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Influence of Aqueous Solution of Eurhodin Treated Mulberry Leaves on the Quality of Cocoons and Silk Filament in Silkworm, *Bombyx mori* (L) Races: Bivoltine Crossbreed [(CSR_6 x CSR_{26}) x (CSR_2 x CSR_{27})] and multivoltine crossbreed [(PM x CSR_2)].

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Abstract

Hundred grams of fresh mulberry leaves were kept immersed in a liter of aqueous solution of eurhodin powder with hundred milligram per liter (100 ppm) strength for half an hour. Such treated mulberry leaves were drained off completely; fed daily to the fifth instar silkworm larvae of bivoltine Cross Breed [(CSR6 x CSR26) x CSR2 x CSR27)] and multivoltine crossbreed (PM x CSR_2) races for first four days after the fourth moult. For each day, four feedings were supplied at the rate 100 grams of mulberry leaves for the group of hundred larvae. Larvae fed with untreated and water treated mulberry leaves were also maintained. Mature larvae were considered for the provision of mountage for spinning the cocoon. The cocoons were harvested on fifth day after the provision of mountage. Treating the mulberry leaves with aqueous solution of eurhodin and feeding fifth instar larvae was found resulted into significant improvement in Tissue Somatic Index (TSI) from 23.855 to 28.499 in multivoltine crossbreed (PM x CSR_2) race and from 24.719 to 29.780 in bltivoltine crossbreed [(CSR6 x CSR26) x CSR2 x CSR27)] race. Cocoons spinned by the larvae fed with eurhodin treated mulberry leaves were found effected into fortification in their shell ratio from 19.741 to 24.107 in multivoltine crossbreed (PM x CSR_2) race and from 20.975 to 33.393 in bltivoltine crossbreed [(CSR6 x CSR26) x CSR2 x CSR27)] race. The fortification through eurhodin treatment was also reflected in the quality of silk filament, the denier scale. The denier scale was reported to significant improvement from 2.049 to 3.003 in multivoltine crossbreed (PM x CSR_2) race and from 3.237 to 4.743 in bltivoltine crossbreed [(CSR6 x CSR26) x CSR2 x CSR27)] race of silkworm, *Bombyx mori* (L). Through feeding a modified diet mulberry leaves treated with eurhodin, a vital dye, the present attempt is reporting production of red tinged silk filament. This kind of approach may help to establish eco-friendly technology for coloring the natural silk.
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Keywords: *Bombyx mori* (L); Eurhodin; Bivoltine; multivoltine; Crossbreed; Denier Scale

INTRODUCTION

Nutrition is having prime role in sericulture. Nutrition reflects on the quality and quantity of silk yield. The *Bombyx mori* species of Silkworm is monophagous insect. It use to derive almost all the needful nutrients from the mulberry leaves (Nasreen *et al*., 1999). The quality and quantity of nutrients in mulberry leaves vary according to the variety mulberry and the quality of soil used for cultivation (Ito, 1978). The ingestion of nutrient by the larvae is supposed to be proportional to the food available. Legay (1958) considering the nutrition as most significant field of studies in sericulture. The studies in silkworm nutrition is an essential prerequisite. This is because, silkworm nutrition as acting as a steering for the journey of sericulture for proper commercial exploitation of the silk (Vitthalrao B. Khyade, *et al*, 2015; Vitthalrao B. Khyade, *et al*, 2015). Nutrition of silkworm is solitory factor in sericulture. Nutrition almost individually concerned with augumenting the quality and quantity of silk produced by larvae of silkworm (Laskar and Datta, 2000). The attempts on this line in recent years include: suplimentation of feed with nutrients such as proteins, carbohydrates, **amino acid**s, vitamins hormones antibiotics etc. for better performance and to get high yield and quantity cocoons (Sannapa *et al*., 2002; Etebari *et al*., 2004). Various salts are reported for significant enhancement in the growth and development of silkworms. For example, nickel chloride has been reported for significant increase in the growth of silkworm larvae (Islam *et al*., 2004). In addition to mulberry leaves, feed supplements are also in practice. Most of the attempts are for availing silkworm for enhanced economic characteristics (Jeyapaul *et al*., 2003; Sheeba *et al*., 2006).

