

Influence of Volatile (*Allium sativum*) on the Pupal Traits of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.)

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ABSTRACT

The silkworm *Bombyx mori* rearing is a traditional industry in Asia. *B. mori* is an important economic insect because it converts leaf protein into silk protein. Present study was carried out to evaluate the effect of volatile (*Allium sativum*) on the pupal length and pupal duration of *B. mori* pupae. The change in the volatile exposure duration and the number of larval treatment influenced the pupal duration and pupal length. The pupal length increased with the increasing number of larval treatment 15, 30 and 45 minute exposure. The maximum pupal length (2.25 ± 0.012 cm) was noticed in case of triple treatment with 45 minute exposure and minimum pupal length (1.71 ± 0.087 cm) was recorded in case of triple treatment with 60 minute exposure duration. The minimum pupal duration was recorded to be 8.15 ± 0.479 days, which showed good development of pupae in case triple treatment with 45 minute exposure duration. If volatile applied tactfully in silkworm rearing it may be useful to improve the production of silk and quality of silk on commercial scale.

KEY WORDS: Garlic• Pupal length• Pupal duration• Mulberry leaves• Pupae

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 pupal length and pupal duration are the important factors, which influence the production of cocoon because pupa is major component of cocoon. The efforts are being made to evolve new technologies that are cost effective, labour saving and eco-friendly. In order to increase the production of silk, Attempts have been made to study the effect of X-rays (Kanarew and Cham, 1985), photoperiod (Mishra and Upadhyay, 1993), temperature (Upadhyay and Mishra, 1991), relative humidity (Mishra and Upadhyay, 2002), ecological factors (Upadhyay and Gaur, 2002), biological study (Mohamed et al., 2013), cocoon refrigeration (Upadhyay et al., 2009), egg magnetization (Upadhyay and Tripathi, 2006), cocoon magnetization (Upadhyay and Prasad, 2010), vitamin C treatment (Balasundaram et al., 2013), pesticides (Kumutha et al., 2013), 20-hydroxyecdysone hormone (Prasad and Upadhyay, 2012), and phytoecdysteroid hormone (Upadhyay and Pandey, 2012; Srivastava and Upadhyay, 2013), also influenced the performance of silkworm. *Aloe vera* herbal tonic 'logen' (Balamurugan and Isaiarasu, 2007), Aloe (Manimitha and Isaiarasu, 2010), and Aloe tonic treated mulberry leaves (Deshmukh and Khyada, 2013), artificial diet (Mona M. Mahmoud, 2013), influenced pupal and growth parameters of *B. mori*. The garlic has antibacterial (Pactiappan et al., 2009), and antimicrobial (Gulson and Erol, 2010), Properties and volatile compound (Yu and Wu, 1989), the garlic also used as controlling silkworm disease (Isaiarasu et al., 2011), and antimyctic activity against pathogenic fungus of white muscardine disease in silkworm *B. mori* linn. (Madana et al., 2007), plant and human disease (Singh et al., 2001). It is hypothesized that if the larvae of *Bombyx 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 pupal length and pupal duration of multivoltine mulberry silkworm (*Bombyx mori* L.).

MATERIAL AND METHOD

Seed Cocoon: 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 (Krishnaswamy et al., 1973), in the silkworm laboratory, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur. The temperature, relative humidity and photoperiod were maintained at $26 \pm 1^\circ\text{C}$, $80 \pm 5\%$ RH and 12 ± 1 hours light a day respectively till the emergence of

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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.

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.*, 1973).

Incubation of eggs and hatching: 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.

Rearing of larvae: 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 $26\pm 1^{\circ}\text{C}$, $80\pm 5\%$ 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 crushed garlic (*Allium sativum*) bulb volatile on the pupal length and pupal duration of (*Bombyx mori* L.). In the present study garlic volatile were taken experiment due to their antifungal, viral, bacterial etc. activity and easily available in market withery low cost. The larvae obtain from the BOD incubator was kept into a glass chamber with garlic volatile (5 ml pure garlic extract as liquid form) on the filter paper into a petridish. In order to maintain volatile concentration with certain limit during the time of experiment, the petridish was replaced after 15 minute. 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 (*Allium sativum*) 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 (*Allium sativum*) 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 min 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 treated 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. All the parameters of observation in the present study were determined from the respective stage obtained from treated larvae.

