Influence of $\alpha$-Pinene and Pine Needles of *Pinus sylvestris* (L) on the Cocoon and Silk Fibres Spinned by Fifth Instar Larvae of Silkworm, *Bombyx mori* (L) (Race: PM x CSR2)

Vitthalrao B. Khyade$^{1*}$, Vishakha S. Chape$^2$ and Priyanka B. Ghadge$^2$

$^1$Sericulture Unit, Malegaon Sheti Farm, Agricultural Development Trust Baramati, Shardonagar, (Malegaon Khurd) Post Box No.- 35 Tal. Baramati, Dist. Pune 413 115 Maharashtra, India
$^2$Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardonagar, Malegaon (Baramati) Dist. Pune – 413115, India

*Corresponding author

**A B S T R A C T**

The pinene has been used as anti-cancer agent in Traditional Chinese medicine, also for its anti-inflammatory, antiseptic, expectorant and bronchodilator properties. Insect juvenoid activity of Terpenes and terpene contents of Pine needles of *Pinus sylvestris* (L) are well established facts. Topical application of ten microliters of one milligram per liter strength acetone solution of $\alpha$-Pinene and Pine needles to the individual fifth instar larvae of multivoltine cross breed race of silkworm, *Bombyx mori* (L) (PM x CSR2) at 48, 54, 60 and 66 hours after the fourth moult was found variously reflected into the prolongation of larval duration (17.011–45.394 % for $\alpha$-Pinene treated groups and 19.078–48.684 % for Pine needle treated groups); improvement in the tissue somatic index (TSI) of silk glands (3.054–3.344 for $\alpha$-Pinene treated groups and 3.071–3.094 % for Pine needle treated groups); cocoon shell ratio (2.791–3.179 for $\alpha$-Pinene treated groups and 2.694–3.631  for Pine needle treated groups) and denier scale of silk filament (0.333–2.359 for $\alpha$-Pinene treated group and 0.466–2.405 for Pine needle treated group). Acetone solution of $\alpha$-Pinene and acetone solution of pine needles, thus chiefly lengthening the larval duration (age) in silkworm, *Bombyx mori* (L). The lengthened larval life is sufficient to prove the juvenoid activity of acetone solution of Pine Needles. Treated group of larvae may be utilizing the lengthened life span for the fortification of cocoon shell through the silk filament. The synergistic activity of $\alpha$-Pinene and Pine needles in the present attempt is hypothesized to be due to changes in the membrane fluidity, interference with membrane bound signaling proteins and cell cycle arrest. Efficient utilization of $\alpha$-Pinene and pine needles, through acetone solvent for topical application to the fifth instared larvae of silkworm, *Bombyx mori* (L) may open a new innovative biotechnological avenue in the sericulture industries.

**Keywords**

$\alpha$-Pinene; Pine needles; TSI; Shell Ratio; Denier Scale of Silk Filament.

