http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (20): 992-996, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.992.996

Tertiary Combination of Freeze-dried Urine of Indian Breeds of Cow with Plant Products Against Snail *Lymnaea acuminata*

Shiv Kumar, D.K. Singh and Vinay Kumar Singh
Malacology Laboratory, Department of Zoology, DDU Gorakhpur University,
Gorakhpur-273009 U.P. India

Abstracts: Snail *Lymnaea acuminata* is the intermediate host of liver fluke *Fasciola gigantica*, which cause endemic fasciolosis among cattle population of eastern Uttar Pradesh. Control of snail population by molluscicides is one of the effective methods to control fasciolosis. In the present study molluscicidal activity of tertiary combination of freeze-dried urine of different Indian breeds of cow Sahiwal, Geer and Tharparkar with *Annona squamosa* seed powder, *Ferula asafoetida* root latex, Azadirachta indica oil and *Camellia sinensis* leaves have been tested against *Lymnaea acuminata*. It was noted that the toxicity of tertiary combination (1:1:5) of cow urine kept for 15 days in sunlight or laboratory condition with different plant products were highly toxic against snail *L. acuminata*. 96 h LC_{50} of tertiary combinations with Sahiwal urine kept for 15 days in sunlight with *A. squamosa*, *F. asafoetida*, *A. indica* oil and *C. sinensis* were 35.47 mg L^{-1}, 37.13 mg L^{-1}, 33.66 mg L^{-1}, respectively higher than the Geer and Tharparkar. The toxicity of Sahiwal urine kept for 15 days in laboratory condition with *A. squamosa* and *C. sinensis* (96 h LC_{50} 28.28 mg L^{-1}) was more potent than the all other combinations. Cow urine in combination with plant product can be used for effective control of snail.

Key words: Cow urine, plant products, *Lymnaea acuminata,* fasciolosis, synergist, snail

INTRODUCTION

Fasciolosis is one of the emerging or re-emerging diseases in recent time (Mas-Coma *et al.,* 2005). *Fasciola hepatica* and *F. gigantica* are the causative agents of the fasciolosis in cattle and human population (Singh and Agarwal, 1981; Mas-Coma *et al.,* 2009; Robinson and Dalton, 2009). According to WHO (2006) 2.4 million people are infected with *Fasciola* species and a further 180 million are at risk of infection. The snail *Lymnaea acuminata* is the intermediate host of the liver flukes *F. gigantica* (Singh and Agarwal, 1981; Agarwal and Singh, 1988). Extensive use of synthetic molluscicides in aquatic environment is not safe to the non target organism, sharing the same habitat with snails. Alternatively, use of native plants and animal products as molluscicide will be safer and user friendly. Plants molluscicides contain several active compounds that are more effective than their synthetic counterpart (Singh *et al.,* 1996). In Indian system of aurveda cow urine has been used in treatment of various diseases (Shastri, 1998). Recently, Kumar *et al.* (2011a, b) have reported that urine obtained from different Indian breeds of cow and their formulations are very effective in killing the snails. In the present study, molluscicidal activity of cow urine of native breed and their tertiary combination with plants products against the snail *L. acuminata* have been studied to explore the use of safe indigenous product to control the fasciolosis.

MATERIALS AND METHODS

Three to four years old Indian breeds of cow i.e., Sahiwal, Geer and Tharparkar were selected in local live-stock keepers. The urine was collected sterilized bottle for experimental purpose.

Preparation of Freeze-dried cow urine (FCU): Sahiwal, Geer and Tharparkar cow urine were kept for 15 days in sunlight (8 h $days^{-1}$) or in laboratory condition. Thereafter, cow urine preparations were freeze dried.

Tertiary combinations (1:1:5) of plant products such as *Annona squamosa* seed powder, Ferula asafoetida root latex powder, *Azadirachta indica* oil with *Camellia sinensis* leaves and freeze-dried cow urine (FCU) were prepared by the method of Shukla *et al.* (2005).

