International Journal of Bioassays
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Original Research Article

UTILIZATION OF THE TOPICAL APPLICATION OF LIMONENE TO THE FIFTH INSTAR LARVAE OF THE SILKWORM, *BOMBYX MORI* (L.) (RACE: PM X CSR2) FOR THE PARAMETERS OF LARVAE, COCOON AND SILK FILAMENT

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Received for publication: December 17, 2014; **Revised:** January 11, 2015; **Accepted:** January 27, 2015

Abstract: The attempt is concerned with topical application of the limonene on the fifth instar larvae of multivoltine silkworm, *Bombyx mori* (L.). The ten microliters of one milligram per liter strength acetone solution of limonene was topically applied to the individual fifth instar larvae of multi voltine cross breed race of silkworm, *Bombyx mori* (L.) (PM x CSR2) variously at 48, 54, 60 and 66 hours after the fourth moult. Topical application of acetone solution of limonene was found variously reflected into prolongation of larval age (23.129-38.775%); improvement in the tissue somatic index (TSI) of silk glands (3.041-7.761); cocoon shell ratio (2.828-4.497); Tex of silk filament (0.038-0.107) and denier scale of silk filament (0.343- 0.962). Topical application of acetone solution of limonene, thus chiefly lengthen the larval age in silkworm, *Bombyx mori* (L.). Time required for eating and amount of mulberry leaves eaten had both been increased and they were practically reflected into the improvement in the quality of cocoon and the silk filament. The terpenoid nature of limonene and delay in the spinning among the larvae, recipient of this limonene in the attempt indicate the possibility of it's insect juvenoid activity in silkworm, *Bombyx mori* (L.). Efficient utilization of limonene, the terpene compound through acetone solvent for topical application to the fifth instared larvae of silkworm, *Bombyx mori* (L.) may open a biotechnological avenue in the sericulture industries.

Key Words: Terpenoids, Limonene, TSI, Shell Ratio, Denier Scale of Silk Filament

INTRODUCTION

Juvenile hormone (JH) serve a key role in the life of insects for metamorphosis of larval instars to the adults. Juvenile hormone active compounds are found in many higher plants, exogenous application through suitable solvents of which exhibited potent activity in the insects (Prabhu and John, 1975). Efficient use of available system, the principle of quality improvement made man to use juvenoids for pest control as well as for the silk yield. Use of juvenoids (synthetic, plant derived and animal derived) in the rearing of silkworm larvae had positive influence, especially in the silk yield (Ching *et al.*, 1972; Nihmura *et al.*, 1972; Muroga *et al.*, 1975; Kamada and Shimada, 1988; Rajashekhar gouda, 1991; Vitthalrao *et al.*, 2002, 2003 and Vitthalrao, 2004). In the metamorphosis, the interplay of the juvenile hormone and ecdysone serve to orchestrate the progression from one instar to the next, with ecdyosteroid regulating the onset and timing of the moult and JH determining whether the moult would be larval-larval or larval-pupal (Gilbert, et *al.*, 1996; Mamatha, et *al.*, 2005). Phytophagous insects like silkworm, *Bombyx mori (L.)* derive their juvenoid nutrients through the plant material available for them (Khyade, *et al.*, 2007). Terpene compounds and or it's analogues, may either be synthesized by the insect tissue or derived from the plant material by the metamorphagic stages of the insects. The specific titer of juvenoids, either topical or through the food, at the specific period of the larval instars of silkworm, *Bombyx mori* (L.) are positively reflected into the retention of larval features long enough enabling the larvae to consume maximum quantity of mulberry leaves and to synthesize paramount silk to be used in spinning the qualitative cocoon (Akai, *et al.*, 1990; Mamatha, *et al.*, 2005, 2006, 2008; Chowdhary, *et al.*, 1990; Miranda, *et al.*, 2002; Mamatha, *et al.*, 2006, 2008). There are no reports on the use of limonene for topical application to the fifth instar larvae of silkworm, *Bombyx mori (L.)*. Terpene structure and possibilities of limonene for insect juvenoid activity are the sole inspiration for the present attempt.

