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Effect of Calcium Chloride on Hatching, Growth and Survival of Snails *Lymnaea auricularia*- Intermediat host of *Fasciola gigantica*

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Abstract

The effect of three different concentration (1.0 , 2.0 and 2.5 g\L) of calcium chloride ($CaCl_2$) on hatching , growth and survival of *Lymnaea auricularia* snails serves as intermediate host of *Fasciola gigantica* parasites.

The current study showed that increasing of calcium chloride concentration lead to lower the rates of eggs hatching and effect on the rate of shell growth which recorded the highest weekly rate length of the snails in week 10 and reached (8.68 , 8.86 and 8.80) mm respectively , compared with control samples and the highest growth rate of the snails was recorded using 2.0 g\ L concentration and reach (8.86) mm and lowest growth rate was recorded in control samples .As noted that calcium chloride effect on the percentage of survival snails, which that reached 30% in 1.0g/L and 10% in 2.0g/L and 2.5g/L compared with control samples 20%.

1- Introduction

Fresh water snail *L. auricularia* belong to the phylum Mollusca , class Gastropoda , order Basmmatophora and family Lymnaeidae (Vinarsk , 2007). Snails of *Lymnaea* spp. is the intermediate host of several types of the digenitic trematods among them *Fasciola* spp. which are the most important parasites causes fascioliasis or liver rot disease , it was common parasitic infection in domestic livestock and considered as one of the dangerous veterinary problems (Zhang *et al.,*2005).If we want to control on fascioliasis we had must provide sufficient information about the

intermediate host, *Lymnaea* spp. is widely distributed in most parts of the world (Itakaji *et al .,* 1998) .In Iraq , the snails *L. auricularia* was distributed in the mid and southern regions and acts as intermediate host for *F.gigantica* while *L. trancitula* is found in north and north east of it and acts as intermediate host for *F. hepatica* (AL-Mashhadani , 1970).

In the southern provenances created an ancient relationship was created between the *F.gigantica* and his intermediate host *L. auricularia* led to the compatibility , which represented by short of the incubation period of the larval stages in the intermediate host , produce large numbers of cercariae and high incidence of infection .However , this relationship influenced by several factors such as the hydrogen ion concentration , temperature , dissolved oxygen , ventilation and dissolved salts as well as of the factors resulting from the ions including SO4 , Cl , NO3 , Mg and Ca (Nduku & Harsson , 1979) .

In Iraq , despite of the frequent using *L. auricularia* in laboratories studies but they had used random different factors in the breeding of these snails without knowledge of their effects on the snails which may be reflect on the parasites life cycle within the intermediate host.

To controlling on fascioliasis we had must provide sufficient information about the intermediate host .Therefore, the current study aimed to know the effect of calcium chloride ($CaCl_2$) on the hatching ,growth and survival of these snails.

2-Materials and Methods

Collection and snails breeding

Snails of *L. auricularia* were collected from Al-Sewaib area north east of the Basrah provenance during September 2009 to March 2010 period and transferred to the Lab. by glass container with capacity 1000 ml containing water and aquatic plants taken from the same collection area , then placed in a glass tank , capacity (60 x 30 x 20) cm and fed dry celery plants , left for one week and during this period egg masses had been collected and distributed on four tanks (20 x 50 x 10) cm , one egg mass (50 – 65 eggs) for each tank , the first tank was containing 1.0 g\L calcium chloride , the second tank was containing 2.0 g\L calcium chloride , the third tank was containing 2.5g\L calcium chloride and the fourth without calcium chloride and served as control , all that concentration were prepared by dissolved each concentration of calcium chloride separately in 1000 ml of declorinated water. These tanks were aerated for 24h and incubated at temperature 26 – 29 C°.

After the eggs hatching, 10 snails of approximately the same size and age were transferred to the four tanks as cited above

, concentrations were replaced every three days.

Measurement of Hatching, Growth and Survival

The effect of Calcium chloride concentration on eggs hatching were recorded weekly and the percentage rate of hatching was calculated using :

Rate of hatch = No. of hatching snails / No. of eggs in egg mass × 100

The growth was determined by the length of the snails in each four tanks were recorded weekly using pair Vernier calipers. The surviving snails were counted weekly and the percentage rate of survival was calculated using :

Rate of survival = No. of survival snails / Initial No. of snails × 100

Statistical analysis

Results were analyzed statistically by using Anova one way analysis and the level of test ($P \leq 0.05$) (AL-Rawi and Khalaf Alla,1980).

3-Results

The results of the current study were showed that the three different concentration of calcium chloride $CaCl_2$ influenced hatching egg masses , it was that when the concentration of calcium chloride increased the percentage rate of eggs hatching decrease Table (1).