Corresponding Author: Vinay Kumar Singh, Malacology Laboratory, Department of Zoology, DDU Gorakhpur University, Gorakhpur-273009 U.P. India Tel:+91-9415855488

Plants used: Plants *A. squamosa* and *F. asafoetida* were collected locally and identified at the herbarium of the Botany Department, DDD Gorakhpur University, Gorakhpur where voucher herbarium specimens (No. 3228 for *A. squamosa* and No. 3815 for *F. asafoetida*) are on deposit. *A. indica* oil was obtained from Indian Herbs Saharanpur, UP, India. *A. squamosa* seed powder was prepared by the method of Singh and Singh (2001).

Test animal: The adult snail *L. acuminata* (2.60±0.30 cm in length) were collected locally from ponds, pools, lakes and low-lying submerged areas located almost adjacent to DDU Gorakhpur University campus. These snails were attached on the ventral surface of the leaves of aquatic plants. The collected snails were acclimatized to laboratory condition in dechlorinated tap water for 72 h and then used for toxicity study.

Experiment: Toxicity experiments were done by the method of Singh and Agarwal (1984). Ten experimental animals were kept in a glass aquarium containing 3 L of de-chlorinated tap water at 22 to 24°C. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.2, 5.2-6.3 and 102.0-105.0 mg L^{-1}, respectively. Snails were exposed continuously for 96 h to different concentration of cow urine formulations with plant products, separately. Six aquaria were set up for each concentration. The control animals were kept in the equal amount of water under similar conditions without treatment. Mortality of snail was recorded at interval of 24 h each up to 96 h The dead animals were removed immediately to avoid contamination in aquarium water.

Statistical analysis: The LC_{50} value, lower and upper confidence limits (LCL and UCL), slope value, t-ratio, g-value and heterogeneity factors were calculated with the help of POLO computer software programme of Robertson *et al.* (2007). This program uses the heterogeneity factor as a correction factor when the value of Pearson's chi-square statistic is significant at $p<0.05$. The regression coefficient between exposure time and different values of LC_{50} was determined by the method of Sokal and Rohlf (1973).

RESULTS

The fresh urine of different Indian breeds of cow was usually pale yellow in colour. It becomes dark brown when kept in sunlight (8 h day^{-1}) for 15 days. Colour of urine kept in laboratory condition for 15 days was light brown. The toxicity of tertiary combination of plant molluscicides and lyophilized cow urine powder of different breeds such as Sahiwal, Geer and Tharparkar against *L. acuminata* was time and concentration dependent. Toxicity of tertiary combination of Sahiwal cow urine kept for 15 days in sunlight+*A. squamosa* seed powder+*C. sinensis* leaves/ *F. asafoetida*+*C. sinensis*/ *A. indica* oil+*C. sinensis* (24 h LC_{50} 90.76, 129.47 and 78.59 mg L^{-1}) was more effective than the Geer (24 h LC_{50} 100.76, 135.08 and 88.08 mg L^{-1}) and Tharparkar (24 h LC_{50} 97.18, 140.13 and 90.33 mg L^{-1}), respectively (Table 1).

Tertiary combination of Sahiwal cow urine kept for 15 days in laboratory condition with+*A. squamosa* seed powder+*C. sinensis* leaves, *F. asafoetida*+*C. sinensis*, *A. indica* oil+*C. sinensis* (24 h LC_{50} 72.33, 99.89 and 82.87 mg L^{-1}) was more potent than the Geer (24 h LC_{50} 80.63, 109.17 and 91.08 mg L^{-1}) and Tharparkar formulations (24 h LC_{50} 82.34, 113.98 and 96.51 mg L^{-1}), respectively (Table 2).

The slope values were steep and separate estimation of LC_{50} based on each of the six replicates was 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 p value was less than 0.5 at all probability levels (90, 95 and 99).

