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Fagara silkworm *(Attacus atlas* L.*)*
spotted on *Cipadessa baccifera*

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Review Paper

GENETICS, CYTOGENETICS, AND GENOMICS OF MULBERRY (*MORUS* SPP.)

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Abstract

Mulberry (*Morus* spp.) is a highly heterozygous, cross-pollinated, and vegetatively propagated perennial tree species, which plays a significant role as a host plant in sericulture. The genetic complexity of *Morus* species arises from their high level of polyploidy, large number of chromosomes, and small chromosomal size. These species display a wide range of chromosome numbers, from balanced sets (2n=14, 28, 56, 84, 112, 140, 168, 196, 224, 252, 280, and 308) to unbalanced numbers (2n=26, 27, 29, 30, 40, 41, 42, 46, 70, 98, 126, 154, 182, 210, 238, 266, and 294), leading to substantial morphological, anatomical, and cytological diversity. Chromosome size decreases with increasing ploidy, with lengths ranging from 0.34 µm to 5.23 µm. The nuclear DNA content varies significantly, ranging from 0.35 pg in diploid forms to as high as 8.34 pg in docosaploid forms, reflecting the extensive genetic variability that results from polyploidy. The estimated sizes of the nuclear, chloroplast, and mitochondrial genomes in *Morus* species are 330.79 Mb to 505.29 Mb, 158,000 bp to 160,000 bp, and 361,546 bp to 395,412 bp, respectively. These genome sizes contribute to the functional complexity of mulberry plants.

Key words: Aneuploidy, chromosomes, cytogenetics, diploid, docosaploid, genome, mulberry, *Morus*, polyploids, silkworm, triploid.

Introduction

Mulberry (*Morus* spp.) is believed to have originated in the border region between the Indo-Chinese area, specifically in the lower slopes of the sub-Himalayan zone (Tikader and Vijayan, 2010). Taxonomically, *Morus* belongs to the family Moraceae within the order Urticales, though molecular phylogenetic studies have now placed the entire Moraceae family under the order Rosales (Sytsma *et al.,* 2002). According to the International Sericultural Commission (ISC), mulberry is cultivated in over 26 countries, and 68 species of *Morus* have been identified, with the majority found in Asia, particularly in China and Japan. The most widely cultivated species for silkworm (*Bombyx mori*) rearing include *Morus alba, M. indica, M. multicaulis, M. bombycis, M. latifolia, M. atropurpurea*, and *M. mizuho*. These species have undergone extensive breeding programs involving open pollination, controlled hybridization, and mutation breeding, resulting in over a thousand varieties, many of which are polyploid. In India, the main species are *M. indica, M. alba, M. serrata,* and *M. laevigata*, which grow naturally in the north of the country (Tikader and Vijayan, 2010). The majority of the cultivated varieties belong to either *M. indica* or *M. alba*, with the total area under mulberry cultivation in India being approximately 253,182 hectares (CSB, 2022). The importance of increasing foliage production is paramount, as it directly impacts silkworm rearing and, in turn, enhances silk production per unit area (Datta, 2000).

Fossil evidence of *Morus* species has been found in the Pliocene record of the Netherlands, indicating the genus has a long history, dating back millions of years (Zhang and Zhong, 2003; Hugo *et al*., 2006). The taxonomy of *Morus* is complex and often debated due to several factors, including its wide geographical distribution, hybridization between species, morphological plasticity, and a long history of domestication. Additionally, the introduction and naturalization of various *Morus* species in different regions further complicate classification (Vijayan *et al*., 2011). Traditionally, *Morus* species have been classified based on specific traits, such as the hairiness of the stigma, the length of the styles, the type of idioblast in the leaf, and various leaf anatomical characteristics (Chakraborti *et al*., 2013). However, despite numerous attempts to classify *Morus*, the taxonomy remains inconsistent, with several species and subspecies being identified. A total of 260 validated names for *Morus* species have been recorded in the International Plant Names Index (http://www.ipni.org/), many of which are considered synonymous. As a result, while up to 68 or even 150 species have been reported, only 10-16 species are widely recognized and accepted by the scientific community. Recent molecular and phylogenetic studies have helped clarify the classification of *Morus*, identifying eight main species: *M. alba, M. rubra, M. insignis, M. notabilis, M. mesozygia, M. nigra, M. serrata*, and *M. celtidifolia* (Zeng *et al*., 2015). The term "mulberry" is commonly used to refer to all species within the *Morus* genus, and these species can be propagated both asexually (stem cuttings/grafting) and sexually (seeds). However, not all *Morus* species or varieties are suitable for use as food for silkworms (*Bombyx mori*). Beyond sericulture, mulberry trees have various other uses, including fruit production, wood, pharmaceuticals, dye production, and landscaping (Chakraborti *et al*., 2013).

The *Morus* genus is a fascinating group of species, notable for its wide range of ploidy levels, which significantly influence the morphology and genetic characteristics of the plants. The study of *Morus* cytology began with Tahara (1909) and was further developed by Osawa (1920), who used garden varieties of mulberry in Japan. Early cytological work led to the discovery that the basic chromosome number for mulberry is $x=14$. Following this, researchers identified a variety of ploidy levels in natural *Morus* species, ranging from haploids to docosaploids. The haploid chromosome number of $n=7$ was first reported in *M. notabilis*. The diploid chromosome set, with $2n=2x=28$, is found in numerous species, including *M. alba, M. atropurpurea, M. acidosa, M. australis, M. bombycis, M. celtidifolia, M. indica, M. ihou, M. kagayamae, M. latifolia, M. laevigata, M. multicaulis, M. macroura, M. microphylla, M. mongolica, M. mesozygia, M. notabilis, M. nigra, M. rotundiloba, M. rubra, M. serrata, M. sinensis*, and

M. wittiorum. Triploid forms (2n=3x=42) have been reported in species like *M. alba, M. australis, M. atropurpurea, M. bombycis, M. boninensis, M. chinensis, M. indica, M. kagayamae, M. latifolia, M. multicaulis, M. mizuho, M. notabilis*, and *M. rotundiloba*. Tetraploid species (2n=4x=56) include *M. alba, M. boninensis, M. bombycis, M. cathayana, M. laevigata, M. macroura, M. teliaefolia, M. rubra*, and *M. wittiorum*. Hexaploid species (2n=6x=84) include *M.cathayana, M.celtidifolia, M. insignis, M. laevigata, M. mongolica, M. teliaefolia, M. rubra*, and *M. serrata*. Interestingly, octoploid species (2n=8x=112) are found in *M. cathayana*, and docosaploidy (2n=22x=308) is reported in *M. nigra* (Tahara, 1909; Osawa, 1920; Janaki Ammal, 1948; Seki, 1952 a; Datta, 1954; Sastry *et al*., 1968; Alekperov *et al.,* 1969; Bedi, 1999; Sarkar, 2009; Zeng *et al*., 2015; Yamanouchi *et al*., 2017).

Cytogenetic studies have also documented the occurrence of polysomaty (Seki and Oshikane, 1957;Das, 1963;Koyama *et al*., 2001) and aneuploidy in certain *Morus* varieties (Sinoto, 1929; Das, 1961; Kundu and Sharma, 1976). However, there are no cytogenetic reports for several other *Morus* species, such as *M. alaisia, M. barkamensis, M. deqinensis, M. formosensis, M. intermedia, M. japonica, M. koordersiana, M. liboensis, M. miyabeana, M. murrayana, M. mexicana, M. nigriformis, M. pendulina, M. philippinensis, M. rabica, M. trilobata, M. tatarica*, and *M. yunnanensis*. Interestingly, normal meiosis occurs in all diploids, hexaploids, octoploids, and docosaploids, while it is slightly irregular in tetraploids and highly irregular in triploids and aneuploids. Irregular meiosis is distinguished by the formation of monads, dyads, triads, pentads, hexads, and polyads, as well as cytomixis. This abnormal meiotic behavior leads to pollen sterility in mulberries (Darvey and Driscoll, 1972; Verma *et al*., 1986a; Dwivedi *et al*., 1989; Kumara and Basavaiah, 2016). The mulberry genotypes show differences in morphological and leaf anatomical characters with the differences of basic sets of chromosome numbers.The optimum level of ploidy considered from a biological point of view is triploidy. Since the leaf surface becomes rougher as the ploidy level increases, the foliage is unsuitable for silkworm (*Bombyx mori*) rearing. As a consequence, only diploid and triploid species of mulberry are commonly used in the sericulture industry (Kumara *et al*., 2021). Genetic variability is essential for the improvement of mulberry, as it plays a critical role in enhancing the plant's yield, leaf quality, stress tolerance, and resistance to pests and diseases. The genetic resources of mulberries form the foundation for crop management strategies, providing a vital gene pool for breeders to develop improved varieties. The collection, introduction, and exchange of *Morus* germplasm can significantly enrich this gene pool, offering breeders a wide range of options to address diverse environmental and sericultural challenges. Among the countries with the most extensive mulberry genetic resources, China leads with over 2,600 accessions, followed by Japan (1,375 accessions), India (1,292 accessions), Korea (615 accessions), Bulgaria (140 accessions), Italy (50 accessions), and France (43 accessions) (Sohn, 2003). Despite this rich diversity of mulberry resources, the genetics of mulberry remain less understood compared to many other crops, primarily due to the historical lack of genomic data and research. However, significant strides have been made in recent years. In 2013, the first full genome sequence of *Morus notabilis* was completed, marking a milestone in mulberry genomics. This achievement, made possible by advances in next-generation sequencing (NGS) technologies, was followed by the sequencing of two more mulberry species' genomes: *M. indica* and *M. alba*. These groundbreaking efforts have expanded our knowledge of the mulberry genome, providing a more comprehensive understanding of the plant's genetic makeup and opening up exciting possibilities for accelerating mulberry breeding programs (Li *et al*., 2014). As a result, I have consolidated and presented the genetic, cytogenetic, and genomic information of the *Morus* genus, providing a comprehensive resource for understanding its complexity. This compilation offers valuable insights into the genetic diversity and potential for *Morus* plant improvement.

Genetics

The mulberry plant exhibits significant phenotypic variation, which is influenced by both its inherent genetic factors and the application of various breeding strategies, including conventional and non-conventional methods. This variation arises due to mulberry's complex heterozygous, cross-pollinated, and polygenic nature, contributing to a wide range of phenotypic outcomes. The species displays both dominant and epistatic characteristics, which can lead to the generation of diverse and valuable variants (Vijayan *et al.*, 2008). When different mulberry genotypes are crossed, the offspring show a high degree of segregation, particularly in economic traits. This is often accompanied by a reduction in the performance of these traits, along with the admixture of polyploidy and mixoploidy lines, which can complicate the breeding process (Chao, 2011).The phenotypic plasticity of mulberry plays a significant role in the expression of its traits. The degree to which environmental factors influence phenotypic variation in mulberry is an important consideration for breeders, as these factors can either enhance or obscure genetic potential (Gray, 1990).

The qualitative and quantitative traits of mulberry leaves are vital for sericulture, as they directly impact silkworm growth and productivity. Understanding the inheritance of these traits is crucial. The leaf shape in mulberry is primarily controlled by a monogenic trait, with the pentalobate shape being dominant over the polylobate shape. In terms of reproductive traits, the long style characteristic is incompletely dominant over the short style, with a linkage action observed between the long style and the polylobate leaf trait, and between the short style and the entire leaf trait (Katsumata, 1982). When crosses between lobed and unlobed genotypes of mulberry are made, the resulting F_1 hybrids exhibit a diverse array of leaf forms, including lobed, unlobed, and intermediate leaves with novel patterns of lobation. Notably, maternal inheritance predominates in these hybrids, with the maternal traits influencing the leaf morphology more strongly than the paternal traits (Kumara and Ramesh, 2016; Kumara and Basavaiah, 2016) (Figure 1). This phenomenon of maternal dominance in leaf traits is consistent with the inheritance patterns observed in other traits in mulberry and is primarily controlled by chloroplast DNA, which is inherited maternally in *Morus* species (Hu *et al.*, 2014). Leaf lobation is more common on juvenile shoots than on mature trees, as observed by Shablovskaya (1982) and further confirmed by Kumara and Ramesh (2016). Additionally, the expression of leaf lobation is significantly affected by environmental factors, with tree age, annual variations, and the interaction between tree genotype and year influencing the distribution of lobed and unlobed leaves (Gray and Richard, 1987). The inheritance of leaf size follows a distinct pattern, where intermediate or small leaves tend to dominate over large leaves, as reported by Cirkov (1966). Similarly, when inter-specific hybridization occurs, the leaf shape in the F_1 progeny shows influences from both parental species, with a blend of traits from each parent (Seki, 1959; Das and Krishnaswami, 1965). Additionally, traits related to the flavonol component in the leaf, which contribute to the plant's chemical composition and possibly its resistance to pests, follow a Mendelian inheritance pattern (Mari *et al.*, 2013), demonstrating typical genetic segregation for this trait.

When comparing the effects of vegetative and sexual hybridization, mulberry hybrids resulting from vegetative hybridization tend to differ morphologically from those produced through sexual hybridization between the same parent components. These vegetative hybrids are often characterized by heterotic vigor, exhibiting superior growth and other desirable traits (Hakimov, 1959). Furthermore, studies by Kuchkarov (1980) showed that phenotypically homogeneous mulberry hybrids could be obtained using inbred lines, which is particularly useful for maintaining desired traits across generations and for creating more stable hybrid populations. In mulberry, various traits have been studied for their genetic inheritance, contributing to a deeper understanding of the species' breeding and improvement. One of the key traits studied is dwarf resistance, which was examined by Murakami (1973). This study helped identify the genetic mechanisms that control resistance to dwarfing, a trait important for maintaining optimal plant height and structure in mulberry cultivars. A particularly interesting trait is the occurrence of winter buds with dark brown stripes, known as striped buds, which is controlled by a single recessive gene designated Si (Hirano, 1981). The weeping characteristics of mulberry stems are controlled by another single recessive gene (Yamanouchi *et al.*, 2009). Resistance to dieback, a serious disease in mulberry, has also been a focus of genetic studies. Okabe (1979) proposed that resistance to this disease is governed by a recessive gene.

According to his hypothesis, when the homozygous recessive condition is present, it results in the phenotypic expression of resistance to dieback. The stipule types in the F_2 progeny of S-30 and Ber S-799 mulberries follow a 9:6:1 ratio, suggesting the character is controlled by two dominant genes that show a similar effect individually but a double effect when both are present (Kumar *et al.*, 1992). Another notable genetic study in mulberry was by Hirano and Naganuma (1979), who explored the inheritance of peroxidase isozymes. Additionally, the study of inter-generic grafting between mulberry and related genera was carried out by Ogure (1983) with respect to morphological variation.

Heritability estimation and genetic advancement studies in mulberry genotypes have provided important insights into the inheritance of key traits associated with leaf yield and other economically significant characteristics. The traits such as plant height, number of branches/plant, branch length, number of leaves/meter, leaf area, and leaf weight exhibited high heritability values, often greater than 70 %. These traits are strongly correlated with leaf yield, which is a critical factor for sericulture and overall crop productivity. High heritability indicates that these traits are largely controlled by genetic factors, meaning that selection for these traits in breeding programs is likely to be effective (Masilamani *et al.*, 2000; Kumara and Ramesh, 2022a). The genetic diversity in mulberry germplasm has been explored through morphological quantitative characters, such as total shoot length, number of branches, internodal length, leaf lamina, leaf weight, and leaf yield. There is considerable variation in these traits across mulberry germplasm from diverse origins, highlighting the potential for selecting superior genotypes for breeding programs. These variations also contribute to the adaptability of mulberry to different environmental conditions and agro-climatic regions (Banerjee *et al.*, 2007). In terms of genetic control of these traits, studies have shown that non-additive genetic effects play a significant role in mulberry, as indicated by the ratio of general combining ability (GCA) to specific combining ability (SCA).The predominance of non-additive genes suggests that mulberry breeding programs may benefit from the use of heterosis or hybrid vigor, where hybrids between genetically diverse parents perform better than their parents, particularly in terms of traits like growth rate and leaf yield (Vijayan *et al.*, 2008).

Furthermore, molecular techniques have become indispensable tools in mulberry genetics, particularly for understanding the genetic relationships among different *Morus* species. The use of molecular markers such as RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), RFLP (restriction fragment length polymorphism), ISSR (inter simple sequence repeat), SSR (simple sequence repeat), SNP (single nucleotide polymorphism), and SRAP (sequence related amplified polymorphism) has enabled researchers to assess the genetic diversity between mulberry species and cultivars (Vijayan, 2007). These molecular markers provide a reliable way to identify and track desirable genetic traits, improve the precision of breeding programs, and enhance the overall genetic resource management of mulberry.

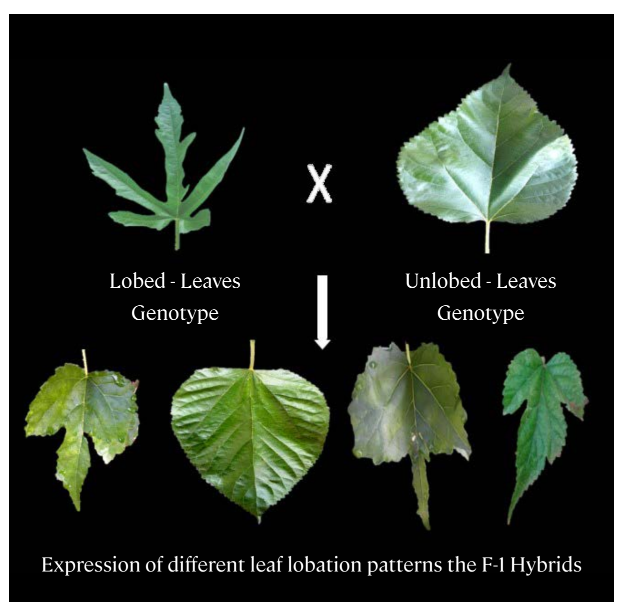

Figure 1: Genetics of leaf shape in mulberry

Cytogenetics

Cytogenetics plays a crucial role in understanding the chromosomal and genetic architecture of plant species, and this is particularly important in mulberry breeding. Studies on the somatic chromosomes and karyotype of mulberry genotypes help establish cytotaxonomic relationships and clarify their evolutionary history. In various *Morus* species, the basic gametic and somatic chromosome numbers provide insights into the ploidy levels and their characteristics. Ploidy in mulberry species ranges from haploidy (2n=14) to docosaploidy (2n=308), with several balanced chromosome numbers reported, including 2n=14, 28, 56, 84, 112, 140, 168, 196, 224, 252, 280, and 308 (Schneider, 1917; Abdullaev, 1965). Additionally, unbalanced chromosome numbers such as 26, 27 (Sinoto, 1929; Das, 1961), 29, 30, 40, 41 (Agaev, 1990a; Minamizawa, 1997), 42 (Abdullaev, 1965), 46 (Agaev, 1990a), 70, 98, 126, 154, 182, 210, 238, 266, and 294 have also been observed (Abdullaev, 1965). These variations in chromosome number contribute to the genetic and phenotypic diversity within the genus *Morus*, with implications for breeding and improvement strategies (Tikader and Kamble, 2008). Moreover, the increase in chromosome number in polyploid varieties is often correlated with an increase in the DNA content in the cell, particularly in the stable chromatin fraction (Talyshinskii, 1980a). However, this increased DNA accumulation is also associated with lower genetic and functional activity compared to other fractions of DNA (Askerova and Kasumov, 1989).

Polyploidy in mulberry plants is associated with significant morphological and physiological changes, many of which are beneficial or detrimental depending on the specific breeding goals (Seki and Oshikane, 1963). The size of pollen grains increases with ploidy: diploids have grains measuring 16-25.7 µm, triploids have 21-28 µm, tetraploids in the range of 23-30.5 µm, hexaploids show pollen sizes of 25-33 µm (Tikader *et al.*, 2000), and docosaploids can have pollen grains as large as 26-50 µm (Halbritter, 2016). Despite the increase in size, polyploids generally have lower pollen germination rates compared to diploids, which can impact fertility and seed production (Dzhaparidze, 1977). Interestingly, there are also notable biochemical changes in polyploid mulberries. Studies have shown that with increasing polyploidization, there is a decline in the content and activity of certain enzymes, such as catalase and peroxidase isozymes (Talyshinskii, 1977). Additionally, the dehydrogenase forms change with higher ploidy levels, indicating altered metabolic pathways in these plants (Talyshinskii, 1980b). Despite these changes, some polyploid forms, such as triploids and tetraploids, exhibit greater potential for protein synthesis than their diploid counterparts (Talyshinskii, 1980b). In addition to morphological and biochemical characteristics, ploidy levels in mulberry plants also affect the number of chloroplasts in stomata guard cells. Higher ploidy plants, such as tetraploids, have a higher number of chloroplasts in their stomatal guard cells, with tetraploid plants showing 18 chloroplasts per guard cell, triploids having 14, and diploids having 10. This characteristic can be used as an indirect marker to identify polyploid plants, as the number of chloroplasts in the stomatal guard cells correlates with ploidy level (Sikdar *et al.*, 1983).

In terms of economic characteristics, higher ploidy levels in mulberry plants are associated with several drawbacks. These include increased leaf coarseness, the development of more leaf hairs, and a decrease in the succulence of leaves, making them less palatable for silkworms (*Bombyx mori*). Additionally, polyploid plants tend to exhibit slower growth rates, poor feeding performance, and lower leaf yield compared to their diploid counterparts. As a result, polyploid mulberries are generally not favored for silkworm feeding, as they do not support optimal silk production (Hamada, 1963; Tojyo, 1985). On the other hand, triploids are particularly notable for their high leaf yield and good leaf quality, making them more suitable for silkworm rearing than higher-ploid forms (Abdullaev *et al.*, 1970). Triploid plants exhibit more vigorous leaf growth in successive phases of development compared to their diploid relatives, making them more attractive for sericulture (Aliev, 1975).

Cytological confirmation of ploidy and chromosome behavior during meiosis is essential in polyploidy breeding for mulberry, as it offers valuable insights into the genetic stability and fertility of different genotypes. This information is crucial for breeders to identify promising genotypes and select the most suitable species and varieties for commercial applications, particularly in sericulture. The ploidy level and its associated characteristics (molecular, cytological, morphological, anatomical, physiological, and biological aspects) have been thoroughly documented in mulberry species, helping guide breeding strategies and improve the quality and yield of mulberry plants used for silkworm production.

Haploid

Haploids in mulberry play a crucial role in advancing genetic improvement through breeding due to their genetic purity, expression of recessive genes, and the ability to generate pure lines via chromosome doubling (Tikader and Kamble, 2007). The male gametes of mulberry are monoploid with a chromosome number of x=14 (Raven, 1975), and this base number is believed to have evolved from an earlier base number of x=7 through chromosomal fusion (Bedi, 1999). *Morus notabilis* S, a natural haploid species found in Sichuan province, China, possesses a chromosome number of n=x=14 and is considered an early divergent form of mulberry (Schneider, 1917; Yu *et al.,* 1995). Interestingly, the mitotic chromosomes of *M. notabilis* 5 and 7 fused into chromosome 5 during diakinesis of meiotic division (Xuan *et al.,* 2017). The chromosomal behavior of *M. notabilis* during meiosis, including chromosome fusion events, highlights its distinct evolutionary lineage from other mulberry species like *M. alba* (Xuan *et al*., 2022). The *M. notabilis* has low vigor, is slow-growing, and is deciduous in nature. It grows up to 9-15 m and possesses greyish-brown bark and orbicular leaves depicting triangular serrated edges with paired male flowers 4-5 cm long (Li *et al*., 2014). Recent advances in tissue culture techniques, such as anther culture (Linn *et al*., 1987), gynogenesis (Lakshmisita and Ravindran, 1991), and unpollinated ovary culture (Dennis *et al*., 1999), have made it possible to regenerate haploid plants, further enhancing the potential of haploid breeding in mulberry.

Diploid

The majority of mulberry varieties found in nature are diploids (Table1), with a chromosome count of 2n=2x=28 (Figure 2). Species identified as diploids include *M. alba, M. atropurpurea, M. acidosa, M. australis, M. bombycis, M. celtidifolia, M. cordifolia, M. indica, M. ihou, M. kagayamae, M. latifolia, M. laevigata, M. multicaulis, M. macroura, M. mongolica, M. microphylla, M. mesozygia, M. notabilis, M. nigra, M. rotundiloba, M. rubra, M. serrata, M. sinensis,* and *M. wittiorum* (Tahara, 1909; Osawa, 1920; Janaki Ammal, 1948; Seki, 1952 a; Datta, 1954; Sastry *et al*., 1968; Alekperov *et al*., 1979; Bedi, 1999; Sarkar, 2009; Zeng *et al*., 2015; Yamanouchi *et al*., 2017). Among these diploid species, leaf yield potential can vary significantly, ranging from 8,000-25,000 kg/ha/yr in varieties like Mysore Local to 65,000-70,000 kg/ha/yr in varieties such as Victory-1. While the chromosome number and vegetative morphology remain similar across diploid varieties, the underlying molecular mechanisms responsible for yield variation remain unclear (Datta, 1954). Cytologically, diploid *Morus* species exhibit regular meiotic behavior, with 14 bivalents, of which one pair is larger than the others. In early prophase, the chromosomes appear as long filaments due to the regular pairing of homologous chromosomes (Datta, 1954). During metaphase I, the bivalents range from 4-12 ring bivalents and 2-10 rod bivalents, indicating a degree of variability in chromosome pairing (Azizian, 2001). Both the first and second anaphases show no lagging chromosomes (Das *et al*., 1970), and telophase is also regular (Azizian, 2001) (Figure 3). This consistent chromosomal behavior contributes to high pollen fertility in diploid mulberries, making them suitable for breeding and propagation (Datta, 1954).

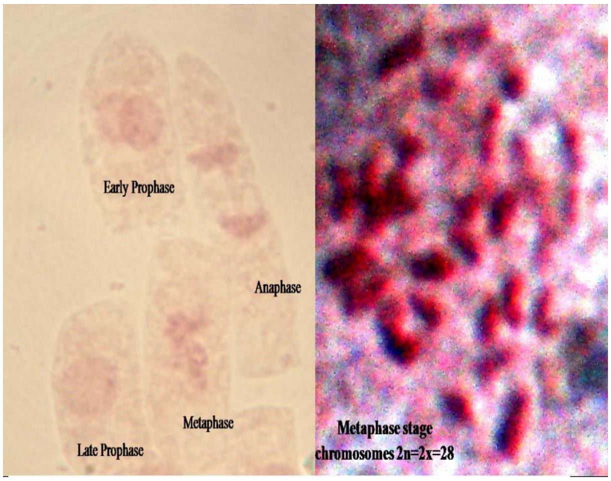

Figure 2: Different stages of mitotic cell division in diploid mulberry

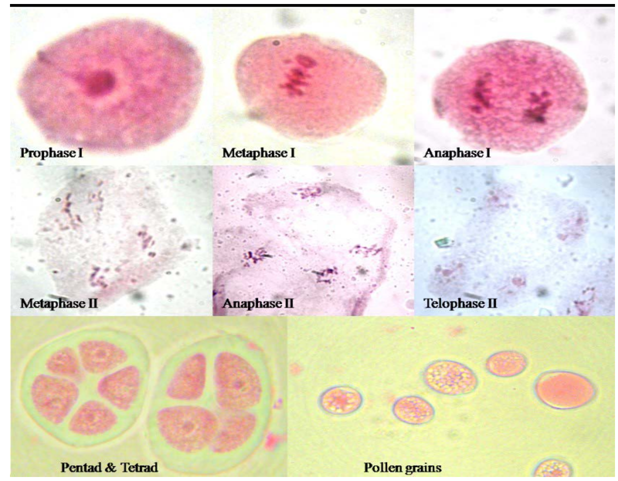

Figure 3: Stages of meiosis and pollen development of mulberry

Table 1: Popular Diploid mulberry varieties in India

Cultivar / Variety	Breeding methods & parents used	Leaf yield (MT/ha/yr)	Recommended region/condition
Mysore Local / Naatikaddi	Land race	08-25	South India under rainfed/irrigated conditions
Kanva-2 / M-5 (Mysore-5)	Open-pollinated hybrid of Mysore Local	30-35	South India under irrigated/rainfed conditions
MR-2 (Mildew Resistance-2)	Selection from open-pollinated hybrids	30-35	South India under irrigated/rainfed conditions
S-13 (Selection-13)	Selection from polycross (mixed pollen) progeny	15-18	South India under rainfed conditions
S-30 (Selection-30)	EMS treatment of Berhampore Local	30-35	South India under irrigated conditions
S-34 (Selection-34)	Selection from polycross (mixed pollen) progeny	13-17	South India under rainfed conditions
S-36 (Selection-36)	EMS treatment of Berhampore Local	35-40	South India under irrigated conditions (red lateritic soil)
S-41 (Selection-41)	EMS treatment of Berhampore Local	30-35	South India under irrigated conditions
S-54 (Selection-54)	EMS treatment of Berhampore Local	40-45	South India under irrigated conditions
RC-1 (Resource Constraint-1)	Hybridization of Punjab Local X Kosen	23-25	South India (limited irrigation & fertilizers)
RC-2 (Resource Constraint-2)	Hybridization of Punjab Local X Kosen	21-23	South India (limited irrigation & fertilizers)
RFS-135 (Rainfed Selection-135)	Open-pollinated hybrid of Kanva-2	10-12	South India under rainfed conditions
RFS-175 (Rainfed Selection-175)	Open-pollinated hybrid of Kanva-2	10-12	South India under rainfed conditions
Sahana	Hybridization of Kanva-2 X Kosen	25-30	South India under irrigated conditions (under coconut shade)
AR-11 (Alkaline Resistance-11)	Open-pollinated hybrid of Kanva-2	15-18	South India under irrigated conditions (alkaline soil)
Anantha	Clonal selection	65-70	South India under rainfed conditions

Cultivar / Variety	Breeding methods & parents used	Leaf yield (MT/ha/yr)	Recommended region/condition
TG-1 (Talaghattapura-1)	Clonal selection	35-40	South India under irrigated conditions
Viswa / DD-1 (Dehra Dun-1)	Clonal selection	35-40	South India under irrigated conditions (high temperature)
V-1 (Victory-1)	Hybridization of S-30 X Ber.C-776	65-70	South India under irrigated conditions
G-2 (Genotype-2)	Hybridization of *M. multicaulis* X S-34	36-38	South India under irrigated conditions (Chawki rearing)
G-4 (Genotype-4)	Hybridization of *M. multicaulis* X S-13	60-65	South India under irrigated conditions (late age rearing)
MSG-2 (Moisture Stress Genotype-2)	Hybridization of BR-4 X S-13	22-23	South India under limited irrigation conditions
AGB-8 (Advanced Generation Breeding-8)	Hybridization of Sujanpur-5 X Philippines and K-2 X Black Cherry	40-47	South India under Sub-optimal irrigated conditions
S-1 (Selection-1)	Open-pollinated hybrid of Mandalaya	30-35	Eastern & North Eastern India under irrigated conditions
S-146 (Selection-146)	Selection from open-pollinated hybrids	30-35	North India & Foot hills of J & K under irrigated conditions
Ber. S-799 (Berhampore Selection-779)	Selection from open-pollinated hybrids	35-40	Eastern & North Eastern India under irrigated conditions
BC2-59 (Back Cross2-59)	Back crossing of hybrid of Matigara Local X Kosen with Kosen twice	18-20	Hills of Eastern India under irrigated/ rainfed conditions
SV-1 (Somaclonal Variant-1)	Somaclonal variant from tissue culture	35-38	Eastern India under irrigated conditions (alkaline soil)
C-763	Hybridization of *M. multicaulis* X Black Cherry	30-32	Eastern India under irrigated/rainfed conditions (laterite soil)
C-776	Hybridization of *M. multicaulis* X Black Cherry	28-30	Eastern India under irrigated/rainfed conditions (laterite soil)
C-2016	Hybridization of Hosur Local X S-162	12-14	Eastern India under rainfed conditions (high temperature)
C-2017	Hybridization of Hosur Local X S-162	35-39	Eastern India under irrigated conditions
C-2028	Hybridization of Chinese White X S-1532	32-36	Eastern India under irrigated conditions (chronic flood prone areas)
C-2038	Hybridization of CF110 X C-763	53-54	Eastern & North Eastern India under irrigated/rainfed conditions
C-2058 (C-9)	Hybridization of Berhampore -A X Shrim-2	34-35	Eastern & North Eastern India under irrigated conditions (low input soils)
C-2060 (Gen-1)	Hybridization of Kajli OP X Victory-1	58-60	Eastern & North Eastern India under irrigated conditions
C-1360 (Ganga)	Hybridization of Hosur Local X S-162	50-57	Eastern & North Eastern India under irrigated conditions
Kosen	Introduction from Japan	10-12	Hills of Eastern & North Eastern India under irrigated conditions
Chinese White	Clonal selection	15-20	North India (temperate)
Chak Majra	Selection from natural variability	25-30	North India (Sub-tropical)
Goshoerami	Introduction from Japan	15-20	North India (temperate)
Sujanpur	Selection from natural variability	20-25	North India (Sub- tropical)

References: (Tikadar and Kamble, 2007; Sarkar, 2009; Vijayan *et al.*, 2012; Chakraborti *et al.*, 2013; Dandin and Giridhar, 2014)

Triploid

Triploid *Morus* species (2n=3x=42) arise both spontaneously and through breeding techniques (Figure 4 and Table 2). Spontaneous triploids have been observed in several *Morus* species, such as *M. alba, M. australis, M. atropurpurea, M. bombycis, M. boninensis, M. chinensis, M. indica, M. kagayamae, M. latifolia, M. multicaulis, M. rotundiloba, M. mizuho*, and *M. notabilis* (Osawa, 1920; Seki, 1959; Yang and Yang, 1991; Zeng *et al.*, 2015; Yamanouchi *et al.*, 2017). In some cases, triploids are developed through crossing diploid (2x) with tetraploid (4x) cytotype (Das *et al.*, 1970; Vijayan and Chakraborti, 1998), or through the production of diploidizing female gametes (Dwivedi *et al.*, 1989). Natural occurrences of triploids can also result from environmental factors, such as late frosts, which cause abnormal chromosomal behavior in pollen mother cells (PMCs) and lead to the production of triploid seeds (Seki, 1956; Seki, 1959).

