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Effect of abiotic factors on the molluscicidal activity of oleoresin of *Zingiber officinale* against the snail *Lymnaea acuminata*

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ABSTRACT

Earlier it has been observed that oleoresin of *Zingiber officinale* is a potent molluscicide against *Lymnaea acuminata*. This snail is the vector of *Fasciola* species, which cause endemic fascioliasis in eastern Uttar Pradesh. As this snail breeds and maintain their population constant through out the year, so that the present study has been designed to find out the effect of variations in some environmental factors in different seasons, on the molluscicidal activity of oleoresin of *Zingiber officinale* and its relative effect on certain enzymes viz., acetylcholinesterase, acid and alkaline phosphatases in the nervous tissue of the snail *Lymnaea acuminata*. In this study temperature, pH, dissolve oxygen, free carbon dioxide, conductivity of the water in control, as well as molluscicide treated water, was measured simultaneously. LC_{50} value of oleoresin was determined in each month of the year. Toxicity of oleoresin in June-July (24 h LC_{50} 16.54-14.28 mgL^{-1}) is highest. Acetylcholinesterase, acid and alkaline phosphatases activity in the nervous tissue of the snails treated with sub-lethal concentration of oleoresin was simultaneously measured. Sig- nificant positive rank correlation, in between the acetylcholinesterase or acid phosphatase activity and LC_{50} of oleoresin was observed. The pre- sent study conclusively shows that variant abi- otic factors can significantly alter the toxicity of oleoresin of *Z. officinale* in *L. acuminata*. The most suitable period for control of *L. acuminata* is June-July.

Keywords: Environmental factors; Acetylcholinesterase; Oleoresin; Temperature; pH

1. INTRODUCTION

It has been reported that, oleoresin of *Zingiber officinale* is a potent molluscicide [1,2]. Fresh water snail *Lymnaea acuminata* is the intermediate host of liver fluke *Fasciola gigantica*, causing an endemic fascioliasis in the cattle population of eastern region of the state of Uttar Pradesh in India [3,4]. An effective method to reduce the incidence of fascioliasis is to control the population of vector snails and, thereby, break the life cycle of these flukes [5-8]. Earlier studies have shown that oleoresin of *Zingiber officinale* has a powerful molluscicidal action on the snail *L. acuminate* [1,2]. It has also been conclusively shown that acetylcholinesterase (AChE), acid and alkaline phosphatase (ACP and ALP) in the nervous tissue of *L. acuminata* are very sensitive parameters influenced by molluscicides [7-10]. The aim of the present study was to explore the possibility whether seasonal changes in abiotic factors, *viz* temperature, pH, dissolved oxygen and carbon dioxide, and conductivity of test water can influence the level of AChE, ACP and ALP assayed in each month of the year 2006-2007 following exposure to sublethal concentrations (40% and 80%) of 24 h LC_{50} of oleoresin of *Z. officinale*.

2. MATERIALS AND METHODS

2.1. Test Materials

Oleoresin was obtained by extraction of prepared dried rhizomes of *Z. officinale* with alcohol. The removal of the solvent under vacuum yields oleoresin of *Z. officinale* [1,11]. Temperature, pH and conductivity of water were measured by thermometer and digital pH and conductivity meters, respectively. Dissolved O_2 and CO_2 were estimated according to the methods prescribe by APHA [12].

2.2. Bioassays for LC_{50}

Adult *L. acuminata* (length 2.25 ± 0.2 cm) were collected from Ramgarh Lake, located in almost adjacent to this university campus. Snails were acclimatized in de-chlorinated tap water for 72 h. The snails were exposed to different concentrations of oleoresin in glass aquaria containing 3 litres of de-chlorinated water. Ten experimental animals were kept in each aquarium. Control animals were kept in equal volumes of de-chlorinated tap water under similar conditions. Mortality of snails was observed after 24, 48, 72, 96 h. No response to a needle probe was taken as evidence of death. Dissolved O_2, CO_2 and conductivity, temperature and pH of treated and control group of water was measured simultaneously with toxicity test at every 24 h of period to 96. Bioassays for the determination of LC_{50} value was performed in each month of the year. Lethal concentration (LC_{50}) values, lower and upper confidence limits (LCL and UCL) and slopes value were calculated by the method of POLO computer program of Robertson *et al.* [13]. The Product moment correlation coefficient was determined between LC_{50} and temperature / pH / conductivity / dissolved O_2 / CO_2, of water in each of the twelve months in order to observe any significant correlation according to the method of Sokal and Rohlf [14].

