Toxicity of Binary Combinations of *Bauhinia variegata* and *Mimusops elengi* with Synergist Piperonyl Butoxide or MGK-264 Against the Fresh Water Snail *Lymnaea acuminata*

Kanchan Lata Singh[1], D. K. Singh[1] & Vinay Kumar Singh[1]*

Malacology Laboratory, Department of Zoology, DDU Gorakhpur University, Gorakhpur-273 009 UP, India
vinaygkpuniv@gmail.com, drvksingh_gpu@yahoo.in;

Abstract: Today fascioliasis is one of the most debilitating parasitic diseases of veterinary and human disease concern globally. Snails serve as intermediate host for parasitic flat warm of *Fasciola* species. One of the possible approaches to eradicate or control this problem is to interrupt the life cycle of the parasitic trematodes by eliminating the snail, which is essential to life cycle. Natural products have an advantage over synthetic products, because such products have eco-friendly, biodegradable and hence are less likely to accumulate in the environment. The molluscicidal activity of column purified fraction of *Bauhinia variegata* leaf, *Mimusops elengi* bark and their active components with synergist piperonyl butoxide (PB) and MGK- 264 were studied in binary combination (1:5) against snail *Lymnaea acuminata*. Binary combination of *B. variegata* leaf column purified and its active component quercetin and *M. elengi* bark column purified and its active component saponin with synergist PB or MGK-264 in 1:5 ratio was used for the determination of molluscicidal activity. Synergistic ratio of column purified fraction of *Mimusops elengi* bark with piperonyl butoxide (PB) (96h: 720 times) or MGK- 264 (96h: 360 times), respectively. Combinations of column purified fraction of *Bauhinia variegata* leaf with piperonyl butoxide (PB) or MGK-264 is more toxic than single treatment. Highest degree of synergism i.e. 199.33 times was observed in the combination of column purified fraction of leaf *Bauhinia variegata* with piperonyl butoxide (PB) at 96h exposure period. Toxicity of binary combination was increased hundreds fold than their individual components indicating synergistic action. It can be concluded from the present study that the use of synergist in binary combination will be more helpful in controlling the aquatic snails, than their individual components and it can be stated from the toxico-dynamic properties of *Bauhinia variegate* and *Mimusops elengi* that they have potential to act as molluscicidal agents.
Researcher 2012; 4(12):66-71]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher. 12

Key words- Fascioliasis, *Bauhinia variegata*, *Mimusops elengi*, piperonyl butoxide, MGK-264, *Lymnaea acuminata*, synergist.

1. Introduction

Fascioliasis is a global veterinary and human disease (Lewin, 2007). There is a very high incidence of fascioliasis in the cattle population of the eastern region of the Uttar Pradesh state, India. Ninety four percent of buffaloes slaughtered in local slaughter houses are infected with this disease (Singh and Agarwal, 1981; Shukla et al., 2006). Snails *Lymnaea acuminata* (Lymnidae) and *Indoplanorbis exustus* (planorbidae) serve as intermediate host for parasitic flat warm of *Fasciola* species (Osman et al., 2007). This disease rank is major cause of morbidity and mortality both in man and lives- stock and contribute to socioeconomic problem (Mas-Coma et al., 2005). One way to reduce the incidence of fascioliasis is to de-link their life cycle of fluke, by destroying the intermediate hosts snail *Lymnaea acuminata* and *Indoplanorbis exustus* (Singh and Singh, 2009; Singh et al., 2010; Kumar et al., 2011). Several attempts have been made to reduce the incidence of fascioliasis by using synthetic molluscicides and plant derived molluscicides (Singh et al., 1996; Kumar and Singh, 2007). The continuous and discriminate use of synthetic pesticides for vector control has created problem of acute and chronic toxicity to environment (Shafer et al., 2005). It has been observed that the plant derive molluscicides are easily available, less expensive and biodegradable in nature rather than their synthetic counter parts. It has been previously reported by that *Bauhinia variegata* and *Mimusops elengi* are potent molluscicides (Singh et al., 2012). In the present study we have evaluated the toxicity of binary combination of *Bauhinia variegata* (Family- Fabaceae) column purified leaf and *Mimusops elengi* (Family- Sapotaceae) column purified bark with synergist piperonyl butoxide (PB) or MGK- 264 against snail *Lymnaea acuminata.*

