



Exploring the Metal Composition of Eri Silkworm Cocoons Reared on Diverse Host Plant Combinations

Priyanka Nayak¹, Shubhasree Dash¹, Bijoy Kumar Mishra¹,
S. Ranjith Kumar², E. Arasakumar³

10.18805/ag.D-6143

ABSTRACT

Background: The objective of this study was to analyze the chemical composition of Eri silk cocoons that were reared on various combinations of host plants. The primary host plant castor and secondary host plants such as kesseru, tapioca, gulancha and papaya were utilized in various combinations for different instars.

Methods: The present analysis was conducted in lab at Department of Entomology, Institute of Agricultural Sciences, Siksha 'O' Anusandhan, Bhubaneswar, Odisha, India during spring season of the year 2022-23. SEM/EDX was used to investigate the cocoons metal elements. Each sample was examined separately to evaluate metal content. Conductive carbon tape attached specimens to stubs. The metal elemental analysis of the scaffold was performed by EDX attached to the scanning electron microscope.

Result: For silk cocoon inner surfaces, the metal elements were ordered as follows: C>O>Mo>K>Cl>Ca>Si>Na>Mg>Al. Some Eri cocoons were produced on different host plants and had somewhat different exteriors, but all had evenly distributed components. C and O were abundant on all silk cocoons' inner and outer surfaces. Cocoons produced on many host plants have higher chemical metal element levels on their exterior and interior surfaces than those raised on a single host plant. The study suggests that chemical factors have a role in silk cocooning, encouraging the creation of β -sheet structure.

Key words: Host plant, Inner surface, Metal elements, Outer surface.

INTRODUCTION

Samia ricini, eri silkworm belongs to the family Saturniidae, is multivoltine (more than two broods of offspring in a year) and the only completely domesticated silkworm other than *Bombyx mori*; (Kom *et al.*, 2023). Silk fiber is among the earliest natural protein fibers utilized by humans and is produced and excreted by the Lepidoptera silkworm, which has become a significant economic insect (Dash *et al.*, 2006). Silk is a protein fiber of significant interest in biomedical and industrial science (Wen *et al.*, 2021; Omenetto and Kaplan, 2010; Herold and Scheibel, 2017). Silk fiber is a kind of fibrous protein which is having remarkable record as second strongest fiber in the world. Silk fibers consist of three protein constituents: fibroin, sericin and more tiny molecular proteins (Xia *et al.*, 2014). Fibroin can be further categorized into silk heavy chain. FibH protein, FibL silk light-chain protein, P25 protein and P25-like protein (Zhou *et al.*, 2001). The conversion of soluble animal silk protein into solid silk fiber is the outcome of a complicated interaction between biological and physical processes (Koh *et al.*, 2015; Heim *et al.*, 2009). There are many reports about the factors affected on the mechanical strength of the silk fiber such as host plants and environmental effect including silk varieties (Shao and Vollrath, 2002). In sericulture, role of environmental conditions for silkworm is of utmost importance and contributes 37.8% for successful cocoon crop. Cocoon crop productivity declines considerably due to slight fluctuations

¹Department of Entomology, Institute of Agricultural Sciences, Siksha 'O' Anusandhan, Bhubaneswar, Khordha-751 003, Odisha, India.

²Regional Sericultural Research Station, Central Silk Board, Koraput-764 020, Odisha, India.

³Regional Sericultural Research Station, Central Silk Board, Sahasapur-248 197, Dehradun, Uttara Khand, India.

Corresponding Author: Priyanka Nayak, Department of Entomology, Institute of Agricultural Sciences, Siksha 'O' Anusandhan, Bhubaneswar, Khordha-751 003, Odisha, India. Email: priyankanayakpn@gmail.com

Orcids: 0000-0002-6514-7295, 0000-0003-0801-1997, 0009-0007-2895-2854, 0000-0002-8885-0805, 0000-0002-8182-6239.

How to cite this article: Nayak, P., Dash, S., Mishra, B.K., kumar, S.R. and Arasakumar, E. (2024). Exploring The Metal Composition of Eri Silkworm Cocoons Reared on Diverse Host Plant Combinations. Agricultural Science Digest. 1-8. doi: 10.18805/ag.D-6143.

Submitted: 05-07-2024 **Accepted:** 25-10-2024 **Online:** 30-12-2024

in temperature and humidity (Chanotra and Angotra, 2022). On the other hand, silk processing method like spinning and reeling is also a major factor on the silk fiber strength (Zhou *et al.*, 2003). The fibrillation of silk proteins constitutes an intricate procedure encompassing numerous endogenous and exogenous variables (Qiu *et al.*, 2019). Silk protein is formed by linking of amino acids together through peptide bonds. The chain contains carbonyl and amino groups, as well as additional side

chains that are connected it through amino, hydroxyl, carbonyl, guanido, imidazole and mercapto groups. These groups can be synchronized with transitional metal ions (Chen *et al.*, 2005). The qualities of silk fiber are greatly influenced by various factors, including metal ions (Chen *et al.*, 2002; Li *et al.*, 2001; Hossain *et al.*, 2003), viscosity and pH (Zhou *et al.*, 2004). Metal ions trigger conformational changes in silk proteins (Zhou *et al.*, 2003).

