Bioactivity of Garlic Exposure on the Larval Traits of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.)

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**Abstract:** *Bombyx mori* L. is primary silk producing insect of great commercial value. Present study was carried out to evaluate the effect of volatile (*Allium sativum*) on the duration and length of *B. mori* larvae. The change in the volatile exposure duration and the number of larval treatment influenced the duration and length of larvae. The larval length increased with the increasing number of larval treatment from single to triple with the garlic exposure duration of 15, 30 and 45 minutes. Maximum larval length (6.85±0.023 cm) was noticed in case of triple treatment with 45 minute exposure duration and minimum larval length (3.87±0.182 cm) of 5 instar larvae was recorded in case of triple treatment with 60 minute exposure duration. The minimum larval duration (22.31±0.325 days) was recorded in case of triple treatment with 45 minute exposure duration. If volatile applied tactfully in silkworm rearing may be useful to improve the production of silk and quality of silk on commercial scale.

**Key words:** Larval Duration · Larval Length · *Allium sativum* · Exposure Duration · Volatile

**INTRODUCTION**

Nistari race is a resistant variety of multivoltine mulberry silkworm *Bombyx mori*, which contributes up to a great extent in the commercial production of cocoon. The larval duration and larval length are the most important factors, which influence the production of cocoon at commercial scale. The efforts are being made to evolve new technologies that are effective, labour saving and eco-friendly. In order to increase the production of silk, efforts have been made to study the effect of X-rays [1], temperature [2], the ecological factors [3, 4], relative humidity [5], photoperiod [6], seasonal variation [7], refrigeration of cocoon [8], cocoon magnetization [9], egg magnetization [10], fed three different variety [11], effect of antibiotic [12], folic acid administration [13], juvenile hormone analogue and phytoecdysteroid [14], royal jelly [15], herbal tonic [16] and nux vomica [17], 20-Hydroxyecdysone also influenced the performance of *B. mori* [18]. The garlic has antibacterial [19] and antimicrobial [20], properties and volatile compound [21], chemistry [22] of garlic. The garlic also used as controlling silkworm disease [23] and antymytic activity against pathogenic fungus of white muscardine disease in silkworm *B. mori* Linn. [24], plant and human disease [25].

It is hypothesized that if the larvae of *B. mori* L. are exposed to garlic volatile in different time duration there may be some beneficial effect on the life pattern of silkworm larvae, keeping this is view, an attempt has been made to investigate the effect of garlic volatile on the length and duration of larvae of multivoltine mulberry silkworm.

**MATERIALS AND METHODS**

The seed cocoon (pupa enclosed in silken case) of multivoltine mulberry silkworm, *Bombyx mori* nistari, a native of west Bengal in India, was taken in the present study. The seed cocoon (pupa enclosed in silken case), obtained from the silkworm grainage Behraich, Directorate of Sericulture Uttar Pradesh and were maintained in the plywood trays (23×20×5 cm) under the ideal rearing condition [26] in the silkworm laboratory, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur. The temperature, relative humidity and photoperiod were maintained at 26±1°C, 80±5% RH and 12±1 hours light a day till the emergence of moth from the seed cocoon. The moths emerged generally in the morning at around 4 am. The trays, in which seed cocoon were kept, were suddenly illuminated by light in the morning at 4 o’clock on 9th and 10th day of spinning.

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The newly emerged moth, from seed cocoons, were quickly picked up and kept sex-wise in separate trays to avoid copulation. The male moths were smaller and more active than the female moths. The whole grainage operation was performed as per description given by Krishnaswamy et al. [26].

The disease free laying (D.F.Ls), thus prepared, were treated with 2% formalin for 15 minutes to increase the adhesiveness of eggs on the paper sheet, with the egg laid on, were thoroughly washed with running water formalin and the eggs were dried in shade. The dried eggs were transferred to the incubator for hatching.

After two consecutive days of hatching, the silkworm larvae were collected with the help of bird’s feather and reared to maintain a stock culture in the silkworm laboratory at 26±1°C, 80±5% RH and 12±1 hour light a day. Four feeding of the small pieces of fresh and clean leaves of *Morus alba* were given to the larvae and care was taken that food always remained in excess in the rearing trays. 3rd, 4th and 5th instar larvae were taken for observation.

