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BIODIVERSITY OF TASAR SILKWORM, BREADING PERFORMANCE, CONSERVATION, GENETICS ANALYSIS AND BIOMEDICAL APPLICATION.

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ABSTRACT

Silk is the most common rearing agro based industries world wise practicing and is not only valuable this is ultimate and wonderful gift of the nature are produced by Sericigenous insects belonging families **Bombycidae** and **Saturniidae** of order Lepidoptera. Silk produced by the silkworm consists of two major kinds of proteins, viz. fibroin and sericin.Tropical tasar silkworm, is one of the most commercial vanyasilk production in the country and presently, about 3.5 lakh families are directly or indirectly associated with tasar culture. There is need for improved races/breeds in order to enhance tasar silk production and bring improvement in its quality. Therefore, genetic resources of tasar silkworm are dire need to be explored, catalogued, conserved and characterized for its commercial utilization by breeders and geneticists. Genetic variations in the natural population and diverse genes in the individuals of the populations of tasar silkworm are the prerequisites for evolving improved breeds/lines. Majority of ecoraces are wild in nature and least amenable to human interference. Among the ecoraces, Raily and Modal female moth lays eggs as high as 485 and silkworm can spin cocoons with very high silk content, sometimes even more than 1800 meter.

Keywords: - Biomedical properties of tasar silk, Antheraea mylitta, tasar silkworm, sricin fibroin and sericin, Tropical tasar silkworm, genetic variation of tasar silkworm etc.

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INTRODUCTION

Silk is the most common rearing agro based industries world wise practicing and is not only valuable this is ultimate and wonderful gift of the nature are produced by Sericigenous insects belonging families **Bombycidae** and **Saturniidae** of order Lepidoptera. Silk produced by the silkworm consists of two major kinds of proteins, viz. fibroin and sericin. The silk fibroin is fibrous in nature forming the core of silk filament and secreted from the posterior part of silk gland. However, sericin is a glue-like protein synthesized in the middle part of the silk gland of silkworm. Sericin plays an important role in the spinning process of the silkworm and thus helps in formation of a robust cocoon shell. Its molecular weight ranges from 11 kDa to 245 kDa with high content of serine, glycine and aspartic acid (Jena et al., 2018a, 2018b). Recently, several unique properties of sericin, such as antioxi-dants (Jena et al., 2018a, 2018b; Dash et al., 2008; Wu et al., 2007), anti-tyrosinase (Jena et al., 2018a, 2018b), anti-coagulation (Tamada et al., 2004), chemoprevention (Zhaorigetu et al., 2001), protective action against alcohol-mediated liver damage (Li et al.,

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2008) and other biomedical properties (Kunz et al., 2016) have also been reported which suggest sericin as a suitable bio-molecule for the development of ideal biomaterials.

Indian tropical tasar silkworm Antheraea mylitta is polyphagous in nature that feeds preferably on Terminalia arjuna, Shorea robusta and T. tomentosa besides secondarily on approximately two dozen of food plants (Dash et al., 1992; Deka and Kumari, 2013). Earlier reports sug-gest that different tasar food plants have diversiied nutritional value in terms of total minerals, crude iber, proteins, carbohydrates, ascorbic acid and phenolic content (Acharya et al., 2017; Sinha et al., 1998). Ahigher level of total minerals and lower amount of crude iber is reported in T. arjuna and T. tomentosa, when compared with S. robusta (Sinha et al., 1998). Similarly, protein content is observed to be higher in T. tomentosa as compared to T. arjuna and S. robusta. However, carbo-hydrates, ascorbic acid and phenolic content are estimated to be higher in S. robusta as compared to T. tomentosa and T. arjuna (Acharya et al., 2017). It is well documented that various characters of cocoon such as shape, size, colour, shell weight, weight of cocoon and silk content are mainly based on variation in climatic conditions, altitude, food habits etc., that may lead to the development of different ecoraces (Acharya et al., 2017; Deka and Kumari, 2013).

