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The Influence of Plant Volatile of *Allium sativum* on the Reproductive Ability of Multivoltine Mulberry Silkworm *Bombyx mori* Linn.

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Abstract: This study was carried out to show the influence of garlic volatile on the reproductive ability of *Bombyx mori* Linn. Variation in the exposure duration with volatile significantly (P_1<0.05) influenced the reproductive ability of *B. mori* in terms of fecundity and hatchability per cent of eggs. In the experiment silkworm larvae were exposed to different exposure duration viz. 15, 30, 45 and 60 minute with garlic volatile as single, double and triple treatment with respect to 3rd, 4th and 5th instars. The fecundity and hatchability gradually increased in single to triple treatment for 15 to 45 minute exposure duration and there after showed notable decline in all treatment of 60 minute exposure duration from single to triple treatment. The maximum fecundity and hatchability per cent was noticed to be 476.53±3.08 and 95.21±0.54 % in case of triple treatment for 45 minute exposure duration and minimum fecundity and hatchability per cent 402.20±3.27 and 86.27±0.63% was recorded in case of triple treatment for 60 minute exposure duration. In conclusion, it may be suggested that exposure of garlic as a plant volatile in sericulture may be useful for boosting up the sericulture industry as well as the economy of silkworm rearing.

Key words: Larvae · Fecundity · Hatchability · Mulberry leaves

INTRODUCTION

In sericulture, it is established fact that several factors contribute in the growth and development of silkworm for the production of quality eggs. Fecundity and hatchability are the two main factors of seed cocoon production. To increase the silk production various efforts have been made to study the effect of ecological factors [1-5] and magnetization of eggs [6, 7] and cocoons [8, 9].

The ecdysone is known to influence the reproductive potential, silk producing potential of *Bombyx mori* [10-17]. Variation in the 20-hydroxyecdysone concentration influenced the reproductive potential of *B. mori* [18].The garlic has antibacterial properties [19], antifungal [20], antithrombotic activity [21] and volatile compound [22]. The garlic also used as controlling silkworm disease [23] and antimycotic activity against pathogenic fungus of white muscordine disease in silkworm *B. mori* Linn. [24]. Garlic also contains vitamins and minerals [25] and trace elements [26]. Quality silkworm seed refers to richness of layings, egg viability, hatching uniformity and more importantly good rearing performance of the progeny [27] and it depends on management practices *i.e.,* rearing temperature, humidity, nutrition and genotype of the breed. The better rearing conditions, environment and nutrition during larval period may leads to higher fecundity by silkworm moths [28, 29]. Certain external factor has been noticed to influence the quality silkworm seed of *B. mori*. Some plant volatiles also influence the life pattern and performance of some insects. Garlic is probably one of the earliest known medicinal plants [30]. Its bulbs (cloves) had been used as a cure-allin ancient Egypt and are mentioned in the Ebers papyrus, one of the earliest treatises on medicinal plants. Garlic contains sulfur compounds. Alliin, is converted to the anti-microbial active allicin, when the bulb is cut or bruised. Keeping this in view it is proposed to undertake comprehensive study on the effect of plant volatiles on the Fecundity and hatchability per cent of *B. mori* eggs.

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MATERIALS AND METHODS

Seed Cocoons: The seed cocoons (pupa enclosed in silken case) of multivoltine mulberry silkworm (*Bombyx mori* nistari), a native of West Bengal in India, were obtained from the silkworm grainage. Directorate of Sericulture, Behraich Uttar Pradesh and were maintained in the plywood trays (23 x 20 x 5cm) under the ideal rearing conditions [31] in the silkworm laboratory, Department of Zoology, DDU Gorakhpur University Gorakhpur. The temperature and relative humidity were maintained at 26 ± 1°C and 80 ± 5% RH till the emergence of moths from the seed cocoons. The moths emerged generally in the morning at around 4 A.M. The tray in which seed cocoons were kept was suddenly illuminated by light in the morning at 4 O'clock on 9th and 10th day of spinning.

The newly emerged moths were quickly picked up and kept sex-wise in separate trays to avoid copulation. The male moths were smaller in size but more active than the female moths which were comparatively larger and less active.

Design of Experiment: To observe the influence of certain plant volatiles on the reproductive ability of *B. mori*, the larvae obtained from the laboratory (BOD incubator) were kept into a glass jar with plant volatile (Garlic extract as liquid form) on the filter paper into a petridish. In order to maintain volatile concentration within certain limits during the course of an experiment, the petridish was replaced after every 15 minute. The experiment was performed with different time interval of treatment with volatile to the 3rd, 4th and 5th instar larvae. Three set of experiment 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 in three replicate of 30 larvae at the initial stage were taken out from the BOD incubator and treated with different time interval like 15, 30, 45 and 60 minute, with garlic extract as a volatile.