2.3. Enzyme Assays

Twenty snails, kept in glass aquaria containing 5 litres of dechlorinated water, were exposed to 40% and 80% of 24 h LC_{50} of oleoresin in each month. Six such aquaria were set up for each concentration. After 24 h treatment, the snails were washed with water and the nervous tissue was dissected out from the buccal mass for the measurement of enzyme AChE, ACP and ALP activities.

2.3.1. Acetylcholinesterase

Acetylcholinesterase (AChE) activity was measured according to the method of Ellman *et al.* [15] as modified by Singh *et al.* [16]. Fifty mg of nervous tissue was homogenized in 1.0 ml of 0.1 M phosphate buffer pH 8.0 for 5 minute in an ice bath and centrifuged at 1000 g for 30 minute at 4℃. Supernatant was used as enzyme source. The change in optical density at 412 nm was recorded for 3 minute after every 30 second interval. Enzyme activity was expressed as μ mol "SH" hydrolyzed / min / mg protein.

2.3.2. Phosphatases

Acid (ACP) and alkaline (ALP) phosphatases activities were measured by the method of Bergmeyer [17] as modified by Singh and Agarwal [18]. Tissue homogenate (2% w/v) was prepared in ice cold 0.9 % NaCl and centrifuged at 5000 g for 20 minute at 4℃. The 4-nitrophenyl phosphate disodium was used as substrate. The acid (ACP) and alkaline phosphatases (ALP) activity has been expressed as μmole substrate hydrolyzed /30 min/ mg protein.

2.3.3. Protein Estimation

Protein was estimated by the method of Lowry *et al.* [19].

2.4. Statistical Analysis

Results have been expressed as mean ± SE of six replicates. Rank correlation was applied in between control and corresponding changes in the enzyme activity in different months of the year [14].

3. RESULTS

There was a significant ($P < 0.05$) time dependent variation in the toxicity of oleoresin *Z. officinale* in different months of the year against *L. acuminata* (**Table 1**); highest toxicity was observed in months of June and July (24 h LC_{50} 14.28-16.54 mgL^{-1}) and lowest (24 h LC_{50} 124.09-126.27 mgL^{-1}) during January and February. A significant positive correlation ($r = 0.89$; $P = 0.001$) between LC_{50} and water pH was noted for each month and at each interval of 24 h exposure (**Table 1**). A similar finding between LC_{50} and dissolved O_2 ($r = 0.82$; $P = 0.001$) was found. Contrastively, significant negative correlation between LC_{50} and dissolved CO_2 ($r = 0.86$; $P = 0.001$) and with water temperature ($r = 0.91$; $P = 0.001$) was noted. No marked correlation was observed between the LC_{50} and conductivity of water. High temperature (36℃), and free CO_2 (30.0 ppm), low pH (7.11) and dissolved oxygen (1.0 ppm) increases the toxicity of oleoresin against *L. acuminata*. The slope values were steep and separate estimations of LC_{50} based on each of the six replicates were found to be with in the 95% confidence limits of LC_{50}. The t-ratio is greater than 1.96 and the heterogeneity factor is less than 1.0. The g value was less than 0.5 at all probability levels.