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**Introduction**

The silkworm is the larva or caterpillar of the domesticated silk moth, *Bombyx mori* (L). It is economically important insect, being a primary producer of silk. The
ladies of silkworm, *Bombyx mori* (L) deserve appreciation for synthesis of silk for their metamorphosis. Sericultural practices are serving a lot to provide the silk fibre. The silkworm, *Bombyx mori* (L) exert a significant influence on the concept of insect metamorphosis through it’s simple life cycle and efficient utilization of the nutrients from the mulberry, *Morus alba* (L). Interplay of juvenile hormone (JH) and moulting hormone (MH) in the insect larval body serves to orchestrate the progression of metamorphosis from one instar to next, with moulting hormone regulating the onset and timing of moulting cycle and juvenile hormone regulating the quality of moulting (Riddiford, 1985, 1994; Sehnal, 1985). During the last larval stadium of holometabolous insects, such as silkworm, *Bombyx mori* (L), a reduction of JH in haemolymph is the necessary step in the initiation of metamorphosis. It has been demonstrated that, haemolymphcycdysteroid and JH level undergo the developmental changes during larval - larval and larval - pupal cycles in silkworm, *Bombyx mori* (L) (Calvez et al., 1976; Kiguchi and Agui, 1981). Juvenoids are well known in prolonging the larval life in the insect and have long been tried for qualitative improvement of silk (Ratnasen, 1988; Granier and Granier, 1983 and Mamatha et al., 1999). There is considerable evidence that juvenile hormone mimics occur in plants, which occasionally leads to economically important consequences in the insect development (Slama, 1979). 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; Rajashekhargouda, 1991; Vitthalrao et al., 2002, 2003 and Vitthalrao, 2004). The terpenes and terpenoids mimics the action of natural insect juvenile hormone. They are widespread in nature and are involved in much more biological activities including morphogenesis, embryogenesis and cellular differentiation. The Pinene is the bicyclic monoterpeneorganic compound of the terpene class, one of two isomers of pinene (Simonsen, 1957).Two structural isomers of pinene are found in nature: α-pinene and β-pinene. As the name suggests, both α-pinene and β-pinene are important constituents of pine resin. They are also found in the resins of many other conifers, as well as in non-coniferous plants such as camphorweed (*Heterotheca*) (Lincoln and Lawrence, 1984) and big sagebrush (*Artemisia tridentata*). Both isomers (α-pinene and β-pinene) are used by many insects in their chemical communication system. The two isomers (α-pinene and β-pinene) of pinene constitute the major component of turpentine. The α-Pinene is an alkene and it contains a reactive four-membered ring. Most of the coniferous plants (including Pine tree) contain α-Pinene. It is also found in the essential oil of rosemary, *Rosmarinus officinalis* and *Satureja myrtifolia* (also known as "Zoufa" in some regions) (Zebib et al., 2015). Both enantiomers are known in nature: (1S, 5S)- or (−)-α-pinene is more common in European pines, whereas the (1R,5R)- or (+)-α-isomer is more common in North America. Racemic mixture is present in some oils such as eucalyptus oil and orange peel oil. At low titer of α-
Pinene serves as a bronchodilator in humans, and is highly bioavailable with 60% human pulmonary uptake with rapid metabolism or redistribution (Russo, 2011). The α-Pinene is an anti-inflammatory via PGE1 (Russo, 2011), and seems to be a broad-spectrum antibiotic. It exhibits activity as an acetylcholinesterase inhibitor, aiding memory (Nissen, 2011). Like borneol, verbenol and pinocarveol (−), -α-pinene is a positive modulator of the GABAA receptors. It acts at the benzodiazepine binding site (Yang et al., 2016). The α-Pinene forms the biosynthetic base for CB2 ligands, such as HU-308 (Russo, 2011).

The terpenejuvenoids are known for disruption of normal developmental pattern leading to the deformities in the insects. Interestingly, the silkworm, Bombyx mori (L) is known to have a stimulatory influence on the administration of exogenous Juvenoids (JHA) in a appropriate quantities at specific time of application. 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). Topical application of acetone solution of retinol has been reported for juvenoid activity and recommended for rearing the fifth instar larvae of silkworm, Bombyx mori (L) (Vitthalrao and Sarwade, 2013; Vitthalrao et al., 2015).

The pine, Pinus sylvestris (L) is conifer belong to the family: Pinaceae. Pinus is the sole genus in the subfamily: Pinoideae. The Plant List compiled by the Royal Botanic Gardens, Kew and Missouri Botanical Garden accepts 126 species names of pines as current, together with 35 unresolved species and many more synonyms. Seed leaves; Juvenile leaves; Scale leaves and the Needles are four different types of Pine leaves. The seed leaves may also be called as cotyledons, which appear on seedlings, born in whorl of 4-24. The juvenile leaves follow immediately on seedlings and young plants. Each juvenile leaf measures 2-6 cm in length. The juvenile leaves are either green or blue (often blue). They are arranged spirally on shoot. And they are produced for six months to five years. The Pine Needles are also called as adult leaves. The pine needles are green, photosynthetic, bundled in clusters (fasciles) of 1-6, commonly 2-5, needles together, each fascicle produced from a small bud on a dwarf shoot in the axil of a scale leaf. These bud scales often remain on the fascicle as a basal sheath. The needles use to persist for 1.5–40 years, depending on species. The damaged needle fascicles are replaced by new. The most abundant terpenoids from the pine needles are reported, which include the monoterpenes (α- and β-pinene, camphene, and δ-carene. Sesquiterpenes analyzed included caryophyllene, humulene, and α-bisabolene) and Diterpenoids(resin acids were quantified in derivatized extractions, including pimaric, isopimaric, levopimaric, palustic, dehydroabietic, abietic, and neoabietic acids) (Anne E. Harman-Ware et al., 2016). There are no reports on use of α-Pinene for rearing of larval instars of silkworm, Bombyx mori (L). Insect juvenoid activity of Terpenes and the terpenecontents of Pine needles of Pinus sylvestris (L) made to plan for the efforts on it’s topical application through the acetone to the fifth instar larvae of silkworm, Bombyx mori (L) (PM x CSR2).The objective of the study is
to analyze the acetone solution of Pine Needles and α-Pinene for the cocoon and silk characters of silkworm, *Bombyx mori* (L) through the Race: PM x CSR2.