For determining the effect volatile exposure duration on pupal length and pupal duration of pupae the ripe worms (fifth instar larvae when stop feeding), reared at $26\pm 1^{\circ}\text{C}$, $80\pm 5\%$ hours light a day, were put on mountages for spinning. Thus, the formation of cocoon takes place and larvae changed in the pupal stage.

For determining the pupal length: The length of pupae (three bathes of 10 pupae in each batch) was recorded for each replicate. Three replicate of each experiments were made. The cocoon shells were dissected to obtain pupae and pupal length was taken on the 3rd day of spinning. There replicates of each experiments were made.

For determining the pupal duration: The time required from the third day of spinning (formation of pupae) to the emergence of moth was considered. For this purpose, 90 cocoons along with their pupae (three batches of 30 cocoons in each batch) were taken for observation. Three replicates of each experiment were made.

Statistical analysis: Results have been expressed as mean \pm SE of three replicates. Results were subjected to analysis of variance by two-way ANOVA (Sokal and Rohlf, 1973) to detect significant changes and Post-hoc test with help using MS Excel software.

RESULTS

Pupal length- The data presented in (table 1a) shows that change in the volatile exposure duration and number of larval treatment influenced the pupal length. With the increasing number of larval treatment from one to three times,

the pupal length increased in case of 15, 30, 45 minute exposure duration of garlic volatile but further increased exposure duration caused notable decline in the pupal length. The trend of increase in the pupal 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 pupal length was noticed to be 2.25 ± 0.012 cm (11.94 % increased as compared to control) in case of triple treatment 45 minute exposure duration and minimum pupal length 1.17 ± 0.087 cm was recorded to be in case of triple treatment of larvae by 60 minute exposure duration.

Two way ANOVA indicates that the volatile exposure duration significantly ($P_1 < 0.01$) influenced the pupal length. While variation in number of larval treatment did not cause significantly effect. The Post-hoc test (**table 1b, HSD = 0.2185**) indicates significant group difference in pupal length. In double treatment of larvae significant group difference was noticed in between 15 and 60 minute, 30 and 60 minute. In triple treatment of larvae significant group difference was noticed in between control and 45 minute, 30 and 60 minute, 45 and 60 minute. In case of single treatment there was no significant group difference.

Pupal duration- The data presented in (table 2a) shows that change in the volatile exposure duration and number of larval treatment influenced the pupal duration. With the increasing number of larval treatment from one to three times, the pupa duration decreased in case of 15, 30, 45 minute exposure duration of garlic volatile but further increased exposure duration caused increased the pupal duration. The trend of decreased in the pupal duration with increasing number of larval treatment has been recorded to be almost similar in case of 15, 30, 45 minute exposure duration. The minimum pupal duration was noticed to be 8.15 ± 0.474 days (16.92 % decreased as compared to control) in case of triple treatment 45 minute exposure duration and maximum pupal duration 11.20 ± 0.529 days was recorded to be in case of triple treatment of larvae by 60 minute exposure duration.

Two way ANOVA indicates that the volatile exposure duration significantly ($P_1 < 0.01$) influenced the pupal duration. While variation in number of larval treatment did not cause significantly effect. The Post-hoc test (**table 2b, HSD = 1.2114**) indicates significant group difference in pupal duration. In double treatment of larvae significant group difference was noticed in between 30 and 60 minute, 45 and 60 minute. In triple treatment of larvae significant group difference was noticed in between control and 45 minute, control and 60 minute, 15 and 60 minute, 30 and 60 minute, 45 and 60 minute. In case of single treatment there was no significant group difference

Table 1a: Effect of volatile exposure on the pupal length (cm) of *Bombyx mori* pupae.

Stage of treatment (larval instar)	Volatile exposure duration (minutes)					F ₁ -ratio n ₁ = 4
	Control (X ₁)	15 (X ₂)	30 (X ₃)	45 (X ₄)	60 (X ₅)	
Single (5 th)	2.01±0.054 (100)	2.04±0.031 (101.49)	2.07±0.018 (102.99)	2.13±0.070 (105.97)	1.96±0.036 (97.51)	
Double (4 th -5 th)	2.01±0.054 (100)	2.06±0.040 (102.49)	2.12±0.018 (105.47)	2.21±0.012 (109.95)	1.85±0.037 (92.04)	9.6336*
Triple (3 rd -4 th -5 th)	2.01±0.054 (100)	2.09±0.053 (103.98)	2.17±0.017 (107.96)	2.25±0.012 (111.94)	1.71±0.087 (85.07)	

F₂ -ratio = 0.0142**

n₂ = 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 pupal 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 1b: Post-hoc test showing effect of volatile exposure on the pupal length (cm) of *Bombyx mori* pupae.

