A Green Approach to Produce Colored Cocoons

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Abstract
Silk is one of the most beautiful and luxurious textiles in the fashion industry. The conventional process of obtaining silk involves killing the silkworm before it gets ready to emerge from the cocoon. But the cocoon formed is naturally white in color, and its wide requirement is in various colors, so in order to obtain silk in various colors, artificial dyes are used on silk, which contain high concentration of toxins, thus resulting in water pollution. There is a high demand for a sustainable and environment friendly option to reduce this damage. Thus, Bombyx mori silkworm larvae are fed with a modified feed of mulberry leaves sprayed with synthetic dye solutions. While some dyes did produce an intrinsically colored silk cocoons and some did not, this process if implied will significantly reduce the need and effects of dyeing which are generally observed in the traditional methods. After carefully evaluating the physical properties of the synthetic dyes used, we have noticed that the balance of hydrophobic and hydrophilic character is extremely necessary for the diffusion of the dye from the alimentary canal of the silkworm larva into the hemolymph and later into the silk glands. These insights are extremely important to be considered in development of novel dye molecules that can be successfully fed to Bombyx mori silkworm larvae for producing colored silk of various colors and shades. Silkworm larvae in their fifth instar are fed with modified diet prepared by dipping mulberry leaves in synthetic dyes. Colomill red, colocid blue, colocid violet, colomill maroon, colocid green, colomill yellow are the synthetic dyes used. Besides these kesar yellow and lemon yellow are the two food colors used in this experiment. Colored cocoons are produced by three experimental groups which are feed with modified diet prepared using colomill red, colomill maroon and colomill yellow.

Keywords: Silkworm, Modified diet, synthetic dyes, colored cocoon, silk glands.

Introduction

Since 4000 years, silk has played an important role in the definition of social and economic life of humans. Occasions like marriages and festivals, bring about the usage of silk, which is considered as the "queen of fabrics".(Baby Joseph et.al 2012). Few of the properties of natural silk such as tensile strength, luster, comfort, texture, adaptation to all climatic conditions and ease of taking up dyes, make it an unequaled textile material. To add to the virtues of the silk fiber, it's believed that it is stronger than a steel fragment, of equal thickness.(Anjana Baburaj et al.2021)

History

Silk produced from silkworm, Bombyx mori has an interesting history. Silk was first discovered by Xilingji, wife of China's third emperor, Huangdi in 2640 B.C. When the empress was having tea in her garden, a silkworm cocoon dropped into her cup of tea and she noticed that the silk fiber got unwound and loosened. Xilingji discovered the means of rearing silkworm and established the silk industry in China which was kept secret for more than 2000 years. The famous silk route helped in spreading this exotic fabric throughout the world. Japan, Korea, Italy, Soviet Union, Brazil and India are chief silk producing countries in the world. India is the 5th largest producer of silk in the world. Sericulture is an employment-oriented industry. Natural silk is in high demand world-wide. Sericulture is believed to be the best sideline activity for those in the agriculture profession especially during the lean period. (researchgate.net)

India produces four types of silk: Mulberry silk, Tasar silk, Muga silk and Eri silk.
1. Mulberry silk: It is produced in the states of Karnataka, west Bengal, Jammu and Kashmir. The silkworms are fed with mulberry leaves.
2.Tasar silk: It is produced by tribes of Madhya Pradesh, Bihar and Orissa. The silkworms are fed on Terminalia tomentosa.
3. Muga silk: It is produced in Assam. The silkworms are fed on salu.
4. Eri silk: The silkworms are fed on castor (Ricinus communis).

Sericulture and its Components

The insects that produce silk are referred to as serigenous insects. Sericulture is the commercial rearing of silk producing insects. It is an agro based industry having three main components.

1. Cultivation of food plants

Silkworms are divided into two categories;
1. Mulberry silkworms - Silkworms which feed on mulberry leaves.
2. Non-mulberry silkworms - silkworms that feed on plants other than mulberry.

Rearing of silkworms involves cultivation of their host plants so that a regular supply of leaves is maintained. DFL’s (Disease Free Larvae) procured from a grainage supplies the worms for rearing.

2. Rearing of silkworms

The larvae of the silk moth are reared in rearing room where temperature and humidity are maintained at an appropriate level suitable for the growth and development of the silkworms. Special trays are used in which the worms are reared. Silkworms are fed on mulberry leaves and continue to grow until they encase themselves inside the cocoons for protection. Cocoons are formed with silk fibers, secreted by the mature larvae by spinning.

3. Reeling and spinning of silkworms

Reeling is a process in which silk yarn is removed from the cocoons. The cocoons are boiled in water to remove the gum and then the filaments are unwound. About 8-10 cocoons are reeled together. Reeling is done by three methods;
1. Charkha
2. Cottage basin
3. Automatic machines

Before weaving, raw silk is boiled in water in order to remove the remaining gum. It is dyed and leached to be woven into garments on handloom.

