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Alteration in Certain Biochemical Parameters Fed to Bait Containing Piperine against *Lymnaea acuminata*: Intermediate Host of *Fasciola gigantica*

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ABSTRACT

Liver fluke infection (fascioliasis) is now neglected tropical disease caused by *Fasciola hepatica* and *F. gigantica*. Snails of family *Lymnaeidae* are the intermediate host of *F. gigantica*. Snail control is considered as one of the best method of choice to eliminate fascioliasis. Bait formulation containing molluscicides would be an effective tool to snail control. There was a significant ($p<0.05$) reduction in protein, amino acids, DNA, RNA and AChE activity in ovotestis/nervous tissue of snail *Lymnaea acuminata*. Feeding of 96 h exposure period of bait containing molluscicides Piperine+Serine+Agar (40% of 24 h LC_{50}) caused maximum reduction in protein (75.27% of control) in month of August, amino acids (78.37% of control) in month of March, DNA (72.47% of control) in month of May and RNA (75.03% of control) in month of October in ovotestis of *L. acuminata*. There was a significant ($p<0.05$) inhibition in AChE activity with the feeding of bait containing plant derived molluscicides Piperine+Serine+Agar (40% of 24 h LC_{50}) of 96 h exposure period. Maximum inhibition in AChE activity (46.42% of control) in month of October was noted in nervous tissue of *L. acuminata*. Notably, the bait containing molluscicides is new technique effectively approaching target snails.

Key words: Bait, attractant, *Piper nigrum*, alkaloids, ovotestis, AChE, snail

INTRODUCTION

Fascioliasis is the most important parasitic disease after malaria in tropical and subtropical countries. It is caused by two trematodes *Fasciola hepatica* (the common liver fluke) and *F. gigantica* (the large liver fluke) (Mas-Coma *et al*., 2005; Singh *et al*., 2012). The global economic loss due to fascioliasis is estimated to be at least US$ 3.2 billion annually mainly through loss of productivity, such as reduction of fertility, milk and meat (Spithill *et al.,* 1999; Odigie and Odigie, 2013). In addition, human fascioliasis is now considered to be an important Food Borne Trematodiasis (FTBs) is neglected in global public health problem. Fresh water snail *Lymnaea acuminata* is the intermediate host of *F. gigantica* (Agarwal and Singh, 1988; Singh *et al*., 2012). This snail breeds year round and lays eggs on the lower surface of the aquatic plants. Treatment of *Fasciola* required high or multiple doses of drugs with frequent side effects (Abdul-Samie *et al*., 2010). Now the introduction of safe drugs for treatment of fascioliasis snail control is remains an important effective tool for combating the disease. It is regarded as a rapid

and efficient method for reducing the snail population by destroying the carrier snails and removing an essential link in life cycle of liver fluke (Mello-Silva *et al*., 2006). Srivastava *et al*. (2009) noted that piperine the active component of *Piper nigrum* is potent molluscicides. Bait formulation of plant derived molluscicides (piperine) with attractant starch/serine would be an effective tool for selective killing of the snail with minimal adverse effect on the non-target animal and ecosystem. In the present study the effects of sub-lethal (40% of 24 h LC_{50}) feeding of piperine molluscicides with attractant starch/serine on the biochemical parameters (free amino acid, protein, nucleic acid and enzyme AChE) in ovotestis/nervous of snail *L. acuminata* were examined in each month of the year Nov. 2011-Oct. 2012.

MATERIALS AND METHODS

Collection of snails: Parent snails (*Lymnaea acuminata* 2.61±0.25 cm in length) were collected naturally from the pond, pools and Lake of Gorakhpur district, U.P., India in each month of the year Nov. 2011-Oct. 2012. The animals were kept in the laboratory and allowed to acclimatize at 25°C for 72 h.

