The Bioscan 5 (1) : 13-18, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

TOXICITY OF BINARY COMBINATION OF *SARACA ASOCA* AND *THUJA ORIENTALIS* WITH SYNERGIST PIPERONYL BUTOXIDE AND MGK-264 AGAINST THE FRESHWATER SNAIL *LYMNAEA ACUMINATA*

ARUNDHATI SINGH, PRADEEP KUMAR, D. K. SINGH AND V. K. SINGH*
Department of Zoology, D. D. U. Gorakhpur University,
Gorakhpur - 273 009, U. P., INDIA
E-mail: vksingh_gpu@yahoo.co.in

KEY WORDS
Fascioliasis
Piperonyl butoxide
MGK-264 (ENT 8184)
Saraca asoca
Thuja orientalis
Lymnaea acuminata

Received on :
05.10.2009
Accepted on :
13.01.2010

***Corresponding author**

ABSTRACT

The molluscicidal activity of leaf/ bark of *Saraca asoca*, leaf/fruit of *Thuja orientalis* and their active components/ column purified fraction with synergist Piperonyl butoxide (PB) and MGK-264 (ENT 8184) were studied in binary combination (1:5) against *Lymnaea acuminata*. Combination of *S. asoca* leaf/ column extract of *S. asoca* leaf or bark with PB or MGK-264 is more toxic than their single treatment. Highest degree of synergism *i.e.*323 times was observed in the combination of *S. asoca* leaf with PB or MGK-264 at 96h exposure period. Combination of *T. orientalis* leaf/ thujone or fruit powder/ column extract of *T. orientalis* fruit with PB or MGK-264 indicate that it enhance the toxicity up to 189.02 times. Toxicity of binary combination was increased hundreds folds than their individual components indicating synergistic action.

INTRODUCTION

Fascioliasis is very common in eastern Uttar Pradesh, 94% buffaloes slaughtered in local slaughter houses are infected with *Fasciola gigantica* (Singh and Agarwal, 1981; Agarwal and Singh, 1988). The fresh water snail *Lymnaea acuminata* serve as intermediate hosts of parasitic flat worm, *Fasciola* and *Schistosoma* species (Mas-Coma *et al.,* 2005; Osman *et al.,* 2007). In human the disease is characterised by hypereosinophilia, abdominal pain and pancreatitis (Saba *et al.,* 2004; Singh and Singh 2009a). One of the solutions to reduce the problem of fascioliasis is to disrupt the life cycle of the fluke by eliminating the vector snails (Godan, 1983; Jaiswal and Singh, 2008). Several attempts have been made to reduce the incidence of fascioliasis by using synthetic molluscicides and plant product molluscicides (Singh *et al.,* 1996; Kumar and Singh, 2007). The continuous and indiscriminate use of synthetic pesticides for vector control has created the problem of acute and chronic toxicity to environment (Shafer *et al.,* 2005; Singh *et al.,* 2009). It has been observed that the plant derived molluscicides are easily available, less expensive and biodegradable in nature than their synthetic counterparts. It has been previously reported by us *Saraca asoca* and *Thuja orientalis* are potent molluscicides (Singh and Singh, 2009b). In the present study we have evaluated the toxicity of binary combination of *Saraca asoca* (Family- Caesalpiniaceae) leaf / bark and *Thuja orientalis* (Family- Cupressaceae) leaf/fruit with synergist piperonyl butoxide (PB) and MGK-264 (ENT 8184) against *Lymnaea acuminata*.

MATERIALS AND METHODS

Test material

Snail *Lymnaea acuminata* were collected locally from the ponds, pools and lakes of Gorakhpur district and used for toxicity testings. These snails either adhere to ventral surface of the leaves of aquatic plants or, lie freely around the vegetation near the banks and are available round the year. Snails are kept in glass aquarium containing dechlorinated tap water and were allowed to acclimatize to the laboratory condition for 72h. Dead animal were removed immediately to prevent any contamination in the aquarium water. The average sized adults of *Lymnaea acuminata* (2.30 $\pm$ 0.25 cm in length) were used as the experimental animals.

Binary combination

Binary combination of crude powder of *S. asoca* leaf/bark and *T. orientalis* leaf/fruit and its active components/column purified fractions (Singh and Singh, 2009b) with synergist PB and MGK-264 in 1:5 ratios were used for the determination of molluscicidal activity.

Molluscicidal activity

The Synergistic toxicity against *L. acuminata* was performed by method of Singh and Agarwal (1984). Ten experimental animals were kept in a glass aquarium, containing 3 lit of dechlorinated tap water. Snails were exposed to different preprations of *S. asoca* leaf/ bark and *T. orientalis* leaf/ fruit singly and binary combinations with synergist PB or MGK-264 (Table 1) to observe their toxicity against *L. acuminata*. Six aquaria were set up for each concentration. Mortality was recorded at 24 h intervals up to 96 h exposure period. Control animals were kept in an equal volume of dechlorinated water under similar condition without treatment.