The silk-producing glands and silk of silkworm and spider proteins comprise of different metallic elements, including potassium, sodium, calcium, copper, manganese, magnesium, zinc and iron. Moreover, metal ions are particularly efficient in modifying the structure and characteristics of silk proteins (Cheng *et al.*, 2018). The metal ions were reported to help in some way to refold the silk protein molecules (Knight and Vollrath, 2001). Metal ions such as Cu_2^+ (Ochi *et al.*, 2002), K^+ , Ca_2^+ and Zn_2^+ were reported to promote the random coil to α -sheet structure transition on the secondary structures (Li *et al.*, 2001). Calcium ions (Ca_2^+) can facilitate the formation of a stable protein network structure from silk proteins in vitro (Laity *et al.*, 2019) and Cu_2^+ can cause the conformational alteration of silk protein solution into β -sheet crystallization (Qiu *et al.*, 2019; Zong *et al.*, 2004). Fe_3^+ can induce a conformational shift in regenerated silk. Protein solution transitions from a helix to a β -sheet, but Fe_2^+ is incapable of this transformation (Liu *et al.*, 2018). Na^+ inhibit silk protein from fibrillation in advance preserves silk protein in a dissolved state during storage; a high concentration of K^+ may stabilize the intermolecular hydrogen bonds of silk fibroin. bond network (Wang *et al.*, 2017). On the other hand, mechanical properties of silk are influenced by both primary structure amino acid sequence and spinning conditions such as temperature, pH, ionic strength, solvent composition and mechanical stress (Shao and Vollrath, 2002; Chen *et al.*, 2001). During the cocoon spinning, a combination of active dehydration, flow-induced forces and many metal ions are involved in the spinning process (Hu *et al.*, 2009). Therefore, in this study, we aimed to systematically test the metal element content of silk fibre in different layer of eri cocoon. Many studied about metallic ions effect on silk fibroin have been reported. However, composition of metal after spinning in cocoon or fiber was rarely published. Hence, it is considered interest to investigate about the metal ions on silk fibre/ cocoon.

Thus, the aim of this study was to analyze the metal content of Eri cocoons which was reared with different combinations of host plants and also to examine the metal composition and its quantity. The findings may provide insight into the metabolic connection between metals and silk fibres. Technology adoption is essential for the success of the sericulture to get more production and better income which will be inferred that better socioeconomic condition is directly related to higher level of adoption (Beula *et al.*, 2016).

MATERIALS AND METHODS

The pre sent analysis was conducted in lab at Department of Entomology, Institute of Agricultural Sciences, Siksha 'O' Anusandhan, Bhubaneswar, Odisha, India during spring season of the year 2022-23. using the three host plants: Castor (*Ricinus communis* L.), which is the primary host plant of the Eri silkworm and two secondary host plants, namely Kesseru (*Heteropanax fragrans*) and Tapioca (*Manihot esculenta*). In addition, two more plants, namely Gulancha (*Plumeria rubraacutifolia*) and papaya (*Carica papaya*), were selected as tertiary host for evaluation. The rearing practices involved the use of five different host plants. The experiment was replicated three times using a total of ten treatments, specifically the host plants. The data were recorded in Microsoft Excel and analysed using a Completely Randomized Design in the Statistical Package for the Social Sciences (SPSS). The standard error and significant differences between results were assessed using Duncan's multiple range test ($P < 0.05$) after conducting a one-way ANOVA. These combinations were evaluated to determine their effects on metal elemental composition of Eri silkworm cocoons.

Treatment combinations are as follows:

- T₁: Castor (1st to 5th instars).
- T₂: Kesseru (1st to 5th instars).
- T₃: Tapioca (1st to 5th instars).
- T₄: Gulancha (1st to 5th instars).
- T₅: Papaya (1st to 5th instars).
- T₆: Castor (1st and 2nd instar) + Kesseru (3rd to 5th instars).
- T₇: Tapioca (1st and 2nd instar) + Kesseru (3rd to 5th instars).
- T₈: Gulancha (1st and 2nd instar) + Kesseru (3rd to 5th instars).
- T₉: Papaya (1st and 2nd instar) + Kesseru (3rd to 5th instars).
- T₁₀: All leaves mixed (1st to 5th instar).

