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Effects of niclosamide and plant extract molluscicides on the different developmental stages of freshwater snails physa acuta

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ABSTRACT

The effect of synthetic molluscicide(Niclosamide) of different concentrations on the survival of fresh water snails, *Physa acuta* (Draparnaud), *Lymnaea auricularia*(L)- [pulmonats] and *Melanopsis praemorsa* (Linnaeus)-[Prosobranchates] in Mosul area was investigated, with emphases on the comparative effects of Niclosamide and botanical molluscicides (*Euphorbia helioscopia* [EE] and *Melia azedirachta* [MA] on fecundity and developmental rate of *Physa acuta*. There were variable lethal effects of different Niclosamide concentrations on the fresh water snails as well as on Mosquito fish(*Gambusia affinis*). The doses used in this study (5ppm – 50 ppm) had more effect on Pulmonate snails (*L.auricularia* and *P. acuta*), that were with out operculum than did on the Prosobranchia snail (*M. praemorsa*) that possess operculum. The Mosquito fish was affected by synthetic Molluscicide in a very short duration of time. The ovicidal action of Niclosamide as well as the botanical molluscicide (E.*helioscopia* and M.*azedirachta*) against the fecundity of *P. acuta* was found to have an negative effect on the eggs survival period, as the molluscicide concentration increased. *Physa acuta* snails treated with (2ppm) Niclosamide showed an increase in shell length during the first four days of the beginning of experiment reaching (1.0,mm). Snails died by the 6th day of the experiment. Snails treated with (5ppm) Niclosamide showed a slight increase in shell length (0.5mm) from the 2nd to the 5th day of experiment after which they all died. Individuals treated with(5ppm) E. *helioscopia* extract showed an increase in the shell length reaching (0.5mm) within a couple of days, then died after 7th day of the experiment, while those treated with same dose of *M. azedirachta*, reached 0.9 mm shell length by the 7th day, but the increase in shell length continued up to the 21st day of the experiment reached 6.74 mm, after which all died. No side effects of the botanical extracts were observed on concomitantly exposed Mosquito fish, however Niclosamide was lethal to the fish within 30 minutes at concentrations that were non-lethal to snails.

KEYWORDS

Molluscicide;
Physa;
Lymnea;
Melanopsis;
Gambusia.

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INTRODUCTION

Various species of fresh water snails acts as intermediate host of many trematode parasite worms which are causing many dreadful diseases in both human as well as in animals are wide spread in over all world, especially in developing countries[12, 28]. *P. acuta* are prevalent in many fresh water bodies in Iraq from North – Mosul[3, 21]; to South – Basra[17, 19]. Control of snails is regarded as one of the best preventive measure in controlling the parasite, where the use of molluscicides has given important results[28, 30].

The biology of fresh water snails and the influence of ecological factors were investigated by many workers[2, 3, 6, 11, 21]. Since the detection of the *P. acuta* population in Mosul area there has been noticeably decline with concomitant growth and expansion of *L.auricularia, M. praemorsa* and *Theodoxus jordani* populations indicating a process of competitive exclusion[3]. The use of Niclosamide and toxic extract of *E heliscopa* and *M. azedirachta* in the control of P.acuta populations could pose harm to native fresh water snails(*L.auricularia, M. praemorsa* and *Theodoxus jordani*) .

Fresh water bodies may be polluted by many insecticides that variously affect co-occurring species[23]. Data on the toxicity of certain molluscicide on natural vegetation necessary for the snails, as well as occurring species (mainly fish and frogs) has recently been considered[14, 24, 28, 29]. Even though reports are available on the toxicity of both Niclosamide[3, 7, 16, 22], and Euphorbia[27]. Detailed, comprehensive and comparative studies of both chemical and natural molluscicide and their effects are lacking and need to be elucidated . The aim of this study was to verify the comparative effects of Niclosamide, and extract of the local growing plants *E.heliscopa* and *M. azedirachta* on *P. acuta* fecundity and developmental rate.

MATERIALS AND METHODS

Fresh water snails were collected from different sites in Mosul area during May–August following procedures of[4]. Live specimens were kept in water aquaria (50x25cm)at room temperature (20-25C°), and fed natural food from habitat for 10 days before experiments started. Eggs laid on aquarium glass during acclimation periods were collected and examined to determine the stage of their development(Gastrula, early trochophore and late trochophore), and used in subsequent experiments. All experiments were carried out under controlled temperature (25 ± 2ºC), using thermostatically controlled water bath with cooling unit.

