Int.J.Curr.Microbiol.App.Sci (2016) *5*(12): 36-55

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 12 (2016) pp. 36-55**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.512.005

Influence of α-Pinene and Pine Needles of *Pinus sylvestris* (L) on the Cocoons 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, Shardanagar, (Malegaon Khurd) Post Box No.- 35 Tal. Baramati, Dist. Pune 413 115 Maharashtra, India
[2]Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Malegaon (Baramati) Dist. Pune – 413115, India
**Corresponding author*

Keywords

α-Pinene;
Pine needles ;
TSI ;
Shell Ratio;
Denier Scale of Silk Filament.

Article Info

Accepted:
08 November 2016
Available Online:
10 December 2016

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 α-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 α-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 α-Pinene treated groups and 3.071 – 3.094 % for Pine needle treated groups); cocoon shell ratio (2.791 – 3.179 for α-Pinene treated groups and 2.694 – 3.631 for Pine needle treated groups) and denier scale of silk filament (0.333 – 2.359 for α-Pinene treated 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. The 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.

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

larvae 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 moult (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, haemolymphecdysteroid 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 terpenoidsmimics 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 pineneare 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 isan 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; (1*S*, 5*S*)- or (−)-α-pinene is more common in European pines, whereas the (1*R*,5*R*)- 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.[11]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, palustric, 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-Pinenefor 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-Pineneand 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_2) race were reared in the laboratory through standard methods (Krishnaswami *et al.*, 1978; Khyade, 2004). α-Pinenewasprocured throughlocal dealer. One mg of α-Pinenewas dissolved in the acetone and the stock solution of one ppm strength was prepared. The pine needles were collected from *Pinussylvestris* 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 instared 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 theB1 group received ten microliters topical application of acetone solution of α-Pinene at 48 and 54 hours after the fourth moult. Individual larva in theC1 group received ten microliters topical application of acetone solution of α-Pinene48; 54and 60 hours after the fourth moult. Individual larva in theD1 group received ten microliters the topical application of acetone solution of α-Pinene at 48; 54; 60 and 66hours after the fourth moult (Table – 1).

Individual larva in the experimental groupA2 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; 54and 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 group 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 timesa 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. 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. 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 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-Pinenegroup 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-Pineneand 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 spinned 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 spinned by the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race : PM x CSR2).The shell ratio of cocoons spinned 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 ofα-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 CSR_2). 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. The juvenoid 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 fenoxycarb 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 phytoecdysteroid 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 (Vitthalarao 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 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. Use of α-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 α-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 α-Pinene is the most popular terpene compound. And Pine Needles are with terpenoid contents. Use of α-Pinene and the terpenoid contents of Pine Needles through acetone solution, for rearing of silkworm larvae is easy method. Use of α-Pineneand 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 microlitere of one ppm) of α-Pinene and pine needles to the fifth instar larvae of silkworm, *Bombyx mori* (L).

Hour after IV Moult→ 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 α-Pinene Treatment
- Groups A2; B2; C2; D2 are for Pine Needles Treatment
- + (Plus Sign) Indicates Treatment.
- - (Minus Sign) IndicatesNo 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.Needles)	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.Needles)	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.Needles)	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.Needles)	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.Needles)	**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.Needles)	**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.Needles)	**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.Needles)	**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).

Parametere→ 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**	**00.255** (±00.098) 40.109**	**0.265**	**2.385**
A.2.(Pine.Needles)	**998** (±35.649) 25.065**	**0.279** (±00.066) 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.Needles)	**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.Needles)	**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.Needles)	**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 and on the larval duration (Hours) in the fifth Instars of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

