

## Fecundity, hatchability and survival of *Indoplanorbis exustus* fed to bait containing attractant and molluscicides

Pradeep Kumar<sup>1</sup>, Kumari Sunita<sup>2</sup>, V.K. Singh<sup>2</sup>, D. K. Singh<sup>2</sup>

<sup>1</sup>Department of Zoology, S.G.N Govt. P.G. College Muhammadabad Gohana Mau, U.P

<sup>2</sup>Malacology Laboratory, Department of Zoology,

DDU Gorakhpur University, Gorakhpur, 273009, U.P. India

E-mail: [dk Singh\\_gpu@yahoo.co.in](mailto:dk Singh_gpu@yahoo.co.in); [pkumar\\_gpu@yahoo.co.in](mailto:pkumar_gpu@yahoo.co.in)

**Abstract:** The effect of sublethal (20% and 60% of 24h LC<sub>50</sub>) feeding of active molluscicide ferulic acid, umbelliferone, eugenol and limonene in bait containing attractant amino acids (tyrosine, lysine) on the, fecundity, hatchability and survival of snail *Indoplanorbis exustus* was studied. Bait containing attractant amino acid and molluscicides significantly reduced the reproductive capacity of the snail *Indoplanorbis exustus*. Maximum reduction in fecundity (55.41 % of control) was observed in snail fed to tyrosine + eugenol. In withdrawal group significant recovery was noted in all bait fed snails (90.48 % of control). The hatching period of egg laid by treated group was prolonged from 11 to 18 days with respect to 10 to 12 days in control snails.

[Pradeep Kumar, Kumari Sunita, V.K. Singh, D. K. Singh. **Fecundity, hatchability and survival of *Indoplanorbis exustus* fed to bait containing attractant and molluscicides.** *N Y Sci J* 2014;7(3):1-5]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 1

**Keywords:** Tyrosine; Lysine; Molluscicides; Bait formulation; Reproduction; *Indoplanorbis exustus*

### 1. Introduction

Fasciolosis is an important disease caused by two trematode species *Fasciola hepatica* and *F. gigantica* (Mas-Coma et al., 2005; Singh and Agarwal, 1981). *Fasciola* is digenetic trematode, having two hosts, a final mammalian hosts and a molluscan intermediate hosts (Mas-Coma et al., 2005). *Indoplanorbis exustus* is acknowledged intermediate host of *Fasciola* (Agarwal & Singh, 1988; Kumar et al., 2011a). At the optimum temperature of 30°C each snail can lay up to 800 eggs (Raut et al., 1992). The best method to control trematodes infection is to control the population of vector snail (Singh et al., 1996; Kumar et al., 2009; Kumar and Singh 2006). Several attempts have been made to reduce the incidence of fasciolosis by using synthetic/plant derived molluscicides against snails (Singh et al., 1996; Kumar and Singh, 2006; Jaiswal and Singh, 2009; Upadhyay and Singh, 2011; Singh et al., 2012; Kumar et al., 2012; Kumar et al., 2013). Direct releases of molluscicides in aquatic environment have played a very significant role in controlling the snail population (Kumar and Singh, 2006; Kumar et al., 2009). The use of bait containing snail attractant and toxicant is a good technique for the species specific management of snails. This method has toxicological and ecological advantage over the use of conventional pesticide (Haniotakis et al., 1991; Abd-El Hamid, 1998; Tiwari and Singh, 2007; Kumar et al., 2011b; Kumar et al., 2012). Earlier, it has been noted by us that ferulic acid, umbelliferone, eugenol and limonene are active molluscicide, when released directly in the water. Attractant amino acid tyrosine

and lysine are very effective against harmful snail *I. exustus* (Kumar et al., 2011a). In the present study effect of feeding bait formulation containing sublethal (20% and 60 % of 24h LC<sub>50</sub>) doses of molluscicides on the fecundity, hatchability and survival of the snail *I. exustus* were studied.

### 2. Materials and Methods

#### 2.1. Experimental Animal

The adult snails *Indoplanorbis exustus* (0.85 ± 0.37 cm in length) were collected from local ponds, lakes and low lying submerged fields and were used as test animals. The snails were acclimatized for 72h in laboratory condition. The pH of the water was 7.1-7.3 and dissolved O<sub>2</sub>, free CO<sub>2</sub> and bicarbonate alkalinity were 6.5-7.2 mg/l, 5.2-6.3 mg/l and 102.0-105.0 mg/l, respectively. Twenty experimental snails were kept in glass aquaria containing 3 L of dechlorinated tap water at 24 to 26°C. *I. exustus* laid their eggs on the lower surface of leaves of the aquatic plants in the form of elongated gelatinous capsules containing 10-600 eggs.

