Effect of *Melia azadirchta* Fruit extract on the eggs, embryo and Juveniles of the fresh water snail *Physa acuta* (Draparnaud) , at different experimental temperatures.♦

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الخلاصة

تم اختبار تأثير المستخلص الكحولي لثمار نبات السبحبح *Melia azadirchta* في الأطـوار الجنينية غير البالغة لقوقع المياه العذبة *Physa acuta* عند درجات حرارة مختلفة . فقد ظهر تأثير معنوي في التطور الجنيني بين درجات حرارة منخفضة 2 ± 10 م° ودرجات حـرارة عالية تصل الى 2 ± 32 م° في مختلف الأطوار (المُعيدة ،) . فعند درجات حرارة 30 م° سجل تأثير معنوي للتطور خلال 3.90 أيام والتي قورنت مع 10 15 م° على التوالي سجلت 13.6 و15.6 يوم على التوالي .

اظهر المبيد النباتي ضد بيض قوقع تأثير عكسي عند زيـادة درجـات الحـرارة *Physa acuta* وعلى الأطوار اللاحقة التي تعرضت للمبيد ومقارنة ذلك مع اطوار الفقـس وقيـاس حجم القواقع الفتية.

Abstract

(Gastrula, Trochophore& hatching) as the incubation temperature increased and it was very The influence of the alcoholic extract of *Melia azadirchta* fruit of different concentrations at different experimental temperatures on the fecundity, embryonic development and hatching of *Physa. acuta* were investigated. There was significant difference between the embryonic development reared at relatively higher temperature (32°C ± 2) and those reared at low temperature(10 °C± 2),their was small acceleration in the rate of development at various developmental stagessignificant (3.90 days) for Trochophore stage at 30°C when it was compared with that at 10&15°C (13.6&15.6 days respectively.

While the molluscicide showed reverse effect as the temperature increase, its effect decrease to certain extend. The botanic molluscicide toxicity against eggs of *P.acuta* was more effective when newly laid eggs exposed to the molluscide , as well as the subsequent developmental stages affected as comparing the duration time of hatching stage & the size of juvenile snails, with those exposed to the molluscicide at Gastrula & Trochophore stages.

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Introduction

It was shown that the plant extracts produce immediate effect against the fecundity and embryonic survival of the snail [5]. An increase in molluscicide concentration at sub lethal levels resulted in a significant reduction in embryo growth rate and embryo hatchability [6]. The effect of crude extracts of neem, *Azadirchta indica* on tropical snails(*Archachatina marginata* and *Limicol aurora*), were determined , responses were measured ,cessation of food intake, cessation of crawling, mucus secretion and lack of response to mechanical stimuli(mortality),[7]. Natural molluscicide have played a significant role in restricting the population of the snail *Lymnaea acuminates* [8and9] , bioactive products of plant origin have become the focus of attention because they are less expensive and less hazardous to environment than their synthetic counterparts [10].

Although the thermal effect on the life cycle parameters of the medically important fresh water snail species Lymnaedae was taken into considerations by many workers [1;3;11;12 and 13] , but the conjugated effect of the combination between the toxin of the molluscicide and temperature on the subsequent life cycle parameters of the fresh water snails elucidated. The aim of this study is to verifying the combination effect of temperature The effect of temperature on the fecundity has been recognized by many workers, a considerable amount of work has been carried out on the effect of temperature on the development and hatching of the fresh water snail eggs . Al Habbib et al[1] found that there was an inverse relationship between the duration time for hatching and incubation temperature of *Lymnaea peregra* . Ali[2] observed that the rate of development of various stages of *Lymnaea auricularia* accelerated as result of temperature increases. Temperature variation , high amount of organic material in water ,high oxygen content in water , and absence of plant-life are factors which could limit the development of the intermediate snail hosts [4].

The molluscicidal activity potency of *Canna indicawas* studied against *Bilinus truncates,* prolonged exposure to sub lethal concentrations and the botanic moullscicide effect on the life cycle parameters of the fresh water snail *Physa acuta*.

Material and Methods

The egg masses used during the present study were obtained from snails collected during April(2005) ,in Mosul area, the differences between field temperatures (15-20°C), and laboratory temperatures(30-32°C) stimulate ovipositions in the snail[3] . The egg masses of *Physa acuta* were Kidney-shaped , the egg proper was found in a relatively large capsule embed in a jelly-like mass Fig(1).The number of egg capsule in each egg mass ranged from 14 - 42 , the egg mass were whitish in colour and transparent ,thus it was easy to study the living embryo in situ.

