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Influence of meroterpene on economic characters of the shell ratio and denier scale in silkworm *Bombyx mori* (L.) (Race: PM x CSR_2)

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Abstract

Present study was undertaken to study the action of Meroterpene through the acetone solution on the economic characters shell ratio of cocoon and denier scale of silk filament in silk worm Bombyx mori (L.) through PM x CSR2 race. The meroterpene selected for the attempt was ferruginol. Three different concentrations of meroterpene - ferruginol compound used in the attempt include: 5ppm; 10ppm and 20 ppm. The larval stages of silkworm, Bombyx mori (L) utilized for the experimentation were the fifth instars. Five groups of larvae, each with hundred individuals were prepared for the study. The groups include: UT (Untreated Control group); STC (Solvent Treated Control group); T-1 (5ppm Meroterpene Treated Group); T-2 (10 ppm Meroterpene Treated Group) and T-3 (20 ppm Meroterpene Treated Group). Each group was consisted of hundred individuals. Separate micropipettes were used for topical application of ten microliteres of each concentration for respective groups. The meroterpene topical application was made to the individual larva of concerned group at 48 hours after the fourth moult. Standard schedule of rearing of the larval instars; provision of mountage to the mature larvae to spin their cocoons; harvesting the cocoons; reeling the cocoons for silk filament were followed. The shell ratio of the cocoons and the denier scale of silk filament from control group was measured 19.331 and 2.170 units respectively. Use of meroterpene ferruginol through acetone solvent for topical application to the larvae was found reflected into: significant improvement in the shell ratio of cocoons (from 21.407 to 22.948) and the silk filament denier scale (2.404 to 3.171). Meroterpene ferruginol at the rate at 5 ppm, 10 ppm and 20 ppm concentrations yielded the heaviest cocoon (with weight: 2.046;2.387;2.924 gm) ; significant weight of cocoon - shell (0.438, 0.541, 0.673 gm), weight of pupa (1.613;1.846;2.252 gm). All three concentrations of meroterpene compound in the study recorded significant cocoon weight; Shell weight; pupal weight; shell ratio and denier scale in comparison with the control group. Specific titer of meroterpene ferruginol through solvent like acetone may lead to welcome new agro-biotechnological era for sericulture.

Keywords: Silkworm, *Bombyx mori*, Meroterpenes, yield of Silk, Cocoon.

Introduction

The metamorphosis in insects is said to be meticulous. The concentration of hormones like moulting hormone (MH) and juvenile hormone (JH) serves to orchestrate it's progression. Being a insect, the life cycle of silkworm include the stages like egg; larval instars; pupa within the silky cocoon and the adult moth. The moulting hormone (MH) and juvenile hormone (JH) are the two significant hormones. They are concerned with control majority of the insect growth and their developmental. The Juvenile Hormone (JH) deserve uniqueness with reference to pleiotropic nature, biochemical structure and action. This juvenile Hormone (JH) has been considered to be exclusive insect hormone and thus has attracted much more attention of researchers. This hormone is a pleiotropic master hormone in the body of insects. It governs most aspects of integrations with the ecosystem and it affects decisive parameters of life cycle of the insects[1]. The Juvenile Hormone (JH) is concerned who regulation of diverse traits in the insects. Some of the traits governed through the action of juvenile hormone (JH) include: synthesis of yolk protein; uptake of the molecule into the developing egg; diapauses in insects; insect flight; insect development, features of insect reproduction; insect dispersal and insect polymorphisms[2-7]. The juvenile hormone is known to alter physiological processes essential for insect development. Therefore, juvenile hormone known to occur in the body of each and every insect including silkworm, *Bombyx mori*(L)[8,9].

