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Topical Application of Acetone Solution of Ferruginol, Meroterpene Compound to the Fifth Instar Larvae of Silkworm *Bombyx mori* (L.) (Race: PM x CSR2) for Quality Improvement in Silk

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A B S T R A C T

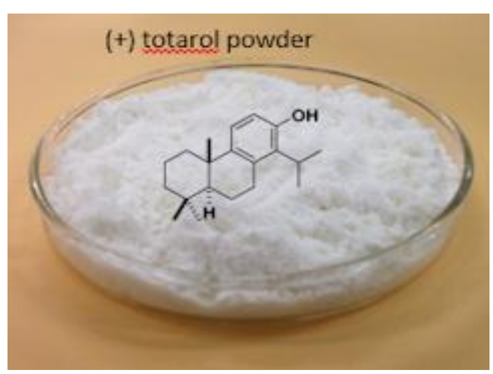
The principle of efficient use of available system for quality improvement is tried to utilize in the present study. The present attempt was undertaken to study the influence of acetone solution of Ferruginol, a natural phenol and a meroterpene compound, on the cocoon characters and silk filament parameters of silk worm *Bombyx mori* (L.) (Race: PM x CSR2). Three concentrations (5ppm; 10ppm and 20ppm) of Ferruginol were prepared. The fifth instar larvae were utilized for the experimentation. Soon after the fourth moult, the fifth instar larvae were grouped into five groups (each with hundred individuals) (Untreated control; Acetone treated control; 5ppm Ferruginol; 10ppm Ferruginol and 20ppm Ferruginol). Ten microliters of each concentration of Ferruginol solution were topically applied to respective group to the individual larva at 48 hours after the fourth moult. The larvae were maintained through standard schedule. Acetone solution of Ferruginol at 5 ppm, 10 ppm and 20 ppm concentrations recorded maximum cocoon weight (2.046; 2.387; 2.924gm), shell weight (0.438, 0.541, 0.673 gm), pupal weight (1.613; 1.846; 2.252 gm). All three concentrations of Ferruginol recorded significant weight of cocoon, shell, and pupal weight in comparison with the control (untreated and acetone treated).There was a gradual increase in the silk yield with an increase in the concentrations of Ferruginol (in acetone) from 5 ppm, 10 ppm and 20 ppm. Shell ratio of the cocoons harvested from the treated group were found with most significant (** $P < 0.005$, ***$P < 0.01$) influence. Similar type of effect was observed for the silk filament parameters. Efficient use of acetone solution of Ferruginol may open a new avenue in the field of sericulture.