There was significant positive rank correlation ($\tau = 0.666$; $P = 0.02$ – 40% of 24 h LC_{50}, $\tau = 0.636$, $P = 0.02$ – 80% of 24 h LC_{50}) between LC_{50} of different months and corresponding anti AChE activity in the sub-lethal treatment (40% and 80% of 24 h LC_{50}) of nervous tissue of snail *L. acuminata*. Maximum inhibition in AChE activity (56.09% of control) was observed in snails exposed to 80% of 24 h LC_{50} of oleoresin in month of July (**Table 2**). There was no significant positive rank correlation between LC_{50} of different months and alkaline phosphatase activity in the sub-lethal treatment (40% and 80% of 24 h LC_{50}) of nervous tissue of snail *L. acuminata* (**Table 3**). Like AChE, there was significant positive rank correlation between LC_{50} and acid phosphatas

Table 1. Alterations in toxicity (LC_{50} mL^{-1}) of oleoresin of *Z. officinale* against *L. acuminata* and different environmental factors in different months of the year 2006-07.

24 h												
Parameters	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.
LC_{50} (mgL^{-1})	39.67	54.54	55.92	58.79	62.18	126.27	124.09	74.93	24.88	21.67	16.54	14.28
Temp (℃) *	30 ± 0.37	30 ± 0.55	29 ± 0.55	26 ± 0.50	22 ± 0.36	17 ± 0.44	20 ± 0.55	22 ± 0.34	34 ± 0.30	29 ± 0.30	35 ± 0.50	36 ± 0.30
pH +	7.09 ± 0.06	8.00 ± 0.05	8.07 ± 0.05	8.09 ± 0.05	8.76 ± 0.05	9.00 ± 0.04	8.90 ± 0.06	8.79 ± 0.05	7.81 ± 0.03	7.50 ± 0.06	7.39 ± 0.04	7.11 ± 0.04
DO(ppm) +	1.5 ± 0.06	2.3 ± 0.05	3.0 ± 0.06	5.0 ± 0.05	5.0 ± 0.05	6.0 ± 0.04	5.0 ± 0.06	4.0 ± 0.03	3.0 ± 0.05	2.5 ± 0.05	1.8 ± 0.04	1.0 ± 0.02
DCO_2(ppm)*	25.0 ± 0.55	20.0 ± 0.45	20.0 ± 0.70	20.0 ± 0.53	15.0 ± 0.50	15.0 ± 0.52	15.0 ± 0.55	15.0 ± 0.40	25.0 ± 0.43	25.0 ± 0.55	25.0 ± 0.50	30.0 ± 0.50
Conductivity (μ mhos/cm)	45.0 ± 0.5	54.0 ± 0.6	46.8 ± 0.5	40.1 ± 0.56	50.0 ± 0.6	57.5 ± 0.6	40.3 ± 0.7	33.2 ± 0.5	52.6 ± 0.7	70.0 ± 0.6	30.3 ± 0.6	40.0 ± 0.4
48 h												
LC_{50} (mgL^{-1})	34.13	20.70	34.79	40.35	42.30	72.79	88.20	61.38	19.21	18.72	11.76	11.62
Temp (℃) *	31 ± 0.40	30 ± 0.55	28 ± 0.60	26 ± 0.23	22 ± 0.36	17 ± 0.44	20 ± 0.50	21 ± 0.36	35 ± 0.35	30 ± 0.33	35 ± 0.50	36 ± 0.33
pH +	7.45 ± 0.05	8.43 ± 0.04	8.45 ± 0.05	8.69 ± 0.04	9.08 ± 0.05	9.33 ± 0.05	9.31 ± 0.56	9.18 ± 0.06	8.00 ± 0.01	7.90 ± 0.05	7.88 ± 0.05	7.50 ± 0.03
DO(ppm) +	1.0 ± 0.02	2.0 ± 0.01	2.5 ± 0.04	4.5 ± 0.03	4.0 ± 0.03	5.0 ± 0.05	4.0 ± 0.05	3.0 ± 0.04	2.5 ± 0.04	2.0 ± 0.04	1.0 ± 0.03	1.0 ± 0.02