The rate of the development of eggs of *P.acuta* (untreated) was determined at low temperature (10°C±2), optimum temperature (15°C±1, resemble field temperature at time of collection) and high temperature (30°C±2, Room

temperature) using thermostaticall controlled water bath, 1 – 3 day old eggs were used, the egg mass were kept at 25/ml beaker filled with dechlornated tap water 2/3 of its volume and kept at the desired temperature. In all experiments the eggs were examined daily and the developmental stage was identified and their duration time recorded, the water was changed every other day, and the temperature checked daily. Finally at each temperature the number of the hatched eggs and hatching percentage were recorded. Plant extract were prepared using modified method recommended by Nirmeijer,et al[14] and Parashar,et al[15] ,alcoholic extracts of *Melia azadirchta* unripe fruit obtained by using, subletheal concentration ,were prepared from vacuum-dried methylonic extract of purified *Melia azadirchta* fruit and used in the test snails(stock solution , final extract concentration was 10%).Stock solution were diluted by distilled water to obtain the desired concentration.

Although water extracts are effective as pesticides , *Melia azadirchta* compounds are not highly soluble in water , the alcohol extracts are about 50 times more concentrated , they may contain 3000 parts per million(ppm) azadirachtin, the most active ingredient .Alcohol extraction is the most direct process for producing *Melia azadirchta* based pesticidal materials in concentrated form. Limonoids are highly soluble in alcohol solvents, the great kernels are usually soaked in ethanol , but some times in methanol. The yield active ingredients varies from 0.2 to 6.2 percent*.(we used 10% in this study),.

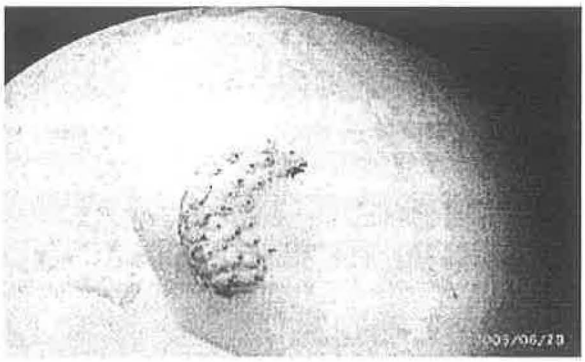
The eggs deposited in a firm gelatinous clutches on the plants, stones and the glass wall of the aquarium .Samples of *P.acuta* brought from field to laboratory at Summer season , egg laying continue and ceased within couple days till the end of the experiment (30 days)[3], stated that *L.auricularia* brought to laboratory during breeding season, many snails were soon engaged in couplatory activity and this was followed later by oviposition take place.

The criteria for the death were the inability for the snail to move and their detachment from the wall of the beaker; they either float on the surface of water or settle down on the bottom of the beaker.

Statistical analysis

Mean duration time and other parameters were analyzed by ANOVA-one way analysis of variance(unstacked) using statistical software Minitab (v11). For mean comparisons , to know the relationship between the molluscicide and the life cycle parameters in study.

*National Research Council ,1992. Report of National Academy press, P37, Washington, D.C.