Chemicals used: Agar-agar, starch, serine and active component piperine were used in bait formulation. The pure active component piperine (1-[5-(1, 3-benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl] piperidine) was purchased from Sigma Chemical Co. (USA). The chemical structure of piperine as shown Fig. 1.

Preparation of bait formulation: Bait containing 20 mM of carbohydrate (starch) or amino acids (serine) with 40% of 24 h LC_{50} of piperine were prepared in 100 mL of 2% agar solution by the method of Madsen (1992) as modified by Tiwari and Singh (2004a, b). These preparations were spread to a uniform thickness of 5 mm and after cooling pellets were cut out using a corer diameter (5 mm). These pellets were used for the evaluation of the food preference against snail *Lymnaea acuminata*.

Assay apparatus and procedure: The bioassay was performed by the method of Tiwari and Singh (2004a, b). The bioassay chamber consists of a clean glass aquarium having a diameter of 30 cm. The aquaria were then filled with 500 mL of dechlorinated tap water to a height of 8 mm and maintained at 25±1°C. At the start of the assay 20 snails were placed in the aquarium. Simultaneously, one of the prepared bait was added in the chamber. Six sets of experiments have been designed with 20 snails in each replicate. Control aquaria were left untreated. After 24 h/96 h of bait feeding the snails were washed with water and the ovotestis/nervous tissue was dissected out from snail and used for the measurement of protein, the total free amino acid, nucleic acid (DNA/RNA) and enzyme AChE activity.

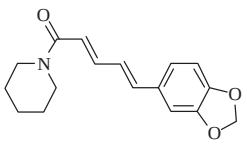

Fig. 1: Chemical structure of piperine

Biochemical estimations

Estimation of protein and free amino acids: Protein measurement (μg mg^{-1}) were made according to the method of Lowry *et al*. (1951) using bovine serum albumin as a standard. Ten percent trichloroacetic acid (TCA: w/v) was used to prepare homogenates of tissue. Total free amino acid (μg mg^{-1}) estimations were made according to the method of Spices (1957).

Nucleic acids: Estimation of DNA and RNA (μg mg^{-1}) were done by the method of Schneider (1957) using diphenylamine and orcinol. Homogenates (1.0 mg mL^{-1}, w/v) of ovotestis were prepared in 10% TCA at 90°C and centrifuged at 5000×g. Supernatants were used for the DNA and RNA estimations.

Enzyme Acetylcholinesterase (AChE): The AChE activity was carried according to the method of Ellman *et al*. (1961) as modified by Singh and Agarwal (1982). Fifty milligram of nervous tissue of *L. acuminata* was taken around the buccal mass and homogenized in 1.0 mL of 0.1 M phosphate buffer pH 8.0 for 5 min in an ice bath then centrifuged at 1000 g for 30 min at 4°C. Enzyme AChE activity was measured in a 10 mm path length cuvette using an incubation mixture consisting of 0.1 mL of enzyme source, 2.9 mL of 0.1 M buffer pH 8, 0.1 mL of chromogenic agent DTNB (5, 5-dithiobis-2-nitrobenzoic acid) and 0.02 mL of freshly prepared ATChI (acetylthiocholine iodide) solution in distilled water. The change in optical density at 412 nm was recorded for 3 min after every 30 sec interval at 25°C. AChE activity has been expressed as μmole 'SH' hydrolyzed per minute per miligram protein.

Statistical analysis: Each experiment was replicated six times and the values were expressed as Mean±SE. Two way analysis of variance and Student's t-test was applied to determine the significant ($p<0.05$) difference between treated and control group of snails at each month of the year Nov. 2011-Oct. 2012 (Sokal and Rohlf, 1995).

RESULTS

Sub-lethal feeding to 40% of 24 h LC_{50} of piperine with starch/serine in bait formulation caused a significant ($p<0.05$) reduction in protein, amino acids, DNA, RNA and AChE activity in ovotestis/nervous tissue of snail *Lymnaea acuminata* at 96 h exposure period (Table 1 and 2).