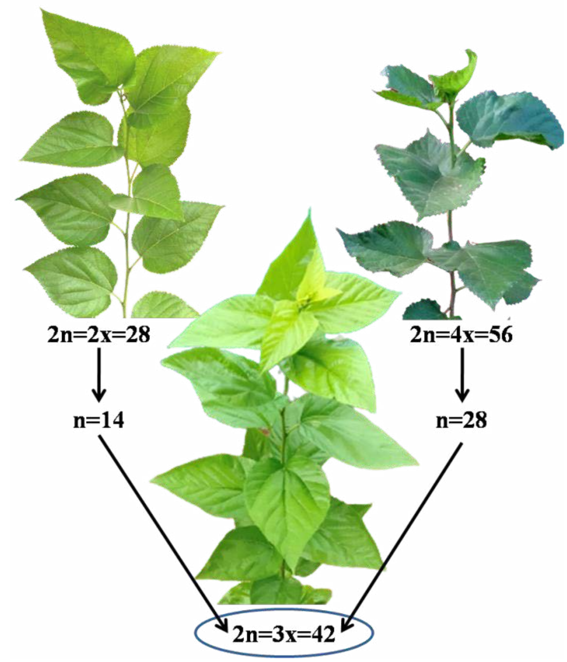

Figure 4: Cytomorphological representation of synthetic triploid development in mulberry

Table 2: Popular Triploid mulberry varieties in India

Cultivar / Variety	Breeding methods & parents used	Leaf yield (MT/ha/yr)	Recommended region/condition
AR-12 (Alkaline Resistant-12)	Hybridization of S-41 (4x) X C-776 (2x)	20-25	South India under irrigated conditions (alkaline soil)
Vishala	Clonal selection	65-80	South India under irrigated conditions
Suvarna-2	Hybridization of M-5 (4x) X Viswa (2x)	58-61	South India under irrigated conditions (Chawki rearing)
Tr-10	Hybridization of T-4 (4x) X Philippines (2x)	12-14	Hills of Eastern & Central India under rainfed conditions
C-1730	Hybridization of T-25 (4x) X S-162 (2x)	15-16	Eastern & Central India under rainfed conditions (red laterite soils)
Tr-23	Hybridization of T-20 (4x) X S-162 (2x)	24-25	Hills of Eastern & North Eastern India under rainfed conditions
S-1635	Selection from open pollinated hybrid (CSRS-1)	40-45	Hills of Eastern & North Eastern India under irrigated & rainfed conditions
S-1608	Selection from open pollinated hybrid (CSRS-1)	30-35	Eastern & North Eastern India under irrigated conditions

References: (Tikadar and Kamble, 2007; Sarkar, 2009; Vijayan *et al.*, 2012; Chakraborti *et al.*, 2013; Dandin and Giridhar, 2014)

Triploid *Morus* species exhibit distinct meiotic behavior due to their aneuploidy, which complicates genetic research and affects fertility. During the early prophase stage of meiosis, pollen mother cells (PMCs) in triploid mulberries display two unequally sized nucleoli, which indicates that the two homologous nucleolar organizers of the diploid complement form a large nucleolus, while the extra nucleolar organizer from the third genome contributes to a smaller nucleolus. The number of nucleoli is typically correlated with the number of secondary constrictions in the chromosomes and the overall ploidy level (Darvey and Driscoll, 1972; Basavaiah *et al*., 1990). In triploid mulberries, the presence of trivalents suggests significant homology between the constituent genomes, which is a characteristic of their autotriploid nature. During anaphase, an unequal segregation of chromosomes to the poles occurs due to irregularities in chromosome pairing and alignment. This results in the formation of monads, dyads, triads, pentads, hexads, and polyads, leading to cytomixis and pollen sterility (Verma *et al.*, 1986a; Dwivedi *et al*., 1989; Kumara and Basavaiah, 2016;Kumara *et al*., 2022).

Despite the sterility of triploids, this characteristic is not a limitation in mulberry breeding, as triploid plants are propagated vegetatively. This allows large-scale commercial use of triploid mulberries with desirable traits, such as high yield, superior leaf quality, and better adaptability to various climatic conditions. The sterility of triploids contributes to their increased vigor, as they exhibit larger cell sizes, thicker leaves, and more rapid DNA and RNA accumulation in their young leaves compared to diploids (Alekperova, 1979; Talyshinskii, 1982; Dwivedi *et al.,* 1986). Additionally, triploids show enhanced photosynthetic and transpiration rates, greater water-use efficiency, and higher chlorophyll content than both diploid and tetraploid genotypes (Vijayan *et al.,* 2000).Triploid mulberry varieties also demonstrate superior sericultural traits, including better rooting ability, higher survival rates, and faster growth with short internodal distances. These traits contribute to a higher yield compared to diploid and tetraploid varieties. The leaves of triploid mulberries are characterized by their dark green color, smooth texture, fewer hairs, and high moisture content, making them particularly suitable for silkworm feeding. The high nutritional value of triploid leaves, including proteins, carbohydrates, vitamins, and minerals, leads to improved silkworm viability, shorter rearing periods, and better cocoon characteristics, all of which contribute to increased silk production (Seki and Oshikane, 1959; Dzhafurov and Alekperova, 1978; Sarkar, 1993; Vijayan *et al.,* 1998). Moreover, triploids are more resistant to cold and disease compared to diploids, and they show greater adaptability to diverse agro-climatic conditions, making them a promising choice for silkworm feed production (Hamada, 1963; Tikadar *et al.,* 1996).

Tetraploid

Tetraploid (2n=4x=56) mulberries exhibit remarkable morphological gigantism, which is primarily characterized by larger, thicker, and coarser leaves (Das *et al.,* 1970). These tetraploids include species like *M. alba, M. boninensis, M. bombycis, M. cathayana. M. laevigata, M. macroura, M. teliaefolia, M. wittiorum,* and *M. rubra* (Janaki Ammal, 1948; Datta, 1954; Seki, 1959; Das, 1961; Yand and Yang, 1991; Yile and Oshigane, 1998; Bedi, 1999; Sarkar, 2009; Zeng *et al.,* 2015; Yamanouchi *et al.,* 2017; Mahshid, 2019) can occur naturally or be induced artificially. Meiosis in both natural and induced tetraploid *Morus* species exhibits slight irregularities, characterized by the formation of multivalents, univalents, and bivalents during the first metaphase (Das, 1961). Quadrivalents and bivalents occur more frequently than trivalents and univalents. During anaphase I, lagging chromosomes are observed in variable numbers, leading to abnormalities that cause size variation in pollen and reduced fertility. In induced tetraploids, fertility is typically halved compared to diploids (Das *et al.,* 1970). The reduced fertility in induced polyploids is primarily attributed to multivalent chromosome associations during synapsis, along other meiotic abnormalities that result in chromatin loss (Dwivedi *et al.*, 1989). While seed size is larger in tetraploids, their germination percentage is lower than that of diploids (Radzhabli, 1962; Shafiei and Basavaiah, 2017). These meiotic and fertility issues complicate the use of induced tetraploids in breeding programs.

Tetraploid *Morus* species are typically induced through treatments with colchicine (0.1-0.4%) (Seki and Oshikane, 1953; Swanson, 1957) or gamma rays (Katagiri, 1976). Following colchicine treatment, tetraploid mulberries often exhibit stunted growth, reduced branching, and shorter internodal distances, although the leaves show significant enlargement due to cellular gigantism (Sastry *et al.,* 1968; Das *et al.*, 1970). This gigantism results in thicker leaf blades, larger sex organs, and greater leaf weight and moisture content. Tetraploid leaves are notably thicker and coarser, with increased cellular tissue density, especially in the xylem and cortex (Tojyo and Watanabe, 1979). There are also significant increases in the size of palisade and spongy parenchyma cells, and the chloroplasts within these cells are 23-50% larger than those in diploid varieties (Tutaiug and Tagyjeva, 1968). Additionally, stomatal size increases by approximately 109 %, though stomatal frequency and cystolith content are lower than in diploids (Chakraborti *et al.*, 2013; Shafiei and Basavaiah, 2015). Despite these structural changes, tetraploids exhibit lower water transport rates compared to diploids (Tojyo, 1966a; 1966b). In terms of mulberry cultivation, tetraploid varieties offer several advantages, particularly in leaf yield. The increased size and thickness of tetraploid leaves contribute to higher foliage yields. Moreover, feeding silkworms with tetraploid leaves improves digestion and results in enhanced economic traits, such as increased silk content, filament length, and cocoon yield (Seki, 1959; Seki and Oshikane, 1959). Tetraploid varieties are also a valuable resource for developing triploid hybrids, which combine the beneficial traits of polyploidy to further optimize both silkworm feeding and leaf production (Das *et al.*, 1970).

Hexaploid

Hexaploid (2n=6x=84) mulberry species include *M. tiliaefolia, M. cathayana, M. serrata, M. australis, M. laevigata, M. celtidifolia, M. insignis, M. mongolica*, and *M. rubra* (Janaki Ammal, 1948; Seki, 1952 a; Basavaiah *et al.*, 1989; Katagiri *et al.*, 1994; Yamanouchi *et al.*, 2017; Mahshid, 2019). These hexaploids are either naturally occurring or induced through colchicine treatment, such as the work by Osawa who produced a hexaploid by treating a triploid plant with colchicine (Hamada, 1960). At the hexaploid level, a decrease in chromosome size is observed (Alizade and Akhundova, 1971), which is one of the characteristic traits of polyploidy. The meiotic behavior of hexaploid mulberries like *M. serrata* and *M. tiliaefolia* is generally regular, with a high frequency of bivalents, indicating a diploid-like behavior. However, there are occasional occurrences of hexa, penta, tetra, and trivalents, suggesting its allopolyploid nature. The regular meiosis and high pollen fertility observed in these hexaploids are indicative of their relatively stable genetic structure (Seki, 1952 b; Basavaiah *et al.*, 1989).

Phenotypically, hexaploid mulberries display distinct characteristics. The leaves, axillary buds, and shoots are different from lower ploidy levels, with hexaploids showing reduced leaf size but increased leaf thickness and weight. The internodes are highly condensed, and there is a noticeable decrease in stomatal frequency, while the size of the stomata and the number of chloroplasts per stoma are increased compared to triploids. In addition to the changes in leaf morphology, the induced hexaploid mulberries show a significant increase in fruit size and weight (by 60.47% and 89.25%, respectively), compared to triploid plants. There is also an enhancement in secondary metabolites, including anthocyanins, carotenoids, flavonoids, and phenolic compounds, which may contribute to the improved fruit quality (Shafiei and Basavaiah, 2018).

Despite these advantageous characteristics for fruit production, the foliage of hexaploid mulberries is generally not considered ideal for silkworm rearing, as it does not possess the same nutritional and digestibility profile as that of triploids and diploids (Hamada, 1960). However, hexaploid mulberry trees are highly valued for their wood, which is used in the manufacture of furniture, sporting goods, agricultural implements, and other products owing to its robustness and desirable properties (Anonymous, 1962). Hexaploid mulberries are also important in breeding programs, particularly as parent plants for generating tetraploids, which can be used for further polyploidy breeding or for developing better silkworm feed varieties (Basavaiah *et al.*, 1989).

Octoploid

A natural octoploid (2n=8x=112) mulberry was reported in *Morus cathayana* H. var. Fengdu-8, with Sichuan, China, being its recorded origin (Janaki Ammal, 1948). This octoploid variety exhibits normal meiotic behavior, as described in the colchioctoploid mulberry variety (Tojyo, 1985), where meiosis proceeds without significant irregularities. Another octoploid variety, Peylyashchii, was developed through inter-specific hybridization (Dzhafarov *et al.*, 1979), further demonstrating the potential for polyploidy to generate new varieties with unique traits. However, efforts to induce octoploid mulberries using colchicine treatment in two tetraploid genotypes have yielded limited success (Shafiei, 2017).

Docosaploid

Morus nigra L., commonly known as "Black Mulberry" and native to West Asia, holds a unique position among flowering plants, exhibiting docosaploidy (22x) with a chromosome number of 2n=308 (Janaki Ammal, 1948; Seki and Oshikane, 1960; Li and Yu, 1989). All of its chromosomes are small and S-type in structure (Agaev, 1984). Janaki Ammal (1948) suggested that *M. nigra* may have originated as an inter-specific hybrid through polyploidization, based on observations of stray polyploid varieties in *M. alba* and *M. cathayana*. Initially, *M. nigra* with 1x =15 emerged through polyploidization, followed by the appearance of a 28-chromosome mulberry (2x=14), with a large metacentric chromosome formed by the fusion of two small chromosomes. *M. nigra* is considered older than all other *Morus* species with 28 chromosomes, offering insight into the evolutionary history of mulberry (Agaev, 1984).

The pollen mother cells (PMCs) of *M. nigra* exhibit 154 bivalents at metaphase I, with regular meiotic divisions and good pollen fertility, reflecting the cytological stability achieved through polyploidy, which helps maintain its chromosomal structure (Agaev and Fedorova, 1970). Among the bivalents, rod-shaped bivalents are more frequent than ring bivalents, univalents, and multivalents (Azizian, 2001), indicating that bivalents outnumber multivalents, and univalents are rare. This suggests that *M. nigra* is an auto-allopolyploid species. Young PMCs typically have 1-4 nucleoli, and the pollen grains are uniform in size, each with 2-5 germpores (Basavaiah *et al.*, 1990). In a cross between *M. nigra* var. Char-tut (22x) and *M. alba* var. Zarif-tut (2x), Agaev and Fedorova (1970) isolated an F_1 hybrid (12x=168), which also demonstrated normal meiosis similar to its parent species. The nuclei size at the leptotene stage was 20.80 µm in *M. nigra* and 8.71 µm in *M. alba*. The absence of apomixis in *M. nigra* is confirmed by the presence of normal meiosis and viable microspores. Additionally, the variety with 308 chromosomes in *M. nigra* has the highest DNA content per cm^3 of leaf tissue, as recorded by Ploskina *et al.* (1978), underscoring its genetic richness.

Morphologically, *M. nigra* resembles other high-polyploid species such as *M. tiliaefolia* and *Broussonetia* sp. It is a giant type with notably large winter buds and thick leaves. The leaves of *M. nigra* have a thick epidermis, and the palisade parenchyma cells are large and irregularly arranged. Stomata in *M. nigra* are larger than those in other *Morus* polyploids (Seki *et al*., 1960). This species is cultivated in many countries for its edible fruits, and its leaves, stems, and fruits contain active secondary metabolites with antioxidant, anti-inflammatory, and blood sugar-lowering properties (Ahlawat *et al*., 2016). While the leaves of *M. nigra* are not favored in the sericulture industry due to their coarse texture and unpalatability to silkworms, the species plays a crucial role in mulberry breeding programs. It is widely used in cross-breeding with other *Morus* species to introduce beneficial traits (Aliev, 1979; Tojyo, 1985).

Aneuploid

Aneuploidy plays a significant role in the evolution of *Morus* species, as demonstrated by the occurrence of aneuploids with chromosome numbers such as 2n=26, 27, and 30 (Sinoto, 1929; Das, 1961; Kundu and Sharma, 1976). Monosomic (2n=28-1=27) mulberry lines have also been reported (Chu, 1986). Varieties like Kokuso-13, Rokokouyoso, and China Peking are aneuploids with 2n=28+2=30 chromosomes, arising from the formation of gametes with unbalanced chromosome numbers (Susheelamma *et al*., 1990). Additionally, a trisomic (2x+1=29) was derived from seedlings of mulberry crosses after temporary grafting onto *Broussonetia kazinoki* and *Cudrania tricuspidata* (Masayoshi, 1987). The chromosome numbers of seedlings obtained by pollinating a triploid (3x) female flower with diploid (2x) pollen, exhibited abnormal chromosomal counts of 29, 30, 40, 41, and 46 (Agaev, 1990a; Minamizawa, 1997). Three primary trisomic plants (2n=29) were isolated from triploid populations resulting from crosses between autotetraploid S-41 and diploid cultivars, such as K-2 and RFS-135. These trisomic plants displayed significant morphological variation, including dwarf growth, a small number of branches, irregular leaf shapes, light green coloration, and poor pollen fertility. Additionally, trisomic plants exhibited variations in leaf peroxidase isozyme banding patterns (Venkateswarlu *et al*., 1989). Despite these abnormalities, an aneuploid hybrid (2n=29) showed some superiority over its diploid parent but was inferior to the triploid parent in certain economic traits (Kumara and Basavaiah, 2016). These aneuploid mulberries serve as valuable cytogenetic tools for studying the independence of linkage groups, assigning linkage groups to specific chromosomes, and conducting genome-wide investigations of aneuploid syndromes (Kumara and Basavaiah, 2016).

Polysomaty

Polysomaty (mixoploids), a phenomenon where different chromosome numbers are found in different cells within the same genotype, has been observed in various polyploid mulberry species (Seki and Oshikane, 1957). This condition is common in vegetatively propagated plants, particularly in genetically unstable genotypes (Smulders *et al.,* 1994). For example, *M. multicaulis* and three strains of *M. alba*, namely China White-1, China White-6, and Mandalaya, exhibit polysomaty. The normal chromosome number for these mulberries is 28, but China White-1 showed cells with 2n=28 and 42 chromosomes, China White-6 exhibited 2n=26, 28, and 46 chromosomes, and Mandalaya displayed 2n=28, 30, and 56 chromosomes (Das, 1963). Similarly, *Morus latifolia* varieties and two strains of *M. alba* have been found to exhibit mixoploidy, with a mixture of 28 and 42 chromosomes (Koyama *et al.,* 2001). Other mixoploid mulberry varieties, such as Koltut 1, 2, and 4, have been reported to have chromosome numbers of 2n=28 and 56 (Agaev, 1990b), and the tetraploid variety Yaan-7 is a mixoploid with both 2x and 6x chromosome numbers (He *et al*., 1989).

et al. (2017) recorded chromosome numbers between 11 and 15, with some cells exhibiting 2n=12 chromosomes at metaphase, and 11-20% of the cells showing mixoploid characteristics. Polysomatic conditions are not limited to these varieties but also occur in chimeric structures, such as periclinal chimeras, which have been documented by Seki and Oshikane (1951) and Sikdar and Jolly (1994).

In some cases, polysomaty has been induced through grafting. For instance, when mulberry was grafted onto *Broussonetia kazinoki,* mixoploid plants with somatic chromosome numbers of 28 and 56 were obtained (Masayoshi, 1987). In these mixoploid plants, the chromosomes from 4x cells were observed to be thinner and longer than those from 2x cells. Phenotypically, mixoploid mulberries often show irregularities in leaf morphology. These plants are characterized by malformed, twisted, and asymmetric leaves, with irregular leaf tips, margins, and veins. The leaf surface is often coarse, uneven, and variegated in color. Anatomically, the leaf's cuticle, epidermal cells, and palisade parenchyma exhibit abnormalities, with irregular arrangements of these layers and variable leaf thickness. The tissues of the veins in mixoploid leaves are also abnormal. Additionally, mixoploid mulberries typically exhibit stunted growth, with short branches, smaller leaves at each order, and delayed, irregular sprouting (Seki and Oshikane, 1957; Tojyo, 1980). These morphological and anatomical traits are often linked to the irregularities caused by polysomaty in the plants' cellular structures.

Cytomixis

Cytomixis, a phenomenon first reported in mulberry by Verma *et al.* (1984), refers to the movement of nuclei from one plant cell to another via specialized intercellular channels, known as cytomictic channels. These channels are more specific and narrower than plasmodesmata, the common cytoplasmic bridges between plant cells (Mursalimov *et al.*, 2013). Cytomixis is particularly frequent during the early stages of meiosis and is often observed in genetically imbalanced plant types, such as hybrids, triploids, haploids, and other plants exhibiting chromosomal disturbances (Leavan, 1941). In mulberry, cytomixis is typically induced by hybridization events, especially those involving tetraploid and triploid plants. These hybrid cells, being genetically unstable and polyploid, are particularly susceptible to cytomictic movement of nuclei. The process of cytomixis leads to several meiotic abnormalities, including the formation of anucleate cells (cells lacking nuclei), multinucleolate cells (cells with more than one nucleolus), and cells with a chromosomal number that is either too high or too low. In addition, this phenomenon results in the formation of abnormal meiotic products such as triads, pentads, and hexads, rather than the usual dyads or tetrads. These abnormalities cause pollen sterility, reducing the fertility of the plant and leading to reproductive challenges in *Morus* species (Dwivedi *et al.*, 1989).

Karyotype

Karyotypic studies (Table 3) in *Morus* species have provided valuable insights into chromosomal organization, sex determination, and evolutionary trends. Early research by Tahara (1909) on *M. indica* and *M. alba* identified two large chromosomes, "alpha" and "beta," and suggested they played a role in sex determination in dioecious species. This idea was later expanded by Osawa (1920) and Sinota (1929) and Seki (1977), who also observed two large chromosomes in the diploid complement of dioecious mulberry species, supporting the hypothesis of sex chromosome involvement. However, Gill and Gupta (1979) found that these large chromosomes were not exclusive to dioecious species and were also present in some monoecious forms, leading them to conclude that the sex mechanism in *Morus* is likely controlled by genes rather than by specific sex chromosomes.

The karyotype of diploid *M. nigra* has been analyzed by Katsumata (1979), who identified a pair of large chromosomes (1.9 μm in length) along with over 13 pairs of smaller chromosomes ranging from 1.1 to 0.8 μm. This karyotype analysis reveals that the diploid mulberry genome consists of 28 chromosomes, with two large chromosomes (likely allosomes or megasomes) that are metacentric and contain nucleolar organizer regions (NORs), measuring 1.6-5.23 μm in different ploidy levels. The other chromosomes include medium-sized, submetacentric pairs (1.0-1.6 μm), while 12 pairs are acrocentric, exhibiting a narrow size variation (0.7-1.0 μm) (Chakraborti *et al.*, 1999). Abdullaev *et al.* (1977) noted that as chromosome number increases above the tetraploid (4x) level, there is a reduction in size and a shift in chromosome morphology. Karyomorphological studies of certain exotic mulberry cultivars have helped establish phylogenetic relationships among different species (Dandin *et al.*, 1987; Susheelamma *et al.*, 1990), with variations in morphological and anatomical characteristics often correlating with changes in the karyotype (Abdullaev, 1965).

Karyotypes of the haploid mulberry species *M. notabilis* have been analyzed by Xuan *et al.* (2017) using fluorescence *in situ* hybridization (FISH), which revealed two pairs of middle-length chromosomes and three pairs of dot chromosomes. The 14 chromosomes were grouped into seven pairs, with lengths ranging from 6.14 μm to 31.85 μm (Figure 5). This study suggests that the basic evolutionary trend in mulberry karyotypes is from symmetry to asymmetry. Primitive *Morus* species tend to have symmetrical karyotypes, while newer mulberry cultivars exhibit more asymmetrical karyotypes (Mahshid, 2019). The analysis of chromosome karyotypes in mulberry species is valuable for genetic studies, species identification, and the analysis of hybrid populations, providing critical insights for breeding programs (Chakraborti *et al.*, 1999).

