TRIALS OF USING *ARTEMISIA CINA L. AGAINST CLEIDODISCUS ACULATUS,* (MONOGENEA: ANCYROCEPHALINAE) OF *CYPRINUS CARPIO* WITH REFERENCE TO PERFORMANCE AND IMMUNE RESPONSE OF FISH

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Abstract

Artemisia cina was effective in the dislodgement and mortality of monogenean parasites, *Cleidodiscus aculatus* of juvenile *Cyprinus carpio* at concentrations ranging from 50 to 200 mg/l. There were positive correlations between the number of parasites dislodged/killed and the concentration of *A. cina* extract, as well as the duration of exposure of affected fish to the substances. Different growth parameters as well as blood parameters were estimated to evaluate the growth performance and immune response of the experimented *C. carpio*. Results revealed that the extract of wormseed plants; *Artemisia cina L.* in the rate of 3% showed significance increase in growth parameters as well as immune response parameters of the examined *C. carpio* . This led to the conclusion that *A. cina* contains substances that are effective against helminthes parasites and increase the performance of *C. carpio*.

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, 1985). 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). Monogenean parasites are hermaphroditic worms which inhabit the skin and gills of fish. Two species, *Gyrodactylus* and *Dactylogyrus,* have been identified as the major causes of mortality in juveniles *Cyprinus carpio* under unfavorable culture conditions. *Cleidodiscus aculatus* are attached to the gill epithelia where they cause serious damage resulting in pathological changes that interfere with gaseous exchange in fish (Ramadan et al., 2009). Skin and gills of affected host are damaged by the attachment hooks resulting in secondary infection by bacteria and fungi. They cause severe economic losses among fish farms (Atallah *et al*., 1999).

Artemisia cina L., also known as annual wormwood and in Egypt known as (Shieh Baladi), belongs to the family Asteraceae. It is a highly aromatic herbaceous plant of Asiatic and Eastern European origin, widely dispersed throughout the temperate region (Abdel-Hadi *et al*., 2008). It was used by Chinese herbalists since A.D. 341 for the treatment of fevers associated with malaria (Hien and White, 1993). Its activity against malarial parasites in primate models was demonstrated in 1971, but the isolation and characterization of the active antimalarial principle, artemisinin, by Chinese scientists were in 1972 (Yeung, 1985). Artemisinin is produced mainly in the leaves and inflorescence of the plant. Its structure has been characterized as acadinane-type sesquiterpene lactone with an endoperoxide bridge. Artemisinin and its semi-synthetically prepared derivatives including dihydroartemisinin, artesunate, and artemether act as blood schizontocidal agents, which effectively inhibit the late stage ring parasites and trophozoites of *Plasmodium*. 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. Interestingly, within the last decade or so, artemisinin and many other bioactive compounds

isolated from *A. annua* have equally displayed unique pharmacological activities against a wide range of bacteria (Bone and Morgan, 1992) including *Enterobacter* and *Klebsiella* species, *Streptococcus faecalis*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Escherichia coli*, and *Pneumocystis carinii* (Chen *et al.*, 1994), an opportunistic pathogen which causes pneumonia in AIDS and other immune-compromised patients. Recent studies have also shown that artemisinin has a therapeutic potential against *Toxoplasma gondii* (Jones-Brando *et al.*, 2006), *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), and Clonorchiasis. Artemisinin destroys the cells of parasitic organisms through the generation of highly reactive oxygen-based free radicals or electrophilic intermediates, by alkylating and oxidizing proteins and lipids of parasite membranes as well as inactivation of channel proteins (Ridley and Hudson, 1998). It has been demonstrated that the effect of artemisinin is equally mediated through disruption of membrane potential by interacting with the electron transport chain in the mitochondrial membrane, resulting in free radical damage and dysfunctional mitochondria (Li *et al*., 2005). It has been demonstrated in earlier studies that supplementing daily rations of poultry with dried pulverized leaves of *A. annua* was found to be effective for the treatment of coccidiosis in chickens (Brisibe *et al*., 2008) without any adverse effects. Moreover, dietary supplementation also has the potential to be an effective anthelmintic treatment in small ruminants destined for the meat market (Hart *et al*., 2007). Where they account for 60% to 70% of the mortality found amongst juveniles in Nigeria (Obiekezie and Taege, 1991). The chemotherapeutic agents currently used for the treatment of fish monogenesis include mebendazole, organophosphate, praziquantel,

closantel, dichlorvos, formaldehyde,etc. (Chisholm and Whittington, 2002).