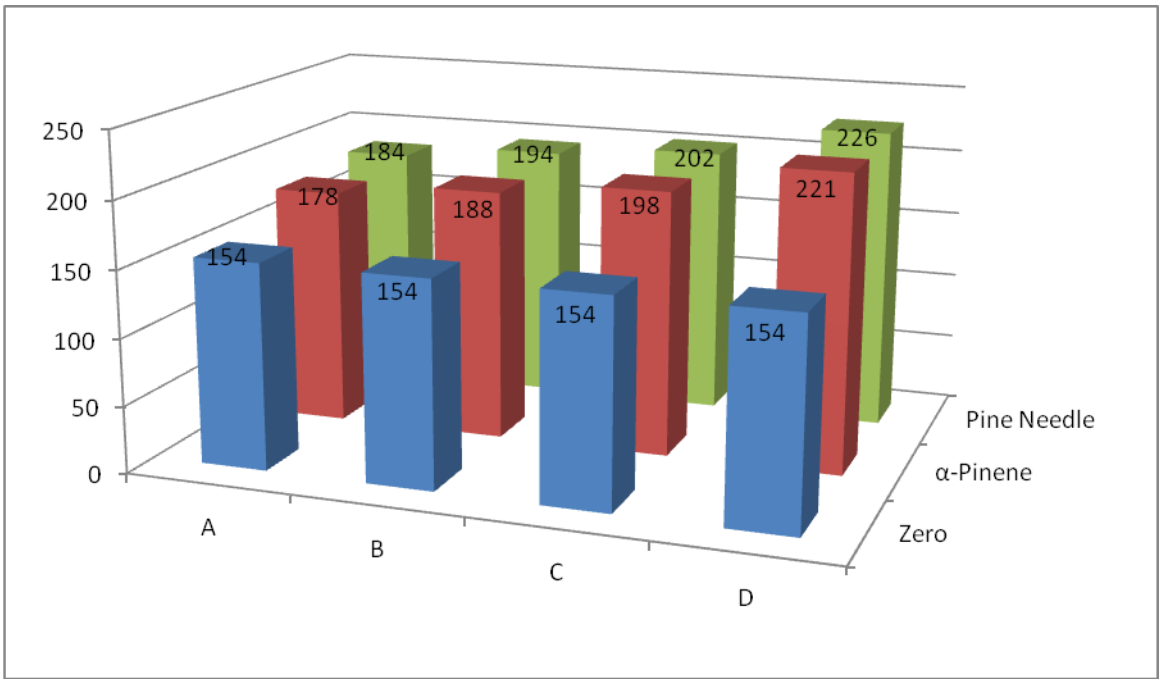


Fig.2 Effect of Acetone Solution of α-Pinene and Pine Needles on the weight (Grams) of fifth Instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

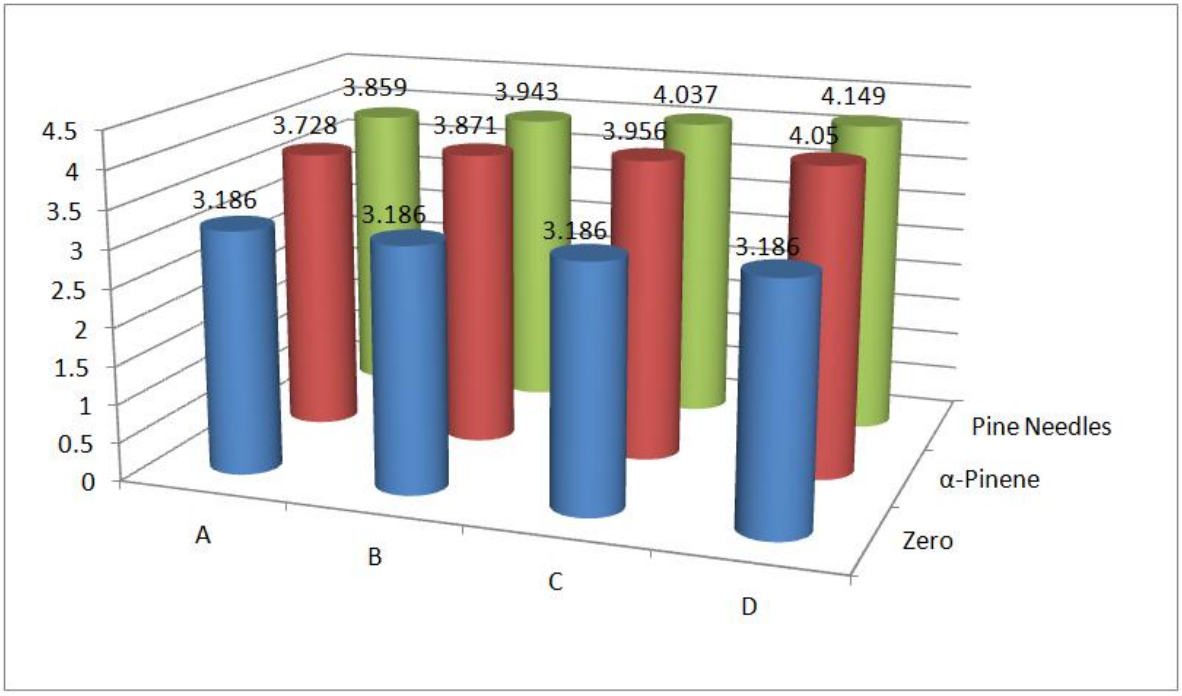


Fig.3 Effect of Acetone Solution of α-Pinene and Pine Needles on the weight (Grams)of silk glands in fifth Instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2)