Natural silk obtained through reeling the cocoons of silkworm, *Bombyx mori* (L) deserve incredibility. The strength of natural silk covers the properties like physical appearance; chemical composition and mechanical tenacity. Lingyue *et al*. (2015) reported the production of resistant silk through feeding titanium dioxide nanoparticles treated mulberry leaves. Treating mulberry leaves with specific dyes with known concentration and feeding silkworm larvae has been reported for obtaining naturally colored silk Tansil, *et al*, 2011; Nisal, *et al*, 2014; Trivedy, *et al*, 2016; Anumol, *et al*, 2017). Color is one of the qualitative parameter for silk filament. Giving the color to silk after reeling the cocoons is exclusively mechanical. It is creating environmental troublesome situations. Artificial silk coloring is affecting the natural and original quality of water (San Diego, 2008). Toxic materials are released in the natural water bodies through the process of coloring the silk filament. Moreover, use of water in large quantity for the purpose to give color to silk is not affordable for existing environmental situations. Robinson, *et al* (2001) reviewed the literature on technology of coloring the silk and recommended some eco-friendly alternatives. Artificial coloring the silk, unique parameter is labeling the sericulture industry as most polluting industry. It require to establish environmentally protected silk coloring technology. The studies on this line should aim the reduction in the production of minimum wastes. This is possible through introducing technology for the production of natural colour silk in the silk gland itself in the silkworm larval body before release for spinning the cocoon.

Concept of production of natural colored silk is not new. It has been handled by many more attempts of researches. Environmentally protected technology for colored silk has been introduced by the authorities of National Chemical Laboratory (NCL) of Pune and Central Sericultural Research and Training Institute (CSRTI) of Mysore (Nisal, *et al*. , 2013). Trivedy, *et al* (2016) reported the production of cocoons with remarkable color change persisting even after degumming. Selection of dye suitable for the life of silkworm and sustainable for silk industry is crucial. On this line of studies, the vital dyes may fulfill the necessities to establish the environmentally protective method of obtaining natural colored silk from the larval instars of silkworm, *Bombyx mori* (L).

The dye named eurhodin is appearing in the literature reviewed as a natural and vital dye. It is also recognized by the common name as neutral red. The labels such as toluylene red and basic red seems to belong to chemical nomenclature. Winckler (1974) reported eurhodin as histological staining. Lysosomes are the organelles stained with this eurhodin stain. This eurhodin stain is used in laboratories of biochemistry as a general stain in histology. It may also be used as a counterstain in combination with other stains. It has also been reported to be used to stain embryonal tissues. It is used together with Janus Green B stain. In hematology, eurhodin is used as supravital stain. It can be also used for staining cell organelles like Golgi apparatus and Nissl granules. Eurhodin is well known for using in the MacConkey agar. The eurhodin, in MacConkey agar help to differentiate bacterial population for lactose fermentation. Repetto, *et al* (2008) reported eurhodin to be used in the study of viability of cells. Lysosomes are stained by eurhodin. That is to say the living cells use eurhodin to incorporate into their lysosomes. The cells that are loosing their life are loosing the ability of incorporation of eurhodin stain. Through such type of studies, one can analyze the pattern of loss of cell viability. Repetto, *et al* (2008), further reporting use of eurhodin stain in cell culture, especially, for plate titration of viral bodies. Use of eurhodin for addition in growth media for bacterial cultures and cell cull cultures is well recognized. Eurhodin is usually is available as a chloride salt. In chemistry laboratories, eurhodin is used as a pH indicator, changing from red to yellow between pH 6.8 and 8.0. Few reports on use of neutral red as food supplement are available (Natalia, *et al*, 2011; Anumol, *et al*, 2018). The attempt of Natalia, *et al* (2011) belong to Singapore Institute recognized as IMRE. This team established green technology to get rid of traditional dying process necessary to obtain colored silk. This attempt claims that, a simple addition of fluorescent dye as the supplements of diet for feeding the silkworms results into colored silk. Consumption of fluorescent dye treated mulberry leaves leads into change the color of silkworms. Soon after maturation, such silkworms spin colored cocoon. The color of silk reeled from such cocoons is matching exactly to the dye used for treating the mulberry leaves. Through the integration of dye material directly into the silk, isn't it environmentally friendly process for adding color to silk ?