Fortunately India produces all the four types of silks namely mulberry, tasar, eri and muga in the tropical and temperate silk producing belts of our country. India rank second as the global producer of tasar silk. Different species of **Antheraea** such as **Antheraea mylitta, Antheraea pernyi, Antheraea roylei, Antheraea yamamai, Antheraea frithi** and **Antheraea proylei** are well known for producing tasar silk of great commercial importance having vast potential of earning much needed foreign currencies. Tasar silkworms are by and large wild, biovoltine/trivoltine and polyphagous in nature are usually reared on the foliages of tasar host plants during the seed crop (July-August) and Commercial crop (September- October) seasons and it follows a long days pupal diapause during the summer and winter season to escape from adverse environment conditions. Among the tasar silk producing insects **Antheraea mylitta** D. is the famous indigenous tropical tasar silk producing insect existing with several eco-races and strains under the diverse eco-habitats of tropical tasar silk producing belts of India.

Tropical tasar silkworm, is one of the most commercial vanyasilk production in the country and presently, about 3.5 lakh families are directly or indirectly associated with tasar culture. There is need for improved races/breeds in order to enhance tasar silk production and bring improvement in its quality. Therefore, genetic resources of tasar silkworm are dire need to be explored, catalogued, conserved and characterized for its commercial utilization by breeders and geneticists. Genetic variations in the natural population and diverse genes in the individuals of the populations of tasar silkworm are the prerequisites for evolving improved breeds/lines. Majority of ecoraces are wild in nature and least amenable to human interference. Among the ecoraces, Raily and Modal female moth lays eggs as high as 485 and silkworm can spin cocoons with very high silk content, sometimes even more than 1800 meter.

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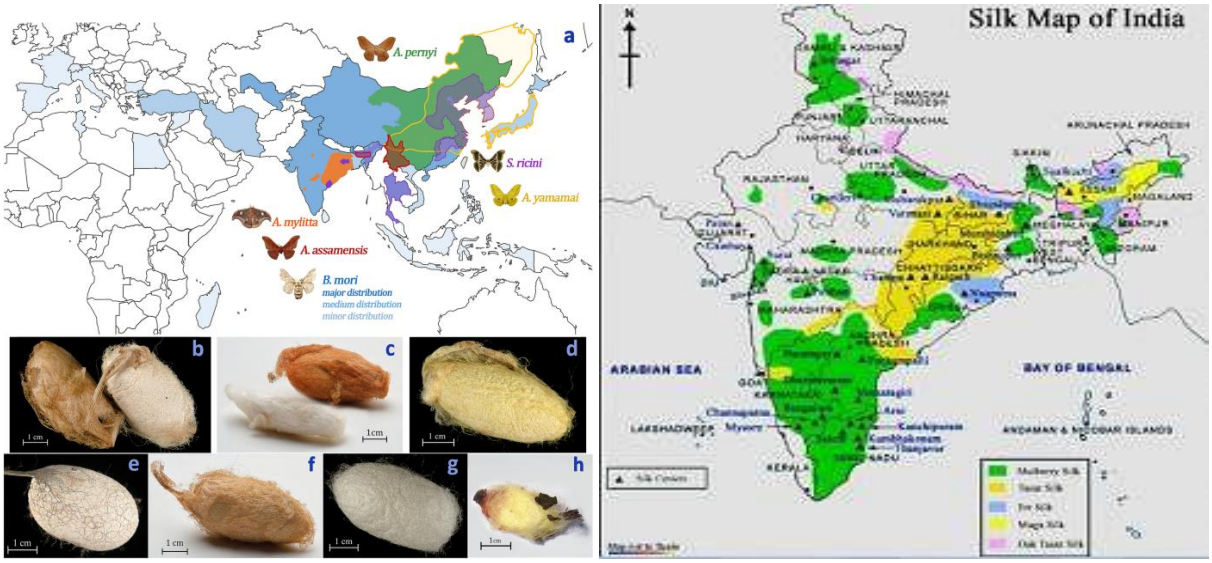
BIODIVERSITY OF TASAR SILKWORM