Table (2) shows the mean of weekly growth rate of *L. auricularia* snails under the influence of three different concentration of calcium chloride (1.0 ,2.0 ,2.5)g/L , it was recorded that the highest rates length in week 10 and reached (8.68 , 8.86 and 8.80) mm respectively , compared with control samples of (6.22)mm . Highest growth rate (8.86) mm was observed on concentration 2.0 g\ L calcium chloride and lowest growth rate of the snails was recorded in control samples reach (6.22)mm.

Statistical analysis results were showed no significant differences among the various concentration when the level of test $p \leq 0.05$.

This study has showed that the effect of different concentrations of calcium chloride on survival rate of snails *L. auricularia* after 10 weeks , it was observed that the percentage rate of survival snails in tank1 (1.0g/L) reach 30% and 10% in the tank2 (2.0 g/L) and tank3(2.5g/L),compared with control samples tank4 (o.og/L) 20% Table (3).

Table(1) The percentage rate of hatching egg masses snails *L. auricularia* under the effect of different concentrations of calcium chloride

Weeks	Tank 1(1.0g/L)	Tank 2(2.0g/L)	Tank 3(2.5g/L)	Tank 4(0.0g/L)
0	0.0	0.0	0.0	0.0
1	17.9	10.7	0.0	80.3
2	8.9	28.6	16	19.7
3	73.2	16.1	4	−
4	−	44.6	80	−
Total	%100	%100	%100	%100

Table (2) mean of growth rate ± stander deviation to lengths (shell diameter in mm) of snails *L. auricularia* under the effect of different concentrations of calcium chloride

Weeks	Tank 1 (1.0g/L)	Tank 2 (2.0g/L)	Tank 3 (2.5g/L)	Tank 4 (0.0g/L)
0	0.000 ± **1.00**	0.000 ± **1.00**	0.000 ± 1.0	0.000 ± 1.0
1	0.054 ± **1.56**	0.894 ± **1.640**	0.836 ± **1.58**	0.010 ± **1.50**
2	0.447 ± **2.02**	0.109 ± **1.680**	0.836 ± **1.98**	0.447 ± **1.580**
3	0.256 ± **2.78**	0.251 ± **3.22**	0.070 ± **3.00**	0.258 ± **2.28**
4	0.258 ± **2.92**	0.504 ± **3.60**	0.241 ± **3.22**	0.593 ± **2.88**
5	0.975 ± **4.020**	0.371 ± **4.26**	0.427 ± **3.56**	1.140 ± **3.40**
6	0.894 ± **5.60**	0.712 ± **5.040**	0.204 ± **4.18**	1.501 ± **4.40**
7	0.937 ± **5.76**	1.193 ± **5.60**	0.249 ± **4.92**	1.548 ± **4.78**
8	1.398 ± **6.90**	1.141 ± **6.74**	0.651 ± **7.10**	1.793 ± **6.140**
9	0.907 ± **8.34**	1.079 ± **7.40**	0.389 ± **7.52**	1.371 ± **6.18**
10	o.739 ± **8.68**	0.512 ± **8.86**	0.200 ± **8.80**	0.192 ± **6.22**

Table (3) percentage survival rate of *L. auricularia* under the effect of different concentrations of calcium chloride

Weeks	Tank 1 (1.0g/L)	Tank 2 (2.0g/L)	Tank 3 (2.5g/L)	Tank 4 (0.0g/L)
0	(10)%100	(10) %100	(10) %100	(10) %100
1	(10)%100	(10) %100	(10) %100	(9) %90
2	(10)%100	(9) %90	(9) %90	(9) %90
3	(7)%70	(9) %90	(9) %90	(6) %60
4	(7)%70	(9) %90	(8) %80	(6) %60
5	(7)%70	(9) %90	(8) %80	(6) %60
6	(5)%50	(5) %50	(4) %40	(5) %50
7	(5)%50	(5) %50	(4) %40	(5) %50
8	(5)%50	(3) %30	(3) %30	(5) %50
9	(4)%40	(3) %30	(3) %30	(4) %40
10	(3)%30	(1) %10	(1) %10	(2) %20

4- **Discussion**

The Calcium ion Ca^{+2} is one of the most chemical factors that affect the growth ,distribution and activity of fresh water snails, which act as intermediate hosts to some trematod that infected human and animals such as , *Fasciola* spp. etc.. . Broderson and Madson (1991) found that *Biomphalaria pfeifferi* was absent in water bodies with high concentration of magnesium (Mg) and present where calcium ion were abundant , the snail intermediate hosts show more tolerance to higher alkalinity than low .When they are found in water with low alkalinity , the number is reduced and they have relatively thin shell (Okwuosa and Ukoli ,1980 ;de Kock *et al*., 2004) .