More recently Albert and Ebiamadon (2010) in Nigeria 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 disease of cultured fish using an extract of *A. cina* instead of chemical-based substances that may not be friendly to the environment, also to throw a light on the effective role of *A. cina* on fish performance.

MATERIALS AND METHODS

Plant material:

Artemisia cina leaves were washed thoroughly in running tap water to remove sand and debris. Here after, 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 and 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 stock and working solutions:

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 (labeled A, B, C, and D 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 hapa made up of mosquito net of tiny mesh size in an outdoor earthen pond for a period of1 week. Examination for the accumulation of monogenean parasites was done from the fourth day of stocking according to Albert and Ebiamadon (2010).

Efficacy testing:

One hundred 1-month-old juvenile *Cyprinus carpio* were stocked in hapa in earthen pond 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 juveniles *Cyprinus carpio* 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 (Albert and Ebiamadon, 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 stone

aeration for production of dissolve oxygen. 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 aquaria contained 5 L aerated tape 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 effective concentration, the mortality of *C. aculeatus* Table (1) 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):

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

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

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

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.

Blood parameters:

Blood samples were taken from the caudal veins of 4 fish per each aquarium (i.e. total of 12 fish or replicates per treatment were sacrificed) for the determination of total and differential leucocytic counts (Dacie and Lewis, 1995), haematocrit values, haemoglobin (Hb) (using commercial colorimetric kits; Randox, Germany) and phagocytic activity using Nitro Blue Tetrazolium (NBT) assay, where the production of oxygen radicals by macrophages was assayed by the reduction of NBT (NBT; Sigma-Aldrich Chemical, St. Louis, MO, USA) according to Rook *et al.* (1985).

Hepato and spleno/somatic indices:

The 6 sacrificed fish from each treatment were dissected. The liver and spleen of each fish were removed, weighed and the hepato/somatic as well as the spleno/somatic indices were calculated according to (Dacie and Lewis, 1995).

Statistical analysis:

All data were analyzed statistically using Analysis of Variance (ANOVA) test. Significant difference between the treatment means was determined at 5% confidence limit (P <0.05) using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

At the end of the study periods, examination of *Cyprinus carpio* fry demonstrated that, while some of the fish that previously harbored parasites were found to be free of some of the parasites, others were

completely free of the parasites. Comparatively, the number of parasites on the body surfaces of fish in the control 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 (1). 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 (1). 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 of fishes taken the upward position in water but some else go down to bottom.

Table (1): Parasite mortality against concentrations (milligrams per liter) of *Artemisia cina* at different time intervals.

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

Table (2): Toxicity test of concentrations (milligrams per liter) of *Artemisia cina* against fish 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*

Results of the toxicity test Table (2) showed that, extract of *A. cina* was well tolerated by juveniles *Cyprinus 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 %.

Evaluation of growth parameters of treated juveniles *Cyprinus carpio*:

Results of Table (3) revealed that juveniles *Cyprinus carpio* treated with *A. cina* in the rate of 5% (treatment 3) had significantly the highest biomass and average body length compared with fish in other treatments and in the control. Fish in treatment 2 and in the control group had the lowest biomass in this experiment. On the other hand, results of Table (4) showed that fish in treatments 3 and 4 gave the highest but non significant estimates of net and daily weight gains as well as relative growth rate. Fish in the same groups also had the lowest feed conversion ratio (FCR). Thus, they significantly, had the best FCR rather than that of the control fish. On the contrary, fish of the control group gave significantly compared with that of fish of treatments 3 and 4.