Chemical preparation

Bayluscide WP70 – powder was purchased from Bayer (Household Garden public Health – Liverkusen-Germany). The molluscicide concentration (Bayluscide) for stock solution was calculated on the basis of the recommended treatment rate 1ppm active ingredient for Bayluscide WP70 (1g a.i /m^3) this concentration was obtained by dissolving 2.22mg/L dechlorinated tap.

Plant extract were prepared using the method described by[20, 25, 26], Regarding E. *helioscopia* . In addition[5] method was used to prepare the alcoholic extracts of *M. azedirachta* fruit.

Effect of niclosamide (Bayluscide) on survival time

The toxicity of Bayluscide was carried out on 10 sexually matured snails and G. affinis individuals of approximately equal size. They were exposed to five different Bayluscide concentrations ranging from 5-50 ppm in addition to untreated control groups of 10 individuals of each type. All experiments were carried out at room temperature (20-25C°). The number of apparently dead was counted and recorded . The survival time were obtained by plotting Bayluscide concentration ppm against survival duration / hours and graphed using Minitab Microsoft program for Windows.

Effect of molluscicides on *P.acuta* eggs survival

Effect of different molluscicides at different concentrations(Niclosamide,2,4,8 ppm (eggs number 48,36 and 34 respectively) ., E. *helioscopia*, 5,10 ppm (eggs number 34and 25)., *M. azedirachta*,2.5,5 ppm (eggs number 30and 32) on *P. acuta* embryonic development stages and eggs survival were examined inside the transparent egg clutch and recorded.

Effect of molluscicides on *P.acuta* developmental rate

With the same molluscicides at the following concentrations(Niclosamide,2,5., E. *helioscopia*; *M.*

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azedirachta, 5ppm) the development rate of *P. acuta* juveniles was evaluated. Individual shell length was measured and growth rate of *P. acuta* was measured in terms of percentage length gain using modifying[9] formula, as given below:

$$\text{Percent length gain} = \frac{\text{Final shell length} - \text{Initial shell length}}{\text{Initial shell length}} \times 100$$

RESULTS AND DISCUSSION

Effect of bayluscide on survival time

Bayluscide (Niclosamide) is a product specially developed for control of fresh water snails that acts as intermediate hosts of casual organisms of bilharziasis(schistosomaiasis), fascioliasis and other diseases caused by trematodes . Bayluscide acts against both snails and their eggs at very low concentrations, killing them within a few hours . As shown in Figure (1), there are variable lethal effects of different Niclosamide concentrations on the survival of the fresh water snails as well as on the Mosquito fish.

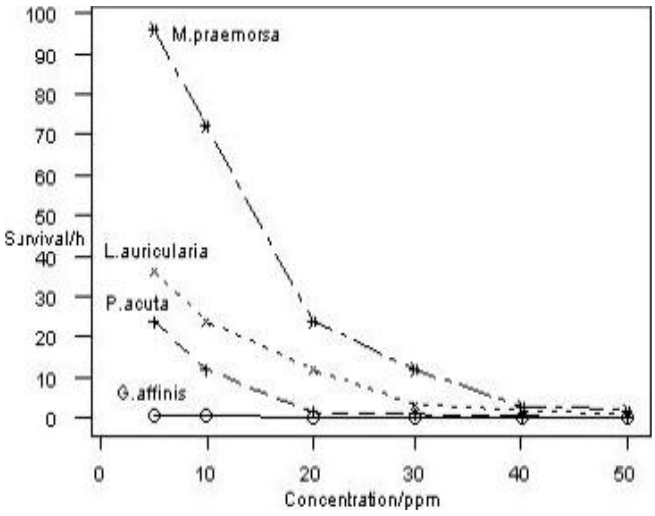

Figure 1 : Survival time of fresh water snails and *G.affinis* exposed to different Niclosamide concentrations

The doses applied in this experiment anticipated situations where dosage would in increased as treatment time is reduced e.g. for 6 hours : 1.5 ppm, for 4 hours : 2 ppm, for 2 hours : 4 ppm or for 1 hour : 8 ppm for Bayluscide WP70.