Feeding of bait containing molluscicides Piperine+Serine+Agar (40% of 24 h LC_{50}) at 96 h exposure caused maximum reduction in protein (75.27% of control) in month of August and amino acids (78.37% of control) in month of March (Table 1). Whereas, feeding of bait containing Piperine+Serine+Agar (40% of 24 h LC_{50}) caused maximum reduction in DNA (72.47% of control) in month of May and RNA (75.03% of control) in month of October in ovotestis of *L. acuminata* at 96 h exposure period (Table 1).

Feeding of bait containing molluscicides Piperine+Starch+Agar (40% of 24 h LC_{50}) caused maximum reduction in protein (76.66% of control) in month of June and amino acids (78.39% of control) in month of September in ovotestis of *L. acuminata* at 96 h exposure period (Table 2). Feeding of bait containing molluscicides Piperine+Starch+Agar (40% of 24 h LC_{50}) caused maximum reduction in DNA (65.03% of control) in month of June and RNA (66.15% of control) in month of August (Table 2).

There was a significant ($p<0.05$) inhibition in AChE activity with the feeding of bait containing derived molluscicides piperine with attractant starch/serine. Feeding of bait containing plant

Table 1: Effect of sub-lethal concentration (40% of 24 h LC_{50}) of piperine+serine+agar fed to bait containing molluscicide on certain biochemical changes in ovotestis and AChE activity in the nervous tissue of *Lymnaea acuminata* at 96 h exposure period