Around 124 species belongs to Antheraeaare distributed in various countries such as United States, Mexico, India, Bhutan, Cambodia, Russia, China, North Korea, Indonesia, Japan, Laos, Brunei, Timor, Taiwan, North Korea, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand and Vietnam. Among 124 species, India comprised of thirteen species, A. mylitta, A. frithi, A. assamensis, A. roylei, A. compta, A. helferi, A. rubicunda, A. paphia, A. andamana, A. insularis, A. cernyi, A. knyvetti and A. meisteri(Table 1). Major production of tasar silk is from tropical tasar silkworm, A. mylitta. A. mylittahas a wide distribution within the country. In India the range of distribution of this species covers Assam, many of which were treated as distinct species though they are ecological populations of the same species (Singh and Srivastava, 1997).

Seri-bio-diversity: Among 34 mega biodiversity countries in the world, India is home to many species of insects with a diverse silk moth fauna. In addition to the diverse silkworm races, there are vast genetic resources of mulberry, tasar, muga and eri host plants spread over diverse geographical locations. This offers a great opportunity for economic utilization of the natural flora and fauna. However, due to deforestation and destruction of habitats, there is a challenge to bring about development without disturbing the ecological balance.



Distribution of Antheraea species World wise

S.NO	COUNTRY	ANTHERAEA SPECIES
1	Indonesia	A. alorensis, A. banggaiana, A. billitonensis, A. borneensis A. cadioui ,A. celebensis, A. cihangiri, A. cordifolia, A. diehli, A. expectata , A. fickei, A. frithi javanensis etc
2	Brunei	A. alleni, A. brune
3	India	A. assamensis, A. compta , A. frithi, A. helferi, A. mylitta, A. paphia, A. rubicunda , A. knyvetti, A. roylei , A. andamana, A. cernyi, A. insularis, A. meisteri
4	Cambodia	A. angustomarginata

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5	Malaysia	A. broschi, A. pahangensis , A. ulrichbrosch
6	Bhutan	A. castanea
7.	Srilanka	A. cingalesa
8	China	A. crypta, A. discata , A. harndti, A. harti, A. pernyi, A. yamamai titan, A. yunnanensis
9	Myanmar	A. myanmarensis paukstadt , A. platessa, A. steinkeorum
10	Thailand	A. frithi pedunculata, A. ranongensis, A. semperi
11	Japan	A. sergestus, A. yamamai, A. yamamai yoshimotoi
12	Taiwan	A. superba
13	North Korea	A. yamamai bergmanni
14	Vietnam	A. frithi tonkinensis, A. luteofrithi, A. rubicunda rubiorientalis
15	Philippines	A. gschwandneri zwicki A. gulata A. hagedorni A. halconensis
16	Laos	A. larissoides
17	Timor	A. lorosae
18	North Korea	A. yamamai bergmanni
19	Russia	A. yamamai ussuriensis
20	America	A. oculea , A. polyphemus
21	Mexico	A. godmani A. montezuma

A. mylitta is widely distributed over a wide range of Indian subcontinent of varied topography, climatic conditions, vegetation and soil conditions it exhibits diversity in the phenotypic, physio-genetic, behavioural and commercial characters (Sengupta and Sengupta 1982; Sengupta et al.,1993; Singh and Srivastava, 1997; Thangavelu et al.,2000; Srivastava et al.,2002 and 2003).

Due to deforestation and anthropogenic activities, there is a constant threat to tasar silkworm biodiversity. Besides, wild/natural populations are supposed to harbour many beneficial alleles developed through centuries by the process of natural adaptation. It should be our duty to conserve the available tasar silkworm genetic resources. Survey, collection, characterization and documentation of tasar silkworm genetic resources should be done in a systematic manner. Further, characterization of the available tasar silkworm genetic resources through molecular tools will not only support the conservation approach but also help in the sustainable utilization through breeding programme. The genetic structure, genetic relatedness, identity and gene flow are the important features of genetic resources need to be assessed.

