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# Environmental Factors and the Toxicity of Eugenol and Quercetin against Snail *Lymnaea acuminata*

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## ABSTRACT

Snails of family Lymnaeidae act as an intermediate host of *Fasciola* species, which caused fasciolosis nearly in all the continents of world. Eugenol and quercetin are active molluscicides against snail *Lymnaea acuminata*. Laboratory evaluation is done to determine the effect of abiotic factors on the toxicity of these molluscicides in the year 2010-2011. Highest toxicity of eugenol (24 h $LC_{50}$ 10.03 mg $L^{-1}$) and quercetin (24 h $LC_{50}$ 129.13 mg $L^{-1}$) was observed in the month of May. Significant correlation was observed between $LC_{50}$ values of eugenol and quercetin with temperature/pH/dissolved oxygen/carbon dioxide. *In vivo* acetylcholinesterase (AChE), alkaline phosphatase (ALP) and acid phosphatase (ACP) activity were measured in the nervous tissue of treated snails in each month of year 2010-2011.

**Key words:** Eugenol, abiotic factors, quercetin, enzyme, *Lymnaea acuminata*

## INTRODUCTION

Fasciolosis is one of the world's most prevalent zoonotic disease caused by trematode parasite of the genus *Fasciola* that infects over 17 million people causing significant morbidity and mortality (Mas-Coma *et al*., 2005; WHO., 2006). Fasciolosis becomes hyperendemic and problematic in regions, where animal reservoir and snail vectors are easily available (Hassan *et al*., 2011). Fasciolosis caused serious economic losses to animal husbandry in the Northern part of India. (Upadhyay and Singh, 2011a) Treatment of *Fasciola* requires high or multiple doses of drugs (Abdul-Samie *et al*., 2010). Snail is considered to be one of the weakest links in *Fasciola*. So management of snail population below a threshold level is advocated for effective control of fasciolosis (Mello-Silva *et al*., 2006). Heavy use of synthetic molluscicides for control of snail population has created serious problem for the aquatic animals. Alternatively, in recent years much attention has been focused on the use of molluscicides of plant origin. Plant molluscicidal appears to be a simple, inexpensive and ecologically safe (Al-Daihan, 2010; Upadhyay and Singh, 2011a,b). Eugenol and quercetin are potent molluscicides found in *Syzygium aromaticum* and *Allium cepa* (Singh *et al*., 1997; Kumar and Singh, 2006).

Earlier studies have shown that various abiotic factors such as temperature, pH, dissolved oxygen, carbon dioxide and conductivity of water affects the toxicity of the synthetic molluscicides (Singh and Singh, 2009). Acetylcholinesterase (AChE), acid phosphatase (ACP) and alkaline phosphatase (ALP) are the sensitive enzymes in the nervous tissue of *L. acuminata*. These enzymes are mainly are inhibited by several potent plant molluscicides (Jaiswal *et al*., 2008; Kumar *et al*., 2009; Upadhyay and Singh, 2011b). The prospective of the present study is to reveal whether

seasonal changes in abiotic factors, viz. temperature, pH, dissolved oxygen and carbon dioxide and conductivity of the test water can influence the activity of AChE, ACP and ALP assayed in each month of the year 2010-2011 as well as toxicity of eugenol and quercetin.

## MATERIALS AND METHODS

**Test materials:** Eugenol is a phenylpropene, an ally chain substitute of guiacol, quercetin is a plant derived flavonoid (3, 3, 4, 5, 7-penta hydroxyl flavones), purchased from Sigma Chemicals Co. U.S.A. Temperature, pH and electrical conductivity were measured by thermometer, digital pH meter and conductivity meter, respectively. Dissolved oxygen and carbon dioxide were estimated according to the method of APHA (2005).

**Bioassays for $LC_{50}$:** Adult snail *Lymnaea acuminata* (length 2.30±0.25 cm) were collected from the different ponds, pools and lake near the Gorakhpur university campus. These experimental snails were acclimatized in dechlorinated tap water for 72 h. Ten experimental snails were kept in a glass aquarium containing 3 L of dechlorinated tap water. Snails were exposed to different concentration of eugenol and quercetin. A set of control animals were kept in an equal volume of dechlorinated tap water under similar conditions. Lethality of the concentrations was observed by number of mortal snails after 24, 48, 72 and 96 h. No behavioral response towards the needle probe is taken as the evidence of the death of snails. Simultaneously with the toxicity test at every 24-96 h temperature, pH, dissolved oxygen, carbon dioxide and conductivity were also measured. Bioassay for the determination of $LC_{50}$ was performed in each month of the year. Lethal concentration ($LC_{50}$) values, lower and upper confidence limits (LCL and UCL) and slope values were calculated by the method of POLO computer programme of Robertson *et al*. (2007). The product moment coefficient was determined between $LC_{50}$ and temperature/pH/conductivity/ dissolved oxygen/carbon dioxide of water in each month to observe any significant correlation.