Months and treatments	24 h LC_{50}	Sub-lethal dose (mg L^{-1})	Protein	Amino acid	DNA	RNA	AchE (µ mole)
			(µg mg^{-1})				
November 2011							
Control	-	-	84.12±0.89 (100)	62.45±0.84 (100)	89.54±0.85 (100)	96.21±0.96 (100)	1.65±0.06 (100)
Pi+Se+Ag	12.21	4.88	78.43±0.83 (93.23)	57.76±0.45 (92.48)	76.41±0.85* (85.33)	86.63±0.85 (90.04)	1.36±0.02* (82.42)
December 2011							
Control	-	-	82.91±0.81 (100)	62.47±0.88 (100)	90.84±0.85 (100)	95.31±0.98 (100)	1.63±0.06 (100)
Pi+Se+Ag	13.52	5.40	78.72±0.61 (94.94)	56.44±0.58 (90.34)	72.43±0.73* (79.73)	83.56±0.89* (87.67)	0.98±0.03* (60.12)
January 2012							
Control	-	-	83.96±0.80 (100)	65.45±0.64 (100)	81.84±0.95 (100)	95.58±0.86 (100)	1.62±0.06 (100)
Pi+Se+Ag	19.63	7.85	74.34±0.62 (88.54)	58.23±0.87 (88.96)	74.42±0.47 (90.93)	81.40±0.48* (85.16)	0.87±0.06* (53.70)
February 2012							
Control	-	-	82.90±0.82 (100)	66.45±0.74 (100)	88.34±0.89 (100)	94.61±0.86 (100)	1.64±0.06 (100)
Pi+Se+Ag	16.32	6.52	78.62±0.49 (94.83)	54.25±0.98* (81.64)	73.80±0.65* (83.54)	82.61±0.98* (87.31)	1.28±0.01* (78.04)
March 2012							
Control	-	-	85.97±0.81 (100)	67.15±0.74 (100)	88.58±0.95 (100)	96.78±0.76 (100)	1.63±0.04 (100)
Pi+Se+Ag	16.86	6.74	68.43±0.64* (79.59)	52.63±0.37* (78.37)	75.48±0.38* 85.21)	82.96±0.75* (85.72)	1.29±0.02* (79.14)
April 2012							
Control	-	-	85.38±0.83 (100)	65.35±0.94 (100)	87.72±0.95 (100)	95.21±0.96 (100)	1.64±0.03 (100)
Pi+Se+Ag	15.23	6.09	63.46±0.92* (74.32)	51.57±0.66* (78.91)	67.93±0.58* (77.43)	82.94±0.98* (87.11)	1.22±0.03* (74.39)
May 2012							
Control	-	-	86.91±0.84 (100)	64.65±0.84 (100)	87.98±0.75 (100)	98.68±0.98 (100)	1.66±0.06 (100)
Pi+Se+Ag	14.74	5.89	64.73±0.84* (74.47)	56.61±0.43* (87.56)	63.76±0.46* (72.47)	74.83±0.62* (75.83)	1.26±0.06* (75.90)
June 2012							
Control	-	-	87.95±0.83 (100)	67.65±0.74 (100)	86.81±0.95 (100)	96.76±0.88 (100)	1.66±0.03 (100)
Pi+Se+Ag	10.36	4.14	66.86±0.75* (76.02)	54.05±0.89* (79.89)	68.44±0.62* (78.83)	72.74±0.80* (75.17)	1.35±0.02* (81.32)
July 2012							
Control	-	-	81.91±0.82 (100)	66.55±0.54 (100)	86.34±0.75 (100)	93.41±0.86 (100)	1.67±0.06 (100)
Pi+Se+Ag	10.84	4.33	64.56±0.63* (78.81)	54.14±0.88* (81.35)	66.20±0.53* (76.67)	77.88±0.75* (83.37)	0.87±0.01* (52.09)
August 2012							
Control	-	-	82.98±0.85 (100)	68.46±0.89 (100)	87.56±0.85 (100)	96.25±0.99 (100)	1.67±0.06 (100)
Pi+Se+Ag	11.63	4.65	62.46±0.61* (75.27)	55.66±0.68* (81.30)	64.72±0.94* (73.91)	76.42±0.78* (79.39)	0.99±0.03* (59.28)
September 2012							
Control	-	-	81.47±0.89 (100)	68.42±0.88 (100)	87.54±0.85 (100)	96.21±0.96 (100)	1.68±0.06 (100)
Pi+Se+Ag	12.53	5.01	64.56±0.69* (79.24)	54.65±0.71* (79.87)	63.60±0.75* (72.65)	76.88±0.63* (79.90)	0.88±0.04* (52.38)
October 2012							
Control	-	-	83.93±0.81 (100)	67.15±0.84 (100)	86.73±0.97 (100)	97.64±0.86 (100)	1.68±0.04 (100)
Pi+Se+Ag	12.82	5.12	66.45±0.75* (79.17)	57.35±0.96* (85.40)	65.52±0.63* (74.68)	73.26±0.67* (75.03)	0.78±0.05* (46.42)

Each experiment was replicated six times and the value of protein, amino acid, DNA, RNA and AChE is the mean of six replicate. Two way of analysis of variance and student t-test were applied in between control and treated group indicate (*) significant ($p<0.05$) test. SH: Hydrolyzed/min/mg protein, Pi: Piperine, Se: Serine, Ag: Agar

Table 2: Effect of sub-lethal concentration (40% of 24 h LC_{50}) of Piperine+starch+agar fed to bait containing molluscicide on certain biochemical changes in ovotestis and AChE activity in the nervous tissue of *Lymnaea acuminata* at 96 h exposure period