**Materials and Methods**

The whole attempt was divided into the parts, which include: Rearing of the silkworm larvae; Topical application of α-Pinene and Pine Needles through the acetone 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<sub>2</sub>) race were reared in the laboratory through standard methods (Krishnaswami et al., 1978; Khyade, 2004). α-Pinene was procured through local dealer. One mg of α-Pinene was dissolved in the acetone and the stock solution of one ppm strength was prepared. The pine needles were collected from *Pinus sylvestris* at Malegaon Sheti farm (East Gate), Baramati (India). Pine needles were shade dried and powdered through the domestic mixture. One mg of Powder of Pine Needles was dissolved in the acetone and the stock solution of one ppm strength was prepared.

Soon after fourth moult, the fifth instar larvae were grouped into one untreated control group; and twelve experimental groups (A0, A1, A2; B0, B1, B2; C0, C1, C2 and D0, D1, D2), each with hundred individuals. Individual larva in the A1 group received ten microliters topical application of acetone solution of α-Pinene at 48 hours after the fourth moult. Individual larva in the B1 group received ten microliters topical application of acetone solution of α-Pinene at 48 and 54 hours after the fourth moult. Individual larva in the C1 group received ten microliters topical application of acetone solution of α-Pinene at 48; 54 and 60 hours after the fourth moult. Individual larva in the D1 group received ten microliters topical application of acetone solution of Pine Needles at 48 hours after the fourth moult.

Individual larva in the group B2 received ten microliters topical application of acetone solution of Pine Needles at 48 and 54 hours after the fourth moult. Individual larva in the group C2 received ten microliters topical application of acetone solution of Pine Needles at 48; 54 and 60 hours after the fourth moult. Individual larva in the group D2 received ten microliters topical application of acetone solution of Pine Needles at 48; 54; 60 and 66 hours after the fourth moult (Table 1).

Individual larva in the Acetone treated control (ATC) group (A0; B0; C0 and D0) received ten microliters of acetone at corresponding time (Table 1). Individual larva in the Untreated control (UTC) group received no topical application.

This topical application was followed by feeding the larvae with tender mulberry
leaves. The schedule of feeding was 100 gms of mulberry leaves for the group of hundred larvae each time. The larvae were feed four times a day (5.00 a.m.; 11.00 a.m.; 5.00 p.m. and 11.00 p.m.). Daily, before the first feeding, larval weight (for each group) 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. Weight of individual larva 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. Weight of individual larva was recorded. The larvae were anesthetized, dissected in insect saline solution and silk glands were separated. The silk glands were bottled and weighed on electronic balance. The reading on weight of silk glands was divided by reading on 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, stopped feeding 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. Cocoons 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 eprouvate. 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). The data collected belong to three successive trials.

**Results and Discussion**

Results on Influence of the α-Pinene and Pine needles on the Larval parameters; cocoon parameters and silk parameters in silkworm, Bombyx mori (L) (Race : PM x CSR2) are summarized in table- 2;3;4 and Figure-1; 2; 3 ;4; 5 and 6.Larval duration of Untreated Control Group and Acetone Treated Group (A0; B0; C0 and D0) was 154 hours. Topical application of ten microliters of one ppm acetone solution of α-Pinene and Pine Needles to the individual fifth instared larva at 48 hours after the fourth was found reflected into extension into the larval period (15.789 – 45.394 percent for α-Pinene group and 19.078 – 48.684 percent for Pine Needles Group) (Table – 2 and Fig. – 1).

