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)

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Abstract

Artemisia cina L. (wormseed plants) and *Allium sativum* (garlic) were tested for the control of Saprolegniosis affecting eggs of carp species; common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*). Twelve spawning funnels each of 50 liters capacity were used for carrying out different experiments. *Artemisia cina L.* (*A.cina*) was used in the form of 5% and 25% stock solutions prepared by pouring boiling water on the herb in a piece of gauze and soaked for an hour. The doses were 0.25, 0.5 and 1 ml/l 3 times every 6 hours in eggs of common carp and twice in eggs of silver carp. Garlic was used as blended garlic and as a stock solution in the rates of 0.25, 0.5 and 1 g/cc per liter of water in spawning funnels. Three replicates were used per each treatment and 3 funnels served as control where malachite green or formalin were applied for comparison. The results revealed that *A. cina* gave the best estimates of fertility, hatchability and viability percentages among the newly hatched larvae. It was safe for larvae in the rates of 5% and 25% where no LC50 was detected for *A. cina*. On the contrary, LC50 for garlic was 3 g/liter and 4 g/l killed all tested larvae in an hour. Although, garlic was safe when used in doses of 0.25, 0.5 and 1 g/l, the viability of the newly hatched larvae was badly affected. *A. cina* is recommended in a dose of 0.5 ml/l of 25% stock solution.

Introduction

Saprolegniosis is a main problem causing severe losses of fish eggs in fish hatcheries. Fish eggs are subjected to fungal infection, which often causes heavy losses of eggs and fry (El-Ashram, 1997). Eighty five fungi species were reported growing on the eggs of 16 fish of several families incubated in the water from various water bodies (Zeczuga and Muszynska, 1999). The control of fungi of the genus *saprolegnia* has long been a major objective of aquaculturists. Once a fungal infection starts, it can spread rapidly from infected to healthy eggs (Pipper *et al*., 1982 and El-Ashram, 1997). Antifungal agents are essential for the maintenance of healthy stocks of fish and their eggs in intensive aquaculture operations. For decades, hatchery personnels have been depending on chemicals to control disease outbreaks on fish eggs. Although it is necessary to control these outbreaks, it is also important to find an alternative disease control method to reduce the use of chemicals as a matter of safety for consumers and environment. Malachite green and Formalin were superior in *in vivo* tests in controlling *Saprolegnia parasitica* in eyed trout eggs (Cline and Post 1972). Caution must be exercised when using formalin at dosages higher than 20 ppt for long term bathes and 200ppt for short term dips because its use may cause hypoxia in fish due to the reduction of dissolved oxygen in tanks water. However, chemical treatment is costly and can itself cause mortality (Jimenez, 1986 and El-Ashram, 1997). Wormseed plants have a powerful biological effect against fungi, bacteria and even some harmful insects (Abo-Zeid, 1988). On the other hand, Garlic has a powerful antifungal and antibacterial effect (Diab, 2002).

Thus, this study was established to meet the following objectives:

1 The effect of wormseed plants and garlic in preventing Saprolegniosis in eggs of carp spp.

2 Investigating the toxicity or side effects induced by the experimental substances on the treated fish's eggs and larvae.

Material and methods

Site of experiments: Twelve funnels with 50 liters capacity each were used for carrying out the experiments at CLAR fish hatchery.

Fish: Broad stock; 3 males and 3 females per each carp species (common and silver carp) were needed. Eggs obtained from individual females were weighed and fertilized with melt obtained from males. The eggs were counted and distributed into hatching funnels (50 liters capacity each) in a rate of 1gm swollen eggs /l. The treatments were applied immediately after distributing the eggs (1g of common carp swollen eggs has 320 eggs, while 1g of silver carp swollen eggs has 25 eggs). Incubation period for common carp eggs ranged from 48-72 hrs, but for silver carp the range is from 18-24 hrs. All hatching funnels were supplied with filtered pond water at a temperature of 25 ºC, pH 8 and mean dissolved Oxygen 5.5 mg/l.

***The experimental substances*:** Wormseed plants; *Artemisia cina L* and bulbs of garlic; *Allium sativum* (native breed) were tested against malachite green and formalin. They were all applied every 6 hs on fertilized egg of common carp till the movement of the embryo took place and twice in case of eggs of silver carp.