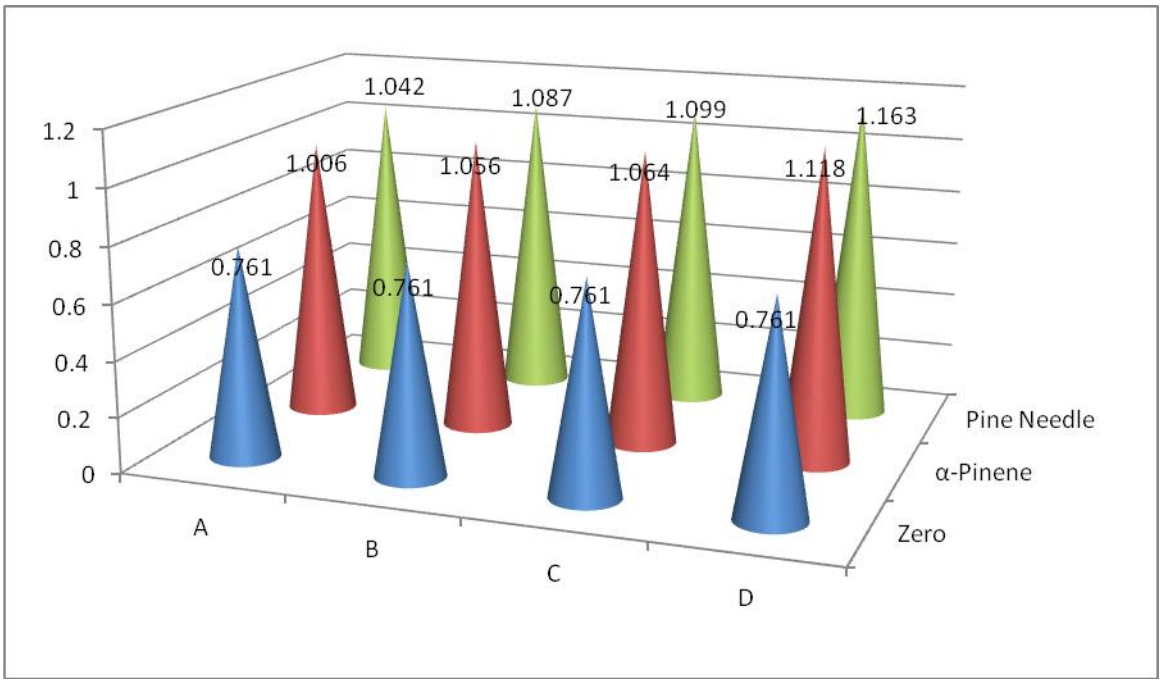


Fig.4 Effect of Acetone Solution of α-Pinene and Pine Needles on the Tissue Somatic Index (TSI) (%) of Silk Glands in the fifth Instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

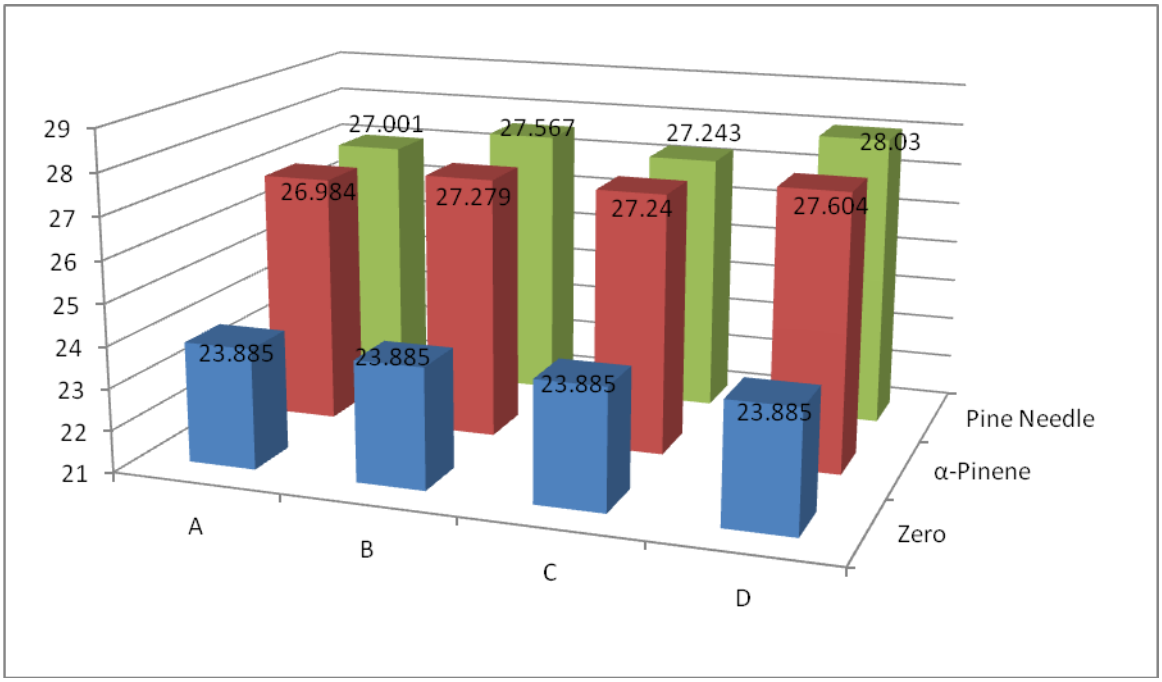


Fig.5 Effect of acetone solution of α-Pinene and Pine Needles on the Shell Ratio (%) of Cocoons spinned by of fifthInstar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

