Toxicity of Ricin and Alkaloid Atropine containing bait formulation against the freshwater snail Lymnaea acuminata

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Abstract: Fascioliasis is the plant-borne trematode zoonoses caused by *Fasciola hepatica* and *Fasiciola gigantica* . One of the possible approaches to control this problem is to interrupt the life cycle of the parasitic trematode by eliminating the intermediate host snail. Baits were prepared from binary combinations of carbohydrate + amino acid (10mM) in 2% agar solution with active molluscicidal component Ricin (*Ricinus communis*), Alkaloid atropine (*Datura stramonium*) and their binary combination in 1:1ratio. The behavioural responses of snails to these binary combinations of carbohydrate and amino acid in bait formulations were examined. Attraction of sails was studied by using clear glass aquaria having diameter of 30 cm. Each aquarium was divided into four concentric Zones; Zone-3 (central Zone), Zone-2 and Zone-1 (middle Zone) and Zone-0 (outer Zone) had a diameter of 13, 18, 24 and 30 cm, respectively. Among all the binary combinations highest attraction of snail (39.23%) was noted towards the bait containing starch + serine + atropine. Starch + serine + binary combination of ricin and atropine bait formulation was most potent molluscicide ($96LC_{50\%}$ 0.10%) against *Lymnaea acuminata*. The present study suggested that the molluscicides of plant origin could be used with varying degrees of success in bait formulation.

Key Words: Carbohydrates, Amino acids, Bait formulation, Molluscicides , *Lymnaea acuminata*

INTRODUCTION: Fascioliasis in a common parasitic disease of live-stock in eastern Uttar Pradesh, (Kumar and Singh, 2006), which is caused by the digenetic trematode of *Fasciola hepatica* and *Fasiciola gigantica*. Snails and mammals are the intermediate and final host of Fasciola. In Live stock, it causes severe reduction in milk and meat yield as well as losses due to decreased fertility. One way to reduce the incidence of fascioliasis is to delink the life cycle of fluke, by destroying the intermediate host. Bait formulation of different molluscicides would be an effective tool for selective killing of the snail with minimal adverse effect on the non target animals and aquatic environment. It is therefore important to identify strong attractant compounds for preparing effective bait formulations. The principal definite host of these parasites is cattle, sheep and goat (Soliman, 2008). However, certain other mammals including human may be infected as an accidental host (Mas-Coma,2004 a b). Among different carbohydrates starch (Tiwari and Singh, 2004) and among different amino acids, proline and serine (Agrahari and Singh, 2010) are the strongest attractant for *Lymnaea acuminata*.

The present study assay the behavioural responses of *Lymnaea acuminata* to different binary combination of carbohydrate + amino acid and identify among them that, which of them could preferably be used as a potent attractant for preparing bait along with molluscicide. Earlier it has been noted that ricin (*Ricinus communis*) and alkaloid atropine (*Datura stramonium*) extract from these

plants are active molluscicide, when released directly in aquatic medium. To reduce the use of these molluscicide in snail control programme, bait formulation of these active compounds/components were used to explore its true potential as safe mollusicicide.

Material and Method:

Collection of animal: Adult *Lymnaea acuminata* snail (average Length 2.6 ± 0.3 cm) collected locally from lakes and low lying submerged field in Gorakhpur were used as test animals. The snails were acclimatized for 72 h in laboratory dechlorinated tap water at 22^0 C to 24^0 c . Ten experimental animals were kept in a glass aquarium containing 3 liters of dechlorinated tap water. The pH of water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.2 mg/L, 5.2-6.3 mg/L and 102.-105.0mg/L respectively.

Chemicals:

Agar-Agar, amino acids, carbohydrates, ricin and atropine were purchased from sigma chemical co (St louis, Mo, USA).

Preparation of bait formulations:

The bait containing binary combination of carbohydrate (glucose, maltose, starch and sucrose 10mm)+ amino acid (alanine, glycine, serine and proline 10mM) in 1:1 ratio were prepared in 100ml 2.0% agar solution by method of Madsen (1992) as modified by Tiwari and Singh (2004),Singh and Singh(2008). After boiling, each selective active components (Molluscicide) was added to the solution in different concentration (Table1) . The mixture was stirred constantly for 30 minutes and was subsequently spread at a uniform thickness of 5mm. After cooling bait was cut into small piece of 5mm in diameter.