**Enzyme assays:** In a glass aquarium containing 3 L of dechlorinated water, twenty experimental snails were exposed to sublethal concentration (60% of 24 h $LC_{50}$) of eugenol and quercetin in each month of the year. After the 24 h exposure the snails were washed with water and the nervous tissue was dissected out for the measurement of AChE, ACP and ALP activity.

**Acetylcholinesterase:** Acetylcholinesterase (AChE) activity was measured according to the method of Ellman *et al*. (1961) as modified by Singh and Agarwal (1983). Fifty milligram of nervous tissue was homogenized in 1.0 mL of 0.1 M phosphate buffer pH 8.0 for 5 min in an ice bath and centrifuged at 1000 g for 30 min at 4°C. Supernatant was used as enzyme source. The change in optical density at 412 nm was recorded for 3 min after every 30 sec interval. Enzyme activity was expressed as micro moles "SH" hydrolysed/min/mg protein.

**Phosphatases:** Acid (ACP) and alkaline (ALP) phosphatases activities was measured by the method of Bergmeyer (1967) as modified by Singh and Agarwal (1989). Tissue homogenate (2%w/v) was prepared in ice cold 0.9% NaCl and centrifuged at 5000 g for 20 min at 4°C. The 4-nitro phenyl phosphate disodium was used as substrate. The acid (ACP) and alkaline phosphate (ALP) have been expressed as micro moles substrate hydrolysed/30 min/mg protein.

**Protein estimation:** Protein was estimated by the method of Lowry *et al*. (1951).

**Statistical analysis:** Results have been expressed as Mean ±SE of six replicates. Rank correlation was applied in between $LC_{50}$ and corresponding changes in enzyme activity in different months of the year 2010-2011 (Sokal and Rohlf, 1973). One way analysis of variance were applied between enzymatic activity in different month of the year.

## RESULTS AND DISCUSSION

There was significant ($p<0.05$) variation in the toxicity of eugenol and quercetin against the *Lymnaea acuminata* in each month of the year 2010-2011. Highest toxicity of eugenol (24 h $LC_{50}$ 10.03 mg $L^{-1}$) (Table 1) and quercetin (24 h $LC_{50}$ 129.13 mg $L^{-1}$) (Table 2) was observed in the month of May and lowest in the month of January (24 h $LC_{50}$ 40.69 mg $L^{-1}$) (Table 1) and quercetin (24 h $LC_{50}$ 430.67 mg $L^{-1}$) (Table 2). A significant positive correlation between $LC_{50}$ and water pH/carbon dioxide, contrary a negative correlation between $LC_{50}$ and dissolved oxygen/temperature was observed in different months of year 2010-2011 (Table 1). There was no marked correlation in between the $LC_{50}$ and conductivity of water. The slope values were steep and separate estimation of $LC_{50}$ based on each of six replicates was found to be within 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 a positive rank correlation between $LC_{50}$ of eugenol in different months and corresponding AChE/ACP/ALP activity in the nervous tissue of treated snail *L. acuminata*. No significant rank correlation was observed between $LC_{50}$ of quercetin and corresponding ACP/ALP activity in the nervous tissue of treated snail.

Maximum inhibition of AChE (52.38% of control) was observed when snails were exposed to 60% 24 h $LC_{50}$ of quercetin followed by eugenol (66.66% of control). Maximum inhibition of ALP (60.15% of control) was observed when snails were exposed to 60% 24 h $LC_{50}$ of eugenol followed by quercetin (90.73% of control) (Table 3). Maximum inhibition of ACP (82.08% of control) was observed when snails were exposed to 60% 24 h $LC_{50}$ of eugenol (Table 3) followed by quercetin (90.72% of control) (Table 4).

Abiotic factors interact with each other to influence a physiological activity of animals (Gertseva and Gertseva, 2006). Pesticides become more lethal when there is an indulgence of abiotic conditions along with variation in pH and temperature (Relyea, 2006). The toxicity of molluscicides may get influenced by the abiotic factors such as temperature, pH, dissolved oxygen, carbon dioxide and conductivity (Singh and Singh, 2009). As we know when temperature is high the toxicity is also high as compare to higher $LC_{50}$ values in winter seasons. Temperature influences the activity of the molluscicides (Oliveira-Filho *et al*., 1999). Higher temperature diminishes the solubility of dissolved oxygen and thus decreases the availability of this essential gas. In normal condition metabolic demand for oxygen increases substantially with temperature (Portner, 2002). *Lymnaea acuminata* is very sensitive to dissolve content of water (Janse, 1981). The temperature and the dissolved oxygen shows inverse correlationship when temperature increases the oxygen dissolving capacity of water system are reduced. At low temperature the water holds more Oxygen ($O_2$) (Ingram *et al*., 1997). Dissolved oxygen is utilized by aquatic organism to accommodate metabolism and excreted as carbon dioxide ($CO_2$). At high temperature the rate of metabolism in snail body increases consequently, it causes release of more carbon dioxide in water. Higher concentration of carbon dioxide effect the pH of water (Toews *et al*., 1995; Berge *et al*., 2006). The high concentration of carbon dioxide in water may synergize the lethality of molluscicides.