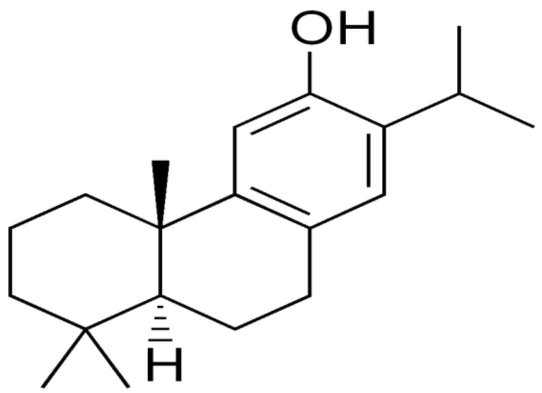
Introduction

The titer of ecdysone and juvenile hormone serves a lot for metamorphosis in insects, like silkworm, *Bombyx mori* (L). The ecdysone and juvenile hormone (JH) are the two major circulating hormones in insects, which control majority of the growth and developmental activities of the insects. The Juvenile Hormone (JH) has been considered to be an exclusive insect hormone and thus has attracted much attention also in plant and grain protection oriented research. The Juvenile Hormone (JH) is clearly a pleiotropic master hormone of insects, which governs most aspects of their integration with the ecosystem and affects decisive life history parameters during their entire life cycles (Hartfelder, 2000). The Juvenile Hormone (JH) also regulates diverse traits in insects such as the synthesis of yolk protein, uptake of the molecule into the developing egg, diapause, flight, development, reproductive features and dispersal polymorphisms (Denlinger, 1985; Nijhout, 1999; Wyatt and Davey, 1996; Era and Cisper, 2001; Wheeler and Nijhout, 2003). The juvenile hormone reportedly alters physiological processes essential for insect development and appears to act especially on insects (Siddall, 1976; Ravindra Chaudhari and Vitthalrao Khyade, 1997).

Juvenile Hormone Analogues (JHAs) are a group of terpenoids that regulate many aspects of insect physiology. They regulate development, reproduction, diapause and polyphenisms (Riddiford, 1994; Nijhout, 1994; Wyatt and Davey, 1996; Khyade and Slama, 2014). The insect Juvenile Hormone Analogues (JHAs) are terpenes and their analogue. The terpenes are a large and diverse class of organic compounds, produced by a number of plants. The terpenes are also produced by some insects, which emit from their osmeteria. The papilionid larvae are distinguished by presence of osmeteria. The osmeterium is a defensive organ found in all Papilionid larvae, in all stages (Chattopadhyay, 2011). The osmeterium is situated in the prothoracic segment. It can be averted when the larva feels threatened. In averted condition, osmeterium resembles a fleshy forked tongue not unlike a snake tongue and this along with the large eye like spots on the body might be used to startle birds and small reptiles. The osmeterial organ remains inside the body in the thoracic region in an inverted position and is averted when the larva is disturbed in any way emitting a foul, disagreeable odour which serves to repel ants (Eisner and Meinwald, 1965); small spiders (Damman, 1986) and mantids (Chow and Tsai, 1989). The composition of secretion from osmeteria varies from species to species. It contains monoterpene hydrocarbons, sesquiterpenic compounds or a mixture of aliphatic acids and esters. Crossley and Waterhouse (1969) studied the fine structure of the osmetrium of *Papiliodemoleus libanius* Fruhstorfer and found to contain 3 types of specialised cells for synthesis, acid secretion. Lu *et al.*, (1991) confirmed the storage of the osmeterial secretion (VitthalraoKhyade, Edvard Moser and May – Britt Moser, 2015; Madhuri Anil Shivpuje *et al.*, 2016).

A meroterpene is a chemical compound having a partial terpenoid structure. The meroterpene compounds are also called as Terpeno-phenolics. The Terpeno-phenolics are compounds that are partly terpenes and party natural phenols. Plants in the genus *Humulus* and *Cannabis* produce terpeno-phenolic metabolites (Page, 2006). Examples of terpeno-phenolics are:Bakuchiol; Ferruginol; Mutisianthol and Totarol. They can also be isolated from animals. The methoxyconidiol, epiconicol, and didehydroconicol, isolated from the ascidian *Aplidium aff. densum*, show anti-proliferative activity (Simon-Levert *et al.*, 2010).





Ferruginol is a natural phenol and a meroterpene (a chemical compound containing a terpenoid substructure) that has been isolated from the needles of the redwood *Sequoia sempervirens*. The terpenoid part is a diterpene of the abietane chemical class. Research published in 2005 found that this and other compound of the class from *Sequoia* have anti-tumor properties, and showed *in vitro* human colon, breast, and lung tumor reduction and reduction in oncogene transformed cells as well. Specific activity of tumorgrowth inhibition (GI) is 2-5 micrograms/milliliter (Son *et al.*, 2005). The Ferruginol has also been found to have antibacterial activity (Smith *et al.*, 2007; Flores, 2001). Gastroprotective effects of ferruginol have also been noted (Areche Carlos, 2008). The totarol is synthesized biologically from *ferruginol.* The Totarol motivates research in drug discovery due to its ability to inhibit numerous microorganisms. The Totarol exhibits antimicrobial properties in numerous species including gram-positive bacteria, nematodes, crustaceous foulers. In addition to inhibiting microorganisms by itself, totarol exhibits inhibitory synergy with currently used antimicrobial drugs, totarol potentiates isonicotinic acid hydrazide against various microbials. There are no reports on use of acetone solution of Ferruginol in rearing the larvae of silkworm for commercial silk yield. In view of to determine the effects of the topical application of acetone solution of Ferruginol on cocoon characters and silk filament parameters, the present study has been planned.