The attempt of Anumol, *et al* (2018) is concerned with consumption and utilization of food material by silkworm, *Bombyx mori* (L). No reports on effect of use of dyes on the quality of cocoons. Therefore, the present study was undertaken to study the influence of aqueous solution of eurhodin treated mulberry leaves on the quality of cocoons and silk filament in silkworm, *Bombyx mori* (L).

MATERIAL AND METHODS

The study was carried out through the steps like: Preparation of Aqueous Solution of eurhodin; Rearing of silkworm larval stages [Race: (CSR6 x CSR26) x CSR2 x CSR27)] and multivoltine crossbreed (PM x CSR_2)]; Mulberry leaves Treatment and feeding ; Analysis of parameters of larval instars; cocoons; silk filament and Statistical analysis of the collected data.

(I). Preparation of Aqueous Solution of Eurhodin:

0.02 weight percent of eurhodin, the neutral red dye is reported for "No Harmful Effects on Silkworm" (Anumol, *et al* , 2018). For the present attempt, 0.01 weight percent of was selected. This eurhodin, the neutral red dye was procured from Nice Chemicals Pvt. Ltd (PB No: 2217, Manimala Road, Edappally, Kochi, Kerala, 682024, India) through local dealer. According to "Percent to ppm conversion table" (https://www.rapidtables.com), 0.01 weight percent is to hundred part per million or hundred milligram per liter. Accordingly, through the use of distilled water, eurhodin solution was prepared. This solution was prepared freshly before the use to treat the mulberry leaves.

(II). Rearing of silkworm larval stages:

The eggs of silkworm in the form of disease free layings were procured from District *Sericulture Office* in Wakad, *Pune* – 411003 through regional sericultural officer. The two races of silkworm, *Bombyx mori* (L) were selected for the present attempt. They include: Double Hybrid bivoltine [(CSR6 x CSR26) x CSR2 x CSR27)] and multivoltine crossbreed [(PM x CSR_2)]. Eggs of both the races were processed for black boxing; early instar rearing; late instar rearing; mountage provision for spinning the cocoon and harvesting the cocoons.

The standard method prescribed by Krishnaswami, *et al* , (1992) and explained by Khyade (2004) for rearing of silkworm larvae was followed. Fresh mulberry leaves of Victory – 1 variety from moriculture garden were utilized for feeding the larvae of silkworm, *Bombyx mori* (L) Races: [(CSR6 x CSR26) x CSR2 x CSR27)] and [(PM x CSR_2)]. Rearing was carried separately for each race.

(III). Mulberry leaves Treatment and feeding:

The last (the fifth) instar larvae are larger in size and easy to handle. Therefore, the last (the fifth) instar larvae were selected for utilization in carrying out the attempt on eurhodin treatment. The last (the fifth) instar start soon after the fourth moult. Therefore, soon after the fourth moult, the last (the fifth) instar larvae were considered for experimentation. For each race, three groups of fifth instar larvae were made. Each group was with hundred larvae. The three groups for each race include: Untreated Control Group; Water Treated Control Group and Eurhodin Treated Group. For the purpose of feeding the group of hundred larvae, hundred grams of fresh mulberry leaves were kept immersed in a liter of aqueous solution of eurhodin with hundred milligram per liter (100 ppm) strength for half an hour. After treatment, the mulberry leaves drained off completely. Such treated mulberry leaves were fed daily to the silkworm larvae of bivoltine Cross Breed [(CSR6 x CSR26) x CSR2 x CSR27)] and multivoltine crossbreed (PM x CSR_2) races of silkworm for first four days after the fourth moult. For each day, four feedings were supplied at the rate 100 grams of mulberry leaves for the group of hundred larvae. Larvae fed with untreated and water treated mulberry leaves were also maintained.The larvae of the group of Untreated Control were received untreated mulberry leaves. The larvae of the group of Water Treated Control were received water treated mulberry leaves. From fifth day onwards, the larvae of all the groups were fed with untreated mulberry leaves through standard methods. Rearing was carried out in the trays of wood. For each day, larvae received four feedings ((at the rate of hundred grams of mulberry leaves for the group of hundred larvae for each feeding). The mountage was made available for spinning the silky cocoon by the mature last larval stages (Khyade , 2004 and Vitthalrao B. Khyade, *et al* (2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017 and 2018).

(IV). Analysis of parameters of larval instars; cocoons; silk filament:

Analysis of parameters of larval instars was carried out on fifth day (120 hours after the fourth moult. Ten larvae from each group were selected randomly. Individual larva was weighed on electronic balance. Individual larva, the anaesthetized with cotton pad soaked in chloroform. It was followed by dissection of larva in insect saline. The two silk glands from the body of individual larva were separated, blotted and used for weighing. The weight of silk glands and the weight of larvae were accounted for calculation of tissue somatic index of silk glands.

The cocoons of each group were separated from the mountage. Separation of cocoons from mountages is called as harvesting. This harvesting of cocoons was carried out on fifth day after the provision of mountage for spinning. Twenty cocoons from each group were selected randomly. They were deflossed. The weight of individual deflossed cocoon was recorded. Each cocoon in the was cut vertically using the blade and weight of pupa was recorded. For knowing the shell weight of individual cocoon, the reading of the weight of pupa was subtracted from weight of respective cocoon. Weight of entire deflossed cocoon; weight of shell of cocoon and weight of pupa were noted. The silk shell percentage (correctly called as shell ratio) was calculated through the use of readings of weight of whole deflossed cocoon and weight of silk shell in cocoon. The mathematical operation of dividing the readings of silk shell weight by readings of weight of whole cocoon without floss was followed. Multiplication operation was carried with hundred with quotient obtained earlier. This yields the shell percentage. In sericulture, this silk shell percentage is called as shell ratio.

Ten cocoons per replication were used for the purpose to reel the silk filament from individual cocoon. The 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 B. Khyade and Abhilasha C. Bhunje, 20015 and 2016).

(V). Statistical Analysis of the data:

The experimentation was replicated for three times. This is for the purpose to get the consistent results. The data was collected and it was subjected for statistical analysis. The statistical parameters considered in the attempt include: mean, standard deviation, percent variation and student "t" – test. Standard statistical methods of analysis prescribed by Norman and Bailey (1955) and explained by Vitthalrao B. Khyade and Manfred Eigen (2018) were followed.

RESULTS AND DISCUSSION

The results on the attempt on the study entitled "Influence of Aqueous Solution of Eurhodin Treated Mulberry Leaves on the Quality of cocoons and silk filament in silkworm, *Bombyx mori* (L) Races: Bivoltine Cross Breed [(CSR_6 x CSR_{26}) x CSR_2 x CSR_{27})] and multivoltine crossbreed [(PM x CSR_2)]" are summarized in table-1, 2, 3 and presented in Fig. 1, 2, 3.

The silk gland tissue somatic index is abbreviated as TSI. It is the calculation of the silk gland mass as a proportion of the total body mass in larval stages of silkworms. It is represented by the formula: TSI = [(Silk Glands Weight) ÷ (Total body weight of Larva)] × 100. This tissue somatic index is a tool for measuring the maturity of larval instars of silkworm in correlation to silk gland development. This somatic index is frequently used as reporting transition time in the metamorphosis in insects like silkworm, *Bombyx mori* (L) (Vitthalrao Bhimasha Khyade and Shinya Yamanaka, 2018).