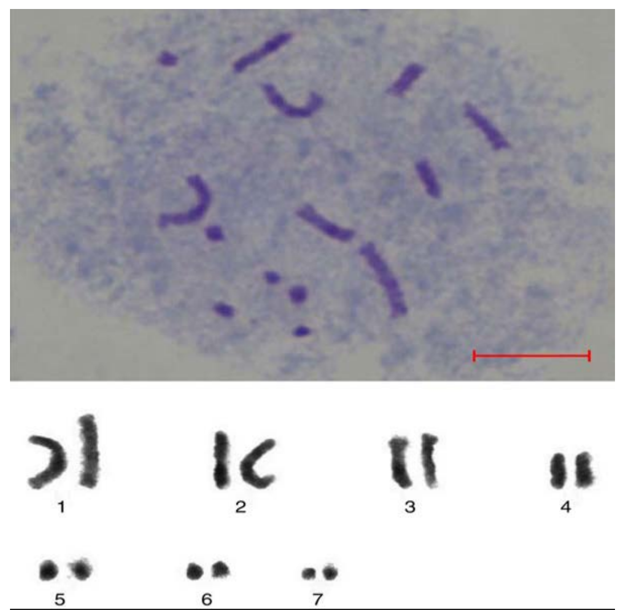

Figure 5: Chromosome karyotyping of *M. notabilis*
(Xuan *et al.*, 2016)

Table 3: Karyotype analysis of different *Morus* species

Mulberry species / variety	Ploidy level	Karyotype formulae	Range of chromosome length (μm)	Chromatin length (μm)	Reference
Morus alba L. (China Peking)	2n=2x=+2=30	$6^{M}+8^{m}+1^{sm}$	0.73-1.48	16.4	Susheelamma *et al.* (1990)
Morus latifolia P. (Kokuso-13)	2n=2x=+2=30	$5^{M}+10^{m}$	0.60-1.48	18.07	
Morus latifolia P. (Rokokuyoso)	2n=2x=+2=30	$10^{M}+5^{m}$	0.84-1.26	15.25	

Mulberry species / varieties	Ploidy level	Karyotype formulae	Range of chromosome length (µm)	Chromatin length (µm)	Reference
Morus latifolia P. (Kokuso-21)	2n=2x=28	$9^{M}+5^{m}$	0.94-1.70	18.34	Chu and Sun (1998)
Morus latifolia P. (Kokuso-27)	2n=2x=28	$17^{M}+4^{m}$	0.62-1.48	20.27	
Morus latifolia P. (Kosen)	2n=2x=28	$13^{M}+1^{m}$	0.84-1.24	14.14	
Morus latifolia P. (Roso)	2n=2x=28	$6^{M}+8^{m}$	0.84-1.48	14.78	
Morus bombycis K. (Kenmochi)	2n=2x=28	$10^{M}+4^{m}$	0.60-1.69	14.50	
Morus bombycis K. (Ichiei)	2n=2x=28	$7^{M}+13^{m}+1^{sm}$	0.95-1.48	25.45	
Morus bombycis K. (Mizusawa)	2n=2x=28	$7^{M}+7^{m}$	0.34-1.48	16.35	
Morus atropurpurea R.	2n=2x=28	$24^{M}+4^{sm}$			
Morus mizuho H.	2n=2x=28	$26^{M}+2^{sm}$	-		
Morus multicaulis P.	2n=2x=28	$24^{M}+4^{sm}$			
Morus alba L. (S-1)	2n=2x=28	$8^{A}+4^{B}+16^{C}$	1.52-3.88	24.94	Chakraborti (1999)
Morus indica L. (S-799)	2n=2x=28	$4^{A+}4^{B}+18^{C}+2^{D}$	1.47-3.28	16.47	
Morus indica L. (C-763)	2n=2x=28	$4^{A}+4^{B}+18^{C}+2^{D}$	1.29-3.18	16.66	
Morus indica L. (C-776)	2n=2x=28	$6^{A}+6^{B}+14^{C}+2^{D}$	1.29-5.23	19.97	
Morus indica L. (BC2-59)	2n=2x=28	$8^{A}+4^{B}+16^{C}$	1.17-3.11	24.98	
Morus indica L. (Local)	2n=2x=28	$12^{A}+8^{B}+8^{C}$	1.23-5.04	24.88	
Morus indica L. (S-1708)	2n=3x=42	$22B^{m}+20B^{sm}$	2.00-3.16	52.33	Venkatesh and Munirajappa (2012)
Morus indica L (RFS-135)	2n=2x=28	$10B^{m}+6B^{sm}+4C^{m}+8C^{sm}$	1.79-2.90	29.20	Venkatesh *et al.* (2013)
Morus indica L. (Mysore Local)	2n=2x=28	$4B^{m}+4B^{sm}+2C^{m}+8C^{sm}$	1.46-2.86	29.80	
Morus cathayana H.	2n=4x=56	$28B^{m}+22B^{sm}+4C^{m}+2C^{sm}$	1.26-3.22	69.52	Venkatesh and Munirajappa (2014)
Morus indica L. (Kollegal)	2n=2x=28	$18B^{m}+2B^{sm}+6C^{m}+2C^{sm}$	1.42-2.83	31.07	
Morus indica L. (S-13)	2n=2x=28	$4B^{m}+2B^{sm}+18C^{m}+4C^{sm}$	1.29-2.31	25.38	Venkatesh and Munirajappa (2015)
Morus indica L. (Victory-1)	2n=2x=28	$18B^{m}+8B^{sm}+2C^{m}$	1.59-2.86	32.43	
Morus indica L. (Tr-8)	2n=3x=42	$18B^{m}+12B^{sm}+6C^{m}+6C^{sm}$	1.49-2.59	45.65	
Morus indica L. (S-41)	2n=3x=42	$10B^{m}+26B^{sm}+4C^{m}+2C^{sm}$	1.40-2.83	46.80	Venkatesh and Munirajappa (2015)
Morus indica L. (Thysong-1)	2n=2x=28	$12B^{m}+8B^{sm}+6C^{m}+2C^{sm}$	1.66-2.70	30.84	

Type A: Chromosomes with sub-median constriction having a centromeric index ranged from 28.56-44.43% and the length of the chromosomes varied from 1.17-5.23 µm; Type B: Chromosomes with median constrictions have a centromeric index of 50% and chromosome length ranged from 1.29-3.94 µm; Type C: Chromosome with sub-terminal constrictions having a centromeric index ranged from 19.55-24.98% and chromosome length varied from 1.29-3.94 µm; Type D: Nucleolar chromosomes with sub-terminal and sub-median constrictions. Sub-terminal constriction has a centromeric index of 16.55%, while sub-median constriction has a centromeric index of 33.33%. Chromosome length varied from 1.5-1.7 µm (Chakraborti, 1999).

Nuclear DNA Content

The 1C DNA value represents the DNA content in the un-replicated haploid nucleus chromosome complement, where "C" stands for "constant," and it provides a baseline for understanding DNA content across various ploidy levels in plants. For diploids, the 2C DNA value corresponds to the total DNA content in a diploid somatic nucleus. To standardize DNA content measurements, 1 picogram (pg) of DNA is equal to 980 megabase pairs (Mbp) of DNA (Tuna *et al*., 2001). In polyploids, the relationship between the 2C DNA content (diploid) and 1C values is not linear: for instance, a triploid has a 2C value corresponding to 3Cx, a tetraploid has 2C=4Cx, a hexaploid has 2C=6 Cx, and the extraordinary docosaploid (2n=308) has a 2C value equivalent to 22Cx. This concept of DNA content measurement is crucial for understanding the genetic makeup of polyploid organisms like mulberry.

Flow cytometry has become a valuable technique in mulberry breeding, enabling breeders to quickly assess and develop new cytotypes, accurately determining DNA content (Table 4), and ploidy levels (Yamanouchi *et al*., 2017). For example, tetraploid mulberries have three times the DNA content of diploid mulberries, while DNA content in higher polyploids, such as hexaploids, increases at a slower rate compared to lower ploidy levels. A highly polyploid form, like the docosaploid mulberry (2n=308), only has 3.5-4 times the DNA content of a diploid, despite the large increase in chromosome number (Alizade and Akhundova, 1977). Moreover, triploids and tetraploids possess greater DNA content than diploids in their nuclei, chloroplasts, and mitochondria, with triploids in particular showing notable differences in DNA content from their diploid and tetraploid counterparts (Talyshinskii, 1982).

Further research by Yamanouchi *et al*. (2008) examined the polysomatic nature of several mulberry species and strains, including *M. acidosa, M. alba, M. bombycis, M. indica, M. kagayamae, M. latifolia*, and *M. rotundiloba*, across various ploidy levels (diploid, triploid, tetraploid, and mixoploid). Their studies also involved the ploidy levels of other species, including *M. laevigata, M. boninensis, M. nigra, M. celtidifolia, M. serrata*, and *M. tiliaefolia*(Yamanouchi *et al*., 2017). The DNA content of *M. nigra* (docosaploid) was determined to be 8.34 pg of DNA (Dalkilic and Dalkilic, 2018), offering further understanding of DNA variation in mulberry species. In addition, studies by Chang *et al*. (2018) analyzed the DNA amounts and ploidy levels of 27 promising mulberry breeding accessions, providing essential information for breeding programs. Kumara *et al*. (2021) also detected significant variation in nuclear DNA content between mother plants and their clonally derived genotypes, highlighting the complexities of genetic inheritance and polyploidy in mulberry.

Table 4: Ploidy status and DNA content of various Morus species

Mulberry species /variety	Ploidy level	Nuclear DNA amount (pg / c)	Reference
Morus alba (Ichinose)	Diploid	0.35 (2C=2Cx)	Yamanouchi *et al.* (2008)
Morus bombycis (Aizujyujima)	Diploid	0.37 (2C=2Cx)	
Morus bombycis (Ichibei)	Triploid	0.36 (2C=3Cx)	
Morus latifolia (Ookaraguwa)	Diploid	0.35 (2C=2Cx)	
Morus latifolia (Popberry)	Diploid in cytochimera	0.35 (2C=2Cx)	
Morus latifolia (Popberry)	Tetraploid in cytochimera	0.35 (2C=4Cx)	

Mulberry species /variety	Ploidy level	Nuclear DNA amount (pg / c)	Reference
Morus rotundiloba (Chiang Kam)	Triploid	0.36 (2C=3Cx)	
Morus rotundiloba (Noi)	Diploid	0.35 (2C=2Cx)	
Morus acidosa	Diploid	0.72 (2C=2Cx)	
	Diploid	0.70 (2C=2Cx)	
	Triploid	1.07 (2C=3Cx)	
Morus atropurpurea	Diploid	0.70 (2C=2Cx)	
Morus bombycis	Diploid	0.72 (2C=2Cx)	
	Triploid	1.10 (2C=3Cx)	
	Tetraploid	1.45 (2C=4Cx)	
Morus indica	Diploid	0.70 (2C=2Cx)	
Morus kagayamae	Diploid	0.73 (2C=2Cx)	
Morus latifolia	Diploid	0.70 (2C=2Cx)	
	Triploid	1.06 (2C=3Cx)	Yamanouchi *et al.* (2017)
Morus notabilis	Diploid	0.70 (2C=2Cx)	
	Triploid	1.04 (2C=3Cx)	
Morus rotundiloba	Diploid	0.70 (2C=2Cx)	
	Triploid	1.07 (2C=3Cx)	
Morus laevigata	Triploid	1.17 (2C=3Cx)	
Morus boninensis	Tetraploid	1. 63 (2C=4Cx)	
Morus celtidifolia	Tetraploid	1.42 (2C=4Cx)	
Morus serrata	Hexaploid	2.02 (2C=6Cx)	
Morus tiliaefolia	Hexaploid	2.48 (2C=6Cx)	
Morus nigra	Hexaploid	2.18 (2C=6Cx)	
Morus nigra (Black Mulberry)	Docosaploid	7.26 (2C=22Cx)	
Morus laevigata (Elongated mulberry No.1)	Docosaploid	8.34 (2C=22Cx)	Dalkilic and Dalkilic (2018)
Morus australis Poir	Triploid	1.06 (2C=3Cx)	
(Acc. 67C001)	Diploid	0.63 (2C=2Cx)	Chang *et al.* (2018)
Morus atropurpurea	Diploid	0.71 (2C=2Cx)	
Morus indica (M-5)	Diploid	0.82 (2C=2Cx)	
Morus indica (TG-1)	Diploid	0.94 (2C=2Cx)	
Morus indica (RFS-135)	Diploid	0.76 (2C=2Cx)	
Morus indica (Anantha)	Diploid	0.81 (2C=2Cx)	Kumara *et al.* (2021)
Morus indica (S-1635)	Triploid	1.23 (2C=3Cx)	
Morus indica (Vishala)	Triploid	1.31 (2C=3Cx)	

Genomics

Genomics is a rapidly advancing field within genetics that focuses on studying the complete genome of organisms, including the precise structure of genes, their functions, and the regulatory mechanisms that control their expression. In recent years, technological breakthroughs have revolutionized our ability to analyze the genomes of a wide range of plants, including mulberry. These innovations have significantly narrowed the gap between genotype (the genetic makeup) and phenotype (the observable traits), providing deeper insights into how genetic variations influence mulberry characteristics. In particular, advancements in high-throughput sequencing technologies, such as next-generation sequencing (NGS), have enabled scientists to sequence the entire genome of mulberry species with greater efficiency and accuracy. This progress has allowed for the identification of specific genes associated with important traits like leaf quality, growth rate, and resistance to diseases or pests, which are crucial for improving mulberry varieties used in silkworm rearing and other applications. By examining the genetic architecture of mulberry genomes, researchers can now pinpoint the underlying genetic factors responsible for key physiological and morphological traits.

Furthermore, genomics has facilitated the identification of genes that are involved in important processes like polyploidy, which is a common feature in mulberry species. Polyploidy plays a significant role in the growth and development of mulberry plants, influencing traits such as leaf size, fruit yield, and overall plant vigor. The ability to isolate genes responsible for polyploidy and mutations opens up new possibilities for breeding programs aimed at developing superior mulberry varieties with enhanced traits for sericultural and horticultural purposes.Overall, the fast-evolving field of genomics has enhanced our understanding of the genetic makeup of mulberry and paved the way for more targeted and efficient breeding strategies, reducing the time and resources needed to improve cultivars and address challenges in mulberry production.

Nuclear Genome

The first whole genome sequence of a mulberry species was completed for the haploid *Morus notabilis* using the shotgun sequencing method. This species has seven distinct pairs of chromosomes in its somatic cells, and its genome size was estimated at 330.79 Mb. The genome contains approximately 29,338 genes, with 27,085 predicted to be protein-coding genes and 2,253 predicted to be partial genes. Notably, about 45 % of the *M. notabilis* genome consists of repetitive sequences, with over 50 % of these being transposable elements, particularly long-terminal retrotransposons like Copia-like and Gipsy-like elements. The GC content of this genome is 35.02 %, which is similar to other eudicots (He *et al*., 2013). In addition to *M. notabilis*, the genomes of other mulberry species have also been sequenced, providing valuable insights into their genetic architecture. For instance, the genome of *M. alba* (cv. Heyebai) was estimated to be 346.39 Mb, with 180.11 MB of repetitive sequences. This genome contains 22,767 protein-coding genes, with an average gene length of 3,209 bp, an average coding sequence length of 1,148 bp, and an average of 5.09 exons per gene. The GC content of this genome is 33.2 % (Jiao *et al.,* 2020). Similarly, the genome of *M. indica* (cv. K-2) has been sequenced, revealing a larger genome size of 505.29 Mb, with 49.2 % repetitive DNA sequences. This species contains 27,435 protein-coding genes and has a GC content of 31.2 % (Jain *et al*., 2022). These genomic studies highlight the diversity in mulberry genome sizes and the complex nature of their genetic makeup. The long-terminal repeat (LTR) retrotransposons play a significant role in driving the divergence and evolution of mulberry genomes, particularly after species have diverged from one another (Xuan *et al*., 2022).

Chloroplast Genome

Chloroplast DNA (cpDNA) is a valuable tool for the identification and evolutionary studies of *Morus* species due to its distinct characteristics, including a small genome size, low nucleotide substitution rate, uniparental inheritance, and highly conserved genomic organization compared to mitochondrial and nuclear genomes. The chloroplast genomes of *Morus* species generally range from 158,000 to 600,000 base pairs (bp), with *M. alba* having the largest chloroplast genome at 159,290 bp, and *M. mongolica* having the smallest at 158,459 bp. These genomes typically contain two identical large single-copy (LSC) regions, two identical small single-copy (SSC) regions, and two inverted repeat (IR) regions. Interestingly, the GC content and IR regions are consistent across the species studied.In terms of variation, *M. cathayana* and *M. alba* possess notably longer LSC regions compared to other species, with lengths of 88,143 bp and 88,065 bp, respectively. In contrast, *M. multicaulis* has the highest single-copy region among the six species examined, with a length of 20,035 bp. Despite these variations in genome size and structure, all *Morus* species share a common set of protein-coding genes within their chloroplast genomes, although differences exist in the sizes of their rRNA and tRNA genes, particularly in *M. indica, M. notabilis*, and *M. alba*.A phylogenetic analysis using chloroplast genomic data revealed that *M. alba* and *M. cathayana* are closely related, reinforcing their genetic similarity. Furthermore, the analysis suggested that the Moraceae family, to which mulberry belongs, is phylogenetically linked to the Ulmaceae family,a relationship that highlights the evolutionary connections between these plant families (Kong and Yang, 2016; Li *et al.*, 2016; Luo *et al.*, 2019).

Mitochondrial Genome

The mitochondrial genomes of plants, known for their distinctive evolutionary trends, typically exhibit large sizes, a high rate of rearrangement, and a relatively low mutation rate. Recent studies on the mitochondrial genomes of *Morus* species, specifically *M. atropurpurea* and *M. multicaulis*, have provided deeper insights into the genetic structure and evolution of mulberry. Liangliang *et al.* (2021) analyzed and assembled the mitochondrial genomes of these two *Morus* species, revealing distinct differences in their genomic architecture. The mitochondrial genome of *M. multicaulis* is a circular structure with a length of 361,546 base pairs (bp). It contains a total of 54 genes, including 31 protein-coding genes, 20 tRNA genes, and 3 rRNA genes. The nucleotide composition of this genome consists of 27.38 % adenine (A), 27.20 % thymine (T), 22.63 % cytosine (C), and 22.79 % guanine (G). On the other hand, *M. atropurpurea* has a longer mitochondrial genome, measuring 395,412 bp, and contains 57 genes in total, which include 32 protein-coding genes, 22 tRNA genes, and 3 rRNA genes. One notable feature of the *M. multicaulis* mitochondrial genome is the presence of sequence repetitions, as well as an RNA editing gene. Additionally, there is evidence of gene migration from the chloroplast (cpDNA) to the mitochondrial genome, a phenomenon that is not uncommon in plant evolution. These findings suggest a dynamic interaction between different organellar genomes in the evolutionary history of *Morus*. Phylogenetic analysis based on the full mitochondrial genomes of *M. multicaulis, M. atropurpurea*, and 28 other species revealed important insights into the evolutionary and taxonomic relationships of mulberry. The analysis showed consistency with traditional molecular classification and taxonomy, supporting the placement of *Morus* within the angiosperm family. It also confirmed the GC content and evolutionary status of *Morus*, highlighting the unique mitochondrial evolutionary trends that distinguish it from other plant species.

Morus Genome Database and its applications

The *Morus* Genome Database (MorusDB), developed by Li *et al.* (2014), is an open-access, web-based platform designed to support mulberry research by providing easy access to large-scale genomic data and facilitating data mining. *Morus*DB offers a comprehensive set of resources, including genomic sequences, gene annotations, and various bioinformatics tools, making it a valuable tool for researchers working on mulberry genomics and related fields.The database includes data on mulberry's genomic sequencing and assembly, with predicted genes, functional annotations, expressed sequence tags (ESTs), transposable elements (TEs), and gene ontology (GO) terms. Additionally, it highlights horizontal gene transfers between mulberry and the silkworm (*Bombyx mori*), as well as ortholog and paralog groups. *Morus*DB also provides an integrated source of these diverse topics, offering easy access to information related to mulberry's genetic and functional landscape.

Researchers can explore and download transcriptome sequencing data for different mulberry tissues, including the root, leaf, bark, winter bud, and male flower, all from the model species *Morus notabilis*. In addition, *Morus*DB is equipped with an analytical workbench that supports genomic research and comparative genomics. This includes tools like BLAST for sequence alignment, Search GO for gene ontology term searches, Mulberry GO for mulberry-specific annotations, and Mulberry GBrowse for visualizing genomic data. Designed to support research on mulberry and other species within the Moraceae family, which includes important fruit crops, *Morus*DB is particularly valuable for studying the interactions between mulberry plants and silkworms. It is a key resource for geneticists and molecular biologists working in plant genomics, crop improvement, and silkworm-breeding research. *Morus*DB is hosted as a basic platform supported by SilkDB, a related resource, and can be accessed via the following URL: http://morus.swu.edu.cn/morusdb.

Conclusions and prospects

Cytogenetic research plays a crucial role in advancing our understanding of mulberry genetics, breeding, evolution, and overall plant development. Despite the significant progress made in cytogenetic studies of mulberries compared to other crops, challenges remain in fully characterizing the genetic diversity of the genus *Morus*. This is primarily due to the complex nature of mulberry plants, which are perennial, arborescent (tree-like), and possess small chromosomes, making traditional cytogenetic methods more difficult to apply effectively. The chromosome numbers of many mulberry genotypes are still underreported, and the complete catalog of germplasm accessions remains lacking.Additionally, the inherent heterozygosity of mulberry complicates genetic studies, as it introduces variability that is difficult to document and analyze. Traditional chromosome preparation methods, such as pressing, often yield suboptimal results due to the small size and large number of chromosomes in *Morus*. This has hampered the ability to fully explore the chromosomal characteristics of mulberry species and varieties.To overcome these challenges, advanced cytogenetic techniques are now being applied. For instance, flow cytometry is widely used to measure DNA content and ploidy levels, offering an efficient and accurate method for determining the genetic constitution of mulberry plants. Chromosome banding techniques (such as G-banding, Q-banding, C-banding, and N-banding) are also valuable for identifying chromosomes based on characteristic banding patterns, helping to distinguish between chromosomal abnormalities and structural rearrangements. Fluorescence *in situ* hybridization (FISH) is another powerful technique that enables researchers to locate specific nucleic acid sequences on individual chromosomes, providing insights into the structural and functional organization of the genome.Karyotype analysis of mulberry species is especially important, as it provides valuable taxonomic information and helps researchers understand the genetic makeup of different species, which can be crucial for breeding programs. However, of the 68 known species of *Morus*, only a few have detailed ploidy-level data. This highlights the need for further cytogenetic research to determine the ploidy levels of the remaining species and to better understand the genetic diversity within the genus. Given the importance of genetic improvement in mulberry, there is significant potential for the integration of modern genomic and molecular biology tools into breeding programs. Techniques such as next-generation sequencing (NGS), CRISPR gene editing, and transcriptomics can expedite the identification of beneficial traits, improve resistance to diseases, and enhance the yield and quality of mulberry crops. The application of these advanced tools is essential for accelerating mulberry genetic improvement and developing varieties that can meet the growing demands of industries such as sericulture, fruit production, and wood products.

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NIRMOOL, AN ECO- & USER-FRIENDLY GENERAL DISINFECTANT FOR SERICULTURE

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Abstract

Disinfection forms an integral activity in sericulture for successful harvest of silk cocoon crops. Majority of Indian sericulture farmers spray 5 % bleaching powder and chlorine dioxide as general disinfectants for the disinfection of silkworm rearing room and rearing appliances. These chlorine-based disinfectants are even though effective against all silkworm pathogens, they also have numerous disadvantages especially by being environmentally harmful and non-user friendly. The present study was undertaken to identify eco-friendly compounds/chemicals that would inactivate all the silkworm pathogens to overcome the drawbacks of the existing popular disinfectants. Two promising chemicals *viz.,* oxidizing and wetting agents were further evaluated by *in vitro* inactivation studies and an effective formulation was identified for the disinfection of silkworm rearing house and rearing appliances. The same was named as Nirmool. The efficacy of Nirmool was further evaluated through elaborate laboratory studies *i.e., in vitro* and *in vivo,* simulated conditions, rearing room disinfection and in-house validation. The performance of Nirmool for all the significant pre- and post-cocoon parameters was on par with 5 % bleaching powder. Nirmool besides being effective disinfectant against all the known silkworm pathogens, is also easily soluble in water, non-corrosive, possesses higher shelf life, eco- and user friendly as well as cost-effective. An Indian patent application (202031050005 dt. 17-11-2020) has been filed to protect the invention. Trademark for Nirmool was also registered as a disinfectant suitable for disinfection of rearing room and rearing appliances in sericulture (Trade Marks Journal India, No: 2073, Class 5: 5146724, 24/09/2021).

Key words: Disinfection, eco- & user-friendly, Nirmool, rearing room, silkworm pathogens.

Introduction

Sericulture is a potential avocation that provides income for the farming community in quite a few countries. Mulberry silkworm, *Bombyx mori* is susceptible to different microorganisms resulting in significant crop losses. Farmers frequently encounter major silkworm diseases such as grasserie (BmNPV), Muscardine *(Beauveria bassiana)*, Pebrine *(Nosema bombycis)* and flacherie, a syndrome inflicted by non-occluded viruses (BmDNV/BmIFV) and bacteria (*Staphylococcus* spp.). It is imperative to prevent and appropriately manage the silkworm diseases for successful cocoon crop harvests (Sivaprasad *et al.*, 2021). Disease management approaches to prevent silkworm diseases in sericulture industry primarily involves the prophylactic measures *i.e.*, disease free silkworm eggs, disinfection of rearing room and rearing appliances, application of silkworm rearing seat/bed disinfectants and maintenance of personal/rearing hygiene. Amongst the above measures, the disinfection of silkworm rearing room and rearing appliances before the commencement and upon completion of each crop plays a very important role in diminishing the degree of disease incidence.

Destruction of disease causing pathogens is termed as 'disinfection' and is accomplished by diverse processes, but the most efficient method is by employing chemicals as disinfectants. Proper disinfection of rearing room, rearing appliances and the areas adjoining the rearing house before the commencement of rearing ascertains the environment devoid of pathogens, which is essentially conducive for silkworm rearing. Pathogen load accumulated over the larval rearing period, if any is sterilized by disinfection upon the completion of rearing. As physical factors such as temperature and humidity play a significant role in pathogen multiplication, the importance of silkworm rearing room disinfection becomes more prominent across the tropics.

The ideal characteristics of a disinfectant includes extensive antimicrobial spectrum, efficiency in the presence of organic matter, non-toxicity to the silkworm, odorless or pleasant, easy solubility in water, effective across wide range of pH, rapid action, non-corrosiveness, non-hazardous, stable upon storage, eco- and user-friendly nature, ease of availability of raw materials and relatively less-expensive. Most of the disinfectants developed and being used in sericulture currently are all either formaldehyde or chlorine or phenol based formulations and each has its own pros and cons. Moreover, they all are developed over a period of timelines. Especially in the Eastern and North Eastern regions of India, 5 % bleaching powder and chlorine dioxide based disinfectants are widely employed for the disinfection of silkworm rearing room and rearing appliances.

Though 5 % bleaching powder and chlorine dioxide are effective against major silkworm pathogens and are used for disinfection across different sectors apart from sericulture, they have certain disadvantages like corrosive nature (Santha *et al.*, 2012; Romanovski *et al.*, 2020; Li *et al.*, 2023), less solubility in water (Bunn *et al.*, 1935), hazardous to the users besides being non-friendly to the environment (Couri *et al.*, 1982; Condie, 1986; Chhetri *et al.*, 2019; Subpiramaniyam, 2021; Parveen *et al.*, 2022). Apart from the scientific literature, the cons of aforementioned disinfectants are also cited by environmental protection agencies of different countries (https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_G-77_1-Sep-91.pdf; https://www2.mst.dk/Udgiv/publications/2015/06/978-87-93352-33-9.pdf; https://iris.epa.gov/static/pdfs/0496tr.pdf). In the quest for developing an effective and efficient general disinfectant for the inactivation of major silkworm pathogens, the paper presents development of an eco- and user-friendly disinfectant formulation, Nirmool for the disinfection of silkworm rearing house and rearing appliances.

Materials and Methods

Screening of eco-friendly chemicals

Seven eco-friendly chemicals including Citric acid (T1; inactivates microbes by lowering the intracellular pH and altering the physiological processes; Su *et al.*, 2014); Glyoxal (T2; denatures microorganism proteins; Kung and Bagutti, 2016); Iodine (T3; retards microbial growth by disruption of electron transport, DNA denaturation or membrane destabilization;

Mc. Donnell and Russell, 1999); Oxidizing agent (T4; oxidizes the critical cellular components); Peracetic acid (T5; denatures proteins, disrupts cell wall permeability, oxidizes sulfhydral and sulfur bonds in microorganisms proteins, enzymes and other metabolites; Mc. Donnell and Russell, 1999); Tetrasodium pyrophosphate (T6; chelates ions leading to disruption of cell wall permeability in microbes; Lorencova *et al.,* 2012); Wetting agent (T7; disrupts microbes membrane integrity) were selected based on their antimicrobial activity and eco-friendliness. These chemicals were screened for their efficacy against all known major silkworm pathogens at different concentrations (1, 2 and 5 %).

Culturing of major silkworm pathogens

The type cultures of causative agents of major silkworm diseases *viz.,* bacteria (*Staphylococcus* spp., *Bacillus thuringiensis*), fungi *(Beauveria bassiana)*, viruses (BmNPV, BmDNV1, BmIFV) and microsporidian *(Nosema bombycis)* maintained at Central Sericultural Research & Training Institute, Central Silk Board, Govt. of India, Berhampore-742101, West Bengal, India were utilized in the present study. The stock inoculums were prepared employing the standard procedures described by Sivaprasad *et al.* (2021) and the same were utilized to prepare working dilutions or known concentrations for further experimentation.

Bacteria: Gram positive pathogenic bacteria *viz.,* non-sporulating *Staphylococcus* spp. and sporulating *Bacillus thuringiensis* were cultured in nutrient broth (g l^{-1}: peptone, 5; beef extract, 1.5; yeast extract, 1.5; NaCl, 5) and incubated at 30+2°C for 36 hours. Bacterial enumeration was determined by serial dilution technique.

Beauveria bassiana was cultured on potato dextrose broth (g l^{-1}: potato infusion, 200; dextrose, 20; pH 5.6±0.2) and incubated at 30±2°C for 72 hours. The conidial concentration was determined by Neubauer haemocytometer.

BmNPV: The haemolymph of grasserie-infected larvae was collected in sterile distilled water, incubated overnight at room temperature and centrifuged at 10,000 rpm for 10 minutes. The pellet was washed 4-5 times with distilled water followed by twice with sterile saline solution. The BmNPV polyhedra were suspended in distilled water and further purified by gradient centrifugation. The polyhedral count was determined by Neubauer haemocytometer and kept for further use as stock BmNPV inoculum in sterile distilled water.

BmDNV1: The midguts of BmDNV1-infected larvae were collected, macerated in phosphate buffered saline, pH 7.4 (9 ml/g fresh weight) and filtered using a muslin cloth. The filtrate was centrifuged at 10,000 rpm for 10 minutes and the supernatant was subjected to 0.22µ anti-microbial filters for removing the bacterial contaminants. The filtrate was stored at -20°C as stock BmDNV1 inoculum (10^{-1} dilution) for further utilization.

BmIFV: The midguts of BmIFV-infected larvae were collected, macerated in phosphate buffered saline, pH 7.4 (9 ml/g fresh weight) and filtered through a muslin cloth. The filtrate was centrifuged at 10,000 rpm for 10 minutes and the supernatant was subjected to 0.22µ anti-microbial filters for removing bacterial contaminants. The filtrate was stored at -20°C as stock BmIFV inoculum (10^{-1} dilution) for further utilization.

Nosema bombycis: Pebrine inoculated larvae were collected, macerated in K_2CO_3 solution (0.6%; 3ml/g fresh weight) and filtered through muslin cloth. The filtrate was centrifuged at 2500 rpm/10 minutes and the pellet containing microsporidian spores was collected, washed thrice with saline followed by sterile water. The isolated spores were purified employing iso-density equilibrium centrifugation with Percoll. The spore count was determined by Neubauer haemocytometer and *N. bombycis* spores were stored in the refrigerator as stock inoculum.