Materials and Methods

The experimentation was divided into the parts like: Preparation of Acetone solution of Ferruginol; Rearing of silkworm larvae; Topical application of Acetone solution of Ferruginolto the fifth instar larvae Analysis of economic parameters and statistical analysis of the data.

Preparation of Acetone Solution of Ferruginol: The Ferruginol powder (Commercially with trade name: Totarol) was procured through the local dealer. According to instructions (Vitthalrao Khyade and Bhunje, 2015), the acetone solution of Ferruginol was prepared. It was dissolved in acetone solvent. Three different concentrations (5 ppm; 10 ppm and 20 ppm) of acetone solutions of Ferruginol were freshly prepared before use.

Rearing of silkworm larvae: The rearing of silkworm larvae has been carried out through standard methods suggested by Krishnaswami *et al.*, (1992) and explained by Khyade (2004) and Vitthalrao Khyade *et al.*, (2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015 and 2016). The disease free layings of multivoltine cross breed race (PM x CSR2) of silkworm, *Bombyxmori* (L) were procured through the "Dr. APIS" Laboratory and processed for black boxing, rearing of early instars, rearing of late age instars, provision of mountage for spinning the cocoon and cocoon harvesting through the standard methods of Krishnaswami *et al.*, (1992) and Sharad Jagtap (2012).

Topical application of Acetone solution of Ferruginol to the fifth instar larvae: The fifth instar larvae were utilized for the experimentation. Soon after the fourth moult, the fifth in star larvae were grouped into five groups (each with hundred individuals). The groups include: Untreated control; Acetone treated control; 5 ppm Ferruginol; 10 ppm Ferruginol and 20 ppm Ferruginol. Ten microliters of each concentration of Acetone solution of Ferruginol were topically applied to respective group to the individual larva at 48 hours after the fourth moult. The larvae were maintained through standard schedule. Rearing was conducted in wooden trays with four feedings per day. The provision of mountage was made to the mature fifth larvae for spinning their cocoons (Khyade, 2004 and Vitthalrao Khyade *et al.*, (2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015 and 2016).

Analysis of economic parameters: The cocoons from the mountage were harvested on fifth day after the provision of mountage for spinning. Twenty cocoons from each group were selected randomly, deflossed and used for recording the weight of entire cocoon. Each cocoon was cut vertically using the blade. Weight of entire cocoon; weight of shell of cocoon and weight of pupa were noted. Through the use of readings of weight of entire cocoon and weight of shell of cocoon, the shell ratio was calculated. The reading of weight of shell was divided with reading of weight of entire cocoon. The quotient thus obtained was multiplied with hundred for getting the shell ratio of individual cocoon. Ten cocoons per replication were reeled and length in meter (A) of unbroken silk filament was obtained by using eprouvate. Weight in gram of silk filament (B) from individual cocoon was recorded. Length (A) and weight (B) of silk filament were accounted for the calculation of Denier scale. The reading of weight of silk filament (B) was divided by the reading of length of silk filament (A). Quotient thus obtained was multiplied by 9000 for the purpose to get the denier scale of silk filament Vitthalrao Khyade and Abhilasha Bhunje, 2015 and 2016).