The chemical compounds that mimic the action of natural insect juvenile hormone (JH) are recognized as the Juvenile Hormone Analogues (JHAs) or juvenoids. According to Williams, Juvenile Hormone Analogues (JHAs) or the juvenoids terpepinoid compounds and specific for arthropods[10-12]. The Juvenile Hormone Analogues (JHAs) or the juvenoids are known to regulate development, reproduction, diapause, andpolyphenisms[13]. The most of the terpene and terpenoid are mimicking the action of natural insect juvenile hormone. The special features of terpenes lies in their large and diverse class of organic nature[14]. Large number of plants are known for synthesis of terpene compounds for no reason. Some of the

insects are also known for their ability of synthesis of the terpenes. The insects emit the terpenes through their osmeteria. The larvae of swallow butterflies of family: Papilionidae are recognized for their *osmetaria.* These *osmetaria* are the defensive organs for the Papilionid larvae, in all stages in their life cycle[15]. The osmeteria are situated in the segments of prothoracic region. In threatened condition, the papilionid larvae use to avert their osmeteria. In this averted condition, osmeteria looks like 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 odor which serves to repel ants; small spiders and mantids[16]. Chemical composition of secretion from osmeteria varies from species to species. The osmeterial sectretion contains the monoterpene hydrocarbons, sesquiterpenic compounds or a mixture of aliphatic acids and esters. In 1969, Crossley, A.C. and Waterhouse D.F. tried their best to study structure of the osmetrium in swallowtail butterfly *Papilio demoleus libanius* Fruhstorfer[17]. In this study, the observed three types of cells that are specialized for the purpose to synthesize the acid secretion. Lu, Chow-Chin; Yien Shing Chow confirmed the storage of the osmeterial secretion[18-22]. The meroterpene compound deserve terpenoid part as well phenol part in it's chemical structure. This may be possible reason for "Terpeno-phenolics" recognition for the meroterpenes. Natural terpenes and natural phenols seems to the essential pillars of the meroterpenes. *Humulus* is the genus of plants well known for synthesis of metabolites of terpeno-phenolics. So also is the case of plant genus *Cannbis*[23]. The meroterpenes like Bakuchiol; Ferruginol; Mutisianthol and Totarol are some of the examples of "Terpeno-phenolics". These terphenolics can also be isolated from animals. Nunez-Pons, *et al* reported the meroterpene compounds in the *Aplidium*, a genus of colonial sea squirts, tunicates in the family Polyclinidae. The mereterpenes of this species include: methoxyconidiol, epiconicol, and didehydroconicol[24-29].

The ferruginol is a chemical compound with phenol and terpenoid parts in it's structure. The needles of redwood *Sequoia sempervirens* (L) (family: Cupressaceae) is the best source of ferruginol. The terpenoid part in the structure of ferruginol is a diterpene, which belong to the abietane chemical class. The ferruginol isolated from the needles of redwood *Sequoia sempervirens* (L) (family: Cupressaceae) is exhibited anti-tumor activities reported antibacterial activity in the ferruginol. Gastroprotective effects of ferruginol have also been reported by Areche Carlos[30-38]. The totarol seems to modified form of *ferruginol. This* Totarol exhibited antimicrobial properties and therefore, it motivated research in drug discovery. The Totarol exhibited 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 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 attempt was carried out through the parts like: Preparation of Meroterpene Ferruginol solution through the use of Acetone solvent; Rearing of silkworm larval instars of silkworm, *Bombyx mori* (L); Treating larval instars with Acetone solution of Ferruginolto; Analysis of cocoon characters and silk filament characters and analysis of the data through statistical methods.

Preparation of Meroterpene Ferruginol solution through the use of Acetone solvent: The meroterpene Ferruginol in the form of powder was procured through the local supplier. For the preparation of solution, acetone was selected as solvent. Instructions narrated by Vitthalrao Khyade and A.C. Bhunje, were followed and the acetone solution of Meroterpene Ferruginol was prepared. The known quantity of powder of meroterpene ferruginol was dissolved in known volume of acetone solvent. Strengths of meroterpene ferruginol solution in acetone used in the present study include: 5 mg/Lit. ; 10 mg/Lit and 20 mg/Lit[39].