Assay apparatus and procedure:

The bioassay was performed by method of Tiwari and Singh (2004). The bioassay chamber consists of clear glass aquarium having a diameter of 30cm. Each aquarium was divided into four concentric Zones, Zone 3 (central Zone), Zone 2 and Zone1 (middle Zone) and Zone 0 (outer Zone) had a diameter of 13, 18, 24 and 30cm, respectively. A small annular elevation of 9mm and 1.5 cm diameter was made in the centre of the aquarium (Zone 3). The aquarium was then filled with 500 ml dechlorinated tap water to height of 8 mm and maintained at 25 $\pm 0^0$C. At the start of the assay 10 individually marked snails of uniform size were placed on the circumference of Zone-0. The distance between two snails was 66 mm. simultaneously the attractant bait (food pellet) was added in the centre of Zone-3. The location of each snail was noted every15 min for 2 hour. For each combination six set of replicates with 10 snails (for both molluscicides and their binary combination) was used in this study, each at the required concentration. A proportion of snails were arcsine transformed for each replication in zone-3 (Sokal an Rohlf ,1973).

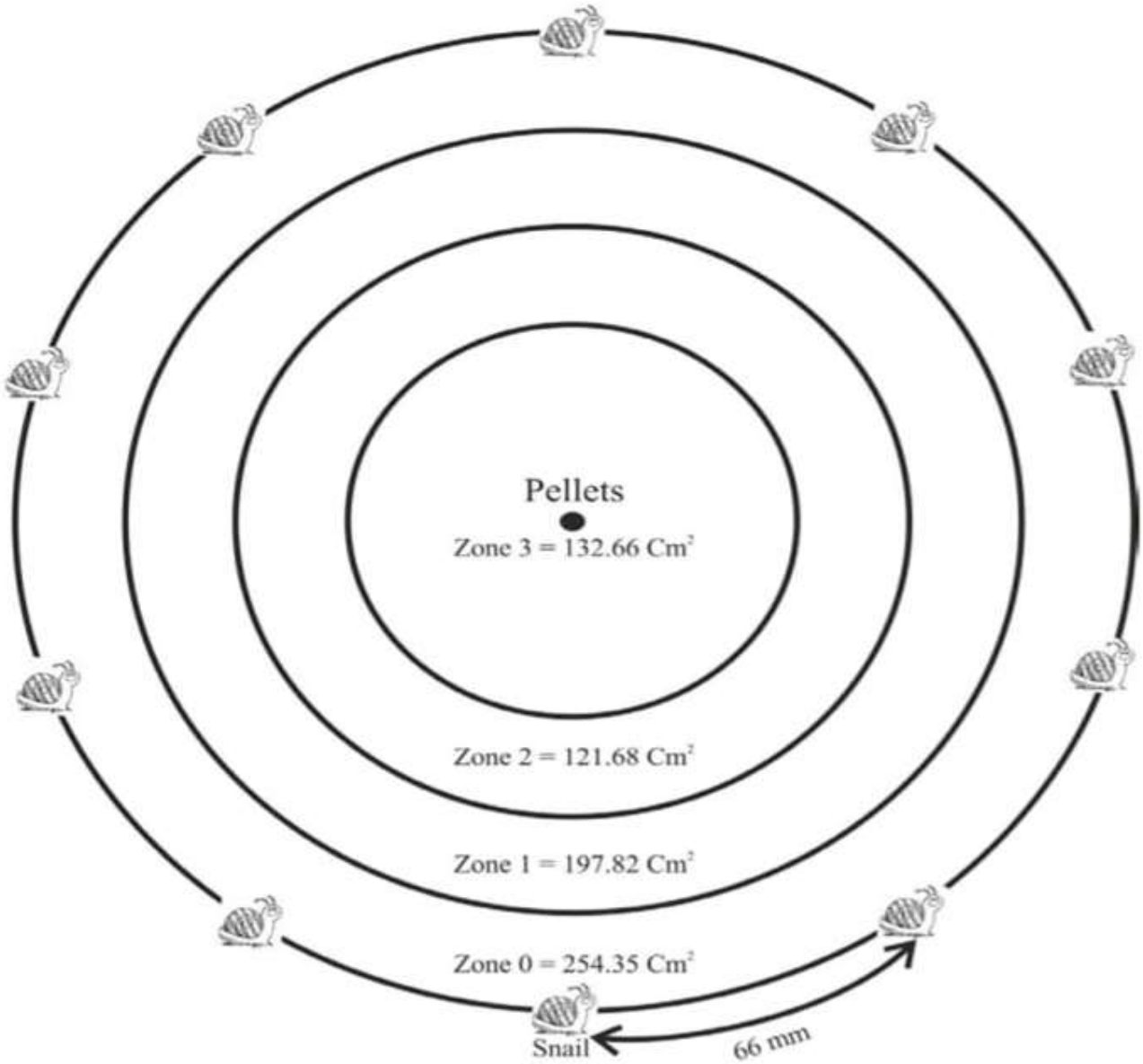

Figure : Experimental design of the aquarium for the study of attraction of snails by bait pellet. Diameter of Zone-3, Zone-2, Zone-1 and Zone-0 were 13, 18, 24 and 30 cm respectively. Pellet was placed in the center of Zone-3, whereas 10 marked snails were placed at periphery of Zone-0. The distance between the two snails was 66 mm.

Statistical Analysis:

The mortality data were observed after every 24h upto 96h. Lethal values (LC_{50}), lower and upper confidence limit (LCL and UCL), slope values, t-ratio, g values, and heterogeneity factor were calculated using POLO computer programme. Two way ANOVA and product moment correlation coefficient was applied to observe significant variation, if any.

Result:

Release of bait containing starch + serine and different concentration of ricin/atropine/ their binary combination caused significant attraction of *Lymnaea acuminata* after 1h and 2h .Snails fed with bait containing starch +serine + 0.7% ricin have least attraction (10.46%) in Zone 3 after 2h. Attraction of snails towards bait containing molluscicide +attractant was lower than control ie bait containing only agar +attracant (Starch+ Serine). Molluscicidal activity of bait containing active components against *L.acuminata* was time and dose dependent (Table 2). Snails were attracted more toward bait containing lower concentration of active components. Bait containing binary combination of ricin and atropine is highly toxic (96 LC_{50}-0.10%). Lowest toxicity was noted in bait containing Ricin (24hLC_{50}-2.1%) .96h toxicity of Ricin 0.26% and atropine 0.29% with starch +serine in bait was noted against *L.acuminata*. The slope value were steep and separate estimations of LC_{50} based on each of 6 replicates were found within the 95% confidence limits . The t-ratio was greater than 1.96 and the heterogeneity factor was less than 1. The g-value was less than 0.5 at all possibility lent there.

Table-1: Mean number of snail *Lymnaea acuminata* in zone-3 in contact with the snail attractant pellet (SAP) or bait of starch+serine that contain different concentrations of Ricin , Atropine alone and their binary combinations in 1:1 ratio after one and two hours from starting of experiment.

Exposure period	Molluscicide	Control	0.1%	0.3%	0.5%	0.7%	1%
1h	St+Sr+Ric.	4±0.36 (39.33)	2±.36 (26.57)	2±0 (26.57)	1.33±.21 (21.38)	.33±.21 (10.46)	-
2h	St+Sr+Ric.	5± .36 (45)	1.33±.21 (21.38)	1.33±.21 (21.38)	.33±.21 (10.46)	0 ± 0 (0 .0)	-
1h	St+Sr+Atr.	5.67±.21 (48.85)	-	3.3±.21 (35.06)	3±0 (33.21)	2.67±.21 (31.11)	1.67±.21 (24.12)
2h	St+Sr+Atr.	5.67±.21 (48.85)	-	4.0 ± 0 (39.23)	3.3±.21 (35.06)	2.67±.21 (31.11)	1.67±.21 (24.12)
1h	St+Sr+Bin.	5±0.96 (45)	2.33±0.21 (28.86)	2±0 (26.57)	1.33±.21 (21.38)	.66±21 (14.88)	-
2h	St+Sr+Bin.	5.33±1.17 (46.89)	2 ± 0 (26.57)	1.67±.21 (24.12)	1±0 (18.43)	.66±.21 (14.88)	-

- Values in parentheses are percentages of snails in zone -3(in contact with attractant food pellet) with snail in zone1and 2.
- Statistically significant ($p<0.05$) when two way ANOVA was applied in between different molluscicide and their different concentration.
- Abbreviations:St-starch,Sr-SerineAtr.-Atropine,Ric-Ricin,Bin-Binary.

Table-2 P:Toxicity of Ricin,Atropine and their Binary combination in 1:1 ratio with Starch+Serine Bait against snail *Lymnaea acuminata* at different time exposure.