2. Materials and Methods

2.1Test material-

Adult *Lymnaea acuminata* (2.30± 0.25 cm in length) were collected locally from the ponds, pools, and Ramgarh lakes and low lying submerged areas of Gorakhpur district, U.P., India and used as experimental animals. These snails adhere to ventral surface of the leaves of aquatic plants, lie freely around the vegetation near the bank and are available around the year. Snails are kept in glass aquarium containing dechlorinated tap water and were allow acclimatize to the laboratory condition for 72h.

Experimental animals kept in the glass aquaria containing dechlorinated tap water at 22-24 °C. The pH, dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 7.1-7.3, 6.5-7.3 mg/l, 5.2-6.3 mg/l and 102-105 mg/l, respectively. Dead animal were removed immediately to prevent any contamination to the aquarium water.

2.2Binary combination

Binary combination of *Bauhinia variegata* leaf column purified fraction and its active component quercetin and *Mimusops elengi* bark column purified fraction and its active component saponin (Singh et al., 2012) with synergist piperonyl butoxide (PB) or MGK-264 in 1:5 ratio was used for the determination of molluscicidal activity.

2.3Molluscicidal activity

The synergistic toxicity against *Lymnaea acuminata* was performed by the method of Singh and Agarwal (1984). Ten experimental animals were kept in glass aquarium, containing 3 liter of dechlorinated tap water. Snail were exposed to different preparations of *Bauhinia variegate* leaf column purified fraction and its active component quercetin and *Mimusops elengi* bark column purified fraction and its active component saponin single and binary combinations with synergist PB or MGK-264 (Table -1) to observed their toxicity against *Lymnaea acuminata*. Six aquaria were setup for each concentration. Mortality was recorded at 24h intervals up to 96h exposure period. Control snails were kept in an equal volume of dechlorinated tap water under similar condition without treatments.

2.4 Statistical analysis

Concentration mortality data for each group of snails were analyzed using the probit analysis program, POLO-PC (LeOra Software) Russell et al. (1977) to estimate the LC_{50} of the 95% confidence intervals for these concentrations. The regression coefficient between exposure time and different values of LC_{50} was determined by the method of Sokal and Rohlf (1973).

3. Results

Toxicity 24h LC_{50} of binary combination of column purified fraction of *Bauhinia variegata* leaf with PB or MGK-264 were 0.08 and 0.11, respectively against *Lymnaea acuminata* than their single treatment. At 96h exposure period toxicity of column purified fraction of *Bauhinia variegata* leaf with PB or MGK-264 were enhanced 199.33 and 149.50 times more toxic against *Lymnaea acuminata*, respectively (Table-2). Binary combination of quercetin with PB or MGK-264 was 7.01 and 7.09 times more toxic against *Lymnaea acuminata* than their single treatment at 24h. At 96h exposure period toxicity of with PB and MGK-264 were enhanced as synergistic ratio was 9.62 and 8.55 times, respectively (Table-2).

Binary combination of column purified fraction of *Mimusops elengi* bark with PB and MGK-264 were 229.25 and 262.00 times more toxic against *Lymnaea acuminata* than their single treatment at 24h. At 96h exposure period toxicity of column purified fraction of *Mimusops elengi* bark with PB and MGK-264 were enhanced 720.00 and 360.00 times more toxic against *Lymnaea acuminata*, respectively (Table-3). Binary combination of saponin with PB and MGK-264 were 10.44 and 9.26 times more toxic against *Lymnaea acuminata* than their single treatment at 24h. At 96h exposure period toxicity of with PB and MGK-264 were enhanced as synergistic ratio was 11.81 and 13.00 times, respectively (Table-3).