**Experimental Design:** To observe the effect of garlic (*Allium sativum*) bulb volatile on the duration and length of *B. mori* larvae. In the present study garlic volatile were taken experiment due to their antifungal, viral, bacterial etc. activity and easily available in market with very low cost. The experiments were performed with different time duration 15, 30, 45 and 60 minute with respect to the treatment of 3rd, 4th 5th instar larvae. Three sets of experiments were designed viz, single double and triple treatment of larvae.

**Single Treatment of Larvae:** Single treatment of larvae was performed at the initial stage of fifth instar larvae. Just after fourth moulting 90 larvae of fifth instar at initial stage were taken out from the BOD incubator and treated with garlic volatile with 15 min exposure duration.

**Double Treatment of Larvae:** Double treatment of larvae was started from the initial stage of fourth instar. In the first treatment 90 larvae of fourth instar, were taken out from the BOD incubator and treated with garlic volatile with 15 min exposure duration. The treated larvae were transferred in BOD incubator for rearing and development. Further similar second treatment for the same larvae was given at the initial stage of fifth instar larvae, thus in double treatment fourth and fifth instar larvae were treated.

**Triple Treatment of Larvae:** For the triple treatment the third instar larvae in the initial stage were taken out from BOD incubator. In the first treatment 90 larvae of third instar were treated with garlic volatile 15 minute exposure duration and kept in BOD for general rearing and development. The second treatment of the same larvae was done just after third moulting i.e. at the initial stage of fourth instar larvae and transferred in BOD incubator for rearing. Third treatment was given at initial stage of fifth instar, i.e. just after fourth moulting of the same treatment larvae as earlier, Thus in the triple treatment third, fourth and fifth instar larvae were treated.

Similar experiments were performed by 30, 45 and 60 minute exposure duration of garlic volatile. A control set was always maintained with each set of experiments.

**Larval Duration:** The time required from the hatching of treated larvae to the third day of spinning by the fifth instar larvae was considered. For this purpose, 90 larvae (three batches of 30 larvae in each batch) were taken for observation. Three replicates of each experiment were made.

**Larval Length:** The length of 30 larvae (three batches of 10 larvae in each batch) was recorded for each replicate. Three replicates of each experiment were made. The larval length of fifth instar larvae was taken as its normal length on the day when fifth instar larvae stop feeding.

**RESULTS**

**Larval Duration:** The data presented in Table 1a clearly indicates that change in the garlic volatile exposure duration and the number of larval treatment influenced the larval duration with the increasing number of larval treatment from one to three times. The larval duration decreased in case of 15, 30 and 45 minute garlic volatile exposure duration but further increase in exposure duration caused increase in larval duration The trend of decrease in the larval duration with the increasing number of larval treatment has recorded almost similar in case of 15, 30 and 45 minute garlic volatile exposure duration. The minimum larval duration was noticed to be 22.31±0.350 days (15.97% decreased as compared to control) in the triple treatment with 45 minute exposure duration and maximum larval duration was recorded 27.12±0.096 days in case of triple treatment with 60 minute exposure duration.
Table 1a: Effect of garlic volatile exposure on the duration (days) of *Bombyx mori* Larvae

<table>
<thead>
<tr>
<th>Stage of treatment (larval instar)</th>
<th>Control (X₀)</th>
<th>(X₁) 15</th>
<th>(X₂) 30</th>
<th>(X₃) 45</th>
<th>(X₄) 60</th>
<th>F-ratio n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single (5₀)</td>
<td>26.55±0.317</td>
<td>25.76±0.656</td>
<td>25.48±0.310</td>
<td>25.23±0.380</td>
<td>26.91±0.318</td>
<td>9.9832*</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(97.02)</td>
<td>(95.97)</td>
<td>(95.03)</td>
<td>(101.36)</td>
<td></td>
</tr>
<tr>
<td>Double (4₀ - 5₀)</td>
<td>26.55±0.317</td>
<td>25.48±0.268</td>
<td>24.79±0.422</td>
<td>24.40±0.493</td>
<td>26.70±0.216</td>
<td>3.3778*</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(95.97)</td>
<td>(93.37)</td>
<td>(91.90)</td>
<td>(100.56)</td>
<td></td>
</tr>
<tr>
<td>Triple (3₀-4₀-5₀)</td>
<td>26.55±0.317</td>
<td>24.33±0.422</td>
<td>24.18±0.373</td>
<td>22.31±0.350</td>
<td>27.12±0.096</td>
<td>5.7198*</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(91.64)</td>
<td>(91.07)</td>
<td>(84.03)</td>
<td>(102.15)</td>
<td></td>
</tr>
</tbody>
</table>