DISCUSSION

It is evident from the present study that tertiary formulation (1:1:5) of cow urine with *A. squamosa* seed powder/ *F. asafoetida* root latex/ *A. indica* oil and *C. sinensis* leaves are potent molluscicide against *L. acuminata*. Tertiary combination of Sahiwal cow urine kept for 15 days in sunlight (8 h day^{-1}) or laboratory condition with *A. squamosa* seed powder/ *F. asafoetida* root latex/ *A. indica* oil+*C. sinensis* leaves were more potent than the Geer and Tharparkar. Cow urine is considered to be the effective animal product used in general health improvement (Khanuja *et al.*, 2005). It contains various volatile and non volatile compounds which has significant antimicrobial activity (Shaw *et al.*, 2007; Sathasivam *et al.*, 2010). Uric acid noted in cow urine has a strong antibacterial activity (Kumar *et al.*, (2011a, b). Cow urine distilled has been patented as an activity enhancer and availability facilitator for bio molecules including anti-infective and anti-cancer agents (Khanuja *et al.*, 2002-US Patent No 6410 059/2002). Probably, in the present study cow urine act as bio-enhancer with plant products, therefore higher titer of active molluscicidal components of plants reaches at active site in snail body. In Ayurvedic system cow urine is widely used in combination with drugs and

Table 1: Toxicity of freeze-dried cow urine powder (FCU) , urine kept for 15 days in sunlight of different breeds cow Sahiwal, Geer, Tharparkar and its tertiary combination (1:1:5), *A. squamosa, F. asafoetida* and *A. indica* oil with *C. sinensis* against *L. acuminata* at different exposure period

Exposure period (h)	Treatment	C_{50} mg L^{-1} (w/v)	Limits LCL-UCL	Slope value	T-ratio	p-value	Heterogeneity
	Sh.+A.S.+C.S.	90.76	72.65-150.93	1.98±0.49	3.98	0.24	0.22
	Gr.+A.S.+C.S.	100.76	82.48-160.33	2.98±0.47	3.65	0.18	0.28
	Th.+A.S.+C.S.	97.18	79.96-151.16	2.18±0.47	3.64	0.18	0.22
24	Sh.+F.S.+C.S.	129.47	91.73-415.33	1.74±0.52	3.34	0.17	0.34
	Gr.+F.S.+C.S.	135.08	101.75-420.81	2.61±0.46	3.96	0.24	0.46
	Th.+F.S.+C.S.	140.13	102.51-416.15	1.96±0.46	3.03	0.17	0.34
	Sh.+A.I.+C.S.	78.59	65.16-111.23	2.12±0.49	4.50	0.18	0.20
	Gr.+A.I.+C.S.	88.08	75.91-121.18	2.12±0.46	3.41	0.14	0.25
	Th.+A.I.+C.S.	90.33	78.91-123.29	1.89±0.52	3.75	0.23	0.25
	Sh.+A.S+C.S.	69.23	55.76-101.24	1.72±0.47	3.45	0.21	0.28
	Gr.+A.S+C.S.	79.48	65.17-111.28	1.92±0.52	3.17	0.17	0.21
	Th.+A.S.+C.S.	78.55	64.14-110.98	2.16±0.36	3.92	0.16	0.27
	Sh.+F.S.+C.S.	77.34	62.99-122.18	2.24±0.48	3.75	0.16	0.34
48	Gr.+F.S.+C.S.	83.08	67.98-127.34	1.80±0.46	3.90	0.17	0.22
	Th.+F.S.+C.S.	88.59	72.55-132.36	1.96±0.24	3.50	0.15	0.46
	Sh.+A.I.+C.S.	58.57	47.79-73.57	1.97±0.47	4.17	0.22	0.57
	Gr.+A.I.+C.S.	68.33	57.65-83.18	2.98±0.46	4.34	0.15	0.22
	Th.+A.I.+C.S.	65.98	55.99-82.34	2.46±0.48	4.75	0.17	0.23
	Sh.+A.S+C.S.	49.04	34.48-62.36	1.72±0.46	3.55	0.31	0.40
	Gr.+A.S+C.S.	59.14	44.43-72.78	2.61±0.24	3.46	0.21	0.24
	Th.+A.S.+C.S.	55.47	40.56-68.24	2.36±0.15	3.81	0.18	0.50
72	Sh.+F.S.+C.S.	56.34	44.55-71.78	1.81±0.46	3.86	0.25	0.31
	Gr.+F.S.+C.S.	61.47	50.18-76.98	1.89±0.49	3.46	0.31	0.34
	Th.+F.S.+C.S.	64.82	52.48-79.76	1.61±0.46	4.30	0.34	0.40
	Sh.+A.I.+C.S.	41.71	29.05-51.13	1.92±0.47	4.03	0.23	0.57
	Gr.+A.I.+C.S.	51.33	39.76-60.98	1.32±0.47	4.34	0.18	0.23
	Th.+A.I.+C.S.	53.34	41.16-63.34	1.74±0.46	4.17	0.14	0.27
	Sh.+A.S+C.S.	35.47	25.51-42.34	2.42±0.49	4.92	0.11	0.40
	Gr.+A.S+C.S.	44.96	35.78-52.54	3.41±0.48	4.90	0.21	0.57
	Th.+A.S.+C.S.	40.46	30.76-47.54	2.13±0.46	3.91	0.16	0.28
96	Sh.+F.S.+C.S.	37.13	25.91-44.98	2.16±0.72	4.50	0.18	0.24
	Gr.+F.S.+C.S.	42.04	30.48-50.93	2.42±0.54	4.46	0.31	0.26
	Th.+F.S.+C.S.	45.23	38.41-52.18	1.96±0.49	3.87	0.16	0.50
	Sh.+A.I.+C.S.	33.66	23.75-40.45	2.46±0.49	4.95	0.15	0.81
	Gr.+A.I.+C.S.	43.16	31.98-40.34	2.32±0.47	4.62	0.18	0.23
	Th.+A.I.+C.S.	35.28	25.01-42.64	2.30±0.54	4.87	0.12	0.40