Diversity in A. mylitta is the result of adaptation of a species to a variable eco factor and interaction of genetic constitution. So far, 44 ecoraces of tasar silkworm have been reported (Table 2). It can be seen from the table that maximum number of ecoraces are available in Jharkhand followed by Chhattisgarh. Odisha is also bestowed with six known ecoraces.

Diversity of A. mylittain relation to primary and predominant food plants is presented in Table-4. Wide range of phenotypic variation is observed in nature grown cocoons. It may be appropriate here to mention that since a long time proper survey and collection has not been done, so there is

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the chance that many of the ecoraces might have been extinct due to deforestation and habitat loss. On the other hand, tasar populations may also be available in new zones or ecopockets which need to be surveyed and documented because the natural populations are the treasure of genes and alleles developed through centuries through the process of natural evolution.

BREADING PERFORMANCES

Tasar silk producing insect existing in the tropical tasar producing belts in the states of Jharkhand, Orissa, Madhya Pradesh and Maharashtra in India. Jolly(1967) reported three mutant strains of Daba ecotype of Antheraea mylitta D. namely Daba-yellow; Daba-blue and Daba-almond based on three different larval body colours existing in the tropical tasar silk producing belts in India. Pandey (1969) mentioned that the three mutant strains of Antheraea mylitta D. in spite of having the same chromosomal number (n=31) differ among themselves in their behavioural and biochemical manifestations. A comprehensive picture in relation to behavioural manifestations of non-mulberry silk insects has been presented by Jolly et.al. (1979). Pandey (2012) mentioned the significant impacts of seasonal changes and subtropical environment on the productivity and quality of tasar cocoons. Renuka et.al. (2015) mentioned that the rich biological diversity of Antheraea mylitta D. is mainly due to its wide range of distribution, climatic factors and food plants etc., which lead to variations in their ethology, physiology and commercial traits. It is further known that the area occupied by the tasar silkworm, Antheraea mylitta D. is highly diversified geographically as such the population from the diverse sources has not evolved uniformly.

It is an admitted fact that the genetic diversity is inversely proportional to isolation and that it is directly correlated with population size thus the genetic diversity of population responds to environmental heterogeneity. Arora et.al. (1979) carried out taxonomic studies of non-mulberry silk moths with essential details. Kumar et.al. (2017) reported evident variation in the coupling behavior of tasar moths under different conditions.

RESULTS AND DISCUSSION

Results obtained as per the table clearly indicate that the three mutant strains of Antheraea mylitta D. differ among themselves in their relative rearing performances in respect of productivity and quality of tasar cocoons. Table reveals that the mutant strains such as Daba-yellow, Daba-blue and Daba-almond evidently present variations in respect of E.R.R. (28.70%, 25.55% and 22.10%), cocoon weight (12.10gm, 11.32gm and 10.26gm), shell weight (1.43gm, 1.30gm and 1.21gm) and shell ratio (12.20%, 10.82% and 10.35%) during the seed crop season and also the E.R.R. (31.80%, 29.10% and 24.30%), cocoon weight (12.42gm, 11.93gm and10.83gm), shell weight (1.53gm, 1.40gm and 1.32gm) and shell ratio (12.10%, 11.95% and 11.80%) during the commercial crop season. It is further evident that the rearing performances of all the three mutant strains of Daba ecotype of Antheraea mylitta D. in spite of relative differences are by and large relatively better during the commercial crop season than the seed crop season.