The doses used in this study (5ppm – 50 ppm) had more lethal effect in shorter duration of time on Pulmonate snails (*L. auricularia* and *P. acuta*), which were with out operculum than that of Prosobranchia snail (*M. praemorsa*) that possess operculum. Moreover *M. praemorsa* of size (13 -14 mm) recovered within 24-h after they were rinsed a few times with distilled water, the operculum may be the reason of the delay in the molluscicide effect. Results in Figure (1), also showed that the Mosquito Fish, was affected by Molluscicide in a very short duration of time(30 minutes) as compared with fresh water snails even at the lowest molluscicide concentration(2.0 ppm), Smaller fish individuals were more resistant to the toxin than larger individuals These observations showed a sharp decline in the operculum movement from 70 /min to 3 per min among snails 33 mm in length exposure to 50 ppm treatments, whereas the opercular rate declined from 80 to 60/ minute among smaller snails (23 mmin length)dropping in the operculum movement. This was with agreement of[1] observation that the development of *Biomphalaria glabrata* was arrested by Niclosamide (0.625-0.31 mg/l).

The concentration used in this study is higher than that mentioned by[15], in which Bayluscide was used with two different concentrations on adults and eggs as well as fish larvae and worms for flowing and stagnant water, but among his results the organism balance was restored after one month of using the molluscicide in the field.

Effect of different molluscicides on eggs of *Physa acuta*

Disintegration of embryos or absence of movement of the embryo, was considered for calculating the percent mortality of eggs. TABLE (1), illustrates the effect of molluscide on survival of *P. acuta* eggs. There was an increase in the relationship between treatment concentration and survival rate. The toxicity of Niclosamide and botanical molluscicides (*E.helioscopia and M.azedirachta*), to eggs of *P. acute* were found to reduce survival time in a dose-dependent manner . For Niclosamide the survival time declined from 24-h (2ppm) to 4-h (8ppm), while for *E.helioscopia*, it declined from 440-h (5ppm) to 36-h, (10 ppm),and for *M .azedirachta* it was dropped from 36- h at (2.5ppm) to 12- h at (5ppm) regardless of egg age. This may be due to the susceptibility of the eggs to different sub-

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stances and the ability of molluscicide penetrations. Ovicidal action of synthetic molluscicide (Niclosamide) and other botanic molluscicides(*E.helioscopia* and *M.azedirachta*) against *P. acuta*, showed that the compound to be to be more toxic to more mature eggs.

TABLE 1 : Effect of different molluscicide concentration on the eggs of *P. acuta*

Molluscicide	Conc.	Egg no.	Duration/ hour
Niclosamide	2.0 ppm	48	24
	4.0 ppm	36	8
	8.0 ppm	34	4
EE*	5.0 ppm	34	440
	10.0 ppm	28	36
MA°	2.5 ppm	30	36
	5.0 ppm	32	12

(E.heliscopia* and M.azedirachta°)

Most gastropods lay eggs covered by a gelatinous coating (Figure 2a). that is thought to protect the eggs from bacteria and fungi, although[28] and[16], reported that the toxicity of Niclosamide increased as L luteola eggs age in contrast to observations of[13]. They found that the gel coating was readily penetrated by the molluscicide. The deformation of the earlier stages (especially cell division and gastrula development) found in results of this study Figure (2b) supports this observation and those of[31] on the toxicity of bromaoacetamide to Oncomelania snail eggs[8]. also found that cadmium impaired development of early life stages of fresh water snails *P.acuta* (the 24-h and 48-h Lc50 for early embryo were 1.27mg/l and 0.85 mg/l) respectively, the 24 hand 48 h LC_{50} for juveniles were (1.32 mg/l and 1.05mg/l), respectively and with that of[27] in which the extracts of some plant molluscicide as toxic against freshly laid eggs of *L. luteola*.

Tawaij, *et al* l,(1988)[29] determined that high concentrations (50 to 100 mg/L) extracts of *Tribulus terrestris* were required in treating *Bulinus trunccatus*. Similarly *M.azedirachta* extract was of lesser toxicity than extracts tract *E.helioscopia* or Bayluscide used in this study.