***Experimental design*:** LC_{50} and safety of both wormseed plants and garlic solutions were detected by adding 20 ml of sheih in the rates of 5% and 25% as well as of garlic solutions in the rates of 1, 2, 3 and 4 g/l in 6 petri dishes. One Petri dish contained 20 ml of distilled water and served as a control. Twenty newly hatched larvae of silver carp were added to each of the 7 petri dishes and observed for viability.

Wormseed plants' solution (Sheih Baladi) was prepared in the rates of 5% and 25% by adding 1 liter of boiling distilled water to beakers containing 50 and 250 g respectively of the herb, wrapped in a piece of gauze. The mouth of the beaker was covered and the herb was left for an hour for proper soaking. The used rates in vivo were 0.25, 0.5 and 1 ml (of sheih, 25%) per liter of water of hatching funnels with 50 liters capacity in 3 replicates per each dose.

Blended (minced) Garlic was incubated at 37 ºC for an hour to allow the activation of the inactive Allinin to the active Allicin (Diab, 2002). Three treatments of garlic (0.25, 0.5 and 1 gram/liter of water in the funnels) were ttested and compared with 1 treatment of formalin (0.1 ml/l of funnel's water) with 3 replicates per each treatment. Total amounts of 12.5, 25 and 50 grams of these incubated garlic were put in pieces of gauze and then held for 18 hours per 50 liters of water of 9 funnels containing fertilized eggs of common carp (*Cyprinus carpio*) and compared with 3 replicates of eggs treated with malachite green 0.5 mg/l (Laszlo *et al*., 2002). Fertility, hatchability and survivability ratios were estimated for all treatments. The same doses of minced garlic were experimented on eggs of silver carp (*Hypophthalmichthys molitrichus*) but compared with formalin regarding the same parameters of evaluation.

Garlic solution was prepared by soaking the blended garlic (contained in a piece of gauze) in distilled water in the rate of 1 gram of garlic per 1 cubic centimeter of water (100% stock solution). The solution was then incubated for 1 hour at 37 ºC. After that, the garlic was squeezed inside the piece of gauze into the water present in the same beaker (the beaker was broad enough and with a wide inlet). Garlic solution was drained by a sterile plastic syringe and distributed into the experimented funnels

containing fertilized eggs of silver carp. Four treatments were tested using garlic solution in the rate of, 0.1 ml/liter, 0.2 ml/l and 0.4 /l of water of the funnels. Formalin was added to 3 funnels containing eggs from the same batch in the rate of 0.1 ml/l. Fertility, hatchability and viability ratios per 1 liter for each group were estimated for all treatments according to Al-Ashram, (1997).

Statistical analysis: Results were expressed as a mean ± S.E and analyzed using one-way analysis of variance (ANOVA). Duncan's test was used to compare the differences of treatment means. The statistical analysis was conducted using SPSS Statistical Software at differences of $P < 0.05$.

Results and discussion

LC_{50} and safety of wormseed plants and garlic on larvae of carp spp.

Wormseed plants' solution in the rate of 5% and 25% was found safe on larvae of examined silver carp, which tolerated and lived for 24 hours in the stock solution of 25% and for 4 days in 5% Sheih solution. Thus, no LC_{50} was detected for wormseed plants. On the other hand, garlic caused the death of 50% of larvae in a concentration of 3 g/l (LC_{50}). Garlic in a concentration of 4 g/l killed all examined larvae while no deaths were detected among larvae when garlic was used in the rate of 2 g/l for 2 hs. However, the larvae were unable to tolerate garlic in the rate of 1 g/l when applied for 24 hours where all larvae died.

Examination of both plants on egg of carp spp.

Results of table (1) revealed that wormseed plants in the rate of 0.5 ml/l applied on eggs of common carp, showed significant higher estimates of fertility, hatchability and survivability percentages (%) than other treatments of the same herb and also malachite green. In case of eggs of silver carp, wormseed plants (0.5 ml/l) and formalin (0.1 ml/l) gave significantly, higher figures of hatchability and survivability% than other doses of sheih. However, no significant difference was found between all treatments with respect to fertility% (table 2).

On the other hand, blended garlic (0.5 g/l) and malachite green (0.5 mg/l) applied on eggs of common carp, showed relatively higher estimates of fertility, hatchability and survivability% but with no significant differences between all treatments (table 3). While in case of eggs of silver carp, blended garlic (0.5 g/l) and formalin (0.1 ml/l) revealed significantly higher estimates of fertility, hatchability and survivability% than other doses of blended garlic (0.25 and 1 g/l) (table 4).

