgan, 1992). Effect of *Artemisia annua* L. leaves essential oil and ethanol extract on behavioral assays (Perazzo *et al*.,2008), an opportunistic pathogen which causes in AIDS and other immune-compromised patients. artemisinin has also a therapeutic potential against *Toxoplasma gondii* (Jones-Brando *et al*., 2006 and Ferreira,2009), *Trypanosoma*, and *Schistosoma* species (Mishina *et al*., 2007), which cause toxoplasmosis that is associated with behavioral abnormalities in patients, human trypanosomiasis or "sleeping sickness," and schistosomiasis, respectively, as well as other pathogens responsible for cryptosporidiosis, amoebiasis, giardiasis, leishmaniasis (Ma *et al*., 2004). The chemotherapeutic agents currently used for the treatment of fish monogenesis include mebendazole, organophosphate, praziquantel, closantel, dichlorvos, formaldehyde,etc Keiser (2010) and Aberham *et al*., (2010).

Ferreira and Luthria (2010) and Ferreira *et al*., (2010) assess the antioxidant capacity of *Artemisia annua* L. leaves and their potential synergism with artemisinin against malaria and cancer.

Recently Ekanem and Brisibe (2010); Albert and Ebiamadon (2010) ; Elango and Rahuman (2011) and Squires *et al*. (2011) try to evaluate

effects of extract of *Artemisia annua* L. against monogenean parasites of *Heterobranchus longifilis* and we take the same idea and try to screen our Egyptian *A.cina on* juvenile *Cyprinus carpio* to find an alternative means for treatment of monogenean diseases of cultured fish using an extract of *A. cina* instead of chemical-based substances that may not be friendly to the environment.

In the present paper, we try to make a screening experiment to discover new natural anthelmintic agents derived from natural resources for oral administration with medicated feed to be much more practical and to ensure comparable and realistic results and asses of both prophylactic and therapeutic effect and provide a knowledge can be explored in the aquaculture industry to eliminate the use of conventional synthetic organic drugs that may be detrimental to consumers of aquaculture products.

MATERIALS AND METHODS

Parasites and hosts

Cyprinus carpio were maintained in constantly aerated tanks containing 500 L water at 20°C. The pH of the water was 7.0± 0.05; its NO2 content was zero, and the NO3 content was 10 mg/l. with regard to flow rate, aeration and feeding rate for *C. carpio* was employed for all the experiments.

500 individuals, one year old weight 100-150 g hatched at laboratory were maintained in a2 ton tank. Gills, branchial cavity, wall and skin surface of 10 fish were randomly examined under microscope to confirm that the fish were free from parasites before each experiment.

Laboratory infections were produced by placing infections uninfected fish in a tank with 2-3 infected fish for 1-3 days. Prevalence and intensity of were detected seasonally and by size in specimens taken monthly.50 fish were selected randomly and the number of parasites presented on fins and body surfaces were counted. 25 individuals,0ne year old, weight 150-250g infected with *G.rysavyi* hatched at our laboratory, were maintained a 100L tank uninfected fish with no recorded of previous infection with parasites,were periodically mixed into the infected fish. Excised gills from these fish infected with parasites larvae 2-6 cm long were used for in vitro studies. . The hatched larvae were used for challenge trials.

A whole gills infected with more than 5 parasites per gill arch were immersed with each 50 ml drug

solution in a tissue culture dish at 20°C according to **Noritaka and Taro (2000)**. The behavior and release of the larvae from gills were observed every hour for 10 h under a microscope to determine the drug efficacy. Parasite infected gills immersed in filtered water without the drug at the same temperature conditions acted as a control.

Drugs

Parziquantel were obtained from Sigma (Deisenhofen, Germany) BILTRICIDE® (praziquantel) is a trematodicide provided in tablet form. Drug was dissolved in filtered water at 20 mg/L and was made up to a volume of 50 ml.

Plant material

Artemisia cina leaves were washed thoroughly in running tap water to remove sand and debris; they were dried by spreading under the sun for 3 days and finally in a hot air oven at 60°C for 8 hrs. The dried leaves were crushed to powder in a mortar with pestle and subjected to Soxhlet extraction with 70% ethanol as extracting solvent. The solvent was exhausted from the extract with the help of a rotary evaporator. The extract was stored in a refrigerator until required for use.

Preparation of an ethanol extract of *Artemisia cina*

The ethanolic extract of *A. cina* was used for the preparation of a stock solution from which the working solution used for the efficacy testing was prepared. The stock solutions were obtained by dissolving 1 g of the extract in 5 ml of dimethyl sulfoxide (DMSO) and made up to 100 ml with de-ionized water. Four working solutions that were represented by concentrations of 50, 100, 150, and 200 mg/l, respectively were prepared from the stock solutions. A preliminary test was carried out to guide in the selection of the concentration of the test solutions. One-week-old fry of *C. carpio* obtained by induced breeding were stored in were maintained in a1ton tank for a period of one week. Examination for the accumulation of monogenean parasites was done from the fourth day of stocking according to Ekanem and Brisibe (2010).