Eugenol (Kumar and Singh, 2006; El-Din, 2006) and Quercetin (Singh *et al*., 1997; Lahlou, 2004) is potent molluscicides. The time dependant effect of these plant products may be

Table 1: Alterations in toxicity ($LC_{50}$ mg $L^{-1}$) of eugenol against *Lymnaea acuminata* and different environmental factors in pond water in different months of the year 2010-2011

| Parameters | May | June | July | August | September | October | November | December | January | February | March | April |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 h $LC_{50}$(mg $L^{-1}$) | 10.21 | 11.4 | 15.49 | 18.18 | 18.88 | 29.51 | 30.11 | 34.15 | 40.69 | 23.48 | 22.10 | 20.70 |
| Temperature (°C)* | 37±0.51 | 38±0.49 | 35±0.71 | 32±0.51 | 33±0.43 | 31±0.56 | 25±0.33 | 16±0.36 | 16±0.36 | 20±0.34 | 23±0.49 | 34±0.21 |
| $pH^{+}$ | 7.54±0.02 | 7.35±0.02 | 7.81±0.03 | 7.70±0.05 | 7.64±0.02 | 7.96±0.07 | 8.14±0.05 | 8.12±0.04 | 8.12±0.04 | 8.29±0.05 | 8.27±0.03 | 8.15±0.07 |
| DO ( ppm)* | 1.5±0.06 | 1.2±0.06 | 2.1±0.05 | 1.9±0.04 | 2.2±0.05 | 1.9±0.04 | 2.4±0.06 | 7.3±0.08 | 7.3±0.08 | 4.1±0.02 | 4.9±0.07 | 2.3±0.03 |
| $DCO_2$( ppm) $^{+}$ | 21.8±0.81 | 23.8±0.63 | 23.8±0.63 | 21.7±0.53 | 19.5±0.45 | 17.6±0.70 | 13.8±0.43 | 15.50±0.40 | 15.5±0.40 | 13.1±0.23 | 20.8±0.31 | 18.2±0.85 |
| Conductivity (µmhos $cm^{-1}$) | 29.4±0.06 | 40.2±0.04 | 40.2±0.04 | 44.7±0.06 | 47.5±0.05 | 48.2±0.07 | 47.9±0.03 | 33.5±0.08 | 33.5±0.08 | 30.2±0.07 | 36.4±0.04 | 31.7±0.03 |
| 48 h $LC_{50}$(mg $L^{-1}$) | 10.03 | 5.85 | 13.52 | 15.93 | 9.02 | 25.53 | 22.46 | 28.17 | 30.56 | 16.89 | 8.90 | 20.04 |
| Temperature (°C)* | 36±0.65 | 38±0.49 | 35±0.71 | 30±0.36 | 33±0.02 | 32±0.53 | 26±0.45 | 21±0.08 | 17.1±0.41 | 20±0.36 | 23±0.49 | 34±0.21 |
| $pH^{+}$ | 8.81±0.02 | 7.30±0.02 | 7.83±0.03 | 7.89±0.05 | 7.63±0.02 | 8.01±0.07 | 8.20±0.05 | 8.40±0.04 | 8.08±0.04 | 8.29±0.36 | 8.21±0.04 | 8.18±0.02 |
| DO (ppm)* | 1.1±0.08 | 1.0±0.04 | 2.0±0.006 | 1.4±0.04 | 1.8±0.05 | 1.7±0.06 | 1.9±0.01 | 3.1±0.08 | 6.5±0.04 | 4.1±0.23 | 4.50±.07 | 2.2±0.04 |
| $DCO_2$ (ppm)$^{+}$ | 22.6±0.71 | 28±0.56 | 16.1±0.16 | 31.8±0.54 | 22.1±0.46 | 21.8±0.80 | 15.8±0.86 | 21.8±0.41 | 18.8±0.01 | 14.2±0.11 | 23.1±0.31 | 19.5±0.07 |
| Conductivity (µmhos $cm^{-1}$) | 30.4±0.06 | 41.2±0.03 | 38.6±0.04 | 34.7±0.06 | 47.5±0.05 | 30.1±0.07 | 47.6±0.03 | 40.6±0.13 | 38.0±0.08 | 41.3±0.16 | 38.4±0.3 | 32.5±0.09 |
| 72 h $LC_{50}$ (mg $L^{-1}$) | 8.17 | 3.56 | 8.58 | 12.15 | 6.93 | 18.75 | 16.44 | 21.59 | 21.77 | 12.61 | 3.99 | 15.15 |