The weight (gm) of body of fifth instar larva on fifth day after the fourth moult; silk gland weight (gm) and it's Tissue Somatic Index (TSI) in water treated control group of multivoltine Crossbreed [(PM x CSR_2] race was 3.123 (±0.456); 0.745 (±0.021) and 23.855 respectively. The weight (gm) of body of fifth instar larva on fifth day after the fourth moult; silk gland weight (gm) and it's Tissue Somatic Index (TSI) in Eurhodin treated group of multivoltine Crossbreed [(PM x CSR_2] race was 3.786 (±0.457); 1.079 (±0.348) and 28.499 respectively.

The weight (gm) of body of fifth instar larva on fifth day after the fourth moult; silk gland weight (gm) and it's Tissue Somatic Index (TSI) in water treated control group of bivoltine Crossbreed (Double Hybrid) – [(CSR6 x CSR26) x CSR2 x CSR27)] race was 3.564 (±0.523); 0.881(±0.024) and 24.719 respectively. The weight (gm) of body of fifth instar larva on fifth day after the fourth moult; silk gland weight (gm) and it's Tissue Somatic Index (TSI) in Eurhodin treated group of bivoltine Crossbreed (Double Hybrid) – [(CSR6 x CSR26) x CSR2 x CSR27)] was 4.053 (±0.614); 1.207 (±0.394) and 29.780 respectively.

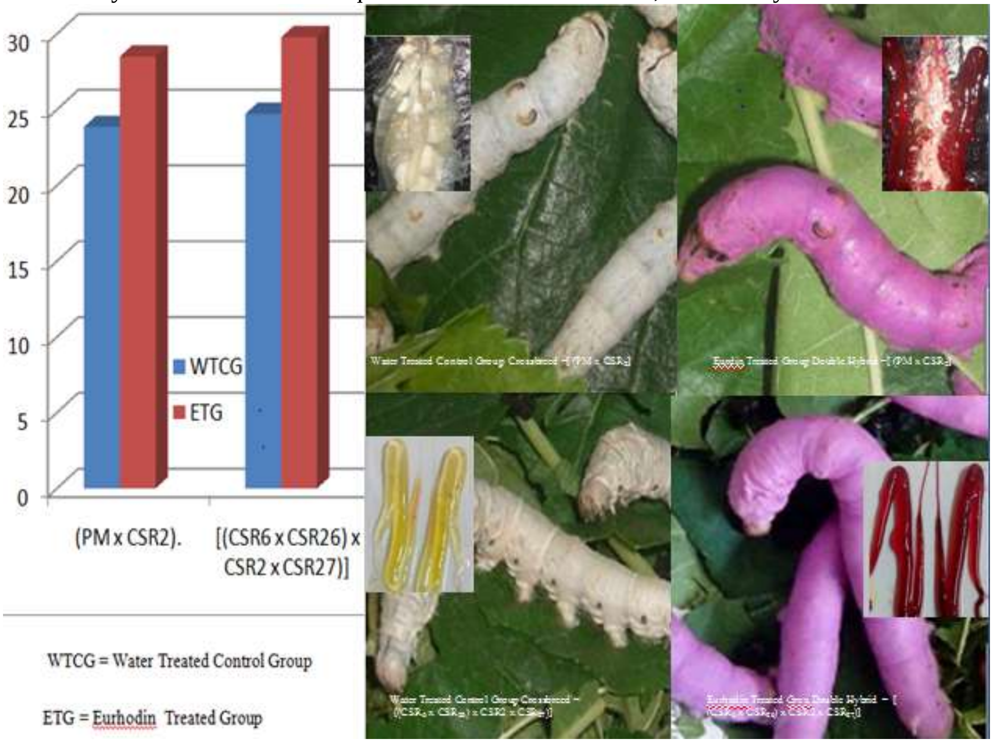
The silk gland tissue somatic index of larvae of both the races in the attempt was found significantly improved through feeding the leaves of mulberry treated with aqueous solution of eurhodin with hundred milligram per liter (100 ppm) strength.