Months and treatments	24h LC_{50}	Sub-lethal dose (mg L^{-1})	Protein	Amino acid	DNA	RNA	AchE (µ mole)
			(µg mg^{-1})				
November 2011							
Control	-	-	84.12±0.89 (100)	62.45±0.84 (100)	89.54±0.85 (100)	96.21±0.96 (100)	1.65±0.06 (100)
Pi+St+Ag	15.25	6.10	76.28±0.57 (90.67)	52.23±0.77* (83.63)	75.67±0.72* (84.50)	79.68±0.64* (82.81)	0.88±0.03* (53.33)
December 2011							
Control	-	-	82.91±0.81 (100)	62.47±0.88 (100)	90.84±0.85 (100)	95.31±0.98 (100)	1.63±0.06 (100)
Pi+St+Ag	16.48	6.59	72.71±0.67* (87.69)	58.26±0.28 (93.26)	82.58±0.85 (90.90)	81.86±0.94* (85.88)	0.85±0.04* (52.14)
January 2012							
Control	-	-	83.96±0.80 (100)	65.45±0.64 (100)	81.84±0.95 (100)	95.58±0.86 (100)	1.62±0.06 (100)
Pi+St+Ag	23.52	9.40	76.85±0.58 (91.53)	56.45±0.79 (86.24)	77.75±0.66 (95.00)	75.46±0.30* (78.94)	1.49±0.03* (91.97)
February 2012							
Control	-	-	82.90±0.82 (100)	66.45±0.74 (100)	88.34±0.89 (100)	94.61±0.86 (100)	1.64±0.06 (100)
Pi+St+Ag	20.37	8.14	75.74±0.84 (91.36)	55.19±0.77* (83.05)	76.53±0.77* (86.63)	75.41±0.75* (79.70)	1.38±0.01* (84.14)
March 2012							
Control	-	-	85.97±0.81 (100)	67.15±0.74 (100)	88.58±0.95 (100)	96.78±0.76 (100)	1.63±0.04 (100)
Pi+St+Ag	21.74	8.69	73.46±0.89* (85.44)	62.52±0.88* (93.10)	70.75±0.79* (79.87)	73.81±0.76* (76.26)	1.34±0.04* (82.20)
April 2012							
Control	-	-	85.38±0.83 (100)	65.35±0.94 (100)	87.72±0.95 (100)	95.21±0.96 (100)	1.64±0.03 (100)
Pi+St+Ag	20.74	8.29	71.52±0.85* (83.76)	61.83±0.54 (94.61)	74.94±0.76* (85.43)	71.80±0.74* (75.41)	0.86±0.01* (52.43)
May 2012							
Control	-	-	86.91±0.84 (100)	64.65±0.84 (100)	87.98±0.75 (100)	98.68±0.98 (100)	1.66±0.06 (100)
Pi+St+Ag	18.86	7.54	65.35±0.76* (75.19)	59.40±0.58 (91.87)	76.83±0.75* (87.32)	72.67±0.67* (73.64)	0.98±0.02* (59.03)
June 2012							
Control	-	-	87.95±0.83 (100)	67.65±0.74 (100)	86.81±0.95 (100)	96.76±0.88 (100)	1.66±0.03 (100)
Pt+St+Ag	13.72	5.48	67.43±0.75* (76.66)	58.68±0.72 (86.74)	56.46±0.87* (65.03)	66.44±0.67* (68.66)	0.96±0.02* (57.83)
July 2012							
Control	-	-	81.91±0.82 (100)	66.55±0.54 (100)	86.34±0.75 (100)	93.41±0.86 (100)	1.67±0.06 (100)
Pt+St+Ag	13.93	5.57	67.84±0.66* (82.82)	56.83±0.77* (85.39)	57.68±0.78* (66.80)	67.71±0.86* (72.48)	0.98±0.01* (58.68)
August 2012							
Control	-	-	82.98±0.85 (100)	68.46±0.89 (100)	87.56±0.85 (100)	96.25±0.99 (100)	1.67±0.06 (100)
Pi+St+Ag	15.59	6.23	64.74±0.85* (78.01)	55.77±0.48* (81.46)	57.48±0.66* (65.64)	63.67±0.79* (66.15)	1.26±0.02* (75.44)
September 2012							
Control	-	-	81.47±0.89 (100)	68.42±0.88 (100)	87.54±0.85 (100)	96.21±0.96 (100)	1.68±0.06 (100)
Pi+St+Ag	16.47	6.58	66.81±0.73* (82.00)	53.64±0.74* (78.39)	69.76±0.73* (79.68)	68.77±0.74* (71.47)	1.15±0.03* (68.45)
October 2012							
Control	-	-	83.93±0.81 (100)	67.15±0.84 (100)	86.73±0.97 (100)	97.64±0.86 (100)	1.68±0.04 (100)
Pi+St+Ag	16.89	6.75	66.82±0.54* (79.61)	56.52±0.65* (84.16)	72.66±0.48* (83.77)	68.69±0.88* (70.35)	0.97±0.01* (57.73)