Inactivation of silkworm pathogens

1ml silkworm pathogen suspensions (Bacteria: *Staphylococcus* sp. & *B. thuringiensis* at 1.7 x 10^7 cfu/ml; Fungi: B. *bassiana* at 2 x 10^7conidia/ml; Microsporidia: N. *bombycis* at 1 x 10^7 spores/ml; Viruses: BmNPV at 1x10^7 polyhedra/ml) were centrifuged at 10,000 rpm for

10 minutes and the pellets were suspended in 1 ml disinfectant chemical/formulation(s) at 1, 2 and 5 % concentrations. The pathogens were exposed to the disinfectant solution for a period of 10 minutes at room temperature (25±1°C). On the completion of inactivation period, the pathogen-disinfectant suspensions were centrifuged and the supernatants were discarded. The pellets were washed thrice with sterile distilled water to remove the traces of disinfectants. Finally, the pellets were suspended in 1ml sterile distilled water and used for further experimentation. In the case of BmDNV1 and BmIFV, the stock inoculums were diluted to 10^{-2} concentration using the disinfectant solution directly for imposing the inactivation treatment. The suspensions were centrifuged at 10,000 rpm for 10 minutes and the supernatants were retained for further experimentation. Pathogens treated with distilled water were maintained as negative controls or mock inocula in the experimentation. The *in vitro* inactivation studies were conducted in three replicates.

Microscopic examination of inactivated pathogens

A smear of pellets of bacterial, fungal, BmNPV polyhedra and microsporidian spores followed by inactivation were individually examined by light microscopy (600X) on a clean glass slide with a glass cover slip. Any changes in the cellular morphology and physical integrity of the pathogens were recorded upon treatment with disinfectant chemicals/formulations.

in vitro studies on culturing of inactivated bacterial & fungal pathogens

The inactivated-pathogen suspensions especially bacteria and fungi were inoculated in nutrient agar and potato dextrose broth (a loop-full in each case), respectively for conducting *in vitro* culture experiments. Bacterial pathogens (*B. thuringiensis* and *Staphylococcus* spp.) were streaked onto the agar plates; while the fungal pathogen, *B. bassiana* was inoculated in broth. The microbial cultures were incubated under standard culture conditions and the growth of microbial pathogens was recorded (7 replications/ treatment). Sterile distilled water and untreated samples were maintained as experimental controls.

in vivo studies of inactivated pathogens

The disinfectant chemical treated pathogen suspensions (BmNPV, BmDNV1, BmIFV, *B. thuringiensis, Staphylococcus* spp. and *N. bombycis*) were smeared onto the mulberry leaves individually and air-dried for conducting the *in vivo* studies. The treated mulberry leaves (1 ml/100 larvae; 25 larvae each in 7 replications/ treatment) were fed to the III instar silkworm larvae (Nistari x SK6.SK7). The batches of larvae fed with untreated mulberry leaves and untreated pathogen inoculums were maintained as experimental controls. *B. bassiana,* the fungal pathogen suspension was smeared directly onto the silkworm larval surface as it infects *via* per cutaneous route. The silkworm larvae were reared up to 10 days following standard rearing conditions. The observations were recorded on the development of disease symptoms and larval mortality in each treatment/replication. The incidence of silkworm diseases was also recorded for respective treatments based on typical disease symptoms, microscopic examination of haemolymph/midgut and immunodiagnosis in case of BmDNV1 and BmIFV. The remaining silkworms were also tested for the presence of different pathogens 10 days post-treatment to determine the healthiness of silkworm larvae.

Development & evaluation of eco-friendly rearing room disinfectants

The chemicals proven to be effective against all the major silkworm pathogens based on the pathogen inactivation studies (microscopic observation, *in vitro* and *in vivo* studies) were selected and their effective concentrations determined to develop the disinfectant formulation. A general disinfectant titled “Nirmool” was developed for disinfection of silkworm rearing house and rearing appliances in sericulture. Further experiments with regard to simulated contamination, rearing room disinfection, in-house and field evaluation were undertaken to prove the efficacy of Nirmool.

Simulated contamination studies

The silkworm pathogen suspensions (BmNPV, BmDNV1, BmIFV, *B. thuringiensis, Staphylococcus* sp., *B. bassiana* and *N. bombycis*) were individually smeared onto three sets of paraffin sheets and air-dried for conducting the simulated contamination studies. One set of paraffin paper sheets were disinfected with 5 % bleaching powder (popular disinfectant), another with Nirmool (2% conc.) and the third with pathogen suspension treated with distilled water (control) were maintained and rearing was performed on these paraffin sheets following standard rearing conditions. Nistari x SK6.SK7 silkworm larvae were brushed onto the paraffin sheets (one dfl each; 7 replications/each treatment). The overall rearing performance including the cocoon harvest details was recorded. The incidence of silkworm diseases was recorded for the respective treatments based on typical disease symptoms, microscopic examination of haemolymph/midgut and immunodiagnosis in case of BmDNV1 and BmIFV. The data for each treatment were pooled for analytical purposes.

Rearing room disinfection studies

One rearing house along with the rearing appliances was disinfected with 5 % bleaching powder and another rearing house along with rearing appliances with 2% Nirmool. 5 dfls each of crossbreed (Nistari x SK6.SK7) were reared following standard rearing conditions across three seasons inside the rearing houses. The overall rearing performance including the cocoon harvest details was recorded.

In-House evaluation of Nirmool

In-house evaluation of Nirmool was conducted at different laboratories (Multivoltine Breeding & Genetics; Bivoltine Breeding & Genetics) of CSRTI-Berhampore and P2 Basic Seed Farm (NSSO), Karnasubarna, West Bengal. The rearing house along with rearing appliances was disinfected with 2% Nirmool. Different silkworm breeds/hybrids (5-100 dfls/batch) were reared following the standard rearing conditions. The overall rearing performance including the cocoon harvest details was recorded.

Field evaluation of Nirmool

Field evaluation of Nirmool was conducted by disinfecting the silkworm rearing rooms along with the rearing appliances of different sericulture farmers in West Bengal, India. The disinfection of rearing houses was conducted under the supervision of researchers. Farmers have conducted silkworm rearing of different silkworm breeds/hybrids (100-400 dfls/batch) following the standard rearing conditions. The overall rearing performance in terms of cocoon harvest details was recorded.

The personnel involved in in-house and field evaluation of Nirmool were also queried for collecting the data on the efficacy of the product in terms of its ease in usage, solubility in water, non-corrosive properties and user friendliness apart from the cocoon harvest details.

Statistical & Economical Analysis

Appropriate experimental designs were followed for testing Nirmool with other disinfectants. Data were analyzed with descriptive statistics, t-test and ANOVA for logical inferences (Ramesh *et al.,* 2020). Partial budgeting technique (Pande *et al.,* 2018) was performed for tech-economic feasibility of developed technology, Nirmool.

Results and Discussion

The seven eco-friendly chemicals screened against major silkworm pathogens by pathogen inactivation followed by microscopic observation, *in vivo* and *in vitro* studies exhibited variations in their antimicrobial activity. Citric acid was not effective against any of the silkworm pathogens even at the highest concentration tested (5 %). Glyoxal was effective only against *Staphylococcus* spp. even at 1%; Iodine was effective against *Staphylococcus* spp. at 5% concentration and also inhibited the growth of *B. bassiana* even at 1%.

Peracetic acid was not effective against *B. thuringiensis* and *N. bombycis.* Tetrasodium pyrophosphate was effective against all the silkworm pathogens except *B. thuringiensis.* Only T4 (Oxidizing Agent; OA) and T7 (Wetting Agent; WA) were effective against all the known major silkworm pathogens (Table 1).

Table 1: Screening of eco-friendly chemicals against silkworm pathogens

Treatment	Test Compounds	Conc. (%)	*Bacillus thuringiensis*	*Staphylococcus* spp.	*Beauveria bassiana*	*Nosema bombycis*	BmNPV	BmDNV1	BmIFV
T1	Citric Acid	5	-	-	-	-	-	-	-
		1	-	+	-	-	-	-	-
		2	-	+	-	-	-	-	-
T2	Glyoxal	5	-	+	-	-	-	-	-
		1	-	-	+	-	-	-	-
		2	-	-	+	-	-	-	-
T3	Iodine	5	-	+	+	-	-	-	-
		1	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+
T4	Oxidizing Agent	5	+	+	+	+	+	+	+
		1	-	+	+	-	+	+	+
		2	-	+	+	-	+	+	+
T5	Peracetic Acid	5	-	+	+	-	+	+	+
		1	-	+	+	-	+	+	+
		2	-	+	+	-	+	+	+
T6	Tetrasodium pyrophosphate	5	-	+	+	+	+	+	+
		1	+	+	+	+	+	+	+
T7	Wetting Agent	2	+	+	+	+	+	+	+
		5	+	+	+	+	+	+	+
T8 (Control)	5% Bleaching Powder	5	+	+	+	+	+	+	+

+ indicates effectiveness to inactivate the silkworm pathogens, while - indicates ineffectiveness.

The OA destroys the microbial cell walls thereby bringing about the sterilizing effect. The key advantage of employing the OA is that it yields environment-friendly by-products. The eco-friendly WA forms an extremely basic solution in water, which disrupts the microbial membrane integrity. Previous studies also depicted differential activity of disinfectants against diverse groups of microbes across sectors including health, poultry and sericulture (Mc. Donnell and Russell, 1999; Jiang *et al.,* 2018; Balavenkatasubbaiah *et al.,* 1994, 1999, 2006).

The effective concentration of efficient disinfectant chemicals *viz.*, OA and WA at 1% concentration (w/v) against major silkworm pathogens was deciphered. However, combining OA and WA at 1% concentration as a disinfectant formulation led to augmentation in stability, wetting characteristics as well as destaining properties. A new eco- and user-friendly disinfectant formulation, Nirmool was developed which is suitable for disinfection of silkworm rearing house and rearing appliances. For preparation of 100 litres of disinfectant solution, 2 kg Nirmool powder is dissolved in 100 litres of water. The rearing room and appliances must be drench-sprayed employing a power sprayer. The quantity of disinfectant solution required for disinfection of rearing house, rearing surroundings and rearing appliances is 1.5 l/sq.m floor area or 140 ml/sq.ft. floor area of rearing house (room height of 3 m or 10 ft.) + 10% of total quantity for disinfection of rearing house surroundings. An additional quantity of 500 ml/sq.m or 14 ml/sq.ft of disinfectant must be sprayed for every m or 1 ft increase in the height of rearing room. The efficacy of Nirmool is illustrated in detail by the elaborate studies conducted which included pathogen inactivation followed by microscopic observation, *in vivo* and *in vitro* experiments, simulated contamination studies, rearing room disinfection and in-house evaluation trials.

The new innovation, Nirmool induced variations in cellular morphology of different pathogens. BmNPV polyhedral bodies were completely dissolved and no polyhedra could be observed. *N. bombycis* spores structural integrity was not noticed and however, disintegrated spore remains were recorded. In the case of *B. bassiana,* the conidial shape was distorted and structural integrity was lost. Distortion of bacterial cells was observed in *Staphylococcus* spp. Aggregation of *B. thuringiensis* cells was observed. The control samples (silkworm pathogens treated with distilled water) retained the physical integrity upon inactivation-exposure (Figure 1). The cellular morphology variations in major silkworm pathogens leading to their inactivation on disinfectant exposure were also described previously and the results in the present study corroborate with the same (Josepha *et al.,* 2018).

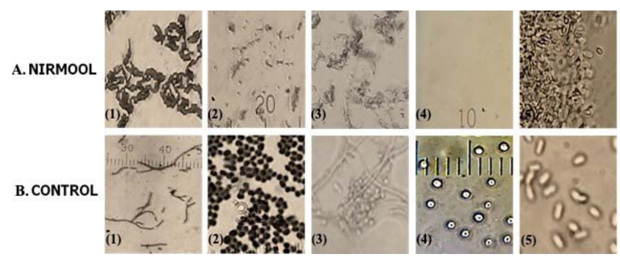

Figure 1: Inactivation of silkworm pathogens and effects on cellular morphology by Nirmool. Images in Series A represents the silkworm pathogens treated with Nirmool disinfectant solution and Series B with distilled water; (1) *B. thuringiensis,* (2) *Staphylococcus* sp., (3) *B. bassiana,* (4) BmNPV and (5) *N. bombycis.*

The effectiveness of Nirmool against bacterial and fungal pathogens was determined by *in vitro* studies (Figure 2), which reveal that Nirmool could effectively inhibit the growth of bacteria (*B. thuringiensis* & *Staphylococcus*) and fungus (*B. bassiana*). The efficacy of Nirmool through *in vivo* studies indicates its effectiveness in inactivating all the silkworm pathogens tested as no silkworm larvae was affected with any disease similar to the untreated mulberry leaves (Table 2).

Table 2: Efficacy of Nirmool against silkworm pathogens by in vivo studies

	Disease Incidence (%)						
Treatment	Nuclear polyhedrosis	Denso-nucle-osis	Infectious Flacherie	Bacterial Toxicosis	Bacterial Flacherie	White Muscardine	Pebrine
Nirmool Treated	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Healthy Mulberry	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Untreated Pathogens	88.00 ± 2.31	81.33 ± 9.61	69.33 ± 7.06	74.67 ± 5.81	76.00 ± 4.62	84.00 ± 4.62	92.00 ± 2.31

Note: Mean ± Standard Error

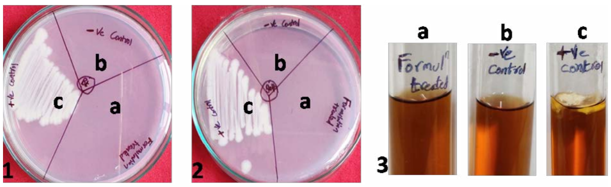

Figure 2: Effect of Nirmool on the growth of bacterial and fungal pathogens of silkworm depicted by culture images. 1: *B. thuringiensis*, 2: *Staphylococcus* sp. and 3: *B. bassiana;* a: Nirmool Treated, b: Negative Control (Sterile Distilled Water) and c: Positive Control (Untreated)

Comparative evaluation of the efficacy of Nirmool and the existing popular disinfectant, 5 % bleaching powder through simulated contamination studies indicated on par effectiveness and prevented the occurrence of silkworm diseases during the silkworm rearing as represented by ERR by number, which indicates the number of live cocoons produced for 10,000 larvae (Table 3). The rearing room disinfection studies also indicated that Nirmool was effective in the management of silkworm rearing across the seasons as all the important pre- and post cocoon parameters recorded are on par with the popular formulation, 5% bleaching powder (Table 4).

Table 3: Comparative efficacy of Nirmool and 5 % bleaching powder against silkworm pathogens under simulated contamination conditions

	Disease Incidence (%)								ERR				
Treatment	Nuclear Polyhedrosis	Densonucleosis	Infectious Flacherie	Bacterial Toxicosis	Bacterial Flacherie	White Muscardine	Pebrine	Total	By No.	By kg	Cocoon Wt. (g)	Shell Wt. (g)	Shell (%)
5 % Bleaching Powder	6.27	0.18	0.17	0.28	4.58	0.02	0.00	11.50	8623	12.62	1.45	0.24	16.55
Nirmool	6.72	0.09	0.02	0.15	4.80	0.04	0.00	11.82	8590	12.21	1.42	0.23	16.19
Control	15.99	0.18	0.11	6.92	11.57	9.01	0.68	44.46	5320	6.71	1.21	0.18	14.87
SEm±	0.54	0.04	0.03	0.69	0.68	0.37	0.11	0.79	162.2	0.31	0.06	0.01	0.36
CD @ 5 %	1.8	NS	NS	2.31	2.27	1.23	0.37	2.61	756.4	1.443	NS	NS	NS

Table 4: Comparative efficacy of Nirmool and 5 % bleaching powder in the disinfection of silkworm rearing house and appliances

Treatment	5 % Bleaching powder	Nirmool	P Value	Significance @ 5 %
ERR by No.	8303	8293	0.93	NS
ERR by Wt. (kg)	12.28	12.61	0.02	*
Cocoon Wt. (g)	1.453	1.501	0.25	NS
Shell Wt. (g)	0.244	0.253	0.23	NS
Shell percentage	16.79	16.85	0.87	NS
Filament Length (m)	638	648	0.87	NS
Denier	2.63	2.62	0.62	NS
Reelability (%)	73	73	1	NS

In-house evaluation to prove the efficacy of Nirmool also indicated its effectiveness in successful harvest of silkworm crops (in terms of ERR by number) and the other variations in pre-cocoon parameters recorded could be attributed to the seasonal influence. Effective rate of rearing data reveals that Nirmool was more effective or on par with the crops reared where disinfection process was conducted with 5 % bleaching powder during the similar period (Table 5). Evaluation studies performed at the farmers' level also indicated that Nirmool based disinfection was effective resulting in successful silkworm crops as signified by on par cocoon yields recorded with 5 % bleaching powder. The slight variations could be attributed to the seasonal variations and egg number per dfl (fecundity) in the batches (Table 6).

Table 5: In-house evaluation on efficacy of Nirmool in the disinfection of silkworm rearing house and rearing appliances

#	Unit	Silkworm Breed/ Hybrid	Disinfection with										
				Nirmool					5 % Bleaching Powder				
			Month	ERR By No.	ERR by Wt. (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Shell (%)	ERR By No.	ERR by Wt. (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Shell (%)
1	Multivoltine Breeding Lab CSRTI-Berhampore	Nistari	January 2020	9767	13.08	1.342	0.200	14.90	9347	10.01	1.078	0.115	10.66
		M12W		9433	11.03	1.175	0.150	12.76	9366	10.98	1.181	0.153	12.95
	P2-BSF (NSSO) Karnasubarana West Bengal	Nistari	March 2020	9227	9.60	1.060	0.130	12.26	9391	9.50	1.060	0.126	11.89
			Average	9476	11.24	1.19	0.160	13.31	9368	10.16	1.11	0.13	11.83
	Percent change over 5 % Bleaching Powder			1%	11%	8%	22%	12%			--		
2	Bivoltine Breeding Lab CSRTI-Berhampore	SK6	April 2020	8240	11.56	1.426	0.243	17.04	8286	10.96	1.308	0.222	16.94
		SK7		9010	12.36	1.404	0.236	16.80	8964	11.99	1.304	0.220	16.87
		BCon1		8640	12.63	1.489	0.249	16.72	7630	10.70	1.308	0.226	17.27
		BCon4		8060	11.00	1.372	0.250	18.22	6397	8.69	1.277	0.222	17.38
			Average	8488	11.89	1.42	0.24	17.20	7819	10.59	1.30	0.22	17.12
	Percent change over 5 % Bleaching Powder			9	12	10	10	0			--		

Table 6: Efficacy of Nirmool in disinfection of silkworm rearing house and rearing appliances with the selected farmers

#	Farmers (Village)	Silkworm Hybrid	Dfls Reared (Nos.)	Yield/100 dfls (kg)	
				Disinfection with Nirmool	Disinfection with 5 % Bleaching Powder
1	Mr. Anisur Rahman (Bankipur)		100	60	61
2	Mr. Abul Karim (Dangapara)	Nistari x SK6.SK7	400	52	48
3	Mr. Mojammel SK (Sahebnagar)		200	54	56
4	Mr. Abu Jakkar SK (Derul)		400	55	55
5	Mr. Aswini Kumar Ghosh (Paschim Medinipur)	SK6 x SK7	100	61	63
		Average or Total	1200	56.4±1.75	56.6±2.62
	P-value of t-stat @ 5 % level of significance			0.95	

Figure 3: Trademark Registration of Nirmool, a disinfectant suitable for disinfection of rearing room and rearing appliances in sericulture (Trade Marks Journal India, No: 2073, Class 5: 5146724, 24/09/2021).

Validation studies of Nirmool were carried out at different Central Silk Board and Departments of Sericulture units across eight states of Eastern & North Eastern India through On Station Trials. An average ERR% of 82.80 was recorded in silkworm rearings across different seasons in 27 test locations. Effective grainage operations were recorded during the validation trials in the grainages at Silkworm Seed Production Centre, Berhampore (NSSO), Berhampore Regional Grainage and Kotasur Sericulture Complex (DoS-West Bengal). The feedback on Nirmool was encouraging as the end-users opined that it is easily soluble in water, fast to prepare and non-corrosive. Currently, the On Farm Trials of Nirmool are in progress for further popularization of eco- and user-friendly disinfection technology with the sericulture farmers of Eastern & North Eastern India.

The efficacy of Nirmool against the pathogens of major silkworm diseases and its effectiveness in disinfecting silkworm rearing house and rearing appliances is proven by the above experimental studies. Nirmool besides being effective against all the major silkworm pathogens, it is eco-friendly and has an illustrious technological profile. As a result, the patent application was filed to safe guard the intellectual property rights of Nirmool successfully (202031050005 dt. 17-11-2020) and Technical Know-How is transferred to National Research Development Corporation (NRDC), New Delhi, India for commercialization purposes. Further, trademark for Nirmool was also registered as a disinfectant suitable for disinfection of rearing room and rearing appliances in sericulture (Trade Marks Journal India, No: 2073, Class 5: 5146724, 24/09/2021). Nirmool is user-friendly unlike the chlorine and formaldehyde based existing disinfectants, which are known to instigate respiratory and skin hazards to the users. In addition, Nirmool is non-corrosive, possess higher shelf-life and is immediately soluble in water which aids in rapid preparation of the disinfectant solution. Nirmool based disinfection of silkworm rearing house and appliances is easy and the product being cost effective is an added advantage. The techno economic impact of Nirmool is presented in Table 7 and the cost-benefit ratio (1.93) is quite favourable. Nirmool encompasses all the user-desirable properties and certainly outplays its counterparts. Nirmool is an effective and efficient new general disinfectant formulation for harvesting successful silk cocoon crops in sericulture.

Table 7: Techno-economic impact of Nirmool (for 100 dfls silkworm rearing in a 400 sq.ft rearing room along with rearing appliances)

#	Particulars	Cost (Rs.)
A.	Additional costs for the Nirmool solution spray (twice for each rearing)	
	(i) NIRMOOL (4 kg)	260
	(ii) Sprayer depreciation cost	200
	(iii) Person days for spraying (2 person days)	500
B.	Reduced returns from Control (No disinfection)	
	Cocoon yield (23.38 kg @ Rs.350/kg)	8183
C.	Reduced costs for general cleaning	
	Person days for cleaning (1 person day)	250
D.	Additional returns from Nirmool	
	Cocoon Yield (49.61 kg @ Rs.350/kg)	17363
E.	Total Debit Side (A+B) or Added Costs	9143
F.	Total Credit Side (C+D) or Gains	17613
G.	Net returns by NIRMOOL (Credit - Debit)	8470
H.	Benefit Cost Ratio (Credit/Debit)	1.93

Conclusion

Nirmool is an eco-friendly general disinfectant for silkworm rearing room & appliances. It is highly effective against all the major silkworm pathogens and on par with 5 % bleaching powder. It has an illustrious technological profile with characteristics such as being easily soluble in water, non-corrosive, having a longer shelf life, being economical and user friendly.

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DIFFERENTIAL EFFECTS OF CASTOR (*RICINUS COMMUNIS*) VARIETIES ON PRODUCTIVITY OF ERI SILKWORM (*SAMIA CYNTHIA RICINI*), IN THIKA, KENYA

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Abstract

Production of Eri silk is determined by the quality as well as quantity of feed consumed by eri silkworms (*Samia cynthia ricini*). Five varieties of the primary host plant, castor (*Ricinus communis*), namely M2, M3, M4, M12 and S11 were investigated at National Sericulture Research Center laboratory in Thika, Kenya, to determine their differential effects on growth, development and cocoon productivity of eri silkworms. The target population were 1000 worms which were divided into five treatments each comprising of 50 worms and replicated 4 times in a completely Randomized design (CRD). Data were collected and analyzed from a sample of 20 % of the entire population consisting of 10 worms picked randomly from each replication. The results showed statistically significant differences among the five varieties for larval, egg and cocoon parameters which include larval duration (26 to 28.75 days), larval survival (73.5 to 100 %), effective rate of rearing (61.5 to 98.5 %), larval weight (4.27 to 4.78 g), fecundity (283.88 to 380), hatchability rate (86.50 to 95.6 %), cocoon weight (2.35 to 2.73 g), shell weight (0.27 to0.33 g) and shell percentage (12.11 to 11.50 %). Upon analysis of the results, the varieties, M4 and M3 were identified as the top performers in terms of cocoon productivity and are thus proposed for further research.

Key words: Castor plant, cocoon weight, eri silk, eri silkworm, shell weight.

Introduction

Silk farming exists as an agricultural based business that involves the raising of silk producing insects which are arthropods (Ahmed *et al*., 2015). Eri silkworm was introduced in Kenya by Tosheka Ltd. (a social enterprise textile company) and KALRO to evaluate their practicability as far as their commercial aspect was concerned. Bindroo *et al.* (2007) highlighted the ability of eri silkworms to undergo multiple breeding cycles per year (multivoltine) and feed on a diverse range of host plants (polyphagous). The insect depends on various kinds of plants for food although castor is the primary one. The other alternative plants include *Kesseru* and Tapioca(Phukon, 1983). Jayaraj (2004), described how oil production is not affected when castor leaves are harvested for eri silkworm feeding. The maximization of superiority of castor foliage influences the efficiency and effectiveness of silk production compared to other food plants (Govindan *et al*.,1992). The growing process, improvement and rate of production of silk is highly influenced by availability as well as the quantity of extremely nutritious host plants for eri silkworm. (Krishnaswami *et al*., 1970).

The countries which have successfully reared eri silkworms include India, Ethiopia, Japan and Arabia (Kedir *et al*., 2014). They suit to most of the developing countries in view of the hardiness and disease resistance of eri silkworms as an advantage over mulberry silkworm especially under the typical rearing conditions in rural households which are not highly sanitary. Further, the capital requirement is minimal and the equipment required can be constructed locally (Kedir *et al*., 2015). According to Shubhangi, (2015) the technicality involved in sericulture and labour requirements can easily be handled at household level providing occupation to a recommendable section of the population with considerable financial earnings in return.

There are various products manufactured out of the protein fiber which includes fishing lines, garments, jackets, insulation coil for electric and telephone wire, sieve for flour mill and tyres of racing cars (Rajasri and Lakshmi, 2015). The leftover foliage can be used as manure and also the branches obtained after pruning can be utilized as fuel (Manjunath, 2014).

The reports of National Sericulture Research Centre (NSRC, 2016) reassured the development of the venture on the countryside on condtion that the Kenyan authority would offer incentives to advance the cultural performance as well as strucural procedures, such as improved techniques and intensive research.

In Kenya, castor is grown by individual farmers, private institutions and government organizations and is the most preferred in coastal, Central and parts of Eastern regions of Kenya (NSRC, 2016). KALRO is a government organization which concentrate in breeding castor varieties for oil production rather than raising of eri silkworm larvae (NSRC, 2016).

Consequently, there has not been any test to determine the viability of the various cultivars for raising the eri worms. As there is no organized cultivation of castor in Kenya, a few eri farmers use wild castor leaves available in the bushes, along the river banks and around their homestead not putting into consideration, the type, quality and variety of the castor leaves. Thus, there is a need to identify the best performer among the castor varieties for enriched silk production, its progression, advancement and maximization of returns. Therefore, the study aims at determining the progression as well as advancement and cocoon productivity portrayed by eri worms (*Samia cynthia ricini*, Boisduval) (Lepidoptera: Saturniidae) towards nourishment on various castor selections in Thika, Central Kenya.

The study was conducted with the objective of identifying the best variety of castor plant in terms of preference and eri silk productivity in Kenya, based on bioassay and comparative analyis of results thereof.

Materials and Methods

The investigation was carried out in the research laboratory at National Sericulture Research Center, Thika. Different varieties of castor (*Ricinus communis* L.) *viz*., M2, M3, M4, S11 and M12 were considered as treatments in this experiment. The target population were 1000 eri worms (*Samia cynthia ricini*), which were divided into five treatments each comprising of 50 worms and replicated 4 times in a completely Randomized design (CRD). Leaves from each variety were weighed separately and an equal amount were used to feed the worms in each treatment in the rearing room. Similar designs have been used in the past to study eri worms species and population density (Kedir *et al*., 2014; Tilahun *et al*., 2017; Pallavi and Sannappa, 2018).

Cleaning and sterilization of the room and equipment where the silkworm nurturing took place was done in advance. The hands were cleaned with a detergent and rinsed with clean water to prevent larval contamination during the operation.

Treatment details were as follows:

Treatment-1, 2, 3, 4 and 5 consisted of Eri silkworms fed on leaves of castor variety M2, M3, M4, M12 and S11, respectively.

The replications in each treatment were fed with the assigned castor variety. The larvae in the first two instars were maintained in small separate trays for each replication since tiny larvae stay very close to each other with very minimal movement up to the 3rd instar (Chowdhury, 1982), after which they were transferred to bigger trays since the increment in size of eri worms often results in overcrowding and insufficient spacing, which can lead to malnutrition and suboptimal environmental conditions, thereby rendering them vulnerable to various ailments (Tilahun *et al*., 2017).

For each replication, newly hatched worms were fed with whole tender leaves of assigned castor variety until they molted to 2nd instar. The 3rd and 4th instars were fed with semi – mature leaves of the same variety and the 5th instar worms were fed on mature leaves. Feeding was done every morning at 8.30 a.m. and in the evening at 4.00 p.m. The food quantity was increased with increase in the age of the larva which ensured proper growth and development (Lorhit, 2000; Tilahun *et al*., 2017).

The worms were observed to stop feeding when they peel the skin during the moulting process. Immediately after molting, lime powder was applied to dry the bed so as to prevent the growth of pathogens and the resultant infections (Usama *et al*., 2020). Feeding was resumed when 95% of the worms were out of molt. Weighing the leaves before feeding ensured that each treatment receives same quantity of leaves. Perforated paraffin paper was used to cover the feed to prevent fast drying of the leaves and to ensure freshness of the leaves for longer periods (Lorhit, 2000). Room temparature and relative humidity were maintained at 25– 28 ^{o}C and 70-80 %, respectively (Venu and Munirajappa, 2013). Data were collected and analyzed from a sample of 20 % of the entire population consisting of 10 worms picked randomly from each replication (Pallavi and Sannappa, 2018). Olfactory technique was used to determine the most preferred variety by eri silkworms whereby leaves of the five varieties were arranged at a radius of 10 cm for the silkworms in 1st, 2nd and 3rd instars and 20 cm for 4th and 5thinstars. One hundred and twenty newly hatched silkworms were placed at the center where they were allowed to move to the castor leaf of their preference. This method was repeated for every first feeding after moulting in each instar.