F₀ -ratio = 3.3778**

Each value represents mean±S.E. of three replicates

X₀, X₁, X₂, X₃ and X₄ are the mean values of larval duration in control, 15, 30, 45 and 60 minute exposure duration respectively

Figures in parentheses indicate percent value when control was taken as 100%

Table 1b: Post-hoc test showing effect of garlic volatile exposure on the duration (days) of *Bombyx mori* larvae

<table>
<thead>
<tr>
<th>Stage of treatment</th>
<th>Mean difference in between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
</tr>
<tr>
<td>X₀ – X₀</td>
<td>0.790</td>
</tr>
<tr>
<td>X₀ – X₁</td>
<td>1.070</td>
</tr>
<tr>
<td>X₀ – X₂</td>
<td>1.320</td>
</tr>
<tr>
<td>X₀ – X₃</td>
<td>0.360</td>
</tr>
<tr>
<td>X₀ – X₄</td>
<td>0.280</td>
</tr>
<tr>
<td>X₁ – X₃</td>
<td>0.530</td>
</tr>
<tr>
<td>X₁ – X₄</td>
<td>1.150</td>
</tr>
<tr>
<td>X₁ – X₅</td>
<td>0.250</td>
</tr>
<tr>
<td>X₂ – X₃</td>
<td>1.430</td>
</tr>
<tr>
<td>X₂ – X₄</td>
<td>1.680</td>
</tr>
</tbody>
</table>

Honesty significant difference (HSD) = q √(MS within / n) = 5.04 √⁶⁹ / 3 = 1.9515

MS = Mean square value of ANOVA Table
q = Studentized range statistic
n = No. of replicates
* = Shows significant group difference
X₀, X₁, X₂, X₃ and X₄ are the mean values of larval duration in control, 15, 30, 45 and 60 minute exposure duration respectively

Two way ANOVA indicates that the garlic volatile exposure duration significantly (P < 0.05) influenced the larval duration. The Post-hoc test (Table 1b, HSD = 1.9515) indicates significant group difference in larval duration. In double treatment of larvae significant group difference was noticed in between control and 45 minute and 45 and 60 minute. In triple treatment, significant group difference was noticed in between control and 15 minute, control and 30 minute, 15 and 45 minute, 15 and 60 minute, 15 and 45 minute and 45 and 60 minute exposure. In case of single treatment there was no significant group difference.

Larval Length: The data presented in Table 2a shows that change in the garlic volatile exposure duration and number of larval treatment influenced the larval length. With the increasing number of larval treatment from one to three times, the larval length increased in case of 15, 30, 45 minute exposure duration of garlic volatile but further increased exposure duration caused notable decline in the larval length. The trend of increase in the larval length with increasing number of larval treatment has been recorded to be almost similar in case of 15, 30, 45 minute exposure duration. The maximum larval length was noticed to be 6.85±0.023 cm (37.27% increased as compared to control) in case of triple treatment 45 minute exposure.
Table 2a: Effect of garlic volatile exposure on the length (cm) of *Bombyx mori* larvae

Volatile exposure duration

<table>
<thead>
<tr>
<th>Stage of treatment (larval instar)</th>
<th>Control (X₁)</th>
<th>15 (X₂)</th>
<th>30 (X₃)</th>
<th>45 (X₄)</th>
<th>60 (X₅)</th>
<th>F-ratio</th>
<th>n = 41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single (&lt;sup&gt;5&lt;/sup&gt;)</td>
<td>4.99±0.20</td>
<td>5.20±0.26</td>
<td>5.35±0.272</td>
<td>5.41±0.153</td>
<td>4.45±0.10</td>
<td>11.6070 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(104.21)</td>
<td>(107.21)</td>
<td>(108.42)</td>
<td>(89.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double (&lt;sup&gt;4&lt;/sup&gt; -&lt;sup&gt;5&lt;/sup&gt;)</td>
<td>4.99±0.20</td>
<td>5.30±0.10</td>
<td>5.50±0.10</td>
<td>6.28±0.148</td>
<td>4.20±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(106.21)</td>
<td>(110.22)</td>
<td>(125.85)</td>
<td>(84.17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triple (&lt;sup&gt;3&lt;/sup&gt; -&lt;sup&gt;5&lt;/sup&gt;)</td>
<td>4.99±0.20</td>
<td>5.61±0.20</td>
<td>5.92±0.17</td>
<td>6.85±0.02</td>
<td>3.87±0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(112.42)</td>
<td>(118.64)</td>
<td>(137.27)</td>
<td>(77.56)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F<sub>1</sub> -ratio = 1.1806  
\( n = 2 \)