Each experiment was replicated six times and the value of protein, amino acid, DNA, RNA and AChE is the mean of six replicate. Two way of analysis of variance and student t-test were applied in between control and treated group indicate (*) significant ($p<0.05$) test. SH: Hydrolyzed/min/mg protein, Pi: Piperine, St: Starch, Ag: Agar

molluscicides Piperine+Serine+Agar (40% of 24 h LC_{50}) at 96 h exposure period caused maximum inhibition in AChE activity (46.42% of control) in month of October, whereas snail fed to Piperine+Starch+Agar (40% of 24 h LC_{50}) caused maximum inhibition of AChE activity (52.41% of control) in month of December in the nervous tissue of *L. acuminata* (Table 1 and 2).

DISCUSSION

It is evident from the results that active component piperine (*Piper nigrum*) in bait formulation were more effective in killing the *Lymnaea acuminata*. Parmar *et al*. (1997) reported that *Piper nigrum*(Piperaceae) contain amides as the major secondary metabolites and responsible for the insecticidal properties (Parmar *et al*., 1997). The active principle of *Piper nigrum* is piperine (alkaloids) (Anonymous, 1995). Alkaloids may also produce striking physiological effect which induces paralysis, stimulation of CNS, change in blood pressure (Kalsi, 1983). Amide derivative of piperine were very effective caused 95.5% mortality against Brazilian insect *Ascia monuste*, *Acanthoscelides obtectus* and *Brevicoryne brassicae* (De Paula *et al*., 2000). Reduction in the levels of protein in the ovotestis, may be due to direct or indirect interference of the drug with protein synthesis. Amino acids are the building block structural protein and certain enzyme. Amino acids levels in the ovotestis of exposed to different preparation were significantly lower than the control. It indicates that they also interfere with the biosynthesis of amino acids in the cell (Singh *et al*., 1998). Purine and Pyrimidine are essential component of nucleic acids (DNA/RNA) is synthesized from amino acids (Jigyasu *et al*., 2010). Elhag (2000) reported that extract of *Piper nigrum* fruit caused 12.4 and 24.5% reduction in F_1 and F_2 progeny of *Callosobruchus naulatus*. Piperine inhibits P-glycoprotein and the major drug metabolizing enzyme CYP 3A4 (Bhardwaj *et al*., 2002). Both are protein and are expressed in enterocytes and hepatocytes and contribute to a major extent to first pass elimination of many drugs. Reduction in the DNA levels in the ovotestis of *L. acuminata* with the treatment of piperine is due to its genotoxic effect. Karekar *et al*. (1996) reported the genotoxic effect of piperine on germ cells of the Swiss albino mice. The decrease in DNA contents may also be because of cascade of cell death caused by toxic aldehyde resulting from rate of lipid peroxidation. Data emerging from result section demonstrate that treatment of sublethal dose of piperine decline the level of RNA. It may be possible that reduced RNA causes reduction in the new protein synthesis. More over researches have proved the alcoholic extract of Black pepper to geno-toxic as it alters the sister chromatids exchange in chromosomes (Madrigal-Bujaidar *et al*., 1997). Chu *et al*. (1994) in his study found that piperine promotes DNA damage and are cytotoxic. Any agonistic or antagonistic activity of a plant derived molluscicides at nerve cell signal and at synapses will usually affect the physiology of the corresponding animal substantially. The AChE activity is one of the known biomarker, frequently used in ecotoxicology. The enzyme is responsible for the breakdown of ACh in cholinergic synapses, preventing continuous nerve firing, which is vital for normal cellular neurotransmitter functioning (Fabbri and Capuzzo, 2010). The AChE inhibition result in accumulation of acetylcholinesterase at the nerve synapses so that the post synaptic membrane is in a state of permanent stimulation producing paralysis, ataxia and general lack of coordination in neuromuscular system and eventual death (Matsumura, 1985; Singh *et al*., 2009). The causes of these might be due to interference with sulphydryl group of enzyme AChE (Schmeller *et al*., 1997).