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
X ₁ ~ X ₂	0.03	0.05	0.08
X ₁ ~ X ₃	0.06	0.11	0.16
X ₁ ~ X ₄	0.12	0.20	*0.24
X ₁ ~ X ₅	0.05	0.16	0.30
X ₂ ~ X ₃	0.03	0.06	0.08
X ₂ ~ X ₄	0.09	0.15	0.16
X ₂ ~ X ₅	0.08	*0.21	0.38
X ₃ ~ X ₄	0.06	0.09	0.08
X ₃ ~ X ₅	0.11	*0.27	*0.46
X ₄ ~ X ₅	0.17	0.36	*0.54

$$\begin{aligned} \text{Honesty significant difference (HSD)} &= q \sqrt{\frac{\text{MS within}}{n}} \\ &= \frac{5.05 \sqrt{0.00562}}{\sqrt{3}} \\ &= 0.2185 \end{aligned}$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

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

Table 2a: effect of volatile exposure on the pupal duration (days) of *Bombyx mori* pupae.

Stage of treatment (larval instar)	Volatile exposure duration (minute)					F ₁ -ratio n ₁ = 4
	Control (X ₁)	15 (X ₂)	30 (X ₃)	45 (X ₄)	60 (X ₅)	
Single (5 th)	9.81±0.714 (100)	9.58±0.422 (97.66)	9.4±0.348 (95.82)	9.29±0.205 (94.70)	10.15±0.360 (103.47)	
Double (4 th -5 th)	9.81±0.714 (100)	9.37±0.157 (95.51)	9.16±0.110 (93.37)	8.84±0.200 (90.11)	10.37±0.336 (105.71)	8.3863*
Triple (3 rd -4 th -5 th)	9.81±0.714 (100)	9.29±0.072 (94.70)	8.84±0.533 (90.11)	8.15±0.474 (83.08)	11.20±0.529 (114.17)	

F₂ -ratio = 0.2729

n₂ = 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 pupal 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 2b: Post-hoc test showing effect of volatile exposure on the pupal duration (days) of *Bombyx mori*.

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
X ₁ ~ X ₂	0.230	0.440	0.520
X ₁ ~ X ₃	0.410	0.650	0.970
X ₁ ~ X ₄	0.520	0.970	*1.660
X ₁ ~ X ₅	0.340	0.560	*1.390
X ₂ ~ X ₃	0.180	0.210	0.450
X ₂ ~ X ₄	0.290	0.530	1.140
X ₂ ~ X ₅	0.570	1.000	*1.910
X ₃ ~ X ₄	0.110	0.320	0.690
X ₃ ~ X ₅	0.750	*1.210	*2.360
X ₄ ~ X ₅	0.860	*1.530	*3.050

$$\begin{aligned} \text{Honesty significant difference (HSD)} &= q \sqrt{\frac{\text{MS within}}{n}} \\ &= \frac{5.05 \sqrt{0.17264}}{\sqrt{3}} \\ &= 1.2114 \end{aligned}$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

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

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. Variation in volatile (*Allium sativum*) exposure duration and the number of larval treatment influenced the pupal length, with the increasing number of larval treatment from one to three times. The pupal length increased in case of 15, 30 and 45 minute exposure of *A. sativum* volatile but in case of 60 minute *A. sativum* volatile exposure caused adverse effect on the pupal length with increase in the number of larval treatment from single to triple. The maximum pupal length was noticed in case of triple treatment of larvae at 45 minute *A. sativum* volatile exposure and the minimum pupal length was recorded in case of triple treatment of larvae at 60 minute *A. sativum* volatile exposure duration. The silkworm Chinese strain and Japanese strain have more pupal length than normal (Alimurong, 1986). Large pupal size or weight has been associated with greater longevity (Bloem et al., 1994). The puparial length increased as puparial weight increased (Alfredo et al., 2006). Relationship between pupal length, width or size and weight of female pupae of *Bombyx mori* were noticed (Rithinam et al., 1991). The morphometric growth of mean length, width and weight of the pupae of *B. mori* fed with Vitamin C treated MR2 leaves were found to be more than that of the larvae fed with control MR2 leaves (Balasundaram et al., 2013). Mulberry leaves sprayed with linseed oil, hemp oil and milk influenced the length and weight of larvae, pupae, raw cocoon, and shell weight parameter (Zah et al., 2011). 8% cocoon weight and three time larval weight increased when mulberry leaves treated with bovine milk (Konala et al., 2013). Treatment of phytoecdysteroid influenced the pupal length of mulberry silkworm (*Bombyx mori* L.) (Upadhyay and Pandey, 2012).