Rearing of silkworm larval instars of silkworm, *Bombyx mori* (L): The rearing of larval instars of silkworm larvae was carried out through standard methods of Krishnaswami *et al* [40-50], explained by V. B. Khyade[34] and Vitthalrao B. Khyade *et al* [51-73]. The egg cards or disease free layings (DFLs) were brought through the "Dr. APIS" Laboratory at Malegaon colony (Baramati Dist. Pune India). The egg cards were belonging to the race: multivoltine crossbreed PM x CSR_2. The egg cards were used for processing through the standard methods of rearing of the larval instars of silkworm, *Bombyx mori*(L). Various steps followed in the attempts include: black boxing (for incubation) rearing of instars of early age (I; II and III instars), rearing of instars of late age (IV and V instars). The mature larvae were considered for provision of mountage for spinning the cocoon. The cocoon harvesting was carried out on fifth day after the provision of muontage. The standard methods prescribed by Krishnaswami *et al*[40] and narrated by Sharad G. Jagtap[41].

Treating larval instars with Acetone solution of Ferruginol to the fifth instar larvae: The fifth stage larval instars were considered for utilization in the present attempt of studies. Soon after the fourth moult, the age for fifth stage instar larvae was considered as zero hour and the they were grouped into five different groups. Each group in the attempt was consisted of hundred larvae. Two control groups (untreated and solvent treated) were made. The treated or experimental groups in the study were include: 5 ppm Meroterpene-Ferruginol Treated

group; 10 ppm Meroterpene-Ferruginol Treated group and 20 ppm Meroterpene – Ferruginol Treated group. The volume used for topical application to individual larva was ten microlitres. The time of topical application of acetone solution of meroterpene – ferruginol was 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[34,42-73].

Analysis of cocoon characters and silk filament characters: The harvesting of cocoons from the mountage was carried out on fifth day after the provision of mountage for spinning. From each group of the study, twenty cocoons were used were for random selection. Each cocoon was subjected for deflossing. Weight of such deflossed entire cocoon was recorded though the use of electronic balance. Then, the cocoon was processed for vertical cutting. Common razor blade was used for vertical cutting of the cocoon. The weight of shell of cocoon and the pupa was recorded separately. The readings of weight of entire deflossed cocoon and cocoon shell were accounted for the calculation of shell ratio of the cocoon. The reading of cocoon shell was divided with reading of weight of entire deflossed cocoon. The quotient thus obtained was used for the calculation of shell ratio through multiplication by hundred. Ten cocoons from each group of the study were used for reeling on eprouvate.

The length (in units of meter) of continuous silk filament was obtained by using eprouvate. The length of silk filament was designated as: (A). The silk filament from single cocoon from each group in the study was separated and used for knowing the weight on electronic balance. Weight in gram of silk filament was designated as: (B). The reading of length (A) of silk filament and the reading of weight (B) of silk filament were used for the calculation of denier scale. Standard mathematical formula was used to calculate the denier scale of individual silk filament obtained from each group in the study. 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[39].

Analysis of the data through statistical methods: All the attempts in the study were subjected for repetition. The purpose of repetition was to obtain the consistency in the results of the experimentation. The data from each attempt was collected. Mathematical statistical methods of analysis were followed for the calculation of the mean; standard deviation; percent change and tests of significancy.

The statistical method used in the study belongs to Norman and Bailey[73]. The student "t" – test was utilized for knowing the significant changes in the treated groups and comparison with the control group. Experimentation was repeated for thrice for the purpose of consistency in the results.

Results and discussion

The weight of the cocoon weight (gm); weight of the cocoon shell (gm); weight of Pupa weight (gm); ratio of the cocoon shell to the entire cocoon; length of Silk Filament (SFL in meters); Weight of the Silk Filament (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 acetone solution of meroterpene - Ferruginol of strength 5 mg/Lit. to individual fifth stage instar larva at 48 hours after the fourth moult was found reflected into increase in the weight of entire (13.983 percent); weight of Cocoon- Shell (26.224 percent); weight of Pupa (11.395 percent). The shell ratio of the cocoon harvested from the 5 ppm (5 mg/Lit.) 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 (mg/Lit.) acetone solution of Ferruginol to the fifth stage 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.