The cocoon obtained from the different host plants after rearing were used for the study. The initial germplasm of Eri silkworm was acquired from the Eri Silkworm Production Centre in Hosur, Tamil Nadu, for the purpose of performing the research. Silk cocoons for investigated were selected and sent to Institute of Minerals and Materials Technology, CSIR-IMMT, Bhubaneswar, Odisha.

Preparation of silk fibroin blends and a spinnable solution

The SF solution was prepared with slight modifications to a protocol previously described by (Liu *et al.*, 2011). In brief, eri silk cocoons were cut into small pieces and boiled in 0.5% w/w Na_2CO_3 (aq) solution at 100°C for 60 min followed by awash in distilled water to remove the sericin glue. The extracted silk fibroin was air dried and dissolved in a ternary mixture of $\text{CaCl}_2/\text{H}_2\text{O}/\text{EtOH}$ (1:8:2 molar ratio) for 40 min at 80°C and dialyzed to remove salts using a Slide-A-Lyzer Dialysis Cassette (Pierce, Thermo Fischer, MWCO 3500 Dalton, Rockford, IL USA) against distilled water for 3 days at room temperature. The SF solution was filtered and concentrated against PEG followed by freeze drying to prepare a regenerated SF powder. SF powders from eri and tasar were mixed in a weight ratio of 70:30 (w/w) with

formic acid and chloroform (60:40 (v/v)) at a stirring rate of 300 rpm at 55°C to prepare a spinnable solution.

Preparation of a blended nanofibrous scaffold by electrospinning

The 3D nanofibrous mat from the SF blend was prepared by electrospinning using a 5 mL syringe and a needle (diameter of 0.55 mm) mounted in a parallel plate geometry by applying a high electrostatic potential of 20–22 kV. A constant volume flow rate of 0.5 mL/h was maintained using a syringe pump and the tip-collector distance was maintained at 15 cm. The fibers were collected on a grounded parallel plate collector consisting of an aluminum sheet mounted over a glass plate. Electrospun non-woven mats from SF blend solutions were immersed in a solution of methanol: water or ethanol: water (90:10 v/v) for 10 min to induce the amorphous to β -sheet conformational transition of silk fibroin. The fiber was then washed with water for 24 h at room temperature to remove the elec-trospun fibrous mat from the aluminum sheet.

Scanning electron microscope and energy dispersive X-ray spectroscopy analysis

Scanning electron microscopy (SEM, JEM2010, JEOL) was used to observe the morphology and fiber size of the electrospun nanofibrous scaffolds. The nanofibrous scaffolds were cut into small pieces (0.5 × 0.5) cm² and attached with Carbon tape before imaging. The metal elemental analysis of the scaffold was performed by EDX attached to the scanning electron microscope.

RESULTS AND DISCUSSION

The results of the recent study on the metal binding capacity of Eri silk fibers reveal significant insights into the metal elemental composition and structural characteristics of the fibers from different cocoon treatments. Notably, the study

employed scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX) to analyze the inner and outer surfaces of cocoons, demonstrating variations in surface smoothness and metal elemental presence across treatments.

Surface texture variations

The SEM images show that Eri silk fibers from the inner surface of the cocoon exhibit a more uniform surface compared to those from the outer surface (Fig 1). Interestingly, fibers from T3 Tapioca (1st to 5th instars) cocoons displayed greater surface unevenness, which was also evident in fibers from T4: Gulancha (1st to 5th instars), T5: Papaya (1st to 5th instars), T6: Castor (1st and 2nd instar) + Kesseru (3rd to 5th instars), T7: Tapioca (1st and 2nd instar) + Kesseru (3rd to 5th instars) and T8: Gulancha (1st and 2nd instar) + Kesseru (3rd to 5th instars) cocoons (Table 1).

Metal elemental composition

The EDX analysis indicated the presence of several key metal elements, including carbon (C), oxygen (O), calcium (Ca), magnesium (Mg), silicon (Si), molybdenum (Mo), sodium (Na), potassium (K) and aluminum (Al). The inner surface of the T8: Gulancha (1st and 2nd instar) + Kesseru (3rd to 5th instars) cocoon had the highest carbon content at 77.075%, followed by the T9: Papaya (1st and 2nd instar) + Kesseru (3rd to 5th instars) cocoon at 74.166% (Fig 2). In terms of metal elemental abundance, the order was C > O > Mo > K > Cl > Ca > Si > Na > Mg > Al. Notably, the T5: Papaya (1st to 5th instars) cocoon lacked sodium, magnesium and aluminum (Table 2).

Consistency across surfaces

The metal elemental composition of the outer surface mirrored that of the inner surface, with the same order of abundance. T8: Gulancha (1st and 2nd instar) + Kesseru

Table 1: Types and contents of metal elements founded in the outer surfaces of Eri cocoon.