Concerning garlic solution applied on eggs of silver carp, results of table 5 revealed that garlic solution in the rate of 0.1 ml/l and formalin (0.1 ml/l) showed significantly, higher figures of fertility% than obtained by garlic solution (0.4 ml/l). At the same time, garlic solution in the rate of 0.2 ml/l gave relatively higher fertility figures than the garlic dose of 0.4 ml/l. Thus, the lower the garlic concentration, the better is the fertility (%). On the contrary, garlic solution in all examined concentrations had a lethal effect on the newly hatched larvae of silver carp, where percentages of hatchability and survivability were zero.

It could be concluded that, wormseed plants in the rate of 0.5 ml/l of 25% solution gave the best estimates of fertility, hatchability and survivability% among the examined eggs and larvae of carp species and recommended for practical application in carp hatcheries to replace the currently used chemicals; malachite

green and formalin with their well known environmental and public health hazards. Although blended garlic in the dose of 0.5 g/l resulted in relatively good results regarding the same measured parameters, the use of garlic with eggs of carp species is not recommended in this study because of its lethal effect on the treated larvae.

Table 1: Wormseed plants compared with Malachite green on common carp eggs.

Survivability (No of larvae/l)	of % hatchability	No of hatched larvae after 72 h	of % fertility after 12 h	No of fertilized eggs after 12 h	No of eggs /funnel	Amount of eggs/funnel (g)	substances and doses
48	35	2420	45	7200			
30	17	1520	50	8000			
11	16	572	70	11200			
180	**95**	**9020**	**60**	**9600**			
		8	**5400**	**0**	**600**		
			50	**6400**	**00**	**00**	
			12	50			
200					0	600	
0	0	000				00	70
3200	0	0	0				
35	1505	5	4300	0			
	67	35	60	60			

ilver carp compared with formalin.

er carp ed with formalin.	urvivability	(No of l) ability	ty of larvae ter 24 h	er 24 h after 6 h	of rtilized	eggs fter 6 h o of eggs	funnel nt
unnel (g)	el (g)	(g)	substance	stances and	ces	and doses	d doses
		2		0	**es and**	**and doses**	**doses**
					s and	**nd doses**	**doses**
688							
65	777					1	0
0	**0**	**50**					
		0		**00**			
13	**80**	**672**	**75**	**840**	**00**		
12	80	600	60	750	**0**		
		12	0	00			
			0	5	600		
0				6	00	60	60

70
0

875
75

e 3: arlic ompared	th lachite	on common p eggs ivability	lity No of ae/l) hatchabilit	atchability hatched vae after 72 h	fter 72 2 h f %	tility after er 12 h of fertilized	ertilized ggs after
h	of eggs	eggs /funnel	/funnel	unnel	Amou mount	unt of nt of	of of

132	75	6600	55	8800			
179	70	8960	80	12800			
189	**80**	**9472**	**74**	**11840**			
230	**86**	**11520**	**90**	**14400**			
124	**75**	**7200**	**60**	**9600**			
141	80	7040	55	8800			
		6	8800	0	800		
			60	5760	00	00	
			79	80			
200					80	8960	
0		000			0	960	60

11520

lver carp compared with formalin

ompared ormalin	bility y	ae/l) chability of hatched	tched larvae r 24 h	fertilized eggs	rtilized ed gs	6 h eggs l	ount of s/funnel
ubstances	tances and	ces and	and	doses	es		
600					**s**		**12**
1	0	25	0	50	es		**2**
10	80	496	50	620			
8	**0**	**00**		**250**			
	20	**90**	**012**		0		
			80	**900**			
0				14	**0**	**00**	**0**
0	875					**0**	
0	10	50	20			**900**	
50	310		620				
		0		00	0		

c solution eggs of silver carp	rp mpared	ormalin. vivability o of larvae/l)	arvae/l) l) chability ity	ty f hatched after 24 hs	s % tilized	eggs No ed eggs	ggs hs
/funnel	funnel	nnel	el	Amount of	t of	f	ggs/funnel
nel	l	(g)	)	ubstances	**of**		**gs/funnel**
d doses	doses	ses	s		not of	**eggs/funnel**	**gs/funnel**
	000	**0**					
0	0	**85**		**62**			
0	0	0	**75**	**938**			
0	0	0	80	1000			
			0			00	
				0	1800	1000	
8						000	
					0		70
5						0	**0**

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