Six batches of 10 snails were exposed different concentration of the above molluscicides with different breeds of cow urine. Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments. Ts-testing significant of the regression coefficient: Sh.+A.S.+C.S.-14.17+; Gr.+A.S.+C.S.-15.88+; Th.+A.S.+C.S.-10.21+; Sh.+F.A.+C.S.-15.62++; Gr.+F.A.+C.S.-6.54++; Th.+F.A.+CS-7.60++; Sh.+A.I.+C.S.-9.47++; Gr.+A.I.+C.S.-44.15+; Th.+A.I.+C.S.-13.79++. +: linear regression between *x* and y, ++: non–linear regression between log x and log y. Abbreviations; A.I:.*Azadirachta indica,* A.S: *Annona squamosa,* C.S: *Camellia sinensis,* F.A:.*Ferula asafoetida,* Gr:Geer, Sh: Sahiwal, Th:Tharparkar, LCL: Lower confidence limit UCL: upper confidence limit

pharmaceuticals. Cow urine contains few essential component such as estrogen, nitrogen, phosphorus, pheromone, potassium, chloride, calcium and urinary protein (Biddle *et al.,* 2007; Yan *et al.,* 2007; Bravo *et al.,* 2003). Carbolic acid, which is mixture of phenol and cresol in cow urine, has sufficient disinfection activity (Kelly, 1997; Khanuja *et al.,* 2002). Annonaceous acetogenins are cytotoxic and it has antitumor activity (Fang *et al.,* 1993). Kumar *et al.,* (2011a,b) have reported that binary combination of cow urine with *A. squamosa* seed/ *F. asafoetida* root latex/ *A. indica* oil is toxic against *L. acuminata.* Sublethal exposure of cow urine and their formulations with plant products significantly block the reproductive capacity of snail *L. acuminata* (Tripathi *et al.,* 2010). Higher toxicity of cow urine formulations at 96 h exposure periods, may be due to accumulation of higher titer of molluscicidal components on active site or degradation of plant products in more toxic form in 96 h. The toxicity of tertiary combination of different breeds of cow urine and plant molluscicides may be due to action of three components at three different sites in the snail body, which ultimately resulted higher toxicity against the snail *L. acuminata.*