Exposure Period	Treatment	LC_{50}	Limit		Slope Value	t-ratio	g-value	Heterogeneity
			LCL	UCL				
24h	Ricin	2.1	1.14	20.88	1.57 ± 0.46	3.36	0.33	0.43
	Atropine	1.25	0.981	2.12	2.51 ± 0.54	4.63	0.17	0.94
	Binary	0.61	0.46	1.01	1.43 ± 0.29	4.85	0.16	0.26
48h	Ricin	1.3	0.76	6.1	1.11 ± 0.30	3.64	0.28	0.17
	Atropine	0.796	0.672	1.04	2.35 ± 0.46	5.05	0.15	0.20
	Binary	0.40	0.30	0.59	1.27 ± 0.27	4.68	0.17	0.21
72h	Ricin	0.64	0.42	1.6	0.96 ± 0.27	3.55	0.30	0.18
	Atropine	0.545	0.414	0.696	1.67 ± 0.43	3.86	0.25	0.10
	Binary	0.20	0.15	0.33	1.16 ± 0.26	4.43	0.19	0.18
96h	Ricin	0.26	0.13	0.43	0.84 ± 0.25	3.24	0.36	0.19
	Atropine	0.294	0.169	0.378	2.00 ± 0.46	4.33	0.20	0.13
	Binary	0.10	0.4	0.15	1.29 ± 0.27	4.71	0.17	0.43

- Six batches of ten snails were exposed different concentration of the above molluscicide inside the snail attractant pellets (SAP). Mortality was determined after every 24h.
- Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments.

Discussion:

Present study clearly demonstrates that the snail *L. acuminata* is significantally attracted toward the different baits formulation. Earlier, it has been noted that gastropods detects the amino acids or carbohydrates as stimulant of their food. Among all the bait formulation starch +serine +atropine caused highest attraction in Zone-3 (39.23%). Snails like other gastropods, are able to detect their food sources by using chemical sense of carbohydrate and amino acid as sign for the presence of their food. Gastropod molluscs are attracted to some of the chemical diffusion out from dead and living aquatic organisms into the modular system of snail. Attraction of the snail *L. acuminata and Biomphalaria alexandrina* towards different carbohydrate/ amino acids in bait formulation has been reported (Abdel- Hamid (1996) and Tiwari and Singh 2004 a, b). Starch is recognized more rapidly by the chemoreceptor of snail/ present in snails (Madsen, 1995). It may be possible that difference in % attraction of *L. acuminata* and other snails towards different amino acids and carbohydrates may be due to differences in feeding patterns / presence of chemoreceptors, and / metabolism in different snail species. Significant variation in the number of the zone-3 attracted by different carbohydrate +amino acid in bait clearly demonstrate that snails are capable of differentiating type of carbohydrate + amino acid in bait formulation.

The toxicity of ricin (*Ricinus communis*) , alkaloid atropine *(Datura stramonium)* and binary combination of ricin and atropine is time and concentration dependent .Snails were less attracted towards the molluscicide containing bait formulation with respect to their control contain only attractant, it indicate that molluscicides in bait have some repellent action. Active component ricin and atropine are very effective molluscicide ; when released directly in aquatic environment. (Sharma et al

2009 and Mahmoud et al 2011). The bait formulation in the present study is very effective in the snail control programme because in bait formulation amount of molluscicide is less released than their direct in the water. Among all the bait formulation binary combination of ricin and atropine containing is more effective in killing the snails.

The steep slope value indicates that a small increase in the concentration of different molluscicide causes higher snail mortality. A t-ratio value greater than 1.96 indicates that the regression is significant. Heterogeneity factor values less than 1.0 denote that in the replicate tests of random sample the concentration response curve would fall within the 95% confidence limit and thus the model fits the data adequately. The index of significance of the potency estimation indicates that the value of the mean is within the limit at all probability level (90, 95 and 99) since it is less than 0.5. The present study reveals that molluscicides of plant origin could be used with varying degree of success in bait formulation. This concept is a new approach and technique for the control of harmful snails.

Reference:

Abdel-Hamid A Z. Amino acids as attractant to *Biompalaria alexandrina* snails intermediate host for attractant *Schistosoma mansoni.* Egypt J bilhariziasis 1996;18:13-26.