Efficacy testing

One hundred 1-month-old juvenile *C.carpio* were stocked in a1ton tank for 7 days to accumulate parasites. The approximate number of parasites per fish was confirmed by counting the number of parasites attached to body surfaces and the gills

with stereo-microscope before being exposed to the extract under in vivo conditions. Parasitized fish were also placed in de-ionized water containing 5 ml of DMSO in plastic Petri dishes to serve as control. The bioactivity of the extract was conducted in plastic Petri dishes with three replications and controls. Parasites were merely dislodged from their attachment organs and killed some hours later in the same concentration of *A. cina*. Fish were exposed for periods ranging from 30 to120 min in both the test and the control treatments according to Albert and Ebiamadon (2010). They were re-examined individually at the expiration of the exposure period for the presence of parasites. The experiments with the substances were replicated three times. The homogeneity of the replicates was checked by Kruskal–Wallis test before the data of the replicates were pooled together (Albert and Ebiamadon 2010).

Toxicity test

Toxicity of the extract of *A. cina* to *C. carpio* juveniles was tested for 24, 48, 72, and 96 hrs, respectively, at higher concentrations (250, 300, 350, 400, and 500 mg/l) to ascertain the safety margin of the substance against the fish host (Zibaee,2010). Glass aquaria of 10 L capacity were used, and each tank was filled with 3 L of the test solution and stocked with ten fish under aeration. The setup was replicated three times with a set of controls under the same experimental conditions. Observation for fish mortality and abnormal swimming behavior was made for 96 hrs according to Albert and Ebiamadon (2010).

Anthelmintic activity

The experiments were performed in glass aquaria with 8 L capacity. Each aquarium contained 5 L aerated tap water and ten previously infected fish. The water temperature was constant at 25±1°C and the water pH ranged from 7.0 to 7.5. Fractions of ethanol extract added in glass aquaria at a different series of concentrations. The blank control group with no extract was used under the same experimental conditions. All treatment and control groups were conducted in triplicate. After 48 h, all fish were biopsied, and the lamella branchialis were placed on glass slides for counting the number of surviving parasites under a stereomicroscope. The mortality of *G. rysavyi* and the mortality of fish were used to evaluate the anthelmintic efficacy of each treatment. The optimal anthelminthic concentration was the concentration which led to the highest mortality of

parasite with no intoxication of fish. The drug concentration which resulted in less than 20% mortality of parasite was considered ineffective concentration. No parasite or dead one on gills represented 100% mortality of parasite. The mortality of parasite of each treatment was calculated according to the following formula (Wang *et al*., 2010):

$$\mathrm{MD}(\%) = \frac{B - T}{B} \times 100\% \quad (1)$$

Where MD is % of the mortality of *Gyrodactylus rysavyi*, B is the average number of surviving *G. rysavyi* in the blank control, and T is that in the treatment. The mortality of fish was also calculated by the follow equation:

$$\mathrm{MF}(\%) = \frac{B - E}{B} \times 100\% \quad (2)$$

Where MF is % of mortality of fish, B is the average number of surviving fish in the beginning of test, and E is that at the end of test.

Artemisia cina were tested by in laboratory trials. The agent was dissolved in filtered water at 80 mg/L and was made up to a volume of 50 ml. Excised gills infected with more than 5 parasites per gill arch were immersed with each 50 ml drug solution in a tissue culture dish at 20°C. The behavior and release of the larvae from gills were observed every hour for 1h under a microscope to determine the efficacy according to Noritaka and Taro (2000). As a control, excised gills infected with more than 30 parasites per gill arch were immersed with each 50 ml without natural agents in a tissue culture dish at 20°C.

Feeding trials

Parziquantel which were determinate to be the most effective in laboratory trials were evaluated for their efficacy in vivo challenge trials. Approximately, 200 hatched larvae (body length 100-200 um) were kept into a 100L tank containing 24 L freshwater with aeration., 10 uninfected fish weighting approximately 150 g were added . These fish exposed to the larvae for 2h at 20°C with no running water to complete the infection. Then, fish divided into 5 groups and were transferred to 100L tank.

Each group was fed diet supplemented with the drugs at 2 doses, 2g drug/kg basal diet throughout the trials. Each drug was mixed into diet and the pellet was formed by passing the past through a disc pellerter. The normal diet was dried at 40°C for 3 h and was stored at 4°C,

with an average of 20.9 °C, during the trials. The effectiveness of each treatment was determined by comparing the number and growth (body gain of the parasite=body length=growth) of the parasite on gills.