Variation in volatile (*Allium sativum*) exposure duration and the number of larval treatment influenced the pupal duration. With the increasing number of larval treatment from one to three times. The pupal duration decreased in case of 15, 30 and 45 minute exposure of *A. sativum* volatile exposure but in case of 60 minute *A. sativum* volatile exposure caused adverse effect on the pupal duration with increase in the number of larval treatment from single to triple. The minimum pupal duration was noticed in case of triple treatment of larvae at 45 minute *A. sativum* volatile exposure and the maximum pupal duration was recorded in case of triple treatment of larvae at 60 minute *A. sativum* volatile exposure duration. The pupal duration of *Bombyx mori* has been noticed to be influenced by the change in varieties of mulberry, given as a food of larvae (Bheemanna et al., 1989). Dietary administration of the vertebrate sex hormone reduces pupal duration (Khan et al., 1997). Larvae of *Bombyx mori* were reared on various kinds of dietary protein (soybean, mushroom and mixture of them) using as semi-artificial diet effected the pupal duration (Mona M. Mahmoud, 2013). The pupal period of control larvae 10.28 days was extended to 11.34 days and 13.42 days at higher concentrations of Dichlorovos and Vijay neem pesticides (Kumutha et al., 2013). Ecological factors as temperature, humidity, photoperiod influenced the pupal duration (Upadhyay and Gaur, 2002). The temperature and humidity in rainy season influenced the pupal duration of *B. mori*. (Mohamed et al., 2013). Phytoecdysteroid reduces larval as well as pupal duration (Trivedy et al., 2003; Upadhyay and Pandey, 2012). Treatment of synthetic juvenoid R394, caused prolongation in the pupal duration (Nair et al., 2004). A juvenile hormone mimic R394, when topically applied on the abdomen tergum of silkworm, improved the pupal duration (Gangwar, 2009). Variation in refrigeration period of silkworm eggs caused considerable influence on the pupal duration of *Bombyx mori*. (Pandey and Upadhyay, 2001). Environmental conditions during embryonic development not only affect the diapauses nature of eggs but also affected pupal duration of *Bombyx mori* L. (Kai et al., 1971). Pupal period was prolonged up to a maximum of 68 days at 7.5°C *Antheraea assamensis* helper (RajKhowa et al., 2011).

In the present investigation, pupal length increased and pupal duration decreased 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 maximum pupal length and minimum pupal duration, it may be possible due to decreasing the microbial pathogens causing silkworm diseases and increasing the feeding behavior of silkworm larvae.

REFERENCES

- Alfredo, J., and V. A. Patricia, 2006. Laboratorio de Ecología Química, Size, fecundity, and gonadic maturation of *Toxotrypana curvicauda* (Diptera: Tephritidae). Centro de Desarrollo de Productos Bioticos. Florida Entomologist. 89(2): 194-198.
- Alimurong, V. S., 1986. Performance of two strains of silkworm reared under high temperature at CLFO (Central Luzon State University, MUNOZ, Nueva Euja, and Philippine) CLSU. Scientific J. (Philippine). 5(2).