The slope values were steep and separate estimation of LC based on each of six replicate were found to be within 95% confidence limits of LC_{50}. The t-ratio was greater than 1.96 and the heterogeneity factor is less than 1.0. The g-value was less than 0.5 at all probability level (90, 95 and 99).

Table 1. Binary combination (1:5) of different plant products *Bauhinia variegata and Mimusops elengi* and their component with synergist PB or MGK-264 used for the toxicity determination against *Lymnaea acuminata* at different exposure period.

Treatments	**Concentration (mg/l)** ***Lymnaea acuminata***
B.variegata leaf Column Purified	5, 10, 15, 20,
B.variegata leaf Column Purified +PB	0.5, 0.10, 0.15, 0.20
B.variegata leaf Column Purified +MGK	0.5, 0.10, 0.15, 0.20
Quercetin	5,7,9,12
Quercetin + PB	0.5, 0.7, 0.9, 0.12
Quercetin + MGK	0.5, 0.7, 0.9, 0.12
M. elengi bark Column Purified	7, 9, 12, 15
M. elengi bark Column Purified +PB	0.7, 0.9, 0.12, 0.15
M. elengi bark Column Purified +MGK	0.7, 0.9, 0.12, 0.15
Saponin	1, 3, 5, 7
Saponin +PB	0.1, 0.3, 0., 0.7
Saponin+ MGK	0.1, 0.3, 0.5, 0.7

Abbreviation: PB- Piperonyl butoxide; MGK-MGK-264; B. v. leaf CP- *B.variegata* leaf Column Purified; M. e. bark CP- *M. elengi* bark Column Purified.

Table -2: Toxicity of binary combination (1:5) ratio of column purified fraction of *Bauhinia variegata* leaf powder and active component Quercetin with synergist PB or MGK-264 against *Lymnaea acuminata.*

Exposure period	Treatments	LC_{50}	Synergistic ratio	LCL	UCL	Slope value	t-ratio	g-value	Hetero-geneity
24h	B. v. leaf CP	20.30	-	16.99	27.41	2.72±0.48	4.16	0.12	0.28
	B. v. leaf CP+PB	0.08	253.75	0.068	0.11	1.89±0.48	3.90	0.25	0.11
	B. v. leaf CP+MGK	0.11	184.54	0.89	0.16	2.37±0.55	4.31	0.20	0.08
	Quercetin	12.13	-	10.42	24.73	2.19±0.62	3.59	0.32	0.16
	Quercetin + PB	1.73	7.01	1.26	5.26	1.78±0.63	2.81	0.48	0.24
	Quercetin + MGK	1.71	7.09	1.35	3.04	2.75±0.73	3.75	0.27	0.26
48h	B. v. leaf CP	16.03	-	12.90	22.95	1.79±0.6.	3.16	0.14	0.32
	B. v leaf CP+PB	0.06	267.16	0.03	0.05	1.79±0.46	3.82	0.26	0.13
	B. v. leaf CP+MGK	0.08	200.00	0.07	0.12	1.94±0.49	3.95	0.24	0.09
	Quercetin	9.86	-	8.45	13.75	2.16±0.60	3.60	0.29	0.13
	Quercetin + PB	1.13	8.72	0.93	1.95	1.74±0.59	2.91	0.45	0.22
	Quercetin + MGK	1.47	6.70	1.16	2.79	2.08±0.63	3.27	0.35	0.17
72h	B. v. leaf CP	10.13	-	7.73	17.75	3.82±0.37	3.60	0.14	0.28
	B. v. leaf CP+PB	0.05	202.6	0.05	0.07	1.77±0.46	3.78	0.26	0.11
	B. v. leaf CP+MGK	0.06	168.83	0.05	0.08	1.79±0.47	3.80	0.26	0.10
	Quercetin	6.82	-	4.79	9.93	4.75±0.60	3.39	0.25	0.10
	Quercetin + PB	0.77	8.85	0.61	0.95	1.70±0.58	2.90	0.29	0.15
	Quercetin + MGK	0.99	13.91	0.85	1.29	2.16±0.60	3.60	0.14	0.11
96h	B. v. leaf CP	5.98	-	4.08	9.47	2.42±0.39	5.16	0.13	0.33
	B. v. leaf CP+PB	0.03	199.33	0.02	0.03	2.35±0.49	4.73	0.17	0.37
	B. v. leaf CP+MGK	0.04	149.5	0.03	0.05	1.86±0.01	3.96	0.24	0.19
	Quercetin	5.39	-	4.81	8.41	3.96±0.64	4.55	0.12	0.24
	Quercetin + PB	0.56	9.62	0.43	0.65	2.50±0.61	4.06	0.23	0.52
	Quercetin + MGK	0.63	8.55	0.55	0.70	3.22±0.62	5.14	0.14	0.50