Silk cocoon	Types of metal element and contents (weight %)									
	C	O	Na	Mg	Al	Si	Cl	K	Ca	Mo
T ₁ : Castor (1 st to 5 th instars)	68.10	17.466	0.033	0.033	0.133	0.366	0.633	1.233	3.20	12.30
T ₂ : Kesseru (1 st to 5 th instars)	71.33	20.033	0.166	0.10	0.10	0.166	1.40	1.70	0.20	4.76
T ₃ : Tapioca (1 st to 5 th instars)	76.70	20.366	0.233	0.233	0.066	0.133	0.166	1.233	0.233	1.833
T ₄ : Gulancha (1 st to 5 th instars)	68.416	22.366	0.166	0.15	0.30	0.70	1.35	1.86	0.31	4.366
T ₅ : Papaya (1 st to 5 th instars)	73.533	18.833	0.20	0.10	0.133	0.30	1.266	1.70	0.36	3.63
T ₆ : Castor (1 st and 2 nd instar) + Kesseru (3 rd to 5 th instars)	73	18.80	0.025	0.025	0.025	0.20	0.25	0.35	0.125	7.175
T ₇ : Tapioca (1 st and 2 nd instar) + Kesseru (3 rd to 5 th instars)	74.33	17.033	0.133	0.133	0.133	0.266	1.067	0.933	0.40	5.567
T ₈ : Gulancha (1 st and 2 nd instar) + Kesseru (3 rd to 5 th instars)	74.525	19.40	0.15	0.125	0.35	1.0	0.20	0.70	0.45	3.075
T ₉ : Papaya (1 st and 2 nd instar) + Kesseru (3 rd to 5 th instars)	72.533	21.933	0.20	0.166	0.10	0.266	0.166	0.433	0.433	3.767
T ₁₀ : All leaves mixed (1 st to 5 th instar)	73.34	18.12	0.32	0.32	0.16	0.36	0.20	1.0	0.50	5.66