TABLE 1

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S.N.	Breeding Parameters	Different methods of larval culture			C.D. at 0.5% level for characters
		Indoor Seed : Comm.	Out door Seed : Comm.	Consolidated Seed : Comm.	
1	Av. Emergence of moths (%)	50 : 70	56 : 68	55 : 65	
2	Average coupling (%)	45 : 75	51 : 70	58 : 69	
3	Average No. of eggs laid	180 : 230	190 : 280	193 : 238	
4.	Average Hatching (%)	55: 67	55 : 70	55 : 68	
5.	Av. Life span of female moth (hr.)	8 : 20	9 : 76	18 : 25	

Table showing tasar silk work of *Antheraea mylitta* D on breeding performances during the seed crop and commercial crop seasons.

* = SIGNIFICANT

* * = HIGHLY SIGNIFICANT
NS =NOT SIGNIFICANT

The results obtained become very clear when one takes note of the fact that the genetic diversities and different physio-genetic makeup of three mutant strains are the potent factors for relative variations among the mutant strains in respect of productivity and quality of tasar cocoons. The relatively better rearing performances of Daba-yellow mutant strain as compared to Daba-blue and Daba-almond mutant strains are probably on account of the fact that Daba-yellow is more robust and physio-genetically better fit than the two others mutant strains for desired behavioural manifestations in course of evolution of mutant strains.

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TABLE 2

Sl. No.	Breeding parameters	Consolidate d method of rearing		C.D. at 0.5% level for
	Seed crop		com. crop	Characters
1	Av. Emergence of tasar moths (%)	55.0	65.0	*
2	Avedrage coupling (%)	58.0	69.0	* *
3	Average number of eggs laid	193	238	*
4.	Average hatching (%)	55.0	68.0	* *
5.	Av. life span of female moth (hr.)	18 hr.	25 hr.	*

Table showing breeding performances of tasar moths during the seed crop and commercial crop season.

* = SIGNIFICANT

* * = HIGHLY SIGNIFICANT

NS = NOT SIGNIFICANT

CONSERVATION OF TROPICAL TASAR SILKWORM

Conservation biology is tied closely to ecology in researching the dispersal, migration, demographics, effective population size, inbreeding depression, and minimum population viability of rare or endangered species. To better understand the restoration ecology of native plant and animal communities, the conservation biologist closely studies both their polytypic and monotypic habitats that are affected by a wide range of benign and hostile factors. Conservation biology is concerned with phenomena that affect the maintenance, loss, and restoration of biodiversity and

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the science of sustaining evolutionary processes that engender genetic, population, species, and ecosystem diversity.

Need for Conservation

1. Rampant collection of Cocoons, Threat of complete genetic erosion, Environmental stress changes the climate/environment/ forest ecosystem, Irrational collection & marketing of nature grown cocoon, Deforestation for fuel, livelihood earning by tribal's1, Increase in anthropogenic activities, Unawareness among the tribal on restriction of collection, Extensive losses due to parasite and predators, Climatic attacks of heavy rains, storms, drought etc.
2. Human interference for industrial and housing areas, with increasing human population, Untested/diseased seeds to the core buffer area homeland, To protect and maintain essential tasar host plants in the forest ecosystem, To preserve the existing wild ecoraces and their population in natural habitat, To ensure sustainable utilization of the ecoraces and ecosystem, Large scale collection of cocoons from their ecological ,niches without giving any heed towards natural breeding process for self-perpetuation.

Need of Wild daba Conservation

High value of commercial characters & zero pebrine status, Highly amenable can transform into cultivated ruling , Daba unlike other in situ bred eco-race, Thus the high trait values & pebrine zero can be utilized for tasar seed system, and In situ conservation strategies will multiply the wild Daba in it native eco-niches.