Table-1: The Tissue Somatic Index of Silk glands of fifth instar larvae of silkworm, *Bombyx mori* (L) [Races: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27) and Crossbreed – (PM x CSR_2] separately received mulberry leaves treated with aqueous Solution of eurhodin, the vital dye.

Group and Parameter → Race ↓	**WTCG Larval Body Weight (A)**	**WTCG Silk Gland Weight (B)**	**WTC Tissue Somatic Index [(B÷A) x100]**	**ETG Larval Body Weight (A)**	**ETG Silk Gland Weight (B)**	**ETG Tissue Somatic Index [(B÷A) x100]**
Crossbreed – [(PM x CSR_2]	**3.123 (±0.456) 00.000**	**0.745 (±0.021) 00.000**	**23.855 00.000**	**3.786*** (±0.457) 21.229**	**1.079*** (±0.348) 44.832**	**28.499 3.619**
Double Hybrid – [(CSR6 x CSR26) x CSR2 x CSR27)]	**3.564 (±0.523) 00.000**	**0.881 (±0.024) 00.000**	**24.719 00.000**	**4.053*** (±0.614) 13.720**	**1.207*** (±0.394) 37.003**	**29.780 2.061**

- 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.
WTCG = Water Treated Control Group; ETG = Eurhodin Treated Group; * : $P < 0.05$;** : $P < 0.005$; ***: $P < 0.01$

Fig-1: The Tissue Somatic Index of Silk glands of fifth instar larvae of silkworm, *Bombyx mori* (L) [Races: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27) and Crossbreed – (PM x CSR_2] separately received mulberry leaves treated with aqueous Solution of eurhodin, the vital dye.



The weight (gm) of whole cocoon (deflossed); shell weight of cocoon (gm) and it’s shell ratio in water treated control group of multivoltine Crossbreed [(PM x CSR_2] race was 1.854 (±0.276); 0.366 (±0.087) and 19.741 respectively. The weight (gm) of whole cocoon (deflossed); shell weight of cocoon (gm) and it's shell ratio in Eurhodin group of multivoltine Crossbreed [(PM x CSR_2] race was 2.269 (±0.786); 0.547 (±0.043) and 24.107 respectively. The weight (gm) of whole cocoon (deflossed); shell weight of cocoon (gm) and it's shell ratio in water treated control group of bivoltine Crossbreed (Double Hybrid) – [(CSR6 x CSR26) x CSR2 x CSR27)] was 1.969 (±0.387); 0.413 (±0.061) and 20.975 respectively. The weight (gm) of whole cocoon (deflossed); shell weight of cocoon (gm) and it's shell ratio in Eurhodin group of bivoltine Crossbreed (Double Hybrid) – [(CSR6 x CSR26) x CSR2 x CSR27)] was 2.758 (±0.553); 0.921(±0.058) and 33.393 respectively.The shell ratio of the cocoon spinned by larvae of both the races in the attempt was found significantly improved through feeding the leaves of mulberry treated with aqueous solution of eurhodin with hundred milligram per liter (100 ppm) strength.

Table-2: The Parameters of Cocoon Spinned by Mature fifth instar larvae of silkworm, *Bombyx mori* (L) [Races: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27) and Crossbreed – (PM x CSR_2] separately received mulberry leaves treated with aqueous Solution of eurhodin, the vital dye.