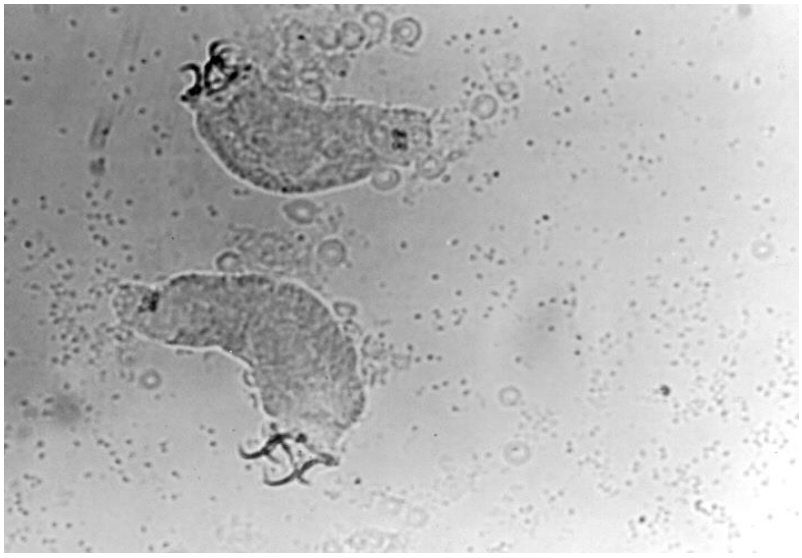

Fig (1). Monogenean parasites *Cleidodiscus aculatus* of juvenile *Cyprinus carpio*.

Table (3): Total biomass, body weight, total length and survival rate of *Cyprinus carpio*.

***A.cina* treatment (T)**	**Total biomass (g)**	**No. of fish**	**Total body weight (g)**	**Total body length (cm)**	**Mortality**	**Survival rate (%)**
1	322.8± 0.003^{c}	19.0 ± 0.004	16.8 ± 0.004^{c}	14.3± 0.004^{b}	2.0 ± 0.004	90.0 ± 0.021^{ab}
2	261.8± 0.126^{d}	15± 0.0	18± 0.009^{bc}	15.2± 0.004^{ab}	6± 0.0	70±0^{d}
3	378.0± 0.041^{a}	18.0± 0.0	19.4± 0.002^{ab}	15.3± 0.001^{a}	1.0 ± 0.0	95.0 ± 0.0^{a}
4	349.4± 0.062^{bc}	17.0± 0.009	18.4± 0.005^{abc}	15.0 ± 0.003^{ab}	2.0 ± 0.009	90.0 ± 0.042^{ab}
Control	307.3± 0.092^{bc}	15.0± 0.004	20.5± 0.0004^{a}	14.7± 0.001^{ab}	5.0 ± 0.004	75.0 ± 0.021^{cd}

$^{a-d}$ Means having the same superscript letters in the same column are not significantly different at $P < 0.05$.

Table (4): Body weight, net weight gain, daily gain, relative growth rate, condition factor & FCR of *Cyprinus carpio* fed with *A. cina* mixed basal diet.

A. cina treatment	Initial Body wt (g)	Final Body wt (g)	Net wt gain (g)	Daily gain (g)	Relative growth rate (%)	Condition factor (k)	Feed conversion ratio (FCR)
1	12.9± 0.0009	17.8± 0.004	4.9± 0.005^{a}	0.163± 0.0002^{a}	41.3± 0.046^{a}	0.58± 0.0003^{abc}	2.6± 0.002^{ab}
2	13.5± 0.002	19± 0.009	5.5± 0.007^{a}	0.184± .0002^{a}	43.7± 0.052^{a}	0.51± 0.0001^{c}	3.2± 0.004^{ab}
3	14± 0.001	19.9± 0.002	6.4± 0.002^{a}	0.213± 4.871^{a}	49.0± 0.008^{a}	0.54± 0.0002^{bc}	1.97± 0.0003^{b}
4	13.03± 0.001	19.8± 0.005	6.4± 0.004^{a}	0.213± 0.0001^{a}	52.8± 0.029^{a}	0.55± 0.0002^{bc}	1.97± 0.0003^{b}
Control	14.7± 0.008	20.5± 0.0004	5.8± 0.009^{a}	0.194± 0.0003^{a}	41.1± 0.081^{a}	0.65± 0.0001^{a}	3.7± 0.009^{a}

Evaluation of immune response of treated juveniles *Cyprinus carpio* :

Survival rate:

As demonstrated in Table (3), fish in treatment -3 had significantly high survival rate compared with that of fish in the control group and treatment -2.survival rate of fish in treatments -1 & 4 were equal.