Infection sources

Approximately 100 hatched larvae were kept into a 100L tank containing 20 L water with aeration. Forty uninfected 100g fish weighting were added, and exposed to the larvae for 2h at 15°C with no running water and then were transferred to 500 L tank where they maintained for 7weeks. Water temperature ranged between 17-20°C with an average 18.5°C. The fish was fed the basal diet. The parasites were matured 50 day later according to **Noritaka and Taro (2000)**. These infected fish were used for the sources of infection and the assessment of the therapeutic effect of the agents.

Forty parasites-free fish weighting approximately 100g were divided into 4 groups and were maintained in a 100L tank. Fish were fed the experimental diets before the challenge trials. Each natural agent was adsorbed to the basal diet at 2.5 g agent / kg. The diet supplemented with 4g Parziquantel/ kg diet was used as positive control and the diet alone as a negative control. Seven days after starting to feed the experiment diets 6 fish infected with mature parasite 114.6+8.5 parasites per fish from 10 fish randomly sampled were added to each group. Each group was fed the experimental diets continuously and prevention of horizontal infection was assessed together with assessment of the therapeutic effect on newly added infected individuals (Noritaka and Taro 2000). The pectoral fins on the right side of initially infected fish were removed to distinguish the second group.

Challenge trials were made for more than 9 weeks. Water temperature ranged between 19-22°C, with an average of 20°C during the trails. Four fish of each group were randomly sampled and the number of infected parasites on gill was counted 30 days after beginning of the challenge. Mortalities were recorded daily and the parasites on the gills and branchial cavity wall of the dead fish were counted. At he end of the experiment, the number of the parasites on the survivor fish was counted.

Statistical analyses

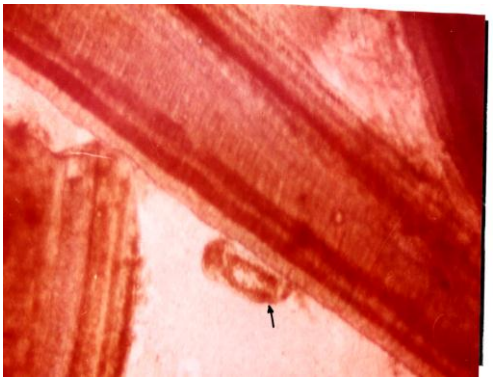
The results were statistically analyzed using analysis of variance (F-test) and Duncan's multiple range tests to determine the differences in means (6).

RESULTS

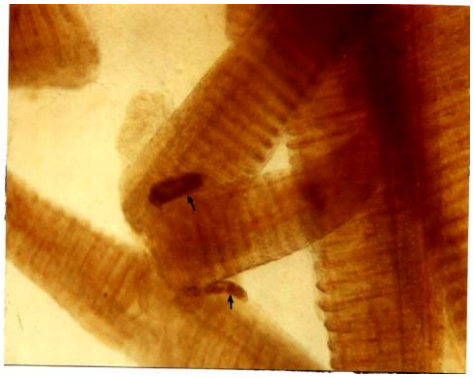
Parasite morphology: Fig (A,B, C, D) and Table (1)

Bodies are elongate 521 (418–590) µm long, 171 (138–226) µm wide. Mouth opening is 35 (31- 39) µm wide; Intestinal crura extending beyond the anterior edge of the testes, 1000 (900-1200) µm long. Haptor is roughly rectangular, 230 (210-250) µm long × 280 (260-290) µm wide. Total length of hamuli 89 (86–91) µm. Total length of marginal hooks 70 (65-80) µm; marginal hook blade 18 (16-19) µm long. The gaffing action of the marginal hooklets is a predominant feature in *G. rysavyi.* The haptor seems to be formed of two functionally different compartments; the marginal hooklets are arranged in an amazing manner, creating a paddle-like system driving the parasite during migration into the water column, whereas the hamuli are packed into an independent tegumantal envelope. The total length of the hamulus measured 89 to 100 µm. The opisthaptor was conspicuous with 16 marginal (lateral) hooks with long shafts arranged in 3 groups. Two groups consisting of 4 hooks each were located anterolaterally, and 8 hooks formed a posterior group. The marginal hooks were flexible with long shafts, comprising proximal and distal parts.