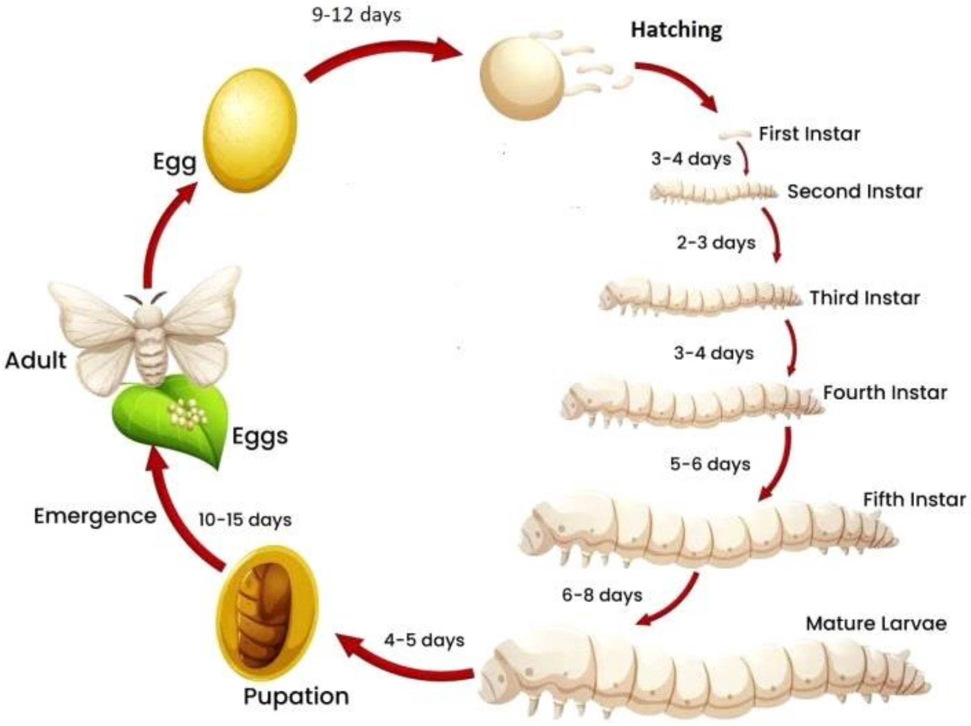
Mulberry Cultivation

Cultivation of mulberry plants is called moriculture. Out of 20 species of mulberry, the common varieties are Morus alba, Morus.indica, Morus.serrata, Morus.latifolia. Cultivation is done by planting cuttings of 23cms long with 3-4 buds and pencil thick from mature stem. In order to induce growth and new shoots to sprout pruning is done on regular basis. The larvae are fed with leaves which are harvested in three ways: Leave picking, branch cutting and top shoot harvesting. 3rd instar larvae are fed with leaves with the entire branch. 4th and 5th instar larvae are fed with shoots that are clipped from the top. In a year about 20-25 thousand kg of leaves are harvested per hectare. About 350-400 kg of leaves are required to rear 20 thousand eggs. The most extensively characterized silk is from the domesticated silk worm Bombyx mori and spiders. Silk worm is an eco-friendly and beneficial insect reared commercially for the production of silk. In order to understand how silk is produced by the silkworms it is important to observe the different stages in the life cycle of silkworm.

LIFE CYCLE OF SILK WORM

Bombyx mori - the domesticated silkworm is originally derived from Bombyx mandarina-moore.
The mulberry silkworm belongs to the following taxonomic classification:
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Bombycida
Genus: Bombyx
Species: mori
Complete metamorphosis is seen in silkworm Bombyx Mori. The four stages in its life cycle are egg, larva, pupa and moth.

Figure 1: Life cycle of a Silkworm (Source: takshilalearning.com)



SILK GLAND

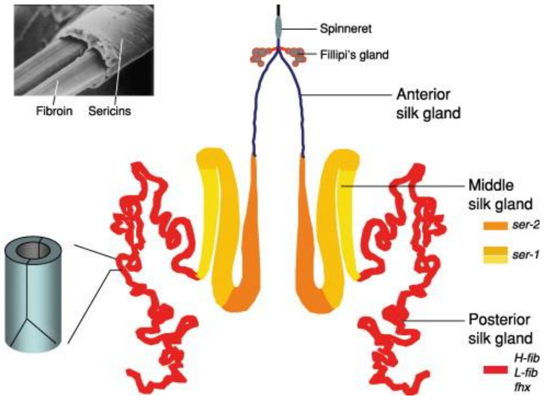
• Silk gland is a dermal gland derived from the invagination of labial ectoderm.

• The silk glands are tubular, cylindrical with branched nuclei. These glands are situated under the mid-intestine. (Textbook of sericulture, Madan Mohan Rao)

• The wall of the silk glands is composed of three layers: the outer tunica propria, the middle gland cells and the inner tunica intima enclosing the cavity of the gland. The tunica intima is renewed at each moult. (Baby Joseph et.al 2012)

• The silk glands can be divided into 1) Anterior 2) Posterior 3) middle region.(suzuki 1977)

Figure 2 : Silk Gland (Source: ScienceDirect.com)



The silk glands unite with each other at the anterior region in head and connect with spinneret oflabium. The anterior region of the silk glands does not secrete any material and is only a passage for flow of silk substance. The middle region of silk gland is the largest and folded in the shape of S.

The middle region can be further divided into anterior, middle, and posterior regions. The middle region acts as a reservoir of fibroin and fibroin matures in this region during storage period. Further this region secretes the sericin around the fibroin. The sericin is subdivided into Sericin-I, Sericin-II and Sericin-III. The posterior part of the silk gland mainly secretes the fibroin. A pair of Filipi's gland open into the silk gland at the junction where the right and left anterior parts of glands unit. It is assumed that this gland secretes some waxy material which covers the silk filament. Those genes that encode the silk proteins are actively expressed during the developmental stage, particularly at the fifth instar of larval development (Suzuki 1977; Prudhomme & Couble 1979).