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 α-Pinene and Pine Needle contents through the acetone solution. Extension of larval age is one of the distinguishing influence of exogenous juvenoid compound. Corresponding to the extension in the larval duration, an increase in the larval growth by the body weight (17.011 – 28.775 % for α-Pinene treated groups and 21.123 -31.923 % for acetone solution of Pine Needles Groups) was observed (Table-2 and Figure-2). Tissue somatic index (TSI) of silk glands designate the status of silk glands and signify their percentage in whole body of fifth instar larva in the present attempt. Tissue somatic index (TSI) of the silk glands of Untreated Control Group and Acetone Treated Group (A0; B0; C0 and D0) in the present study was found measured 23.917. Treating the larvae with one ppm α-Pinene solution and Pine Needle powder 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-2 and figure-4). For α-Pinene treated groups, the TSI of silk glands was improved from 3.067 to 3.694 and for Pine Needle treated groups, it was 3.084 – 4.120. Extension of larval life through the topical application of acetone solution of α-Pinene and Pine Needles must be responsible for significant TSI of Silk Glands in the present attempt.

The economic or commercial parameter in sericulture is the cocoon spun by the mature fifth instar larvae of silkworm, *Bombyx mori* (L). The Cocoon is the most important aspect in sericulture as it is used for reeling the commercial silk fibre. The cocoon weight; Shell weight; Pupal weight and shell ratio in Untreated Control Group and Acetone Treated Group (A0; B0; C0 and D0) of larvae were measured 1.712 grams; 0.303 grams; 1.409 grams and 17.698 percent respectively. Topical application of acetone solution of α-Pinene and Pine Needles was found effected into the improvement in the shell ratio of the cocoons spun by the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2). The shell ratio of cocoons spun by α-Pinene treated groups was found measured 20.341 to 22.445 units. The acetone solution of Pine Needles was found influencing the increase in the shell ratio (minimum 20.396 unit and maximum 22.697 unit). Most significant (p < 0.001) shell ratio belonged to cocoons harvested from the group of larvae treated with one ppm acetone solution of α-Pinene and Pine Needles at 48; 54; 60 and 66 hours after the fourth moult (Table-3 and Figure-5).

The silk filament is sole aim in sericulture. Length and weight of entire silk filament are the qualitative measurements to be accounted for the Tex and Denier scale. Present attempt is reporting significant improvement in both the parameters (Tex and Denier) of silk filament through the topical application of acetone solution of α-Pinene and Pine Needles to the fifth instared larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2). The α-Pinene and Pine Needles through acetone were found resulted into fortified silk filament, with reference to Tex and Denier scale. The silk reeled from the cocoons belong to the group C (Topical application of acetone solution of α-Pinene and Pine Needles at 48; 54; and 60 hours after the fourth moult) and group D (Topical application of acetone solution of α-Pinene and Pine Needles at 48; 54; 60 and 66 hours after the fourth moult) (Table-4 and Figure-6) were exhibited most significant improvement. The tex and Denier scale of silk filament reeled from the cocoons of Untreated Control Group and Acetone Treated Group (A0; B0; C0 and D0) measured 0.228 and 2.052 units respectively. The α-Pinene treatment was found influencing the tex to improve from 0.265 to 0.490 units. And treating the larvae with acetone solution of Pine Needles resulting the silk filament of 0.279 to 0.495 units of Tex. Likewise, the Denier was also found improved from 2.385 to 4.411 units for α-Pinene treatment and from 2.518 to 4.457 units for treating with acetone solution of Pine Needles.

Prolonged larval duration in the larvae treated with acetone solution of α-Pinene and Pine Needles in the present study is as good as tendency of larvae retaining their larval stage. Extension of larval duration is one of the distinguishing features of insect larvae recipient of exogenous juvenoid (Akai and Kobayashi, 1971). α-Pinene is deserving well established insect juvenoid activity. As the Pine Needles are with terpene contents, it may have had the insect juvenile activity (at least in silkworm, *Bombyx mori* (L). The larvae of all the
treated groups in the present attempt were found increasing in their body weight. The α-Pinene (and may be other terpenes) the contents of Pine Needles received by larvae through the acetone topical application, may influence the appetite, nutrition and absorption of digested food. This may be responsible for accelerated and significant growth of silk glands.