Abdel-Hamid AZ Madsen H. Chemoreception of *Biomphalaira alexandria* snails to different sugar. Proceeding international conference of schiostosomiosis March 13-16 Cairo, Egypt 1995.

Agrahari P. and Singh D.K. Behavioral responses of the snail *Lymnaea acuminata* to carbohydrates & amino in bait pellets. Mannuals of Tropical Medicine & Pararsitology, Vol 104, No 8, 667-671 (2010)

Agrahari Pooja, V.K.Singh & D.K. Singh : Toxicity of Snail attractant pellet containing eugenol with respect to abiotic factors against the vector snail *Lymnaea acuminata Biological Agriculture and Horticulture, Vol28,No3 September 2012,156-166.*

Audesirke; Audesirk G.J. Behaviours of gastropods molluscs. In: The Mollusca vol.B, Dennis Willow, A.O, ed, Academic Press. London, 1985;pp.1-94.

Chauhan S., Singh A.: Molluscicidal potential of *Lantana indica & Alstonia scholaris* Plants against fresh water snail *Lymnaea acuminata*. The Internet Journal of Toxicology. 2009 Vol 7 November 2.

Chauhan Saroj & Singh Ajay: Molluscicidal & ovicidal activity of euphorginol against two harmful fresh water gastropods: Indian Journal of Natural Products & Resources Vol 2(4), December 2011, pp.452-457.

Chauhan Saroj,Shahi Jaya and Singh Ajay: Ecofriendly management of *Lymnaea acuminata*, Snail Vector of Fascioliasis in livestock in Eastern Uttar Pradesh. Global verterinaria7(1):10-18,2011.

Croll R.P. Gastropod chemoreception. Boil Rev 1983; 22: 293-319.

Echenique-Euzondo M. Amondarain J, Liron De Robues C. Case report. fascioliasis: An exceptional cause of acute pancreatitis J Pancreas 2005;6:36-39.

Esteban, JG, Gargues MD, Mascoma S. Geographical distribution, diagnosis & treatment of human fascioliasis. A review.res rev parasital 1998;58:13-42

Evaluation of molluscicidal activity on *Biomphalaria glabrata* (Say, 1818) and phytochemical characterization of hydroalcoholic extract of leaves of *Ricinus communis* L. (Euphorbiaceae), Experimental Parasitology Vol 247,April 2023,108481.

F. Hanif and D.K.Singh (2012) Molluscicidal activity of *Morus nigra* against the Freshwater snail *Lymnaea acuminata*.J.Biol.Earth Sci.2(2):B54-B62

Godan D. Pests slug & snail biology & control, Edited by Dora Godan, translated by Sheila Gruber. Springer Verlog, Berlin, Heidelberg, New York: 1983.

Hanif F., Kumar P., Singh D.K. Behavioral responses of *Lymnaea acuminata* against apigenin, moursin & quercetin in bait pellets. Scientific Journal of Zoology(2013)2(5)40-45ISSN2322-293X.

Kpikpi J.E.K. and Thoman JD. A Study of the sugar chemoreception nitches of the two bulinid snail hosts of Schiostosomiasis. Ann Trop Med Parasitol 1992;86:181-198.

Kumar Anil, Samanta Krishanu A Review of Phytochemical and pharmacological activity of *Ricinus communis* (Castor) Plant. International Journal of multidisciplinary Research & Growth Evaluations. ISSN (Online): 2582-7138 Volume : 4 Issue: 02 March. April 2023 Page No 10-15

Kumar P., Singh D.K., Amino acids & carbohydrate binary combinations an attractant in bait formulation against the snail *Lymnaea acuminata* Malays Appl Boil 2010:39:7-11.

Kumar P., Singh D.K. use of amino acids & their combinations as an attractant in bait formulations against the snail *Lymnaea acuminata*. Appl Biosci 2009;35:63-66.

Kumar P., Singh V.K. Singh D.K., Combination of Molluscicides with attractant carbohydrates & amino acids in bait formulation against the snail *Lymnaea acuminata*. European Review for medical & Pharmacological Science 2011; 15: 550-556.

Madsen H., A Comparative study on the food locating ability of *Helisoma duryi*, *Biomphalaria comerunensis* & *Bulinus truncates* (Pulmonate Planorbidae). J Appl Ecol .1992;29:78-78.

Marston A., Hostetman Plant mollucicides. Pytochemistry 1985:24:639-652.