Blood parameters:

Results of Table (5) revealed that fish in all treatments of *A. cina* showed the highest figures of blood haemoglobin, Packed Cell Volume, (PCV) and NBT (treatments 2, 1 and 3 respectively) especially, fish in treatment -2, which gave significantly, higher estimates than those of other treatments and the control group. Fish in treatment 4 occupied the next rank after *A. cina* in this regard.

Hepato/somatic and spleno/somatic indices:

Fish in all treatments of wormseed plants had the significant high hepato/somatic indices (treatments -2, 1 & 3 respectively), fish in

treatment 2, which had significantly, higher indices than those of fish in treatments 4 as well as in the control group. On the contrary, fish in treatment 2 showed the lowest spleno/somatic index compared with all other treatments and even the control group. However, fish in other treatments of wormseed plants (1 & 3) had the significant high spleno/somatic indices (Table 5).

Table (5): Total and differential leucocytic counts, haematocrit values, haemoglobin and NBT estimates of *Cyprinus carpio* fed with *A. cina* mixed basal diet.

***A. cina* treatment**	**Total Leucocytic count**	**Differential Leucocytic count**			**Hemoglobin (Hb)**	**Hematocrit Value (PCV)**	**NBT estimates (mg/mL)**
		Neutrophils	**Monocytes**	**Lymphocytes**			
1	21366.7± 4.5^{abcd}	6.2± 0.003	3± 0.004	88.8± 0.003	7± 0.002^{ab}	21.96± 0.66ab	0.42± 0.001^{a}
2	20633.3± 9.2^{cd}	5.3± 0.004	2± 0.004	95.7± 0.004	7.8± 0.003^{a}	24.58± 1.004^{a}	0.42± 0.0003^{a}
3	20950± 6.6^{bcd}	6.7± 0.005	2.3± 0.002	94± 0.007	6.7± 0.004^{bc}	20.38± .١.11^{b}	0.3± 0.001^{ab}
4	24416.7± 16.1^{abc}	7.3± 0.006	2.2± 0.003	90.8± 0.006	6.7± 0.003^{abc}	20.38± 1.32b	0.26± 0.0002^{bc}
Control	20300± 28.9^{d}	5.7± 0.016	3.3± 0.005	91± 0.017	6.2± 0.004^{bc}	19.1± $^{1.39}$b	0.26± 0.0001^{bc}

Table (6): Total body, spleen and liver weights as well as spleno/somatic and hepato/somatic indices of *Cyprinus carpio* fed with *A. cina* mixed basal diet.

***A.cina* treatment**	**Total body weight (g)**	**Liver weight (g)**	**Spleen weight (g)**	**Hepato/ somatic index**	**Spleno/ somatic index**
1	17.8± 0.020	0.20± 0.0003	0.022± 4.95	1.031± 0.0004^{ab}	0.137± 0.0003^{a}
2	21.50± 0.021	0.24± 8.335	0.01± 0.00	1.5± 0.001^{a}	0.078± 0.003^{b}
3	18.18± 0.02	0.15± 0.0002	0.018± 4.17	0.98± 0.0009^{abc}	0.18± 0.0003^{a}
4	18.59± 0.09	0.15± 0.0002	0.017± 2.19	0.87± 0.0006^{bc}	0.088± 0.0001^{ab}
Control	21.98± 0.012	0.17± 0.0002	0.012± 1.73	0.757± 0.001^{c}	0.054± 0.002^{b}