The following formulae were used to estimate the various parameters as suggested by Singh and Benchamin (2002):

Hatchability (%) = No. of eggs hatched/Total number of eggs x 100

ERR % = Number of worms spinning cocoon/Number of worms brushed x 100

Larval survival % = Number of survived worms/ Number of worms brushed x 100

Shell % = Weight of the cocoon shell/weight of whole cocoon x 100

The data obtained were subjected to one way Anova to compare means at 5 % level of significance. Significant means were separated using Least significant difference (LSD). The data were tested for normality using MS Excel software. The results were presented using tables.

Results and Discussion

Determination of the relative feeding preference of eri silkworm towards different castor varieties

There was no significant difference among the number of worms that preferred each of the five varieties used ($F_{4,20}$=0.917, P=0.473). However, variety M4 was the most preferred (mean=29.4±6.651) followed by M3 and M2 (25.6±2.863, 20.0±3.899, respectively). M12 and S11 recorded the least preference each (18.0±5.109). Probably, the difference might be due to the variations in palatability of the leaves owing to many characteristics. Various larval stages portrayed varied preferences. Newly hatched eri silkworms have shown high preference for M4 whereas they hardly prefered S11 and M12. During the late stages, the variation was not significant (Table 1). Similar findings were recorded by Lorhit (2000) in his experiment on nutritional efficiency of *Samia cynthia ricini* reared on different castor varieties.

Table 1: Differential preference (No.) of castor variety by eri silkworms

	Castor variety				
Larval instar	M2	M3	M4	M12	S11
I	33	31	53	0	0
II	25	31	33	20	20
III	15	23	15	29	32
IV	13	17	25	26	32
V	14	23	20	15	16
Mean ± SE	20.0 ± 3.899	25.0 ± 2.863	29 ± 6.261	18.0 ± 5.109	18 ± 5.109
$F_{4,20}$	0.917				
P =	0.473				

Preference of castor varieties by eri silkworms at 0.05 significance level

Effects of castor varieties on growth and development of eri silkworms

The results on the determination of eri silkworms' growth and development in terms of larval duration, larval survival, and effective rate of rearing, mature larval body weight, fecundity and hatchability are presented and discussed below.

Larval duration

Significant differences ($F_{4, 15}$= 64.25, P<0.001) in larval duration were recorded in eri silkworms provided with different castor varieties. The least larval duration was observed in M2 and S11 which took 26 days followed by M12 and M4 (27.00 and 28.25 days, respectively). M3 took the longest period of 28.75 days (Table 2). The observed variation might be due to the differences in the nutrient composition of the leaves of the castor varieties. Results in this line (26.60 – 29.15 days) were recorded by Swathiga *et al*. (2019).) on rearing performance of c2 breed of eri silkworm, *Samia cynthia ricini*, fed with different castor genotypes.

Larval survival

Significant differences ($F_{4, 15}$=38.92, P<0.001) in larval survival rate were recorded among treatments of castor varieties. M2 and M4 showed equivalent larval survival of 100 % each. S11 followed closely with 99.5 % followed by M12 with 98.5 %. M3 had the least survivability of 73.5 % compared to the rest of the varieties. (Table 2). Similar to our findings, Sannappa *et al.* (2007) observed comparable larval survival rates in eri silkworms when investigating the influence of various castor genotypes on larval and cocoon characteristics.

Effective rate of rearing

The percentage of the number of cocoons harvested in relation to the number of larvae brushed (ERR) was significantly different ($F_{4, 15}$=9.435, P<0.001) for eri silkworms fed on different castor varieties. Eri silkworms fed on M2 recorded the highest ERR (98.5 %,) followed by M12 and S 11 (91.5 and 91.0 %, respectively). M4 had it, 84.0 %. M3 recorded the least ERR value (61.5 %) (Table 2). The variations recorded in ERR was due to the mulberry varietal impact on the growth and development of silkworm. Prasanna *et al.* (2013) reported variations in ERR during the study on evaluation of castor varieties based on the performance of eri silkworm *Samia cynthia ricini*.

Mature larval body weight

Significant differences on the weight of mature larva were recorded ($F_{4, 15}$=2.881,P<0.001) among varieties. However, M3 recorded the highest mean weight (4.78 g,) followed closely by S11, M4 and M12 (4.70, 4.64, and 4.58 g, respectively). M2 recorded the least weight (4.27 g). The variation might be attributed to the differences in the composition of foliar nutrients of those varieties. The results are in conformity with those of Patil *et al.* (2000) for their study on the performance of eri silkworms on different castor varieties.

Fecundity

A significant difference ($F_{4, 15}$=4.179, P=0.005) in fecundity was recorded when they were fed on different castor varieties. Silkworms fed on M4 expressed the highest mean fecundity (380) followed by M3, M12, and S11 (343.00, 339.89 and 327.38, respectively). M2 recorded the least fecundity (283.88 eggs) among the varieties (Table 2). Clossely related values (275 to 360) had been reported earlier (Swathiga *et al.*, 2019) on rearing performance of c2 breed of eri silkworm, *Samia cynthia ricini*, fed with different castor genotypes.

Hatchability

There was no significant difference ($F_{4, 15}$=1.616 P=0.221) in hatching percentage of eri silkworms fed on different varieties of castor. The highest hatching percentage was recorded in S11 (95.6 %) followed by M12 (93.2 %), M4 (89.47 %) and M2 (88.9 %). M3 recorded the lowest hatchability (86.50 %) (Table 2). Deviation in the hatchability of eri silkworms was also recorded by Govindan *et al.* (2002) while studying the influence of castor genotypes on economic traits of eri silkworms.

Table 2: Effect of Castor varieties on eri silkworm's larval and egg parameters

	Castor variety (Mean ± S.E.)					$F_{4,15}$	P-Value
Parameter	M2	M3	M4	S11	M12		
Larval duration (days)	26 ± 00.00	28.75 ± 0.25	28.25 ± 0.25	26.00 ± 0.00	27.00 ± 0.00	64.25*	<0.001*
Survival rate (%)	100 ± 0.00	73.50 ± 4.03	100 ± 0.00	99.50 ± 0.50	98.50 ± 0.96	38.92*	<0.001*
ERR (%)	98.5 ± 0.96	61.5 ± 2.87	84.0 ± 4.40	91.0 ± 4.20	91.5 ± 7.85	9.435*	<0.001*
Larval weight (g)	4.27 ± 0.04	4.78 ± 0.05	4.64 ± 0.04	4.70 ± 0.04	4.58 ± 0.04	2.881	<0.001
Fecundity (No.)	283.88 ± 15.96	343.00 ± 15.96	380.00 ± 17.07	327.38 ± 15.96	339.89 ± 15.96	4.179*	0.018*
Hatching %	88.92 ± 5.46	86.50 ± 5.46	89.47 ± 5.83	95.6 ± 5.46	93.22 ± 5.46	1.616	0.221

Data are Mean ± S.E. of 4 replications; * Significant (P < 0.05)

Cocoon production

According to the observations, eri silkworms fed on various castor varieties portrayed various results. There was significant difference among the cocoon weight of eri silkworms fed on different varieties of castor leaves ($F_{4, 15}$=5.568, p=0.0059). The larvae fed on castor variety M3 recorded the highest cocoon weight (2.73 g) followed by M12, M4, S11 and M2 (2.63, 2.62, 2.57 and 2.35 g, respectively). The results revealed statistical significance with regard to shell weight ($F_{4, 15}$=3.07, P= 0.001). M3 portrayed the highest mean shell weight (0.33 g), followed by M4, M12, S11 and M2 (0.32, 0.31, 0.30 and 0.27 g, respectively). Significant difference was noted between the means of the castor varieties when shell percentage was put into consideration ($F_{4, 15}$=7.17, P>0.001. The experimental results showed that variety, M3 had the highest shell percentage (12.11) followed by M4, S11, M2, and M12 (11.87, 11.77, 11.53, and 11.50, respectively) (Table 3).These results are in agreement with those of Swathiga et al. (2019) who investigated the rearing performance of c2 breed of eri silkworm, Samia cynthia ricini, fed with different castor genotypes.

Table 3: Cocoon parameters of eri silkworms reared on different castor varieties

Castor variety (Mean ± S.E.)						$F_{4,15}$	P-Value
Parameter	M2	M3	M4	S11	M12	5.568	0.005*
Cocoon weight (g)	2.35 ± 0.034	2.73 ± 0.035	2.62 ± 0.035	2.57 ± 0.035	2.63 ± 0.035	5.568	<0.001*
Shell weight (g)	0.27 ± 0.015	0.33 ± 0.021	0.32 ± 0.022	0.30 ± 026	0.31 ± 0.018	3.07	0.001*
Shell %	11.53 ± 0.128	12.11 ± 0.223	11.87 ± 0.119	11.77 ± 0.272	11.50 ± 0.134	7.17	0.001*

Data are Mean ± S.E. of 4 replications; * Significant (P < 0.05)

Correlation analysis revealed varied results among larval and cocoon parameters. Larval duration and cocoon weight had a very weak negative association, as demonstrated by the correlation coefficient of -0.0392. The p-value was 0.8768 which was greater than 0.05, and f-value 0.001545 which suggested no statistically significant correlation. The correlation coefficient between cocoon weight and survival was 0.3535, which showed a moderate positive correlation between the two variables. The p-value was 0.1211 and the f-value was 1.972, which indicated no statistically significant relationship between the two variables. The correlation coefficient between cocoon weight and ERR was 0.0198, (p = 0.949, f = 0.000395) which demonstrated a very weak positive correlation between the two variables which was not statistically significant. Based on the results, there was a moderate positive correlation between cocoon weight and larva weight (r = 0.451), but it was not statistically significant (p-value = 0.657, f = 3.095). The correlation coefficient between cocoon weight and hatchability was 0.0798 (p-value = 0.629, f-value = 0.248) which showed a very weak positive correlation between the two variables which was not statistically significant. The correlation between cocoon weight and fecundity was moderately positive (0.5319, p = 0.0202, f = 6.579) which was statistically significant. The correlation coefficient between cocoon weight and shell percentage was -0.430 (p = 0.033, f = 3.270) which showed a significant negative relationship between the two variables. The correlation coefficient between cocoon weight and shell weight was 0.73 (p-value = 0.000354, f-value = 10.19) which demonstrated a moderate positive correlation which was statistically significant. The correlation coefficient between shell percentage and shell weight was -0.3887 (p-value = 0.497,f -value = 0.2976) thereby indicating a weak negative correlation that was not statistically significant at conventional significance level of 0.05. The correlation coefficient between shell weight and larva duration was -0.01918, which demonstrated a very weak negative correlation between the two variables. The p-value of 0.9256 and f-value of 0.0001071, suggested no statistically significant correlation between shell weight and larva duration.

The correlation coefficient between shell percentage and larva duration was 0.20709 (p-value = 0.427, f-value = 0.8238) which demonstrated a very weak positive correlation between the two variables which was not statistically significant. The correlation coefficient between shell weight and larva weight was 0.5802, (p-value = 0.00751, f-value = 10.81) which indicated a statistically significant, moderate positive correlation between the two variables. There was a significant weak positive correlation between shell weight and fecundity (r=0.0909, p = 0.0099, f = 4.115). The correlation coefficient between shell weight and survival was 0.146 (p-value = 0.6166, f-value = 0.02749) indicating a weak positive correlation which was not significant. The correlation coefficient between shell weight and hatchability was -0.217, p-value = 0.4692, f-value = 1.409) which indicated non-significant weak negative correlation between the two variables. The correlation between shell weight and ERR gave a value of -0.259 (p-value = 0.00277, f-value = 5.783). This indicated a significant moderate negative correlation between the two variables. The correlation coefficient between shell percentage and larva weight was -0.534 (p-value = 0.165, f-value = 4.079), which demonstrated a non-significant moderate negative correlation between the two variables. Correlation coefficient between shell percentage and ERR was -0.318 (p value = 0.375, f value = 1.201) which showed a weak negative correlation between the two variables which was not statistically significant at the 0.05 significance level. The correlation coefficient between shell percentage and survival was 0.023, (p-value = 0.8614, f-value = 0.0324) which indicated no significant correlation between the two variables. Correlation coefficient between shell percentage and fecundity was found to be -0.19 (p-value = 0.46, f-value = 1.63) which indicated a weak negative correlation that was not statistically significant. There was a weak negative correlation coefficient between shell percentage and hatchability r = -0.485, p = 0.166, f = 0.885) which was not statistically significant at the 5 % level of significance since it was greater than the conventional threshold of 0.05. The findings are consistent with those of Subramanianan *et al.* (2013) who studied the rearing performance of the eri silkworm (*Samia cynthia ricini*) under various seasonal and host plant circumstances and discovered a variety of findings about the relationship between cocoon characteristics and larval growth. (Table 4).

Table 4: Correlation between larval and cocoon characteristics of eri silkworm

Parameter	Cocoon weight			Shell weight			Shell percentage		
	r	p	F4,15	r	p	F4,15	r	p	F4,15
Cocoon weight									
Shell weight	0.73	0.00035*	10.19						
Shell percentage	-0.430	0.033*	3.270	-0.388	0.497	0.2976	-	-	-
Larval duration	-0.0392	0.9699	0.001545	-0.019	0.9256	0.0001	0.2070	0.4271	0.8238
Survival rate	0.3535	0.1798	1.972	0.146	0.6166	0.02749	0.023	0.8614	0.0324
ERR	0.0198	0.985	0.000395	-0.259	0.37	5.783	-0.318	0.375	1.201
Larval weight	0.451	0.657	3.095	0.5802	0.00754*	10.81	-0.534	0.165	4.079
Fecundity	0.5319	0.0202*	6.579	0.0909	0.0099*	4.115	-0.19	0.46	1.63
Hatchability	0.0798	0.629	0.248	-0.217	0.4692	1.409	-0.485	0.166	0.885

r = Correlation tested at $F_{4,15}$ degree of freedom; * Significant (P < 0.05).

Conclusion

According to the results of the current study, selecting the right castor variety for eri silkworm rearing is essential for maximizing the productivity of the cocoons since different castor variations have varying impacts on eri silkworm growth, development, and silk production. In terms of cocoon features, the castor cultivars, M3 and M4 showed the most promise. To increase their chances of survival, it is strongly advised that in the future, worms be raised indoor during the early stages, from the first instar to the third instar.

It is strongly advised to do additional study to support the current findings taking into account the following factors; The different nutritional values of the various castor varieties grown in Kenya, the role that these nutrients play in the growth and development of eri silkworms and silk production, the pests and diseases that affect castor leaves and their management, and the availability of a substitute crop for eri silkworm production in Kenya. Further investigation is needed to develop methods for controlling the invasive spread of castor, which is categorized as an invasive species.

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Research Paper

PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF PECTIN EXTRACTED FROM MULBERRY SERICULTURE RESIDUES

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Abstract

The study was aimed to extract and analyze the physiochemical properties of pectin from a few sericulture residues. Fresh mulberry leaves, bark of mulberry plant, left-over leaves and silkworm *(Bombyx mori)* excrement were used for extraction of pectin at varied pH (pH 1.0, 1.5, and 2.0) followed by acid hydrolysis at 80 ºC. At low pH 1.0, the yield was higher, *i.e.* 2.90 % in fresh leaves, 5.74 % in bark, 8.14 % in silkworm excrement, and 9.26 % in left-over leaves than at higher pH. The comparative yield *i.e.,* percentage difference (PD) was 65.74 % in bark, followed by 94.92 % in silkworm excrement and 104.60 % in left-over leaves. Thin Layer Chromatography (TLC) of pectin showed retention factor (Rf) value of 0.63 to 0.64. The pectin extracted from waste residues showed similar results in different qualitative assays in comparison with standard pectin. The extracted pectin equivalent weight (EW) ranged from 500 to 625 mg/ml; the methoxyl content (MC) from 5.00 % to 6.20 %; anhydrouronic acid content (AUA) from 70.40 % to 77.44 %; and the degree of esterification (DE) from 38.09 % to 45.45 %. Along with pectin protein (1.60-1.90 %), total sugar (6.16-8.70 %), and ash (3.30-3.60 %) were also recorded. Further, the extracted pectin was authenticated using Fourier-transform infrared spectroscopy (FTIR) and powder X-ray diffraction (P-XRD). In P-XRD, the 2θº (theta) peak values of 12.16, 21.74, 25.32, 28.75, and 41.43 confirmed its crystalline structures and FTIR analysis revealed the presence of functional groups having different biological activities.

Key words: *Bombyx mori,* characterization, excrement, extraction, mulberry, pectin, silkworm.

Introduction

Pectin is one of the biocompatible polysaccharides that manifests various natural biological activities that have different structures based on its source and extraction procedures. The structure-wise percentage of pectin varies among plants, within a plant over different durations, and in different parts. It is the principal polysaccharide of the cell wall that allows the extension and growth of the plant. In the course of fruit ripening and in the abscission zone of petioles of deciduous plants, pectin is fragmented by the enzymes pectin esterase and pectinase, in whose action the fruit becomes mushy and pulpy as the middle lamellae are broken down and cells get separated from each other in leaf fall due to the breakdown of pectin (Crandall *et al.,* 1978). Extraction of pectin from various industrial by-products appears to be a green resource for agro-industrial residue beneficiation by producing a high commercial value product. Moreover, pectin is safe and non-toxic. Their products can be made available with low production costs and high availability (Henriquez *et al.,* 2019). Pectin is vulnerable to chemical, physical, and enzymatic changes. Many of the functional groups that exist in the structure can produce various functionalities. A few modifications can allow pectin to have innumerable applications in food, drugs, biomedical research, and agriculture.

Currently, it is a trend to make use of pectin to manufacture edible coatings to preserve foodstuffs. Pectin coating plays a significant role in controlling water loss and reducing fruit decay. It also extends the shelf-life of the foodstuffs and reduces their respiration rate (Ciolacu *et al.,* 2014). Popularly, pectin is used in the preparation of jams, desserts, fruit juices, jellies, and dairy products, and therefore, the gelling property of pectin is well known (Jindal *et al.,* 2013). As a stabilizing agent, pectin in colloidal dispersions differs between foods fortified with antioxidants, emulsions, fruit-based drinks with high protein content, and acidified milk beverages (Zhang and Zhang, 2015). Pectin is also used in the biomedical field for gene delivery, drug delivery, and reduction of cholesterol; wound healing, fabrication of the membranes used in the manufacture of contact lenses, tissue engineering, catheters, and the production of artificial corneas. They have relatively low molecular weight, which can be easily absorbed by the body, and have shown anticancer properties by inhibiting metastasis (Maxwell *et al.,* 2012; Wang *et al.,* 2018, Martau *et al.,* 2019; Munarin *et al.,* 2012; Mata *et al.,* 2018).

Like other agricultural sectors, sericulture also derives a vast number of by-products since mulberry leaves are used as the sole food for rearing silkworms, enabling the production of a huge volume of phytochemicals to be retrieved from the plant-based residues. Pectin is one of them. It can be extracted from mulberry leaves, bark, fruit, and also silkworm excrement. These products are obtained as a left-over product of silkworm rearing, are easy to obtain, handle, preserve, and are eco-friendly in nature. The unique feature of these residues is their composition of phytochemical derivatives such as alkaloids, polyphones, flavonoids and anthocyanins, and they serve as a raw material for different bioactive material synthesis. Due to its ease of availability, the present study focused on the extraction and characterization of pectin.

Materials and Methods

Collection and processing of materials

Sericulture residues such as mulberry leaves and bark from mulberry garden and silkworm excrement and the left-over leaves were collected from silkworm rearing bed during the mandatory bed cleaning in the rearing house. All the collected materials were dried in a hot air oven at 85^{0}C for 24-48 h and ground into a fine powder using a mixer grinder. The powder was stored in desiccators.

Extraction of pectin

Pectin present in the dried powder of leaves, bark, left-over leaves, and excrement was extracted by following water-based acidic media at different pHs of the water: 1.0, 1.5, and 2.0 using concentrated sulphuric acid (H_2So_4) by the method of Yeoh *et al.,* 2008. The reaction was started by heating and continuously stirring the mixture in a boiling water bath at 80^{o} C for one hour, followed by cooling overnight. After 24 h, the gelatinous pectin flocculants in the upper part were seen floating on the top layer and the sediment at the bottom was separated using muslin cloth. The upper pectin layer solution was collected and centrifuged at 4000 rpm for 15 minutes and subjected to further separation process. Since the acidic pectin liquid may contain debris, particles of leaf or bark, or excrement they were separated through the method described by Javeed *et al.,* (2020). To the centrifuged flocculants, an equal amount of 99% ethanol was added and incubated at 4ºC for 3 h. The precipitate (ethanol-insoluble fraction) was recovered through filtration and centrifugation. The sediment was collected and and initially dried at room temperature for 12-24 h and was further dried in a hot air oven at 80 °C for 2 h. The purified pectin powder was collected, the yield was calculated for different PH, and the product was utilized for physico-chemical characterization.

Isolation by Thin-layer chromatography (TLC)

10μl of the standard pectin (sourced from Central Drug House, New Delhi) and an extracted pectin sample were loaded onto the pre-coated 60 F 254 aluminum silica gel plates. The solvent systems used were butanol: formic acid: water and butanol: water: acetic acid in the ratio of 2:3:1 and 3:2:1 respectively. The gel plate was placed in the chamber and was run under appropriate conditions. Spots were detected using iodine spray. By identifying the spot of the solute on the TLC plate and the distance traveled by the solvent, the R_f values were calculated and compared with the standard values.

Physicochemical characterization

The dried pectin obtained from all the materials was subjected to various qualitative and quantitative analyses.

Qualitative tests of Pectin

i) Color: This test was done by visual observation.

ii) Solubility of dry pectin: Extracted pectin (1g) was dissolved in 5ml of 95 % ethanol and 25 ml of distilled water. The mixture was thoroughly shaken to form a suspension which was heated for 15 min (Fishman *et al.,* 1984).

iii) Precipitation test: An equal volume of ethanol was added to the pectin solution. The alcohol insoluble pectin developed a stringy, gelatinous deposit, which indicates a positive result (Marshall *et al.,* 2000).

iv) pH determination: The pH was determined by preparing a buffer at pH 7.0 and adjusting the temperature to 28 °C, standardized the glass electrode with a standard buffer solution, and the electrode was rinsed with distilled water before inserting into the pectin solution (Enkuahone, 2018).

v) Stiff gel test: The extracted powdered pectin was heated with 9 ml of water in a boiling water bath until it dissolved and formed a solution and formation of stiff gel on cooling (Sharma and Gupta, 2014).

vi) Potassium hydroxide test: To 5 ml of pectin 1 % w/v solution, 1ml of KOH 2 % w/v solution was added and incubated for 15 minutes. A transparent gelatinous liquid (semi gel) formation was observed. The acidification and shaking of the above gel with dilute HCl formed a voluminous, colorless gelatinous precipitate, which upon boiling became white and flocculent (Azad *et al.,* 2014).

vii) Iodine test: To 5 ml of boiled and cooled 2 % w/v solution of pectin, 0.15 ml of iodine solution was added. The absence of blue color indicates the presence of pectin (Sumita and Aneri, 2020).

Quantitative analysis

1. Yield: Pectin yield was calculated following Kanmani *et al.* (2014). This is an important indicator which helps to understand the recovery rate and the effects of extraction conditions. It is calculated using the following formula:

Y_{PEC} (%) = P/Bi x 100
Where, P = the quantity of extracted pectin
Bi = the initial quantity of material used.

2. Equivalent weight determination: Pectin sample (0.5 g) was dissolved in 5 ml of ethanol. To this, 100 ml of distilled water and 1 g of sodium chloride were added. To this solution, 6 drops of phenol red indicator was added and was titrated against 0.1 N NaOH until pale pink was observed as an endpoint (Ranganna, 1995). The values were then used to calculate the equivalent weight using the following formula:

$$\text{Equivalent weight} = \frac{\text{Weight of sample (g) x 1000}}{\text{Volume of alkali x normality of alkali}}$$

3. Methoxyl content determination (MeO): Determination of MeO was carried out by using the method of Ranganna, (1995). 25 ml of neutral solution from equivalent weight determination was taken. To this, 25 ml of 0.2N NaOH was added and stirred continuously, and the solution was kept at room temperature for 30 min. To this solution, 25 ml of 0.25N HCl was added and titrated against 0.1N NaOH using phenol red as an indicator until the presence of pink color. The volume of alkali was used to calculate the methoxyl content by using the following formula:

$$\text{Methoxyl content} = \frac{\text{Volume of alkali x normality of alkali}}{\text{Weight of sample (g)}}$$

4. Total Anhydrouronic acid content (AUA): AUA was calculated by using the values of equivalent weight and methoxyl content of pectin by using the below formula as described by Ranganna, (1995).

$$\text{AUA (\%)} = \frac{176 \text{ x } 0.1 \text{ x A x } 100}{\text{w x } 1000} + \frac{176 \text{ x } 0.1 \text{ x B x } 100}{\text{w x } 1000}$$

Where,
A = ml of NaOH from equivalent weight
B = ml of NaOH from methoxyl content
w = weight of the sample

5. The Degree of esterification (DE): The DE of extracted pectin was measured by following Sumita and Aneri, (2020) based on the values from Methoxyl content and Anhydrouronic acid content using the following formula.

$$\text{DE (\%)} = \frac{\text{176 x Methoxyl content (\%)}}{\text{31 x AUA (\%)}} \text{ x } 100$$

6. Determination of protein content: A pectin sample (25 g) was mixed with 1 ml of 10 % TCA. The mixture was centrifuged at 1800 rpm for 15 minutes. The supernatant was discarded, and the residue was collected. The residue was dissolved in 1 ml of 1N NaOH. The mixture (0.02 ml) was taken and mixed with 4 ml of alkaline copper reagent and 0.4 ml of folin phenol reagent. The absorbance of the solution was measured in a UV-Vis spectrophotometer at a wavelength of 600 nm (Lowry *et al.,* 1951). The values were expressed in mg/g. The weight of pectin was assessed and converted into a percentage.

7. Determination of total sugars: Pectin sample (0.2g) was mixed with1.5 ml of 75 % H_2SO_4. The mixture was heated in a boiling water bath at 25^0 C for 25 mins. After cooling the mixture at room temperature, 10 ml of distilled water was added. From this solution, 3 ml of solution was pipetted into a test tube and 0.06 ml of 85 % phenol was added. 1 ml of H_2SO_4 was added again and left for incubation at room temperature for 20 min. The mixture was subjected to UV spectrophotometry to measure the absorbance at 490 nm, expressed in mg/g of pectin, which was then converted into percentage (Lim *et al.,* 2012).

8. Determination of ash content: The ash content of extracted pectin was determined by Isobel *et al.* (2021). Five grams of each of the samples was accurately weighed into a weighed empty crucible separately. The crucible was transferred to a furnace set at 60 °C to burn off all the organic matter. The carbon was charred and then burned off as carbon dioxide, leaving dark ash; this process lasted for 24 h. The crucible was taken out of the furnace and placed in desiccators to cool. The crucible, after cooling, was reweighed again. Ash content was calculated using the following formula:

$$\text{Ash Content (\%)} = (\text{Weight of Ash} / \text{Weight of Sample}) \times 100$$

Biological characterization of pectin

1. Fourier transform infrared spectroscopy (FTIR) The extracted pectin was subjected to FTIR analysis and compared with the standard pectin for the functional group analysis. The compounds were separated based on their peak ratios and determined with the respective wave numbers of the samples. The spectra were recorded in the range from 4000 to 500 cm^{-1} (Nandiyanto *et al.,* 2019).

2. Powder X-Ray Diffraction (P-XRD) The P-XRD analysis was conducted for pectin samples at 40KV, 15 mA, and the scan range angle of 5-80 ° with the detector D/teX Ultra2 scan axis 2θ. The distance between the planes of atom (D (A°)) and peak height cps (count per second) were comparatively analyzed.

Results and Discussion

Pectin, a complex set of polysaccharides, exists in most primary cell walls and is most abundant in non-woody segments of terrestrial plants and algae. It is soluble in pure water, as are monovalent cation salts such as pectinic and pectic acids, but weakly or insoluble in bivalent and trivalent cations. Identification of raw material, processing, and extraction is the most important process in the commercial production of pectin. Different methods such as chemical and enzymatic methods were applied and hydrolyzed insoluble protopectin was into soluble pectin which leached out the pectin from the tissues. In the present investigation, the acidic water media of different pH ranges were used for the pectin extraction followed by separation from its impurities using ethanol. Since the method is efficient, easy to carry out, uncomplicated, brisk, adopted for sericulture residues. Determination of yield is an important parameter for the adoption of any technology with the significance of economic benefit.

In the present investigation, the water-based extraction process with different p^H showed variations in their yields. The fresh leaves have shown a 2.90 % yield at p^H 1.0, 2.60 % at 1.5 p^H, and 1.25 % at 2.0 p^H. The reduction in the yield was noticed when the p^H was increased in the water (a percentage difference (PD) of 10.90 % decrease at p^H 1.5 and 79.51 % at pH 2.0) (Table 1). A similar trend was noticed for all the raw materials. In bark, a yield of 5.74 % was noticed at pH 1.0, 4.40 % at pH 1.5, and 3.40 at pH 2.0. The PD was found to be 26.43 % decrease at pH 1.5 and 51.20 % decrease at pH 2.0. It is noteworthy that the yield from the leftover leaf was 9.26 % at the pH of 1, 8.45 % at pH 1.5, and 6.50 at a pH of 2.0. The PD of 9.147 % decrease was noticed at pH 1.5 and 35.02 % decrease at pH 2.0. Similarly, in silkworm excrement, the obtained yield was 8.14 % at pH 1.0, 7.20 % at pH 1.5, and 5.26 % at pH 2.0, and the PD observed was 12.25 % decrease at pH 1.5, followed by 42.98 % decrease at pH 2.0. From the results, it is clear that, with the low PH, the breaking of pectin-linked bonds favors the extraction of pectin from sericulture residues. Our results align with that of Javeed et al. (2020) the lemon peel pectin extraction gave better yield with the low pH condition. The assessments of the percentage difference of maximum yields of all the tested sources were compared. Among test raw materials, fresh leaves showed low yield at pH 1.0, and increased yield of 65.74 % in bark, 94.92 % in silkworm excrement, and 104.60 % in left-over leaf. Among the tested residues, the left-over leaves and silkworm excrement were the best sources for pectin extraction.