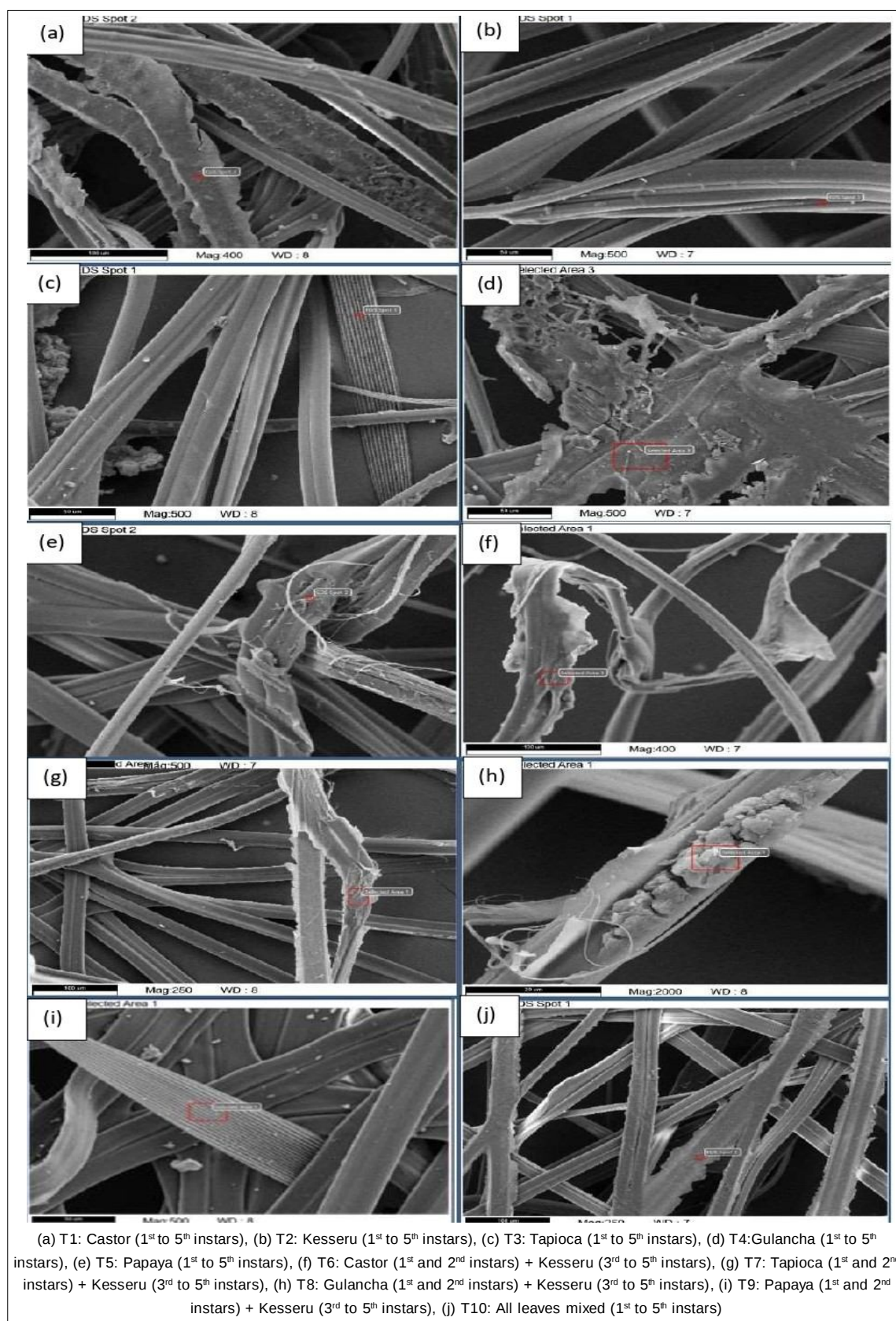


Fig 1: Longitudinal view of Eri silk fiber from outer surface of cocoon by scanning electron micrographs.

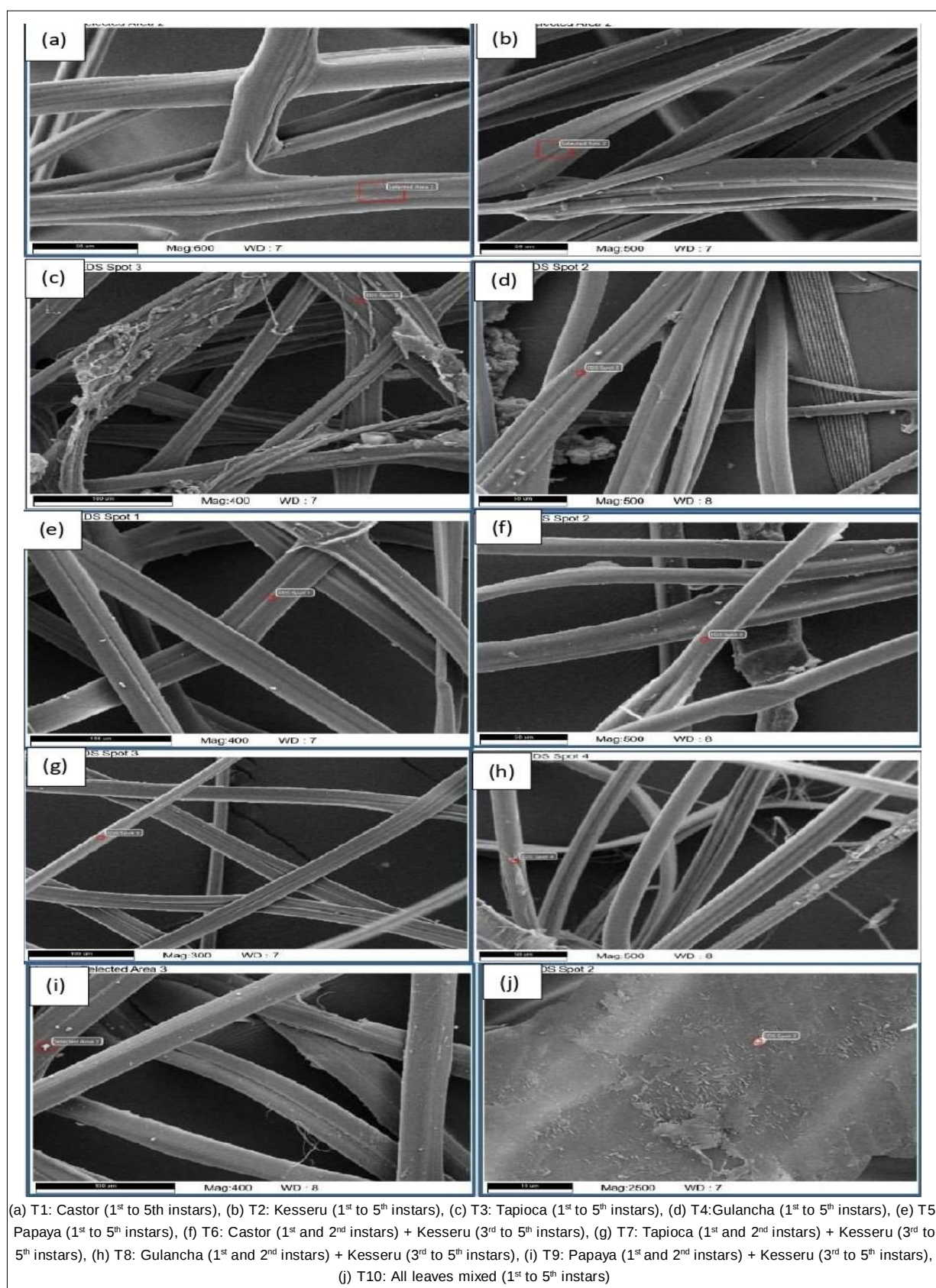


Fig 2: Longitudinal view of Eri silk fiber from inner surface of cocoon by scanning electron micrographs.