Each six batches of ten snails were exposed to different concentration. Concentrations given are the final Concentration (mg/l w/v) in the aquarium water. Mortality was recorded at every 24h intervals up to 96h. Synergistic ratio calculated of LC_{50} of column purified fraction of *B. variegata* leaf powder and active component Quercetin with synergist PB or MGK. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments. Ts-testing significant of the regression coefficient: B. v. leaf CP+PB -2.55++, B. v. leaf CP- 20.37+, B. v. leaf CP + MGK-264- 13.27 +, Quercetin- 4.96+ Quercetin + CP- 10.10++, Quercetin + MGK-11.71++.

+: linear regression between x and y.

++: non-linear regression between log x and log y.

Abbreviations: *B. v.* leaf CP – *Bauhinia variegata* leaf column purified; PB-- piperonyl butoxide; MGK- MGK-264; LCL- Lower confidence limit; UCL- Upper confidence limit.

Table -3: Toxicity of binary combination (1:5 ratio) of column purified fraction of *Mimusops elengi bark* powder and active component saponin with synergist PB or MGK-264 against *Lymnaea acuminata.*

Exposure period	Treatments	LC_{50}	Synergistic ratio	LCL	UCL	Slope value	t-ratio	g-value	Hetero-geneity
24h	M. e. bark CP	18.3	-	10.50	57.75	2.19±0.34	3.49	0.31	0.22
	M. e. bark CP+PB	0.07	262.00	0.05	0.15	1.05±0.27	3.78	0.26	0.15
	M. e bark CP+MGK	0.08	229.25	0.06	0.16	1.16±0.29	4.00	0.24	0.12
	Saponin	15.57	-	13.68	19.88	4.02±0.78	5.72	0.14	0.26
	Saponin +PB	1.49	10.44	0.86	6.68	0.97±0.29	3.30	0.35	0.26
	Saponin +MGK	1.68	9.26	0.91	9.79	0.94±0.29	3.15	0.38	0.24
48h	M. e. bark CP	15.71	-	8.07	17.82	1.76±0.28	2.70	0.15	0.16
	M. e. bark CP+PB	0.04	392.75	0.03	0.06	0.95±0.26	3.63	0.29	0.16
	M. e. bark CP+ MGK	0.50	31.42	0.03	0.08	1.01±0.26	3.78	0.26	0.10
	Saponin	13.90	-	11.78	17.82	3.20±0.28	3.70	0.18	0.19
	Saponin +PB	0.41	33.90	0.29	0.69	0.87±0.26	3.34	0.39	0.19
	Saponin +MGK	0.89	15.61	0.55	3.30	0.18±0.27	3.00	0.42	0.20
72h	M. e. bark CP	10.60	-	9.18	12.41	4.76±0.28	5.70	0.11	0.26
	M. e. bark CP+PB	0.02	530.00	0.01	0.05	1.86±0.01	3.96	0.24	0.19
	M. e. bark CP+MGK	0.03	353.33	0.02	0.03	0.94±0.26	3.62	0.29	0.18
	Saponin	4.25	-	2.99	7.06	1.89±0.26	3.41	0.33	0.20
	Saponin +PB	0.20	21.25	0.12	0.27	1.01±0.26	3.90	0.25	0.21
	Saponin +MGK	0.21	20.23	0.13	1.80	0.13±0.29	3.85	0.26	0.25
96h	M. e. bark CP	7.20	-	5.74	8.15	3.84±0.28	3.70	0.14	0.31
	M. e. bark CP+PB	0.01	720.00	0.005	0.01	0.99±0.26	3.77	0.2	0.23
	M. e. bark CP+MGK	0.02	360.00	0.008	0.02	1.17±0.26	4.42	0.16	0.25
	Saponin	1.30	-	0.27	2.16	0.69±0.25	2.69	0.53	0.19
	Saponin +PB	0.11	11.81	0.06	0.15	1.29±0.27	4.71	0.17	0.59
	Saponin +MGK	0.10	13.00	0.59	0.14	1.40±0.27	5.02	0.15	0.61

