

# Review on Different Algorithms and Techniques Used in Classification of Gender in Silkworm

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## Article history

Received: 02-07-2024

Revised: 22-11-2024

Accepted: 15-12-2024

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**Abstract:** Silkworm seed is the key factor for the success of sericulture industry. Silk seed production process at grainages centre mainly involve procurement of seed cocoon, cutting of seed cocoon for pupae, separation of male and female pupae, emergence of moth, coupling of silk moth and further egg laying by female moth. Separation of male and female is very much crucial and vital step in silk seed production which usually occurs at pupal stage and requires highly skilled and trained workers with good eyesight, but there are chances of error which may lead to poor quality silk seed production. There is also wastage of silk due to cutting of cocoon for obtaining pupa. It is also a fact that male cocoon silk is of finer quality as compared to female cocoon silk, so it is of great importance to detect the sex of pupa without cutting of cocoon so as to minimize the damage caused to silk by cutting of cocoon. In this review study various techniques including both destructive and non-destructive methods regarding the gender detection are discussed with their future scope and limitations. A variety of techniques were investigated, including optical penetration techniques, fluorescence spectrometry, DNA, X-ray imaging, MRI, hyper spectral imaging, near infrared spectroscopy, physical observations, multisensory systems, and computer vision etc. According to recent studies, relatively few non-destructive techniques have been noticed to classify silkworm's gender which is very much essential in dealing with living material in connection to that present review study is conducted.

**Keywords:** Silkworm, Sex Detection, Gender Classification, Sericulture, Machine Learning

## Introduction

Sericulture refers to the practice of producing silk through the rearing of silkworms. Sericulture is an agro-based industry which provides unique opportunity for socioeconomic progress for developing countries where sericulture is feasible. Silk has always been of considerable importance and has been referred as the queen of textiles. Silk is also mentioned in Rig-Veda. The Chinese term "Su (Si)" which means "Silk" and the English word "Culture" which means "Rearing" are the roots of the word "Sericulture" (Taufique and Hoque, 2021). Sericulture is a labor-intensive work so that the entire family can participate in any of the operations, including mulberry plantations, leaf gathering, raising and reeling procedures, etc. for their earnings (Madan Mohan Rao, 1999). The life cycle of Silkworm consists of stages: The egg, larva, pupa and adult moth Figure (1).

The female moth deposits her eggs after mating with the male. The egg hatches into a larva that feeds for about four weeks on mulberry leaves before entering the

pupal stage and then stops feeding. After that, it develops a cocoon-like outer shell by its secretion (Matsuura *et al.*, 1968). The heart of any sericulture industry is the silkworm seed. The quality of the silkworm seed is entirely responsible for the commercial production of silkworm cocoons and, in turn, the maintenance of high-quality cocoon production for silk yarn. To meet the current demand for silk seed in various areas, there are now not only government grainage centres but also licensed seed producers in the private sector (Madan Mohan Rao, 1999). Grainage faculties allow couples to reproduce and lay silkworm eggs by gender-separation. Farmers make use of these seeds to produce cocoons and after that, the cocoons are either given to grainage or other locations according to their quality and criteria for enhanced seed output or delivered to be reeled in order to make raw silk (Muruges Babu, 2013). The production of silkworm seeds is a delicate and complex process that demands meticulous planning and execution. The secret to success in the manufacture of silk seeds is error-free gender detection and precise gender segregation.

Separation in the grainage centres is done manually with the help of trained professionals under bright lights so the chances of error may be there in physical observations. Although it sometimes happens in the cocoon stage which is very much difficult, but mostly it is done usually in the pupal stage. One of the crucial steps in the manufacturing of Bivoltine silkworm seeds is the separation of male and female pupae, which calls for technical proficiency and utmost care. Bivoltine moths emerge simultaneously as both the male and female, therefore there is always an opportunity of selfing which results in decreasing the quality of laid eggs if proper care is not taken in a time bound manner. The Sex separation during the pupal stage, which is primarily done to prepare bivoltine hybrids, ensures the production of 100% genuine hybrids. Cut the cocoons off at one end, about a fifth of the way down, to produce bivoltine hybrids without damaging the pupae inside. When enough pupae have gathered in trays, they are moved to a different area for sex separation. Pupae usually are manually divided into male and female groups based on various characteristics such as sexual markings as well as form, size and weight. Figure (2) illustrates the basic steps in silk seed production.