Statistical analysis of the data

The experimentation was repeated for thrice for the purpose of consistency in the results. The data was subjected for analysis. 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 on the topical application of acetone solution of Ferruginol to the individual fifth in star larvae of silkworm, *Bombyx mori* (L) are presented table-1. The cocoon weight (gm); shell weight (gm); Pupal weight (gm); Shell Ratio; Silk Filament Length (SFL in meters); Silk Filament Weight (SFW in grams) of the Untreated Control group and Acetone Treated group were measured 1.795 (±0.084); 00.347 (±0.009);1.448; 19.331; 758.81 (±9.159); 0.183 (±0.035) and 2.170 respectively. Topical application of 5 ppm acetone solution of Ferruginol to the fifth instar larvae at 48 hours after the fourth moult was found increasing in the entire cocoon weight (13.983 percent); Cocoon Shell Weight (26.224 percent); Pupal weight (11.395 percent). The shell ratio of the cocoon harvested from the 5 ppm acetone solution of Ferruginol group was found measured 21.407. It was significant ($P < 0.05$) over the control. The denier scale of silk filament reeled from the 5 ppm acetone solution of Ferruginol group was found measured 2.404, which was also significant ($P < 0.05$) over the control group.

Topical application of 10 ppm acetone solution of Ferruginol to the fifth instar larvae at 48 hours after the fourth moult was found increasing in the entire cocoon weight (32.980

percent); Cocoon Shell Weight (55.907 percent); Pupal weight (27.486 percent). The shell ratio of the cocoon harvested from the 10 ppm acetone solution of Ferruginol group was found measured 22.664. It was significant over the control. The denier scale of silk filament reeled from the 10 ppm acetone solution of Ferruginol group was found measured 3.165, which was also significant (P < 0.005) over the control group.

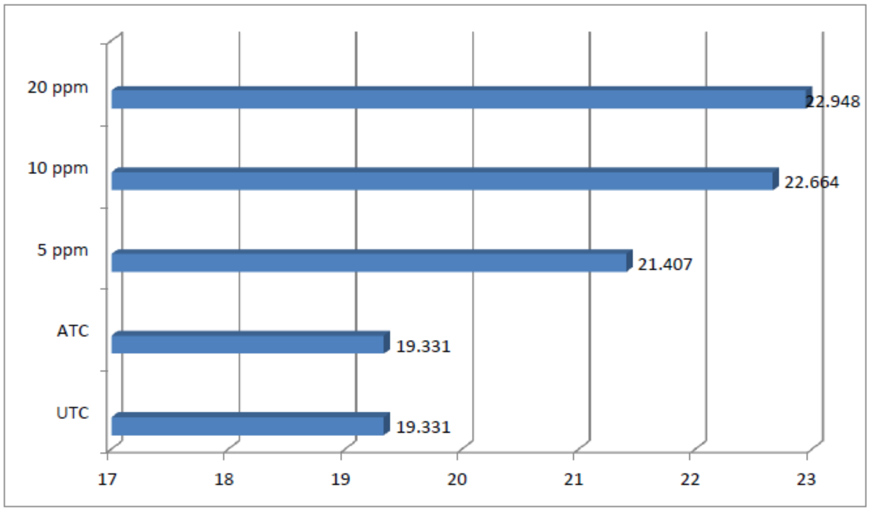
Topical application of 20 ppm acetone solution of Ferruginol to the fifth instar larvae at 48 hours after the fourth moult was found increasing (P < 0.01) in the entire cocoon weight (62.896 percent); Cocoon Shell Weight (93.948 percent); Pupal weight (55.524 percent). The shell ratio of the cocoon harvested from the 20 ppm acetone solution of Ferruginol group was found measured 22.948. It was significant (P < 0.01) over the control. The denier scale of silk filament reeled from the 20 ppm acetone solution of Ferruginol group was found measured 3.171, which was also significant (P < 0.01) over the control group.