Each six batches of ten snails were exposed to different concentration. Concentrations given are the final Concentration (mg/l w/v) in the aquarium water. Mortality was recorded at every 24h intervals up to 96h. Synergistic ratio calculated of LC_{50} of column purified fraction of *Mimosops elengi* bark powder and active component saponin with synergist PB or MGK. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments. Ts-testing significant of the regression coefficient: M. e. bark CP+PB -6.32+, M .e. bark CP- 12.02+, M. e. bark CP+MGK- 0.82++, saponin- 4.96+ , saponin + CP- 62.02++, saponin + MGK- 4.82+.

+: linear regression between x and y.

++: non-linear regression between log x and log y.

Abbreviations: M. e. bark CP- *Mimusops elengi* bark column purified; PB-- Piperonyl butoxide; MGK- MGK-264; LCL- Lower confidence limit; UCL-Upper confidence limit.

4. Discussion-

Results clearly demonstrate that the binary combination of *B. variegata* and *M. elengi* with PB or MGK-264 synergize molluscicidal activity than their single treatment against the snail *Lymnaea acuminata*. The molluscicidal activity of these combinations was

time and concentration dependent. A number of studies on binary combination of plant derived molluscicides with PB and MGK-264 have been reported against harmful snails (Singh et al., 2005; Shukla et al., 2005; Singh et al., 2010).

Action of binary combination mixture is non-interactive (Plackett and Hewlett, 1952) as the component do not affect the transport and final concentration of each other at the site of action. Piperonyl butoxide and MGK- 264 alone are not toxic to snail *Lymnaea acuminata* (Singh and Agarwal, 1989; Sahay et al., 1991). Piperonyl butoxide and MGK- 264 are commonly used with carbamates, organophosphates, pyrethroids pesticides and certain plant derived molluscicides to increase their efficiency against different pests (Sahay et al., 1991; Rao and Singh, 2001). They exert the synergistic action mainly by inhibiting the mixed function oxidase (MFO) activity, which detoxify xenobiotics (Matsmura, 1985; Rao and Singh, 2001). The penetration of the toxicant also have greater significant for aquatic environment, because here the whole body is bathed in a diluted solution of the toxicant. To have maximum effect the synergistic must penetrate the organism and transported to active sites rapidly. It is due to rapid penetration synergist through soft foot of snails.