At the end of the study periods, examination of *Cyprinus carpio* demonstrated that, while some fish that previously harbored parasites were found to be free of some of the parasites, others were completely free. Comparatively, the number of parasites on the body surfaces of fish was the same throughout the test period. The concentration of *A. cina* in which 50% of the parasites were killed was 100 mg/l within 60 min, and a significant number (about 85%) were killed in 200 mg/l Table (2). Interestingly, monogenean parasites were all dislodged from their attachment sites before the occurrence of mortality following treatment with *A. cina*. It was also observed that the parasite loads were reduced with increasing concentrations of *A. cina* extract as shown in Table (2). There was a positive correlation between the number of parasites dislodged from the body surfaces of fish and the time of exposure of fish to the extract. In addition to all of these, an increased agility in the swimming of fish freed from parasites was also observed when compared to their counterparts in the control with all parasites remaining intact. Some fish taken the upward position in water but some else go down to bottom.



(A)



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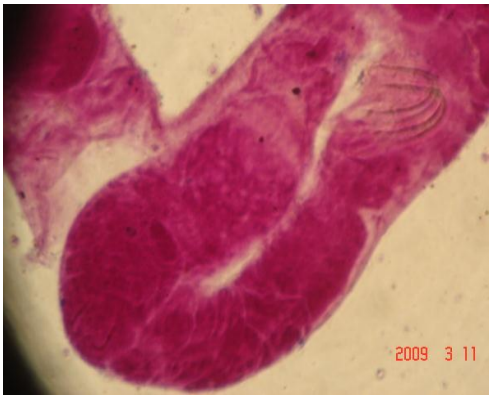
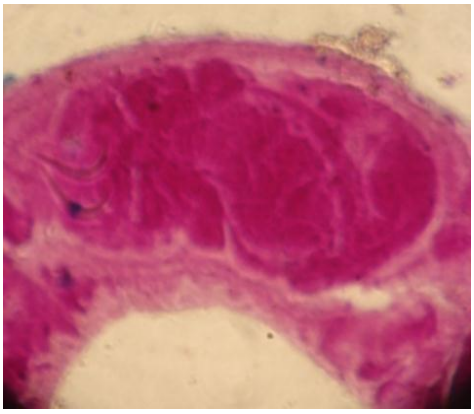


(C)



(D)

Table (3) showed that, extract of *A. cina* was well tolerated by juveniles *C.carpio*. A minimal mortality observation was made throughout the 96-h period of exposure of fish to the extract. A few fish showed weak swimming activity in 350 to 500 mg/l of the test solutions. The highest percentage mortality observed after 96 h in the highest concentration was 36 %.

In the media containing Paraziquantel our result show that the larvae contracted immediately and 100% of larvae were released from the gills 1h after starting the immersion (Fig A&*B*).in the media containing *Artemisia cina* the larvae were drop off from the gills during the observation after1-3 minutes and 100% of larvae were released from the gills 1h too.

Table (1): *Gyrodactylus rysavyi* measurements

Feature	Range (µm)	Mean (n = 10)
Total length	418–590	521
Total width	138–226	171
Width of suction disc	31- 39	35
Hamulus\ Total length	86–91	89
Length of shaft	49–59	53
Deep root to curve of blade	64–71	69
Length of point	11–15	14
Length of deep root Length of superficial	12–17	15
Root	34–42	39
Ratios Superficial root:shaft	1:1.1–1.7 1:	1.4
Deep root:shaft	1:2.8–4.7	1:3.4
Length of transverse bar	23–29	25
Marginal hooks Total length	63–75	71
Length of sickle	7–9	8
Proximal width of sickle	5–6	5.2
Distal width of sickle	8–10	8.4
Length of distal part of shaft	26–29	28

Table (2): ***Parasite mortality against concentrations (milligrams per liter) of Artemisia cina at different time intervals***

Time intervals/ mortalities(min)	Concentrations (mg L)			
	50	100	150	200
30	7	15	19	28
60	16	22	28	31
90	26	31	32	31
120	32	35	39	35

Table (4) showed that, the number of parasites on the gills of the fish fed on the diet containing 4g Paraziquantel / kg diet for 20 days was significantly fewer (89.07± 18.80)than negative control(298.07± 123.76). The growth of the parasite was affected and the length of the parasites from this group was significantly shorter (1.06±0.80) than negative control (3.46±6.80). No Statistical difference between 2gm Paraziquantel /kg diet group and negative control group, but the length of the parasites was significantly shorter (2.86±3.80).