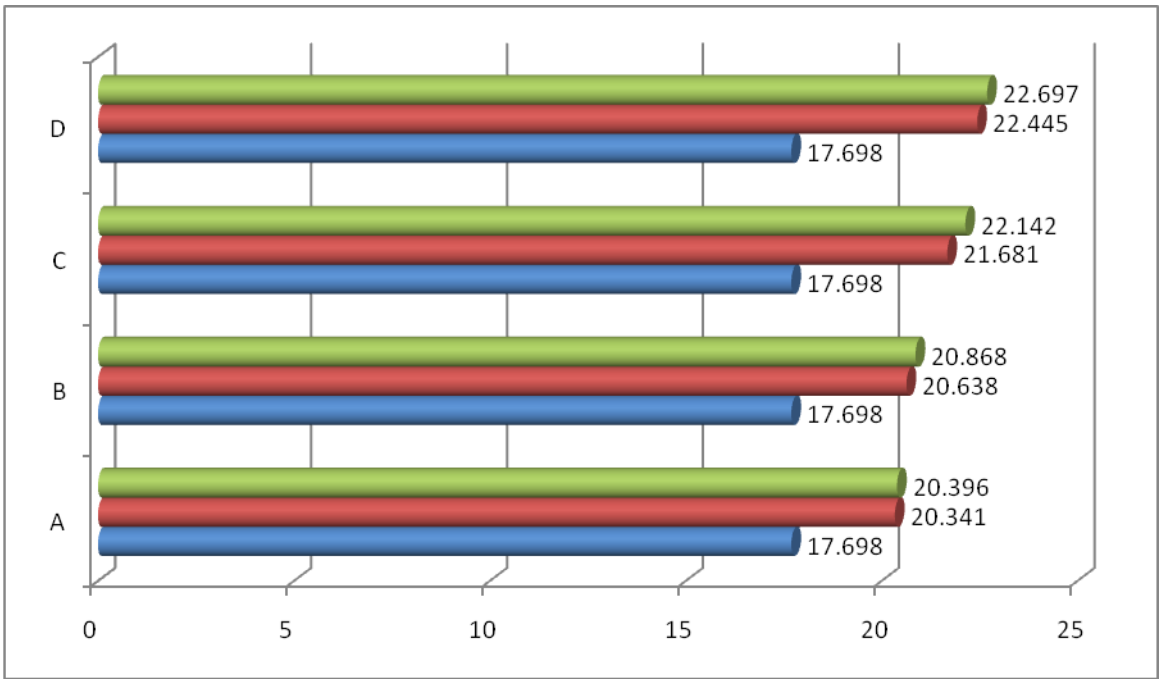
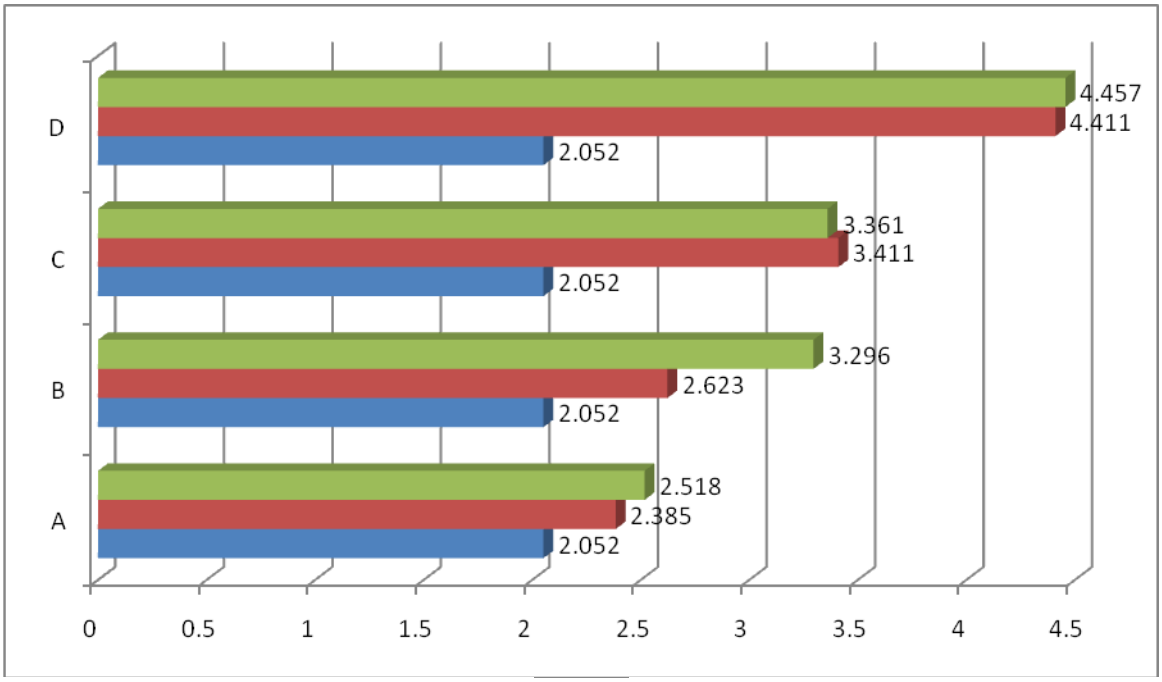


Fig.6 Effect of Acetone solution of α-Pinene and Pine Needles on the Denier Scale of Silk Filament Reeled from the Cocoons spinned by of fifth Instars of silkworm, *Bombyx mori*(L) (Race: PM x CSR2).