Toxicity of column purified fraction of *B. variegata* leaf with PB or MGK-264 (96h LC_{50}- 0.03 mg/l and 0.04 mg/l). *M. elengi* bark with PB or MGK-264 (96h LC_{50}- 0.01 mg/l and 0.02 mg/l) against the *Lymnaea acuminata*. In the present study is very high in comparison to synthetic pyrethroid pesticides viz. permethrin (96h LC_{50}- 0.37 mg/l), cypermethrin (96h LC_{50}- 0.36 mg/l) and organophosphate pesticide trichlorofan (96h LC_{50}- 0.30 mg/l) (Singh and Agarwal 1991). Combinations of column purified fraction of *B. variegata* leaf with PB or MGK-264 is more toxic than their single treatment. Highest degree of synergism is 267.16 times was observed in the combination of purified fraction of *B. variegata* leaf with PB at 48h exposure period. PB or MGK-264 is the mixed function oxidase inhibitors (Wilkilson, 1976; Matsmura, 1985). Due to inhibition of mixed function oxidase the active component is detoxified and there is high titer of molluscicidal component at target site. In the present study activity of quercetin, identified as molluscicidal component in *B. variegata*. It is detoxified by the mixed function oxidase. Toxicity data showed a significant positive correlation between the mortality of snail and exposure periods. Other factors which, may be singly or jointly, uptake of active moiety leads to a progressive increase in the titer of the active moiety and its effect in the snail's body.

Combination of column purified of *M. elengi* bark and active component saponin with PB or MGK-264 indicate that it enhanced the toxicity up to 720.00 times. Higher potency of molluscicidal activity of column purified fraction *M. elengi* bark PB or MGK-264 clearly indicate that in contrast to saponin. It demonstrates that MFO inhibitor PB or MGK-264 detoxify of saponin in the snail body, which cause several times more toxicity against the *Lymnaea acuminata.* Highest concentration of PB (300.54 mg/l) and MGK- 264 (260.50 mg/l) are not toxic to snail as reported earlier by Singh and Agarwal, (1989) and Sahay et al., (1991).

The steep slope values indicate that even a small increase in the concentration causes higher snail mortality. Values of t-ratio higher than 1.96 indicate that the regression is significant. Values of heterogeneity factor, less than 1.0, denotes that in the replicates test of random sample the concentration response curves fall with the 95% confidence limit and thus the model fit the data adequately. The index of significance of potency estimate g-value indicate that the value of the mean is within the limits at all probability (90, 95, 99) since it is less than 0.5.

It can be concluded from the present study that the use of synergist in binary combination will be more helpful in controlling the aquatic snails, than their individual components. The effective toxic concentration of each component is lower and would be safer in aquatic environment.

Acknowledgments:

Authors are thankful to the University Grants Commission (UGC), New Delhi, India for financial assistance (F. No. 39-590/2010 (SR).

Correspondence to:
Dr. Vinay Kumar Singh,
Department of Zoology,
DDU Gorakhpur University,
Gorakhpur-273009, UP, India.
Telephone-+91-9415855488
Emails- vinaygkpuniv@gmail.com;
drvksingh_gpu@yahoo.in.

References

1. Kumar P, Singh DK. Binary combination of the some common spices against harmful snails J. Appl. Bio. Sci. 2007; 33(2): 167-70.
2. Kumar S, Singh DK, Singh VK. Binary combination of different breeds of freeze- dried cow urine (FCU) with some plant molluscicides against *Lymnaea acuminata*. Vector of Fascioliasis. Advances in Life Sciences, 2011; 1(1): 24-29.