Lethal concentration value (LC_{50}), upper and lower confidence limit (UCL and LCL) and slope values were calculated according to POLO computer programme of Robertson *et al.,* (2007). The regression coefficient was determined between exposure time and different values of LC_{50} (Sokal and Rohlf, 1976).

RESULTS

Binary combination of *S. asoca* leaf powder with PB or MGK-264 was 16.35 and 22.2 times, more toxic against *L. acuminata* than their single treatment at 24h. At 96h exposure period toxicity of *S. asoca* leaf powder with PB or MGK-264 were enhanced to 26.64 or 25.29 times, respectively. Binary combination of column purified of *S. asoca* leaf powder with PB or MGK-264 was 99.62 or 132.83 times, more toxic against *L. acuminata* than their single treatment at 24h. At 96 h exposure period's toxicity of column purified of *S. asoca* leaf powder with PB or MGK-264 were enhanced 323 times (Table, 2).

The 96h toxicity of column purified of *S. asoca* leaf powder with PB (LC_{50}- 0.01 mg/L) and the column purified of *S. asoca* leaf powder with MGK-264 (LC_{50} – 0.01 mg/L) were same against *L. acuminata*, whereas *S. asoca* leaf powder with PB

Table1: Concentration of different plant product and their components with synergist PB and MGK-264 used for the toxicity determination against *L. acuminata*

Treatments	Concentration(mg/L)
S. asoca leaf powder (LP)	30,50,70,90
LP + MGK-264	1,2,3,4
LP + PB	1,2,3,4
Column purified (CP)	2,4,6,8
CP + MGK-264	0.009,0.03,0.05,0.07
CP + PB	0.009,0.03,0.05,0.07
S. asoca bark powder (BP)	-
BP + MGK-264	10,20,30,50
BP + PB	10,20,30,50
Saponin	5,10,20,25
Saponin + MGK-264	1,3,5,7
Saponin + PB	1,3,5,7
T. orientalis leaf powder (LP)	200,300.400,500
LP + MGK-264	3,4,5,6
LP + PB	3,5,7,9
Thujone	5,10,20,25
Thujone + MGK-264	7,9,12,15
Thujone + PB	7,9,12,15
T. orientalis fruit powder (FP)	200,300,400.500
FP + MGK-264	1,2,3,4
FP + PB	1,2,3,4
Column purified (CP)	3,5,7,9
CP + MGK-264	0.1,0.3,0.7,0.9
CP + PB	0.1,0.3,0.7,0.9

Abbreviation: LP- Leaf powder, BP- Bark powder, FP- Fruit powder, SP- Seed powder MGK- MGK-264; PB- Piperonyl butaoxide, CP- Column purified

Table 2: Toxicity of binary combination (1:5 ratio) of *Saraca asoca* (leaf powder) and column purified fraction with synergist MGK-264 or PB against *Lymnaea acuminata*

Exposure period/ Molluscicides	LC_{50} mg/L	Synergistic ratio	Limits LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24h Leaf powder	84.24		74.26	102.33	3.62 ± 0.60	6.03	0.10	0.27
Leaf powder + MGK	3.79	22.2	3.23	4.88	2.88 ± 0.49	5.85	0.11	0.36
Leaf powder + PB	5.15	16.35	3.94	9.19	2.13 ± 0.46	4.60	0.18	0.46
Column purified	7.97		6.76	10.41	2.95 ± 0.51	5.79	0.11	0.35
Column purified + MGK	0.06	132.83	0.05	0.09	3.65 ± 0.70	5.20	0.14	0.40
Column purified + PB	0.08	99.62	0.06	0.16	1.57 ± 0.32	4.85	0.16	0.81
48h Leaf powder	69.53		61.15	82.45	3.04 ± 0.51	5.88	0.11	0.17
Leaf powder + MGK	2.94	23.64	2.84	3.71	2.27 ± 0.40	5.58	0.12	0.21
Leaf powder + PB	3.60	19.31	2.87	5.48	1.81 ± 0.40	4.54	0.18	0.23
Column purified	6.35		5.24	8.50	2.05 ± 0.40	5.11	0.14	0.26
Column purified + MGK	0.04	158.75	0.03	0.06	1.59 ± 0.28	5.68	0.11	0.26
Column purified + PB	0.05	127.00	0.04	0.09	1.36 ± 0.27	4.98	0.15	0.28
72h Leaf powder	53.05		45.19	61.39	2.68 ± 0.48	5.51	0.12	0.10
Leaf powder + MGK	2.17	24.44	1.84	3.12	2.15 ± 0.35	5.48	0.12	0.27
Leaf powder + PB	2.42	21.92	1.94	3.10	1.79 ± 0.37	4.73	0.17	0.27
Column purified	4.39		3.54	5.41	1.93 ± 0.38	5.08	0.14	0.23
Column purified + MGK	0.02	219.5	0.02	0.03	1.41 ± 0.25	5.46	0.12	0.12
Column purified + PB	0.03	146.33	0.02	0.04	1.24 ± 0.25	5.89	0.16	0.34
96h Leaf powder	39.96		32.45	46.01	2.95 ± 0.49	5.93	0.10	0.16
Leaf powder + MGK	1.58	25.29	1.29	1.84	2.66 ± 0.40	6.61	0.08	0.76
Leaf powder + PB	1.50	26.64	1.22	1.74	2.29 ± 0.40	5.84	0.11	0.81
Column purified	3.23		2.60	3.80	2.48 ± 0.39	6.28	0.09	0.61
Column purified + MGK	0.01	323.00	0.011	0.02	1.51 ± 0.25	5.86	0.11	0.22
Column purified + PB	0.01	323.00	0.012	0.02	1.62 ± 0.26	6.21	0.10	0.80