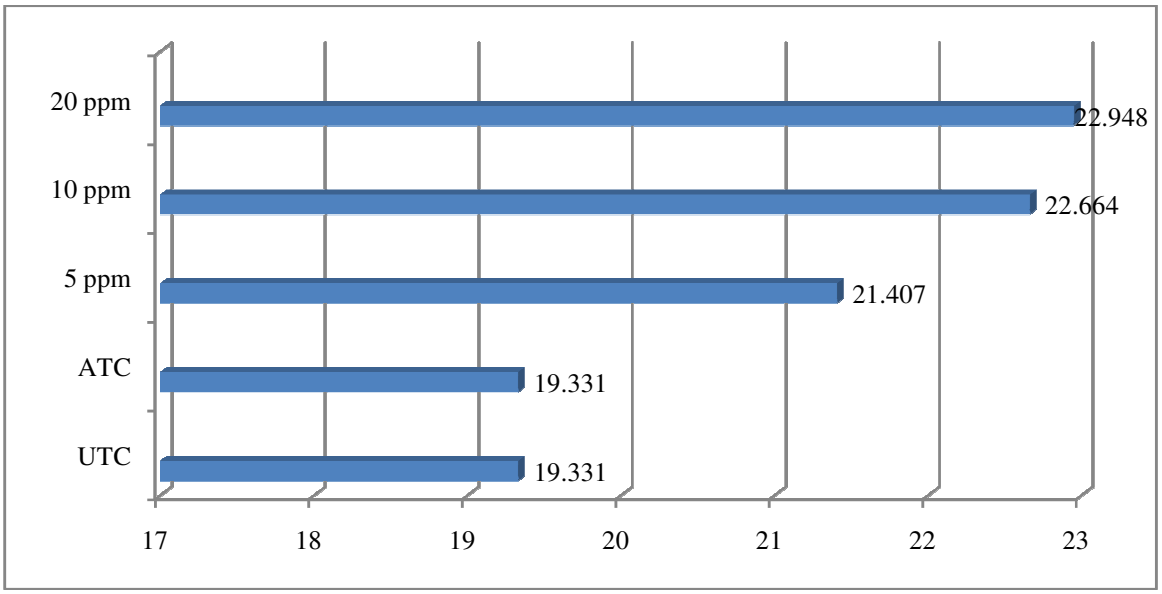
Topical application of 20 ppm acetone solution of Ferruginol to the fifth stage instar larvae at 48 hours after the fourth moult was found increasing ($P < 0.01$) in the entire cocoon weight (62.896 percent); Cocoon Shell Weight (93.948 percent); Pupal weight (55.524 percent). The shell ratio of the 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. The economic parameter in sericulture is the cocoon spinned by the mature fifth stage 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. Weight of the cocoon; weight of the cocoon - shell and thereby the shell ratio, all were found in effected by the treating the larvae through topical application of acetone solution of meroterpene - Ferruginol to the fifth stage instar larvae of silkworm, *Bombyx mori*(L). The range of percent increase in the weight of cocoon weight and weight of cocoon - shell in the experimental (treated) groups was 13.983 to 62.896 and 26.224 to 93.948 respectively. Shell ratio of the cocoons in the study was found improved in the corresponding groups of treatment. Most significant ($p<0.001$) shell ratio belonged to the cocoons belong

to the group of larvae treated with twenty ppm (mg/Lit) acetone solution of Ferruginol at 48 hours of age of the fifth instar larvae. 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.

Table-1: The economic parameters of the cocoons (and silk filament) 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.