Table.1 The economic parameters of the cocoons (and silk filament) spinned by mature fifth instar larvae of silkworm, *Bombyx mori* (L) (Race PM x CSR2) received topical application of acetone solution of Ferruginol at 48 hours after the fourth moult

Parameters→ Group↓	**Cocoon Weight (gm)**	**Shell Weight (gm)**	**PupalWeight (gm)**	**Shell Ratio**	**S F L (m) (A)**	**S F W (gm) (B)**	**Denier Scale of S F = (B÷A) x 9000**
UTC	1.795 (±0.084) 00.000	00.347 (±0.009) 00.000	1.448 0.000	19.331 0.000	758.81 (±9.159) 00.000	0.183 (±0.035) 00.000	2.17 00.00
ATC	1.795 (±0.089) 00.000	00.347 (±0.009) 00.000	1.448 0.000	19.331 0.000	758.81 (±9.159) 00.000	0.183 (±0.035) 00.000	2.17 0.000
5 ppm	2.046* (±0.013) 13.983	00.438** (±0.022) 26.224	1.613**	21.407 *	1051.73** (±13.694) 38.602	0.281** (±0.042) 53.551	2.404**
10 ppm	2.387*(±0.018) 32.980	00.541* (±0.094) 55.907	1.846**	22.664 **	1358.82** (±17.693) 79.072	0.478** (±0.077) 161.202	3.165**
20 ppm	2.924***(±0.187) 62.896	00.671*** (±0.103) 93.371	2.252***	22.948 ***	1393.31*** (±18.142) 83.617	0.491*** (±0.083) 168.306	3.171***

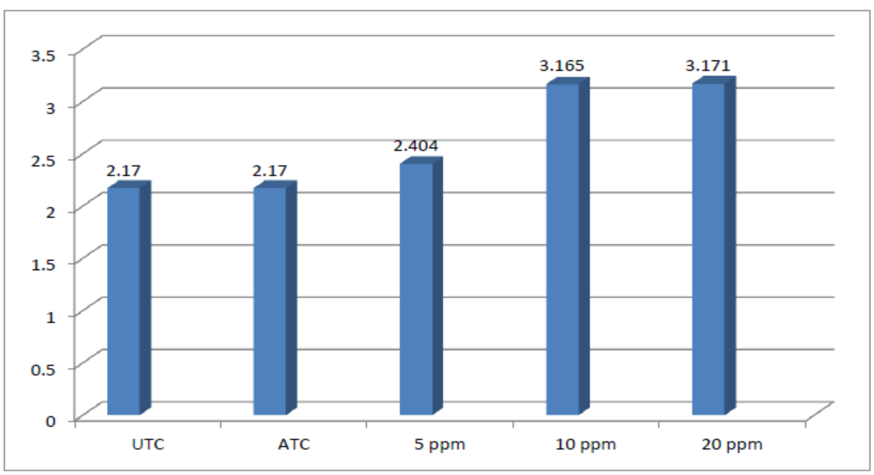
-Each figure is the mean of the three replications.
-Figure with ± sign in the bracket is standard deviation.
-Figure below the standard deviation is the increase for calculated parameter and percent increase for the others over the control. UTC=Untreated Control; ATC =Acetone Treated Control; SFL= Silk Filament Length; SFW= Silk Filament Weight
* : P < 0.05;** : P < 0.005; ***: P < 0.01

Fig.1 The Shell Ratio of the cocoons spinned by mature fifth instar larvae of silkworm, *Bombyx mori* (L) (Race:) received topical application of acetone solution of Ferruginol at 48 hours after the fourth moult



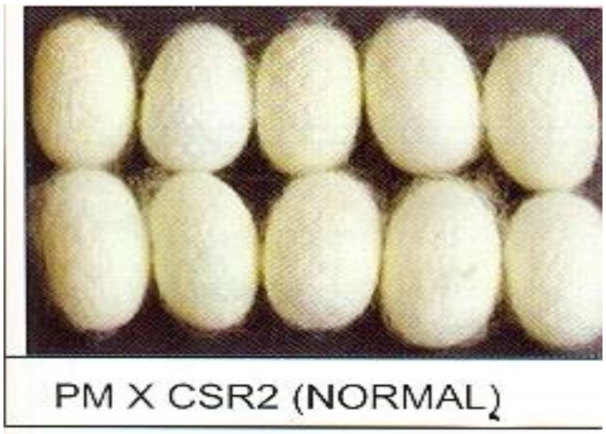
UTC=Untreated Control; ATC =Acetone Treated Control