Cocoon is the material used for reeling the commercial silk fibre. It is in fact, a protective shell made up of a continuous and long proteinaceous silk filament spun by mature silkworm prior to pupation for self protection from adverse climatic situations and natural enemies. Juvenile hormone (JH) controls insect metamorphosis. High JH titers maintain the larval state while a decrease in the JH titer initiates the pupation sequence as well as a change in tissue commitment away from synthesis of larval tissues to pupal tissues at the pupal stage. The extra JH titer at the beginning of the last larval instar in the Lepidoptera appears to be due to a combination of increased metabolism. Thejuvenoid titer (endogenous and / or exogenous) in the body of larvae stimulate hypermetabolism (Slama, 1971). The terpenoids are known to arrest the cell cycle at G1, S and G2 M- phases Zore et al., 2011). It may be referred that the treatment of larvae with low phytojuvenoid concentration caused an increase in the cellular activity of silk gland resulting in the increased thickness of cocoon shell; whereas, higher concentration may cause stress response, lessening the thickness of cocoon. The seasonal variation influenced the production of cocoon and shell ratio (Thiagrajan et al., 1993) and the reduced amount of food intake by the parasitized silkworm larvae reduced the silk production of Bombyx mori (Nath et al., 1990). The plant growth regulators significantly increased the shell ratio of Bombyx mori (Das and Vijayraghavan, 1993) and temperature variation also influenced the shell ratio in Bombyx mori (Mishra and Upadyay, 1995). The exposure of silkworm larvae in the magnetic field of 3500 gauss caused an increase in the silk yield due to the increasing protein level in the silk gland (Chaugale and More, 1992). Magnetization of eggs considerably influenced the silk producing potential of Bombyx mori. The administration of plant growth hormone Indloe-3-acetic acid increased the shell ratio (Bharathi and Miao, 2003). Methoprene and fenoxy carb treated Bombyx mori showed increased shell percentage (Mamatha et al., 2006). JHA Labomin at various age, hours and season affected the shell ratio (Trivedy et al., 1993). JHA R394 at different hours of treatment increased the shell ratio. Twenty four juvenile hormone mimicking compounds showed improvement in the shell ratio (Nair et al., 1999). BPE epoxide treatment on the Bombyx mori significantly increased the shell ratio (Nair et al., 2001). R394 has no significant difference in the shell ratio except that in the 48h treatment in PM x NB4D2 (Nair et al., 2004). The phytocedysteroid were administered at different ages of 5th instar larvae of Bombyx mori, which influenced the shell ratio (Nair et al., 2004). The combined administration of JH and PE increased the shell ratio (Nair et al., 2004). Mulberry leaves treated with chemical, biofertilizers and their mixture increased shell ratio of B. mori cocoon (EL-Khayat et al., 2013). Similar results are noted in earlier studies (Viththalarao and BrijKishor, 2016). In the present investigation, the shell ratio increased with the increase in phytojuvenoid concentration up to 30%. The treatment of larvae with phytojuvenoid, extended the larval period and larvae consumed more food which is utilized in the synthesis of more protein increasing both the cocoon weight and cocoon shell weight. Thus, shell ratio
increased at low concentrations while a higher phytojuvenoid concentration caused stress response and the shell ratio decreased.

The synergistic activity of $\alpha$-Pinene and Pine Needles in the present attempt is hypothesized to be due to changes in the membrane fluidity, interference with membrane bound signaling proteins and cell cycle arrest. Use of $\alpha$-Pinene and Pine Needles through the acetone for topical application, thus chiefly reflected into lengthening fifth instar larval duration. The time required for eating and amount of mulberry leaves eaten both may have been increased and were practically reflected into the improvement of cocoon quality, shell ratio and silk filament quality. The $\alpha$-Pinene and the contents of Pine Needles topically applied through acetone may be utilized by the silkworm larvae for the extra synthesis of silk. The $\alpha$-Pinene is the most popular terpene compound. And Pine Needles are with terpenoid contents. Use of $\alpha$-Pinene and the terpenoid contents of Pine Needles through acetone solution, for rearing of silkworm larvae is easy method. Use of $\alpha$-Pinene and contents of Pine Needles through acetone solution may open a “Biotechnological Avenue” in sericulture for the qualitative cocoon and silk filament.