| Temperature (°C)* | 37±0.51 | 38±0.49 | 35±0.71 | 31±0.36 | 32±0.53 | 32±0.53 | 26±0.48 | 21±0.05 | 17.1±0.01 | 20±0.96 | 24.1±0.51 | 34±0.21 |
| $pH^{+}$ | 8.91±0.02 | 7.32±0.04 | 7.85±0.02 | 7.9±00.05 | 8.09±0.08 | 8.09±0.08 | 8.27±0.06 | 8.56±0.03 | 8.15±0.07 | 8.39±0.36 | 8.19±0.03 | 8.20±0.04 |
| DO (ppm)* | 1.0±0.08 | 0.9±0.05 | 1.9±0.06 | 1.4±0.04 | 1.5±0.08 | 1.5±0.08 | 1.6±0.02 | 2.6±0.08 | 5.9±0.08 | 3.5±0.05 | 3.3±0.05 | 2.0±0.02 |
| $DCO_2$ (ppm)$^{+}$ | 29.0±0.81 | 32±0.61 | 16.2±±0.01 | 31.8±0.54 | 22.6±0.07 | 22.6±0.07 | 20.1±0.07 | 23.8±0.02 | 22.1±0.01 | 19.3±0.13 | 31.1±0.31 | 21.6±0.03 |
| Conductivity (µmhos cm)$^{-1}$ | 31.8±0.06 | 41.2±0.03 | 40.2±0.05 | 34.7±0.06 | 35±0.07 | 35±0.07 | 49±0.03 | 41.6±0.12 | 40.0±0.08 | 42.1±0.11 | 40.3±0.04 | 32.5±0.03 |
| 96 h $LC_{50}$ (mg $L^{-1}$) | 4.98 | 2.01 | 5.34 | 8.22 | 4.52 | 14.26 | 12.94 | 20.32 | 15.33 | 8.85 | 1.30 | 10.29 |
| Temperature (°C)* | 38±0.51 | 38±0.49 | 35±0.71 | 31±0.03 | 33±0.02 | 32±0.53 | 26±0.48 | 20±0.75 | 17±0.01 | 20±0.18 | 24.1±0.01 | 34±0.21 |
| $pH^{+}$ | 8.80±0.03 | 7.34±0.05 | 7.85±0.02 | 7.95±0.04 | 7.41±0.02 | 8.10±0.02 | 8.29±0.01 | 8.60±0.08 | 8.09±0.21 | 8.29±0.01 | 8.11±0.02 | 8.20±0.06 |
| DO (ppm)* | 0.9±0.08 | 0.5±0.05 | 1.3±0.1 | 0.9±0.03 | 1.2±0.01 | 1.1±0.06 | 1.3±0.05 | 1.0±0.06 | 5.6±0.01 | 3.2±0.06 | 2.9±0.02 | 1.9±0.23 |
| $DCO_2$ (ppm)$^{+}$ | 30.0±0.91 | 35±0.52 | 18.2±0.01 | 38±0.01 | 28.3±0.16 | 27.1±0.07 | 28±0.08 | 26.6±0.08 | 23±0.01 | 24±0.08 | 37.2±0.31 | 22.0±0.23 |
| Conductivity (µmhos cm)$^{-1}$ | 32.8±0.06 | 41.3±0.02 | 42.2±0.05 | 40.0±0.09 | 28.1±0.16 | 36±0.06 | 50±0.03 | 42.2±0.12 | 45±0.001 | 46±0.09 | 42.3±0.01 | 35±0.04 |

Each experiment was replicated six times and values are the mean of six replications, Temperature, pH, DO: Dissolved oxygen, $DCO_2$: free carbon dioxide and conductivity were measured at intervals of 24 h up to 96 h. Product moment correlation coefficient in between $LC_{50}$ and different abiotic parameters indicate significant ($p<0.05$) positive (+) and negative (*) correlation. Testing significance of the regression coefficient May $3.68^{+}$, June $8.86^{++}$, July $9.16^{+}$, Aug $11.89^{+}$, Sep$12.74^{++}$, Oct $14.24^{++}$, Nov $8.73^{+}$, Dec $7.36^{++}$, Jan $14.44^{+}$, Feb $10.36^{+}$, Mar $6.10^{++}$, Apr $4.95^{+}$. +: Linear regression between X and Y. ++: Non linear regression between log X and Y. Months show highest and lowest toxicity of eugenol against *Lymnaea acuminata*