#### 2.2. Pure chemical compounds

Agar-agar, amino acid (tyrosine and lysine) and active molluscicide ferulic acid, umbelliferone, eugenol and limonene were used in the bait formulations. The pure active components ferulic acid (4-Hydroxy-3-methoxycinnamic), umbelliferone (7-Hydroxy coumarin; 7-hydroxy-2H-1-benzopyran-2-one), eugenol (2-Methoxy-4-(2-propenyl) phenol) and limonene (R)-4-Isopropenyl-1-methyl-1-cyclohexene) were purchased from Sigma Chemical Co. (USA).

### 2.3. Formulations of bait with molluscicides

Formulations of bait containing amino acid (tyrosine and lysine 10mM) and sub-lethal (20% and 60% of 24h and 96h LC<sub>50</sub>) molluscicides ferulic acid, umbelliferone, eugenol and limonene were prepared in 100 ml of 2% agar solution by the method of Madsen (1992) and modified by the Kumar et al., (2012). Concentrations of amino acids/molluscicides were based on the earlier reports of Tiwari and Singh (2004a,b) and Kumar et al., (2012), respectively. These solutions were spread at a uniform thickness of 5mm. After cooling the bait containing sublethal molluscicides were cut out a corer measuring 5 mm in diameter. Six replicates were prepared for each concentration. Control group of snails were fed with bait without molluscicide.

### 2.4. Experimental procedure

The experimental procedure was performed by the method by Tiwari and Singh (2004a,b). The experimental equipments consist of a clean glass aquarium having a diameter of 30 cm. Each aquarium was divided into four concentric zones; Zone 3 (Central zone), 2, 1 (Middle zone) and zone 0 (Outer zone) had diameters of 13, 18, 24 and 30 cm, respectively. A small annular elevation of 9 mm height and 2.4 cm diameter was made in the centre of aquarium (Zone 3). Zone 0 had an area of 254 cm<sup>2</sup> on the periphery of aquarium. The aquaria were then filled with 3L of dechlorinated tap water to a height of 8 mm and maintained at 26±1<sup>o</sup> C. At the start of the experiment twenty snails of uniform size were placed on the circumference of zone 0. Simultaneously, one of the prepared bait of different active component (molluscicides) was added on the small annular elevation in the center (Zone 3). Six sets of experiments have been designed with twenty snails in each replicate.

### 2.5. Treatments

Snails were fed to bait containing sublethal (20% and 60% of 24h LC<sub>50</sub>) concentration of ferulic acid/umbelliferone/eugenol/limonene and amino acid tyrosine/lysine as attractant and their effect on the reproduction was studied by the method of Kumar et al., (2012). Groups of 20 snails in 3L water were fed to sublethal concentrations (20% and 60% of 24h LC<sub>50</sub>) of different combinations of molluscicides with amino acids.

### 2.6. Fecundity, Hatchability and Survivability of Snail

These experiments were performed according to the method of Kumar et al., (2013). The total number of eggs laid by bait fed and control snails were counted after every 24h for 96h. Since it is difficult to detect the mother snails for particular spawn, capsules containing eggs from each feeded group were incubated at 30°C in covered petridishes. The development of embryos at regular intervals was observed under binocular microscope until hatching. Per cent hatching was studied only with eggs laid after the 24h feeding period. Dead embryo was removed to avoid any contamination. Survival of young snails was observed up to 72h. Snails were transferred to fresh water after a 96h feeding period to observe the effect of the above bait after withdrawal.