Table 1: Yield (%) of pectin at different pH

Extraction pH	Source			
	Fresh leaves	Bark	Left-over leaf	Silkworm excrement
1.0	2.90 (±0.252) *	5.74 (±0.120) *	9.26 (±0.020) *	8.14 (±0.098) *
1.5	2.60 (±0.152) *	4.40 (±0.152) *	8.45 (±0.142) *	7.20 (±1.020) *
2.0	1.25 (±0.201) *	3.40 (±0.025) *	6.50 (±0.042) *	5.26 (±0.120) *

Values represent the mean ± standard deviation (SD) of three separate observations. *Statistically significant ($P < 0.005$).

The pectin extracted was subjected to thin-layer chromatography (TLC), which serves as one of the important chromatographic techniques to isolate compounds in a mixture and helps to identify them referring to their Rf value with the standard as a reference. Out of the two systems used, butanol: water: acetic acid (3:2:1) elicited the desired compound and was found to be the better mobile solvent mixture compared to butanol: formic acid: water (2:3:1). The R_f value of all the pectin ranged between 0.63 and 0.64, *i.e.,* the fresh leaves, bark and left-over leaves showed R_f value of 0.64, whereas the silkworm excrement showed values of 0.63. The values of pectin were found to match the standard pectin with an R_f value of 0.63. This result is in accordance with the preceding work carried out on pectin extraction from apple and citrus pomace (Sood and Mathur, 2014). Similarly, Sumita and Aneri (2020) carried out pectin extraction from apple, lemon, carrot, citron, and French bean and found the R_f values ranged between 0.61 and 0.64, which was in agreement with the value of standard pectin (0.63). The R_f values of each pectin and their profile on the gel plate are presented in (Table 2).

Table 2: Rf values of pectin extracted from different sources

Pectin source	Distance travelled by		R_f value
	Solvent	Solute	
Standard pectin	9.5	6.0	0.63
Fresh leaves	9.5	6.1	0.64
Bark	9.5	6.1	0.64
Left-over leaf	9.5	6.1	0.64
Silkworm excrement	9.5	6.0	0.63

A few qualitative tests were conducted for the pectin derived from different sources and the results are displayed in Table 3. The experimental results of the color test shows that the pectin color falls in the range between dark brown and yellow (Petra *et al.,* 2011). As relative to that, all the pectin extracted from different sources was observed in brown color, which was similar to that of standard pectin. The images of the extracted pectin from different sources are placed in Figure 1. Pectin is highly soluble in pure water. The dry pectin powder tends to hydrate rapidly when added to the water and NaOH. The monovalent cations of pectic acids are more soluble in water compared to di and trivalent salts (Rolin and Vries, 1993). In the current study, all the pectin showed positive results since they all got solubilized both in water and NaOH. Pectin is more prone to alcoholic precipitation because it is less solvated by water molecules. The precipitation also helps to increase the yield of pectin with its polarity. In the current investigation, the pectin extracted from all the sericulture residues showed positive results for alcoholic precipitation. Usually, the optimum pH of pectin ranges between 2.8 and 4.7 and is always on the acidic side due to its best solubility nature in this range. In a recent study by Mohamed, (2016) it was revealed that the pH of pectin ranges from 3.5 to 4.5 for citrus fruits. In the present investigation, the pectin extracts from different sources showed a pH range between 2.9 and 3.8. The pH of pectin extracted from the fresh leaf, left-over leaf, silkworm excrement, and bark was 2.9, 3.5, 3.8, and 3.8, respectively.

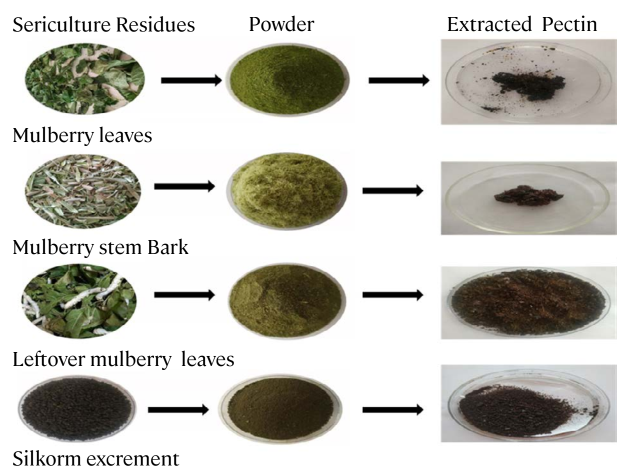

Figure 1: Extracted pectin from different sericulture residues

Table 3: Qualitative tests of extracted pectin from different sources

Qualitative test	Standard Norms	Pectin source			
		Fresh leaves	Bark	Left-over leaf	Silkworm excrement
Colour	White/ pale yellow/ brown	brown	brown	brown	brown
Solubility water/ NaOH	soluble	✓	✓	✓	✓
Precipitation	white precipitate	✓	✓	✓	✓
PH	2.8 to 4.7	2.9	3.8	3.5	3.8
Stiff gel test	formation of stiff gel	✓	✓	✓	✓
Test with KOH	formation of gelatinous precipitate	✓	✓	✓	✓
Iodine test	Absence of blue colour	✓	✓	✓	✓

The most prominent physical property of pectin is its ability to form gels. This results when the polymer chain interacts over a portion of its length to form a three-dimensional network. When pectin is dissolved in water at a high temperature, it forms gels. In this test, all the pectin exhibited a positive result in the formation of gels. In the KOH testing, hydroxide reacts with pectin, which helps them to improve gel strength, whereas potassium reacts to promote gel formation. Hence, it can be said that potassium hydroxide (KOH) helps pectin in gel formation (Bernhard *et al.,* 2004). In this study, all the extracted pectin formed a transparent semi-gel, which indicated a positive result. Similarly, the iodine test conducted for different sources of pectin showed a positive result with the absence of blue colour and the same was confirmed with standard pectin.

The qualitative tests fall under its quality determination and diversify or categorize its proper utilization in different fields. The equivalent weight of pectin always depends upon the source. Usually, the equivalent weight of pectin varies between 400.0 and 795.0 mg (Arshi *et al.,* 2021). A previous study has shown that the equivalent weight of pectin from Nephrolepis biserrate was 1250 g and reported that the higher the equivalent weight, the better the gel-forming ability (Halifah *et al.,* 2019). In this study, when compared to all the sources of pectin, the bark and silkworm excrement showed the highest equivalent weight of 625 mg/ml each, whereas the fresh leaf and left-over leaf showed about 500 mg/ml and 555.5 mg/ml, respectively. The equivalent weight of the standard pectin was found to be 416.66 mg/ml, which was on the lower side when compared to all the pectin sources (Table 4).

Table 4: Physicochemical properties of pectin extracted from different sources

Pectin source	Eq.wt. (mg/ml)	Methoxyl content (%)	AUA (%)	DE (%)
Standard pectin	416.66 (±0.12)*	7.44 (±0.21)*	84.48 (±0.04)*	50.00 (±0.08)*
Fresh leaf	500.00 (±0.02)*	5.00 (±0.23)*	77.44 (±0.14)*	38.09 (±0.23)*
Bark	625.00 (±0.21)*	5.60 (±0.14)*	70.40 (±0.15)*	45.00 (±0.12)*
Left-over leaf	555.50 (±0.10)*	5.00 (±0.12)*	70.40 (±0.12)*	40.00 (±0.21)*
Silkworm excrement	625.00 (±0.12)*	6.20 (±0.21)*	73.92 (±0.22)*	45.50 (±0.22)*

Values represent the mean ± standard deviation (SD) of three separate observations. *Statistically significant ($P < 0.005$).

Methoxyl content is one of the impacting factors which affects the setting time of pectin and determines the potential of the pectin in gelation. Pectin is regarded as high in methoxyl content if it is equal or higher than 7 %. If it is less than 7 %, then it is classified as pectin with low methoxyl content. Previous studies have shown that the methoxyl content of apples is the maximum at 6.840 % and carrots with low methoxyl content at 2.348 % (Sumita and Aneri, 2020). Usually, the methoxyl content of pectin varies between 0.2 and 12.5 % depending on the mode and source of extraction. In the current study, the methoxyl content varied from 5.00 % to 6.20 %. Pectin extracted from fresh leaves and left-over leaves showed the value of 5.00 %, followed by the bark at 5.60 % and silkworm excrement at 6.20 %. From the results, pectin derived from all the sources is considered as low methoxyl pectin since their values have fallen below 7 %. The standard pectin used in the laboratory showed a value of 7.44 %, which indicates its high methoxyl content (Table 4).

Anhydrouronic acid (AUA) content in pectin is an important characteristic that promises the suitability of pectin in food industry for the preparation of jams, jellies, etc. (Virk and Sogi, 2004). The AUA content indicates the purity of extracted pectin with an endorsed value of not less than 65 %. If the AUA value is less than 65 %, then it is considered that the pectin is of low purity and it cannot be used commercially. Results of this study showed that the pectin extracted from all the sources are falling within the purity range in terms of AUA content, with pectin extracted from fresh mulberry leaves showing the maximum value of 77.44 %, followed by the silkworm excrement with 73.92 %, whereas the left-over leaves and bark showed the lowest of 70.40 %. The standard pectin showed an AUA value of 84.48 %, which also reflected the purity of pectin. Our results are consistent with that of Sumita and Aneri (2020), where pectin derived from sources like apple, carrot, lemon, citron, etc., had an AUA value between 72.70 % and 80.95 (Table 4).

The degree of esterification is the proportion of the esterified galacturonic acid groups to the total galacturonic acid groups existing. It is one of the major characteristics that determine the gelling quality of pectin (Altaf et al., 2015). Theoretically, the degree of esterification (DE) can range from 0 % to 100 %. Pectin with a DE of more than 50 % is considered high methoxyl (HM) pectin, whereas less than 50 % is considered low methoxyl (LM) pectin (Walter, 1991). In the present study, the lowest DE value was noticed in fresh mulberry leaves followed by left-over leaves and bark with 38.09 % and 40.00 %, respectively. The bark and silkworm excrement showed the highest DE value of 45.00 and 45.50 %. Since all the pectin extracts have a DE value below 50 %, they can be considered as low methoxyl (LM) pectin and the results coincide with the methoxyl content estimation (Table 4).

The extracted pectin was subjected to the purity assessment. A few non-pectin compounds such as proteins, neutral sugars and ash content were also assessed, which have a distinctive role in jelling ability. In pectin, proteins are observed in very meager quantities. For every 100 g of pectin, only about 0.03 g of protein may be identified. Studies have shown that 2.4 % of protein is present in dry lemon waste (Ketema and Akuma 2021). Our results revealed that mulberry leaves contain about 1.3 g of protein per 100 g, the bark showed a relatively high protein content of 1.90 %, silkworm excrement showed 1.70 %, whereas left-over leaves and fresh leaves showed 1.80 % and 1.60 %, respectively as an impurity in the pectin. All the derived pectin from different sources was in closeness with the standard pectin having a protein content of 2.00 %. The protein values of all the pectin and their percentage difference with respect to standard are represented in Table 5. In pectin, proteins are widely used for the desired conversion of physicochemical properties. The blending of protein moiety to pectin is usually conferred with its excellent emulsifying and emulsion-stabilizing properties and widely used in the food industry for fat incorporation and texture development (Ngouemazong *et al.,* 2015).

Since pectin is made up of galacturonic acid, a sugar derived from galactose, the level of total sugar in pectin ranges from a minimum of 4 % and a maximum of 25 %. Studies have shown that the total sugar content in dry lemon waste ranges between 14.6 and 20.50 %, the pectin extracted from green tea leaves showed up the total sugar level to be around 7 % (Ketema and Akuma 2021). In the current study the total sugar content, bark, and the left-over leaf showed maximum values of 8.70 % and 8.32 % followed by silkworm excrement and fresh leaf at 6.86 % and 6.16 % respectively. The total sugar content in standard pectin was 8.35 % which was in closeness with all the pectin samples, especially with that in the left-over leaf (Table 5).

Table 5: Percentage of protein and total sugar content in pectin extracted in different sources

Pectin source	Protein content (%)	Total sugar content (%)	Ash content (%)
Standard	2.0 (±0.12) NS	8.35 (±0.13)*	4.50 (±0.14)*
Fresh leaves	1.6 (±0.14) NS	6.16 (±0.15)*	3.30 (±0.13)*
Bark	1.9 (±0.02) NS	8.70 (±0.04)*	3.50 (±0.15)*
Left-over leaf	1.8 (±0.01) NS	8.32 (±0.14)*	3.45 (±0.42)*
Silkworm excrement	1.7 (±0.24) NS	6.86 (±0.12)*	3.60 (±0.23)*

Values represent the mean ± standard deviation (SD) of three separate observations. *Statistically significant ($P < 0.005$), NS-Nonsignificant.

The purity of the pectin is determined by ash content. A recent study has shown that the ash content in pectin varies from 2.40 % to 5.65 % (Cagatay *et al.,* 2017). In this study, all the pectin samples showed relatively close values for ash content. The fresh leaf showed the highest content of about 3.30 %, followed by the left-over leaf at 3.45 %, bark at 3.50 % and silkworm excrement at 3.60 %. The ash content of the standard pectin was 4.5 %, and all are within the preferred range for commercial purposes (Table 5).

The biophysical analyses such as P-XRD and FTIR were assessed and found that the results are promising. Extracted pectin's crystalline structures were assessed and found to have distinctive peaks of 2θ at 12.16, 21.08, 21.74, 25.32, 28.75, 29.60, 31.72, 33.30, 37.74, and 41.43, revealing the presence of some crystalline structures of pectin (Table 6 and Figure 2). A series of sharp peaks in general represents crystalline material and seems to be more evident in the extracted pectin than in the standard. The scientific reports of Supreetha *et al.,* (2021), defined the peaks at 13.22, 21.78, and 25.28 as pure pectin. Similarly, the functional analysis FTIR analysis of extracted pectin showed that, a similar number of peaks and functional groups match with that of the standard (Figure 3). The peaks at wave numbers such as 419.96, 1010.34, 1512.30, 1746.88, 2310.87, and 2377.90 cm-1 have confirmed the presence of aryl, methylene, silicon oxy compounds, phosphate ions, organic sulfates sulfates, aromatic secondary amine, hetero-oxy compounds, carbonyl compounds (Table 7), as it is evident from the earlier findings of Nisar *et al.,* (2019) and Wathoni *et al.,* (2019) on Citrus and Garcinia mangostana extracted pectin. The biological activities of identified functional groups such as aryl, hetero oxy, aromatic secondary amine, carbonyl compound, and phosphine are associated with antibacterial, metabolic regulation, precursors for many biological compounds, various pharmaceuticals, and agrochemicals. phosphine are associated with antibacterial, metabolic regulation, precursors for many biological compounds, various pharmaceuticals, and agrochemicals.

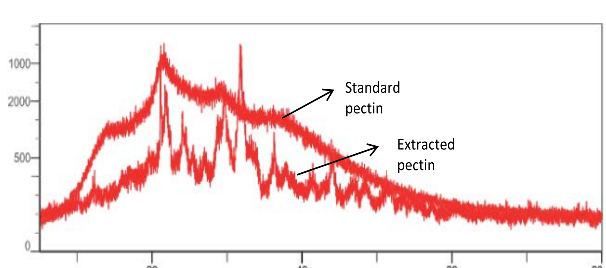

Figure 2: P-XRD pattern of standard and extracted pectin

Table 6: P-XRD tabulated major peak angles, D values and heights of standard and extracted pectin

Standard pectin			Extracted pectin		
2 θ°	D (A°)	Height (cps)	2 θ°	D (A°)	Height (cps)
13.62 (7)	6.50 (3)	224 (10)	12.16 (4)	7.28 (2)	26 (3)
21.28 (4)	4.173 (9)	416 (15)	21.088 (5)	4.2094 (4)	408 (23)
29.12 (5)	3.065 (5)	98 (4)	21.74 (3)	4.085 (5)	210 (14)
38.19 (15)	2.355 (9)	97 (4)	25.32 (2)	3.514 (3)	58 (5)
62.9 (3)	1.477 (7)	42 (3)	28.75 (3)	3.103 (3)	128 (9)
66.5 (3)	1.406 (6)	23.1 (19)	29.604 (17)	3.0151 (17)	198 (3)
69.5 (4)	1.351 (17)	26 (2)	31.729 (17)	2.8179 (15)	309 (18)
73.4 (2)	1.288 (4)	27 (2)	33.30 (3)	2.689 (2)	81 (6)
			37.74 (4)	2.382 (2)	54 (4)
			41.43 (10)	2.178 (5)	36 (3)

Numbers in parentheses are SD of the least units cited.

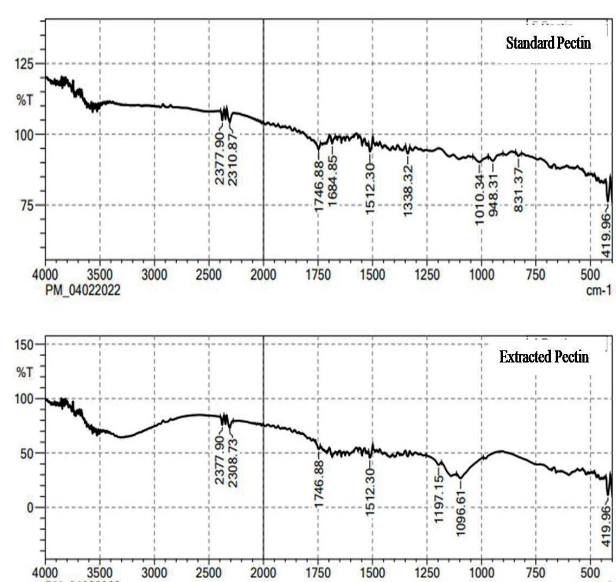

Figure 3: FTIR spectra of standard and extracted pectin

Table 7: FTIR spectroscopy analysis of pectin's functional groups and biological activity

S.No.	Wave Number (Cm-1)		Responsible functional group/Assignment	Biological activity	References
	Standard Pectin	Extracted Pectin			
1	419.96	419.96	Unknown	-	-
2	831.37	-	Aromatic ring (aryl)	Antibacterial	Chaudhary *et al.,* 2006
3	948.31	-	Methylene (Skeletal C-C vibrations)	Antimicrobial	Thesnnar *et al.,* 2021
4	1010.34	-	Simple hetero-oxy compounds (Silicon oxy compounds)	Metabolic regulation	Nicholas *et al.,* 2021 brown
		1096.61	Common inorganic ions (Phosphate ion)	Wide range of biological function related to growth and metabolism	Oves and Iqball, 2019
		1197.15	Simple hetero-oxy compounds (organic sulphates)	Growth, development, intermediary metabolism Used in pharmacological drugs, proteoglycans and steroids	Dawson, 2013
5	1338.32	-	Aromatic amino (Aromatic secondary amine, CN stretch)	precursors of many biological compounds essential for normal functioning of organism	Krzysciak, 2011
6	1512.30	1512.30	Simple hetero-oxy compounds (Nitrogen-oxy compounds)	precursors for the synthesis of various pharmaceuticals and agrochemicals	Gulati *et al.,* 2021
7	1684.85	-	Carbonyl compound (Quinone or conjucated ketone)	Quinones are electron carriers playing a role in photosynthesis. As vitamins, they represent a class of molecules preventing and treating several illnesses such as osteoporosis and cardiovascular diseases.	Nahed *et al.,* 2011
8	1746.88	1746.88	Carbonyl compound (Ester)	Antioxidants and anti microbial	Wintola and Afolayan, 2017
9	2310.87	2308.73	Phosphine	Anticancer activity used in drugs and pharmaceutical products	Berners-Price and Salder, 1988
10	2377.90	2377.90			

Conclusion

Pectin is a structural heteropolysaccharide found in the cell walls of mulberry plant residues and silkworm excrement. It is widely used as an excipient in drugs, as a stabilizer in a wide range of food products, and as a source of dietary fiber. The extracted pectin from the sericulture residues has the same physicochemical properties as commercial pectin. Because raw materials are readily available and easy to preserve and extract, emphasis can be placed on putting them to use. Its application can be heavily diverted to the biomedical and food processing industries, which can provide significant value addition to sericulture sector.

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VULNERABILITY OF SERICULTURAL FARMERS AGAINST CLIMATE CHANGE IN WEST BENGAL

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Abstract

Marginal land holding farmers constitute a significant portion of the West Bengal sericultural production. The climate change is increasingly becoming an important consideration and expected to disproportionately affect the marginal holders. The changing climate might affect the livelihoods of silkworm cocoon producers even more precarious; however, there is limited information on their overall vulnerability and adaptation needs. Keeping the above facts, a total of 100 cocoon producers were randomly selected for survey to characterize the vulnerability of the marginal sericulture farmers and their coping strategies through social vulnerability index (SVI) approach. The findings revealed that, the respondents are more vulnerable to climate change having recorded SVI of 0.47. Economic factors (low income level and less diversified sources of income) and social factors(low level of ownership of land and low level of access to climate change information) influenced the vulnerability level of farmers in West Bengal. Most engaged coping strategies were avoiding brushing or discarding the silkworms during extreme climate vagaries. Crop diversification or shifting to vegetable production and engagement in non-farm jobs are the major coping strategies followed in the study area. The study makes the recommendations on importance of adaptive capacity and resilience of marginal sericultural farmers towards climate change impacts.

Key words: Adaptation, climate change, sericulture, Social Vulnerability Index (SVI), vulnerability.

Introduction

The existing studies on climatic impact highlight that, the sericulture sector is susceptible to change in climate, particularly in developing nations like India (Blaikie *et al.*, 1994).A more significant proportion of people, especially in the sericulture sector are small and marginal land holders and their primary source of livelihood is sericulture farming.The rise of annual mean temperature, irregular rainfall, humidity, unpredictable monsoon, accumulation of anthropogenic greenhouse gases in the atmosphere, and lack of management practices will lead to a reduction in mulberry leaf yield and quality cocoon production. Climate change is therefore likely to have negative welfare implications now and forever on sericulture farmers (Burton *et al.*, 2002).

Vulnerability

The Intergovernmental Panel on Climate Change (IPCC) defines vulnerability as the degree to which a system is susceptible to or unable to cope with adverse effects of climate change, including climate variability and extremes. It is a function of the character, magnitude and rate of climate variation to which a system is exposed, its sensitivity and adaptive capacity(Burton *et al.*, 1993).Vulnerability to climate change varies greatly among regions, sectors and social groups due to climatic and geographic heterogeneity, and differences in physical and social factors that influence a system or people's adaptive capacity. The existing political, economic, social and environmental factors within a system play a critical role in either increasing or decreasing vulnerability arising out of climate change.

The vulnerability to climate change has both physical and social dimensions. The physical dimension of vulnerability relates to exposure and measures the natural hazard or environmental stressor(s) (Donatti *et al.*, 2019).The social dimension of vulnerability termed 'social vulnerability'(Eriksen *et al.*, 2007; Dumenu and Obeng, 2016)'contextual vulnerability' (Gbetibouo and Ringler, 2009), or 'second generation adaptation research' (IPCC, 2001) focuses on the capacity or existing state of the system and is related to the socio-economic, demographic and institutional factors that characterize the capacity of the system, population or individuals (Kane and Shogren, 2000; Kelly and Adger, 2000; O'Brien *et al.*, 2007; Nanita and Shilpa, 2022). This paper adopts Dumenu and Obeng definition of social vulnerability to climate change as the degree to which a system is susceptible to the effects of climate change due to socio-economic and demographic factors (Dumenu and Obeng, 2016). The present study, therefore, examines causes of locally driven vulnerability and also understands current adaptation options, followed in the sericulture sector.

Adaptation

Adaptation has been recognized as an effective option to reduce the negative impact of climate change. In the climate change discourse, adaptation means an adjustment in human and natural systems to actual or expected climatic stimuli,which can reduce the negative impacts of climate change (Parameswaranaik *et al.*, 2015). A wide range of studies has emerged to estimate both macro and micro level adaptation in agriculture and allied sectors. The poor and the vulnerable people have used different adaptation measures (*e.g.*, on-farm strategies and off-farm strategies) to smoothen vagaries of climate change. However, there is a serious lack of information on understanding locally driven vulnerability, micro-level sustainable adaptation practices, and relevant constraints with respect to sericulture. Interestingly, such information is much more relevant for the successful implementation of adaptation strategies in sericulture at the micro level. In this context, it is emphasized on understanding the relationship between present-day coping and vulnerability, which will assist policymakers to design appropriate adaptation policies.

Methodology

The study was conducted in three districts, namely Murshidabad, Malda, and Nadia of West Bengal. These districts have been selected purposively because of their major contribution towards raw silk-production in the state. Eventually from each district, proportionate sericulture farmers were selected for the data collection. In total, 100 marginal holder sericulture farmers were interviewed for data collection with pretested semi-structured interview schedule.

Social Vulnerability Index was adopted with the indicator approach used by Dumenu and Obeng. Based on the extensive literature review, three main components *i.e.*, Demographic, Social and Economic factors, and six indicators were selected to assess the social vulnerability of the districts. The indicators include household size, literacy level, diversified sources of income, level of income, access to climate change information, and ownership of media gadgets (radio or television, or smart phone). Accordingly, this study integrates demographic, economic, and social factors to assess social vulnerability to climate change through a set of indicators that represent the factors of social vulnerability as expressed below:

$$SV = f \cdot 1/n(DF + EF + SF)$$

Where, SV is social vulnerability, DF is demographic factors, EF is the economic factors and SF is social factors, and n is number of social vulnerability factors.

Results and Discussion

Vulnerability of sericulture farmers against climate change

The results of the social vulnerability index (SVI) revealed that marginal holder sericulture farmers in West Bengal are vulnerable to climate change having recorded an SVI of 0.47 (Table 1). In terms of social vulnerability factors, economic factors largely influenced the high vulnerability level of the farmers followed by social factors, with demographic factors being the least.

Low-income level, less diversified sources of income and low level of access to media gadgets (radio or TV or smart phone) contributed to the economic and social factors influencing the vulnerability level of marginal holder sericulture farmers in study areas. The high literacy level and medium household size of the respondents were the least contributing to the social vulnerability of the farmers.

The economic factors have recorded a higher index score (0.72). The contributing factors (sub-components) had low levels of income indicative of an index score of 0.86. This means a large percentage of households in the study area did not have extra income left to cover other expenses after satisfying basic or important household needs. The income levels should be high enough to cover extra household needs, thereby reducing the vulnerability of marginal holder farmers in this region. This finding is similar to that of Gbetibouo and Ringler in 2009 who found that farming regions in South Africa deemed to be most vulnerable to climate change/variability did not always overlap with the most vulnerable populations. The results indicate that vulnerability to climate change is indeed socially differentiated by socio-economic characteristics and the particular context of social groupings. This drives home the point that the most vulnerable people may not be in the most vulnerable places (Ram *et al.*, 2016).

On the other hand, diversified sources of income were significantly limited with the marginal holder sericulture farmers in West Bengal (0.57). Less diversified sources of income coupled with a low-income levelscontributed to its high index score under economicfactors. Limited sources of income restrict people to very narrow range ofresources from which livelihood can be drawn. Thus, in times of shocks or crisis, they are unable to draw on other resources (should the main source of income fail) to secure their needs and minimize the resultant impacts of adverse events such as climate change and variability (Schroter *et al.*, 2005).

Concerning social factors, the critical indicator contributing to the higher index score for social factors responsible for higher climate change vulnerability level of marginal holder sericulture farmers was low-level ownership of mass media gadgets. The study areas have had a small proportion of the marginal holder farmer population owning these important media gadgets. This is indicative of an index score of 0.49.

These gadgets are imperative means of communication for dissemination of information on climate change, forewarning, and adaptation techniques. Singh *et al.*, in 2014 and Donatti *et al.*, in 2019 pointed that, early warning systems as a technological measure that improves the adaptive capacity of farmers. Communication gadgets such as smart phones or TV or radio serve as means of transmitting the information generated for example by early warning systems for the benefit of farmers. Consequently, the low availability of media gadgets in households limits accesses to climate change information thus, rendering people more vulnerable to climate change. Vincent in 2004, pointed out that, limited access to relevant climate change information increases vulnerability. Access to climate change information is critical to developing appropriate and effective responses to climate change impacts.

Table 1 : Indexed sub-components, major components and overall social vulnerability index of marginal holder farmers in West Bengal

Social vulnerability factors (major components)	Indicators (sub-components)	Sub-component indices	Major component index
Demographic	Household size	0.38	0.27
	Literacy	0.17	
Economic	Income level	0.86	0.72
	Diversified sources of income	0.57	
Social	Access to climate change information	0.37	0.43
	Ownership of media gadgets (Radio/TV/Smart phone *etc.*)	0.49	
	Overall social vulnerability index	0.47	

Adaptation Strategies followed by the respondents

Adaptation to climate change and variability is now considered an important subject of research and assessment, not simply to guide the selection of the best mitigation policies, but rather to reduce the vulnerability of populations to the impacts of climate change (Turner *et al.*, 2003; Vincent, 2004). This has, in part, stemmed from a realization that a certain amount of climate change will occur and that society can take concrete steps to minimize the net losses (including taking advantage of opportunities for gains) (Watts and Bohle, 1993).