DISCUSSION

The results of investigation shows that the ethanolic extract of *A.cina* are also effective against monogenean parasites *Cleidodiscus aculatus* of juveniles *C.carpio*. Their application led to the reduction of the parasite load and even eliminated the parasites completely in some cases within the duration of exposure. And so remove the drastic role of *Cleidodiscus aculatus* from juveniles *C.carpio* in Abbassa. Several reports have shown the effectiveness of artemisinin, the active substance in *A. annua* against protozoan and helminthic parasites in human, poultry, and small ruminants, respectively (WHO, 1981; Hart *et al.*, 2007 and Brisibe *et al*., 2008). When the parasitized fish fry were exposed to low concentrations of *A. cina* extract for a short period of time (for example, 50 mg/l for 30 min), the extract had little effect on the parasitized fish. Using this same concentration with an increased time of exposure (50 mg/l for 90 min), parasitic load was further reduced, but the parasites were still present. However, increasing the time of exposure further (50 mg/l for 120 min) led to a drastic reduction in the parasitic load to a minimal level. This observation tacitly implies that a low dosage of *A. cina* extract can still be effective for dislodging ectoparasites from fish as long as the duration of exposure to the treatment is increased. All of these results have been reported before by (Albert and Ebiamadon, 2010).

This obviously has several advantages over treatments with high dosages using short exposure time. Of particular interest and significance was the fact that fish exposed to the *A. cina* extracts appeared more active and agile in behavior than their counterparts in the control. This could possibly be explained by the fact that the immune system of these fish may have been boosted as they were free from the effects of the parasites. Monogenetic trematodes are some of the most threatening parasites of the juvenile carp involved in fish importation (Mortensen *et al*., 2006). Huge economic losses have been reported in situations where appropriate

chemotherapeutic agents are not readily employed (Faruk *et al*., 2004). The commonly used chemical agents for the treatment of this parasite include mebendazole, organophosphate, praziquantel, closantel, dichlorvos, formaldehyde, etc. (Chisholm and Whittington, 2002). However, the use of synthetic organic substances in the treatment of diseases in food fish should be discouraged (Committee on Mutagen city of Chemicals in Food, Consumer Products and the Environment, 1999). Apart from the fear of accumulation in the tissues; their discharges into the aquatic environment may contribute to habitat destruction or degradation. Consequently, there is an urgent need for the development of cheap, nontoxic, and environmentally benign agents for the treatment of such parasite of food fish. Although the extract of *A. cina* has a wide range of tolerance, administration of the extract in the dislodgement of ectoparasites of fish is done in holding tanks in the hatchery, and the fish are returned to the pond when they have fully recovered from parasites and effects of treatment. Other aquatic organisms do not stand a chance of exposure to the substance. The extract used in high amount as shown in this study is because it has a wide range of tolerance. The dislodgement of fish parasites was affected from 50 mg/l of the extract. It was also observed that within1 h of exposure at 200 mg/l, 85% of parasites were killed and dislodged from the host without any host mortality. It is also possible to reduce the concentration of extract during treatment but increase the exposure time of fish to the extracts. The reduction in the parasite burden would enhance fast recovery and may pave way for the boosting of host protective immunity (Clark and Dickerson, 1997).

Our results in respect to the anthelmintic activity of *A. cina* coined with Albert and Ebiamadon (2010) in using *A. annua* against monogenean parasites of *Heterobranchus longifilis* in Nigeria.

Juveniles *Cyprinus carpio* treated with *A. cina* in the rate of 5% had significantly the highest biomass and average body length compared with fish in other treatments and in the control. This could be attributed to the high mortalities among the juveniles *Cyprinus carpio* in these 2 groups and accounted for the higher but insignificant average body weight gained by fish of the control group (fewer fish received the same amount of food of other treatments with higher number of fish) the highest but non significant estimates of net and daily weight gains as well as relative growth rate but had the lowest feed conversion ratio (FCR). Thus, they significantly, had the best FCR rather than that of the control fish. On the contrary, fish of the control group gave significantly compared with that of fish of treatments 3 and 4. This might be probably because of the same reason of high mortality.

The higher survival rate of fish treated with *A. cina,* could be attributed to the immune-stimulant effect induced by *A. cina* on the non-specific immune response of the fish under study. These results were supported by Abdel-Hadi *et al.* (2008).