The components of silk are sericin and fibroin. Sericin and fibroin are secreted by the gland cells in the central region and the posterior area, respectively. A pair of primary filaments (brin) known as silkworm fibers are spontaneously extruded from two silkworm glands. These primary filaments are then glued together to form a bave by sericin proteins.

The amino acids that make up silk are woven into pleated sheets. While side chains are above and below the H-bond network's plane, inter-chain H-bonds develop. The fibers are robust and resistant to stretching, and the large fraction (50%) of glycine allows for tight packing (Altman etal. 2003).

Three different types of proteins—high chain, low chain, and glycoprotein—are found in the silkfibroin released by the posterior silk gland of the B. mori (Shimura et al. 1982)

SILK AND SILK PROTEIN CHARACTERISTICS

Unlike many synthetic fibres, silk has a smooth, silky texture that is not slippery. The triangular cross-section and rounded corners of the B. mori silkworm's fibres give them their distinctive shape. They are 5–10 m broad. Due to a 59-mer amino acid repeat sequence with slight variants, the fibroin heavy chain is primarily made up of β -sheets.
Silk has a natural sheen because of the numerous angles at which the flat surfaces of the fibrils reflect light. A pair of primary filaments (brin) known as silkworm fibres are spontaneously extruded from two silkworm glands. These primary filaments are then glued together to form abave by sericin proteins. (Baby Joseph et.al 2012)
The amino acids that make up silk are woven into β- pleated sheets. While side chains are above and below the H-bond network's plane, inter-chain H-bonds develop. The fibres are robust and resistant to stretching, and the large fraction (50%) of glycine allows for tight packing (Altman et al. 2003).
The substantial hydrogen bonding is what gives silk fibres their stability, because there is a lot of hydrogen bonding, a lot of the protein is hydrophobic, and there is a lot of crystallinity, silk fibres are very stable. In most solvents, silk fibres are not soluble (Kaplan et al. 1994).
One of the strongest natural fibers is silk, but it loses up to 20% of its strength when it gets wet. If it is exposed to too much sunshine, it can weaken. Due to its weak electrical conductivity, it is prone to static cling. The majority of mineral acids do not dissolve silk, however sulfuric acid does. (Cook 1964; Lizuka 1996)

SERICIN

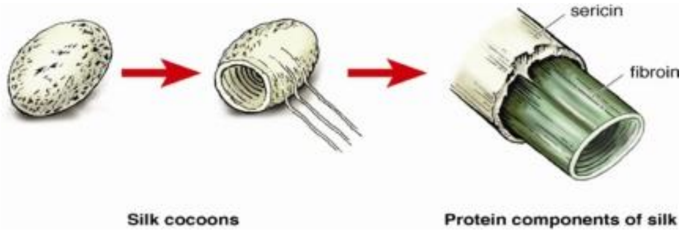
Sericin can be divided into three portions, A, B, and C, based on its solubility. Nitrogen and amino acids make up 17.2% of sericin A's composition. The top layer, it is insoluble in hot water. The middle layer, sericin B, is hydrolyzed by acid to produce tryptophan as well as sericin A's amino acids. 16.8% of the substance is nitrogen. The innermost layer, which is next to fibroin, is called sericin C. (Shaw & Smith 1951; Sprange 1975).
• Sericin's molecules become less soluble in water when they change from a random coil conFigureuration to a -sheet structure (Kataoka 1977).
• Due to its simple dissolution into water at 50–60°C and subsequent return to gel form upon cooling, it has the sol–gel property. (Zhu et al.1996)

FIBRONIN

• Two equimolar protein subunits of fibroin, a glycoprotein, are covalently joined together by disulfide bonds to form the entire molecule. Crystalline and amorphous domains are both present in fibroin filament. Alanine, glycine, and serine are present in significant concentrations in the crystalline domains, whereas amino acids are present in the
amorphous regions (Magoshi 1996; Lesile et al. 2003).
• The crystalline aspect of the silk fiber is due to the anti-parallel -sheet structure generating micro fibrils. The micro fibrils are arranged into fibril bundles, and a number of bundles eventually lead to a single silk thread. (Voegeli et al. 1993)

About 75% of the strand is silk, or fibroin, and 22.5% is sericin. The remaining components of the strand are made up of fat and wax (1.5%), ash of silk fibroin (0.5%), and mineral salts (0.5%).
Scouring is a procedure used to treat raw silk so that the sticky materials and other impurities can be removed. Following that, the silk fiber is gathered and delivered for use in the weaving process that creates silk fabric. (Meer Md Rasel khan et al.2015)

Figure 3. Silk fiber (source: ScienceDirect.com)



Naturally the cocoon that is formed is white in color, however it is observed other strains of silkworms that have produced a variety of other color cocoons like pink, yellow, brown or even green, these varieties are hard to domesticate, considering their rarity factor.