Group and Parameter → Race ↓	**WTCG Whole Cocoon Weight (gm)(A)**	**WTCG Shell Weight (gm)(B)**	**WTC Shell Ratio [(B÷A) x100]**	**ETG Whole Cocoon Weight (gm)(A)**	**ETG Shell Weight (gm)(B)**	**ETG Shell Ratio [(B÷A) x100]**
Crossbreed – [(PM x CSR_2]	**1.854 (±0.276) 00.000**	**0.366 (±0.087) 00.000**	**19.741 00.000**	**2.269** (±0.786) 22.384**	**0.547*** (±0.043) 49.453**	**24.107 4.366**
Double Hybrid – [(CSR6 x CSR26) x CSR2 x CSR27)]	**1.969 (±0.387) 00.000**	**0.413 (±0.061) 00.000**	**20.975 00.000**	**2.758*** (±0.553) 40.071**	**0.921*** (±0.058) 123.002**	**33.393 12.418**

- 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.
WTCG = Water Treated Control Group; ETG = Eurhodin Treated Group; * : P < 0.05;** : P < 0.005; ***: P < 0.01

Fig-2: The Shell Ratio of Cocoons Spinned by Mature fifth instar larvae of silkworm, *Bombyx mori* (L) [Races: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27) and Crossbreed – (PM x CSR_2] separately received mulberry leaves treated with aqueous Solution of eurhodin, the vital dye.

The length (meter); weight (mg) and denier scale of silk filament reeled from cocoons of water treated control group of multivoltine Crossbreed [(PM x CSR_2] race was 786 (±23.011); 0.179 (±0.021) and

2.049 respectively. The length (meter); weight (mg) and denier scale of silk filament reeled from cocoons of Eurhodin treated group of multivoltine Crossbreed [(PM x CSR_2] race was 889.88 (±27.053); 0.297 (±0.036) and 3.093 respectively.

The length (meter); weight (mg) and denier scale of silk filament reeled from cocoons of water treated control group of bivoltine Crossbreed (Double Hybrid) – [(CSR6 x CSR26) x CSR2 x CSR27)] race was 1142.49 (±98.389); 0.411 (±0.088) and 3.237respectively. The length (meter); weight (mg) and denier scale of silk filament reeled from cocoons of Eurhodin treated group of bivoltine Crossbreed (Double Hybrid) – [(CSR6 x CSR26) x CSR2 x CSR27)] race was 1292.01 (±113.26); 0.681 (±0.104) and 4.743 respectively. The denier scale of silk filament reeled from the cocoons spinned by larvae of both the races in the attempt was found significantly improved through feeding the leaves of mulberry treated with aqueous solution of eurhodin with hundred milligram per liter (100 ppm) strength.

Fig-3: The Denier Scale of Silk Filament Reeled from the Cocoon Spinned by Mature fifth instar larvae of silkworm, *Bombyx mori* (L) [Races: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27) and Crossbreed – (PM x CSR_2] separately received mulberry leaves treated with aqueous Solution of eurhodin, the vital dye.