MATERIAL AND METHODS

The entire experimentation was divided into the parts, which include: Rearing of the silkworm larvae; Topical application of limonene through the acetone solution to the fifth instar larvae; Analysis of parameters (Larval, cocoon and silk filament) and Statistical analysis of the data. The larvae of silkworm belongs to polyvoltine cross breed (PM x CSR_2) race were reared in the laboratory through standard methods (Krishnaswami *et al.*, 1978). The limonene was procured from through dealer. One mg of limopnene was dissolved in the acetone and the stock solution of one ppm (mg per liter) was prepared. Soon after fourth moult, the fifth instared larvae were grouped into one control group; one acetone treated group and four experimental groups, each with hundred individuals. Ten microliters of acetone solution of limonene was used for topical application to the individual larva in each group separately. The experimental group: "A" received the topical application of limonene at 48

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hours after the fourth moult. The experimental group: "B" received the topical application of limonene at 48 and 54 hours after the fourth moult. The experimental group: "C" received the topical application of limonene at 48; 54 and 60 hours after the fourth moult. And the experimental group: "D" received the topical application of limonene at 48; 54; 60 and 66 hours after the fourt moult. Topical application was followed by feeding the larvae with tender mulberry leaves. The hundred grams of fres mulberry leaves were used for feeding the group of hundred larvae for each time, each day (6.00 a. m.; 11.00 a. m.; 4.00 p. m. and 10.00 p. m. of each day) (Vitthalrao B. Khyade and Sucheta S. Doshi, 2012). Acetone treated control and untreated control groups of larvae were also maintained. Daily larval weight was recorded. For the purpose to calculate tissue somatic index (TSI) of silk glands, ten larvae from each group were selected at random on the fifth day, anesthetized, dissected and silk glands were separated. The silk glands were blotted and weighed on electronic balance. The weight of silk glands was divided by weight of larva. The quotient thus obtained was multiplied by 100. Weight of silk glands and larval body weight, thus, were accounted for the calculation of tissue somatic index (TSI) of silk glands. The matured larvae (having transparent skin, feeding stopped and moving its head in specific manner for searching the surface for attachment of fluid silk) were transferred to the mountage for spinning the cocoon. The larval duration (right from zero hour of fifth instar to fifty percent spinning) was recorded. The cocoons were harvested on sixth day after mounting the mature larvae on the mountage. Cocoon weight, shell weight and pupal weight were recorded. Shell ratio was calculated. Ten cocoons per replication were reeled and length (m) of unbroken silk filament was obtained by using epprouvate. Weight of silk filament from individual cocoon was recorded. Length (m) and weight (gm) of silk filament were accounted for the calculation of Denier scale. The experimentation was repeated for thrice for the purpose of consistency in the results. The statistical methods were employed to calculate the mean, standard deviation, percent variation and student "t" – test (Norman and Bailey, 1955).

RESULTS AND DISCUSSION

The results are summarized in tables (1, 2 and 3). The parameteres analyzed include: Larval Parameters; Cocoon parameters and the silk fibre parameters. The larval parameters get reflects on the quality of the cocoon and silk fibre. Extension of fifth instar larval period was observed in groups of larvae topically applied with one ppm acetone solution of limonene (Table 1). The extension of fifth instar larval period was 23.129 – 38.775 % in all the treated groups of larvae. Maximum increase in the larval duration was recorded in larvae received four times (at 48, 54, 60 and 66 hours after the fourth moult) the topical application of limonene. Corresponding to the extension in the larval duration, an increase in the larval growth by the body weight (17.05 – 24.536 %) was observed in all the treated groups. Tissue somatic index (TSI) signify the percentage of tissue in entire body. Tissue somatic index (TSI) of the silk glands of treated larvae in the percent study was found significantly increased. Treating the larvae with one ppm limonene solution through acetone at 48, 54, 60 and 66 hours after fourth moult was found variously reflected into most significant improvement in the TSI of silk glands (Table 1). The economic parameter in sericulture is the Cocoon spinned by the mature fifth instar larvae of silkworm, *Bombyx mori (L.)*. Cocoon is the most important aspect in sericulture as it is used for reeling the commercial silk fibre. Cocoon weight, shell weight and thereby the shell ratio were found influenced by the topical application of acetone solution of limonene to the fifth instar larvae of silkworm, *Bombyx mori (L.)* (Table 2). The range of percent increase in the cocoon weight and shell weight in the experimental (treated) groups was 43.886 to 50.507 and 66.371 to 87.905 respectively. Shell ratio of the cocoons was found improved in the corresponding groups of treatment. Most significant ($p < 0.001$) shell ratio belonged to cocoons harvested from the group of larvae treated with one ppm acetone solution of limonene at 48 hours after the fourth moult. Silk filament is sole aim in sericulture. Both the parameters (Tex and Denier) of silk filament were found influenced through treating the larvae with retinol solution (one ppm). The limonene through acetone was found resulted into fortified silk filament, with reference to Tex and Denier scale. The silk reeled from the cocoons belong to the group C and D (Table 3) were exhibited most significant improvement. Prolonged larval duration in the larvae treated with acetone solution of limonene in the present study is as good as tendency of larvae retaining their larval stage (Vitthalrao B. Khyade, 2014). Limonene topically applied may be utilized by the silkworm larvae for the extra synthesis of silk. The limonene is one of the most popular terprne compound used in pharmacology. Utilization of limonene through acetone for rearing of silkworm larvae is much more easy method. Use of terpene compounds like limonene may open a new biotechnological avenue through green revolution in sericulture for the qualitative cocoon and silk filament.