Double Treatment of Larvae: Double treatment of larvae was started from the initial stage of fourth instar larvae. In the first treatment 90 larvae of fourth instar in three replicate of 30 larvae were treated with different time interval like 15, 30, 45, 60 minute with garlic extract as a volatile. 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 Larvae: For the triple treatment the third instar larvae in the initial stage were separated from BOD incubator. In the first treatment 90 larvae of third instar (three replicate of 30 larvae) were treated with different time interval like 15, 30, 45, 60 minute with volatile and kept in BOD for rearing. The second treatment was done just after third molting i.e. at the initial stage of fourth instar larvae and transferred in BOD incubator for rearing. The third treatment was given at the initial stage of fifth instar. Thus, in the triple treatment third, fourth, fifth instar larvae were treated.

A control set was always maintained with each set of experiment. The experiment was conducted on normal rearing condition i.e. 26±1°C Temperature, 80±5% relative humidity and 12±1 hours photoperiod a day. The parameters fecundity and hatchability percent of eggs were determined.

Fecundity: To observe the effect of garlic volatile on the egg laying ability of moths obtained from the experimental larvae, the early emerged moths were kept sex-wise in separate trays to avoid copulation. Three batches of five males and five females were prepared and they were allowed to mate. After four hours of mating, the paired moths were decoupled manually. Further, female moths were allowed to lay eggs on the sheet of paper. The egg laying moths were covered by open plastic cellules to prevent the intermixing of egg masses deposited by different female moths and after proper processing the eggs were transferred chronically to BOD incubator. For determining the fecundity, 15 layings (three batches of five layings in each batch) were taken. Thus, average of five layings was taken as representative number of eggs per laying, laid by female moth in each experiment.

Hatchability: For determining the influence of garlic extract as a volatile on the hatchability per cent of silkworm eggs, various sets of DFLs were considered. The eggs were transferred chronically to BOD incubator maintained at 26±1ºC temperature, 80±5% RH and 12±1 hours of photo period of a day. At head pigmentation stage, the eggs under incubation were black boxed and exposed to diffused light in the morning on the day of hatching. After completion of the incubation period, the

larvae started hatching which was completed within three days. After completed hatching (third day from the beginning of larval hatching) the DFLs were counted to obtain the data in respect of the total number of eggs laid per female moth, number of unfertilized eggs and number of hatched eggs per laying. The average hatching of 10 layings were taken representative hatchability percentage per laying in case of each batch of the study 30 layings (three batches of the 10 laying in each batch) were counted for each replicate. Three replicate of each experiment were made the hatchability was calculated.

RESULTS

Fecundity: The data presented in table-1a shows that change in the exposure duration of treatment with garlic extract as a volatile and the number of larval treatment influenced the fecundity. With the increasing number of larval treatment by garlic extract from single to triple, the fecundity increased in case of 15, 30 and 45 minute treatment but 60 minute treatment with garlic as a volatile caused notable decline in the fecundity in all treatment from single to triple. The maximum fecundity was noticed to be 476.53±3.08 eggs (17.19 % increased as compared to control) in case of triple treatment for 45 minute exposure duration with garlic as a volatile and the minimum 402.20±3.27 eggs (1.09 % decreased as compared to control) was recorded in case of triple treatment for 60 minute exposure duration with garlic extract as a volatile.

Two way ANOVA indicates that variation in the garlic volatile exposure duration significantly ($P < 0.05$) influenced the fecundity of *B. mori* moth (Table 1a). The Post-hoc test (Table 1b) indicates significant group difference in the fecundity in between control and 45 minute exposure duration with garlic extract as a volatile in case of double treatment. In the triple treatment significant group difference in the fecundity was found in between control and 15 minute, control and 30 minute, control and 45 minute, 15 and 45 minute, 15 and 60 minute and also in 30 and 60 minute, 45 and 60 minute exposure duration.

Table1a: Effect of plant volatile (*Allivum sativum*) exposure on the fecundity of *Bombyx mori*.