DCO_2(ppm)*	32.0 ± 0.20	19.0 ± 0.44	18.0 ± 0.3	18.0 ± 0.45	18.0 ± 0.53	17.0 ± 0.56	18.0 ± 0.54	18.0 ± 0.44	20.0 ± 0.56	20.0 ± 0.45	30.0 ± 0.46	35.0 ± 0.54
Conductivity (μ mhos/cm)	40.2 ± 0.04	45.0 ± 0.05	43.7 ± 0.4	40.0 ± 0.6	45.0 ± 0.5	47.0 ± 0.4	38.7 ± 0.5	33.3 ± 0.6	42.0 ± 0.7	50.3 ± 0.7	30.7 ± 0.3	34.2 ± 0.6
72 h												
LC_{50} (mgL^{-1})	28.72	15.29	24.56	25.59	27.33	43.32	44.87	38.56	13.92	13.96	8.46	8.15
Temp (℃) *	31 ± 0.40	30 ± 0.55	28 ± 0.56	26 ± 0.20	22 ± 0.36	17 ± 0.30	20 ± 0.50	21 ± 0.36	35 ± 0.33	30 ± 0.47	35 ± 0.55	36 ± 0.32
pH +	8.35 ± 0.06	8.25 ± 0.06	8.42 ± 0.04	8.66 ± 0.03	9.15 ± 0.05	9.34 ± 0.04	9.18 ± 0.04	9.10 ± 0.07	7.97 ± 0.4	8.47 ± 0.03	7.82 ± 0.03	8.00 ± 0.08
DO(ppm) +	1.0 ± 0.02	2.0 ± 0.01	2.5 ± 0.04	4.0 ± 0.02	4.0 ± 0.03	4.5 ± 0.04	4.0 ± 0.05	3.0 ± 0.04	2.2 ± 0.06	2.0 ± 0.04	0.8 ± 0.09	0.5 ± 0.01
DCO_2(ppm)*	32.0 ± 0.20	21.0 ± 0.40	22.0 ± 0.6	21.0 ± 0.43	20.0 ± 0.56	18.0 ± 0.54	20.0 ± 0.30	18.0 ± 0.44	25.0 ± 0.43	25.0 ± 0.45	35.0 ± 0.37	40.0 ± 0.67
Conductivity (μ mhos/cm)	35.2 ± 0.05	35.4 ± 0.6	33.7 ± 0.6	33.4 ± 0.67	32.0 ± 0.6	40.3 ± 0.5	40.7 ± 0.5	30.3 ± 0.6	33.0 ± 0.3	40.3 ± 0.6	32.0 ± 0.4	34.2 ± 0.7
96 h												
LC_{50} (mgL^{-1})	24.25	11.79	19.13	18.87	19.49	22.44	24.34	25.76	11.35	11.36	7.03	7.27
Temp (℃) *	30 ± 0.30	30 ± 0.55	29 ± 0.40	26 ± 0.10	22 ± 0.36	17 ± 0.30	20 ± 0.50	21 ± 0.30	34 ± 0.21	30 ± 0.44	36 ± 0.65	35 ± 0.40
pH +	9.35 ± 0.07	8.25 ± 0.06	8.32 ± 0.03	8.69 ± 0.03	9.00 ± 0.04	9.49 ± 0.03	9.40 ± 0.03	9.50 ± 0.04	7.95 ± 0.02	8.00 ± 0.02	7.84 ± 0.05	8.43 ± 0.05
DO(ppm) +	1.0 ± 0.02	2.0 ± 0.01	2.3 ± 0.07	2.1 ± 0.03	2.5 ± 0.04	3.0 ± 0.03	3.5 ± 0.06	3.0 ± 0.04	1.8 ± 0.04	2.0 ± 0.04	0.5 ± 0.04	0.5 ± 0.01
DCO_2(ppm)*	30.0 ± 0.25	30.0 ± 0.56	25.0 ± 0.5	21.0 ± 0.48	20.0 ± 0.62	19.0 ± 0.55	20.0 ± 0.30	18.0 ± 0.44	30.0 ± 0.50	30.0 ± 0.37	40.0 ± 0.35	38.0 ± 0.66
Conductivity (μ mhos/cm)	34.2 ± 0.06	35.4 ± 0.70	23.7 ± 0.5	33.2 ± 0.5	22.0 ± 0.7	31.3 ± 0.3	32.7 ± 0.6	30.3 ± 0.5	32.0 ± 0.7	40.3 ± 0.4	30.0 ± 0.5	30.2 ± 0.8

Each experiment was replicated six times and values are the mean of six replications. Temperature, pH, dissolved oxygen, free carbon dioxide and conductivity were measured intervals of 24 h to 96 h. Product moment correlation coefficient in between the LC_{50} and different parameters indicate significant ($P < 0.05$) (+) positive / (*) negative correlation.