• To understand the coloration of cocoons we need to correlate the genes required for transport of certain pigments from the mulberry leaves into the silk cocoon, upon degumming the color is often lost.
• Carotenoids, carotenes, and xanthophylls, which are obtained from mulberry leaves and are exclusively present in sericin, which coats the fibroin base, are responsible for the hues of the cocoons. (Kanika Trivedy et.al 2016)
• Cocoon colors are due to differences in the dye permeability in different parts of the silkworm.
• Color appearance is an important attribute of silk. Synthetic dyes are used to produce a wide range of colorful garments. The textile industry demands a palette of vivid colors of silk fibers to later form attractive textiles. But the sustainability factor always comes up consciously, keeping that in mind, various methods to get colored silk in an environment friendly manner has been developed. One way is to use non-toxic natural dyes, which will help cut down the use of hazardous chemicals.
• The production of 60 billion kilograms of fabric yearly and the use of up to 9 trillion gallons of water make the textile sector one of the major global environmental problems.
• Large amounts of unfixed dye are released during coloration into water bodies, and 10- 15% of the dye is lost to the environment as waste water.
• Additionally, as a result of production pressures brought on by competition in the textile sector, more synthetic dye combinations are being used, which has increased the amount of dye waste water produced.
• Due to its excellent thermal photo stability and capacity to withstand biodegradation, dye can last for a long time in the environment.
• It is extremely harmful to all living organisms, including man and other animals, to release dye effluent into sea water and river water. (Aravin Prince Periyasamy et.al 2020)
• The textile effluent is extremely toxic due to chromium compounds, heavy metals like copper, arsenic, lead, cadmium, mercury, nickel, and cobalt, as well as several auxiliary chemicals.
• Other dangerous substances that may be found in the water include non-biodegradable dyeing agents, chlorinated stain removers, hydro carbon-based softeners, and formaldehyde-based color fixing agents.

These organic compounds interact with various disinfectants, particularly chlorine, and create undesired byproducts (DBP'S) that are frequently carcinogenic. These have allergic responses frequently.

• The presence of colloidal matter, in addition to the colors and oily scum, causes the turbidity to grow, making the water cloudy and smell terrible, and it hinders sunlight from penetrating, which is important for the process of photosynthesis.

• The oxygen transfer mechanism at the air-water interface is then hampered, which affects marine life and the water's ability to purify itself. If this effluent is allowed to flow through the fields, it plugs the soil pores and reduces soil production.

• It affects the quality of drinking water in hand pumps, making it unfit for human consumption, if permitted to run in rivers and drains. Prior to final disposal, it is crucial to eliminate these pollutants from the waste waters. (Rita Kant, 2012)

Figure 4: Commercial dyeing of Silk

ACID DYES

Acid dyes contain acid groups like -COOH, -SO3H which have affinity to slightly basic –NH group in the amide links of wool, silk and nylon. The functional group such as -SO3Na ,or SO3- are responsible for dye solubility in water they also form ionic bonds with protein fibre.

• Acid dyes are of different types. Major group of acid dyes are sulphonated azo dyes consisting of mono and bis-azo compounds with color range from yellow through red to violet and brown.

• Navy blue bis azo dyes accumulate to produce black color.

• The substantivity of azo dyes for protein and polyamide fibers is greater.

• High molecular weight dye has a smaller number of sulphonate groups per dye molecule. Anthraquinone acid dyes complement the azo dyes and ranging in colour from violet, blue to green. These dyes possess excellent light fastness.

• The acid dyes used in this experiment are colomill red, colocid blue, colomill maroon, colocid green, colocid violet, colomill yellow.

• Acid dyes are frequently categorized based on their dyeing behavior, particularly in relation to the dying pH, their capacity for migration during dyeing, and their washing fastness.

• The level of sulphonation and molecular weight of the dye molecule determine its dyeing ability. (Md Koushicuddin et al.2010)

• Although, in acid dyes the functional group like SO3Na or SO3 are responsible for dye solubility in water (Solubilizing group) and also create ionic bond with protein fiber (e.g.: Silk).

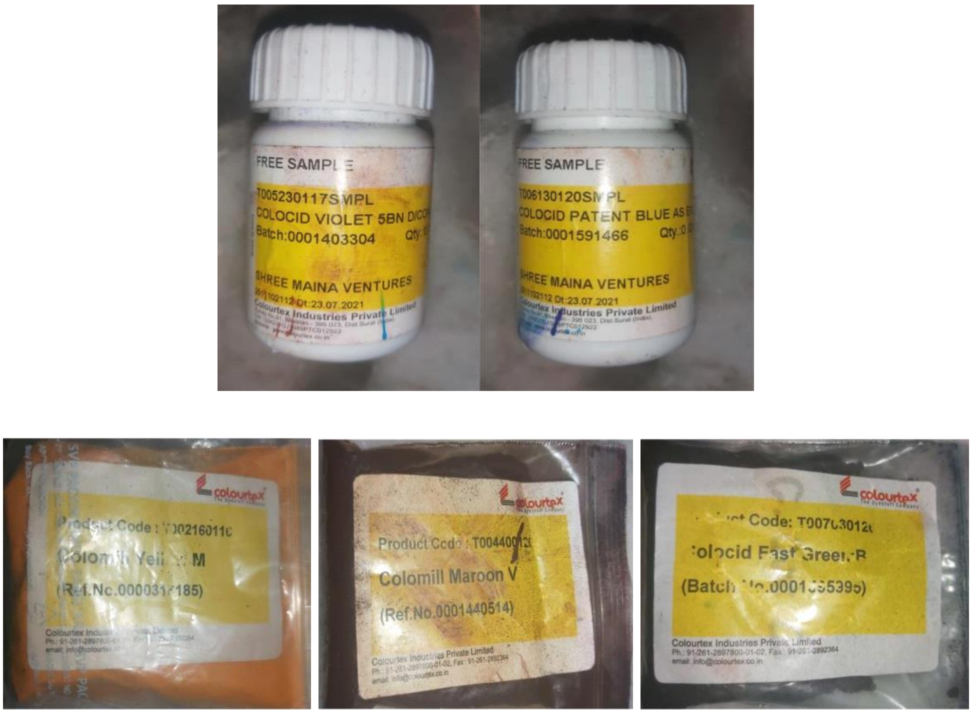
• The reaction between silk fiber and acid dyes is given below:

Fibre-NH2(S) + H+ (aq) + HSO4- (aq) → Fibre-NH3+HSO4-

Fibre-NH3HSO4(S) + Dye-SO3-(aq) → Fibre-NH3+DyeSO3-(S) + HSO4-(aq)

(Meer Md Rasel Khan et al .2015)

Figure 5. Acid Dyes



The dyes used in this experiment are from colourtex Industries Private limited. colomill red, colocid blue, colomill maroon, colocid green, colocid violet, colomill yellow are the different dyes used.

LITERATURE REVIEW

• Colored cocoons can be produced without disturbing nutritional traits like ingesta, digesta, growth rate, digestibility percentage when a selected dye in selected concentration is used. The dyes used did not have any adverse effect on physiological and biochemical aspects. (Anumol Anto et.al 2018)

• High concentrations of rhodamin and thionin in the modified diet showed physiological disorders in silkworms. When appropriate amount of dye was used silkworms grew without any physiological disorders. As soon as modified diet was fed to the silkworms, its body showed color and subsequently colored cocoons and eggs were produced. The dye induced color was not inherited to the next generation. (Pil Don Kang et.al 2011)

• Dye can be introduced to silkworm in the last two days of 5th instar and depending upon the concentration, the color intensity of the cocoon varied. For producing pink colored silk cocoons rhodamin B was used in different concentrations. The dye used was nonlethal to the silkworm. The performance of the dye depends on its structure, surface properties and particle size. Taking these aspects in mind we can select novel dye molecules to produce different colors and shades. (Kanika Trivedi, et.al 2016)

• Not all azo dyes produced intrinsically colored cocoons. A balance of hydrophobic and hydrophilic character is essential for diffusion of dye into the hemolymph from the alimentary canal and later into the silk glands. Molecular weight less than 400g/mol was required in order to transport the dye in the biochemical pathway of the silkworm to produce naturally dyed silk fiber. (Anuya Nisal et.al 2013)

• Compared to the synthetic dyes, food colors are cheaper and use of these colors showed non-significant change in the parameters of the silkworm. (Shimaa et.al 2022)
• Silk proteins are produced in special glands where they are stored in the lumen before spinning. Silk fibers are made up of these proteins. (Baby Joseph et.al 2012)
• The silk dyeing effluents induced mitotic abnormalities and act as potential mutagents in Allium Cepa root system. (R. Sudhakar et.al 2001)
• Organic pollutants such as dyes and phenolic compounds are extensively used in the textile, paper, cosmetic, pharmaceutical, paint, leather and plastic industries etc contribute to severe water pollution. Dyes are toxic, mutagenic and carcinogenic. They cause harm to the human body as well as affect the process of photosynthesis in aquatic plants. (Muhammed Farhan Hanafi et.al 2021)
• Synthetic dyes are a threat to the environment due to the negative impact on living organisms. Natural dyes which are produced from bark and root of some plants like Zizipus jujube, Ficus benghalensis are eco-friendly and can be used to prevent the harmful affect caused by water pollution. (Shamsheer et.al 2021)
• Due to seasonal availability of natural resources, tiresome process of dye extraction, contamination of land and resources by metallic moderants, high cost of production natural dyes cannot be used for textile dyeing. (Hana Krizova et.al 2015)
• Intrinsically colored and luminescent silk is produced directly from silkworm for a wide range of applications. Molecular properties can be incorporated into silk while it's being produced in the silk gland. (Natalia C. Tansil et.al 2012)
• Fluorescent silk which had exceptional mechanical properties was produced by feeding the silkworm with graphene quantum dots. The silk produced had application in optics, photonics and biomedical engineering. (Cheng et al 2019)

Aim

To adopt a green method to produce colored cocoons by feeding silkworms larvae with modified diet.

Objectives

1.To obtain the color cocoons by feeding modified diet.
2. To compare the cocoons produced by experimental groups with that of control.