Stage of treatment (larval instar)	Volatile exposure duration (minute) Control (X_1)	15(X_2)	30(X_3)	45(X_4)	60(X_5)	F_1-ratio n_1= 4
Single (5th)	406.60±3.38 (100)	417.66±1.59 (102.72)	422.73±3.32 (103.96)	436.80±4.73 (107.42)	415.66±4.47 (102.22)	
Double (4th -5th)	406.60±3.38 (100)	433.60±3.11 (106.64)	436.40±5.35 (107.32)	454.00±2.78 (111.65)	422.26±4.21 (103.85)	8.5212*
Triple (3rd -5th)	406.60±3.38 (100)	442.00±3.76 (108.70)	452.26±3.73 (111.22)	476.53±3.08 (117.19)	402.20±3.27 (98.91)	

F_2 –ratio = 2.4676** n_2 = 2

*$P_1 < 0.05$

**Non Significant

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

X_1, X_2, X_3, X_4 and X_5 are the mean values of fecundity in control, 15, 30, 45 and 60 minute exposure duration respectively.

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

Table 1b: Post-hoc Test showing effect of plant volatile (*Allivum sativum*) exposure on the fecundity of *Bombyx mori*.

Mean difference in between groups	Stage of treatment Single	Double	Triple
$X_1 \sim X_2$	11.06	24.00	*35.40
$X_1 \sim X_3$	16.13	29.80	*45.66
$X_1 \sim X_4$	30.20	*47.40	*69.92
$X_1 \sim X_5$	09.06	15.66	04.40
$X_2 \sim X_3$	05.07	05.80	10.26
$X_2 \sim X_4$	19.14	23.40	*34.53
$X_2 \sim X_5$	02.00	08.34	*39.80
$X_3 \sim X_4$	14.07	17.60	24.27
$X_3 \sim X_5$	07.07	14.14	*50.06
$X_4 \sim X_5$	21.14	31.74	*74.33

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

$= 5.05\sqrt{\frac{134.944}{3}}$

$= 33.87$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are the mean values of fecundity in control, 15, 30, 45 and 60 minute exposure duration respectively.

Table2a: Effect of plant volatile (*Allium sativum*) exposure on the hatchability (%) of *Bombyx mori* eggs.

Stage of treatment (larval instar)	Volatile exposure duration (minute) Control (X_1)	15 (X_2)	30 (X_3)	45 (X_4)	60 (X_5)	F_1-ratio n_1= 4
Single (5th)	87.34±0.84 (100)	88.59±1.08 (101.43)	90.10±0.80 (103.16)	91.09±0.43 (104.29)	88.27±0.63 (101.06)	
Double (4th -5th)	87.34±0.84 (100)	89.78±0.66 (104.98)	91.69±0.51 (104.98)	92.72±0.33 (106.15)	88.49±0.70 (101.31)	9.0389*
Triple (3rd -5th)	87.34±0.84 (100)	90.42±0.69 (103.52)	93.42±0.41 (106.96)	95.21±0.54 (109.01)	86.27±0.63 (98.77)	

F_2 –ratio = 1.4017** n_2 = 2

*$P_1 < 0.05$

**Non Significant

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

X_1, X_2, X_3, X_4 and X_5 are the mean values of hatchability in control, 15, 30, 45 and 60 minute exposure duration respectively.

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

Table 2b: Post-hoc Test showing effect of plant volatile (*Allium sativum*) exposure on hatchability (%) of *Bombyx mori* eggs.

Mean difference in between groups	Stage of treatment Single	Double	Triple
$X_1 \sim X_2$	1.25	2.44	3.08
$X_1 \sim X_3$	2.76	*4.35	*6.08
$X_1 \sim X_4$	3.75	*5.38	*7.87
$X_1 \sim X_5$	0.93	1.15	1.07
$X_2 \sim X_3$	1.51	1.95	3.00
$X_2 \sim X_4$	2.50	2.94	*4.79
$X_2 \sim X_5$	0.32	1.29	*4.14
$X_3 \sim X_4$	0.99	1.03	1.79
$X_3 \sim X_5$	1.83	3.20	*7.15
$X_4 \sim X_5$	2.82	*4.23	*8.94

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

$= 5.05\sqrt{\frac{1.895}{3}}$

$= 4.01$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are the mean values of hatchability in control, 15, 30, 45 and 60 minute exposure duration respectively.

Hatchability: The data presented in table 2a indicates that change in the exposure duration of treatment with garlic extract as a volatile and the number of larval treatment influence the hatchability of *B. mori* eggs. With increase the number of larval treatment by garlic extract from single to triple treatment, the hatchability of egg increased in case of 15, 30 and 45 minutes exposure and reached to maximum level 95.21±0.54 % (9.01 % increased as compared to control) in case of triple treatment for 45 minute exposure duration garlic extract as a volatile treatment but 60 minute treatment caused notable decline in the hatchability of egg in all treatment from single to triple. The minimum hatchability 86.27±0.63% (1.23 % decreased as compared to control) was recorded in case of triple treatment for 60 minute exposure duration with garlic extract as a volatile.