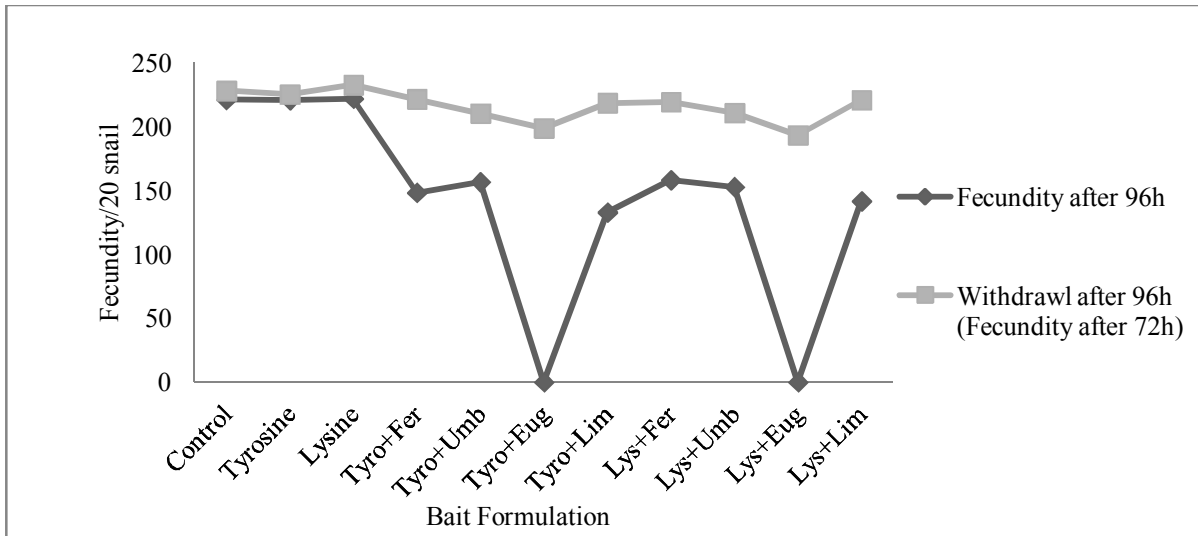
### 2.7. Statistical analysis

Each experiment was replicated at least 6 times. Values were expressed as Mean±SE. Students' t' test was applied to determine the significant (P<0.05) difference between bait feeded and control group of the animals. Product moment correlation coefficient was applied in between exposure time and different values of fecundity/ survival of hatched snails (Sokal and Rohlf, 1973).

### 3. Results

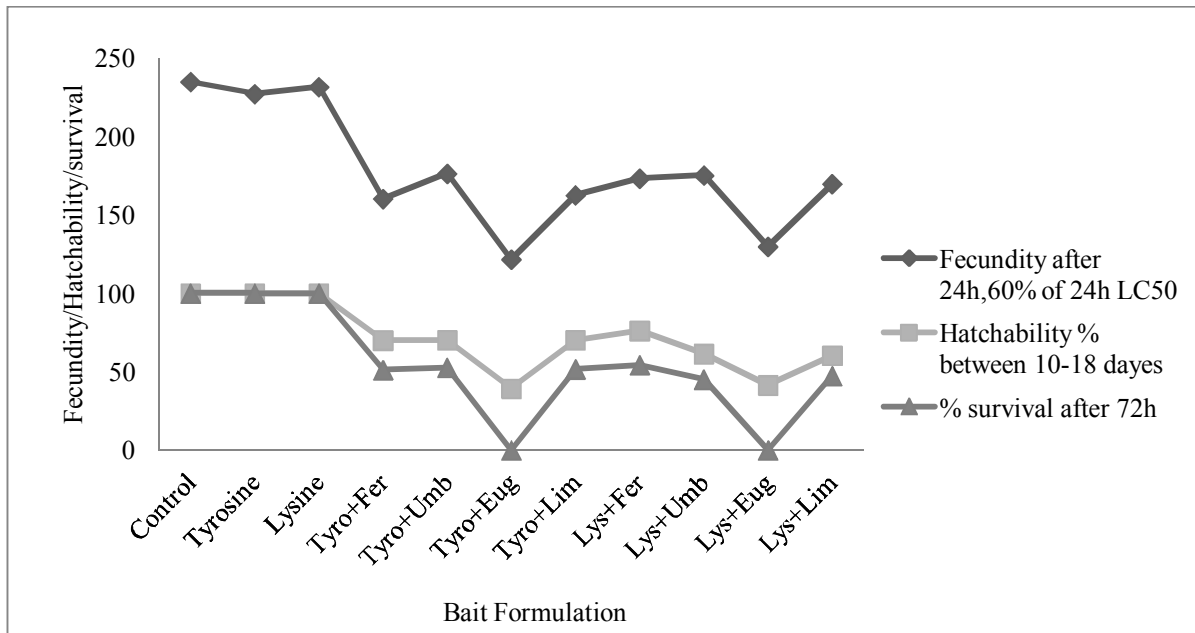
In control groups of 20 snails laid 230-235 eggs/day. There was a significant (p<0.05) reduction in the fecundity of snail *I. exustus* feeding to 20 and 60 % of LC<sub>50</sub>/24h of ferulic acid, umbelliferone, eugenol and limonene with amino acids (tyrosine/lysine) as a snail attractants in bait formulations (Figure-1). No egg lying after 96h feeding, was observed in snails feed to 60% of 24h LC<sub>50</sub> of bait formulations of tyrosine + eugenol or lysine + eugenol. The hatching period was prolonged in treated group (11-18 days) with respect to control group (10-12 days) (Figure-2). Withdrawal of snail after 96h feeding of baits for next 72h in fresh water caused a significant (P<0.05) recovery in the fecundity of snails with respect to their corresponding treatment (Figure-1).