CONCLUSION

It can be concluded from the above study that the bait containing plant molluscicides (piperine) with attractant (starch/serine) can significantly alter the biochemical of snail *Lymnaea acuminata*

in each month of the year Nov. 2011-Oct. 2012. This concept is a new technique and approach for the effective control of harmful snails, without using more active molluscicide directly in the water and attracting specifically the particular target snail.

REFERENCES

Abdul-Samie, R.E., E.O. Soliman, H. El-Nemr and A. Masou, 2010. Study of Il1b, Il4, Il5 and IgE before and after Mirazid Therapy in children with intestinal schistosomiasis and fascsioliasis. N. Y. Sci. J., 3: 116-122.

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

Anonymous, 1995. Wealth of India, Raw Materials. Publication and Information Directorate, CSIR, New Delhi, India, ISBN-13: 9788185038209, Pages: 591.

Bhardwaj, R.K., H. Glaeser, L. Becquemont, U. Klotz, S.K. Gupta and M.F. Fromm, 2002. Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. J. Pharmacol. Exp. Therapeut., 302: 645-650.

Chu, C.Y., J.P. Chang and C.J. Wang, 1994. Modulatory effect of piperine on benzo[α]pyrene cytotoxicity and DNA adduct formation in V-79 lung fibroblast cells. Food Chem. Toxicol., 32: 373-377.

De Paula, V.F., L.C.A. Barbosa, A.J. Demuner, D. Pilo-Veloso and M.C. Picanco, 2000. Synthesis and insecticidal activity of new amide derivatives of piperine. Pest Manage. Sci., 56: 168-174.

Elhag, E.A., 2000. Deterrent effects of some botanical products on oviposition of the cowpea bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Int. J. Pest Manage., 46: 109-113.

Ellman, G.L., K.D. Courtney, V. Andres Jr. and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.

Fabbri, E. and A. Capuzzo, 2010. Cyclic AMP signaling in bivalve molluscs: An overview. J. Exp. Zool. Part A: Ecol. Genet. Physiol., 313: 179-200.

Jigyasu, H.V., D.K. Singh and V.K. Singh, 2010. The effect of abiotic factors on certain biochemical changes in ovotestis of snail *Lymnaea acuminata*: Intermediate host of trematode diseases. Global Veterinaria, 4: 523-531.

Kalsi, P.S., 1983. Chemistry of Alkaloids. In: Chemistry of Natural Product, Kalsi, P.S. (Ed.). Kalyani Publishers, New Delhi, India, pp: 536-572.

Karekar, V.R., A.M. Mujumdar, S.S. Joshi, J. Dhuley, S.L. Shinde and S. Ghaskadbi, 1996. Assessment of genotoxic effect of piperine using *Salmonella typhimurium* and somatic and somatic and germ cells of Swiss albino mice. Arzneimittel-Forschung, 46: 972-975.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Madrigal-Bujaidar, E., S.D. Barriga, P. Mota, R. Guzman and M. Cassani, 1997. Sister chromatid exchanges induced *in vitro* and *in vivo* by an extract of black pepper. Food Chem. Toxicol., 35: 567-571.