Experimental Design

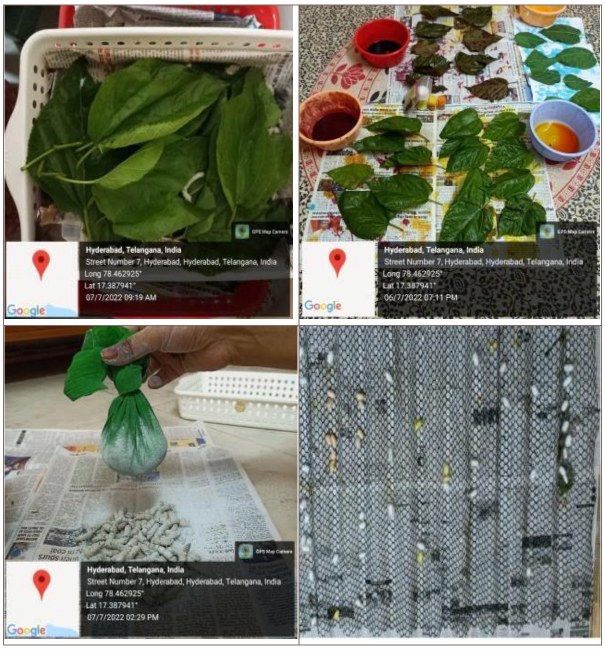
• Procuring silkworm larvae in their third instar from a sericulture farm.
• Preparing modified diet using dyes.
• From the second day of fifth instar, the silk worm larvae will be divided into five groups.
• Out of the five groups, four groups will be experimental and one group will serve as control.
• The experimental groups will be fed with modified diet, whereas the control groups will be fed with unmodified mulberry leaves.
• Cocoon formation will be observed in all the experimental groups and control group.

MATERIALS & METHODOLOGY

• Mulberry leaves
• Plastic Trays

- Rack
- Tubs
- Cotton towel
- Dyes
- Hydrated Lime Powder
- Newspapers
- Wire mesh
- Disinfectant
- Chandrika
- Plastic Bottles
- Spray Bottles
- Paint Brushes

Figure 6: Materials Required



REARING OF SILK WORMS

Rearing equipment's:

Rearing house: As the silkworms are very sensitive to weather conditions like humidity and temperature, the rearing house should be maintained accordingly. Optimum temperature, proper humidity, proper ventilation should be maintained in the rearing room. Bright sunlight, strong wind and stagnation of air should be avoided.

Rearing trays: Medium sized plastic trays are used for rearing the worms.

Rearing stand: A plastic rack with four rows of plastic trays is used for rearing the experimental groups.

Chopstick: Young larvae are picked with chopsticks in order to maintain hygiene.

Leaves chamber: The leaves procured were stored in a plastic tray wrapped with a wet cotton cloth.

Mountages: Plastic Chandrikes available from the sericulture lab is used to support silkworms for spinning.

Rearing Practices

Silkworm must be reared with utmost care since they are susceptible to diseases. Good sanitation and hygienic rearing techniques should be followed to prevent diseases. The appliances and rearing room should be thoroughly cleaned and disinfected with 2-4 % formaldehyde solution. Room temperature should be maintained around 25 °C.

Feeding: Leaf quality plays an important role in the production of quality cocoons. The young age worms are fed with tender, succulent leaves which contain sugar, less amount of fibre, starch but high moisture and protein for chowki worms. Mature leaves are fed to late age worms. The leaves which were used in this experiment were of V1 variety which had high protein content and moisture. The leaves were shiny and had a strong petiole. The quantity of mulberry leaves consumed during each instar increases gradually. Maximum leaves are consumed in the fifth instar.

Bed Cleaning: Periodical removal of left-over leaves and worms' excreta is referred to as bed cleaning. It is necessary for proper growth and hygiene. The beds are cleaned daily. Rearing bed should be kept dry, for this purpose application of lime powder after each moult is done.

Spacing: Provision of adequate space is of great importance for vigorous growth of silkworms. As the worms grow in size, the density in the rearing bed increases and conditions of overcrowding are faced. Normally it is necessary to double or triple the space by the time of moult from one to other instar stage.

Mounting: Transferring of mature 5th instar larvae to mountages is called mounting. When larva is fully mature, they become translucent, their body shrinks and they stop feeding and start searching for corners to attach themselves for cocoon spinning and pupation. They are picked up and put on montages' continuous movement of head, silk fluid is released in minute quantity which hardens to form long continuous filament. After the compact shell of the cocoon is formed the larva wraps itself and detached itself from the shell to form pupa. The spinning is completed in 3-4 days.

Harvesting: The larva undergoes metamorphosis inside the cocoon and becomes pupa. In early days, pupal skin is tender and ruptures easily. Thus, early harvest may result in rupture of pupa, and this may damage the silk thread. Late harvest has the risk of threads being broken by the emerging moth. Therefore, the cocoons should be harvested on time. Cocoons are harvested by hand.

METHODOLOGY

1. Bivoltine silkworm larvae in their third instar are collected from Shadnagar and Nalgonda and are reared in two separate trays following the standard protocol for rearing.
2. When the larvae entered fifth instar stage after undergoing moulting they are separated into groups for experimental purpose.
3. The experiment is done in two batches of silkworm larvae collected from two different sources.
4. Each batch consisted of five groups of which four are experimental and one serves as control.
5. The experimental groups are fed with mulberry leaves dipped in dye solution whereas the control is fed with mulberry leaves without dipping in dye solution.
6. Leaves are air dried to evaporate moisture and are fed to the worms.
7. Feeding is done following the feeding timing according to the table given.

Figure 7: Rearing of Silkworms

Table 1: Feeding Plan

Stage	Number of Feeds	Number of Mulberry Leaves
3rd Instar	3-4 Times	5-8
4th Instar	3-4 Times	10-15
5th Instar	4-5 Times	20-25

Table 2 : Feeding Time

S.NO	1st FEEDING	2nd FEEDING	3rd FEEDING	4th FEEDING	5th FEEDING
1.	6-6.30 a.m.	10-10.30 a.m.	2-2.30 p.m.	6-6.30 p.m.	10- 10.30 p.m.