**Table.1** Schedule of topical application of acetone solution (ten microliters of one ppm) of $\alpha$-Pinene and pine needles to the fifth instar larvae of silkworm, *Bombyx mori* (L).

| Hour after IV Moul ≤ Group ↓ | 48 | 54 | 60 | 66 |
|-------------------------------|----|----|----|----|
| UTC                          | -  | -  | -  | -  |
| A. (Zero)                    | +  | -  | -  | -  |
| A. (1)                       | +  | -  | -  | -  |
| A. (2)                       | +  | -  | -  | -  |
| B. (Zero)                    | +  | +  | -  | -  |
| B.1                          | +  | +  | -  | -  |
| B.2                          | +  | +  | -  | -  |
| C. (Zero)                    | +  | +  | +  | -  |
| C.1                          | +  | +  | +  | -  |
| C.2                          | +  | +  | +  | -  |
| D. (Zero)                    | +  | +  | +  | +  |
| D.1                          | +  | +  | +  | +  |
| D.2                          | +  | +  | +  | +  |

- Groups A. (Zero); B. (Zero); C. (Zero); D. (Zero) are for Acetone Treatment
- Groups A1; B1; C1; D1 are for $\alpha$-Pinene Treatment
- Groups A2; B2; C2; D2 are for Pine Needles Treatment
- + (Plus Sign) Indicates Treatment.
- - (Minus Sign) Indicates No Treatment.
- A. (Zero); B. (Zero); C. (Zero) and D. (Zero) Groups were treated with Acetone.
Table 2. Effect of Acetone Solution of α-Pinene and Pine Needles on the larval parameters of silkworm, *Bombyx mori* (L).

| Parameter→ | Group↓ | Larval Duration (Hrs.) | Larval Weight (Grams) | Silk Glands Weight (Grams) | Tissue Somatic Index (TSI) of Silk Glands |
|------------|--------|------------------------|-----------------------|-----------------------------|------------------------------------------|
| UTC        | 154 (±26.786) 00.000 | 3.186 (±0.457) 00.000 | 0.761 (±0.019) 00.000 | 23.885                       |
| A.(Zero)   | 154 (±21.663) 00.000 | 3.186 (±0.457) 00.000 | 0.762 (±0.019) 00.000 | 23.917                       |
| A.1.(α-Pinene) | 178** (±24.203) 15.584 | 3.728** (±0.592) 17.011 | 1.006** (±0.293) 31.931 | 26.984                       |
| A.2.(Pine.Needs) | 184** (±24.117) 19.480 | 3.859** (±0.739) 21.123 | 1.042** (±0.239) 36.925 | 27.001                       |
| B.(Zero)   | 154 (±21.663) 00.000 | 3.186 (±0.457) 00.000 | 0.762 (±0.019) 00.000 | 23.917                       |
| B.1.(α-Pinene) | 188** (±23.678) 23.684 | 3.871** (±0.706) 21.500 | 1.056** (±0.127) 35.505 | 27.279                       |
| B.2(Pine.Needs) | 194** (±24.122) 27.631 | 3.943** (±0.581) 25.373 | 1.087** (±0.238) 44.547 | 27.567                       |
| C.(Zero)   | 152 (±21.663) 00.000 | 3.145 (±0.457) 00.000 | 0.752 (±0.019) 00.000 | 23.910                       |
| C.1.(α-Pinene) | 198*** (±24.913) 30.263 | 3.956*** (±0.913) 25.786 | 1.064*** (±0.228) 41.489 | 27.240                       |
| C.2.(Pine.Needs) | 202*** (±24.967) 32.894 | 4.037*** (±0.853) 28.362 | 1.099*** (±0.286) 46.143 | 27.243                       |
| D.(Zero)   | 152 (±21.663) 00.000 | 3.145 (±0.457) 00.000 | 0.752 (±0.019) 00.000 | 23.910                       |
| D.1.(α-Pinene) | 221*** (±25.012) 45.394 | 4.050*** (±0.823) 28.775 | 1.118*** (±0.311) 43.882 | 27.604                       |
| D.2.(Pine.Needs) | 226*** (±25.289) 48.684 | 4.149*** (±0.894) 31.923 | 1.163*** (±0.356) 54.654 | 28.030                       |