Table 2: Types and contents of metal elements founded in the inner surfaces of Eri cocoon.

Silk cocoon	Types of metal element and contents (weight %)									
	C	O	Na	Mg	Al	Si	Cl	K	Ca	Mo
T ₁ : Castor (1 st to 5 th instars)	65.95	14.25	0.025	0.025	0.025	0.15	0.825	1.15	0.325	17.25
T ₂ : Kesseru (1 st to 5 th instars)	70.8	23.76	0.36	0.20	0.166	0.233	0.533	0.70	0.233	3.033
T ₃ : Tapioca (1 st to 5 th instars)	71.25	23.825	0.325	0.275	0.15	0.225	0.275	0.575	0.075	2.95
T ₄ : Gulancha (1 st to 5 th instars)	64.42	25.95	0.12	0.20	0.28	0.52	0.98	1.72	0.22	5.58
T ₅ : Papaya (1 st to 5 th instars)	71.066	18.733	nd	nd	nd	0.066	0.766	2.366	0.40	6.60
T ₆ : Castor (1 st and 2 nd instar)	70.775	21.85	0.05	0.025	0.05	0.175	0.375	0.275	0.175	6.25
+ Kesseru (3 rd to 5 th instars)										
T ₇ : Tapioca (1 st and 2 nd instar)	72.866	17.667	0.20	0.20	0.1667	0.333	0.766	1.30	0.50	6.00
+ Kesseru (3 rd to 5 th instars)										
T ₈ : Gulancha (1 st and 2 nd instar)	77.075	17.00	0.10	0.075	0.10	0.325	0.450	0.825	0.250	3.80
+ Kesseru (3 rd to 5 th instars)										
T ₉ : Papaya (1 st and 2 nd instar)	74.166	19.966	0.4667	0.1667	0.1333	0.30	0.80	0.40	0.1667	3.036
+ Kesseru (3 rd to 5 th instars)										
T ₁₀ : All leaves mixed (1 st to 5 th instar)	74	17.42	0.18	0.14	0.12	0.38	0.56	0.70	0.38	6.08

(3rd to 5th instars) again recorded the highest carbon content (76.70%), while T4: Gulancha (1st to 5th instars) had the highest oxygen content at 22.366% (Table 2).

The findings align with prior research that has explored the role of metal ions in silk protein structure and function. Goto and Suyama (2000) highlighted the potential of utilizing natural protein fibers for metal binding, setting the groundwork for exploring Eri silk's unique properties. Arai *et al.* (2001) emphasized the significance of incorporating metal ions into fibrous polymers, further establishing the context for the current study.

The ability of silk proteins to interact with metal ions has been well documented. For instance, Chen *et al.* (2005) and Taddei *et al.* (2003) identified how the various amino acids in silk, characterized by multiple functional groups, can effectively bind with transition metals. This interaction is not only crucial for the structural integrity of the fibers but also for enhancing their functional properties. Additionally, earlier studies have demonstrated that metal elements such as copper (Cu) and calcium (Ca) are vital for the formation and stability of silk proteins. Lim *et al.* (1999) found that copper induces the formation of β -sheet structures in silk, while Ruan *et al.* (2008) noted that magnesium plays a crucial role in silk fiber formation and its structural folding. Furthermore, Ji *et al.* (2009) reported that ferric ions can facilitate conformational changes in silk fibroin, highlighting the impact of metal ions on protein structure.

In this study, contents and types of metal elements were greatly differed from earlier study. This may be due the different rearing practices, host plant and location of ericulture, since it has been reported that these parameters were affected the silk fiber characteristics. There is one strong evidence that metals are concerned in the silk spinning process including the transition of the secondary structure of silk protein. It is very interesting to about the mechanism of those metal involved the process as well as the action of them. Furthermore, this work was done to know how the metal elements were scattered in each silk variety that is significantly different.

CONCLUSION

The SEM with EDX results revealed the characteristic spectrum of the metal elements which were arranged in following order of C>O>Mo>K>Cl>Ca>Si>Na>Mg>Al for inner surfaces of cocoons. For the outer surfaces, all metal elements were distributed in all types of Eri cocoons. Among them, C and O were found in high percentage in all silk cocoons both inner and outer surfaces. Cocoons from combined host plants showed higher chemical metal element with higher percentage, than cocoons obtained from rearing on single host plants. It is a promising fact that metal elements may be involved the secondary structure of silk fiber to change the helix into β -sheet form. However, the information about the action of that metal element has not been clear nowadays.