PREPARATION OF MODIFIED DIET

1. Silkworm Larvae from second day of fifth instar are fed with this diet until they start spinning.
2. Modified Diet is prepared by mixing 1gm of Dye in 40ml of water. Mulberry leaves are dipped in this dye mixture.
3. The synthetic textile dyes used are Colomill Red F2R and Colocid Patent Blue. Food colors used in the experiment are Kesar Yellow and Lemon Yellow.
4. The leaves are air dried. However, care should be taken to feed leaves without any moisture content.
5. This procedure is done half an hour prior to feeding leaves to the silk worms.
6. Silkworm larvae are fed with this diet five times daily. The feeding timings are followed according to the feeding chart.

Figure 8: Modified Diet

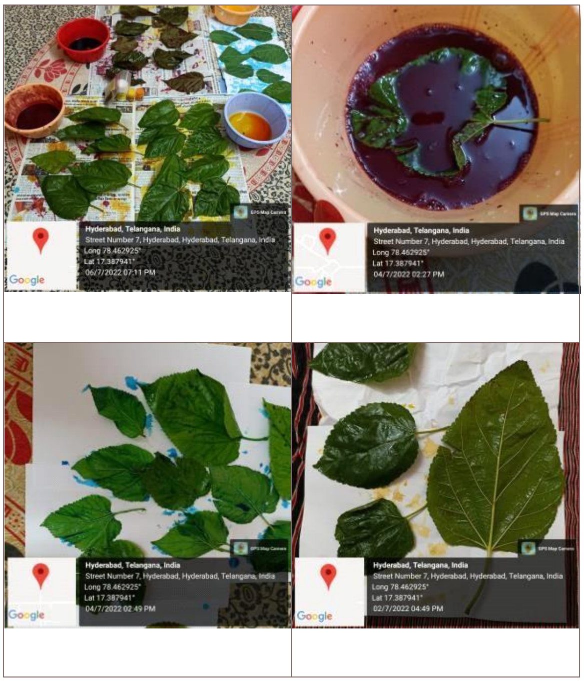


Table 3: Dyes used for Batch 1

Groups	**Dyes used**	**Amount of dye**	**Amount of water**
control 1	-	-	-
group 1	Lemon Yellow	1 g	40 ml
group 2	Kesar Yellow	1 g	40 ml
group 3	Colomill Red	1 g	40 ml
group 4	Colocid Yellow	1 g	40 ml

Table 4: Dyes used for Batch 2

Groups	**Dyes used**	**Amount of dye**	**Amount of water**
control 2	-	-	-
group 5	Colocid Purple	2 g	20 ml
group 6	Colomill Yellow	2 g	20 ml
group 7	Colomill Maroon	2 g	20 ml
group 8	Colocid Green	2 g	20 ml

group 9	colomill Red	1g	40 ml

Table 5: Diet Plan of 5th Instar Larvae-Batch 1

Date	Day of 5th Instar	Modified Diet	No. of Leaves	No. of Feeds
02-07-2022	2nd	Mulberry leaves dipped in dye solution	10-12	5
03-07-2022	3rd	Mulberry leaves dipped in dye solution	10-12	5
04-07-2022	4th	Mulberry leaves dipped in dye solution	10-12	5
05-07-2022	5th	Mulberry leaves dipped in dye solution	15-20	5
06-07-2022	6th	Mulberry leaves dipped in dye solution	15-20	5
07-07-2022	7th	Mulberry leaves dipped in dye solution	15-20	5

Table 6: Diet Plan of 5th Instar Larvae-Batch 2

Date	Day of 5th Instar	Modified Diet	No. of Leaves	No. of Feeds
06-07-2022	2nd	Mulberry leaves dipped in dye solution	10-12	5
07-07-2022	3rd	Mulberry leaves dipped in dye solution	10-12	5
08-07-2022	4th	Mulberry leaves dipped in dye solution	10-12	5
09-07-2022	5th	Mulberry leaves dipped in dye solution	15-20	5
10-07-2022	6th	Mulberry leaves dipped in dye solution	15-20	5
11-07-2022	7th	Mulberry leaves dipped in dye solution	15-20	5

RESULT

Batch 1

- Colored cocoons are produced by only one experimental group.
- The remaining groups produce white cocoons similar to that of control.
- The experimental group (group 1) which is feed with a modified diet using colomill red produces yellow color cocoons.
- The length and weight of the cocoons are taken and tabulated.

Table 7: Results of Batch 1

GROUPS	DYES	COLOR OBSERVED IN THE COCCON
GROUP 1	COLOMIL RED	YELLOW
GROUP 2	COLOCID BLUE	WHITE
GROUP 3	KESAR YELLOW	WHITE
GROUP 4	LEMON YELLOW	WHITE

Batch 2

• Coloured cocoons are produced by three experimental groups (group 7, group 8 and group 10) when they are feed with mulberry leaves dipped in dyes.

• The cocoons produced by the above group are musk, light musk and yellow in color.

• The experimental group 10 which is feed with modified diet using colomill red produced yellow cocoon which is similar to batch one result.

• The remaining groups produce white cocoons similar to that of control.

• The length and weight of the cocoons are tabulated.