Each figure is the mean of the three replications.
- Figure with ± sign in the bracket is SD.
- Figure below the standard deviation is the percent increase over the control.

* - P < 0.05; ** - P < 0.005; *** - P < 0.01
Table 3 Effect of Acetone Solution of α-Pinene and Pine Needles on the cocoon parameters of silkworm, Bombyx mori (L)

| Parameter→ Group↓          | Entire Cocoon Weight (Gram) | Shell Weight (Gram) | Pupal Weight (Gram) | Shell Ratio |
|---------------------------|-----------------------------|---------------------|---------------------|-------------|
| UTC                       | 1.712 (±0.351) 00.000       | 0.303 (±0.081) 00.000 | 1.409               | 17.698      |
| A. (Zero)                 | 1.712 (±0.323) 00.000       | 0.303 (±0.089) 00.000 | 1.409               | 17.698      |
| A.1 (α-Pinene)            | 2.463** (±0.369) 43.866     | 0.501** (±0.085) 65.346 | 1.962               | 20.341      |
| A.2 (Pine.Needs)          | 2.471** (±0.371) 44.334     | 0.504** (±0.092) 66.336 | 1.967               | 20.396      |
| B. (Zero)                 | 1.712 (±0.323) 00.000       | 0.303 (±0.089) 00.000 | 1.409               | 17.698      |
| B.1 (α-Pinene)            | 2.505** (±0.446) 46.320     | 0.517** (±0.126) 70.602 | 1.988               | 20.638      |
| B.2 (Pine.Needs)          | 2.511** (±0.606) 46.670     | 0.524** (±0.183) 72.607 | 1.987               | 20.868      |
| C. (Zero)                 | 1.712 (±0.323) 00.000       | 0.303 (±0.089) 00.000 | 1.409               | 17.698      |
| C.1 (α-Pinene)            | 2.523*** (±0.786) 47.371    | 0.547*** (±0.115) 80.528 | 1.976               | 21.680      |
| C.2 (Pine.Needs)          | 2.791*** (±0.554) 63.025    | 0.618*** (±0.123) 103.960 | 2.173               | 22.142      |
| D. (Zero)                 | 1.712 (±0.323) 00.000       | 0.303 (±0.089) 00.000 | 1.409               | 17.698      |
| D.1 (α-Pinene)            | 2.593*** (±0.856) 51.460    | 0.582*** (±0.138) 92.079 | 2.011               | 22.445      |
| D.2 (Pine.Needs)          | 2.921*** (±0.913) 70.619    | 0.663*** (±0.111) 118.81 | 2.258               | 22.697      |

- Each figure is the mean of the three replications.
- Figure with ± sign in the bracket is SD.
- 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
Table 4 Effect of Acetone Solution of α-Pinene and Pine Needles on the silk filament parameters of silkworm, *Bombyx mori* (L).