Table (3): ***Toxicity test of concentrations (mg per liter) of Artemisia cina against*** *C. carpio* ***mortality (%)***

Concentrations (milligrams per liter)	Fish mortality (%)			
	24h	48h	72h	96h
250	0	0	0	17
300	0	12	14	16
350	8	21	18	31
400	7	28	25	28
500	24	31	33	36*

Table (4): ***Determination of parasites number in 4g Praziquantel/ kg diet***

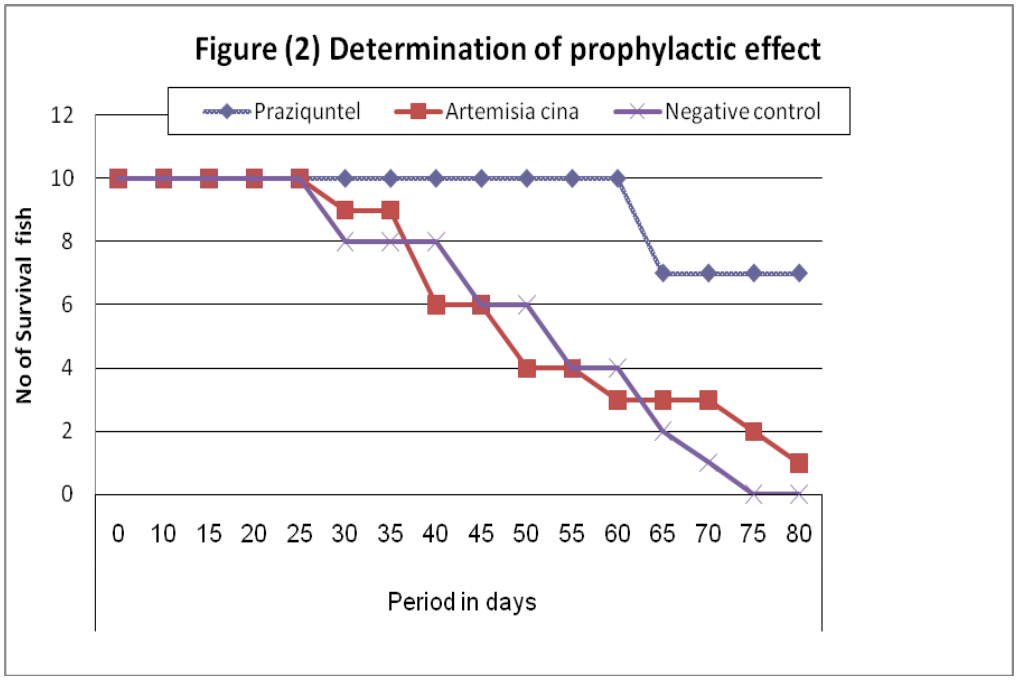
Groups	Mortalities			Surviving fish		
	No	Mature	Larvae	No	Mature	Larvae
Paraziquantel	**2**	**0**	**55**	**5**	**0**	**22.6 ±44.0**
Artemisia cina	**8**	**56.1 ±50.9**	**841.3±1153.3**	**1**	**99**	**83**
Negative control	**5**	**132.7±53.1**	**286.1± 866.9**	**0**	**-**	

After thirty days of the challenge, the number of parasites was significantly fewer on the gills of the fish examined to asses the prophylactic effect on the groups treated with Praziquantel than negative control group No mature parasites were observed o the banchial cavity wall of examined fish.

Fig (2) showed that, mortalities of the fish in prophylactic group occurred earliest in the negative control group when 100% of mortalities were recorded by day 75 after starting the trial. While the survival of the groups treated with Praziquantel was significantly higher than negative control. However, the differences in the survival between treated with *Artemisia cina* and negative control group was statistically significant.

Examination of gills of dead fish revealed that the infection of the parasites larvae was heavier in the negative control groups and the groups treated with *A. cina* compared with the infection in the groups treated with Praziquantel (Table, 4). The infection of the surviving fish of the treated groups with *Artemisia cina* was low compared with the dead fish, but the number of mature parasites in the group treated with Praziquantel was apparently fewer than those in the groups treated with *A. cina*. The groups treated with Praziquantel was not infected by any mature parasites and only few parasite were found

As with prophylactic groups, heavy infestation were observed in dead negative control fish and in the groups treated with *A. cina*. The



number of mature parasites in the branchial cavity wall of the fish that survived in the group treated with Praziquantel decreased from the initial infections rates was the differences between these groups and initial infection rates was statistically significant, even through the survival of the groups treated essential oil was as low as the negative control, there were few mature parasites on the branchial cavity wall of the surviving fish in these groups. The number of larvae parasites on the surviving fish of the groups treated, *A. cina* and Praziquantel was less than the negative control.