Fig(1).*P.acuta* egg mass

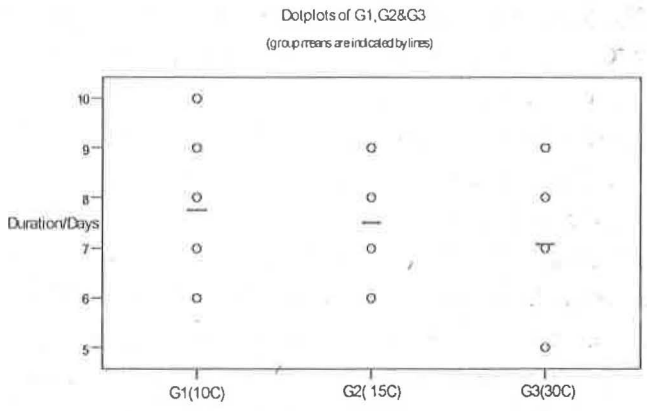
Results and Discussion

The mean duration of Gastrula, Trochophore & Hatching of *P.acuta* eggs at different temperatures(10,15, and 30°C,which simulate field & laboratory conditions at the period of study April-August) are shown in Table(1). It is clear from the result that their was small acceleration in the rate of development at various developmental stages (Gastrula, Trochophore and hatching) as the incubation temperature increased, and it was very significant (3.90 days) for Trochophore stage at 30°C when it was compared with that at 10 and 15°C (13.6 and 15.6 days respectively).This is obvious by the statistical analysis expressed (Figs.2,3 and 4) ,and the acceleration pronounced for hatching stage at 15 and 30°C(15.2 and 12 .3 days)when it compared with those at 10°C(22.2 days)although the elevation of temperature influence the rate of development but the size of the hatching juveniles and hatching percent could be effected negatively(Fig.5), and seem to that the suitable temperature for positive maturation was at 15°C, and this was the field temperature of the breeding season(April)during collection period. These duration time is shorter when it was compared with [3] results on of the co-occurrence fresh water snail *Lymnaea auricularia* , the oviposition was proportionally related to body size and the highest egg laying capacity was observed in large snails. [3].maturity is not always correlated with shell size , very small specimens of *physa acuta* occasionally produce capsules which are generally small , therefore temperature is a basic through indirect factor governing oviposition ,since it control the rate of development apart from this temperature can have a more direct influence , there being a maximum value above which oviposition does not take place. The mean duration at 10˚C for Gastrulation , trochophore and hatching (11.1; 20.5 and 54.9 days respectively), and at 32 ˚C (1.5; 3.5 and 8.5 days respectively), this has an ecological adaptation in which both species inhabit the same ecological nitch.Like all physiological process, animal development take place only within a definite temperature range [3].Incubation at low temperature had much more pronounced delay affect duration time of developmental stage (gastrula , trochophore and hatching)and the rate of development was accelerated as result of temperature increased and the pattern of reduction in the percentage of hatching with small sized juveniles with increasing the incubation temperature similar observation also made by Al Habbib et al, [1]and Schmid et al,[2] on Lymnadae . The rate of development of

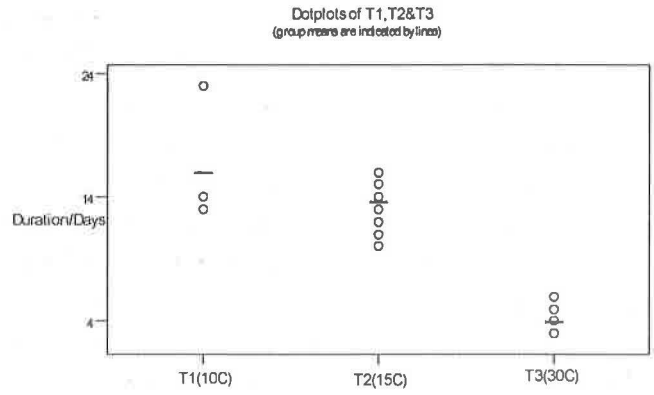
Lymnaea peregra eggs at various temperatures showed small but statistically significant acceleration reared from low to high temperatures and found to be that the snail acclimated to different temperatures express paradoxical adaptation Aziz and Raut [11] and Sukumaran et al,[16] results showed that the percentage of survival after 1h at 40°C is lowest at the larval trochophore stages and hatching embryo of the young, from the early cleavage stage onwards a high percentage of embryos can withstand high temperatures ,while the heat resistant depends on the stage of development , prehatch snail were less susceptible than the juvenile and adult.

Table (1). The duration time of various developmental stages of *Physa acuta* at different temperatures(S: Survival).

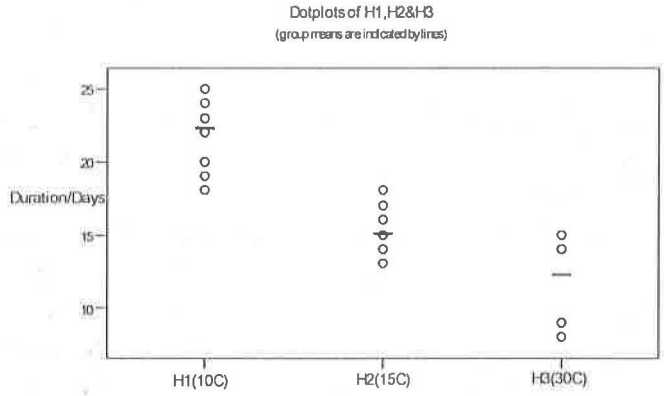
Temp. °C	Mean Duration(Days) For Gastrula	S %	Trochophore	S%	Hatching	% Hatch.	Sell size.mm
10 ± 2	7.750±1.125	95	15.60±3.851	88	22.214±2.455	80	0.69000 ±0.05701
15± 1	7.556±1.149	100	13.643±1.781	90	15.214±1.311	80	0.85050 ±0.0394
30 ± 2	6.833±1.424	90	3.929±0.997	80	12.33±2.82	60	0.84600 ±0.04135