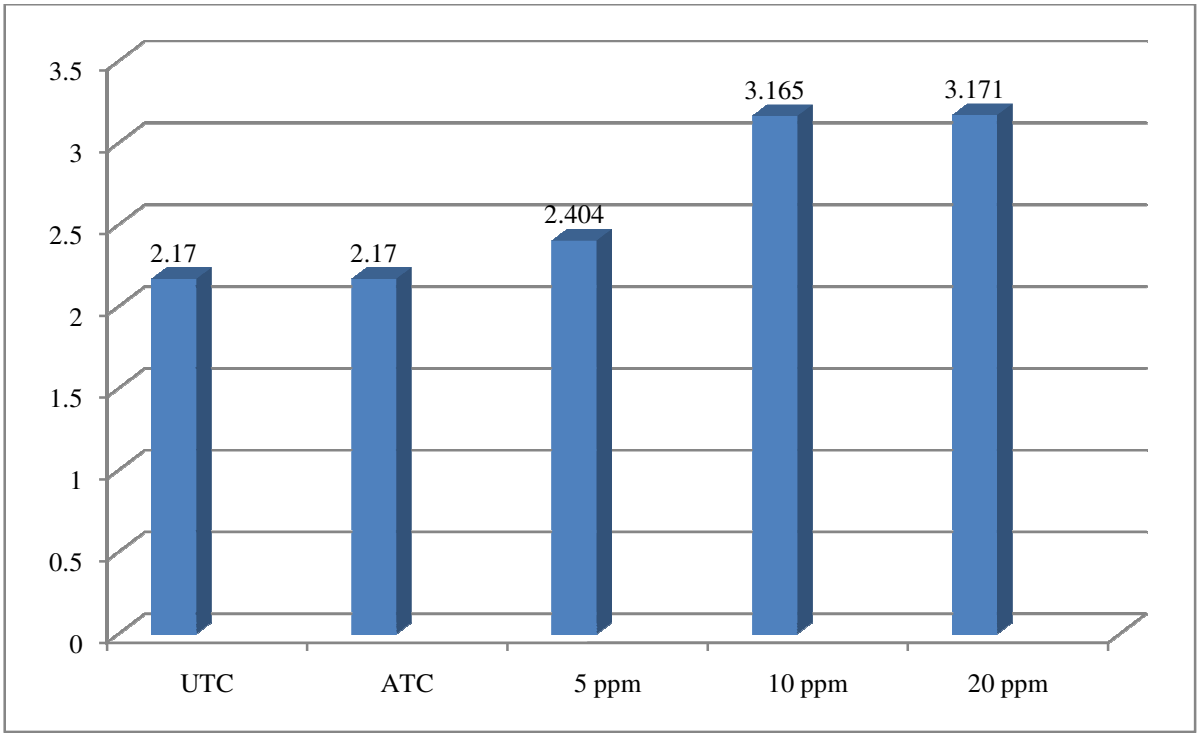
Parameters→ Group ↓	Cocoon Weight (gm)	Shell Weight (gm)	Pupal Weight (gm)	Shell Ratio	S F L (m) (A)	S F W (gm) (B)	Denier Scale of S F = B÷A x 9000
UTC	1.795(±0.084) 00.000	00.347(±0.009) 00.000	1.448 0.000	19.331 0.000	758.81 (±9.159) 00.000	0.183 (±0.035) 00.000	2.17 00.00
ATC	1.795(±0.089) 00.000	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
5ppm	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 **
10ppm	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 **
20ppm	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. TC=Untreated Control; ATC =Acetone Treated Control; SFL= Silk Filament Length; SFW= Silk Filament Weight, *: P < 0.05; **: P < 0.005; ***: P < 0.01.



UTC=Untreated Control; ATC =Acetone Treated Control

Figure-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

Figure-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.

Most of the terpene compounds used for treating the larval instars of silkworm are the Juvenoids[72]. 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[42-74]. Cocoon is the material used for reeling the commercial silk fibre. It is in fact, silky shell meant for the protection of the pupa and for provision of conditions essential for metamorphosis of pupa into the adult. The shell of the cocoon made by silk protein, single, continuous and long silk filament made by mature fifth stage instar larva of silkworm. This silky shell preparation prior to pupation and meant for self protection from adverse climatic situations and natural enemies.

The juvenoid titre (endogenous and / or exogenous) in the body of larvae stimulate hypermetabolism[75]. Use of Ferruginol through the acetone for topical application, thus chiefly reflected into the improvement of cocoon quality, shell ratio and silk filament quality. 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 be the opportunity for sericulture to enter into the biotechnological a new avenue in sericulture for the qualitative cocoon and silk filament.

Conclusion

Topical application of meroterpene ferruginol compound through acetone solvent to the fifth instar larvae of silkworm, *Bombyx mori* (L) of PM x CSR_2 race at 48 hours after the moult in the study recorded significant cocoon weight; Shell weight; pupal weight; shell ratio and denier scale of silk filament. Ferruginol topically applied may be utilized by the silkworm larvae for the extra synthesis of silk.

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