Collection of nature grown cocoons

Harvesting of cocoons raised through rearing of Laria silkworm In the former, there is no human andling and all the life cycle activities like moth emergence, coupling, decoupling, egg laying, hatching and growth of larvae, cocoon formation are fully dependent on natural environmental conditions without human interfernce, whereas in case of later, feed and growth of larvae and cocoon formation are out-doors and remaining activities are indoor wherein large scale pupal mortality, erratic emergence, unsynchronised emergence and pairing were noticed in the model grainage house. Observations revealed that Laria seed cocoons preserved under Sal forest of Bhusur, regular moth emergence was significantly delayed, emergence period was reduced and emerged male and female moths were more synchronised for coupling. The present study may lead to change in preservation methodology of seed cocoons of Laria. This concept came to solve the problem of large scale preservation of seed cocoons of Laria in outdoor conditions under shade, in or nearer to Sal forest Genetic analysis.

Thus, it can be concluded that information generated from the above study may pave the way for future planning of preservation of seed cocoons and preparation of Laria seed to suit the requirement of the farmers. Cultivation of Laria is an important co-discipline of applied forest biology that needs special attention to promote conservation and sustainable utilization of its host plant.

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Since, A. mylittaprimarily inhabits forested habitats, it is expected that with the gradual depletion of forest cover due to surge in the human activities the habitat lost its continuity and resulted in geographic isolation. This isolation might have allowed the populations to continue separately for generations, result-ing in different ecoraces. There is quite a bit of ambiguity in naming of these ecoraces. As the boundaries between the ecoraces are often fuzzy and the races do not obey the concept of static boundaries, the racial identity seems to be arbitrary at times. Quite surprisingly, two crops of the same population are sometimes named as two different ecoraces .

There is a lack of well-characterized molecular markers to study A. mylitta ecoraces. Although few studies have been carried out with RFLP 13, RAPD 14 , SCAR 15 , ISSR 12,16 and other DNA markers 17 , except for RFLP, the rest are dominant markers, and hence the estimation of allele frequencies are based on the assumption that the loci are in Hardy-Weinberg equilibrium. Also, barring a couple of reports on Antheraea assama 18–20, there are no reported studies that describe the detailed population genetics of saturniid silkmoths, using SSR markers.

Conclusion

A. Mylittaand used them to study the genetic structure of its different ecoraces. Like most other species studied, we observed dinucleotide microsatel-lites to be the most abundant, followed by tri- and tetra nucleotide. Among the dinucleotide microsat-ellites, those with (CA) motifs were the most abundant. CA/GT repeats are generally the most common dinucleotide repeat in a wide variety of vertebrates and arthropods25,26. Insects 27,28, including lepidopter-ans 29–37 are no exception.

There have been studies investigating the nature and distribution of genetic variation in wild lepidop-terans. However, these have mainly focused on threatened or declining species 38–40 or pests 41–44. Among other silkworms the genetic structure of A. assama, a species with a very restricted distribution, has been studied and its populations were found to be reasonably differentiated 18,20. Different strains and lines of the domesticated silkworm B. morihave also been subjected to this kind of analysis , however these studies are arguably not comparable to wild lepidopteran species.

In this current work, we present a detailed genetic study of eight different ecoraces of a wild silkworm A. Mylitta. However, one should keep in mind that we do not have replicates of the ecoraces in our data-set, i.e., each ecorace has been sampled only from a single locality and hence the effect of ecorace and locality might be confounded in the pattern we have observed.

Genetic variation.

Descriptive statistics. Considerable variation was observed at all microsatellite loci. Except Bhandara, which was monomorphic at the locus Amysat013, all other populations showed polymorphism at all the 10 loci Supplementary data 1). (Number of alleles ranged from 1 (Locus: Amysat013, Population: Bhandara) to 15 (Locus: Amysat023, Population: Modal) across the ten

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micro-satellite loci studied among the eight ecoraces. The average number of alleles per locus ranged between 2.5 ± 0.76SD (Amysat013) and 11.75 ± 2.05SD (Amysat023). The minimum average number of alleles for a population across all 10 loci was 3.7 ± 1.77SD for Sukinda and the maximum was 6.6 ± 4.24SD for Modal. Average observed heterozygosity per locus (Ho) taking all the populations together ranged from 0 (Amysat013) to 0.81 ± 0.12 (Amysat023). The minimum average Ho across all the loci was observed in the population Daba Trivoltine (0.25 ± 0.32) and maximum in Sukinda (0.37 ± 0.34).