Fish in all treatments of *A. cina* showed the highest figures of blood haemoglobin, Packed Cell Value, (PCV) and NBT (treatments 2, 1 and 3 respectively) especially, fish in treatment 2, which gave significantly, higher estimates than those of other treatments and the control group. These results might indicate a more positive effect of *A. cina* on immune response of experimental catfish. Similar results and figures of haemoglobin in catfish of the control group were reported by Abdelhamid *et al.* (2009). On the contrary, estimates of haematocrit value in fish of the control group disagreed with those recorded by Adedeji *et al.* (2009) who recorded significant high figures of PCV.

Fish in all treatments with *A. cina* had the significant increase of hepato/somatic indices thus, *A. cina* enhanced the development of liver and spleen; the main blood forming organs in fish (in addition to the fore-

kidney) and as a consequence, stimulated the immune response of fish. Taken together, the results of the present study could lead to the conclusion that the ethanolic extract of *A. cina* leaves has an antiparasitic efficacy against monogenean parasites *Cleidodiscus aculatus* of cultured juveniles *C. carpio,* possibly due to the medicinal properties of the plant and It's recommended to add *A. cina,* (wormseed plants) in the rate of 3% and 5% to the artificial feeds of *C. carpio* and it needs to apply in earth ponds as agreed with that of Osama *et al.* (2010). Consequently, further investigation is recommended on the use of the plant against fish protozoan and other parasites responsible for fish mortalities under culture conditions.

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محاولات استخدام نبات الشيح (أرتميسيا سينا) ضد طفيل كليودسكس اكيولاتس (الديدان المفلطحه وحيدة العائل) الذى يصيب اسماك المبروك العادى مع اشارة الى الاداء الوظيفى والأستجابه المناعيه للاسماك

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الملخـص العربـى

يتجنب الباحثون في مجال الاستزراع السمكي استخدام الأدوية والكيماويات التى بقدر مالها من مميزات وكفاءة فى زيادة الأنتاج،الى أنها لها تأثير سلبى على صحة المستهلك وكذلك على البيئة والتوازن البيولوجى بها. والأتجاه الان إلى إستخدام الأعشاب والنباتات الطبيعية التى ترفع من كفاءة الجهاز المناعى للأسماك مما ينعكس بالإيجاب على معدلات التحول الغذائى والنمو وزيادة الأنتاج، والأهم من ذلك أنه امن للأستهلاك الأدمى وليس له تأثير سلبى على البيئة المحيطة. وفى هذا البحث تم دراسة تأثير مستخلص نبات الشيح على طفيل كليودسكس اكيولاتس الذى يصيب اسماك المبروك العادى مع متابعة اثرة فى السلوك الوظيفى و الإستجابة المناعية غير المتخصصة للاسماك. وقد أجريت الدراسة على أصبعيات المبروك العادى. تم تقسيم الأسماك الى ٦ مجموعات فى ثلاث مكررات. تم إستخدام مستخلص نبات الشيح و بمعدلات ١، ٢، ٣ % ثلاث معاملات لكل منهم مع الإحتفاظ بمجموعة بدون معاملة (المجموعة الخامسه) لكل مكرر. وتم تغذية الأسماك المختبرة بمعدل ٣% من وزنها الحى لمدة شهر. تم حساب القياسات المختلفة للنمو و صورة الدم وذلك لتقييم معدل النمو والأستجابة المناعية للاسماك المختبرة. وقد أوضحت النتائج ان مستخلص نبات الشيح له أثر فعال في فصل الطفيليات من أنسجه الاسماك وقتلها بنسبة تركيزات من٥٠- ٢٠٠مليجرام للتر كذلك فقد وجد ارتباط ايجابى بين عدد الطفيليات المنفصله والمقتوله وكلا من تركيز المستخلص، ومدة التعرض للأسماك المتأثرة لهذه المواد . واستعمال الشيح بمعدل أضافة١-٣% قد أعطى أعلى نسب إعاشة ومعدل تحويل غذائى وأعلى معدل نمو وإستجابة مناعية للمبروك العادى. ولا يترك رائحة فى عضلات الاسماك لان الاسماك تنتقل بعد العلاج الى مياة طبيعيه للتخلص من الرائحة. ومن دراسة مكونات الدم لتقييم أداء النمو واستجابة جهاز المناعة يتضح ان هذا النبات يحتوي على مواد فعالة ضد طفيليات الديدان وزيادة أداء الاسماك.