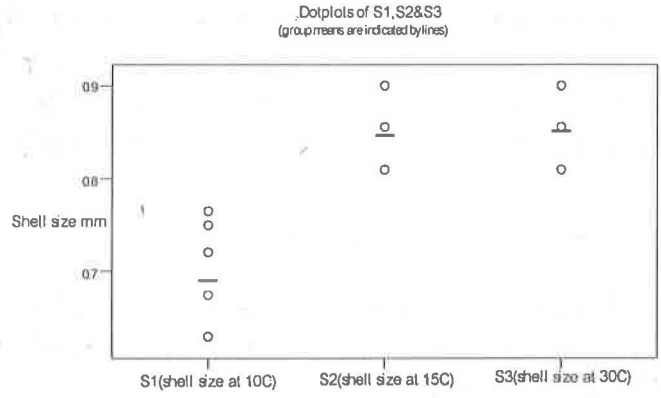
Fig(2). Mean duration of Gastrula stage at 10°C,15°C and 30°C (F:1.16)



Fig(3).Mean duration of Trochophore stage at 10,15 and 30°C(F:86.60)



Fig(4). Mean duration of Hatching stage at 10,15 and 30°C(F:70.73)



Fig(5) Mean shell size of Juveniles hatched at 10,15 and 30°C (F: 38.48)

Table (2), Shows that botanic molluscicide toxicity(alcoholic extracts of *Melia azadirachta*) against eggs of *P.acuta* was more effective when newly laid eggs exposed to the molluscide , as well as the subsequent developmental stages affected as comparing the duration time of hatching stage and the size of juvenile snails, when the fresh laid eggs exposed to the botanic molluscicide(MA) ,with those exposed to the molluscicide at Gastrula and Trochophore stages. Fig.(5) shows a small but almost statistically significant differences among the hatching duration time while Fig (6) shows a highly significant difference between the shell size of newly hatched juveniles of the above embryonic stages treated with the molluscicides . This may be due to the embryonic histological changes during cleavage process allow the molluscicide to affect the jelly-like material that protect the egg capsules in the freshly laid egg mass(Fig.1), although these results in contrary with Gillet and Bruax[17]and Sukumaran et al,[18] in that their results indicate that some plant extracts like *Euphorbia splendens* exhibit lower toxicity towards earlier developmental stages than the adult, but it is nevertheless in agreements with Gillet and Bruax [17] speculations that the jelly-like egg protactant covering the ova in both *Lymnaea matalensis* and *P.acuta* was easily penetrated by molluscicide, also Sukumaran et al ,[18] reported that extracts of some plant molluscide was toxic against freshly laid eggs of *lymnaea luteola* .

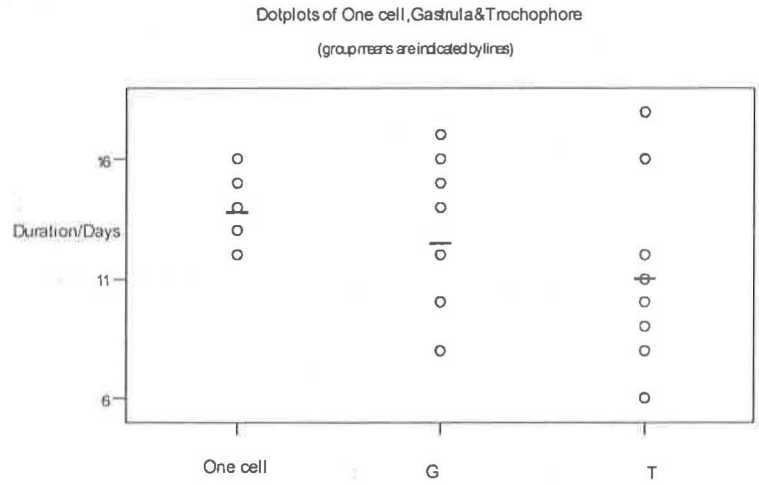
Singh and.Singh[7],results clearly indicate that the bark of *Nirium indicum* is an important source of botanical molluscicide and revealed that the toxic component of *Nirum indicum* bark is soluble in both water and ethanol and the extract may be used as a potent molluscicide since it was used to kill *Lymnaea acuminate* snail, Ebens [5] results showed that the crude extracts of different part of neem at different concentrations produce mortality after the exposure of tropical snails, as well as Cheung and Lam[4] results suggest that embryonic growth and hatchability are useful endpoints in chronic toxicity test.

The development and hatching of 1day o-1day old eggs could not be prevented but was prolonged by continuous exposure to molluscicide,prehached snail were less susceptible than the juvenile and adult snails . Farag and Khalil[3] stated that the prolonged exposure to sub lethal concentration of the plant extract produce immediate effect against fecundity and embryonic survival , the embryonic mortality increased to about 50% of the snail *Bulins trencatus*, while a compensatory response has been demonstrated in *Lymnaea auricularia*, though it was manifested within only few hours rather than over many hours [19].