Table 2. Effect of 24 h exposure of 40% and 80% of 24 h LC_{50} of oleoresin of *Z. officinale* in different months of the year 2006-07 on acetylcholinesterase activity in the nervous tissue of *L. acuminata.*

Months	24 h LC_{50} mgL^{-1}	AChE-μ mole "SH" hydrolyzed / min / mg protein		
		Control [a]	40% of 24h LC_{50}	80% of 24h LC_{50}
August	39.67	0.093 ± 0.01 (100)	0.091 ± 0.01 (97.84)	0.088 ± 0.01 (94.62)
September	54.54	0.139 ± 0.01 (100)	0.136 ± 0.01 (97.84)	0.132 ± 0.01 (94.96)
October	55.92	0.101 ± 0.02 (100)	0.099 ± 0.01 (98.02)	0.097 ± 0.02 (96.04)
November	58.79	0.106 ± 0.01 (100)	0.103 ± 0.02 (97.17)	0.101 ± 0.01 (95.28)
December	62.18	0.109 ± 0.00 (100)	0.107 ± 0.01 (98.17)	0.105 ± 0.01 (96.33)
January	126.27	0.190 ± 0.00 (100)	0.189 ± 0.01 (99.47)	0.186 ± 0.01 (97.89)
February	124.09	0.176 ± 0.01 (100)	0.175 ± 0.00 (99.43)	0.171 ± 0.00 (97.16)
March	74.93	0.147 ± 0.02 (100)	0.142 ± 0.01 (96.60)	0.134 ± 0.01 (91.16)
April	24.88	0.141 ± 0.02 (100)	0.137 ± 0.01 (93.66)	0.133 ± 0.01 (84.51)
May	21.67	0.104 ± 0.01 (100)	0.101 ± 0.02 (75.92)	0.099 ± 0.03 (70.37)
June	16.54	0.087 ± 0.03 (100)	0.083 ± 0.02 (62.19)	0.074 ± 0.02 (59.75)
July	14.28	0.082 ± 0.02 (100)	0.073 ± 0.01 (63.41)	0.071 ± 0.01 (56.09)

Values are mean ± SE of six replicates. Value in parenthesis indicates % enzyme activity with untreated control taken as 100%. Rank correlation coefficient in between LC_{50} and AChE activity in treated group indicate significant ($P < 0.05$) positive (+) correlation. a, Significant ($P < 0.05$) when one way of ANOVA was applied in between the enzyme activity in different months of the year in control group without treatment.

Table 3. Effect of 24 h exposure of 40% and 80% of 24 h LC_{50} of oleoresin of *Z. officinale* in different months of the year 2006-07 on alkaline phosphatase activity in the nervous tissue of *L. acuminata.*

Months	24 h LC_{50} mgL^{-1}	ALP-μ moles / 30 min / mg protein		
		Control	40% of 24 h LC_{50}	80% of 24 h LC_{50}
August	39.67	2.27 ± 0.01 (100)	2.17 ± 0.01 (95.59)	2.14 ± 0.01 (94.27)
September	54.54	3.34 ± 0.01 (100)	3.31 ± 0.01 (99.10)	3.27 ± 0.01 (97.90)
October	55.92	3.27 ± 0.01 (100)	3.23 ± 0.02 (98.77)	3.19 ± 0.02 (97.55)
November	58.79	3.36 ± 0.02 (100)	3.32 ± 0.02 (98.80)	3.27 ± 0.00 (97.32)
December	62.18	3.11 ± 0.00 (100)	3.06 ± 0.02 (98.39)	3.02 ± 0.02 (97.10)
January	126.27	2.89 ± 0.01 (100)	2.85 ± 0.00 (98.62)	2.81 ± 0.01 (97.23)
February	124.09	3.07 ± 0.01 (100)	3.02 ± 0.01 (98.37)	2.98 ± 0.01 (97.06)
March	74.93	3.10 ± 0.00 (100)	3.07 ± 0.00 (99.03)	3.02 ± 0.01 (97.41)
April	24.88	3.45 ± 0.01 (100)	3.42 ± 0.02 (99.13)	3.37 ± 0.02 (97.68)
May	21.67	2.94 ± 0.02 (100)	2.89 ± 0.00 (98.29)	2.86 ± 0.03 (97.28)
June	16.54	1.86 ± 0.03 (100)	1.74 ± 0.02 (93.55)	1.64 ± 0.02 (88.17)
July	14.28	2.36 ± 0.01 (100)	2.23 ± 0.01 (94.49)	2.16 ± 0.02 (91.52)