Six batches of ten snails were exposed to different concentration of above combination. Mortality was determined every 24h. Concentration given are the final concentration (mg/L, w/v) in the aquarium water. Synergistic ratio (LC_{50} of *S. asoca* leaf powder or column purified/ LC_{50} of binary combination of *S. asoca* leaf powder or column purified with synergist MGK-264 or PB). Abbreviations: LCL- lower confidence limits; UCL- Upper confidence limits; MGK- MGK264. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments, testing significance of the regression coefficient. *S. asoca* leaf powder + MGK (19.43^{+}) *S. asoca* leaf powder + PB (11.13^{+}), column purified of *S. asoca* + MGK (1.54^{+}) column purified of *S. asoca* + PB (5.63^{+}). + : linear regression between x and y; + + : non regression between log x and log y.

(LC_{50} - 1.50 mg/L) was more effective than *S. asoca* leaf powder with MGK-264 (LC_{50} -1.58 mg/L) (Table 3). There was a negative regression between the LC_{50} of different combination of *S. asoca* leaf powder with PB /MGK-264 and different exposure period 24h LC_{50} of binary combination of *S. asoca* bark powder with PB or MGK-264 was 48.06 or 45.00 mg/l, respectively, whereas single treatment up to1500 mg/L of *S. asoca* bark powder caused no mortality in snail *L. acuminata*. Binary combination of saponin with MGK-264 was only 1.07 times more toxic against *L. acuminata* than their single treatment at 24h. At 96h exposure period toxicity of saponin with PB or MGK-264 were not enhanced as synergistic ratio was 0.48 or 0.39, respectively. There was a negative regression between the LC_{50} of different combination of *Saraca asoca* bark powder with PB /MGK-264 and different exposure period.

Binary combination of *Thuja orientalis* leaf powder with PB or MGK-264 was 51.42 or 82.45 times, more toxic against *L. acuminata* than their single treatment at 24h (Table 4). At 96h exposure period toxicity of *T. orientalis* leaf powder with PB or MGK-264 was enhanced 59.51 or 66.63 times, respectively. Binary combination of thujone with PB or MGK-264 was 2.4 or 1.16 times, more toxic against *L. acuminata* than their single treatment at 24h. At 96h exposure period toxicity of thujone with PB or MGK-264 were enhanced 27.5 or 21.15 times, respectively. The 96h toxicity of *T. orientalis* leaf powder with MGK-264 (LC_{50}-3.76 mg/L) against *L. acuminata* was more effective than the *T. orientalis* leaf powder with PB (LC_{50} -4.21 mg/L), thujone with MGK-264 (LC_{50} – 8.13 mg/L) or PB (LC_{50} - 7.57 mg/L), respectively (Table 4). There was a negative regression between LC_{50} of the different combination of *T. orientalis* leaf powder with PB /MGK-264 and different exposure period.

Binary combination of *Thuja orientalis* fruit powder with PB or MGK-264 was 70.98 and 173.88 times, more toxic against *L. acuminata* than their single treatment at 24h. At 96h exposure period toxicity of *T. orientalis* fruit with PB or MGK-264 were enhanced 133.58 and 187.58 times, respectively. Binary combination of column purified fraction of *T. orientalis* fruit with PB or MGK-264 was 10.74 or 7.43 times, more toxic against *L. acuminata* than their single treatment at 24h. At 96h exposure period toxicity of column purified of *T. orientalis* fruit powder with PB or MGK-264 was enhanced 21.75 or 19.77 times, with respect to their single treatment. The 96h toxicity of column purified fraction of *T. orientalis* fruit powder with PB (LC_{50}-0.20 mg/L) against *L. acuminata* was more effective than the column purified fraction of *T. orientalis* fruit powder with MGK-264 (LC_{50} – 0.22 mg/L), *T. orientalis* fruit powder with MGK-264 (LC_{50} -1.36 mg/L) and PB (LC_{50} - 1.91 mg/L) (Table 5). There was a negative regression between the LC_{50} of different combination of *T. orientalis* fruit powder with PB / MGK-264 and different exposure period.