Non survival after 72h was noted in young snails fed with 20 % or 60 % of 24h LC<sub>50</sub> of tyrosine + eugenol or lysine + eugenol (Figure-2). There was a significant (P<0.05) negative correlation between the feeding time and survival of young snails hatched from eggs laid by snail feeded to 20%, 60% of 24h LC<sub>50</sub> of different formulations of bait (Figure-2).



**Fig 1.** Effect of sublethal feeding (60% of 24h LC<sub>50</sub>) of molluscicides ferulic acid/umbelliferone/eugenol/limonene and amino acid (Tyrosine/Lysine) as attractant in bait formulation on the fecundity after 96h of the snail *Indoplanorbis exustus*.

Each value is mean ± SE of six replicates. Each replicates represents the egg laid by the group of 20 snails. Student “t” test was applied to treated and control groups. Product moment correlation coefficient showed that there was significant (P<0.05) negative correlation in between the exposure period and fecundity of snail *Indoplanorbis exustus*. Abbreviation- Tyro=Tyrosine, Lys=Lysine, Fer=ferulic acid, Umb=umbelliferone, Eug=eugenol, Lim=limonene



**Fig 2.** Effect of sublethal feeding (60% of 24h LC<sub>50</sub>) of molluscicides ferulic acid/umbelliferone/eugenol/limonene and amino acid (Tyrosine/Lysine) as attractant in bait formulation on the fecundity, % hatchability and % survival after 72h of the snail *Indoplanorbis exustus*.

Each value is mean ± SE of six replicates. Each replicates represent the egg laid by the group of 20 snails. Significant (P<0.05) when student “t” test was applied to treated and control groups. Product moment correlation coefficient showed that there was significant (P<0.05) negative correlation in between survival period and survival of the snail *Indoplanorbis exustus*.

#### 4. Discussion

Result of the present study clearly indicates that sublethal feeding of bait containing molluscicide (20% and 60% of 24h LC<sub>50</sub> and attractant) and attractant tyrosine/lysine significantly reduced the reproductive capacity of snail *I. exustus*. Bait formulations with 20% or 60% of 24h LC<sub>50</sub> of tyrosine + eugenol / lysine + eugenol significantly ( $P < 0.05$ ) reduced the fecundity of *I. exustus* within 72h.

However, the plant derived active molluscicidal component ferulic acid, umbelliferone, eugenol, and limonene have dose dependent influence on the fecundity of snails (Kumar et al., 2009), when directly given in aquarium water against snail *Lymnaea acuminata*. A number of plant products have been effectively used for control of snail reproduction (Marston and Hostettmann, 1985; Singh et al., 2004; Bacchetta et al., 2002; Mello-Silva et al., 2007). Although, the 0.01 % concentration of eugenol caused a significant decrease in the fecundity of potato tuber moth (*Phthorimaea aperculla*) and decreased the percentage of egg hatchability (Sharaby et al., 2009). Kumar et al., (2012) have reported the bait containing 60 % of 96h LC<sub>50</sub> of eugenol with starch + histidine, starch + methionine; respectively inhibit alkaline phosphatase (ALP) (20 % of control) and acetylcholinesterase (AChE) (49.49 % of control) activity in the nervous tissue of *L. acuminata*. In the present study the mode of entry of molluscicides into the snail *Indoplanorbis exustus* body through the digestive track, which may affect the caudodorsal cells (CDSs) in brain and ultimately decrease the release of the ovulation hormone that resulted a decrease in the fecundity of bait fed snails. Roubos et al., (1981) has reported that the caudodorsal cell is responsible for the fecundity of snail *Lymnaea*. Kumar et al., (2011c) reported that there was a depletion of amino acid/protein and nucleic acid levels in the ovotestis of *L. acuminata*. Alkaline phosphatase play a critical role in protein synthesis (Pilo et al., 1972), shell formation (Timmermans, 1969) and other secretory activity (Ibrahim et al., 1974) and its inhibition may result in the reduction of protein level in gastropods (Singh and Singh, 1995).