It is evident that the steep slope value that a small increase in the tertiary combination of plant derived molluscicides such as *A. squamosa* seed powder, *A. indica* oil, *F. asafoetida* root latex and *C. sinensis* leaves with freeze dried powder of different Indian breeds cow urine kept for 15 days laboratory and sunlight (8 h $days^{-1}$) was causes a higher mortality in snail. A t-ratio value greater than 1.96 indicated that the regression is significant. Heterogeneity factor value is less than 1.0 denoted that in the replicate test of random sample, the concentration response line would fall within

Table 2: Toxicity of freeze-dried cow urine powder (FCU) , urine kept for 15 days in laboratory condition of different breeds cow Sahiwal, Geer, Tharparkar and its tertiary combination (1:1:5), *A. squamosa, F. asafoetida* and *A. indica* oil with *C. sinensis,* against *L. acuminata* at different exposure period

Exposure period (h)	Treatment	LC_{50} mg L^{-1} (w/v)	Limits LCL-UCL	Slope value	T-ratio	p-value	Heterogeneity
	Sh.+A.S.+C.S.	72.33	60.19-97.91	2.67±0.52	4.11	0.14	0.16
	Gr.+A.S.+C.S.	80.63	69.98-104.74	3.16±0.47	4.62	0.17	0.18
	Th.+A.S.+C.S.	82.34	70.49-107.96	2.67±0.48	4.75	0.16	0.17
24	Sh.+F.S.+C.S.	99.89	79.51-168.72	2.16±0.52	4.16	0.22	0.22
	Gr.+F.S.+C.S.	109.17	89.19-178.96	3.24±0.52	4.05	0.20	0.50
	Th.+F.S.+C.S.	113.98	93.15-182.27	3.16±0.38	4.87	0.23	0.49
	Sh.+A.I.+C.S.	82.87	69.33-115.01	2.35±0.50	4.63	0.17	0.34
	Gr.+A.I.+C.S.	91.08	78.99-124.18	2.38±0.46	3.63	0.34	0.49
	Th.+A.I.+C.S.	96.51	83.57-129.72	2.74±0.30	3.96	0.17	0.49
	Sh.+A.S+C.S.	55.33	45.99-72.55	2.67±0.52	4.34	0.14	0.16
	Gr.+A.S+C.S.	65.98	55.28-82.55	2.30±0.48	4.56	0.19	0.22
	Th.+A.S.+C.S.	61.55	51.99-81.34	2.46±0.34	4.87	0.18	0.22
48	Sh.+F.S.+C.S.	68.54	57.91-87.85	2.24±0.56	4.62	0.22	0.29
	Gr.+F.S.+C.S.	73.82	62.96-92.18	1.80±0.46	3.98	0.34	0.50
	Th.+F.S.+C.S.	81.61	70.55-100.85	3.01±0.52	4.34	0.16	0.27
	Sh.+A.I.+C.S.	67.58	56.64-87.60	2.13±0.48	4.42	0.19	0.17
	Gr.+A.I.+C.S.	77.53	66.91-97.96	2.32±0.48	4.17	0.15	0.22
	Th.+A.I.+C.S.	75.97	64.19-95.58	2.34±0.16	4.24	0.18	0.31
	Sh.+A.S+C.S.	39.67	30.49-48.55	2.32±0.47	3.87	0.16	0.17
	Gr.+A.S+C.S.	47.63	38.47-56.34	2.30±0.34	4.33	0.22	0.18
	Th.+A.S.+C.S.	49.98	40.58-58.46	2.16±0.38	4.81	0.23	0.23
	Sh.+F.S.+C.S.	55.58	44.27-69.64	1.88±0.46	4.01	0.18	0.35
72	Gr.+F.S.+C.S.	58.67	47.91-72.55	2.12±0.49	4.17	0.23	0.31
	Th.+F.S.+C.S.	66.82	55.91-80.34	2.67±0.52	4.65	0.22	0.17
	Sh.+A.I.+C.S.	50.34	39.89-60.62	2.06±0.33	4.38	0.20	0.22
	Gr.+A.I.+C.S.	57.33	46.96-67.13	2.46±0.47	4.22	0.23	0.40
	Th.+A.I.+C.S.	53.58	43.96-65.64	2.88±0.48	4.33	0.18	0.40
	Sh.+A.S+C.S.	28.28	20.05-34.64	2.75±0.56	4.54	0.23	0.21
	Gr.+A.S+C.S.	38.24	30.91-44.65	2.12±0.47	5.12	0.12	0.23
	Th.+A.S.+C.S.	43.67	35.01-49.64	2.12±0.49	5.17	0.12	0.21
96	Sh.+F.S.+C.S.	33.98	22.38-41.79	2.74±0.52	4.45	0.19	0.22
	Gr.+F.S.+C.S.	41.82	30.96-48.34	3.16±0.33	4.68	0.22	0.49
	Th.+F.S.+C.S.	45.54	34.99-53.55	2.58±0.36	4.12	0 .16	0.25
	Sh.+A.I.+C.S.	36.73	27.68-43.43	2.22±0.55	5.23	0.14	0.40
	Gr.+A.I.+C.S.	48.86	39.18-57.97	2.96±0.47	4.76	0.17	0.22
	Th.+A.I.+C.S.	39.28	30.01-46.85	3.01±0.34	4.34	0.12	0.36