Table 8: Results of Batch 2

GROUPS	DYES	COLOR OBSERVED IN THE COCCON
GROUP 5	COLOCID PURPLE	WHITE
GROUP 6	COLOMIL YELLOW	MUSK
GROUP 7	COLOMIL MAROON	PALE MUSK
GROUP 8	COLOCID GREEN	WHITE
GROUP 9	COLOMILL RED	YELLOW

Figure 9 : Results of Batch 2

Figure 10: Cocoons Formed In Experimental groups(Batch 1)

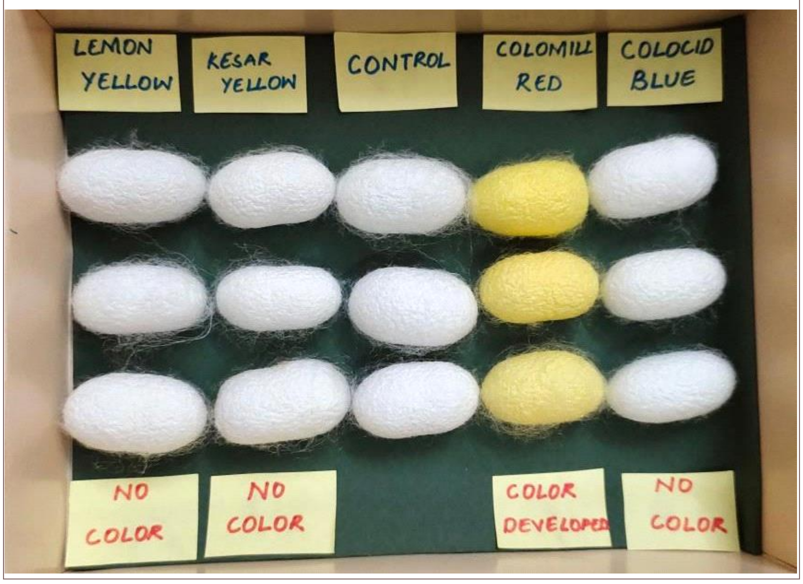


Figure 11: Cocoons Formed In Experimental groups(Batch 2)

Table 9: Length and Weight of Cocoons-Batch 1

S.NO	DYES	WEIGHT OF COCOON	LENTH OF COCOON
01	Control	0.65	3.7
02	Kesar Yellow	0.63	3.3
03	Lemon Yellow	0.57	3.4
04	Colocid Blue	0.54	3.5
05	Colomil Red	0.72	3.3

Table 10: Length and Weight of Cocoons-Batch 2

S.NO	DYES	WEIGHT OF COCOON	LENGTH OF COCOON
01	Control	0.84	3.6
02	Colocid Green	0.62	3.1
03	Colomil Yellow	0.56	3.3
04	Colomil Maroon	0.54	3.2
05	Colocid Purple	0.66	3.1
06	Colomil Red	0.91	3.2

DISCUSSION

- Green method is the best option to produce naturally colored silk as it is eco-friendly and feasible method that silk dyeing can depend upon in the sericulture industry.
- More research using different eco-friendly dyes is required to produce colored silk that are easily available in the market.
- The dyes used in this experiment did not harm the silkworm.
- In the present research work done, out of total eight dyes used in two batches only three dyes are able to

diffuse in the silk glands of the silkworm to some extent and the cocoons formed are colored.
Colomill Red - Yellow cocoon
Colomill Maroon - Light Musk cocoon
Colomill Yellow - Musk cocoon
• The experiment is repeated using colomill red dye in modified diet in the second batch.
• Formation of yellow cocoon confirms that the experimental group fed with colomill red containing modified diet produces yellow cocoon for the second trial.
• The setbacks recorded during previous researches such as poor color retention on degumming silk fiber can be overcome by experimenting on various parameters of the dye that are considerably associated with silk fibroin.
• Further, the intensity of color can be enhanced through increasing the concentration of dye.
• When azo dyes like acid orange, moderant black, direct acid-fast red are used while preparing modified diet intrinsically colored bright orange, light violet and pink colored are obtained. (Anuya Nisal et. al)
• The results do not coincide with the results obtained by (Shimaa et. al) in their experiments where colored cocoons were obtained when food colorants were used instead of synthetic dyes in preparing modified diet.
• Pink colored cocoons are obtained when rhodamine B was used to color mulberry leaves before feeding them to the silkworms. This dye used did not harm the silkworm. (Kanika Trivedy et. al)

Table 11: Characteristics of an ideal dye for natural silk dyeing

Partition Coefficient	Positive, but not too high for it to be very hydrophobic which reduces its solubility.
Nature	Amphiphilic balance of (hydrophobicity and hydrophilicity)
Lipophilicity	High ,aids in diffusion of dye into silk glands and increases the dye association with fibroin.
Molecular Weight	Less than 400g/mol,

• The possible reason for the uptake of dyes in the silk gland is due to its hydrophobic nature i.e., as the hydrophobicity of the dye increased the dye was transported to the hemolymph and then to the silk gland, and finally to the cocoons.
• Dyes with molecular weight more than 400 g/mol could not cross the alimentary canal into the hemolymph as a result the cocoons were white in color.
• Therefore, keeping in mind all these aspects dyes should be selected for better results.

CONCLUSION

Production of colored silk using conventional method involves the use of toxic chemicals which is hazardous to the environment. Hence, a sustainable way to obtain colored silk is by feeding the silkworm with dye-dipped mulberry leaves. Objectives of this research work are met partially as colored cocoons are obtained through few dyes only. Further research with different colored dyes in increased concentration may give more insight in achieving the set objectives.

Figure 12: Colored Cocoons

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