Madsen, H., 1992. A comparative study on the food-locating ability of *Helisoma duryi*, *Biomphalaria camerunensis* and *Bulinus truncatus* (Pulmonata: Planorbidae). J. Applied Ecol., 29: 70-78.

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

Matsumura, F., 1985. Toxicology of Insecticides. 2nd Edn., Plenum Press, New York, Pages: 598.

Mello-Silva, C.C., M.C. de Vasconcellos, J. Pinheiro and M.L.A. Rodrigues, 2006. Physiological changes in *Biomphalaria glabrata* say, 1818 (Pulmonata: Planorbidae) caused by sub-lethal concentrations of the latex of *Euphorbia splendens* var. hislopii N.E.B (Euphorbiaceae). MemOrias Instituto Oswaldo Cruz, 101: 3-8.

Odigie, B.E. and B.O. Odigie, 2013. Fascioliasis in cattle: A survey of abattoirs in Egor, Ikpoba-Okha and Oredo local government areas of Edo State, using histochemical techniques. Int. J. Basic Applied Innov. Res., 2: 1-9.

Parmar, V.S., S.C. Jain, K.S. Bisht, R. Jain and P. Taneja *et al*., 1997. Phytochemistry of the genus *Piper*. Phytochemistry, 46: 597-673.

Schmeller, T., B. Latz-Bruning and M. Wink, 1997. Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. Phytochemistry, 44: 257-266.

Schneider, W.C., 1957. Determination of Nucleic Acid in the Tissue by Pentose Analysis. In: Methods in Enzymology, Colowick, S.P. and N.O. Kaplan (Eds.). Vol. 14, New York Academic Press, New York, Pages: 680.

Singh, D.K. and R.A. Agarwal, 1982. *In vitro* inhibition of acetylcholinesterase by carbamate and organophosphorous pesticide in the snail *Lymnaea acuminata*. Comput. Physiol. Ecol., 7: 191-196.

Singh, D.K., V.K. Singh and P. Kumar, 2012. Pestiferous Gastropods and Their Control. LAP LAMBERT Academic Publishing GmbH and Co., Germany, ISBN: 978-3-659-15840-7, Pages: 152.

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

Singh, S., V.K. Singh and D.K. Singh, 1998. Effects of active molluscicidal agents of common spices on biochemical parameters in the ovotestis of *Lymnaea acuminata*. Malaysian Applied Biol., 27: 45-49.

Sokal, R.R. and F.J. Rohlf, 1995. Biometry: The Principles and Practice of Statistics in Biological Research. 3rd Edn., W.H. Freeman and Co., New York, USA., ISBN: 0-7167-2411-1, Pages: 887.

Spices, J.R., 1957. Colorimetric Procedure for Amino Acids. In: Methods in Enzymology. Calowick, S.P. and N.O. Kalpan (Eds.). Academic Press, New York, pp: 468.

Spithill, T.W., P.M. Smooker and D.B. Copeman, 1999. *Fasciola gigantica*: Epidemiology, control, immunology and molecular biology. In: Fasciolosis, Dalton, J.P. (Ed.). CAB International, Wallingford, Oxon, UK., ISBN: 0-85199-260-9, pp: 465-525.

Srivastava, P., P. Kumar, V.K. Singh and D.K. Singh, 2009. Molluscicidal activity of *Piper nigrum* (black) against the snail *Lymnaea acuminata* and *Indoplanorbis exustus* in the control of fascioliasis. J. Herbal Med. Toxicol., 3: 81-86.

Tiwari, F. and D.K. Singh, 2004a. Attraction to amino acids by *Lymnaea acuminata*, the snail host of *Fasciola* species. Braz. J. Med. Biol. Res., 3: 587-590.

Tiwari, F. and D.K. Singh, 2004b. Behavioural responses of the snail *Lymnaea acuminate* to carbohydrates in snail-attractant pellets. Naturewissenschaften, 91: 378-380.