In conclusion, exogenous Juvenile Hormone Analogues (JHA) areserving 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 – 3.179for 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-Pineneand 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.

Acknowledgement

Academic support and inspiration received from Punyamayee (Savitribai Phule Pune University). The work has been supported by Agricultural Development Trust, Baramati India; International Science Congress Association, Indore (India) through Hon. Dr. Dipak Sharma and it's dignitaries.

References

Akai, H. and Kobayashi, M. 1971. Introduction of prolonged larval instar by juvenile hormone in *Bombyx mori*(L) (Lepidoptera : bombycidae). *Appl. Entemol. Zool*, 6: 1938 – 1939.

Angelica Nakamura, RenataStiebler, Marcelo, R., Fantappié, Eliane Fialho, Hatisaburo Masuda and Marcus, F. Oliveira 2007. Effects of retinoids and juvenoids on moult and on phenoloxidase activity in the blood-sucking insect, *Rhodniusprolixus (L).Acta Tropica*, 103: 222–230.

Anjang, Tan, Hiromasa, Tanaka, Toshiki Tamura and Takahiro Shiotsuki. 2005. Precocious metamorphosis in transgenic silkworms over expressing juvenile hormone esterase. *Proc. Natl. Acad. Sci. USA*, 102(33): 11751 – 11756. http://genepath.med.harvard.edu/~perrimon/papers/2013_Zirin_DevBio_Ecdysone.pdf

Anne, E., Harman-Ware, Robert Sykes, Gary, F. Peter and Mark, Davis. 2016. Determination of Terpenoid Content in Pine by Organic Solvent Extraction and Fast-GC Analysis. Front. Energy Res, 25 January 2016 http://dx.doi.org/10.3389/fenrg.2016.00002http://journal.frontiersin.org/article/10.3389/fenrg.2016.00002.

Bharthi, D. and Miao, Y. 2003. Administration of Plant growth hormone, Indole-3-acetic on growth and economic charaters of silkworm.(*Bombyx mori* L). *J. Adv. Zool.*, 24 (1&2): 1-7.

Bowles, J., Knight, D., Wihlem, D., Richman, J., Mamiya, S., Yashiro, K., Chawengsaksphak, K., Wilson, M. J., Rossant, J., Hamada, H. and Koopman, P. 2006. Retinoid signaling determines germ cell fate in mice. *Sci.*, 312: 596 – 600.

Calvez, B., Hiren, M. and Reddy, M. 1976. Progress of developmental programme during the last larval instar *Bombyx mori* L): Relationships with food intake, ecdysteroids and juvenile hormone. *J. insect physiol.,* 24*(*4): 233-239.

Chaugale, A.K. and More, N.K. 1992. Effect of magnetisation on the developmental period and cocoon characters of the silkworm, *Bombyx mori* L. *Indian J. Seric.*, 31*(*2): 115 – 122.

Ching, F.C., Mukakeshi, S. and Tamura, S. 1972. Giant cocoon formation in the silkworm, *Bombyx mori* L) treated with methyloneoxyphenyl derivatives. *Agar. Biol. Chem.,* 36: 692-694.

Das, P.K. and Vijayaraghavn, K. 1993. Studies on the effect of different mulberry varieties and season on the larval development and cocoon characters of silkworm, *Bombyx mori* L. Indian J.Seric., 29(1): 44-53.

Dhas, L. S.,Latpate, C. B., Sonkambale, M. M. and Desai S. D. 2014. Effect of Azotobzcter and Vermicompost on Mulberry in relation to economic traits of Silkworm, *Bombyx mori* L. The Ecoscan: Special Issue, Vol. VI: 375 – 378.

EL-Khayat, E.F., Gaaboud, L.A., Omer, R.E.M., Ghazey, U.M. and EL-Shewy, A.M. 2013. Impact of bio and organic fertilizer treatment on economic traits of mulberry silkworm B. mori L.) *Academic J. Entomol.,* 6(1): 01-06, 2013.

Gajanan, B., Zore, Archana, D., Thakre, Sitaram Jadhavand, S., Mohan Karuppayil. 2011. Terpenoids inhibit Candida albicans L) growth by affecting membrane integrity and arrest cell cycle. *Phytomed.,* 18: 1181 – 1190. www.elsevier.de/phytomed

Granier and Granier. 1938. Fenoxycarb, a fairly new growth regulator: A review of its effects on insects. *Ann. Appl. Biol.*, 122: 369-403.

Hamberger, B. and Bak, S. 2013. Plant P450s as versatile drivers for evolution of species-specific chemical diversity. *Philos Trans R SocLond B Biol Sci.*, *3*68(1612):20120426. doi: 10.1098/rstb.2012.0426.