Six batches of 10 snails were exposed different concentration of the above molluscicides with different breeds of cow urine. Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments. Ts-testing significant of the regression coefficient: Sh.+A.S.+C.S.-8.61++; Gr.+A.S.+C.S.-22.86+; Th.+A.S.+C.S.-9.12+; Sh.+F.A.+C.S.-15.40++; GrCU+FA+CS-15.23++; ThCU+FA+CS-10.12+; ShCU+AI+CS-7.30++; GrCU+AI+CS-15.28+; h CU+AI+CS-7.10++.: inear regression between x and y; ++: non–linear regression between log x and log y. A.I: *Azadirachta indica,* A.S: *Annona squamosa,* C.S: *Camellia sinensis,* F.A: *Ferula asafoetida,* Gr: Geer, Sh-Sahiwal, Th: Tharparkar, LCL: Lower confidence limit UCL: uper confidence limit

95% confidence limits and thus the model fits our data adequately. The index of significant of protein estimation p-value indicates that the value of mean is within the limits at all probability level of 90, 95 and 99%.

CONCLUSION

Conclusively, it can be stated that the urine of Indian cow breeds and their formulations with different native plants can be used as potent molluscicides. These products are easily available and ecologically and culturally more acceptable to live-stock keepers and farmers of this region than their synthetic molluscicides. Toxicity of Sahiwal urine formulations is more potent than Geer and Tharparkar.

ACKNOWLEDGMENT

One of the authors (Shiv Kumar) is thankful to Rajiv Gandhi National Fellowship (RJNF), University Grants Commission, New Delhi letter No. F. 14-2 (SC)/2010 (SA-III) for financial assistance.

REFERENCES

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

Biddle, S., P. Teale, A. Robinson, J. Bowman and E. Houghton, 2007. Gas chromatography-mass spectrometry/mass spectrometry analysis to determine natural and post-administration levels of oestrogens in bovine serum and urine. Anal. Chim. Acta, 586: 115-121.

Bravo, D., D. Sauvant, C. Bogaert and F. Meschy, 2003. Quantitative aspects of phosphorous excretion in ruminants. Reprod. Nutr. Dev., 43: 285-300.

Fang, X.P., M.J. Rieser, Z.M. Gu, G.X. Zhao and J.L. McLaughlin, 1993. Annonaceous acetogenins: An updated review. Phytochem. Anal., 4: 27-49.

Kelly, J.F., 1997. The urine cure and other curious medical treatments. http://wfmu.org/LCD/19/urine.html.