As Shown in Figure (3), the treatment with *E.heliscopa* (EE) and *M. azedirchta* (MA) caused gradual decrease in the heart beat rate of *P. acuta* embryos in egg batches). The peak of the heart beat rate reached (88/Min) in the first week of treatment, then decreased to (58/Min.) at the 21st day in both botanic

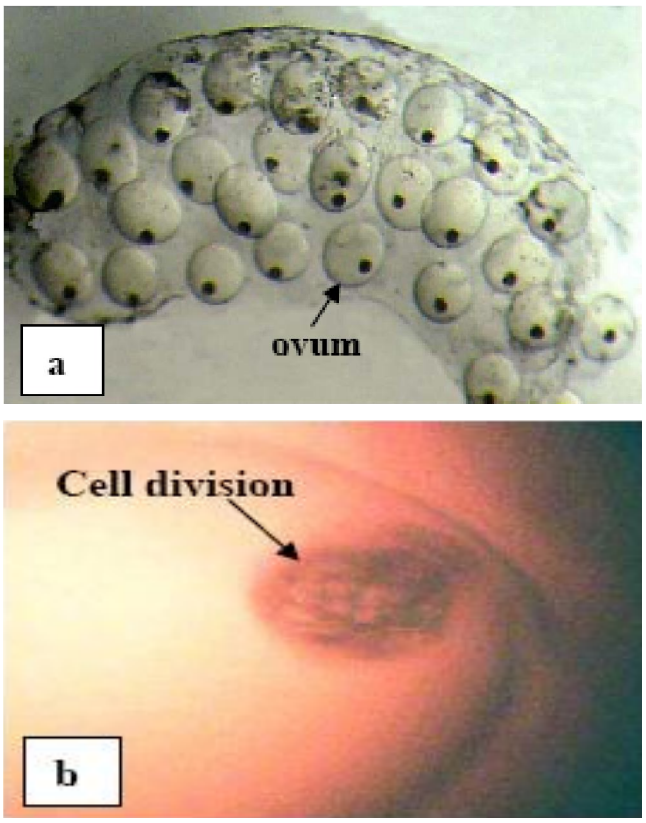

Figure 2 : (a)Egg batches (cell division -black dots). (b) embryonic development

molluscicides . This relation between the molluscicides and heart rate in contrast to controls whose average heart rate was70/min[10]. determined that *Bulinus africanus* eggs and neonates were considerably more sensitive than the adults.

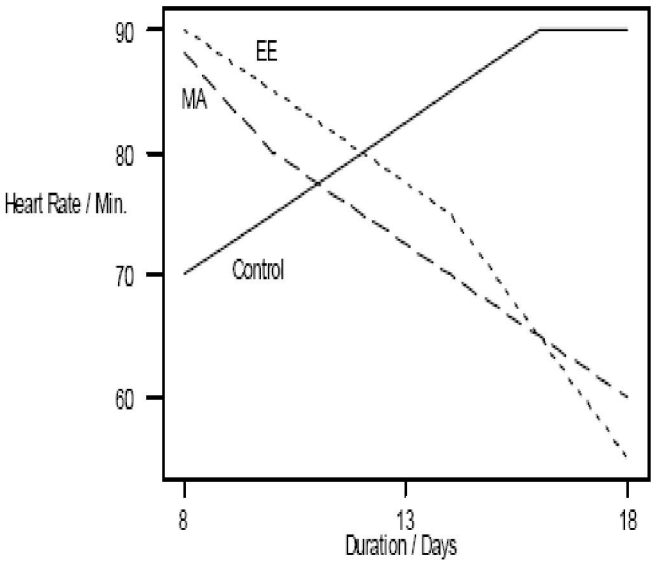

Figure 3 : *P.acuta* embryo heart Rate within the egg capsule treated with *E. heliscopa* (EE) and *M. azedirechta* (MA).

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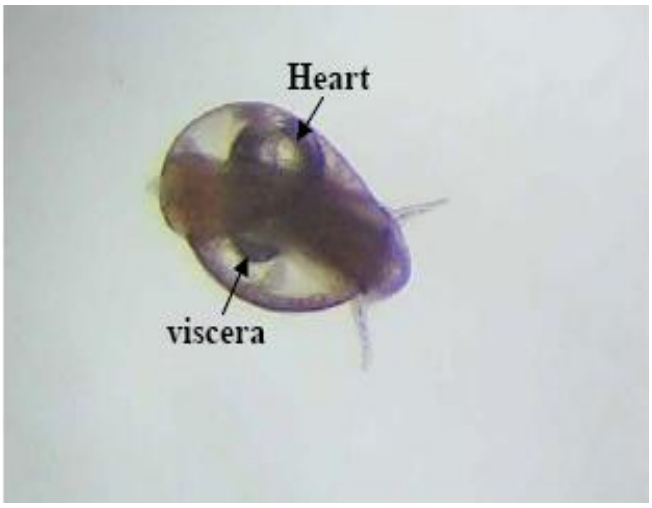