The slope values were steep and separate estimation of LC based on each six replicates were found with in the 95% confidence limits of LC_{50}. The't' ratio was greater than 1.96 and the heterogeneity factor was less than 1.0, the 'g' value was less than 0.5 at all probability (90, 95, 99) levels.

Table 3: Toxicity of binary combination (1:5 ratio) of *Saraca asoca* (bark powder) and active component saponin with synergist MGK-264 or PB against *Lymnaea acuminata*

Exposure period/ Molluscicides	LC_{50} mg/L	Synergi-stic ratio	Limits LCL	UCL	Slope value	t-ratio	g-value	Hetero-geneity
24h Bark powder	-		-	-	-	-	-	-
Bark powder + MGK	45.00		37.03	61.69	2.34±0.39	5.89	0.11	0.27
Bark powder + PB	48.06		39.32	67.37	2.40±0.41	5.77	0.11	0.58
Saponin	7.04		2.49	9.10	2.12±0.38	5.53	0.12	0.26
Saponin + MGK	7.00	1.07	5.72	11.24	1.91±0.38	5.45	0.12	0.40
Saponin + PB	6.79	1.03	5.45	9.63	2.10±0.36	5.72	0.11	0.35
48h Bark powder	-		-	-	-	-	-	-
Bark powder + MGK	41.78		32.58	65.91	1.63±0.34	4.69	0.17	0.32
Bark powder + PB	36.41		29.94	48.83	1.99±0.35	5.59	0.12	0.55
Saponin	5.18		4.77	6.04	1.68±0.32	5.17	0.14	0.27
Saponin + MGK	5.20	1.07	3.95	7.81	1.41±0.28	4.97	0.15	0.34
Saponin + PB	4.96	1.12	3.95	6.75	1.73±0.30	5.77	0.11	0.38
72h Bark powder	-		-	-	-	-	-	-
Bark powder + MGK	25.17		20.40	31.49	1.81±0.33	5.35	0.13	0.71
Bark powder + PB	17.81		7.72	13.21	2.60±0.44	5.09	0.11	0.76
Saponin	1.13		0.16	2.12	1.39±0.30	4.55	0.18	0.28
Saponin + MGK	3.29	0.34	2.32	4.68	1.19±0.26	4.48	0.19	0.46
Saponin + PB	2.96	0.38	2.21	3.86	1.47±0.27	5.44	0.13	0.26
96h Bark powder	-		-	-	-	-	-	-
Bark powder + MGK	16.24		12.49	19.56	2.08±0.35	5.93	0.10	0.99
Bark powder + PB	7.70		4.12	7.06	2.35±0.35	6.65	0.38	0.36
Saponin	0.80		0.06	0.98	2.13±0.32	6.49	0.09	0.67
Saponin + MGK	2.02	0.39	1.49	2.52	1.81±0.27	6.49	0.09	0.78
Saponin + PB	1.66	0.48	1.10	2.17	1.60±0.27	5.81	0.11	0.66

Six batches of ten snails were exposed to different concentration of above combination.Mortality was determined every 24h. Concentrations given are the final concentration (mg/L, w/v) in the aquarium water. No mortality up to 1500 mg/L. Synergistic ratio (LC_{50} of *S. asoca* bark powder or column purified/ LC_{50} of binary combination of *S. asoca* bark powder or column purified with synergist MGK-264 or PB). Abbreviations: LCL- lower confidence limits; UCL- Upper confidence limits; MGK- MGK264.Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments, testing significance of the regression coefficient. *S. asoca* bark powder + MGK (10.09⁺) *S. asoca* bark powder + PB (4.81⁺⁺), saponin + MGK (1.04⁺⁺) saponin + PB (16.73⁺). +: linear regression between x and y; ++: non regression between log x and log

Table 4: Toxicity of binary combination (1:5 ratio) of *Thuja orientalis* (leaf powder) and active component thujone with synergist MGK-264 or PB against *Lymnaea acuminata*