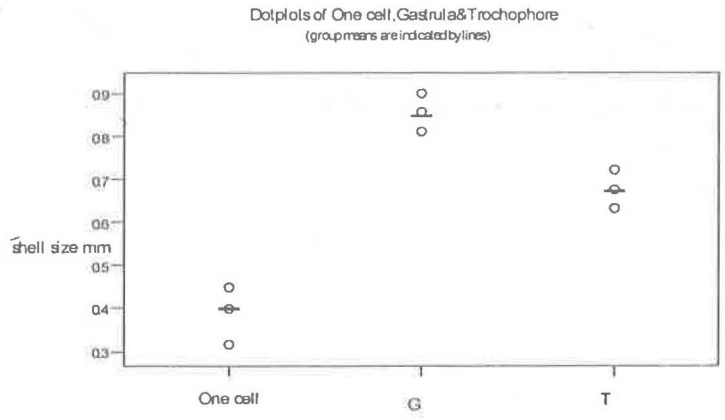
This contradictory results should be clarified in the forth coming studies , to find out whether the type of molluscides or different snail species play a role in the molluscicide susceptibility.

Table (2).The duration time of various developmental stages of *Physa acuta* treated with 2.5ppm *Melia azadirachta* at constant temperatures(15° C).

Mean Duration(Days) For				% Hatch	Shell size mm
Egg toGastrula	Gastrula to Trochophore	Trochophore to Hatching	N		
9.272 ±1.127	7.0 ± 1.032	13.750±1.389	20	40	0.39950±0.06162
———	6.625 ± 0.808	12.462±2.757	18	77.8	0.84600±0.03550
———	———	11.000±4.641	20	72.3	0.67050±0.03320

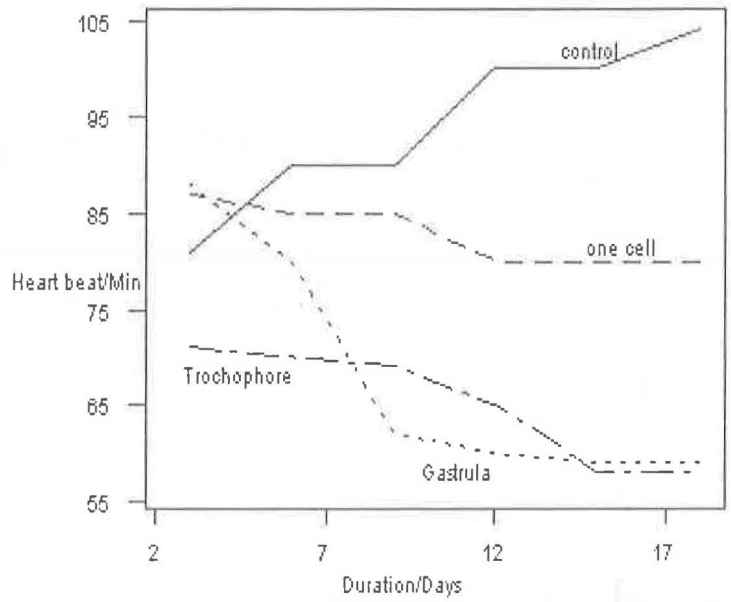


Fig(6) Mean duration to hatching of different developmental stages pre-treated with 2.5ppm(MA)/ (F: 1.67)



Fig(7) Mean shell size of Juveniles Hatched after different developmental stages have been exposed to 2.5ppm(MA) / F: 246.45.

There were a significant decline in heart beat , there was a vibration in the movement of embryos inside the developing egg with gradual drop in their activities , especially heart beat ,as the egg a gelatinous coating shrink due to the penetration of the extract solution, the peak of the heart beat rate start to decline within a couple days of the exposure for different stage of development have been treated day of treatment((88/ min.) , then decrease at the 21st day (58/min.), the most susceptible seems to be at gastrula & Trochophore stage ,while Juveniles hatched after havc been exposed to molluscicide at freshly laid eggs were the least susceptible . The mechanism by which the extracts cause retardation not exactly known and will be require further studies for elucidation.



Fig(8). Heart beat rate of Juveniles ,after have been treated with Molluscicide at different developmental stages

The results obtained demonstrate the effect f temperature and extracts of *Melia azadirachta* on development & hatching in *P.acuta* and provide information in the targets affected (control of organogenesis of the embryos). It is possible to predict the probability of survival of the species in an environmental polluted with molluscicides & to compare it with the effects of other pollutants in the same or other species.

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