Table 2: Alterations in toxicity ($LC_{50}$ mg $L^{-1}$) of quercetin against *Lymnaea acuminata* and different environmental factors in pond water in different month of the year 2010-2011

| Parameters | May | June | July | August | September | October | November | December | January | February | March | April |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 h $LC_{50}$ (mg $L^{-1}$) | 129.13 | 130.90 | 134.95 | 192.27 | 198.27 | 329.45 | 375.45 | 410.63 | 430.67 | 228.36 | 211.82 | 162.77 |
| Temperature (°C)* | 35±0.02 | 37±0.07 | 36±0.07 | 35±0.08 | 35±0.08 | 24±0.04 | 24±0.04 | 20±0.12 | 16±0.63 | 20.0±0.67 | 23±0.27 | 33±0.61 |
| $pH^{+}$ | 7.15±0.01 | 7.18±0.08 | 7.16±0.07 | 7.01±0.07 | 7.82±0.07 | 7.84±0.01 | 7.84±0.01 | 8.67±0.73 | 8.68±0.90 | 8.68±0.81 | 8.75±0.96 | 8.54±0.82 |
| DO (ppm)* | 4.2±0.04 | 4.6±0.06 | 5.4±0.04 | 6.8±0.06 | 5.7±0.07 | 5.1±0.53 | 5.7±0.53 | 4.3±0.91 | 4.2±0.09 | 4.6±0.09 | 5.6±0.21 | 3.2±0.51 |
| $DCO_2$ (ppm)$^{+}$ | 20.8±0.31 | 15.5±0.04 | 18.2±0.85 | 22.7±0.05 | 20.5±0.04 | 17.6±0.05 | 13.8±0.07 | 27.8±0.63 | 18.8±0.04 | 20.1±0.03 | 20.0±0.08 | 13.8±0.47 |
| Conductivity (µmhos $cm^{-1}$) | 40.3±0.4 | 39±0.08 | 34.2±0.3 | 41±0.04 | 43.7±0.5 | 40.1±0.10 | 40.1±0.10 | 41.6±0.08 | 41.3±0.07 | 42.3±0.96 | 40.3±0.5 | 36.7±0.21 |
| 48 h $LC_{50}$ (mg $L^{-1}$) | 96.85 | 104.27 | 82.67 | 160.91 | 148.54 | 278.71 | 258.14 | 330.92 | 319.45 | 193.96 | 165.88 | 134.87 |
| Temperature (°C)* | 35±0.01 | 37±0.08 | 35±0.05 | 35±0.09 | 35±0.06 | 34±0.08 | 24±0.08 | 19±0.11 | 16±0.71 | 20±0.63 | 23.1±0.13 | 33±0.71 |
| $pH^{+}$ | 7.82±0.63 | 7.22±0.09 | 7.20±0.00 | 7.08±0.07 | 7.73±0.07 | 8.44±0.08 | 8.70±0.08 | 8.56±0.5 | 8.69±0.72 | 8.69±0.09 | 8.73±0.82 | 8.66±0.78 |
| DO( ppm)* | 3.9±0.08 | 4.3±0.07 | 4.2±0.05 | 4.1±0.09 | 4.9±0.05 | 4.2±0.08 | 4.2±0.03 | 3.9±0.71 | 3.8±0.86 | 4.0±0.87 | 4.0±0.36 | 3.2±0.90 |
| $DCO_2$( ppm)$^{+}$ | 22.1±0.31 | 20.5±0.04 | 19±0.03 | 24.8±0.04 | 22.6±0.03 | 20.1±0.52 | 15.9±0.009 | 32.0±0.04 | 23.7±0.04 | 24.1±0.04 | 22±0.08 | 16.0±0.57 |