DISCUSSION

In this study, the Praziquantel anthelmintic efficacy against, *G. rysavyi* in *Cyprinus carpio* was established and it was used as a positive control to compare the efficacy of natural anathematic agents *Artemisia cina*. Praziquantel is widely used to control trematode and cestode infections in mammals (Martin *et al.*,

1997) and the recommended oral dose of Paraziquantel is 20-40 mg/kg BW/day in human (Kitahara,1995).In fish, Paraziquantel has been used in experimental to treat monogean disease by bath treatment (Kim *et al*.1998 who reported that the oral administration of Paraziquantel by intubation into the stomach of rockfish *Sebastes schlegeli* had an effect against *Microcotyle sebaste*). The present study clearly showed that feeding a diet containing Praziquantel at high dose to fish (40 mg/kg BW/day) had an anathematic efficacy against *G. rysavyi* larvae on gills and body in *Cyprinus carpio*. Also, showed that the growth of the parasite was affected in both groups of high and low doses. Thus, the Praziquantel in feed was chosen for the effective positive control. The recommended oral dose of Albendazole is 20-40 mg/kg BW/day in human to control gastrointestinal nematode infections (Miller *et al.*, 1978). In the previous studies application of Albendazole which hardly absorbed from GIT and act directly on nematode parasite, so we try to test levamisol at a high dose and found that the drug reach the target. These results to some extent go in with the in vitro assay reported by Schmahl and Mehlhorn (1985*)* on rainbow trout *Oncorhynchus mykiss*,for the best of our knowledge no publication in Praziquantel and Albendazole pharmacokinetics in fish so it is assumed that Praziquantel may reach the parasites more easier than Albendazole via peripheral blood of host and thus has anathematic efficacy, this is coined with that reported by (Hirazawa *et al*.,2000) on *Heterobotherium okamoto* in Tiger puffer *Takifugu rubripes* in Japan.

Hirazawa *et al.* (2000) reported that, Praziquntel (4gm/kg diet) is screened for their anthelmintic efficacy and so select the drug most suitable as an effective positive control for the trials of natural sources. Tubbs and Tingle (2006) evaluate an oral PZQ dosing strategy, the pharmacokinetics of a dissolved and in feed oral PZQ preparation (40 mg kg–1 body weight) were compared with an intravenous bolus in kingfish plasma and skin using HPLC.Also,Leslie and Whittington (2002) who recorded that, Praziquantel is faster than the post-oncomiracidia and juveniles, which were attached between the secondary lamellae of these tissues. This behavior apparently improves parasite survival in treatments of short duration and high concentration. Two 40-h baths (48 h apart) in 5 mg/L Praziquantel in seawater were required to remove all parasites from branchial and nasal tissues.

Our result showed that the larvae treated with Praziquantel contracted immediately and 100% of larvae were released from the gills 1h after starting the immersion .In the media containing *Artemisia cina* were the larvae treated with contracted after 1-3 minutes and 100% of larvae drop off from the gills during the observation 1h after starting the immersion.

Our results agreed with Ekanem and Brisibe (2010); Albert and Ebiamadon (2010) ; Elango and Rahuman (2011) and Squires *et al.* (2011)who reported that, the Mortality was best achieved by ethanolic extracts of Artemisia which, at 2 mg/mL, killed S. mansoni and E. caproni in 20 h or less (except for wormwood), *F. hepatica* between 16 and 23 h . Also, with Alawa *et al*., (2003).who found that result indicates that only *A. senegalensis* showed promising anthelmintic activity especially with use of ground whole plant materials as used by some farmers.

Artemisia cina had an effect against *G. rysavyi* larvae by in vitro trials. The parasite contracted quickly and the degree of contraction was strong compared with the trials for anthlemintic drugs, possibly to the higher dose of *A.cina*. Therefore, the in vitro trials for *A.cina* were made for a short period compared with the trials for the anthlemintic drugs; fish survival was as low as in the negative control group and clear effect in preventing horizontal infection in the challenge trials. However, the number of parasites on the surviving fish was fewer compared with those on the negative control fish. Higher doses of *A. cina* may have a strong effect. But at doses 3g/kg BW/day, *C.carpio* lost their appetite.

Thus the incorporation of tested natural agents such as herbs with antiparasitic ability is hereby encouraged, as they will not only reduce the challenge of toxicity if used at recommended doses but will also reduce the incidence of fish mortality due to parasites.

REFERENCES

Abdel-Hadi, Y.M.; Saleh, O.A. and A.M. Akar (2008): Study on the use of *Artemisia cina* L. (wormseed plants) and *Allium sativum* (garlic) in the control of Saprolegniosis in egg of *Cyprinus carpio* (common carp) and *Hypophthalmichthys molitrix* (silver carp). Proceedings of the 3oth Malaysian Symposium on Microbiology (MSM), 16-19,

Hyatt Regency Resort, Kuantan, Malaysia.

Aberham A, Cicek SS, Schneider P, Stuppner H (2010) Analysis of sesquiterpene lactones, lignans, and flavonoids in wormwood (*Artemisia absinthium* L.) using high-performance liquid chromatography (HPLC)-mass spectrometry, reversed phase HPLC, and HPLC-solid phase extraction-nuclear magnetic resonance. J Agric Food Chem 58(20):10817–10823.