Reduction in percent hatchability of eggs laid by *I. exustus* fed to the different baits may be due to interference of molluscicides with the embryonic growth and development of the snails. In bait fed snails, young larvae were weak, unable to break the egg capsule, and died owing to starvation. Young snails hatched from the treated egg masses showed delay in attaining maturity in comparison with the control groups. Most of these eggs were attached to the wall of the aquarium and were apathetic toward feeding. In general, the egg shells were thinner, and the hatchlings had shorter tentacles and slower

movement and were smaller in size as compared with control group. Mortality and low reproduction in the bait fed snails suggest the active molluscicidal components in bait formulation was able to reduced the snail population by inhibiting development at any stage of growth.

Transfer of mother snails to fresh water for the next 72h after 96h bait feeding all the feeded snails leads to a significant recovery in the fecundity. Thus, reversibility of the effects would be an added advantage in their use against aquatic target snails as they would cause only short-lived effects. This concept is new approach to use the bait formulations with sublethal molluscicides and snail attractant. It will be helpful in control of snail population without using direct more active molluscicide in the natural aquatic habitats.

#### Address for correspondence

\* Prof. D.K. Singh

Malacology Laboratory Department of Zoology, DDU Gorakhpur University, Gorakhpur-273009, U.P., India  
E-mail: [dk Singh\\_gpu@yahoo.co.in](mailto:dk Singh_gpu@yahoo.co.in);  
[pkumar\\_gpu@yahoo.co.in](mailto:pkumar_gpu@yahoo.co.in)  
Phone: +91-551-2202187 (Office); +91-9454211574 (Mobile)

#### References

1. Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-borne trematode zoonoses, *Int Parasitol* 2005; 35: 1255-1278.
2. Singh O, Agarwal RA. Toxicity of certain pesticides to two economic species of snails in northern India, *Journal of economic Entomology* 1981; 74: 568-571.
3. Agarwal RA, Singh DK. Harmful gastropods and their control, *Act Hydrochim Hydrobiol* 1988; 16: 113-138.
4. Kumar P, Singh VK, Singh DK. Bait formulation of molluscicides with attractant amino acid against the snail *Indoplanorbis exustus*, *Pharmacologyonline* 2011a; 3: 30-36.
5. Raut SK, Rahman MS, Samanta SK. Influence of temperature on survival, growth and fecundity of the freshwater snail *Indoplanorbis exustus* (Deshayes), *Memorias do Instituto Oswaldo Cruz* 1992; 87(1): 15-19.
6. Singh A, Singh DK, Mishra TN, Agarwal RA. Molluscicide of Plant origin, *Bio Agric and Horti* 1996; 13: 205-252.
7. Kumar P, Singh VK, Singh DK. Kinetics of enzyme inhibition by active molluscicidal against ferulic acid, umbelliferone, eugenol and limonene in the nervous tissue of snail *Lymnaea acuminata*, *Phytotherapy Research* 2009; 23: 172-177.
8. Kumar P, Singh DK. Molluscicidal activity of *Ferula asafoetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against