| Conductivity (µmhos $cm^{-1}$) | 44.2±0.5 | 38±0.08 | 32.1±0.4 | 41±0.04 | 41±0.04 | 48±0.08 | 41.3±0.20 | 41.8±0.00 | 42.3±0.08 | 42.1±0.18 | 41.2±0.5 | 34.1±0.33 |
| 72 h $LC_{50}$ (mg $L^{-1}$) | 63.82 | 75.55 | 68.35 | 135.64 | 106.76 | 203.09 | 200.47 | 233.73 | 209.74 | 155.05 | 121.50 | 80.87 |
| Temperature (°C)* | 35±0.02 | 37±0.06 | 36±0.08 | 35±0.08 | 35±0.08 | 350.09 | 24±0.00 | 16±0.12 | 16±0.82 | 20±0.19 | 22±0.77 | 33±0.61 |
| $pH^{+}$ | 8.20±0.63 | 7.94±0.23 | 7.41±0.04 | 7.25±0.02 | 7.42±0.4 | 8.610.06 | 8.64±0.64 | 8.81±0.54 | 8.39±0.76 | 8.39±0.04 | 8.96±0.11 | 8.86±0.81 |
| DO (ppm)* | 3.5±0.06 | 3.1±0.08 | 4.0±0.04 | 5.8±0.05 | 5.5±0.05 | 5.20.81 | 4.2±0.33 | 3.9±0.86 | 4.0±0.94 | 4.3±0.89 | 5.2±0.62 | 3.0±0.89 |
| $DCO_2$ (ppm)$^{+}$ | 24±0.31 | 22.3±0.04 | 20.3v0.09 | 26.9±0.45 | 25.8±0.03 | 21.1±0.73 | 22±0.08 | 31.0±0.04 | 24.8±0.04 | 25.1±0.04 | 25±0.63 | 20±0.57 |
| Conductivity (µmhos $cm^{-1}$) | 48.0±0.6 | 29±0.10 | 32.3±0.5 | 41.0±0.23 | 45.1±0.05 | 48±0.00 | 42.2±0.11 | 41.3±0.09 | 46.1±0.09 | 42.0±0.00 | 44.2±0.4 | 37.6±0.087 |
| 96 h $LC_{50}$ (mg $L^{-1}$) | 47.53 | 47.37 | 51.38 | 92.91 | 84.00 | 151.05 | 158.55 | 171.80 | 186.210 | 116.52 | 82.26 | 68.39 |
| Temperature (°C)* | 35±0.03 | 37±0.08 | 35±0.06 | 35±0.06 | 35±0.06 | 35±0.93 | 24±0.07 | 15±0.12 | 16±0.16 | 20±0.18 | 22±0.95 | 33±0.18 |
| $pH^{+}$ | 7.82±0.48 | 7.42±0.08 | 7.54±0.05 | 7.29±0.08 | 7.50±0.05 | 8.68±0.07 | 8.70±0.73 | 8.73±0.35 | 8.83±0.18 | 8.83±0.02 | 8.91±0.27 | 8.43±0.31 |
| DO (ppm)* | 2.8±0.08 | 2.7±0.06 | 3.2±0.02 | 5.5±0.05 | 3.7±0.03 | 4.4±0.04 | 3.1±0.93 | 3.9±0.08 | 3.9±0.03 | 4.2±0.52 | 5.0±0.67 | 3.0±0.79 |
| $DCO_2$ (ppm)$^{+}$ | 26±0.32 | 23.9±0.05 | 21.3±0.25 | 28.0±0.45 | 26.6±0.03 | 22.2±0.22 | 24±0.09 | 31.0±0.05 | 25.8±0.04 | 28.3±0.03 | 28.3±0.03 | 22±0.66 |
| Conductivity (µmhos $cm^{-1}$) | 45.0±0.4 | 30.0±0.11 | 31.6±0.06 | 42±0.04 | 46±0.06 | 50±0.09 | 43.0±0.00 | 41.6±0.09 | 46.6±0.08 | 42.0±0.29 | 47.0±0.3 | 37.6±0.48 |