Medical applications.

The pronounced phenotypic and behavioral variation of A. mylittaecoraces has made it difficult for researchers to identify ecorace specific phenotypic markers. Therefore, there is a need to identify genetic markers for a specific phenotype to differentiate ecoraces. Are these ecoraces genetically distinct from each other? Do the ecoraces form structured population? Are any of these ecoraces in decline? These questions are of considerable importance to the biology of A. mylitta. With these points in mind, we developed 32 microsatellite markers and screened eight A. mylittaecoraces collected from different geographical locations across India to obtain insights into the population genetics of these ecoraces .

Silkworm cocoons are biological structural materials constructed by silkworm larvae and provide protection from the natural environment, parasitism, or predators of silkworm pupae. The silkworm cocoon could be used as protective materials, sorbent materials, gas filters, and biosensors considering its porous hierarchical structure, good impact resistance, sorption capacity, temperature- and humidity-dependent electrical properties, and photoelectrical properties. Increasing attention has been paid to modifying the microstructure and improving the properties of silkworm cocoons without damaging their biological structures for further applications to the field of composites and biomimetic materials.

It is estimated that the new cases of cancer in the year 2040 will be 29.4 million per year globally. Sericin, an adhesive protein of silk cocoon, is a potential protein in various biomedical applications including cancer therapeutics. The present study evaluates the anticancer property of sericin prepared f rom cocoons of Antheraea proylei J. (A. proylei) against human lung cancer (A549) , cervical cancer (HeLa) , and prostate cancer (PC3) cell lines. This is the rest report of the anti-cancer activity of the non-mulberry silkworm A. proylei.

Silk protein, sericin have anti-cancer property, that binds together the silk fibroin fibers to form the cocoon. Silk textile industry targets only the silk fiber obtained after the process of sericin removal, through degumming. Depending upon the species from where the sericin is obtained, the amino acid composition varies considerably. Sericin f rom wild silkworms has a higher content of threonine, glutamic acid, cysteine and phenylalanine and a lower content of serine, proline, methionine, glucosamine, galactosamine, and histidine. Owing to the dif ference in the proportions of amino acid compositions in the domesticated silkworm, Bombyx .mori and the wild type silkworm, Antheraea sp., there may be variation in the bioactivity.

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Sericin stands as a promising anti-cancer agent that inhibits the growth of cancer cells. The effect of sericin was studied in the colon cancer mice models induced by Dimethlhydrazine (DMH) . The studies concluded that sericin supplemented diet reduced the formation of colonic aberrant crypt foci. Further Zhaorigetu et al., 2001 reported that sericin suppresses the development of colonic tumors by reducing oxidative stress, cell proliferation, and nitric oxide production. The strong antioxidant activity of sericin and its resistance to intestinal proteases prolongs its sustainability in the colon thereby lowering oxidative stress and tumorigenesis in the colon. Yet in another study, sericin was reported to suppress skin tumorigenesis in a mice cancer model induced by dimethybenz (α) anthracene (DMBA) and O- tetradecanoylphorbol acetate (TP A) by reducing oxidative stress, inflammatory responses and endogenous tumor promoter (TNF-α) It was also observed that sericin from B. mori induced apoptosis through the caspase pathway and downregulation of Bcl-2 expression in human colorectal cancer cells (SW480) . Most of the previous reports related to the prevention and treatment of colon cancer are concerned with sericin obtained from the commonly domesticated silkworm, Bombyx mori which has been extensively studied as compared to the wild silkworms including Antheraea sp. The oak Tasar silkworm, A. proylei is reared in several sericulture farms in Manipur , India and adjoining states and feeds on leaves of oak (Quercus sp.) Which are naturally grown in the region? The sericin from A. proylei silkworm has yet to be explored for its prospective anti-cancer properties and health benfits.

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