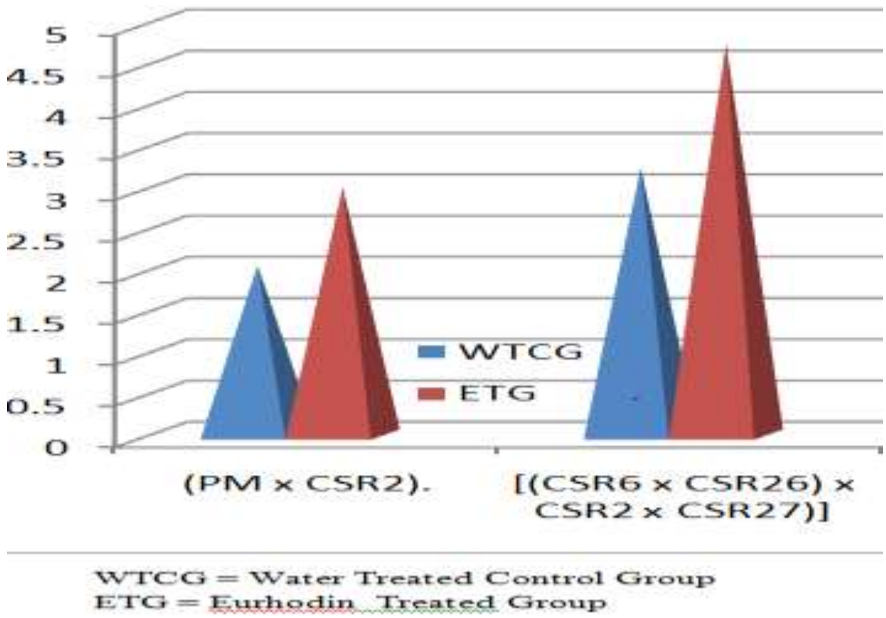


Table-3: The Parameters of Silk Filament Reeled from the Cocoon Spinned by Mature fifth instar larvae of silkworm, *Bombyx mori* (L) [Races: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27) and Crossbreed – (PM x CSR_2] separately received mulberry leaves treated with aqueous Solution of eurhodin, the vital dye.

Group and Parameter → Race ↓	**WTCG Silk Filament Length (meter) (A)**	**WTCG Silk Filament Weight (gm) (B)**	**WTC Denier Scale of Silk Filament [(B÷A) x 9000]**	**ETG Silk Filament Length (meter)(A)**	**ETG Silk Filament Weight (mg)(B)**	**ETG Denier Scale of Silk Filament [(B÷A) x 9000]**
Crossbreed – [(PM x CSR_2]	**786 (±23.011) 00.000**	**0.179 (±0.021) 00.000**	**2.049 00.000**	**889.88** (±27.053) 13.216**	**0.297*** (±0.036) 65.921**	**3.003 0.954**
Double Hybrid – [(CSR6 x CSR26) x CSR2 x CSR27)]	**1142.49 (±98.389) 00.000**	**0.411 (±0.088) 00.000**	**3.237 00.000**	**1292.01*** (±113.26) 13.087**	**0.681*** (±0.104) 65.693**	**4.743 1.506**

- 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.
WTCG = Water Treated Control Group; ETG = Eurhodin Treated Group; * : $P < 0.05$;** : $P < 0.005$; ***: $P < 0.01$

CONCLUSION

Treating the mulberry leaves with aqueous solution of eurhodin and feeding fifth instar larvae was found resulted into significant improvement in Tissue Somatic Index (TSI) from 23.855 to 28.499 in multivoltine crossbreed (PM x CSR_2) race and from 24.719 to 29.780 in bltivoltine crossbreed [(CSR6 x CSR26) x CSR2 x CSR27)] race. Cocoons spinned by the larvae fed with eurhodin treated mulberry leaves were found effected into fortification in their shell ratio from 19.741 to 24.107 in multivoltine crossbreed (PM x CSR_2) race and from 20.975 to 33.393 in bltivoltine crossbreed [(CSR6 x CSR26) x CSR2 x CSR27)] race. The fortification through eurhodin treatment was also reflected in the quality of silk filament, the denier scale. The denier scale was reported to significant improvement from 2.049 to 3.003 in multivoltine crossbreed (PM x CSR_2) race and from 3.237 to 4.743 in bltivoltine crossbreed [(CSR6 x CSR26) x CSR2 x CSR27)] race of silkworm, *Bombyx mori* (L). Through feeding a modified diet mulberry leaves treated with eurhodin, a vital dye, the present attempt is reporting production of red tinged silk filament. This kind of approach may help to establish eco-friendly technology for coloring the natural silk. The change in the body color of silkworm larvae; the cocoon and silk filament of both the races noticed in the present attempt confirms that, the eurhodin, the neutral red dye is efficiently carried across the gut wall into haemolymph and then into the silk glands.

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12 December is birthday of Hon. Sharad Govindrao Pawar (The Global Agro-academic Culture Raising Personality). Through the best compliments from Scientific Research Center, this attempt on Influence of Aqueous Solution of Eurhodin Treated Mulberry Leaves on the Quality of cocoons and silk filament in silkworm, *Bombyx mori* (L) Races: Bivoltine Cross Breed [(CSR_6 x CSR_{26}) x CSR_2 x CSR_{27})] and multivoltine crossbreed [(PM x CSR_2)] is wishing his kind self Happy Birthday.

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