MAS-Coma, Estesan JGMD Bargues. Epidemiology of human fascioliasis: a Review and proposed new classification, Bull WHO 1999; 77:340-346.

Mas-Coma S., Valeroma, Bargues MD, Fasciola, Lymnacids and human fascioliasis with a global overview on disease Transmission, Epidemiology, Evolutionary genetics. Molecular epidemiology & control. In :David Rollinson & Smian lain Hay, Editor: Advances in parasitology, Burlington: Academic press 2009; 69:141-146.

Mohammed J.L. Al-Obaidi, Ali H. Abbas ,Ahmed A.M.Al-Azzauy and Aliaa Waael :Survival rates of *Bulinus truncatus* as a way to determine the molluscicidal activity of *Ricinus communis* extracts.Iraqi Journal of Biotechnology,2015,vol.14,no.2,32-53.

Momeana B.Mahmoud,Wafaa L. Ibrahim, Basma M.Abou-El-Nour, Mohamed A.El-Emam and Alaa A. Youssef: Biological and biochemical parameters of *Biomphalaria alexandrina* snails exposed to the plants *Datura stramonium* and *Sesbania sesban* as water suspensions of their dry powder, Pesticide Biochemistry and physiology, Vol 99, Issue 1, January 2011, pages 96-104.

Ndamba J. Response of mollucicidal berry plant *Phytolacca dodecandra* to different climatic & edphic condition: Trop Agric 1995:72:135-140.

Reema Srivastiva, Pankaj Srivastiva. The medical significances of *Datura stramonium*: A Review. Biomed J Science & Tech Register 29 (2)-2020.BJSTR. MS.ID. 004761.

ROBERTSON J L. Russel R M, Preisier H.K, Savin NE Bioassay with arthropods POLO computer programme for analysis data 2nd Eds. Taylor &Francis, CRC Press, 2007;pp.l-224.

Sahshikant Patel, Piyush Yadav, Priyanshu Maurya, Sushil kumar Yadav, Vikash Kr. Gupta: CastorBean: *Ricinus communis* 2021 IJCRTI Volumes 9, Yore I January 2021/ Issn:2320-2882.

SAYYED, A; SHAH, M. PHYTOCHEMISTRY, Phamacologicol & traditional uses of *Datura stramonium* L. RAV. J. PHARMA. PHYTOCHEM. 2014,2,123-125.

SHARMA M,M; DHALIWAL, I; RANA, K; DELTA A.K. KAUSHI K,P. Phytochemistry, Pharmacology & Toxicology of *Datura* Species- A Review. Antioxidants 2021, 10, 1291. https:// doi. org/10.3390/ antiox 10081291.

Singh A., Singh D.K., Misra T. N., Agrawal R A, Molluscicide of plant origin. Biol Agric Hortic 1996;13:205-252.

Singh P., Kumar P., Singh V.K, Singh D.K. Effect of snail attractant pellet containing Plant molluscicides on certain enzymes in the nervous tissue of *Lymnaea acuminata* (Lamarck). Bioscan 2009: 4: 395-8

Singh P.and Singh D.K. Binary Combination of carbohydrates & amino acids as snail attractant pellets containing Molluscicides against the snail *Lymnaea acuminata*. Pestic Biochem Physiol 2008;92:120-124.

Sokal R R, Rohlf F J. Introduction of biostatistics W.H.Freeman, San Francisco 1973; pp185-207.

Tiwari F., Singh D.K. Behavioral responses of the snail *Lymnaea acuminata* to carbohydrates in snail attractant pellet .Naturwissensch aften 2004:91:378-380.

TIWARI F. SINGH D.K., Attraction of amino acids by *Lymnaea acuminata* snail host of fasciola species. Broz j Med Biol Res 2004:37:587-590

Tiwari Farindra and D. K. Singh, Toxicity of plant derived molluscicides in Attractant food pellet against Snail, *Lymnaea acuminata*. Indian Journal of Pharmacology & Therapeutics IJPT6 : 103-107,2007.

Tiwari Farindra, Bait formulation Toxicity of plant derived molluscicides in Attractants food pellet Against Vector snail, *Lymmaea acuminata*. World Journal of Zoology 7 (1) : 55-59,2012

Uhayhl, Tanaka RO, Maclnnis, AJ. *Schistosoma mansoni*: Identification of chemical that attract or trap its snail Vector *Biomphalaria Glabarata*. Science 1978; 201: 924-926.