Each experiment was replicated six times and values are the mean of six replications, Temperature, pH, DO: Dissolved oxygen, $DCO_2$: free carbon dioxide and conductivity were measured at intervals of 24 h up to 96 h. Product moment correlation coefficient in between $LC_{50}$ and different abiotic parameters indicate significant ($p<0.05$) positive (+) and negative (*) correlation. Testing significance of the regression coefficient May $90.79^{++}$, June $9.87^{+}$, July $13.84^{++}$, Aug $13.12^{+}$, Sep $8.87^{+}$,Oct $17.79^{+}$, Nov $16.37^{++}$, Dec $17.42^{+}$, Jan $7.42^{++}$, Feb $50.67^{+}$, Mar $13.76^{+}$, Apr $6.28^{+}$. +: Linear regression between X and Y. ++: Non linear regression between log X and Y. Months show highest and lowest toxicity of quercetin against *Lymnaea acuminata*

Table 3: Effect of 24 h exposure of 60% of 24 h $LC_{50}$ of eugenol in different months of the year 2010-2011 on acetylcholinesterase, alkaline and acid phosphatase activity in the nervous tissue of *L. acuminata*

| | | AChE -µ moles 'SH' hydrolyzed/30min/mg protein | | ACP-µ moles substrate/30min/mg protein | | ALP-µ moles substrate/30min/mg protein | |
|---|---|---|---|---|---|---|---|
| Months | 24 h $LC_{50}$ (mg $L^{-1}$) | Control[a] | 60% of 24 h $LC_{50}$ | Control[a] | 60% of 24 h $LC_{50}$ | Control[a] | 60%of 24 h $LC_{50}$ |
| May | 10.03 | 0.048±0.01(100) | 0.032±0.05(66.66) | 3.53±0.01 (100) | 3.12±0.00(88.38) | 4.93±0.01(100) | 3.06±0.02(62.06) |
| June | 11.14 | 0.097±0.01(100) | 0.066±0.05(68.04) | 4.13±0.01(100) | 3.39±0.02(82.08) | 3.89±0.01(100) | 2.34±0.01(60.15) |
| July | 15.49 | 0.091±0.03(100) | 0.065±0.01(71.42) | 3.67±0.005(100) | 3.07±0.16(83.65) | 2.12±0.01(100) | 1.33±0.39(62.73) |
| August | 18.18 | 0.095±0.02(100) | 0.071±0.00(74.73) | 3.83±0.004(100) | 3.33±0.05(86.94) | 4.05±0.01(100) | 2.72±0.07(67.16) |
| September | 18.88 | 0.049±0.01(100) | 0.041±0.03(83.67) | 3.96±0.007(100) | 3.45±0.00(89.18) | 2.27±0.05(100) | 1.67±0.09(73.57) |
| October | 29.51 | 0.152±0.06(100) | 0.132±0.00(86.84) | 2.59±0.006(100) | 2.43±0.01(93.82) | 4.47±0.03(100) | 3.18±0.05(71.14) |
| November | 30.11 | 0.193±0.01(100) | 0.170±0.01(88.08) | 3.20±0.009(100) | 3.00±0.01(93.75) | 3.17±0.23(100) | 2.53±0.08(79.18) |
| December | 34.15 | 0.148±0.01(100) | 0.133±0.00(89.86) | 3.41±0.00(100) | 3.35±0.04(98.19) | 3.43±0.06(100) | 2.85±0.01(83.09) |
| January | 40.69 | 0.200±0.01(100) | 0.182±0.00(91.00) | 2.88±0.05(100) | 2.41±0.05(83.68) | 3.17±0.01(100) | 2.08±0.05(82.33) |
| February | 23.48 | 0.154±0.02(100) | 0.138±0.00(89.61) | 3.38±0.001(100) | 3.20±0.05(94.67) | 2.53±0.01(100) | 2.72±0.1(82.22) |
| March | 22.10 | 0.186±0.03(100) | 0.165±0.07(75.00) | 3.75±0.007(100) | 3.43±0.03(78.23) | 1.35±0.06(100) | 1.19±0.01(82.22) |
| April | 20.70 | 0.042±0.07(100) | 0.032±0.06(76.19) | 4.11±0.06(100) | 3.78±0.06(91.97) | 4.15±0.03(100) | 2.95±0.02(71.08) |

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/ALP/ACP activity in treated group indicate significant ($p<0.05$) positive (+) correlation a, Significant ($p<0.05$) when one way ANOVA was applied in between the enzymatic activity in different month of the year in control as well as treated group

Table 4: Effect of 24 h exposure of 60% of 24 h $LC_{50}$ of Quercetin in different months of the year 2010-2011 on acetylcholinesterase, alkaline and acid phosphatase activity in the nervous tissue of *L. acuminata*