3. Lewin, MR. The Johns Hopkin and microbiology Newsletter, 2007; 26(03). http://www.Hopkinsmedicine.Org/microbiology (Newsletter/index 2007.html).
4. Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-borne trematode zoonosis. Int. J Parasitol. 2005; 35: 1255-78.
5. Matsumura F. Toxicology of insecticides. 2nd Ed. Plenum Press, 1985; New Yark.
6. Osman EA, Mohmad EM, Abu Elreesh BI, Elegami AA. Molluscicidal activity of *Combretum glutinosum*. International J. of Mol. Med. Sci. B. 2007; 14: 141-163.
7. Plackett RL, Hewlett PS. Quantal responses to mixture of poison, J. R. Statist. Soc. B. 1952; 14: 141-63.
8. Rao IG, Singh DK. Combination of *Azadirachta indica* and *Cedrus deodara* oil, with piperonyl butoxide, MGK-264 and *Embelia ribes* against *Lymnaea acuminata.* Chemosphere 2001; 14: 1691-95.
9. Russell RM, Robertson JL, Savin NE. POLO: A new Computer Program for probit Analysis. Bulletin of the Entomological society of America 1977; 23, 209- 13.
10. Safer TJ, Mayer DA, Crofton KM. Developmental neurotoxicity of pyrethroid insecticides: Critical review and future research needs. Environ. Health Persp. 2005; 113: 123-36.
11. Sahay N, Singh DK, Agarwal RA. Synergistic effect of piperonyl butoxide on the toxicity of synergistic pyrethroid in the *Lymnaea acuminata.* J. Med. Appl. Malacol. 1991; 3: 107-11.
12. Shukla S, Singh VK, Singh DK. Binary and tertiary combinations of plant derived molluscicides against the vector snail *Lymnaea acuminata* and *Indoplanorbis exustus* Malays. Appl. Biol., 2005; 34(2): 15-24.
13. Shukla S, Singh VK, Singh DK. Effect of singly, Binary and tertiary combinations of few plant derived molluscicides alone or in combination with synergist on different enzymes in nervous tissue of the fresh water snail *Lymnaea* (Radix) *acuminata* (Lamark). Pesticide Biochemistry and Physiology 2006; 85: 167-73.
14. Singh A, Kumar P, Singh DK and Singh VK. Toxicity of binary combination of *Saraca asoca* and *Thuja orientalis* with synergistic piperonyl butoxide and MGK-264 against the fresh water snail *Lymnaea acuminata*. The Bioscan 2010; 5(1): 13-18.
15. Singh A, Singh DK, Mishra TN, Agarwal RA. Molluscicide of plant origin. Biol Agaric Hortic. 1996;13:205-52.
16. Singh A, Singh VK. Molluscicidal activity of *Saraca asoca* and *Thuja orientalis* against the fresh water snail *Lymnaea acuminata*. Veterinary Parasitology 2009; 164: 206-10.
17. Singh DK, Agarwal RA. Action site of cypermethrin, a synthetic pyrethroid in the snail *Lymnaea acuminata.* Acta Hydrochim Hydrobiol. 1991; 19 (4) 425-30.
18. Singh DK, Agarwal RA. Correlation of the anticholinesterase and molluscicidal activity of latex of *Euphorbia royleana* on *Lymnaea acuminata*. J. Nat. Pro. 1984; 42: 702-05.
19. Singh DK, Agarwal RA. Toxicity of piperonyl butoxide carbaryl synergism on the snail *Lymnaea acuminata*. Int Revue Ges Hydrobiol 1989; 74(6): 689-99.
20. Singh KL, Singh DK, Singh VK. Characterization of the molluscicidal activity of *Bauhinia variegata* and *Mimusops elengi* plant extract against the *Fasciola* vector *Lymnaea acuminata*. Rev. Inst. Med. Trap. Sao Paulo 2012; 54(3) 135-40.
21. Singh O, Agarwal RA. Toxicity of certain pesticides to two economic species of snail in northern India. J. Econ. Entomol. 1981; 74(5): 568-71.
22. Singh P, Singh DK. Toxicity of binary combination of some plant derived molluscicides with MGK-264 and Piperonyl butoxide on the reproduction of the snail *Lymnaea acuminata* Pes. Manag. Sci. 2003; 61: 204-08.
23. Singh P, Singh VK, Singh DK. Effect of binary combination of some plant-derived molluscicides with MGK-264 or piperonyl butoxide on reproduction of snail *Lymnaea acuminata*. Pest Management Science, 2005; 61: 204-08.
24. Singh VK, and Singh DK. Molluscicidal activity of pre- and post-harvest *Allium sativum* (Garlic). Biol Agric and Horticult. 1996; 12:311-18.
25. Sokal RR, Rohlf FJ. Introduction to biostatistics. San Francisco: W.H. Freeman; 1996.
26. Wilkinson CF. Insecticide synergism .In. R.L. Metcalf and J.J. Mckelvey Jr. (eds). Insecticides for the Future: Needs. And Prospects Wiley New York, 1976; p. 195-218.