Exposure period/ Molluscicides	LC_{50} mg/L	Synergistic ratio	Limits LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24h Leaf powder	465.89		418.01	550.46	4.15±0.09	6.02	0.10	0.26
Leaf powder + MGK	5.65	82.45	5.16	6.55	4.88±0.86	5.62	0.21	0.24
Leaf powder + PB	9.06	51.42	7.68	12.20	2.87±0.55	5.14	0.145	0.18
Thujone	8.03		4.72	9.49	1.93±0.36	5.23	0.14	0.18
Thujone + MGK	6.89	1.16	14.66	22.40	4.26±0.84	5.04	0.15	0.42
Thujone + PB	3.33	2.4	12.05	15.56	4.21±0.73	5.69	0.11	0.14
48h Leaf powder	391.41		351.02	450.62	3.56±0.61	5.84	0.11	0.19
Leaf powder + MGK	4.97	78.75	4.57	5.54	4.70±0.80	5.83	0.11	0.18
Leaf powder + PB	7.45	52.53	6.22	10.16	2.13±0.48	4.37	0.20	0.16
Thujone	6.59		4.61	8.56	1.74±0.32	5.29	0.13	0.19
Thujone + MGK	4.68	1.4	12.64	20.16	3.02±0.70	4.27	0.21	0.20
Thujone + PB	1.15	5.73	9.97	12.79	3.40±0.68	4.98	0.15	0.16
72h Leaf powder	321.17		280.57	362.95	3.15±0.59	5.42	0.13	0.21
Leaf powder + MGK	4.31	74.51	3.95	4.70	4.66±0.78	5.96	0.10	0.24
Leaf powder + PB	5.55	57.86	4.42	6.96	1.88±0.46	4.01	0.23	0.10
Thujone	4.62		2.26	7.16	1.35±0.30	4.40	0.19	0.26
Thujone + MGK	0.22	21.0	8.86	11.75	2.96±0.67	4.41	0.19	0.24
Thujone + PB	0.18	25.66	7.89	10.27	3.36±0.68	4.92	0.15	0.20
96h Leaf powder	250.55		215.99	278.38	4.05±0.62	6.54	0.09	0.28
Leaf powder + MGK	3.76	66.63	3.45	4.03	5.93±0.84	7.05	0.07	0.65
Leaf powder + PB	4.21	59.51	3.45	4.83	2.89±0.49	5.04	0.11	0.55
Thujone	2.75		1.88	4.44	2.12±0.33	6.43	0.09	0.76
Thujone + MGK	0.13	21.15	7.14	8.89	4.69±0.76	6.17	0.10	0.80
Thujone + PB	0.10	27.5	6.37	8.41	4.29±0.75	5.66	0.12	0.39

Six batches of ten snails were exposed to different concentration of above combination.Mortality was determined every 24h. Concentrations given are the final concentration (mg/L, w/v) in the aquarium water. Synergistic ratio (LC_{50} of *T. orientalis* leaf powder or Thujone/ LC_{50} of binary combination of *T. orientalis* leaf powder or thujone with synergist MGK-264 or PB). Abbreviations: LCL- lower confidence limits; UCL- Upper confidence limits; MGK- MGK264. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments, testing significance of the regression coefficient. *T. orientalis* leaf powder + MGK (29.41^{+}) *T. orientalis* leaf powder + PB (22.31^{+}), thujone + MGK (9.17^{+}) thujone + PB (21.21^{+}). +: linear regression between x and y; + +: non regression between log x and log y.

Table 5: Toxicity of binary combination (1:5 ratio) of *Thuja orientalis* (fruit powder) and column purified fraction with synergist MGK-264 or PB against *Lymnaea acuminata*

Exposure period/ Molluscicides	LC_{50} mg/L	Synergistic ratio	Limits LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24h Fruit powder	638.15		495.71	1324.7	2.32±0.63	3.66	0.28	0.26
Fruit powder + MGK	3.67	173.88	3.08	4.87	2.49±0.44	5.57	0.12	0.20
Fruit powder + PB	8.99	70.98	6.49	17.36	1.62±0.34	4.76	0.16	0.28
Column purified	8.70		7.47	11.26	3.02±0.55	5.43	0.13	0.48
Column purified+MGK	1.17	7.43	0.81	2.28	1.43±0.28	5.09	0.14	0.46
Column purified+ PB	0.81	10.74	0.63	1.18	1.67±0.28	5.97	0.10	0.59
48h Fruit powder	460.44		385.83	670.75	2.28±0.58	3.91	0.25	0.17
Fruit powder + MGK	2.95	156.08	2.37	4.10	1.75±0.38	4.54	0.18	0.27
Fruit powder + PB	5.53	83.26	4.07	9.24	1.28±0.28	4.55	0.18	0.25
Column purified	6.69		5.84	7.97	2.83±0.50	5.63	0.12	0.24
Column purified+MGK	0.71	9.42	0.51	1.20	1.21±0.24	5.50	0.12	0.31
Column purified+ PB	0.57	11.73	0.43	0.83	1.31±0.23	4.69	0.17	0.65
72h Fruit powder	355.37		297.77	441.01	2.23±0.56	3.94	0.24	0.19
Fruit powder + MGK	1.88	189.02	1.35	2.40	1.55±0.37	4.19	0.21	0.27
Fruit powder + PB	3.18	111.75	2.38	4.28	1.44±0.27	5.31	0.13	0.38
Column purified	5.53		4.59	6.17	2.76±0.48	5.67	0.11	0.39
Column purified+MGK	0.48	11.52	0.33	0.80	1.52±0.22	4.24	0.21	0.80
Column purified+ PB	0.38	14.55	0.24	0.58	1.14±0.22	5.06	0.21	0.27
96h Fruit powder	255.12		210.59	289.29	3.22±0.59	5.46	0.12	0.29
Fruit powder + MGK	1.36	187.58	1.00	1.65	2.23±0.39	5.66	0.12	0.93
Fruit powder + PB	1.91	133.57	1.34	2.44	1.65±0.27	6.00	0.10	0.72
Column purified	4.35		3.71	4.91	3.75±0.50	6.55	0.09	0.66
Column purified+MGK	0.22	19.77	0.09	0.34	1.26±0.22	5.59	0.26	0.96
Column purified+ PB	0.20	21.75	0.07	0.33	1.31±0.22	5.05	0.33	0.97