Adams, J.G., (1978): Pharmacokinetics of Levamisole. J. Rheumatol. Suppl. 4, 137-142.

Alawa,C.B., Adamu A.M., Gefu JO, Ajanusi O.J., Abdu P.A., Chiezey N.P., Alawa J.N., Bowman, D.D.,,. **(2003)**: In vitro screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. Vet Parasitol 113:73–81

Albert, PE, and Ebiamadon, A.B. (2010) Effects of ethanol extract of *Artemisia annua* L .Against monogenean parasites of *Heterobranchus longifilis* . *Parasitol Res* , 106, 1135: 1139

Andrew J. Mitchella & Melissa S. Hobbsa (2007): The Acute Toxicity of Praziquantel to Grass Carp and Golden Shiners North American Journal of Aquaculture Volume 69, Issue 3, pages 203-206

Bone, K., Morgan, M. (1992) Clinical *Applications of Ayurvedic and Chinese Herbs: Monographs for the Western Herbal Practitioner* .Phytotherapy Press, Warwick, pp. 7-12.

Ekanem A. and Brisibe EA. (2010): Effects of ethanol extract of *Artemisia annua*; L. against monogenean parasites of *Heterobranchus longifilis*. Parasitol Res 106(5):1135–1139

El-Abbassy, S. A. (2001): Biological and histochemical studies on some monogenean parasites of the catfish *Clarias gariepinus* inhabiting Nile Delta Waters. M. Sc. Thesis, Faculty of Sciences, Mansoura University, Mansoura, Egypt.

El-Naggar,M.M. and M.M Serag,(1987): The monogernean *Quadriacantus kearnin*.sp and *Quadriacantus claridis claridis* Paperna 1979 reported on the gills of Clarias lazera in Nile

Delta. Journal of Egypt. Soc. Parasitol. 15(2):497 - 492.

Elango G, Rahuman A (**2011**): Evaluation of medicinal plant extracts against ticks and fluke. Parasitol Res 108(3):513–519

Ferreira,J.F.S. (2009): Artemisia species in small ruminant production: their potential antioxidant and anthelmintic effects. In: Morales M (ed) Appalachian Workshop and Research Update: improving small ruminant grazing practices, Beaver, WV, 2009. Mountain State University/USDA, p 53–70

Ferreira J.F.S. and Luthria D.L., (2010): Drying affects artemisinin, dihydroartemisinic acid, artemisinic acid, and the antioxidant capacity of *Artemisia annua* L. leaves. J Agric Food Chem 58(3):1691–1698

Ferreira JFS, Luthria DL, Sasaki T, and Heyerick A. (2010):Flavonoids from A*rtemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules 15(5):3135–3170

Hirazawa, N. and Ohtaka, T. (2000): Challenge trials on anthelmintic effectof drugs and natural agents against the monogenean *Heterobotherium okamotoi* in tiger puffer *Takifugu rubripes* .Aquaculture 1881-13

Jones-Brando, L., D'Angelo, J., Posner, GH, and Yolken, R. (2006) In vitro inhibition of *Toxoplasma gondii* by four new derivatives of artemisinin *Antimicrob .Agents Chemother* 50, 4208-4206

Ma,Y., Lu D., Lu, X., Liao L and Hu, X., (2004): Activity of dihydroartemisinin against *Leishmania donovani* both in vitro and vivo. Chin Med J 117:1271–1273

Mishina, Y.V, Krishna, S., Haynes, RK, and Meade, JC (2007) Artemisinins inhibit *Trypanosoma cruzi* and *Trypanosoma brucei* rhodesiense in in vitro growth .*Antimicrob .Agents Chemother* .208,51,1852-1854

Keiser J. (2010): In vitro and in vivo trematode models for chemotherapeutic studies. Parasitology 137:589–603

Kim, K.H., Choi, E.S (1998): Treatment of *Microcotyle sebastis* (monogenea) on gills of cultured rockfish *Sebastis schelegeli* with oral adminsteration of mebendazole and mebendazole and bithinol. Aquaculture (167)115-121.

Kitahara, M. (1995): Drugs of Parasites,.manual of therapeutic agents, Japan, pp787-795.

Martin, R.J; Robertoson, A.P; Bjorn, H. (1997): Target sites of Anthelmntics. Parasitology 4, 111-124.

Miller, M.J.; Farahmandian, I; Arfaa, F. Winsor, E. and Bennett, E. (1987): An evaluation of Levamisole for treatment ascariasis .South.Med.j.71, 137-140

Mohamed, A. M.; Ismaiel, M. M.; Kenawy, A. K. and Abd El-Ghany, O. A. (2010): Impact of experimental infection with Gyrodactylus species on the density of skin mucous in fries of catfish (Clarias gariepinus) with emphasis on the pathological changes. Global Veterinaria, 4(1): 67-73.