- the snail *Lymnaea acuminata*, Chemosphere 2006; 63: 1568-1574.
9. Jaiswal P, Singh DK. Molluscicidal activity of Nutmeg and Mace (*Myristica Fragrans* Hoult.) Against the vector snail *Lymnaea acuminata*, Journal of Herbs Spices & Medicinal plants 2009; 15: 117-186.
  10. Upadhyay A, Singh DK. Molluscicidal activity of *Sapindus mukorossi* and *Terminalia chebula* against the freshwater snail *Lymnaea acuminata*, Chemosphere 2011; 83: 468-474.
  11. Singh KL, Singh DK, Singh VK. Characterization of the molluscicidal activity of *Bauhinia variegata* and *Mimusops elengi* plant extracts against the *Fasciola* vector *Lymnaea acuminata*, Rev Inst Med Trop Sao Paulo 2012; 54(3): 135-140.
  12. Kumar P, Singh VK, Singh DK. Enzyme activity in the nervous tissue of *Lymnaea acuminata* fed to different bait formulations, American Journal of Chemistry 2012; 2 (2): 89-93.
  13. Kumar P, Singh VK, Singh DK. Reproduction of *Lymnaea acuminata* fed to bait containing binary combination of amino acid with molluscicide, J Biology and Earth Sciences 2013; 3(1): B65-B71.
  14. Haniotakis G, Kozyrakakis M, Fitsakis T. An effective mass trapping method for the control of *Dacus oleae* (Diptera: Tephritidae), J Econ Entomol 1991; 84 (2): 564-569.
  15. Abd-El Hamid AZ, Development of bait formulation for control of intermediate host of African Schistosome species, J Appl Toxicol 1998; 17(6): 391-395.
  16. Tiwari F, Singh DK. Toxicity of plant derived molluscicides in attractant food pellets against snail *Lymnaea acuminata*, Iranian J Pharmacology and Therapeutics 2007; 6: 103-107.
  17. Kumar P, Singh VK, Singh DK. Combination of molluscicides with attractant carbohydrates and amino acids in bait formulation against the snail *Lymnaea acuminata*, European Review for Medical and Pharmacological Sciences 2011b; 15: 550-555.
  18. Madsen H. A comparative study on the food-locating ability of *Helisoma duryi*, *Biomphalaria camerunensis* and *Bulinus truncates* (Pulmonata: planorbidae), J Appl Ecol 1992; 29: 70-78.
  19. Tiwari F, Singh DK. Attraction of amino acids by *Lymnaea acuminata*, snail host of *Fasciola* species, Brazilian Journal of Medical & Biological Research 2004a; 37: 587-590.
  20. Tiwari F, Singh DK. Behavioural responses of the snail *Lymnaea acuminata* to carbohydrates in snail attractant pellets, Naturwissenschaften 2004b; 91: 378-380.
  21. Sokal RR, Rohlf FJ. Introduction of biostatistics, W. H. Freeman, San Francisco, 1973 ; 185-207.
  22. Marston A, Hostettmann K. Plant molluscicides, Phytochemistry 1985; 24: 639-652.
  23. Singh S, Singh VK, Singh DK. Effect of spices ginger (*Zingiber officinale*) and ajowan (*Trachyspermum ammi*) on the reproduction of the vector snail *Lymnaea acuminata*, Biol Mem 2004; 30 (1): 14-19.
  24. Bacchetta R, Mantecca P, Vailati G. Oocyte degeneration and altered ovipository activity induced by paraquat in the fresh water snail *Physa fontinalis* (Gastropoda: Pulmonata), J Moll Stud 2002; 68: 181-186.
  25. Mello-Silva CC, Vilar MM, Bezerra JCB, de Vasconcellos MC, Pinheiro J, Lerdes, Rodrigues M. Reproductive activity alterations on the *Biomphalaria glabrata* exposed to *Euhporbia splendens* var. hislopi latex, Mem Inst Oswaldo Cruz 2007; 102 (6): 671-674.
  26. Sharaby A, Abdel-Rahman H, Moawad S. Biological effects of some natural and chemical compounds on the potato tuber moth, *Phthorimaea operculella* Zell. (Lepidoptera:Gelechiidae), Saudi Journal of Biological Sciences 2009; 16: 1-9.
  27. Roubos EW, Boer HH, Schot LPC. Pesticidogenic neurons and the control of neuroendocrine activity in the freshwater snail *Lymnaea stagnalis* L, Proc Int Sym Neurosecret 1981; 8: 119-127.
  28. Kumar P, Singh VK, Singh DK. Bait formulations of molluscicides and their effects on biochemical changes in the ovotestis of snail *Lymnaea acuminata* (Mol-lusca; Gastropoda:Lymneidae), Rev Inst Med Trop Sao Paulo 2011c; 53 (5): 271-275.
  29. Pilo B, Asnani MV, Shah RV. Studies on wound healing and repair in pigeon liver III. Histochemical studies on the acid and alkaline phosphatases during the processes, J Animal Morphology and Physiology 1972; 19(2): 205-212.
  30. Timmermans LPM. Studies on shell formation in mollusks, Netherlands Journal of Zoology 1969; 19: 17-36.
  31. Ibrahim AM, Higazi MG, Demian ES. Histochemical localization of alkaline phosphatase activity in the alimentary tract of the snail *Marisa coruarielis*, Bulletin of the Zoological Society of Egypt 1974; 26: 94-105.
  32. Singh K, Singh DK. Effects of Neem (*Azadirachta Indica*) on fecundity, hatchability and survivality of snail (*Lymnaea acuminata*), Malays Appl Biol 1995; 24(1): 19-22.