Six batches of ten snails were exposed to different concentration of above combination.Mortality was determined every 24h. Concentrations given are the final concentration (mg/L, w/v) in the aquarium water. Synergistic ratio (LC_{50} of *T. orientalis* fruit powder or column purified/ LC_{50} of binary combination of *T. orientalis* fruit powder or column purified with synergist MGK-264 or PB). Abbreviations: LCL- lower confidence limits; UCL- Upper confidence limits; MGK- MGK264. Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments, testing significance of the regression coefficient. *T. orientalis* fruit powder + MGK (11.27^{+}) *T. orientalis* fruit powder + PB (6.81^{+}), column purified of *T. orientalis* + MGK (5.38^{+}) column purified of *T. orientalis* + PB (5.07^{+}). +: linear regression between x and y; + +: non regression between log x and log y.

DISCUSSION

Binary combination of *S. asoca*, and *T. orientalis* with PB and MGK-264 show effective molluscicidal activity than their single treatment against the snail *L. 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 conducted against harmful snails (Singh *et al.,* 1998a b; Singh and Singh 2003; Singh *et al.,* 2005; Shukla *et al.,* 2005)**.**

Action of binary mixture is non-interactive (Plackett and Hewlett, 1952) as the components 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 *L. 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 pesticides to increase their efficacy against different pests (Casida, 1970; Sahay *et al.,* 1991; Rao and Singh, 2001). They exert their synergistic action mainly by inhibiting the mixed function oxidase (MFO) activity, which detoxify xenobiotics (Metacalf, 1967; Matsumura, 1985; Rao and Singh, 2001) or it may increase the penetration of the toxin which resulted high titer of toxin at active sites (Rao and Singh, 2001). The penetration of the toxicant also have a greater significant for the aquatic environment, because here the whole body is bathed in a diluted solution of the toxicant. To have maximum effect, the synergist must penetrate the organism and transported to active sites rapidly. It seems that high level of synergism in snails may be due to rapid penetration of synergists through soft foot of snails.

Toxicity of *S. asoca* leaf with PB or MGK-264 (96h LC_{50} -1.50 mg/L and 1.58 mg/L), *B. nigra* seed with PB or MGK-264 (96h LC_{50} - 2.06 mg/L and 1.63 mg/L), *T. orientalis* leaf with PB or MGK-264 (96h LC_{50} - 4.21 mg/L and 3.76 mg/L) and *T. orientalis* fruit with PB or MGK-264 (96 LC_{50} – 1.91 mg/L and 1.36 mg/L) against the snail *L. acuminata* in the present study is very high in comparison to synthetic pesticides viz. carbaryl (96h LC_{50} -14.4 mg/L), phorate (96h LC_{50}-15.0 mg/L), aldicarb (96 LC_{50} - 11.5 mg/L), and farmothion (96h LC_{50} - 8.56 mg/L) (Singh and Agarwal, 1983a, b). Toxicity of column extract of *S. asoca* leaf with PB or MGK-264 (96h LC_{50} -0.01 mg/L and 0.01 mg/L), and column extract of *T. orientalis* fruit with PB or MGK-264 (96h LC_{50} -0.20 mg/L and 0.22 mg/L) against the snail *L. acuminata* is very high in comparison to synthetic pyrethroids pesticides permethrin (96h LC_{50}-0.37 mg/L), cypermethrin (96h LC_{50}-0.36 mg/L), and organophosphate pesticide trichlorfon (96h LC_{50} -0.30 mg/L), (Singh and Agarwal, 1991, 1986a, b,). Combination of *S. asoca* leaf/ column extract of *S. asoca* leaf or bark with PB or MGK-264 is more toxic than their single treatment. Highest degree of synergism *i.e.*323 times was observed in the combination of *S. asoca* leaf with PB or MGK-264 at 96h exposure period. Contrarily, combination of PB or MGK-264 with saponin caused no significant synergistic activity. It clearly demonstrates that either the molluscicidal activity of *S. asoca* leaf/bark may be due to saponin as well as other component whose activity is enhanced by the combination of PB or MGK-264. PB or MGK-264 is the mixed function oxidase inhibitors (Wilkinson, 1976; Matsumura, 1985). Due to inhibition of mixed function oxidase the active components is not detoxified, and there is high titer of molluscicidal component at target site. Although, in the present study activity of saponin is identified as molluscicidal component, yet no synergistic action with PB or MGK-264 demonstrate that inside the snail body it is not detoxified by the mixed function oxidase. At higher exposure period there is some antagonistic action as the synergistic ratio is less than 1.0. It also indicates that saponin/ PB/MGK-264 target in snail body may be same, so that the toxicity of saponin is antagonized by PB or MGK-264.