Kamada, A. and Shimada, S. 1988. Effects of methoprene on growth profile of larval organs of silkworm, *Bombyx mori* L. *J. sericulture sci., Jpn.* 48 2): 129-136.

Karlberg, Ann-Therese, Magnusson, Kerstin, Nilsson, Ulrika. 1992. "Air oxidation of d-limonene the citrus solvent) creates potent allergens". Contact Dermatitis 26*(*5): 332–40. doi:10.1111/j.1600-0536.1992.tb00129.x. PMID 1395597.

Khyade V.B. 2004. Influence of juvenoids on silkworm, *Bombyx mori* L. Ph.D thesis, Shivaji University, Kolhapur. Maharashtra, India.

Kiguchi, K. and Augi, N. 1981. Ecdysteroid level and development events during larval moulting in silkworm, *Bombyx mori* L. insect physiol. 26: 805-813.

Krishnaswami, S., Narimhan, N., Suryanarayana, S.K and Kumararaja, S. 1978. Manual on sericulture, vol. 2: silkworm rearing, FAO publication, agricultural services bulletin no. 15.

Lincoln, D.E., B.M. Lawrence. 1984. The volatile constituents of camphorweed, *Heterothecasubaxillaris*. *Phytochemistry* 23(4):933-934.

Mamatha, D.M., Cohly, H.P.P., Raju, A.H.H. and Rao Rajeswara, M. 2006. Studies on the quantitative and qualitative characters of cocoons and silk from methopreneand fenoxycrab treated *Bombyx mori* L.) larvae. *African J. Biotechnol.*, 5(15): 1422-1426.

Mamatha, D.N., Nagalalakhamma, K., Vijay, P. and Rajeshwara Rao, A.M. 1999. Impact of selected juvenile hormone mimics on organic constituents of silkworm, *Bombyx mori* L. proceedings of NSTS- 99: 185-186.

Mishra, A.B. and Upadhyay, V.B. 1995. Influence of temperature on the silk producing potential of multivoltine *Bombyx mori* L. race nistari. *Sercologia*, 35(2): 217 – 222.

Moser, Ann, B., Hey, Jody, Dranchak, Patricia, K., Karaman, Mazen, W., Zhao, Junsong, Cox, Laura, A., Ryder, Oliver, A., Hacia, Joseph, G. 2013. "Diverse captive non-human primates with phytanic acid-deficient diets rich in plant products have substantial phytanic acid levels in their red blood cells". *Lipids in Health and Dis.*, 12: 10. doi:10.1186/1476-511X-12-10.PMC 3571895. PMID 23379307.

Muroga, A., Nakajima, M., Aomori,S., Ozawa, Y. and Nihmura, M. 1975. Utilization of synthetic juvenile hormone analogue for the silkworm rearing on the mulberry leaves. *J. Sreic. Sci. Jpn.* 44: 267-273.

Nair, K.S., Kariappa, B.K. and Kamble, C.K. 2008. Impact of individual and co-administration of juvenile hormone analogue and phytoecdysteroid on the crop management and performance of silkworm *Bombyx mori*. L. *J. Biol. Sci.*, 8(2): 470-473.

Nair, K.S., Miao Yun-Gen and Nirmal, K.S. 2005. Differential response of silkworm, *Bombyx mori* L. to phytoecdysteroid depending on the time of administration. *J. Appl. Sci. Environ. Mgt.* 9(3): 81-86.

Nair, K.S., Nair, .J.S, Vijayan, V..A and Trivedy, K. 1999. Juvenilomimic compounds for enhance productivity in silkworm, *Bombyx mori* – A screening. *Indian J. Seric.* 38(2): 119-124.

Nair, K.S., Trivedy, K., Vijayan, V.A., Nair, J.S. and Chinya, P.K. 2004. Efficiency of feed conversion of the last instar silkworm, *Bombyx mori* L. under the influence of a juvenoid, R394. *Indian J. Seric.*, 43(2): 187-193.

Nair, K.S.,Vijayan, V.A., Trivedy, K. and J.S.N., 2001. Improvement in the commercial traits of silkworm, *Bombyx mori* L. in the administration of a Juvenile, R 394. *Int. J. industrial Entomol.*, 3(2): 169-175.

Nath, T.N., Shivanth, Shamsuddin M and SubbaRao G, 1990. Effect of food deprivative on dry matter utilization, shell and cocoon weight and fecundity of multivoltine silkworm, *Bombyx mori* L. race nistari. *Indian J. Seric.*, 29(2): 174-181.

Nihmura, M., Aomori,S., Mori, K. and Matusui, M. 1972. Utilization of synthetic compounds with juvenile hormone activity for silkworm rearing. *Agri. Biol. Chem.*, 36: 882-889.