*P < 0.01  
**Non Significant  

Each value represents mean±S.E. of three replicates  

X₁, X₂, X₃, X₄, and X₅ are the mean values of larval length in control, 15, 30, 45 and 60 minute exposure duration respectively.  

Figures in parentheses indicate percent value when control was taken as 100%

Table 2b: Post-hoc test showing effect of garlic volatile exposure on the length (cm) of *Bombyx mori* larvae

<table>
<thead>
<tr>
<th>Mean difference in between groups</th>
<th>Stage of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
</tr>
<tr>
<td>X₁ - X₂</td>
<td>0.210</td>
</tr>
<tr>
<td>X₁ - X₃</td>
<td>0.360</td>
</tr>
<tr>
<td>X₁ - X₄</td>
<td>0.420</td>
</tr>
<tr>
<td>X₁ - X₅</td>
<td>0.540</td>
</tr>
<tr>
<td>X₂ - X₃</td>
<td>0.150</td>
</tr>
<tr>
<td>X₂ - X₄</td>
<td>0.210</td>
</tr>
<tr>
<td>X₂ - X₅</td>
<td>0.750</td>
</tr>
<tr>
<td>X₃ - X₄</td>
<td>0.060</td>
</tr>
<tr>
<td>X₃ - X₅</td>
<td>0.900</td>
</tr>
<tr>
<td>X₄ - X₅</td>
<td>0.960</td>
</tr>
</tbody>
</table>

Honesty significant difference (HSD) = \( q \sqrt{\frac{MS\text{ within}}{n}} \)

\[
q = 0.1435 \\
MS\text{ within} = \frac{5.05}{3} \\
n = 1.104
\]

DISCUSSION

Volatile and particular biogenic volatile compound (VOCs) is everywhere. They directly and indirectly influence the lives of many plant and insect species and human beings in many ways. It is well known that an ideal race is one, which has a shorter larval duration thus causing low consumption of leaf [27]. Larval duration...
varied significantly due to seasonal changes [7, 28]. The temperature and humidity in rainy season influenced the larval duration of B. mori. [29]. Refrigeration of cocoon influenced larval duration [8]. When the silkworm larvae were exposed to 24 hours lights a day, the larval span was prolonged [30]. The rearing condition was also deciding the larval span and growth [31]. Cocoon magnetization affected larval duration [9]. The magnetization of eggs influenced larval duration [10]. Higher magnetic field strength [32] and reduction in the larval duration under 20 minute exposure at 3500 gauss [33]. The rich nutrients of mulberry leaves enhanced its growth. Three races of larval duration under 20 minute exposure at 3500 gauss treatment with the test compound R394 on B. mori larvae were exposed to 24 hours lights a day, the larval potassium, calcium and copper influenced larval length [46]. Optimum dosage of nitrogen, potassium, calcium and copper influenced larval length [60]. Bovine milk influenced the growth of B. mori [61]. Developmental stages are significantly related to the body length and head capsule width [62-64]. Organic manures have strong hold on the growth and development of silkworm. [52]. Maximum larval length was observed after treatment with the test compound R394 on B. mori larvae [65]. Three races of B. mori larvae fed with three varieties of mulberry leaves show significant increase in larval size [66]. Mulberry leaves sprayed with linseed oil, hemp oil and milk [67], Aloe vera essential oil influenced the length of larvae of B. mori [68]. Production of silk depends upon the larval length and the treated larvae obtained maximum length.

In the present investigation, the larval duration decrease and larval length increased with increasing the number of treatment with different exposure duration of garlic volatile, thus it may be concluded that larvae treated with garlic volatile have minimum larval duration and maximum larval length and, it may be possible due to decreasing the microbial pathogens causing silkworm diseases and increasing the feeding behavior of silkworm larvae. The above observation may be significant and best for silk industry.

REFERENCES