- Balamurugan R., and L. Isaiarasu, 2007. Effect of the herbal tonic “Iogen” on the growth and cocoon parameters of mulberry silkworm, *Bombyx mori* L. Proceedings of the National Seminar on Applied Zoology, ANJA College, Sivakasi.
- Balasundaram, D. P., G. Prabu, Selvisabhanayakam, V. Mathivanan, and V. Ramesh, 2013. Studies on the Nutritional Supplement of Vitamin C treated MR₂ Mulberry leaves fed by Vth instar larvae of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) in Relation to feed Efficacy and Growth rate. International Journal of Research in Biotechnology and Biochemistry 3(1): 11-18
- Bheemanna, C., R. Govindan, J. Ashoka, and T. K. Narayanswamy, 1989. Growth indices for silkworm *Bombyx mori* on some mulberry varieties. Enrichment and Ecology, 7(3): 743-747.
- Bloem, K. A., S. Bloem and D. L. Chambers, 1994. Field assessment of quality: release-recapture of mass-reared Mediterranean fruit flies (Diptera: Tephritidae) of different sizes. Environ. Entomol. 23, 629-633
- Deshmukh R. B., and V. B. Khyade, 2013. Utilization of *Aloe vera* (L) herbal tonic for treating mulberry leaves before feeding the fifth instar larvae of silkworm, *Bombyx mori* L. (Race: PM × CSR₂). IJB, 02(01), 281-285.
- Gangwar, S. K., 2009. Effect of juvenile hormone mimic R394 on silkworm *Bombyx mori* L. growth and development of silk gland. J. of Agriculture and Biological Science, 4(6), 65-66.
- Gulson, G., E. Ayaz, 2010. Antimicrobial effect of Garlic (*Allium sativum*) and traditional Medicine J. Anim. Vet Adv., 9(1): 1-4
- Kai. H., and K. Hasegawa, 1971. Studies on the mode of action of the diapause hormone with special reference to the protein metabolism in the silkworm *Bombyx mori* L. Journal of Sericulture Science of Japan, vol. 40, pp. 199-20
- Isaiarasu, L., N. Sakthivef, J. Ravikumar, P. Samuthiravelu, 2011. Effect of herbal extracts on the microbial pathogens causing flacherie and muscardine disease in the mulberry silkworm, *Bombyx mori* L. Journal of Bio pesticides, 4(2): 150-155.
- Kanarev, G., and G. T. Cham, 1985. Effect of laser irradiation of silkworm eggs on silkworm *Bombyx mori* development and productivity. Zhivotnov Nauki, 22, 47-53.
- Khan, A., S. Rahman, and N. Birendra, 1997. Effect of vertebrate sex hormone, menstranol norethindrone, on growth and development of *Bombyx mori* (Lepidoptera). Bangladesh Journal of zoology, 25(2), 103-109.
- Konala N., P. Abburi, V. R. Bovilla and A. Mamillapalli, 2013. The effect of bovine milk on the growth of *Bombyx mori*. Journal of Insect Science, Vol. 13 Article 98.
- Krishnaswamy, S., M. M. Narasimhanan and S.K. Suryanarayana, (1973). Sericulture Manual 2 silkworm rearing F.A.O. Agric. Services Bull., 15(2), 1-131.
- Kumutha, P.C., K. Padmalatha, A. J. A. Chairman and R. Singh, 2013. Effect of Pesticides on larval and duration of silkworm *Bombyx mori* International Journal of Pharmaceutical and Chemical Science Vol.2 (4)
- Madana, N., S. K. Guota, and P. Mitra, 2007. Antimycotic activity of *Allium sativum* against *Beauveria bassiana* pathogenic Fungus of white muscardine disease in silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Int. J. Indust. Entomol. 14(2): 81-85
- Manimutha M., and L. Isaiarasu, 2010. Influence of herbal tonic Aloe on the overall performance of the mulberry silkworm, *Bombyx mori* L. J.Biopest, 3(3), 567-572.
- Mishra A. B., and V. B. Upadhyay, 2002. Influence of temperature on the silk producing potential of multivoltine *Bombyx mori* L. race nistari Sericologia, 35 (2): 217-222.
- Mishra, A. B., and V. B. Upadhyay, 1993. Nutritional efficiency of bivoltine *Bombyx mori* Linn larvae at different photoperiod, Proc. 80th session. Indian Science Congress Assoc. Goa., PP; 54:55.
- Mohamed T. A., E. A. Elbashir, M. Hassan Rudwan, M. Khafagi, 2013. Biological studies on the wild silkworm, *Epiphora bautinia* (Guerin- Meneville): Lepidoptera: Saturniidae) in Gedarif State; Sudan. Journal of Forest Product, and Industries, 2(2):5-11.
- Mona M. Mahmoud., 2013. Effect of various kinds of Dietary Proteins in Semi- Artificial Diets on the Mulberry Silkworm *Bombyx mori* L. Egypt.Acad.J.Biolog.Sci., 6(1): 21-26
- Nair, K. S., K. Trivedi, V. A. Vijayan, J. S. Nair, and P.K. Chinya, 2004. Efficiency of food conversion of last instar silkworm *Bombyx mori* L. under the influence of juvenoid, R394. Indian, J. Seric., 43(2): 187-193.
- Nair, K.S., K. Trivedy, S. Chintalwar, G. J. Chinya, P. K. Datta, R. K. Chattopadhyay, and A. Banarji, 2002. Ecdysteroid from *Sesuvium portulacastrum* for synchronization of cocoon spinning in silkworm, *Bombyx mori* L. In Advances in Indian Seric. Res. Eds. CSRTI., Mysore, Pp: 247-251.
- Pactiappan, P., M. C. Aruchamy. and S. Ramanna, 2009. Evaluation of Antibacterial Efficiency of certain Batanicals against Bacterial Pathogen Bacillus sp of silkworm, *Bombyx mori* L. Int. J. Inusi. Entomal vol.18 No.1.PP-49-52