| | | AChE -µ moles 'SH' hydrolyzed/30min/mg protein | | ACP-µ moles substrate/30min/mg protein | | ALP-µ moles substrate/30min/mg protein | |
|---|---|---|---|---|---|---|---|
| Months | 24h $LC_{50}$ (mg $L^{-1}$) | Control[a] | 60 % of 24 h $LC_{50}$ | Control[a] | 60% of 24 h $LC_{50}$ | Control[a] | 60% of 24 h $LC_{50}$ |
| May | 129.13 | 0.147±0.01(100) | 0.077±0.00(52.38) | 2.57±0.01(100) | 2.27±0.08(91.53) | 2.91±0.00(100) | 2.75±0.03(94.50) |
| June | 130.90 | 0.061±0.02(100) | 0.035±0.01(57.37) | 2.48±0.16(100) | 2.25±0.08(90.72) | 1.65±0.01(100) | 1.50±0.08(90.90) |
| July | 134.95 | 0.121±0.03(100) | 0.081±0.04(66.94) | 4.05±0.17(100) | 3.73±0.10(92.24) | 2.59±0.08(100) | 2.35±0.30(90.73) |
| August | 192.27 | 0.049±0.03(100) | 0.033±0.02(67.34) | 2.63±0.18(100) | 2.43±0.06(92.39) | 3.03±0.01(100) | 2.81±0.16(92.73) |
| September | 198.27 | 0.057±0.04(100) | 0.040±0.00(70.17) | 3.70±0.02(100) | 3.54±0.16(95.67) | 2.26±0.04(100) | 2.08±0.01(92.03) |
| October | 329.45 | 0.160±0.04(100) | 0.143±0.03(89.37) | 3.82±0.08(100) | 3.54±0.07(94.50) | 2.65±0.01(100) | 2.50±0.02(94.33) |
| November | 375.45 | 0.135±0.06(100) | 0.122±0.06(85.18) | 3.28±0.08(100) | 2.18±0.01(95.61) | 3.95±0.01(100) | 3.74±0.02(94.68) |
| December | 410.62 | 0.207±0.09(100) | 0.185±0.00(92.50) | 3.15±0.08(100) | 3.09±0.01(98.09) | 2.85±0.01(100) | 2.71±0.09(95.08) |
| January | 430.67 | 0.133±0.00(100) | 0.124±0.03(93.23) | 3.46±0.00(100) | 3.39±0.005(97.97) | 3.41±0.02(100) | 3.27±0.03(96.18) |
| February | 228.36 | 0.111±0.08(100) | 0.100±0.08(90.09) | 3.95±0.01(100) | 3.84±0.01(97.21) | 2.72±0.08(100) | 2.63±0.01(96.69) |
| March | 211.82 | 0.135±0.01(100) | 0.115±0.02(85.18) | 3.82±0.08(100) | 3.54±0.01(94.50) | 3.23±0.06(100) | 3.05±0.01(94.42) |
| April | 162.11 | 0.115±0.02(100) | 0.078±0.01(67.82) | 3.81±0.04(100) | 3.54±0.05(92.91) | 3.20±0.05(100) | 2.98±0.03(93.12) |

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/ALP/ACP activity in treated group indicate significant ($p<0.05$) positive (+) correlation a, Significant ( $p<0.05$) when one way ANOVA was applied in between the enzymatic activity in different month of the year in control as well as treated group.

due to the uptake of active moiety which progressively increases the amount of active components in snail body with increase in exposure period or it may be possible that the active component(s) could change into more toxic forms in the aquarium water or in the snail body.

The enzyme acetylcholinesterase occurs in the outer basal lamina of nerve synapses (Taylor, 1980) neuromuscular junction and in certain other tissues (Hall, 1973). Acetylcholinesterase (AChE) inhibition results in the accumulation of acetylcholine at nerve synapses, so that the post snaptic membrane is in a state of permanent stimulation producing paralysis, ataxia and general lack of co-ordination in neuromuscular system and eventual death. Earlier it has been reported that sublethal treatment 60% of 24 h $LC_{50}$ of eugenol caused significant inhibition of AChE activity (70.47% of control) in nervous tissue of *Lymnaea acuminata* (Kumar *et al*., 2009). Earlier it has been shown proved that quercetin is toxic to *L. acuminata* (Singh *et al*., 1997).

Eugenol has a radio protective potential (Tiku *et al*., 2004). It also acts as mixed type inhibitors (Chaieb *et al*., 2005). It acts as the sex attractant in fruit flies (Mahmood *et al*., 2002). It was observed that treatment of methanol (200 ppm) and ethanol (600 ppm) extracts of *S. aromaticum* were toxic against larvae of *Culex pipiens*. They reported that these extracts caused complete inhibition of adult emergence. Kim *et al*. (1998) observed that aqueous extract of *S. aromaticum* induced systematic and phylaxis in rats ($IC_{50}$-31.25 mg $kg^{-1}$). Quercetin is as effective as N-acetylecysteine in protecting rat peripheral lymphocytes against DNA damage (Muthukumaran *et al*., 2008). It improved immune function against external stress and also acts as powerful antioxidant and free radical scavenger (Shin *et al*., 2010).

Acid phosphatase (ACP) a lysosomal enzyme (Aruna *et al*., 1979), which plays an important role in catabolism, pathological necrosis, autolysis and phgocytosis (Abou-Donia, 1978) and alkaline which plays a critical role in protein synthesis (Pilo *et al*., 1972) and shell formation. Positive rank correlation coefficient between $LC_{50}$ value and corresponding AChE/ACP/ALP inhibition indicate that toxicity of eugenol is due to simultaneous inhibition of these enzymes. It indicates that changes in the activity of AChE/ACP/ALP in the nervous tissue of snail can also be the cause of mortality of these snails.

## CONCLUSION

The present study conclusively shows that variant abiotic factors can significantly alter the toxicity of eugenol. It also results for the most suitable period to control the snail in India. It is more potent and cost effective during these months rather than spending the rest months of the year. Out of both the molluscicides namely eugenol and quercetin, eugenol is prove to be more potent because of low values of $LC_{50}$ in comparison to quercetin. Hence eugenol can be used as potent molluscicides in the month of May rather than spending throughout the year against vector snail *Lymnaea acuminata.*

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