Nissen, L, Zatta, A, Stefanini, I, Grandi, S, Sgorbati, B, Biavati, B, *et al*. 2010. "Characterization and antimicrobial activity of essential oils of industrial hemp varieties Cannabis sativa L.)". *Fitoterapia*. 81: 413–419. doi:10.1016/j.fitote.2009.11.010.

Normn, T.J. and Bailey, 1995. Statistical methods in biology.2nd ed., Halsted Press, John Wiley & Co., New York, 216 pp. http://deepblue.lib.umich.edu/bitstream/handle/2027.42/24020/0000269.pdf?sequence=1&isAllowed=y

Pakdel, H. 2001. "Production of dl-limonene by vacuum pyrolysis of used tires". Journal of Analytical and Applied Pyrolysis 57: 91–107. doi:10.1016/S0165-2370(00)00136-4.

Prabhu, V.K.K, John, M. 1975. Juvenomimetic activity in some plants. Experinential,31: 913-914.

Rajashekhargouda, R. 1991. Studies on methods to increase silk yield of *Bombyx mori* L) Lepidoptera : bombycidae. Ph.D. thesis, Tamil Nadu University, Coimbtore, India.

Ratna Sen. 1988. How does juvenile hormone analogue cause more silk yield. *Indian silk,* 21-22.

Riddiford, L. M. and Ashburner, M. 1990. Role of Juvenile hormone in larval development and metamorphosis in *Drosophila melanogaster* L. *Comparative Endocrinol.,* 82: 172-183.

Riddiford, L.M. 1985. Hormone action at Cellular and Molecular actions of juvenile hormone: general considerations for premetamorphic actions. *Adv. Insect physiol.*, 24: 213-214.

Russo, E. B 2011. "Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects". *British J. Pharmacol.,* 163 7): 1344–1364. doi:10.1111/j.1476-5381.2011.01238.x. PMC 3165946. P MID 21749363.

Sehnal, F. and Rambold, H. 1985. Brain stimulation of juvenile hormone production in insect larvae. *Experimentation,* 41: 684-685.

Simonsen, J.L. 1957. *The Terpenes 2nd edition)* Vol. 2 Cambridge:Cambridge University Press, pp 105-191.

Slama, K. 1971. Insect juvenile hormone analogues, *ann. Rev. biochem.*, 40: 1079-1102.

Slama, K. 1985. Pharmacology of insect juvenile hormones. In: Biochemistry and pharmacology Eds G.A Kerkutand L.I Gilbert), vol. 11: 357-394. Pergamon Press, New York.

Slama, K., Wimmer, Z. and Romanuk, M. 1978. Juvenile hormone activity of some glycosidicjuvenogens. *Hopperstyler's Physiol. Chem.*, 359: 1407-1412.

Thiagarajan, V., Bhrgava, S.K., Babu, M.R. and Nagaraj, B. 1993. Difference in seasonal performance of 26 strain of silkworm, *Bombyx mori* Bombycidae. *J. Lepidopterists Soc.*, 47 4): 331 – 337.

Trivedy, K., Nair, K.S., Assan, N.M. and Datta, R.K. 1997. A Juvenile hormone mimic modulated enhancement of silk productivity in silkworm, *Bombyx mori, Indian J. Serc.,* 360: 35- 38.

Trivedy, K., Remadevi, O.K., Magadum, S.B. and Datta, R.K. 1993. Effect of a juvenile hormone analogue, Labomin on the growth and economic characters of silkworm, *Bombyx mori* L. I*ndian J. seri.,* 32(2): 162-168.

Upadhyay VB and Tripathi SK, 2006) Effect of the magnetization of eggs on the silk producing potential of multivoltine mulberry silkworm *Bombyx mori* Linn.. Sericologia, 46(3): 269-278.

Van Den Brink, D. M., Wanders, R. J. A. 2006. "Phytanic acid: Production from phytol, its breakdown and role in human disease". Cellular and Molecular Life Sciences 63 15): 1752–65.doi:10.1007/s00018-005-5463-y. PMID 16799769.

Vencl, Fredric V., Morton, Timothy C. 1998. "The shield defense of the sumac flea beetle, Blepharidarhois Chrysomelidae: Alticinae)". *Chemoecol.*, 8(1): 25–32.doi:10.1007/PL00001800.

Vishakha, S. Chape, Abhilasha, C. Bhunje and Vitthalrao B. Khyade 2016. Efficient Use of Extractive of *Oroxylumindicum* for the improvement of Quality of Silk in Silkworm, *Bombyx mori* L) Race: PM x CSR2. International Conference on "Plant Research and Resource Management" And 25th APSI Silver Jubilee Meet 2016 at T. C. College Baramati 11, 12 and 13 February, 2016. Pages: 304 – 308.

Vitthalrao, B. Khyade and BrijKishor Tyagi. 2016. Utilization of the Acetone Solution of Limonene and Pine Needles of *Pinussylvestris*(L) For the Quality Improvement of Cocoons and Silk Fibers Spin by Fifth Instar Larvae of Silkworm, *Bombyx mori*(L) Race: PM x CSR2.*J. Basic Sci.*, Vol. 4, No. 1:21 – 35.www.skpubs.com

Vitthalrao, B. Khyade, Patil, S.B, Khyade, S.V and Bhavane, G.P 2002. Influence of acetone maceratives of *Vitisvinifera* on the larval parameters of silkworm *Bombyx mori* L. *Indian J. Comparative Animal Physiol.*, vol. 20: 14-18.