ACKNOWLEDGEMENT

We appreciate and thank Council of Scientific and Industrial Research-Institute of Minerals and materials Technology (CSIR-IMMT), Ministry of Science and Technology, Govt. of India for technical support for this study.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

- Arai, T., Freddi, G., Colona, G.M., Scotti, E., Boschi, A., Murakami, R. and Tsukada, M. (2001). Absorption of metal cations by modified *B. mori* silk and preparation of fabrics with antimicrobial activity. *J. Applied Polymer Sci.* 80: 297-303.
- Beula, M., Darshini, P. and Kumari, N.V. (2016). Technology dissemination and adoption of sericulture technology by the farmers of Chittoor district- A case study. *Indian Journal of Agricultural Sciences.* 50(5): 483-486.
- Chanotra, S. and Angotra, J. (2022). Implications of meteorological forecasting for accelerating the success rate in Sericulture: A new avenue in Seri-industry: A review. *Agricultural Reviews.* doi: 10.18805/ag.R-2513.
- Chen, W.X., Lu, S.F. and Yao, Y.Y. 2005. Copper (II)-silk fibroin complex fibers as air-purifying materials for removing ammonia. *Textile Res. J.* 75: 326-330.
- Chen, X., Knight, D.P. and Vollrath, F. (2002). Rheological characterization of Nephilaspidroin solution. *Biomacromolecules.* 3: 644-648.
- Chen, X., Shao, Z.Z., Marinkovic, N.S., Miller, L.M., Zhou, P. and Chance, M.R. (2001). Conformation transition kinetics of regenerated *Bombyx mori* SF membrane monitored by time-resolved FTIR spectroscopy. *Biophys. Chem.* 89: 25-34.
- Cheng L., Huang, H., Zeng, J., Liu, Z., Tong, X., Li, Z., Zhao, H. and Dai, F. (2018). Effect of different additives in diets on secondary structure, thermal and mechanical properties of silkworm silk. *Materials.* 12: 14.
- Dash, R., Mukherjee, S. and Kundu, S.C. (2006). Isolation, purification and characterization of silk protein sericin from cocoon peduncles of tropical tasar silkworm, *Antheraea mylita*. *Int. J. Biol. Macromol.* 38: 255-258.
- Goto, M. and Suyama, K. (2000). Occlusion of transition metal ions by new adsorbents synthesized from plant polyphenols and animal fibrous proteins. *Applied Biochem. Biotechnol.* 84: 1021-1038.
- Heim, M., Keerl, D. and Scheibel, T. (2009). Spider silk: From soluble protein to extraordinary fiber. *Angew. Chem. Int. Ed. Engl.* 48: 3584.
- Herold, H.M. and Scheibel, T. (2017). Applicability of biotechnologically produced insect silks. *Z. Nat. C.* 72: 365.
- Hossain, K.S., Ochi, A., Ooyama, E., Magoshi, J. and Nemoto, N. (2003). Dynamic light scattering of native silk fibroin solution extracted from different parts of the middle division of the silk gland of the *Bombyx mori* silkworm. *Biomacromolecules.* 4: 350-359.
- Hu, X., Kaplan, D. and Cebe, P. (2009). Thermal analysis of protein-metallic ion system. *J. Therm. Anal. Calorim.* 96: 827-834.
- Ji, D., Deng, Y.B. and Zhou, P. (2009). Folding process of silk fibroin induced by ferric and ferrous ions. *J. Mol. Struct.* 938: 305-310.
- Knight, D.P. and Vollrath, V. (2001). Changes in metal element composition along the spinning duct in a *Nephila* spider. *Naturwissenschaften.* 88: 179-182.
- Kom Serto Sophiya, Nakhro Rokoneituo and Sharma Amod. (2023). Sustainable rearing of Eri silkworm (*Samia ricini*) in Bishnupur district of Manipur. *Agricultural Science Digest.* doi 10.18805/ag.D-5574.
- Koh, L.D., Cheng, Y., Teng, C.P., Khin, Y.W., Loh, X.J., Tee, S.Y., Low, M., Ye, E.Y., Yu, H.D., Zhang, Y.W., et al. (2015). Structures, mechanical properties and applications of silk fibroin materials. *Prog. Polym. Sci.* 46: 86.
- Liu, H. X., Li, Zhou, G., Fan, H. and Fan, Y. (2011). Electrospun sulphated silk fibroin nanofibrous scaffolds for vascular tissue engineering. *Biomaterials.* 32: 3784-3793.
- Laity, P.R., Baldwin, E. and Holland, C. (2019). Changes in silk feedstock rheology during cocoon construction: The role of calcium and potassium ions. *Macromol. Biosci.* 19: 1800-188.
- Li, G., Zhou, P., Sun, Y., Yao, W. and Mi, Y. et al. (2001). The effect of metal ions on the conformation transition of silk fibroin. *Chem. J. Chin. Univ.* 22: 860-862.
- Lim, A., Guy, P.A., Makhov, A.M., Saderholm, M.J. and Kroll, M. et al. (1999). Engineering of betabellin 15D: Copper (II)-induced folding of a fibrillar β -sandwich protein. *Lett. Pept. Sci.* 6: 3-14.
- Liu, Q.S., Wang, X., Tan, X.Y., Xie, X.Q., Li, Y., Zhao, P. and Xia, Q.Y. (2018). A strategy for improving the mechanical properties of silk fiber by directly injection of ferric ions into silkworm. *Mater. Design.* 146: 134.
- Ochi, A., Hossain, K.S., Magoshi, J. and Nemoto, N. (2002). Rheology and dynamic light scattering of silk fibroin solution extracted from the middle division of *Bombyx mori* silkworm. *Biomacromolecules.* 3: 1187-1196.
- Omenetto, F.G. and Kaplan, D.L. (2010). New opportunities for an ancient material. *Science.* 329: 528.
- Qiu, W., Patil, A., Hu, F. and Liu, X.Y. (2019). Hierarchical Structure of Silk Materials Versus Mechanical Performance and Mesoscopic Engineering Principles. *Small.* 15: e1903948.
- Ruan, Q.X. and Zhou, P. (2008). Sodium ion effect on silk fibroin conformation characterized by solid-state NMR and generalized 2D NMR-NMR correlation. *J. Mol. Struct.* 883: 884: 85-90.