Combination of *T. orientalis* leaf/ thujone or fruit powder/ column extract of *T. orientalis* fruit with PB or MGK-264 indicate that it enhance the toxicity up to 189.02 times. Potentiation of molluscicidal activity of *T. orientalis* fruit with PB or MGK-264 clearly indicate that in contrast to saponin, *T. orientalis* leaf/ thujone toxicity is synergized more effectively. It demonstrates that mfo inhibitor PB or MGK-264 check the detoxification of thujone in the snail body, which cause several times enhancement in their toxicity against *L. acuminata*. Highest concentration of PB (300.54 mg/L) and MGK-264 (260.50 mg/L) mixture are not toxic to snail as reported earlier by Singh and Agarwal, (1989) and Sahay *et al.,* (1991).

The steep slope indicates 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 is less than 1.0 denote that in the replicates test of random sample the concentration response curves fall within the 95% confidence limits and thus the model fits the data adequately. The index of significance of potency estimation, g-value indicates the value of the mean is within the limits at all probabilities (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 population of snails in threshold level. The effective concentration in combination is certainly lower than their individual component.

REFERENCES

Agarwal, R. A. and Singh, D. K. 1988. Harmful gastropods and their control. *Acta Hydrochim. Hydrobiol.* **16:** 113-138.

Casida, J. E. 1970. Mixed-function oxidase involvement in the biochemistry of insecticide synergist. *J. Agric. Food. Chem.* **12:** 294-304.

Godan, D. 1983. Pest Slugs and Snails, Biology and Control (de. Dora godan) translated by Shelia Gruber, Springer verlog, Berlin, Heidelberg, New York.

Jaiswal, P. and Singh, D. K. 2008. Molluscicidal activity of *Carica papaya* and *Areca catechu* against the fresh water snail *Lymnaea acuminata*. *Vet. Parasitol.* **152:** 264-270.

Kumar, P. and Singh, D. K. 2007. Binary combination of some common spices against harmful snails. *J. Appl. Biosci.* **33(2):** 167-170.

Mas-Coma, S., Bargues, M. D. and Valero, M. A. 2005. Fascioliasis and other plant- borne trematode zoonose. *Int. Parasitol.* **35:** 1255-1278.

Matsumura, F. 1985. Toxicology of Insecticides. 2nd Ed. Plenum Press, New York.

Metacalf, R. L. 1967. Mode of action of insecticide synergists. *Ann. Rev. Entomol*. **112:** 229-256.

Osman, E. A., Mohamed, E. M., Abu Elreesh, B. I. and Elegami, A. A. 2007. Molluscicidal activity of *Combretum glutinosum*. *International J. of Mole. Med. And Adv. Sci.* **3 (4):** 151- 154.

Plackett, R. L. and Hewlett, P. S. 1952. Quantal responses to mixtures of poisons, J. R. *Statist. Soc. B*. **14:** 141-63.

Rao, I. G. and Singh, D. K. 2001. Combination of *Azadirachta indica* and *Cedrus deodara* oil, with piperonyl butoxide, MGK-264 and *Embelia ribes* against *Lymnaea acuminata*. *Chemosphere* **14:** 1691-1695.

Robertson, J. L. Russell, R. M., Preisler H. K. and Savin, N. E. 2007. *Bioassay with Arthropods* POLO computer programme for analysis of bioassay data. 2nd Eds (Talor and Francis, CRC Press) 1-224pp.

Sahay, N., Singh, D. K. and Agarwal, R. A. 1991. Synergistic effect of piperonyl butoxide on the toxicity of synergistic pyrethroids in the *Lymnaea acuminata*. *J. Med. Appl. Malacol.* **3:** 107-111.