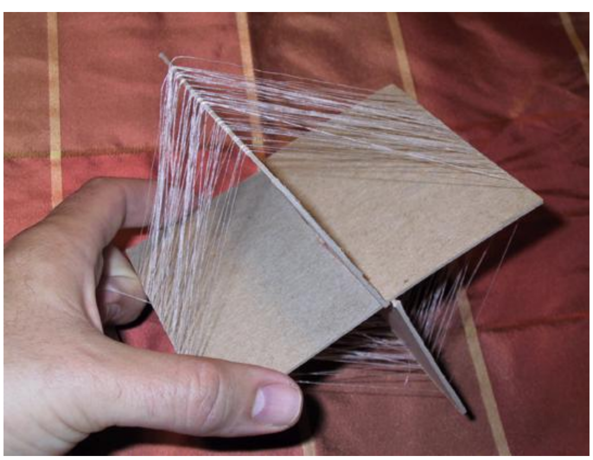
Fig.2 The Denier Scale of the silk filament from the cocoons spinned by mature fifth instar larvae of silkworm, *Bombyx mori* (L) (Race:) received topical application of acetone solution of Ferruginol at 48 hours after the fourth moult



UTC=Untreated Control; ATC =Acetone Treated Control

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 was found influenced by the topical application of acetone solution of Ferruginol to the fifth instar larvae of silkworm, *Bombyx mori* (L). The range of percent increase in the cocoon weight and shell weight in the experimental (treated) groups was 13.983 to 62.896 and 26.224 to 93.948 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 twenty ppm acetone solution of Ferruginol at 48 hours after the fourth moult.





Silk filament is sole aim in sericulture. Length and weight of entire silk filament are the qualitative measurements to be accounted for it's Denier scale. The Denier scale of silk filament was found influenced through treating the larvae with Ferruginol solution. The denier scale of silk filament reeled from the cocoons from control group (both, untreated and acetone treated) was measured 2.170 units. The Ferruginol treatment was found influencing the denier scale of silk filament, measuring 2.404 (for 5 ppm Ferruginol treatment); 3.165 (for 10 ppm Ferruginol treatment) and 3.171 (for 20 ppm Ferruginol treatment) units. The Ferruginol through acetone was found thus, resulted into fortified silk filament, with reference to Denier scale. The silk reeled from the cocoons belong to the 20 ppm Ferruginol treatment.

Most of the terpene compounds used for topical application to the larval instars of silkworm are the Juvenoids (Vitthalrao Khyade and Dhanashri Gaikawad, 2016). Being member of terpene group, the Ferruginol may have Juvenoid activity in silkworm. The Ferruginol received by larvae through the acetone topically, may influence the appetite, nutrition and absorption of digested food. This may be responsible for accelerated 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. The juvenoid titre (endogenous and / or exogenous) in the body of larvae stimulate hypermetabolism (Slama, 1971). Use of Ferruginol through the acetone for topical application, thus chiefly reflected into the improvement of cocoon quality, shell ratio and silk filament quality (Vitthalrao Khyade, *et al.*, (2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015 and 2016). Ferruginol topically applied may be utilized by the silkworm larvae for the extra synthesis of silk. The Ferruginol is one of the most popular meroterpene supplement. Use of Ferruginol through acetone for rearing of silkworm larvae is much more easy method. Use of Ferruginol, a meroterpene may open a new avenue in sericulture for the qualitative cocoon and silk filament.

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