- Pandey, A. K., and V. B. Upadhyay, 2001. Effect of refrigeration of eggs and prerefrigeration periods on the weight and survival of pupae of *Bombyx mori* L. Lepidoptera. J. Anim. Morphol. Physiol., 48(1): 11-16
- Prasad S., and V. B. Upadhyay, 2012. Influence of 20-Hydroxyecdysone on the Larval Performance of Multivoltine Mulberry Silkworm (*Bombyx mori* L.) African J. Basic & Appl. Sci., 4 (5):146-15
- Rajkhowa, G., R. Kumar and R. K. Rajan, 2011. Studies on the long term preservation method of muga cocoon (*Antheraea assamensis helfer*) at low temperature. Mun. Ent. Zool. Vol. 6, No.2, pp: 815-818
- Rithinam, C. M. S., V. Kabita, and J. Solochamchetty, 1991. Relationship between pupal size and egg production in silkworm, *Bombyx mori* L. Environment and Ecology. 9(1), 266-267.
- Singh, U.P., P. Sarma, M. Singh, and A. B. Ray, 2001. Role of garlic (*Allium sativum*) in human and plant diseases. Indian Journal of Experimental Biology Vol. 39. Pp. 310-322
- Sokal, R.R., and F. J. Rohlf, 1995. Biometry: The principle and practice of statistics in biological research, 3rd edition, W.H. Freeman New York pp: 440-447.
- Srivastava K., and V.B. Upadhyay, 2013. Effect of phytoecdysteroid on silk producing potential of multivoltine mulberry silkworm (*Bombyx mori* Linn). The Bioscan, 8(1), 43-47.
- Syed, I. A. S., I. A. Khan, Z. Hussain, M. Shah, A. Usman and A. Sadozai, 2007. Studying the performance of silkworm, *Bombyx mori* L. races fed with different mulberry varieties. Sarhad J. Agric. Vol. 23, no. 4.
- Trivedy, K., K. S. Nair, M. Ramesh, N. Gopal, and S. N. Kumar, 2003. New Semi-synthetic diet ‘‘Nutrid’’- A technology for rearing young instar silkworm in India. Indian J. Seric., 42 (2): 158-161.
- Upadhyay, V. B., and A. B. Mishra, 1991. Nutritional ability of bivoltine silkworm *Bombyx mori* Linn. Larvae at higher temperature regimes. J. Adv. Zool., 12(1): 56-59
- Upadhyay, V. B., and K. P. Gaur, 2002. Effect of ecological factors on the performance of *Bombyx mori* L. larvae. Zool. Sci. India PP: 91-104
- Upadhyay, V.B., and Prasad, S. 2010. Biotechnological importance of cocoon magnetization with particular reference to the reproductive potential of multivoltine mulberry silkworm (*Bombyx mori* Linn). Sericologia, 50(4), 461-672.
- Upadhyay, V.B., and P. Pandey, 2012. Influence of phytoecdysteroid on pupal performance of multivoltine mulberry silkworm (*Bombyx mori* Linn). The Bioscan. 7(3), 401-407.
- Upadhyay, V.B., and S.K. Tripathi, 2006. Effect of the magnetization of eggs on the silk producing potential mulberry silk worm (*Bombyx mori* Linn.) Sericologia, 46 (13): 269-278.
- Upadhyay, V.B., R. Singh and S. Prasad, 2009. Refrigeration of cocoons influences the pupal Characteristics of multivoltine mulberry silkworm (*Bombyx mori* Linn.) J. Appl. Biosci., 35(2): 166-168
- Yu T., C. Wu, Y. Lion, 1989. Volatile compound from garlic. J.Aaric Food chem, 37: 725-30
- Zah, C., L. A. Marghitas, A. Matei, and M. N. Madas, 2011. The effect of dietary supplements on the development of *Bombyx mori* L. silkworms. Animal science and biotechnologies. 44 (1): 153-157.