Values are mean ± SE of six replicates. Value in parenthesis indicates % enzyme activity with untreated control taken as 100%. Rank correlation coefficient in between LC_{50} and ALP activity in treated group indicate non significant ($P < 0.05$) positive correlation. a, Significant ($P < 0.05$) when one way of ANOVA was applied in between the enzyme activity in different months of the year in control group without treatment.

(ACP) ($\tau = 0.606$; $P = 0.05$ – 40% of 24 h LC_{50}, $\tau = 0.606$; $P = 0.05$ – 80% of 24 h LC_{50}) activity in the nervous tissue of *L. acuminata* exposed to sub-lethal treatments of oleoresin in different months. Maximum inhibition in ACP activity (91.00% of control) was observed in snails exposed to 80% of 24 h LC_{50} in July (**Table 4**).

4. DISCUSSION

It is clear from result section that toxicity of oleoresin varies with changes in abiotic environmental factors in the water. Effect of abiotic variants in aquatic environment *i.e.* pH [20], Temperature [21] on the toxicity of different pesticides have been reported. The temperature of water is a significant factor, which alters the toxicity of oleoresin in each month of the year. When the water temperature is higher in summer season June-July, the toxicity of oleoresin is maximum. Contrarily, in winter season, the temperature of water is low and toxicity of oleoresin is less as evident by higher LC_{50} value. Temperature of environment in which the animal resides is a crucial factor, when toxicity of pesticides is determined [22-25]. Osterauer and Kohler [26] reported that the toxicity of diazinon against zebra fish strongly increased at elevated temperature. Dissolved oxygen is also one of the factors, which alter the toxicity of oleoresin. Water in winter season holds more oxygen [27] and as a result, less mortality of snails occurs during this period. At higher water temperature dissolved oxygen concentration decreases which is reflected by higher mortality of the snails. Dissolved oxygen is one of the major components, which is required by snails during metabolic activity [28,29]. Consequently, at higher temperature, increasing rate of metabolism in snail body may release more CO_2, which affects the pH of water [30,31] As the time duration increases concentration of CO_2 increases in the water (released by snails) and it also affects the pH of water. Murphy [32] reported that pesticides belonging to organophosphate and carbamate groups are very sensitive to change in pH. Earlier Vasconcellos [33] observed the influence of pH variation on the molluscicidal activity of *Euphorbia splendens* latex. According to them molluscicidal activity was maximum at pH 5.0 - 6.0 and minimum at pH 7.0 - 8.0. Toxicity of oleoresin is highest at high temperature, CO_2 of water as well as low pH, dissolved O_2 of water. The low concentration of dissolved O_2 act as physical stressor on aquatic animals [34] and in the absence of sufficient dissolved O_2; the snails appear to become more sensitive against the molluscicide. The pungent moieties of oleoresin are gingerol, zingirone and shogaol [35]. It is conceivable that, the active molluscicidal component, present in the oleoresin might get converted into a more toxic form in the aquarium water

Table 4. Effect of 24 h exposure of 40% and 80% of 24 h LC_{50} of oleoresin of *Z. officinale* in different months of the year 2006-07 on acid phosphatase activity in the nervous tissue of *L. acuminata.*