Two way ANOVA indicates that variation in the garlic volatile exposure duration significantly ($P < 0.05$) influenced the hatchability per cent of *Bombyx mori* eggs (Table 2a). The Post-hoc test (Table 2b) indicates significant group difference in the hatchability per cent in between control and 30 minute, control and 45 minute and 45 and 60 minute exposure with garlic extract as a volatile in case of double treatment. In the triple treatment significant group difference in the hatchability was noticed in between control and 30 minute, control and 45 minute, 15 and 45 minute, 15 and 60 minute, 30 and 60 minute and 45 and 60 minute exposure.

DISCUSSION

The fecundity is hereditary character [32]. The fecundity of moths emerged from the pupae of refrigerated eggs [33] and refrigerated pupae [34], has been noticed to be negatively influenced showing the sharp decline in the eggs laying potential of silkworm. Effect of crowding on fecundity of eri silkworm, *Philosamia ricini* [35]. The fecundity of *B. mori* varies basically due to variation in the race of silkworm [36]. Highly significant positive correlation of pupal weight with the fecundity

has been noticed in some other sericogenous moth viz *Antheraea mylita* [37] *P. ricini* [38] and *Samia cynthia ricini* [39]. The heavy fecundity was noticed in the moths, obtained from *B. mori* larvae feeding on ascorbic acid treated mulberry leaves [40]. At high range of relative humidity, the fecundity of *B. mori* declined with the storage duration of male and female moth [41]. The production of eggs has been noticed to be influenced by the mating duration in *B. mori* [42]. The heat treatment of *B. mori* caused an increase in the fecundity of silkworm [43]. The exposure of gamma radiation of *B. mori* eggs caused an increase in the fecundity [44] and cocoon magnetization influences fecundity [8]. In female insects, the steroid hormone 20-hydroxy ecdysone (20 E) plays a major role in activating vitellogenesis, a process required for egg development [45]. Insect reproductive activity is controlled by juvenile hormone [46] and ecdysone hormone [10]. Corpus allatum (CA), the source of JH regulated egg formation and the presence of an active corpus allatum is necessary for successful yolk deposition and egg maturation [46]. JH plays a key role in the ovariole development, oocyte maturation etc; with an equally important role by ecdysone released from prothoracic gland (PG) in silkworm, *B. mori* [11]. The egg laying capability of the *B. mori* reduced when silkworm were treated by an antijuvenile hormone agent, KK-42 [47]. The activity of juvenile hormones considerably influenced the reproductive potential of *B. mori* [48]. Effect of 20-hydroxyecdysone on egg production of silkworm resulted into more large eggs in addition to normal eggs [12]. Addition of feed additives was effective in increasing the fecundity [49].

Variation in the 20-hydroxyecdysone concentration significantly ($P < 0.05$) influenced the reproductive potential of *B. mori* in terms of fecundity and hatchability of eggs [18]. The Phytoecdysteroid are not hypersensitive androgenic, oestrogenic, or anti oestrogenic and do not induce virilisation [50]. Antijuvenile hormone is generally known to prevent egg maturation when applied to feeding adults [51]. Rearing of silkworm larvae at lower levels of RH resulted in lower fecundity, hatchability [52]. Generally mutants are inferior to the normal in several characters such as fecundity and hatching [53]. The survival and development of insects are at the mercy of nature and developmental activities are restricted in accordance with the prevailing ecological conditions and to a certain extent to their genetic built up [54, 55, 31]. The treatment of *B. mori* eggs with HCl (6N) caused higher hatchability of eggs [56, 51]. The increasing duration of refrigeration of *B. mori* eggs caused notable decline in the hatching per cent [57-58]. At higher duration (50 and 70 days) of the refrigeration of *B. mori* eggs, hatchability declined sharply [58]. The cocoon magnetization influences hatchability [8]. Maternal ecdysteroids appear to be required at different titeres for fertilization, embryogenesis and hatching of the silkworm larvae [13]. Ecdysone 20-mono oxygenase in eggs of the silkworm *B. mori* was characterized in relation to embryonic development [59].

In conclusion, the fecundity and hatchability of moths obtained from the larvae (exposed in garlic volatile with different exposure duration) influenced may be due to ultrastructural changes in the cell contents and enzyme activity during larval and pupal stages caused positive effect on the reproductive traits resulting increase in the fecundity and hatchability of *B. mori* eggs. The maximum exposure duration with garlic volatile caused adverse effect on the cellular level, therefore, reproductive potential declined.

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