Vitthalrao, B. Khyade, Patil, S.B, Khyade, S.V and Bhavane, G.P. 2003. Influence of acetone macerative of *Vitisvinifera* on the economic parameters of *silkworm Bombyx mori* L. *Indian J. Comparative Animal Physiol.*, Vol. 21: 28-32.

Vitthalrao, B., Khyade and Anil, N., Shendge. 2012. Influence of *Aloe vera* L) herbal formulation on the larval characters and economic parameters of silkworm, *Bombyx mori* L) Race: PM x CSR2. The Ecoscan Special Issue, Vol.1: 321 – 326. http://theecoscan.in/journalpdf/spl2012_v1-55%20b.%20vitthalrao%20khyade.pdf

Vitthalrao, B., Khyade and Brij Kishor Tyagi. 2016. Utilization of the Acetone Solution of Limonene and Pine Needles of *Pinussylvestris*(L) For the Quality Improvement of Cocoons and Silk Fibers Spin by Fifth Instar Larvae of Silkworm, *Bombyx mori*(L) Race: PM x CSR2.Journal of Basic Sciences, Vol. 4, No. 1:21 – 35.www.skpubs.com

Vitthalrao, B., Khyade and Jiwan, P. Sarwade. 2013. Utilization of Retinol through the topical application to the fifth instar larvae of the silkworm, *Bombyx mori* L) Race: PM x CSR2) for qualitative improvement of the economic parameters. *Int. J. Adv. Life Sci.*, 6*(*5): 532 – 537. www.unitedlifejournals.com

Vitthalrao, B., Khyade, KarelSlama, Rajendra, D. Pawar and Sanjay, V. Deshmukh. 2015. Influence of Various Concentrations of Acetone Solution of Retinol on Pattern of Chitin Deposition in the Integument of Fifth Instar Larvae of Silkworm, *Bombyx mori* L) Pm X Csr2. *J. Applicable Chemistry*, 4)-15: 1434 – 1445. www.joac.info

Watkins, Paul, A., Moser, Ann, B., Toomer, Cicely, B., Steinberg, Steven, J., Moser, Hugo, W., Karaman, Mazen W, Ramaswamy, Krishna, Siegmund, Kimberly D, Lee, D Rick, Ely, John J, Ryder, Oliver A, Hacia, Joseph G 2010. "Identification of differences in human and great ape phytanic acid metabolism that could influence gene expression profiles and physiological functions".BMC Physiology 10:

19. doi:10.1186/1472-6793-10-19. PMC 2964658. PMID 20932325.

Weitkamp, A.W. 1959. "I. The Action of Sulfur on Terpenes.The Limonene Sulfides". *J. American Chemical Society*, 81(13): 3430–3434. doi:10.1021/ja01522a069

Wimmer, Z. and Romanauk, M. 1981. The synthesis of biologically active 2-(4-hydroxyl benzyl)-1- cyclohexanol derivation. *Coll. Czech. Chem. Commun.*, 46: 2573-2586.

Yang, H., Woo, J., Pae, A.N., Um, M.Y., Cho, N.C., Park, K.D., Yoon, M., Kim, J., Lee, C.J., Cho, S. 2016. "α-Pinene, a major constituent of pine tree oils, enhances non-rapid eye movement sleep in mice through GABAA-benzodiazepine receptors". *Mol. Pharmacol.*, doi: 10.1124 / mol. 116 . 105080 PMID 27573669.

Ying, H., Wang, Guirong Wang and Gerold, A., LeBlanc. 2006. Cloning and characterization of the retinoid X receptor from a primitive crustacean, *Daphnia magna* L. General and Comparative Endocrinology. 150: 309 – 318. www.sciencedirect.com and www.elsevier.com/Locate/ygcen

Zebib, Bachar, Beyrouthy, Marc EL, Sarfi, Carl, Merah, Othmane 2015-04-16. "Chemical Composition of the Essential Oil of Saturejamyrtifolia Boiss. &Hohen.) from Lebanon". *J. essential oil-bearing plants JEOP*. 18 1. doi:10.1080/0972060X.2014.890075. ISSN 0972-060X.

How to cite this article:

Vitthalrao B. Khyade, Vishakha S. Chape and Priyanka B. Ghadge. 2016. Influence of α-Pinene and Pine Needles of *Pinus sylvestris* (L) on the Cocoons and Silk Fibres Spinned by Fifth Instar Larvae of Silkworm, *Bombyx mori* (L) (Race: PM x CSR2). *Int.J.Curr.Microbiol.App.Sci.* 5(12): 36-55. doi: http://dx.doi.org/10.20546/ijcmas.2016.512.005