The results of the current study showed that the impact of different concentrations of calcium chloride (1.0 , 2.0 , 2.5)g/L on hatching of egg masses to the *L. auricularia* snails table (1) ,which it was observed that the embryos developed and capable of hatching in different concentrations but, the faster of completion hatching process vary from concentration to another . In control samples were

required two weeks to complete hatching process and three weeks in concentration 1.0g/L and fourth weeks to the concentration (2.0 ,2.5) g/L respectively , may be due to the effect of calcium ion on the process of fertilization and eggs, which the increasing of calcium in some animals lead to eggs activated and started the next evolutionary stages :such as exists in some vertebrates (mammalian) and some invertebrates (*Echinus*) , In contras another animals lead to stop activity of the eggs ,it was observed that when the direct injection of calcium in eggs cytoplasm of *Echinus* (Wolpert *et al.,* 1998) .It seems that the snails *L. auricularia* of the kind that is negative affected by increased concentration of Ca^{+2} and the present study confirmed that .

In general ,it was observed that the effect of Ca^{+2} on the rate of growth of the snails *L. auricularia* were recorded the highest growth rate in 10 week and amounted to 8.86mm at concentration 2.0g/L $CaCl_2$ and the lowest growth rate in control samples and amounted to 6.22mm .The higher Ca ion in the tank lead to increase the rate of growth of the snails , perhaps due to actively and necessary contributions for adequate shell formation and then high rate of growth ,additional to the role in the crams muscle that responsible on a movement of snail feet and that needed to a movement and searching for food (Muyssen *et al.,* 2006) .This results agreed with the findings of Harrison *et al.* (1979) and Okwuosa and Ukoli (1980) that the higher concentration of Ca^{+2}, will faster the rate of growth of the snails , and also agree with the study Olaseninde and Ouerindo (2007) on the effect of three different concentrations of $CaCl_2$ (1.0 , 2.0 and 2.5)g/L on the rate of growth of the snails *B.pfeifferi* which is found that the highest growth rate of it in 10 week and amounted to 1.16 mm at concentration 2.5g/L and the lowest growth rate in control samples and amounted to 1.10 mm .

It is obvious from the results that the survival or mortality of the snail was also affected by concentration of Ca^{+2} in the culture tanks after 10 weeks .The highest percentage survival rate was recorded in tank1 with concentration of 1.0g/L $CaCl_2$ and reached to 30% ,compared with control tank 4 (with no Ca add) 20%, while the highest percentage mortality were recorded in tank2 and tank3 with concentrations (2.0 , 2.5)g/L and reached to 90% ,respectively , may be due to the needing of *L. auricularia* snails natural to Ca ion with low concentration ,and this is confirmed Olaseninde and Ouerindo (2007) with their findings the concentration 1.0g/L $CaCl_2$ was containing 0.36 g/L of Ca , which is identical to the amount of Ca concentration in water

bodies o.37 g/L .Therefore , the calcium in low concentration is necessary for optimum growth and survival of fresh water snails (Okwosa ,1982) this statement is agreed with Olaseninde and Ouerindo (2007) on the *B. pfeifferi* and their finding that the highest percentage survival in concentration 1.0g/L was 50% ,while the highest percentage mortality in concentration 2.5g/L was 75%.

In conclusion ,it has been found that calcium in low concentration (1.0g/L) is necessary for optimum growth and survival of *L. auricularia* . Based on the outcome of this study , calcium chloride can therefore be used as an additional source of the calcium in laboratory culture of *L. auricularia* for adequate shell formation , high rate of growth and survival , which are necessary for the completion of the life cycle of *F. gigantic* , a basis for the eradication of fascioliasis .

5-References

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تأثير كلوريد الكالسيوم على فقس ونمو وبقاء قواقع *Lymnaea auricularia* المضيف المتوسط لطفيلي *Fasciola gigantica*

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الخلاصة

درس تأثير ثلاثة تراكيز مختلفة (1.0 و 2.0 و2.5)غم/ل من كلوريد الكالسيوم $CaCl_2$ على فقس ونمو وبقاء قواقع *L. auricularia* المضيف المتوسط لطفيلي*F. gigantica* .

أظهرت الدراسة الحالية ان زيادة تركيز كلوريد الكالسيوم تخفض نسبة فقس البيض ، كما بينت ان لايون الكالسيوم تأثير على معدل نمو القواقع وقد سجل اعلى معدل طول اسبوعي للقواقع في الاسبوع 10 وبلغ (8.68 و 8.86 و 8.80) ملم على التوالي مقارنة بعينات السيطرة وان اعلى معدل نمو للقواقع 8.86 ملم سجل عند تركيز 2.0 غرام /لتر وادنى معدل نمو 6.22 ملم سجل في عينات السيطرة 0.0غرام/ لتر .كما لوحظ ان لتركيز ايون الكالسيوم تاثير على نسب بقاء قواقع *L. aurcularia* اذ بلغت 30 % عند تركيز 1غرام/لتر و10% في تركيز 2 و 2.5 غرام /لتر على التوالي مقارنة بالسيطرة 20% .