Khanuja, S.P., S. Kumar, A.K. Shasany, J.S. Arya and M.P. Darokar *et al*., 2002. Pharmaceutical composition containing cow urine distillate and an antibiotic. U.S. Patent No. 6410059. http://www.freepatentsonline.com/6410059.html

Khanuja, S.P., S. Kumar, A.K. Shasany, J.S. Arya and M.P. Darokar *et al*., 2005. Use of bioactive fraction from cow urine distillate (go-mutra) as a bio-enhancer of anti-infective, anti-cancer agents and nutrients. U.S. Patent No. 6896907. http://www.directorypatent.com/US/6896907.html

Kumar S., D.K. Singh and V.K. Singh, 2011a. Binary combination of different breeds of Freeze-dried Cow Urine (FCU) with some plant molluscicides against Lymnaea acuminata: Vector of fasciolosis. Adv. Life Sci., 1: 24-29.

Kumar, S., D.K. Singh and V.K. Singh, 2011b. Molluscicidal effect of freeze-dried cow urine of Indian cow breeds against Lymnaea acuminata. Am.-Eurasian J. Toxicol. Sci., 3: 70-74.

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

Mas-Coma, S., M.A. Valero and M.D. Bargues, 2009. Fasciola, Lymnaeids and Human Fascioliasis, with a Global Overview on Disease Transmission, Epidemiology, Evolutionary Genetics, Molecular Epidemiology and Control. In: Advances in Parasitology, Rollinson, D. and S.I. Hay (Eds.). Vol. 69, Chapter 2. Academic Press, Burlington, USA., ISBN: 978-0-12-374795-2, pp: 141-146.

Robertson, J.L., R.M. Russell, H.K. Preisler and N.E. Savin, 2007. Bioassay with Arthropods. 2nd Edn., CRC Press/Taylor and Francis, Boca Raton, FL., USA., ISBN-13: 978-0849323317, Pages: 224.

Robinson, M.W. and J.P. Dalton, 2009. Zoonotic helminth infections with particular emphasis on fasciolosis and other trematodiases. Phil. Trans. R. Soc, Lond. B. Biol Sci., 364: 2763-2776.

Sathasivam, A., M. Muthuselvam and R. Rajendran, 2010. Antimicrobial activities of cow urine distillate against some clinical pathogens. Global J. Pharmacol., 4: 41-44.

Shastri, A.D., 1998. Shushrut Samhita. 11th Edn., Chaukhamba Sanskrit Prakashan, Varanasi, India.

Shaw, S.L., F.M. Mitloehner, W. Jackson, E.J. DePeters and J.G. Fadel et al., 2007. Volatile organic compound emissions from dairy cows and their waste as measured by proton-transfer-reaction mass spectrometry. Environ. Sci. Technol., 14: 1310-1316.

Shukla, S., V.K. Singh and D.K. Singh, 2005. Binary and Tertiary combinations of plant derived molluscicides against the vector snails Lymnaea acuminata and Indoplanorbis exustus. Malaysian Applied Biol., 34: 15-24.

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

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

Singh, A., D.K. Singh, T.N. Misra and R.A. Agarwal, 1996. Molluscicides of plant origin. Biol. Agric. Hortic., 13: 205-252.

Singh, A. and D. K. Singh, 2001. Molluscicidal activity of the custard apple (Annona squamosa L.) alone and in combination with other plant derived molluscicides. J. Herbs Spices Med. Plants, 8: 23-29.

Sokal, R.R. and F.J. Rohlf, 1973. Introduction to Biostatistics. W.H. Freeman and Co., San Francisco, Pages: 365.

Tripathi, R., P. Kumar, V.K. Singh and D.K. Singh, 2010. Cow urine and their formulations with plant molluscicide against the reproduction of vector snail Lymnaea acuminata. Drug Invention Today, 2: 376-380.

WHO, 2006. Report of the WHO informal meeting on use of triclabendazole in fascioliasis control. WHO headquarter, Geneva, Switzerland. 17-18 October.

Yan, T., J.P. Frost, T.W. Keadya, R.E. Agnew and C.S. Mayne, 2007. Prediction of nitrogen excretion in feces and urine of beef cattle offered diets containing grass silage. J. Anim. Sci., 85: 1982-1989.