Months	24 h LC_{50} mgL^{-1}	ACP-μ moles/ 30min / mg protein		
		Control	40% of 24 h LC_{50}	80% of 24 h LC_{50}
August	39.67	3.15 ± 0.00 (100)	3.04 ± 0.01 (95.51)	2.91 ± 0.01 (92.38)
September	54.54	3.64 ± 0.01 (100)	3.58 ± 0.01 (98.35)	3.52 ± 0.01 (96.70)
October	55.92	3.31 ± 0.01 (100)	3.29 ± 0.01 (99.40)	3.24 ± 0.00 (97.89)
November	58.79	3.34 ± 0.02 (100)	3.32 ± 0.02 (99.40)	3.28 ± 0.01 (98.20)
December	62.18	3.28 ± 0.01 (100)	3.23 ± 0.01 (98.48)	3.19 ± 0.01 (97.26)
January	126.27	3.29 ± 0.01 (100)	3.27 ± 0.00 (99.70)	3.21 ± 0.02 (97.87)
February	124.09	3.40 ± 0.00 (100)	3.39 ± 0.01 (99.70)	3.33 ± 0.01 (97.94)
March	74.93	3.46 ± 0.01 (100)	3.40 ± 0.00 (98.27)	3.34 ± 0.01 (96.53)
April	24.88	3.26 ± 0.01 (100)	3.19 ± 0.01 (97.85)	3.09 ± 0.02 (94.79)
May	21.67	3.45 ± 0.02 (100)	3.38 ± 0.02 (97.97)	3.31 ± 0.02 (95.94)
June	16.54	2.95 ± 0.03 (100)	2.86 ± 0.01 (96.95)	2.72 ± 0.03 (92.20)
July	14.28	3.00 ± 0.01 (100)	2.82 ± 0.01 (94.00)	2.73 ± 0.01 (91.00)

Values are mean ± SE of six replicates. Value in parenthesis indicates % enzyme activity with untreated control taken as 100%. Rank correlation coefficient in between LC_{50} and ACP activity in treated group indicate significant ($P < 0.05$) positive (+) correlation. a, Significant ($P < 0.05$) when one way of ANOVA was applied in between the enzyme activity in different months of the year in control group without treatment.

or in the snail body due to variant environmental factors in the month of June and July. Earlier, it has been shown that the treatment of oleoresin of *Z. officinale* caused significant inhibition of AChE, ALP and ACP activity in the nervous tissue of *L. acuminata* [9]. The high anti AChE and ACP activity of oleoresin of *Z. officinale* was observed in months of June-July. The enzyme ALP plays a critical role in protein synthesis [36] and secretary activity [37] is comparatively less inhibited than AChE. Acid phosphatase (ACP), a lysosomal enzyme [38], plays an important role in autolysis and phagocytosis, pathological necrosis, and overall catabolism [8,10,39] was reduced significantly. Earlier, it has been observed that increased activity of ACP causes breakdown of existing protein in *L. acuminata* [18], but inhibition of ACP activity in this study indicates that it is not used in breakdown of cellular protein. The rank correlation coefficient applied between the LC_{50} values of different months and the corresponding inhibition in enzyme activity, point out a positive correlation between the LC_{50} and the inhibition of AChE and ACP. Whereas there was no correlation in between LC_{50} and ALP activity indicate that ALP is not altered by action of oleoresin in different months.

Accurate prediction of molluscicide fate and toxicity in aqueous environment against snails are hindered due to lack of information that how abiotic factors of aqueous environment affect the biological activity and related toxicity of molluscicides. Abiotic factors are not only correlated with the lethality of molluscicide, but with each other also. The present study conclusively shows that variant abiotic factors can significantly alter the toxicity of oleoresin of *Z. officinale* in *L. acuminata*. It is also obvious that the most suitable period for the control of this snail in India is the month of June, July. It is suggested that the treatment of a water body with oleoresin of *Z. officinale* for the control of *L. acuminata* and ultimately fascioliasis, is not only more potent and cost effective during these months than spending more money by using higher concentrations of this molluscicide during the rest ten months of the year.

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