| Parameter→ Group↓ | S.F. length (meter) (X) | S.F. weight (mg) (Y) | Tex = (Y÷X) x 1000 | Denier = (Y÷X) x 9000 |
|-------------------|--------------------------|----------------------|---------------------|-----------------------|
| UTC               | 798 (±26.786) 00.000     | 0.182 (0.047) 00.000 | 0.228 | 2.052 |
| A.(Zero)          | 798 (±27.098) 00.000     | 0.182 (±0.069) 00.000 | 0.228 | 2.052 |
| A.1.(α-Pinene)    | 957** (±33.217) 19.924   | 0.255** (±0.098) 40.109 | 0.265 | 2.385 |
| A.2.(Pine.Needs)  | 998** (±35.649) 25.065   | 0.279** (±0.066) 53.296 | 0.279 | 2.518 |
| B.(Zero)          | 798 (±27.098) 00.000     | 0.182 (±0.069) 00.000 | 0.228 | 2.052 |
| B.1.(α-Pinene)    | 971** (±36.786) 21.679   | 0.287** (±0.043) 57.692 | 0.291 | 2.623 |
| B.2.(Pine.Needs)  | 1013** (±31.007) 26.942  | 0.371** (±0.087) 103.84 | 0.366 | 3.296 |
| C.(Zero)          | 798 (±27.098) 00.000     | 0.182 (±0.069) 00.000 | 0.228 | 2.052 |
| C.1.(α-Pinene)    | 1045*** (±38.786) 30.952 | 0.396*** (±0.071) 117.58 | 0.378 | 3.410 |
| C.2.(Pine.Needs)  | 1067*** (±33.747) 33.709 | 0.517*** (±0.126) 184.06 | 0.484 | 4.361 |
| D.(Zero)          | 798 (±27.098) 00.000     | 0.182 (±0.069) 00.000 | 0.228 | 2.052 |
| D.1.(α-Pinene)    | 1071*** (±111.11) 34.210 | 0.525*** (±0.189) 188.46 | 0.490 | 4.411 |
| D.2.(Pine.Needs)  | 1167*** (±137.94) 46.240 | 0.578*** (±0.157) 217.58 | 0.495 | 4.457 |

- Each figure is the mean of the three replications.
- Figure with ± sign in the bracket is SD.
- 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
**Fig.1** Effect of Acetone Solution of α-Pinene and Pine Needles on the larval duration (Hours) in the fifth Instars of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

![Graph](image1)

**Fig.2** Effect of Acetone Solution of α-Pinene and Pine Needles on the weight (Grams) of fifth Instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

![Graph](image2)
**Fig.3** Effect of Acetone Solution of α-Pinene and Pine Needles on the weight (Grams) of silk glands in fifth Instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

**Fig.4** Effect of Acetone Solution of α-Pinene and Pine Needles on the Tissue Somatic Index (TSI) (%) of Silk Glands in the fifth Instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).
Fig. 5 Effect of acetone solution of α-Pinene and Pine Needles on the Shell Ratio (%) of Cocoons spinned by fifth Instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

In conclusion, exogenous Juvenile Hormone Analogues (JHA) are serving a lot in sericulture for qualitative improvement of silk yield. Insect juvenile Hormone
Analogue (JHA) action of Terpene compounds and terpene contents of Pine needles of *Pinus sylvestris* (L) are well established facts. Topical application of ten microliters of one milligram per liter (1 ppm) strength acetone solution of α-Pineneand Pine needles to the individual fifth instar larvae of multivoltine cross breed race of silkworm, *Bombyx mori* (L) (PM x CSR2) at 48, 54, 60 and 66 hours after the fourth moult was found variously reflected into the prolongation of larval duration (15.789–45.394% for α-Pinene treated groups and 13 – 42 % for Pine needle treated groups); improvement in the tissue somatic index (TSI) of silk glands (3.054 – 3.344 for α-Pinene treated groups and 3.071 – 3.094 % for Pine needle treated groups); cocoon shell ratio (2.791 – 3.179 for α-Pinenetreated groups and 2.694 – 3.631 for Pine needle treated groups) and denier scale of silk filament (0.333 – 2.359 for α-Pinenetreated group and 0.466 – 2.405 for Pine needle treated group).

Acetone solution of α-Pinene and acetone solution of pine needles, thus chiefly lengthening the larval duration (age) in silkworm, *Bombyx mori* (L). The lengthened larval life is sufficient to prove the juvenoid activity of acetone solution of Pine Needles. Treated group of larvae may be utilizing the lengthened life span for the fortification of cocoon shell through the silk filament. Treated group of larvae synergistic activity of α-Pinene and Pine needles in the present attempt is hypothesized to be due to changes in the membrane fluidity, interference with membrane bound signaling proteins and cell cycle arrest. Efficient utilization of α-Pinene and pine needles, through acetone solvent for topical application to the fifth instared larvae of silkworm, *Bombyx mori* (L) may open a new innovative biotechnological avenue in the sericulture industries.

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