Table 2 clearly depicts that, the respondents were following mainly two types of adaptation strategies against climate change *i.e.*, on-farm strategies and off-farm strategies. Skipping/avoiding brushing of DFLs is a majorly adapted on-farm strategy (71.00 %) followed by a shift to vegetable production/crop diversification (54.00 %) and a change in micro-climate in silkworm rearing house (44.00 %). Concerning off-farm strategies, income from off-farm job (61.00 %) is highly adapted off-farm strategy followed by relying on family/friends/ neighbors (23.00 %). The study reveals that farming-related coping strategies dominated the non-farming strategies against climate change.

Despite these admirable efforts on the part of farmers to cope with climate change impacts, the measures taken are mostly coping strategies that reduce current vulnerabilities without necessarily accounting for future climate change impacts. To increase the resilience of communities against future climate change impacts, there should be a concerted effort at

promoting planned or anticipatory adaptation strategies based on planned studies and research. In this regard, adaptation strategies would be effective at reducing present and future vulnerabilities to climate change and increasing resilience (Smit and Pilifosova, 2001; Tompkins and Hurlston, 2005).

Table 2: Adaptation/Coping strategies against climate change (n=100)

Coping strategies	Frequency	Percentage
On-Farm strategies		
Skipping/avoiding brushing of DFLs	71	71.00
Shift to vegetable production/Crop diversification	54	54.00
Change in micro-climate in silkworm rearing house	44	44.00
Off-Farm strategies		
Rely on family/friends/neighbors	23	23.00
Income from off-farm job	68	68.00

Conclusion

This study has investigated social vulnerability of marginal holder sericulture farmers to climate change and the coping strategies adopted by them in West Bengal state. The sericulture farmers in the study area were found to be more vulnerable to climate change. Low-income levels, less diversified sources of income, and low-level ownership of media gadgets (radio or television, or smart phone) contributed to the economic and social factors influencing the high vulnerability level of marginal holder sericulture farmers. On the other hand, the most active coping strategies were skipping/avoiding brushing of DFLs followed by a shift to vegetable production/crop diversification and income from off-farm jobs. Hence, it is essential to develop/identify effective adaptation strategies to cope up with climate change through the research and development process. The popularization of efficacious adaptation strategies among sericulture farmers will make them more resilient to climate change.

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LARGE SCALE FIELD TRIALS OF IMPROVED CROSSBREED OF SILKWORM, *BOMBYX MORI*, 12Y × BFC1 IN EASTERN INDIA

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Abstract

Mulberry silk production in Eastern India is dominated by crossbreeds utilizing indigenous Nistari race, particularly N × (SK6.SK7) which is associated with non-gradable quality silk. The improved crossbreed (ICB), 12Y × BFC1 developed for obtaining higher yields with silk of international grade can be an improved alternative for N × (SK6.SK7). Totally, 2.29 lakh DFLs of 12Y × BFC1 were tested against 1.77 lakh DFLs of N × (SK6.SK7) covering 7 states of East and North East India. Eventually, 12Y × BFC1 performed significantly better than N × (SK6.SK7) with improvement in cocoon productivity (12.49 % at 45.31 kg), cocoon weight (5.69 % at 1.449 g), shell weight (10.22 % at 0.248 g), shell percentage (3.46 % at 17.05 %), filament length (10.62 % at 573 m), raw silk recovery (18.75 % at 13.3 %) and neatness (1.15 % at 88 p). The results of the trial clearly showed a dominant performance of the ICB, 12Y × BFC1 both in terms of cocoon productivity and silk quality. Not only in rearing and reeling traits but 12Y × BFC1 was also superior in grainage parameters studied, with highest pairing percent (32.16 %) and egg recovery (52.11 g/kg). Therefore, the improved crossbreed, 12Y × BFC1 performance was superior to the existing popular cross N × (SK6.SK7) and found suitable for rearing in Eastern India.

Key words: Crossbreed, cocoon productivity, egg recovery, reeling traits, shell percentage.

Introduction

Eastern states of India, especially West Bengal is characterized by fluctuating climatic conditions compelling sericulture farmers to rear traditional multivoltine hybrids or multivoltine × bivoltine hybrids (Crossbreeds) as they are tolerant to abiotic challenges but produce inferior quality silk. The quality of silk can be improved by raising cocoons of bivoltine silkworm hybrids or to some extent improved crossbreeds (ICBs). However, the prevailing hot climatic conditions in Eastern states of India like West Bengal, Tripura, Odisha, Jharkhand, and Chattisgarh are not particularly conducive to rear productive bivoltine silkworm hybrids. On the flip side, the existing traditional crossbreeds of Nistari exhibit lower productivity and inferior silk quality. Therefore, ICBs could be game changers in improving the silk quality and productivity in Eastern India (Chandrakanth *et al.*, 2021).

Previously, attempts to develop new multivoltine silkworm breeds and thereby identifying the ICBs by crossing them with high-yielding and superior quality silk producing bivoltine breeds were undertaken. As a result, an ICB, 12Y × BFC1 was identified as suitable for rearing in Eastern India to improve silk productivity as well as quality. The multivoltine parent, 12Y was developed through congenic breeding approach by using MH1 (Donor) and M.Con.4 (Recipient) as parents for high shell content (Chandrakanth *et al.*, 2021). The main concern in congenic breeding is to minimize the heterogeneity and to develop syngenic lines for developing improved hybrids with higher heterosis (Verma *et al.*, 2016). Congenic lines developed needs to be employed to develop viable hybrids that suit the agro-climatic conditions prevailing in eastern India for commercial exploitation.

Multivoltine silkworms are well adapted to the tropical climatic conditions of India and play a major role in the production of silk in India (Datta, 1984). Nistari is the major multivoltine race utilized for commercial raw silk production in eastern India by rearing in the form of crossbreed. Nistari race forms the base for mulberry sericulture in West Bengal as the traditional rearing for over decades has made it well adapted to the local environmental conditions (Nirmal Kumar, 2015). Though, many crossbreeds were identified by silkworm breeders in the last 50 years, none of them were able to replace the Nistari crossbreeds in the field (Mukhopadhyay et al., 2013; Chandrakanth *et al.*, 2021). Earlier testing with small scale field samples of 12Y × BFC1 showed better performance in comparison to the popular Nistari crossbreed, N × (SK6.SK7). Therefore, in order to affirm the improved performance of new ICB, 12Y × BFC1 over the existing crossbreed, N × (SK6.SK7), large scale field testing of the two crossbreeds was required. Furthermore, it was imperative to evaluate the performance of the newly developed promising ICB, 12Y × BFC1 in different states of eastern India to assess its suitability across the seasons and regions. Therefore, in this study, a total of 2.29 lakh DFLs of 12Y × BFC1 were field tested in two years across the seasons, covering seven states of E & NE India. The pre- and post-cocoon and commercial egg production parameters were assessed to understand the suitability and sustainability of 12Y × BFC1 in different regions and seasons.

Materials and Methods

Maintenance of parents

The parental breeds, 12Y and BFC1 were maintained conforming to their original characters by cellular rearings at Central Sericultural Research and Training Institute (CSRTI), Berhampore. 12Y is a plain multivoltine breed that spins elongated yellow coloured oval cocoons whereas BFC1 is a plain bivoltine breed that spins white dumbbell (mild constriction) cocoons. The parental breeds were maintained in three replications by retaining 250 larvae each after the 3rd moult. Stringent selection was performed in the cocoon stage to restrict the parental breeds to their original characters. Silkworm rearing was conducted following the standard methods under recommended temperature and humidity conditions (Krishnaswami *et al.*, 1973).

Multiplication of parental seed

Mass rearing was conducted for multiplication of

12Y and BFC1 at CSRTI, Berhampore. Fifteen-twenty DFLs of each parent were reared in every crop. After 3rd moult, 250 larvae were retained to obtain optimum growth. After cocoon formation, deformed and defective cocoons were removed and cocoons with the original shape as those of the parents were selected for producing P1 DFLs.

The P_1 DFLs were supplied to the adopted seed rearers (ASRs) for P1 rearing. The seed cocoons produced by the ASRs were procured and utilized for preparing hybrid DFLs of 12Y × BFC1. Data on economically important rearing traits like cocoon yield/100 DFLs, cocoon weight, shell weight, shell percentage and chit (number of cocoons in one kg) in each crop was collected.

Production of hybrid DFLs

The seed cocoons generated by the selected ASRs were procured for producing hybrid DFLs of 12Y × BFC1. 12Y × BFC1 DFLs were produced by undergoing the process of cocoon cutting, separation of male and female pupae and crossing female 12Y with the male BFC1. The data on economically important grainage parameters like pairing percentage (%), DFL recovery (%) and egg recovery (g/kg) were collected.

Field testing

12Y × BFC1 DFLs along with the disinfectants were supplied to the farmers, and active crop monitoring was done to ensure crop success. Since N × (SK6.SK7) is the ruling crossbreed, the data on the performance of N × (SK6.SK7) were recorded as control.

Data collection and statistical analysis

Farmer-wise data on rearing traits like cocoon yield, cocoon weight, shell weight and shell percentage along with cocoon selling rate were collected. For reeling parameters, 3kg of green cocoons per farmer was collected representing one sample, which was dried and assessed for silk reeling and grading parameters. Two-way ANOVA was performed on the rearing traits keeping silkworm hybrids and locations (states) as fixed factors.

Results and Discussion

West Bengal is the highest raw silk producing state among the eastern states of India. The availability of mulberry leaves and climatic factors encourage the farmers of West Bengal to take up five crops in a year. Since the climatic conditions from the month of November to March are favourable to rear silkworm, two commercial crops taken during this period perform well, whereas that from May to September are not particularly favourable to silkworm crops and the performance is adversely affected. Understandably, during the unfavorable seasons, farmers restrict themselves to rearing crossbreeds of Nistari for sustainable cocoon yields, despite lower productivity and inferior silk quality. Multiple attempts made by silkworm breeders to identify suitable crossbreeds resulted in different crossbreeds of Nistari. The first crossbreed popular in the field was Nistari × NB4D2. This combination had high hybrid vigor but was extremely susceptible to diseases (Narayanan *et al.*, 1967; Chandrasekharaiah and Jolly, 1986; Dandin *et al.*, 2004). Later, it was replaced by Nistari × (SK6.SK7) and is presently being commercially exploited for sustainable cocoon yields, but with similar disadvantages (Nirmal Kumar, 2015). In this study, 12Y × BFC1 was selected for a large scale trial because the hybrid performance was superior to Nistari × (SK6.SK7) in the small scale field testing conducted in the eastern states of India across the seasons (Chandrakanth et al., 2021).

Rearing performance

In this study, a total of 2.29 lakh DFLs of 12Y × BFC1 were tested against 1.77 lakh DFLs of N × (SK6.SK7) covering 7 states of E and NE India. Rearing data on cocoon yield, cocoon weight, shell weight and shell percentage were collected for twenty-four crops tested (Table 1). An overall average cocoon yield of 45.31 kg/100 DFLs was recorded in 12Y × BFC1 against 40.28 kg/100 DFLs in the control , resulting in an improvement of 12.49 %. The highest cocoon yield of 57.11 kg for 12Y × BFC1 was recorded in Arunachal Pradesh followed by 50.26 kg in West Bengal and 44.87 kg in Jharkhand. The highest cocoon weight (1.631 g) and shell weight (0.286 g) were recorded in West Bengal, while the highest shell percentage (18.03 %) was recorded in Tripura. The overall average of cocoon weight (1.449 g), shell weight (0.248 g) and shell percentage (17.05 %) have resulted in improvements of 5.69 %, 10.22 % and 3.46 % respectively over the control. Similar results were also observed during the small scale on-farm trials of 12Y × BFC1 as reported by Chandrakanth *et al.* (2021).

In West Bengal, a total of 1.65 lakh DFLs of 12Y × BFC1 were tested against 1.20 lakh DFLs of the control [N × (SK6.SK7)] covering six crops (Table 1). Only one crop was conducted in the unfavorable season (June/July), and the other five crops were in the favorable season. In unfavorable season, 12Y × BFC1 recorded a cocoon yield of 47 kg against 44.5 kg in the control, an improvement of 5.61 %. In favorable seasons, the cocoon yield for 12Y × BFC1 ranged from 44.32 to 54.45 kg and that of the control ranged from 40.24 to 47.5 kg. Similarly, the cocoon weight and shell weight ranged from 1.422 to 1.737 g and 0.231 to 0.337 g for 12Y × BFC1. The overall performance of 12Y × BFC1 in West Bengal showed an improvement of 8.13% in cocoon yield (50.26 kg), 10.41 % in cocoon weight (1.631 g) and 17.21 % in shell weight (0.286 g). The cocoons of 12Y × BFC1 were sold at a higher rate than that of N × (SK6.SK7) by Rs. 55/ kg. A farmer who rears 100 DFLs of 12Y × BFC1 would be benefited from a higher profit of Rs. 5131 when compared to rearing N × (SK6.SK7).

In Tripura, a total of 58000 DFLs of 12Y × BFC1 were tested against 55000 DFLs of control [N × (SK6.SK7)/ PM × CSR2] covering 6 crops (Table 1). Only one crop was conducted in the unfavorable season (June/July) and the other five crops in favorable season. In the unfavorable season, 12Y × BFC1 recorded a cocoon yield of 47 kg. In favorable seasons, the cocoon yield for 12Y × BFC1 ranged from 39.5 to 49.3 kg, and that of the control ranged from 38.4 to 48 kg. Similarly, the cocoon weight and shell weight ranged from 1.257 to 1.75 g and 0.213 to 0.33 g in 12Y × BFC1. The overall performance of 12Y × BFC1 in Tripura showed an improvement of 11.45 % in cocoon yield (42.72 kg), 8.46 % in cocoon weight (1.513 g) and 9.13 % in shell weight (0.275 g) over control. Since all the cocoons produced in Tripura are sold at a fixed price to the state sericulture department, there was no quality-linked price difference in selling the cocoons. In Tripura, the farmer would gain an additional amount of Rs. 1228 by rearing 100 DFLs of 12Y × BFC1 compared to the control.

In Nagaland, when 12Y × BFC1 was tested covering two favorable seasons against N × (SK6.SK7) as control (Table 1), the highest cocoon yield and better cocoon traits were noticed in spring crop when compared to autumn crop. 12Y × BFC1 recorded an average cocoon yield of 40.15 kg against 35.03 kg of control, an improvement of 14.62 %. The other rearing traits like cocoon weight (1.359 g) and shell weight (0.243 g) were increased by 3.03 % and 14.69 %, respectively. 12Y × BFC1 cocoons fetched a higher rate than N × (SK6.SK7) by Rs.20/kg. If the farmer prefers to rear 12Y × BFC1 in place of control, then he would be benefited from a higher profit of Rs. 2237/ 100 DFLs.

When 12Y × BFC1 was tested in Assam covering three favorable seasons against N × (SK6.SK7) as a control (Table 1), the highest cocoon yield was noticed in autumn crop when compared to spring crop. 12Y × BFC1 recorded an average cocoon yield of 40.19 kg against 38.23 kg of control, an improvement of 5.13 %. The other rearing traits like cocoon weight (1.319 g) and shell weight (0.218 g) were increased by 8.03 % and 4.31 %, respectively.

12Y × BFC1 was also tested in small quantity in the eastern state Odisha, covering two favorable seasons, against N × (SK6.SK7) as control (Table 1). The highest cocoon yield was seen in the autumn crop when compared to the spring crop. 12Y × BFC1 recorded an average cocoon yield of 41.88 kg versus 38.32 kg of control with an improvement of 9.29 %. The other rearing traits like cocoon weight (1.456 g) and shell weight (0.217 g) were increased by 0.9 % and 2.84 %, respectively. 12Y × BFC1 cocoons fetched a higher rate than N × (SK6.SK7) by Rs.10/kg. If the farmer preferred to rear 12Y × BFC1 in place of control then he would be benefited from a higher profit of Rs.1130/ 100 DFLs.

A small quantity of DFLs of 12Y × BFC1 was tested in Arunachal Pradesh and Jharkhand states (Table 1). As these states are bivoltine dominant, control was not considered. An average cocoon yield of 57.11 kg and 44.87 kg was recorded in Arunachal Pradesh and

Jharkhand, respectively. cocoon weight and shell weight recorded were 1.531 g and 0.262 g in Arunachal Pradesh and 1.352 g and 0.227 g in Jharkhand.

It is important for the silkworm breeders to understand and recommend stable breeds and hybrids under different environmental conditions to the farmers. Silkworm breeds and hybrids that perform constantly well under different environmental conditions with fewer variations are considered stable. According to Allard and Bradshaw (1964), the performance of the breed or hybrid in a given environment indicates its superiority. Since most of the economic traits in silkworms are qualitative in nature, the phenotypic measurement of such traits is highly influenced by environmental factors (Pillai and Krishnaswami, 1980; Zhang et al., 2002; Ramesha et al., 2010). It is essential to measure the degree of phenotypic differences in economic traits under varied environmental conditions to understand genetic steadiness. In the present study, the phenotypic expression of rearing traits of 12Y × BFC1 was tested in the fields of seven states across seasons against N x (SK6.SK7). A notable increment was observed in the rearing traits of 12Y × BFC1 against the control. Therefore, 12Y × BFC1 is considered superior to N × (SK6.SK7) in all the states and seasons tested.

The stable performance of the 12Y × BFC1 across seasons over the control suggests that there was less interference from unfavorable genes (Chattopadhyay et al., 2001 c). A few studies reported varied expression by different breeds under different climatic conditions (Watanabe, 1928; Ueda and Lizuka, 1962). Different silkworm hybrids expressed differently when evaluated under various seasons (Krishnaswami and Narasimhanna, 1974). The differences in fitness of silkworms in different seasons and regions have long been recognized in Japan and China, and the outcomes were identified as season- or region-specific breeds or hybrids to obtain sustainable cocoon yields (Hirobe and Ooi, 1954; Yokoyama, 1976; Xu Meng Kui et al., 1990; He Yi *et al.*, 1991).

Table 1: State-wise rearing performance of 12Y × BFC1

State	Hybrid	Crop	DFLs	Farmer (No.)	Yield/ 100 DFLs (kg)	Cocoon weight (g)	Shell weight (g)	Shell percent-age	Rate (Rs.)	Average amount/ 100 DFLs (Rs.)
West Bengal	12Y × BFC1	6	165000	1679	50.26	1.631	0.286	17.46	505	25487
	N × (SK6.SK7)		120000	1555	46.48	1.477	0.244	16.54	450	20250
	Improvement (%)		-	-	8.13	10.41	17.21	5.59	55	5131
Tripura	12Y × BFC1	6	58500	1115	42.72	1.513	0.275	18.03	280	11960
	N × (SK6.SK7)		55000	1100	38.33	1.395	0.252	18.02	280	10732
	Improvement (%)		-	-	11.45	8.46	9.13	0.06	0	1228
Nagaland	12Y × BFC1	2	1100	20	40.15	1.359	0.243	17.72	300	12045
	N × (SK6.SK7)		1000	20	35.03	1.319	0.211	16.10	280	9808
	Improvement (%)		-	-	14.62	3.03	14.69	10.12	20	2237
Assam	12Y × BFC1	3	1300	28	40.19	1.319	0.218	16.6	Self reeling	
	N × (SK6.SK7)		1000	20	38.23	1.221	0.209	17.12		
	Improvement (%)		-	-	5.13	8.03	4.31	-3.04		
Arunachal Pradesh	12Y × BFC1	2	800	8	41.88	1.456	0.217	14.96	210	8795
	N × (SK6.SK7)		600	8	38.32	1.443	0.211	14.62	200	7665
	Improvement (%)		-	-	9.29	0.90	2.84	2.33	10	1130
Arunachal Pradesh	12Y × BFC1	2	800	9	57.11	1.531	0.262	17.24	300	17100
Jharkhand	12Y × BFC1	3	1550	38	44.87	1.352	0.227	16.80	205	9225
Total/Average	12Y × BFC1	24	229050	2897	45.31	1.449	0.248	17.05	300	14102
	N × (SK6.SK7)	19	177600	2703	40.28	1.371	0.225	16.48	303	12114
Improvement	12Y × BFC1	24	229050	2897	45.31	1.449	0.248	17.05	300	14102

The results of the two-way ANOVA performed on the rearing traits with silkworm hybrids and locations (states) as fixed factors revealed that there was no significant difference between the cocoon weight of 12Y × BFC1 and that of the control, but the shell weight and cocoon yield/100 DFLs varied significantly among the hybrids, locations (states), and their interactions (Table 2). The reason for the significant difference in shell weight may be attributed to the genetic nature of 12Y breed, as it is a congenic multivoltine breed developed by crossing, continuous backcrossing with the receptor parent, and sib-mating for seven generations by applying directional selection towards higher shell content. In the process, the breed might also have developed higher genetic plasticity. Therefore, the resultant crossbreed performed constantly well with high shell content across the seasons and regions, in line with the earlier reports (Chattopadhyay *et al.*, 2001 a, b).

Table 2: Two-way ANOVA on rearing traits

Rearing trait	Factor	DF	Mean square	P
	State	6	407.5	0.0001
Cocoon yield / 100 DFLs	Hybrid	1	809.5	0.0001
	State × Hybrid	6	28.6	0.0001
	State	6	0.127	0.0001
Cocoon weight	Hybrid	1	0.076	0.076
	State × Hybrid	6	0.132	0.0001
	State	6	0.006	0.001
Shell weight	Hybrid	1	0.003	0.01
	State × Hybrid	6	0.014	0.001

Rearing performance

Seven parameters namely filament length, non-breakable filament length, denier, reelability, renditta, raw silk and neatness were considered for assessing the reeling performance of the silkworm hybrids. Fifty-five batches of 3 kg each of green cocoons were analyzed for post cocoon analysis for 12Y × BFC1 and 26 batches for the control. The overall results of the reeling analysis showed that the 12Y × BFC1 hybrid is superior to the control in many of the parameter studied (Table 3). The filament length of the 12Y × BFC1 was 573 m in comparison to that of the control's 518 m with an improvement of 10.62 %. Similarly, the NBFL of the 12Y × BFC1 was 425 m against the control's 416 m with an improvement of 2.16 %. The denier was better in control than in 12Y × BFC1. The reelability of the 12Y × BFC1 was on par with that of the control at 65 %. The renditta of 12Y × BFC1 was 12.96 % less than that of the control. The raw silk and neatness of the 12Y × BFC1 was 13.3 % and 88 p against 11.2 % and 87 p of the control with an improvement of 18.75 % and 1.15 %, respectively. The improvement in the reeling parameters is primarily attributed to the bivoltine component that is BFC1 with high shell content (19-20 %) whereas the bivoltine component for existing crossbreed is SK6 × SK7 (17-18 %).

Table 3: State-wise reeling performance of 12Y × BFC1

State	Hybrid	No.	Crop	Filament length	Non-broken filament length (m)	Denier (d)	Reel-ability (%)	Renditta (kg)	Raw silk (%)	Neat-ness
West Bengal*	12Y × BFC1	35	3	583	484	2.82	83	9.52	10.72	94
	Control	20		542	439	2.62	79.9	10.2	9.89	91
Tripura*	12Y × BFC1	15	3	631	428	2.77	68.2	9.4	12.1	93
	Control	5		500	297	2.60	59.9	10.2	15.4	87
Nagaland	12Y × BFC1	1	1	634	634	2.64	58	10.99	9.10	90
	Control	1		511	511	2.45	56	12.01	8.33	83
Assam	12Y × BFC1						Reeling by the farmers themselves			
Odisha	12Y × BFC1	1	1	546	274	2.53	62	9.21	10.8	80
Arunachal Pradesh	12Y × BFC1	1	1	456	229	2.36	56	7.55	24.4	81
Jharkhand	12Y × BFC1	2	2	456	229	2.36	56	7.55	24.4	81
12Y × BFC1				**573**	**425**	**2.67**	**65**	**9.4**	**13.3**	**88**
Control N × (SK6.SK7)				**518**	**416**	**2.55**	**65**	**10.8**	**11.2**	**87**
Improvement over control (%)				10.62	2.16	4.71	0.00	-12.96	18.75	1.15

* Represents the data for three seasons

Grainage performance

The P1 rearings were conducted in six crops, of which five were done in West Bengal and one in Andhra Pradesh at the premises of the ASRs attached to NSSO-SSPC units (Table 4). In West Bengal, the average cocoon yield recorded for 12Y was 26.87 kg and that for BFC1 was 36.19 kg. The highest and lowest cocoon yields for 12Y were 33.75 kg and 20.32 kg during the December and May crops, respectively. Similarly, the highest and lowest cocoon yields for BFC1 were 40.47 kg and 20.81 kg during the December and September crops, respectively. The chit for 12Y was ranging from 780 to 908 and that of BFC1 was ranging from 650 to 695. But the one crop taken in Andhra Pradesh showed the highest cocoon yield for 12Y and BFC1 with 52 kg and 60 kg, respectively. The chit recorded for 12Y and BFC1 was 749 and 655 with 16.17 % and 19.56 % shell percentage, respectively. The lowest traits were recorded in the September season because it is the adverse season with high temperature and high humidity, resulting in an increase in silkworm diseases affecting rearing parameters adversely (Hossain *et al.*, 2017).

Table 4: P1 rearing performance of 12Y and BFC1

Seed Crop	Breed	DFLs	Farmer (No.)	Yield/ 100 DFLs (kg)	Cocoon weight (g)	Shell weight (g)	Shell percentage	Chit
West Bengal								
Dec.	12Y	850	8	21.93	1.114	0.143	12.87	797
	BFC1	650	3	40.47	1.48	0.28	19.32	686
Feb.	12Y	800	6	28.44	1.161	0.171	14.77	861
	BFC1	600	3	45.80	1.50	0.30	19.96	664
May	12Y	500	8	20.32	1.198	0.144	12.02	780
	BFC1	200	2	35.09	1.459	0.298	20.42	695
Sep.	12Y	200	2	29.90	1.29	0.20	15.57	820
	BFC1	100	1	20.80	1.538	0.302	19.64	650
Dec.	12Y	2000	35	33.75	1.104	0.137	12.41	908
Total / Avg.	**12Y**	**4350**	**59**	**26.87**	**1.173**	**0.159**	**13.53**	**833**
	BFC1	**2550**	**15**	**36.19**	**1.497**	**0.294**	**19.65**	**669**
		CD @ 1%		8.60	0.17	0.07	3.32	91.58
		CV (%)		26.52	12.72	30.80	19.49	11.86
Andhra Pradesh								
Dec.	12Y	500	1	52	1.36	0.22	16.17	749
	BFC1	300	1	60	1.687	0.33	19.56	655

The grainage was conducted for six crops in West Bengal and once in Andhra Pradesh. 12Y × BFC1 was superior in all the grainage parameters studied vis-à-vis the popular control crossbreed N × (SK6.SK7). Data analysis revealed significant differences in pairing percentage, DFLs recovery and egg recovery between the silkworm hybrids during the October seasons, indicating better performance of 12Y and BFC1 in adverse conditions (September) in comparison to the control (Table 5). The highest pairing percentage (32.16 %), DFLs recovery (24.27 %) and egg recovery (52.11 g/kg) for 12Y × BFC1 were found in the March grainage season. For N × (SK6.SK7) also the highest pairing percentage (32.04 %), DFLs recovery (22.47 %) and egg recovery (50 g/kg) were during the March grainage season. Irrespective of hybrids, the March grainage season was the best for DFLs recovery. But the one crop taken in Andhra Pradesh showed the highest egg recovery of 61.5 g/kg, showing the importance of climatic conditions. Quite understandably, the performance of 12Y × BFC1 was negatively impacted during unfavorable seasons in West Bengal and Tripura states in comparison to favorable seasons due to high temperature and humidity conditions. Similar results with reduced fecundity and fertility were observed in the silkworms exposed to high temperatures and high humidity (Hussain et al., 2011). The performance of 12Y × BFC1 was superior in terms of rearing, reeling and grainage parameters. 12Y × BFC1 crossbreed performed outstandingly in all seven states across the seasons.

Large-scale trial of 12Y × BFC1 demonstrated higher cocoon productivity and shell content than the ruling crossbreed, Nistari × (SK6.SK7). 12Y × BFC1 exhibited consistent and stable performance in the field with regard to cocoon yield and other important economic traits, resulting in additional income for the farmers. The field-testing results showed that 12Y × BFC1 has the potential for rearing under favorable and unfavorable seasons alike and produce gradable silk quality. Commercial exploitation of 12Y × BFC1 can enhance silk productivity in eastern India and gradually replace Nistari-based crossbreeds.

Table 5: Grainage performance of 12Y × BFC1

Unit	Grainage season	Hybrid	Pair %	DFLs recovery (%)	Egg recovery (g/kg)	DFLs by No.	C:D Ratio
Berhampore	Oct.	12Y × BFC1	28.04*	23.04*	46.94*	2300	5.65:1
		N × (SK6.SK7)	20.17*	11.11*	25.4*	-	9.0:1
	Jan.	12Y × BFC1	30.05	21.04	46.89	32900	4.75:1
		N × (SK6.SK7)	31.96	20.11	45.04	-	4.97:1
	Mar.	12Y × BFC1	32.16	24.27	52.11	41500	4.12:1
		N × (SK6.SK7)	32.04	22.47	50	-	4.45:1
	June	12Y × BFC1	27.55	18.68	41.18	14100	5.11:1
		N × (SK6.SK7)	26.98	18.56	42.5	-	5.23:1
	Oct.	12Y × BFC1	26.72*	18.95*	41.81*	13000	5.28:1
		N × (SK6.SK7)	22*	14.09*	32.06*	-	7.1:1
	Jan.	12Y × BFC1	27.3	20.6	46.4	68900	4.86:1
		N × (SK6.SK7)	26.4	18.5	41	-	5.41:1
		CD @ 1%	2.97	4.20	7.21	-	-
		CV (%)	12.64	24.44	19.34	-	-
Madanapalle	Jan. 21	12Y × BFC1	30.1	32.9	61.5	54500	-

C:D ratio = Cocoons : DFLs ratio

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FIRST REPORT OF *AGAMERMIS* SP. PARASITIZING INDIAN TROPICAL TASAR SILKWORM, *ANTHERAEA MYLITTA* D.