Table 1: Effect of limonene on the larval parameters of silkworm, *Bombyx mori (L.)* (Race: PM x CSR_2).

Groups → Parameter ↓	Control	A	B	C	D
Larval duration (hours)	147 (±22.413)	181** (±16.043) 23.129	194** (±4.685) 31.972	198** (±5.651) 34.693	204** (±5.413) 38.775
Larval weight (gm)	3.126** (±0.457)	3.659** (±0.538) 17.050	3.808** (±0.443) 21.817	3.881** (±0.769) 24.152	3.893** (±0.948) 24.536
Weight of silk glands (gm)	0.749 (±0.018)	0.988** (±0.113) 31.909	1.065** (±0.231) 42.189	1.086** (±0.339) 44.993	1.196** (±0.458) 59.679
Tissue somatic index (TSI)	23.960	27.001** 3.041	27.967** 4.007	27.982** 4.022	30.721** 7.761

Table 2: Effect of limonene on the cocoon parameters of silkworm, *Bombyx mori (L.)* (Race: PM x CSR2).

groups→ parameters↓	Control	A	B	C	D
Cocoon weight (gm)	1.873** (±0.281)	2.695** (±0.413) 43.886	2.718** (±0.509) 45.114	2.742** (±0.513) 46.396	2.819** (±0.663) 50.507
Shell weight (mg)	0.339 (±0.081)	0.564*** (±0.143) 66.371	0.571** (±0.108) 68.437	0.588* (±0.113) 73.451	0.637* (±0.182) 87.905
Pupal weight (mg)	1.534 (±0.093)	2.131** (±0.864) 38.917	2.147** (±0.786) 39.960	2.154* (±0.654) 40.417	2.182* (±0.467) 42.242
Shell ratio	18.099	20.927*** 2.828	21.008** 2.909	21.444* 3.345	22.596* 4.497

Table 3: Effect of limonene on the silk filament parameters in silkworm, *Bombyx mori (L.)* (Race: PM x CSR_2)

group→ parameters↓	Control	A	B	C	D
S.F length (m)	795 (±24.246)	963** (±8.619) 21.132	978* (±11.806) 23.018	1189* (±11.714) 49.559	1273* (±36.789) 60.125
S.F weight (mg)	0.181* (0.039)	0.256** (±0.055) 41.436	0.281* (±0.047) 55.248	0.397* (±0.058) 119.337	0.426* (±0.086) 135.359
Tex	0.227	0.265* 0.038	0.287* 0.060	0.333* 0.106	0.334** 0.107
Denier	2.049	2.392** 0.343	2.585* 0.536	3.005*** 0.956	3.011*** 0.962

Foot Note for Table 1, 2 and 3

- Each figure is the mean of the three replications.
- Figure with ± sign in the bracket is standard deviation.
- Figure below the standard deviation is the increase for calculated parameter and percent increase for the others over the control.

* - P < 0.05; **- P < 0.005; ***- P < 0.01

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Cite this article as:
Vitthalrao B Khyade, Vivekanand V Khyade and Randy Wayne Schekman. Utilization Of The Topical Application Of Limonene To The Fifth Instar Larvae Of The Silkworm, Bombyx Mori (L) (Race: Pm X Csr2) For The Parameters Of Larvae, Cocoon And Silk Filament. International Journal of Bioassays, 2015, 4 (02), 3632-3635.

Source of support: Nil
Conflict of interest: None Declared