- Ruan, Q.X., Zhou, P., Hu, B.W. and Ji, D. (2008). An investigation into the effect of potassium ions on the folding of silk fibroin studied by generalized two-dimensional NMR-NMR correlation and Raman spectroscopy. *FEBS J.* 275: 219-232.
- Shao, Z.Z. and Vollrath, F. (2002). Surprising strength of silkworm silk-silk fibers produced by artificial reeling are superior to those that are spun naturally. *Nature.* 418: 741-741.
- Taddei, P., Monti, P., Freddi, G., Arai, T. and Tsukada, M. (2003). IR study on the binding mode of metal cations to chemically modified *Bombyx mori* and Tussah silk fibers. *J. Mol. Struct.* 651-653: 433-441.
- Wang, X., Li, Y., Liu, Q., Chen, Q., Xia, Q. and Zhao, P. (2017). *In vivo* effects of metal ions on conformation and mechanical performance of silkworm silks. *Biochim. Biophys. Acta.* 1861: 567.
- Wen, D.L., Sun, D.H., Huang, P., Huang, W., Su, M., Wang, Y., Han, M.D., Kim, B., Brugger, J. and Zhang, H.X., *et al.* (2021): Recent progress in silk fibroin-based flexible electronics. *Microsyst. Nanoeng.* 7: 35.
- Xia, Q.Y., Li, S. and Feng, Q.L. (2014). Advances in silkworm studies accelerated by the genome sequencing of *bombyx mori*. *Annu. Rev. Entomol.* 59: 513.
- Zhou, C.Z., Confalonieri, F., Jacquet, M., Perasso, R., Li, Z.G. and Janin, J. (2001). Silk fibroin: Structural implications of a remarkable amino acid sequence. *Proteins-Struct. Funct. Genet.* 44: 119.
- Zhou, L., Chen, X., Shao, Z., Huang, Y. and Knight, D.P. (2005). Effect of metallic ions on silk formation in the Mulberry silkworm, *Bombyx mori*. *J. Phys. Chem. B.* 109: 16937-16945.
- Zhou, L., Chen, X., Shao, Z., Zhou, P., Knight, D.P. and Vollrath, F. (2003). Copper in the silk formation process of *Bombyx mori* silkworm. *-FEBS Lett.* 554: 337-341.
- Zhou, P., Xie, X., Knight, D.P., Zong, X.H., Deng, F. and Yao, W.H. (2004). Effects of pH and calcium ions on the conformational transitions in silk fibroin using 2D Raman correlation spectroscopy and ¹³C solid-state NMR. *Biochemistry.* 43: 11302-11311.
- Zong, X.H., Zhou, P., Shao, Z.Z., Chen, S.M., Chen, X., Hu, B.W., Deng, F. and Yao, W.H. (2004). Effect of pH and copper (II) on the conformation transitions of silk fibroin based on EPR, NMR and Raman spectroscopy. *Biochemistry.* 43: 11932.