Figure 4 : Trochophore (transparent embryonic stage – internal viscera visible)

Effect of different molluscicides on the growth rate of *P.acuta*

TABLE (2) Shows that there are variation in the developmental rate of *P. acuta* treated with different concentrations of both synthetic molluscicide (Bayluscide), and botanical molluscicides (*E. heliscopia* and *M.azedirachta*). Individuals treated with.2ppm Niclosamide showed an increase in body length(shell length) during the first week reaching (1mm), they ceased growing by the second week and died when Individuals treated with.5ppm of Niclosamide, *E. heliscopia* and *M.azedirachta*, shows relatively similar increase of shell length reached 0.5mm, but the subsequent survivals found to be dose dependent (dropped from 21 day for 5ppm *M .azedirachta* to 6 days at 5ppm Niclosamide), the inhibitory effect on subsequent growth was less pronounced as compared with that of early stages(eggs in this study) and immature adult (newly hatched juveniles[4] regardless of the molluscicide. The same observation reported by[13] on *L .matalensis* and also[27] reported that the extract of the same plant was toxic against freshly laid eggs of *L. luteola.*

In conclusion the plant extracts as well as the synthetic molluscicides have inhibitory effects on the survival and developmental rate of *P. acuta* . The statistical analysis(using Minitab v11for Window), [ANOVA-one way analysis of variance], showed there were highly significant differences between the

TABLE 2 : Development rate of *P .acuta* treated with different Molluscicides (Niclosamide, *E. heliscopia and *M .azedirachta*°) on newly hatched juveniles.**

parameters	Treatment			
	Niclosamide/ 2 ppm	Niclosamide /5ppm	EE* / 5 ppm	MA° / 5 ppm
Initial shell length /mm	4.8 ± 0.175	5.37 ± 0.1039	3.29 ± 0.166	5.81 ± 0.137
Final shell length / mm	5.77 ± 0.221	5.85 ± 0.126	3.81 ± 0.162	6.74 ± 0.134
Shell length Gain %	19.7*	8.9	15.8	16*

***significant difference $p<0.05$ from Niclosamide /5ppm and MA° /5 ppm**

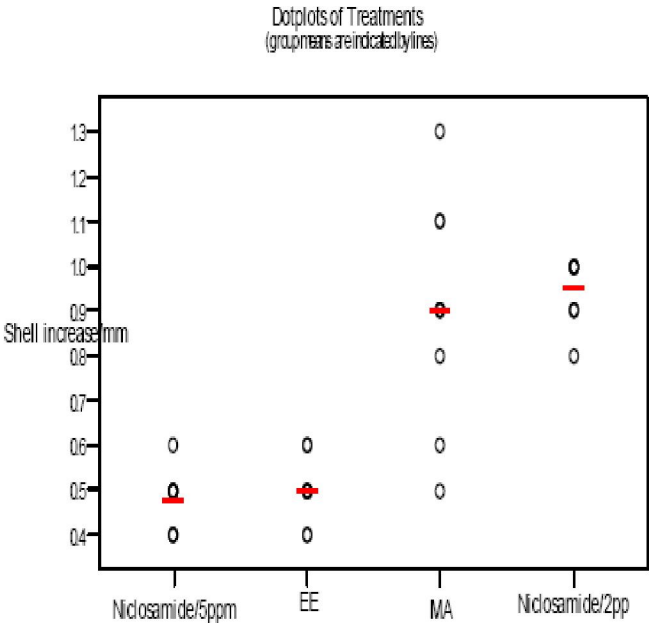

Figure 5 : ANOVA-One way Analysis of variance(unstacked).

four different concentration (F-36.85), individual 95% CIs for mean(Figure 5), indicate that both *M .azedirachta* and Bayluscide (2ppm) were least toxic while both Bayluscide /5ppm and *E. heliscopia* / 5 ppm were most inhibitory Bayluscide was also lethal to fish in any given concentrations. While, the botanic molluscicide is safer this may encourage. The use of plant extracts as molluscicides may be encouraged because they are less toxic to fish and other species potentially exposed during treatment. Bioactive products of plant origin are also less expensive and less hazardous to the environment than the synthetic counterparts.

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