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Abstract

Parasitization by mermithid nematodes causes severe damage to the economically important silkworm, *Antheraea mylitta* in certain potentially important tasar culture regions of India. The host mortality rate varies from mild to severe. Hence, the present study was taken up as it is of paramount importance to identify the mermithid at the species level to devise eco-friendly management measures to avoid economic loss. The mermithids, measure 18 to 36 cm in length and 0.42 to 0.51 mm in width, consisting of a homocephalic mouth with a terminal opening surrounded by six papillae, cuticular crisscross fibers, an S-shaped vagina, paired spicules, and a stub-like appendage at the tail end, but no finger-like appendage as prominently seen in the genus *Hexamermis.* Thus, our findings represent the first detailed morphological documentation of *Agamermis* mermithid parasitizing a lepidopteran insect. Morphological characteristics indicate that this mermithid belongs to the Mermithidae family, genus *Agamermis,* and has been named as a new species, *Agamermis mylittensisantheraea* sp. Cobb, Steiner and Christie, 1923; synonymous with the names of the Professors who first identified the *Agamermis* species and *Antheraea mylitta,* the non mulberry silkworm host.

Key words: *Agamermis, Antheraea mylitta,* crater or stub-like appendage, Mermithid, Tasar silkworm.

Introduction

Tasar silk is a forest produce and has been practiced by the tribal people from time immemorial in the tropical and temperate belts, even before the introduction of mulberry in India. The Indian tropical tasar silkworm, *Antheraea mylitta* is a polyphagous sericigenous insect and feeds mainly on Arjun *(Terminalia arjuna),* Asan *(Terminalia tomentosa)* and Sal (*Shorea robusta*), which are most abundant in various states of Indian natural forests. The tasar culture, with its lowest investment, has immense potential for providing employment opportunities to a large tribal population. Tasar silk alone accounts for nearly 90% of the global non-mulberry silk production, with approximately 1.32 to 1.50 lakh tribal families engaged in tasar culture (Suryanarayana *et al.,* 2007; Hugar *et al.,* 2016).

At present, the tasar culture is facing many problems caused by various biotic and abiotic factors. Among the biotic factors, diseases are the major constraints in the tasar culture. The infectious diseases of the tasar silkworms caused by microsporidians, fungi, bacteria and viruses cause substantial economic loss in tasar cocoon production leading to drastic reduction in raw tasar silk production. In addition to these diseases, parasitic nematodes have been causing considerable damage to the economically important silk producing insect, *Antheraea mylitta* for the last four decades. This nematode is more rampant during the final instars, especially during the first crop in July when is rains frequently. The infective juvenile nematodes enter tasar silkworms through their natural openings, spiracles and feed mainly on different tissues like gut, fat bodies and silkglands. A severe infestation of nematodes has been reported from various tasar silkworm rearing areas in the states of Telangana and Odisha *viz.,* Chinnor and Nabarangpur. Various authors have reported moderate to severe infestations of the nematodes belonging to unidentified genera in tasar silkworms from major tasar rearing areas of India (Griyaghey and Das, 1982; Chaudhuri *et al .,* 1995; Yadav, 2014). Mondal (1986) recorded a nematode parasite and presumed that it might be *Hexamermis* sp. However, the mermithids infesting the tasar silkworm, *A. mylitta* were not identified to the species level by any morphological methods by the same author. Likewise, a brief report was published with an *Agamermis* species infesting a lepidopteran pest, *Nephopteryx leucophaella,* a pest of brinjal in India, without detailed morphological and molecular studies (Mandal *et al.,* 2005).

Nematodes from the family Mermithidae (Enoplea: Mermithida) are obligate endoparasites of insect hosts, mainly terrestrial and aquatic insects (Poinar, 1975). Mermithid parasitism is typically lethal to its host, as the exit of the fully developed nematodes from the body of the host causes irreparable damage (Poinar, 1975). They are known to attack a wide range of insects belonging to the orders Coleoptera, Orthoptera, Diptera, Hemiptera, Hymenoptera, Heteroptera and Lepidoptera (Nickle, 1972; Gradinarov, 2014). There are approximately 200 described mermithid species, but the biology and distributions of only a few are well known (Poinar, 1975). However, given their high diversity and ubiquitous presence, their total number of species is still unknown (Hugot *et al.*, 2001; Blaxter, 2003). As a consequence of their conserved, phenotypic plasticity, cryptic speciation and simple body plan, morphological diagnostics are difficult, laborious and in most cases restricted to experienced nematology taxonomists (Derycke *et al.,* 2010; Vogt *et al.,* 2014). Although the nematode infestation in tasar silkworms has become very severe; alas, no work has been carried out on nematode morphology to date to identify the mermithid at the species level. Hence, the aim of our study was application of morphological methods as part of an integrative taxonomic approach to identify the nematode mermithid species infesting tasar silkworms and devise suitable eco-friendly management methods to avoid financial loss in tasar culture.

Materials and Methods

Post parasitic juvenile mermithids

Live post-parasitic juvenile mermithid nematodes that naturally emerged from the 5th instar tropical tasar silkworm, *Antheraea mylitta* from Chinnor, Telangana State, India were collected for their morphological, scanning electron microscopic (SEM) studies, or preserved in 100 % ethanol for further use. Nematode mermithids observed in the abdominal cavities of dissected insects and those that naturally emerged have been photographically documented.

Light and scanning electron microscopy

Nematodes collected from the infested tasar silkworms were examined for morphological features using light and a scanning electron microscope by using the appropriate taxonomic keys. Briefly, the live mermithids were heat killed by immersion in 70 °C distilled water for 2 min, fixed in 3 % formalin solution and subsequently transferred into 50 ml beaker with 25 ml ethanol-glycerol mixture (70 ml 95 % ethanol; 5 ml glycerol; 25 ml water) which was allowed to evaporate at room temperature (Seinhorst, 1959; Poinar, 1975). Specimens in glycerol were pliable and examined at up to 100x magnification using a Carl Zeiss Axio microscope equipped with a digital camera and specialized software. The scanning electron microscopy (SEM) was carried out according to Zhang *et al.* (2008). The head, middle and tail portions were cut and used for scanning electron microscopy studies. The excised parts of mermithids were first fixed in 3 % glutaraldehyde prepared in 0.1 M cacodylate buffer for 24 h at 4 °C. Post-fixed with 2 % osmium tetroxide solution, then washed in sterile distilled water and dehydrated in an ethanol gradient series; dried in a critical point dryer (EMS-850) and coated with gold in ion sputtering coater device (JEOL-JFC1100-E); mounted on copper stubs and scanned under FEI Quanta 200 scanning electron microscope. The morphological taxonomic distinction of the genus of this nematode was completed using existing taxonomic keys and descriptions available in the published taxonomic literature (Nickle, 1972; Baker and Poinar, 1995; Achinelly and Camino, 2008; Stubbins *et al.*, 2016).

Results

Mermithid nematode parasites were reported to parasitize the larvae of tasar silkworms brushed during the year 2015 and 2017 in the July first crop. The dampness of the tasar rearing areas with relatively high rainfall may be the possible reason for the high incidence of mermithid parasitism on *A. mylitta* larvae. The mermithid infested larvae show the general symptoms of loss of appetite, swollen body, sluggishness, reduced feeding, retardation of growth, inability to defecate since the inside body and coelomic fluid was covered with an interwoven spiral mass of mermithid nematodes and poor development of silkglands. Finally, white thread-like post-parasitic juvenile mermithid nematodes of approximately 25 to 35 in number egress from the host body through the spiracles, mouth, anal opening, or even by rupturing the cuticle (Figure 1 a to c) and eventually lead to death of tasar silkworms, which results in considerable tasar cocoon crop loss in India.

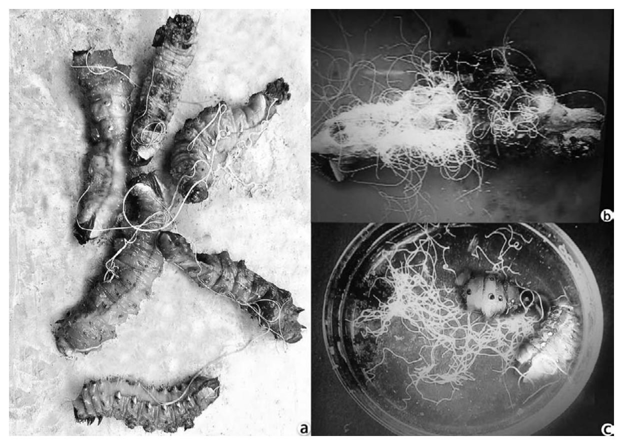

Figure 1: *Agamermis mylittensisantheraea* species infesting fifth-instar tasar silkworm, *Antheraea mylitta.* a. Post-parasitic juvenile nematode mermithids that emerged from host body through the spiracles, mouth, anal opening and by rupturing the cuticle, **b-c.** Numerous white thread-like post-parasitic juvenile mermithids that emerged naturally as well as after dissecting the infested tasar silkworm.

The post-parasitic juveniles are fairly lengthy and the mean body lengths (n = 10) were measured as 30 to 36 cm for females and 18 to 22 cm for males with a circular width of 0.42–0.51 mm (Figure 1 b, c) with a head and tail (Figure 2 a to c). The body color is white, slender and non-segmented (Figure 1 a to c). The mouth is homocephalic in both sexes, with a terminal opening and usually surrounded by six cephalic papillae in one plane (Figure 3 c). The cuticle contains prominent crisscross fibers (Figure 2 g to i); a bluntly rounded tail (Figure 2 d to f) with a crater or stub-like

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appendage (Figure 2 d, f) but not like a finger or hook type appendage as prominently and commonly seen in the genus *Hexamermis;* an S-shaped vagina (Figure 3 a); and a pair of slightly curved spicules (Figure 3 b). After their exit from the silkworm larvae, they enter the bottom soil and undergo moulting. After moulting, mating and oviposition occur after four days. Thousands of unembryonated eggs are deposited in the bottom soil by female mermithids during August and September. The eggs are very small and round in shape, have a smooth surface and are without any hair-like structures or byssi. The development of embryonation usually completes within 8 to 12 days and the eggs contain fully developed mermithid larvae. The eggs do not hatch upon completion of development, but remain dormant for a couple of months. When favorable conditions occur during late July with rain, dampness and mist, the free living pre-parasitic infestive juveniles emerge from the eggs actively. When compared with post-parasitic juveniles, the pre-parasitic infestive juvenile mermithids collected from the soil and live bait method is very small in size, ranging from 5 to 7 mm and 9 to 12 mm, respectively. No hook or finger-like caudal appendage has been observed. When the host plants are wet due to rain, the larvae slowly move towards the trunk, reach the branches and leaves and actively search for the host. When they encounter tasar silkworms, they enter mainly by penetrating through the inter-segmental membrane of the silkworms. Later, the mermithids grow inside the silkworms and after 20 to 25 days of parasitism, very lengthy post-parasitic juveniles egress from the silkworms (Figure 1 a, b). Thus, the identified nematode mermithid completes its life cycle once a year in the host insect, *A. mylitta* in certain tasar silkworm rearing regions in India.

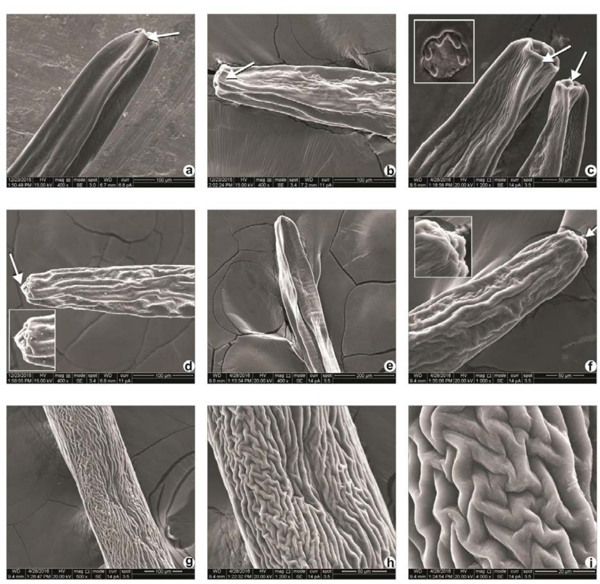

Figure 2: *Agamermis mylittensisantheraea* species infesting fifth-instar tasar silkworm, *Antheraea mylitta* as seen under scanning electron microscope (SEM). a-c. Anterior portion of the mouth opening and inset image showing six cephalic papillae, **d-f.** Bluntly rounded posterior end tail showing the residual crater or stub-like nodal appendage without a hook or finger-like appendage as prominently seen in *Hexamermis* species; clear crater or stub-like nodal appendage with higher magnification is shown in inset, **g-i.** lower to higher magnification of cuticular crisscross fibres.

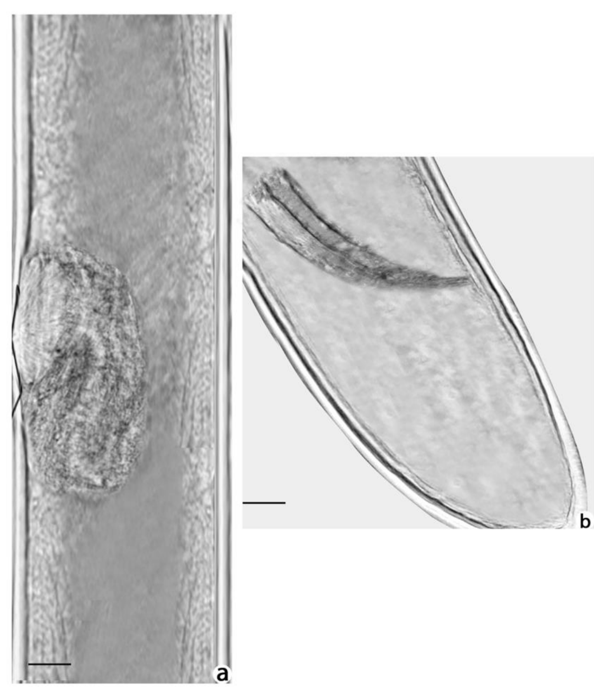

Figure 3: *Agamermis mylittensisantheraea* species infesting fifth-instar tasar silkworm, *Antheraea mylitta*. a. S-shaped female mermithid vagina, b. Male mermithid with paired spicules (scale bar 50 μm).

Discussion

Despite their association with economically important pests of Orthoptera and Hemiptera, hitherto there has been no in-depth, detailed report of *Agamermis* sp. parasitizing insects that belong to the order Lepidoptera. To the best of our knowledge, natural parasitism by a parasitic *Agamermis* mermithid species is reported for the first time from an economically important silk producing Indian tropical tasar silkworm, *A. mylitta* (Lepidoptera: Saturniidae) based on morphological studies. Thus, this is the first detailed morphological documentation of an *Agamermis* mermithid nematode parasitizing a lepidopteran insect. The taxonomic distinction of the genus of this mermithid based on morphological features was completed according to taxonomic keys and descriptions published by different subject experts (Nickle, 1972; Poinar, 1977, 1979; Wouts, 1984; Poinar and Chang, 1985; Stock and Camino, 1992; Baker and Poinar, 1995; Achinelly and Camino, 2008; Stubbins *et al.*, 2016).

Mermithid infestation in different lepidopteran insects, especially *Hexamermis* species is well documented by various researchers (Wouts, 1984; Poinar and Chang, 1985; Mazza *et al.*, 2017). Examination of parasitic mermithids revealed the presence of crisscross fibers; diagnostic characteristic features of the family Mermithidae (Kaya and Stock, 1997; Rizvi, 2010). Furthermore, morphological observations on *Agamermis* sp. obtained from *A. mylitta* revealed that it is not at all a *Hexamermis* sp. as reported by the earlier researcher (Mondal, 1986). In 1937, an anonymous Japanese scientist identified a parasitic nematode from the lepidopteran oak silkworm, *Antheraea pernyi* in Liaoning Province in China and named it *Pernyimermis* sp. Later, two genera and six species of Mermithidae were identified as the parasites of oak silkworms *viz., Amphimermis elegans* (Hagmeier, 1912), *Amphimermis* sp., *Hexamermis micromphidis* (Steiner, 1925), *Hexamermis arsenidea* sp. (Hagmeier, 1912), *Hexamermis Kirjanovae* (Polozhentsev and Artyukhovsky, 1958) and *Hexamermis brevis* (Hagmeier, 1912) as reported by Wang and Li (1987). Members of the genus *Hexamermis* are well-known natural parasites of lepidopteran insects and due to this, Mondal (1986) might have guessed that it might be one of the *Hexamermis* spp. However, except for the level of incidence, he did not study the parasite in detail; not even in comparison with single morphological taxonomic features. In another instance, Mandal *et al.* (2005) published a short one-paragraph report on *Agamermis* species infesting a lepidopteran pest, *N. leucophaella* by studying only a percentage of mermithid infestation, measuring the length and width of the parasite, but not by either single supporting morphological feature or by using molecular studies.

The post parasitic juveniles of *Agamermis* sp. differ from *Hexamermis* species by their length, absence of finger-like tail tip caudal appendage and presence of crater or nodal stub-like appendage. The absence of a tail (posterior end) caudal appendage and the presence of a clear nodal stub, terminal side mouth, S-shaped vagina and two spicules provided robust evidence for *Agamermis* genus identification (Cobb *et al.*, 1923; Nickle, 1972; Poinar *et al.*, 1981; Kaiser, 1991; Stock and Camino, 1992; Baker and Poinar, 1995; Hernandez-Crespo and Santiago-Alvarez, 1997; Achinelly and Camino, 2008; Stubbins *et al.*, 2016). *Hexamermis* and *Agamermis* sp. are usually long nematodes ranging from 3 mm to 30 cm and 3 mm to 46 cm, respectively (Nickle, 1972). The length of the mermithids identified in the present study is found to be between 18 to 36 cm and this characteristic also supports the fact that the mermithid is not a *Hexamermis* sp. In the present study, the lengths of the post-parasitic female juveniles are found to be longer, while the males are considerably small. Hence, the length of the nematodes can also be used as one of the morphological diagnostic characters for the identification of females and males. The chief morphological differences between male and female post-parasitic larvae have been briefly described by Welch (1960) as being the larger size of the females. Christie (1929), Tsai and Grundmann (1969) showed that the size differences among females and males can be used for rapid identification of the sexes of mermithids.

Agamermis sp. parasitic in insects, particularly in Orthoptera and Hemiptera, have been identified in various insect pests like *Orchelimum vulgare, Melanoplus femurrubrum, Phaulaaidium vittatum, Praxibulus exsculptus, Megacopta cribraria, Chinavia hilaris, Euschistus servus, Aelia rostrata, Nilaparvata lugens, Nilaparvata oryzae, Eurygaster maura, Laplatacris dispar and Sogota furcifera* (Cobb *et al.*, 1923; Rubtsov, 1969; Zhao *et al.*, 1987; Choo *et al.*, 1989; Choo and Kaya, 1990; Baker and Poinar, 1995; Stubbins *et al.*, 2015, 2016; Rusconi *et al.*, 2017). There are no detailed morphological records of *Agamermis* nematode mermithids infesting lepidopteran insects. According to Cobb *et al.* (1923), the decaudata races infesting certain hosts are questionable and they opined that the larvae of various races are entering hosts without special host choice. However, for many mermithids, the host species is still unknown (Nickle, 1972). It indicates that the *Agamermis* species do infest not only Orthopteran insects but also Hemipteran insects as well. Likewise, the different *Agamermis* mermithids might have also infested the lepidopteran insects, which were not observed or reported in detail by the researchers to date. *Mermis nigrescens* Dujardin is normally associated with grasshoppers (Orthoptera) (Christie, 1937) but is also reported to occur naturally in earwigs (Dermaptera), beetles (Coleoptera), caterpillars (Lepidoptera) and even honeybees (Hymenoptera) (Capinera, 1987; Presswell *et al.*, 2015) because it is similar in appearance to other species *viz., Amphimermis, Longimermis, Agamermis* and *Hexamermis.* In addition, experimental parasitism was successfully induced in the range caterpillar, *Hemileuca oliviae* Cockerell (Lepidoptera: Saturniidae) by *M. nigrescens* (Capinera, 1987). During the course of habitat fragmentation and evolution, this struggle might have resulted in the "survival of the fittest", by successfully infesting the various insects that belong to different orders with different species of mermithids, with no special host choice in each eco-niche. Results of Ross *et al.* (2010) indicate multiple origins of different species of nematodes in land slugs (Mollusca) and they considered that the morphological diversity changes that occur among these families are due to the rapid evolution that occurred during the evolutionary history of slugs versus parasitic nematodes, suggesting adaptive radiation to fill different niches within the host.

Nematodes are biologically versatile, abundant, dominant and diverse taxon occupying an enormous range of habitats (Megen *et al.*, 2009; Rizvi, 2010; Vogt *et al.*, 2014). Mermithid taxonomy is in a state of confusion and species determination was not feasible for several reasons *viz.*, difficulty in finding adult mermithids in some cases, a few measurable morphological traditional taxonomic approaches, that are not sufficient for describing species status and difficulty in analyzing important aspects of their biology and host range. Classification of species based on morphological attributes may be riddled with problems because morphological features may vary with the environment (Shouche and Patole, 2000). Hence, most of the early descriptions were incomplete and have become inadequate as more species are described. Curran and Hominick (1981) reported that many quantitative characteristics are affected by the environment and suggested that many ratios commonly used in mermithid taxonomy should be rejected. As a result of these classification problems, even the taxonomy of the most extensively studied mermithid, *Romanomermis culicivorax,* remains in doubt. This mermithid was first determined to be an undescribed species of *Romanomermis.* Nickle (1972) subsequently determined Louisiana isolate to be the same as *Reesimermis nielseni* from Wyoming. In a detailed study of this group, Ross and Smith (1976) resurrected the genus *Romanomermis* and described Louisiana isolate as *R. culicivorax.* Due to the lack of certain criteria for assessing the homology of morphological characters regarding many nematodes, the systematics of this phylum has always been contentious and challenging (Ross *et al.*, 2010; Vogt *et al.*, 2014). Because nematodes are very difficult to identify with certainty using morphological techniques. Stock and Camino

(1992) reported that, only 4 and 6% of the population of both juveniles, parasitic males and females of *Hexamermis ovistriata,* a parasite of the grasshopper (*Staurorhectus longicornis* Giglio-Tos (Orthoptera: Acridiidae) possess a tail caudal appendage. This indicates that the presence of a caudal appendage is also not a reliable morphological characteristic feature for the identification of *Hexamermis* species. Hence, identification through both morphological and molecular techniques can help, predict and infer mermithid biology as well as species discrimination and identification. The morphological characters studied in the present study were found to be congruent with molecular data (communicated data) and we have found that a combination of morphological and molecular approaches enhances the efficiency of species delimitation and identification of genus.

An NCBI-BLAST search revealed that the sequences of *cox1* and 18S rDNA have the greatest similarity to Agamermis species identified by Stubbins *et al.* (2015, 2016) from *Megacopta cribraria* (Hemiptera: Plataspidae); whereas 28S rDNA has the closest homology with *Agamermis xianyangensis* identified from China soils by Wang *et al.* (2007) (communicated data). Overall, an integrative taxonomic approach combining both morphological and molecular phylogenetic analysis methods based on *cox1,* 18S and 28S-rDNA gene sequences revealed a shred of evidence that the mermithid nematode described in the present study is indeed *Agamermis* sp. and the species is proposed as *Agamermis mylittensisantheraea* n. sp. Cobb, Steiner and Christie, 1923 in agreement with the names of the professors who first identified the *Agamermis* species based on the morphological features and its insect host, *Antheraea mylitta.*

Since the 1930s, there has been a renewed interest in entomopathogenic nematodes, which could prove pivotal as biological control agents against various insect pests in agriculture (Smart, 1995; Hajek *et al.,* 2007). Furthermore, the epizootics of insect pests caused by mermithid parasitism occur; and population levels of blackflies, mosquitoes, grasshoppers, ants and certain lepidopterans and other insects are held down by these self-perpetuating biological control agents (Nickle, 1972; Poinar, 1979; Micieli *et al.,* 2012). To reduce various insect pests, research has historically been conducted on integrating control tactics, which includes identification and use of natural enemies as biological agents (Bhatnagar *et al.,* 1985; Stubbins *et al.,* 2016). Interestingly, the oak-silkworm parasitic mermithid nematodes have been used by various researchers to control orchard insect pests (Wei and Wu, 1984; Wang and Li, 1987). However, the following statement is out of the purview of the present manuscript; the identified *Agamermis* mermithid species may be a useful, self-sustaining and exceptional potential material as a control agent in a classical or augmentative integrated programme for controlling various major and minor agricultural insect pests of commercial crops.

Conclusion

To the best of our knowledge and published reports about mermithid nematodes so far, the species *Agamermis* is reported for the first time from the insect order Lepidoptera based on its detailed morphology and molecular data (communicated data). The taxonomical features, especially the length, absence of a caudal appendage, presence of paired spicules and prominent crater or stub-like appendage, support the findings that the identified mermithid is indeed an *Agamermis* species. Even molecular phylogenetic analysis based on *cox1,* 18S and 28S-rDNA gene sequences suggests that the mermithid nematode infesting tasar silkworm, *A. mylitta* was most closely related to the genus *Agamermis* that infests various Orthopteran and Hemipteran insects (communicated data). Our results demonstrated the high potential of both morphological and sequencing approaches as more useful diagnostic characteristics for the identification of the new terrestrial nematode mermithid at the genus level and highlighting the applicability of these combined taxonomic approaches in general. The higher percentage of mortality indicates that the mermithid nematode, *Agamermis mylittensisantheraea* sp. Cobb, Steiner and Christie, 1923, plays a significant role in the natural mortality of tropical tasar silkworms in India, which causes a significant economic loss in tasar silk industry.

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NEWS CORNER

International Training in Silk Industry

Two international trainings were conducted in 2024 through the Central Silk Board, Govt. of India, under the sponsorship of Indian Technical and Economic Cooperation (ITEC) programme of Ministry of External Affairs, Govt. of India.

A) Training on Sericulture and Silk Industry

The training on "Sericulture and Silk Industry" was conducted at Central Sericultural Research and Training institute, Central Silk Board, Mysuru, India, for four weeks from 3rd to 30th September 2023. Twenty eight candidates from 13 countries were trained on different aspects of sericulture and silk industry, which includes mulberry cultivation, silkworm rearing, silkworm egg production, young-age silkworm rearing, post cocoon technology, *etc.* Besides the practical oriented training activities, the trainees were exposed to various commercial activities of sericulture and silk industry by visiting farmer's fields, cocoon market, post cocoon and post yarn activities, etc. The trainees also visited historically important sites to learn about India's culture and history.

B) Training on Post Cocoon Technology

The training on "Post Cocoon Technology" was conducted at Central Silk Technological Research Institute, Central Silk Board, Bengaluru, India for four weeks from 5th November to 2nd December 2023. Eighteen trainees from 11 countries participated in the programme. The practical oriented training was provided on various aspects of the post cocoon industry, such as reeling, weaving, wet processing, testing and grading, craft making, *etc.* As part of their training, the participants were exposed to various aspects of sericulture and the silk industry, from pre-cocoon activities, such as chawki rearing, mulberry cultivation, and silkworm rearing, to visits of cocoon markets and industrial facilities. To further broaden their understanding, they also visited sites of historical importance to learn about Indian culture and history.

Exposure Visit on Silk Industry

Under the capacity building programme of International Sericultural Commission, 10 days exposure visit in Thailand was organized for the country delegates of ISC on the corporate sericulture practices established in Thailand. The Queen Sirikit Department of Sericulture (QSDS), Thailand has organized the programme during the period from 20th to 29th May 2023. Sixteen delegates from ISC Member Countries participated in the programme. The delegates visited the sericulture facilities at Nakhon Ratchasima, Chul Thai Silk Co. ltd., industrial sericulture farmers at Phetchabun, *etc.*, to have a detailed insight on corporate sericulture practices in Thailand. Chul Thai Silk Company's organic and corporate farming models exemplify the successful establishment of the silk industry in Thailand. The use of natural dye, sericulture products, such as mulberry tea, mulberry juice, edible products of silkworm pupae *etc.*, are unique contributions from Thailand to the global silk industry.

An exposure visit cum training was also organized for the ISC delegates on Chinese silk industry during the period from 25th May 2024 to 6th June 2024. Twenty delegates participated in the training cum exposure visit to the industrial sericulture activities practiced in China. The visit to National Silk Museum of China, Sericulture Research Institute, Zhejiang Academy of Agricultural Sciences, Cathaya group corporate sericulture model and silk showroom, Zhejiang Sci-Tech University, automatic reeling factory, weaving industry and silk showrooms, Mulsun Biotech, *etc.* provided valuable information and insights. The visit has facilitated the country delegates to understand the structure of Chinese silk industry that help them to replicate these successful models in their respective countries for enhancing the productivity and quality of silk. During the visit, interactive meetings were organized between the delegates and the Senior officials of International Silk Union and CEOs of many companies as part of establishing collaborative activities and business relations among the countries.

Executive Committee

An executive Committee meeting was conducted at Pattaya, Thailand on 21st May 2023 and on 28th May 2024 at Hangzhou, China. A few of the important points deliberated in these meetings were;

1) Evaluating the progress of Global LCA study on Silk,
2) To identify the International Silk Day,
3) Empanel volunteer experts under the Volunteer Expert Programme (VEP),
4) Organization of Conference and Congress,
5) Renewal of Silk Standards and Grading and the formulation of Technical Committee for silk testing standards, etc.

ISC Conference

The 27^{th} Conference of ISC was held at Bucharest, Romania on 12^{th} October 2024 to elect the new Secretary General of ISC for the tenure of January 2025 to December 2027. Dr. Adela R. Moise, Romania chaired the conference and Dr. Shuichiro Tomita, Japan, functioned as the Election officer for conducting the SG election proceedings. The Indian nominee Mr. P. Sivakumar, was elected as the Secretary General of International Sericultural Commission for the period. Besides the election, the Conference discussed about the progress of ISC activities. The Conference also finalized to conduct an exposure visit to ISC delegates on the silk industry developments in Europe during May 2025.

INFORMATION TO CONTRIBUTORS

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