Perazzo FF, Lima LM, Maistro EL, Carvalho JE, Rehder VLG, Carvalho JCT (2008): Effect of *Artemisia annua* L. leaves essential oil and ethanol extract on behavioral assays. Braz J Pharmacog 18:686–689

Ramadan, A.M., (2000): Morphological and Immunological studies on ectoparasites of some freshwater fishes, Ph. D. Thesis. Parasitology, Suez Canal University.

Schamhl, G.; Mehlhorn, H (1985): Treatment of fish Parasites: 1- Praziquntel effective against Monogenea (*Dactylogyrus vasatator, D.extensus and Diplozoon paradoxum*). Z. paraitenkd. 71,727- 737.

Squires JM, Ferreira JFS, Lindsay DS, Zajac AM (2011): Effects of artemisinin and Artemisia extracts on *Haemonchus contortus* in gerbils (*Meriones unguiculatus*). Vet Parasitol 175(1–2):103–108.

Wang G.X.;Zhou Z.; Jiang D.X.; Han, J., Wang, J.F., Zhao L.W. and Li, J. (2010) In vivo anthelmintic activity of five alkaloids from Macleayamicrocarpa (Maxim)

Fedde against *Dactylogyrus intermedius* in *Carassius auratus*. Vet Parasitol 171:305–313.

Zibaee A, Bandani AR. (2010): A study on the toxicity of a medicinal plant, *Artemisia annua* L. (Asteracea) extracts to the sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). Journal of Plant Protection Research. 50(1):79–85.

أستخدام نبات الشيح (أرتميسيا سينا) ضد طفيل الجيرودكتيلس ريسيفيي فى المبروك العادى المصاب بالمقارنة بعقار (البرازيكنتيل)

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المعمل المركزى لبحوث الثروة السمكية بالعباسة- مركز البحوث الزراعية

فى هذا البحث تم دراسة تأثير مستخلص نبات الشيح على طفيل الجيرودكتيلس ريسيفيي الذى يصيب اسماك المبروك العادى. وقد أجريت الدراسة على أصبعيات المبروك العادى. تم تقسيم الأسماك الى 6 مجموعات فى ثلاث مكررات. تم إستخدام مستخلص نبات الشيح و بمعدلات 1، 2، 3 % ثلاث معاملات لكل منهما مع الإحتفاظ بمجموعة بدون معاملة (المجموعة الخامسه) لكل مكرر. وتم تغذية الأسماك المختبرة بمعدل 3% من وزنها الحى لمدة شهر. وقد أوضحت النتائج ان مستخلص نبات الشيح له أثر فعال في فصل الطفيليات من أنسجه الاسماك ونفوقها بنسبة تركيزات من50-200مليجرام للتر كذلك فقد وجد ارتباط ايجابى بين عدد الطفيليات المنفصله والنافقه وكلا من تركيز المستخلص ، و مدة التعرض للأسماك المتأثرة لهذه المواد و استعمال مستخلص نبات الشيح بمعدل أضافة1-3% قد أعطى أعلى نسب إعاشة للمبروك العادى. ولا يترك أثر ولا رائحة فى عضلات الاسماك لان الاسماك تنتقل بعد العلاج الى مياة طبيعيه للتخلص من الرائحة. تم استخدام عقار (البرازيكنتيل) لاظهار الكفاءةِ ضدّ يرقات الجيرودكتيلس ريسيفيي وبالتالى انتخاب العقار المنتخب مناسب الايجابية فعّال للسيطرة لتجارب التحدي باستخدام مصادرِ الطبيعيةِ(مستخلص نبات الشيح) فى كلا من التجارب المعمليه وتجارب الاعطاء عن طريق العليقه بمعدل (4جرام لكل كيلو جرام عليقه) والتى استخدمت للسيطرة الايجابيه (Positive control) فى تجارب التحدى,عند ثبوت كفأءة المصدر الطبيعى (2.5جرام لكل كيلو جرام عليقه) فى التجارب المعمليه يتم اعطائة للاسماك فى العليقه.

أوضحت النتائج:-

ان (البرازيكنتيل) تمنع تماما العدوى الافقيه بين الاسماك.

2-الاحياء من الاسماك المعالجه بهم(البرازيكنتيل) يكونوا افضل من الاسماك غير المصابه.

3- عدد الاطوار البالغه فى التجويف الخيشومى تقل عن المجموعات الاخرى.

4- تطبيق مستخلص نبات الشيح الاستفادة منه في صناعة تربية الأحياء المائية للقضاء على استخدام العقاقير التقليدية العضوية الاصطناعية التي قد تكون ضارة للمستهلكين من منتجات تربية الأحياء المائية.