Saba, R. Korkmaz, M., Inan, D., Mamikoglu, L., Turhan, O., Gunseren, F., Cevikol, C. and Kabaalioglu, A. 2004. Human fascioliasis. *Clinical microbial. Infection* **10:** 385-387.

Shafer, T. J., Meyer, D. A. and Crofton, K. M. 2005. Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. *Environ. Health Persp.* **113:** 123-136.

Shukla, S., Singh, V. K. and Singh, D. K. 2005. Binary and tertiary combinations of plant derived molluscicides against the vector snails *Lymnaea acuminata* and *Indoplanorbis exustus*. *Malys. Appl. Biol.* **34(2):** 15-24.

Singh, A., Singh, D. K., Mishra, T. N. and Agarwal, R. A. 1996. Molluscicide of Plant origin. *Bio. Agric. and Horti*. **13:** 205-252.

Singh, D. K. and Agarwal, R. A. 1984. Correlation of the anticholinesterase and molluscicidal activity of the latex of *Euphorbia royleana* on the snail *L. acuminata*. *J. of Nat. Prod.* **47:** 702-705.

Singh, D. K. and Agarwal, R. A. 1986a. Synergistic effect of sulfoxide with carbaryl on the *in vivo* acetylcholinesterase activity and carbohydrate metabolism of the snail *L. acuminata*. *Acta Hydrochim. Hydrobiol*. **14(4):** 421-427.

Singh, D. K. and Agarwal, R. A. 1986b. Piperonyl butoxide synergism with two synthetic pyrethroids against *L. acuminata*. *Chemosphere.* **15:** 493-498.

Singh, D. K. and Agarwal, R. A. 1989. Toxicity of piperonyl butoxide-carbaryl synergism on the snail *L. acuminata*. *Int. Revue. Ges. Hydrobiol*. **74(6):** 689-699.

Singh, D. K. and Agarwal, R. A. 1991. Action sites of cypermethrin, a synthetic pyrethroid in the snail *L. acuminata*. *Acta Hydrochim. Hydrobiol*. **19(4):** 425-430.

Singh, K., Singh, A. and Singh, D. K. 1998a. Synergism of MGK-264 and piperonyl butoxide on the toxicity of plant derived molluscicides. *Chemosphere.* **36:** 3055-3060.

Singh, K., Singh, A. and Singh, D. K. 1998b. The use of piperonyl butoxide and MGK-264 to improve the efficacy of some plant derived molluscicides. *Pestic. Sci.* **54:** 145-149.

Singh, O. and Agarwal, R. A. 1981. Toxicity of certain pesticides to two economic species of snails in northern India. *J.Econ. Entomol*. **74:** 568-571.

Singh, P. and Singh, D. K. 2003. Toxicity of binary combinations of plant derived molluscicides with MGK-264 and piperonyl butoxide against *L. acuminata*. *Biol. Memoirs*. **29(2):** 78-84.

Singh, P., Singh, V. K. and Singh, D. K. 2005. Effect of binary combination of some plant derived molluscicides with MGK-264 or piperonyl butoxide on the reproduction of the snail *L. acuminata*. *Pes. Manag. Sci.* **61:** 204-208.

Singh, V. and Singh, D. K. 2009a. The effect of abiotic factors on the toxicity of cypermethrin against the snail *Lymnaea acuminata* in the control of fascioliasis. *J Helminthology* **83:** 39-45.

Singh, A. and Singh, V. K. 2009b. Molluscicidal activity of *Saraca asoca* and *Thuja orientalis* against the fresh water snail *Lymnaea acuminata*. *Vet. Parasitol.* **164:** 206-210.

Singh, P., Kumar, P., Singh V. K. and Singh, D. K. 2009. Effect of snail attractant pellets containing plant molluscicides on certain enzymes in the nervous tissue of *Lymnaea acuminata*. *The Bioscan* **4(3):** 395-398.

Singh, D. K. and Agarwal, R. A. 1983a. *In vivo* and *in vitro* studies on synergism with anticholinesterase pesticides in the snail *Lymnaea acuminata*. *Arch. Environ. Cantam. Toxicol.* **12:** 483-487.

Singh, D. K. and Agarwal, R. A. 1983b. Inhibition kinetics of certain organophosphorous and carbamate pesticides on acetylcholinesterase from the snail *Lymnaea acuminata*. *Toxicology Letters* **19:** 313-319.

Sokal, R. R. and Rohlf, F. J. 1976. Introduction to Biostatistics. Freemann, W. H., San Francisco. p.368.

Wilkinson, C. F. 1976. Insecticide synergism. In: R. L. Metcalf and J. J. Mckelvey (eds). The future for insecticide need and prospects. New York, Awily Interscience, p.195.