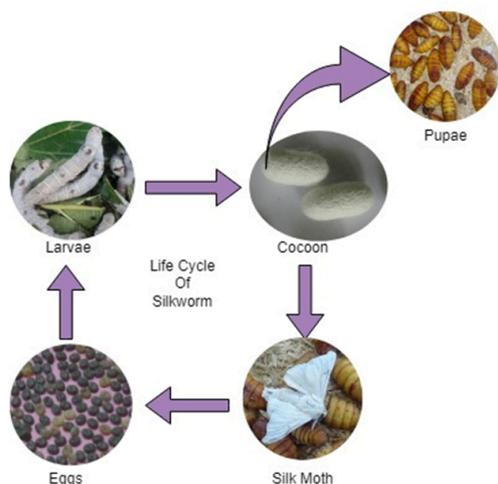


Fig. 1: The silkworm's life cycle

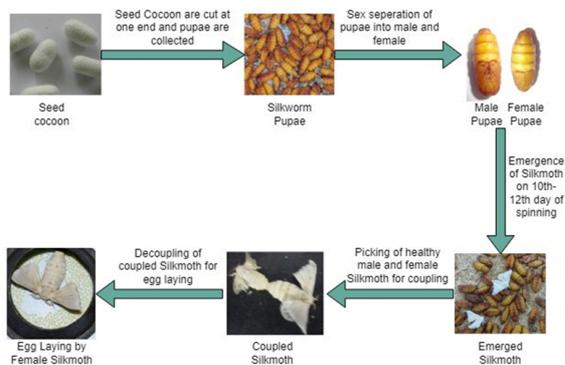


Fig. 2: The basic steps in silkworm seed production at grainage centres

Sericulture has a multidisciplinary nature and the post cocoon sector is almost entirely automatic. The demand of the present time requires automation of the pre cocoon sector, particularly the seed sector. In light of this, here we conduct a review of gender detection of silkworm because mostly it is done by trained labourer manually and it forms an integral part of the silk seed production process. Deep learning and its algorithms are modern technologies that can significantly improve sericulture (Pal *et al.*, 2023).

### Literature Review

Basically, methods of gender detection broadly can be categorized into two approaches, namely destructive and non-destructive. The non-destructive approach does not involve any kind of harm to the living material, Figure (3) illustrates various methods of gender detection in silkworm.

Key stages of sex identification include Larva, pupae and adult stage (Sakai *et al.*, 2014). Differentiation of gender in the egg stage is practically not possible. Sex may be differentiated in the 5<sup>th</sup> stage of larva, but it is not an easy job due to the continuous mobile nature of larva. At grainage centres, observers determine the sex in the pupal stage, identifying females by a vertical line on the 8<sup>th</sup> abdominal region and a small spot on the 9<sup>th</sup> abdominal region identifies males. Currently, trained workers manually perform sex separation, but it can be error-prone and time-consuming.

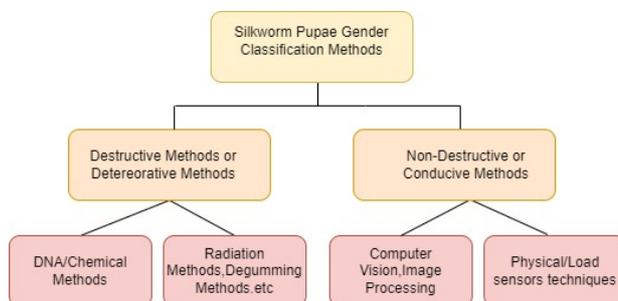


Fig. 3: Methods of gender detection in silkworm

Liu *et al.* (2008) investigated the intrinsic stillness time of bound and free water in silkworms. A silkworm relaxation model was developed based on the ratio of bound to free water in the silk glands of the midsection. The T2 relaxation model accurately represents the inherent disparity in silk tissue from glands within the silkworm's midsection. The tissue's T2 sensitivity is translated into image contrast using T2-weighted MRI, effectively highlighting tissue variations. This method was also used to determine the gender of silkworms with precision. Sumriddetchkajorn and Kamtongdee (2012) described and experimentally demonstrated an optical penetration-based gender sensor for silkworm pupae. The system uses near-infrared light to safely and effectively penetrate the pupa's body. The gender gland is highlighted while unwanted image noise is removed

using standard image processing techniques such as blob filtering, thresholding, and inversion.

Kamtongdee *et al.* (2013a) addressed the issue of image noise leading to inaccurate gender classification. They introduced a transparent support with a rectangular hole that acts as a region of interest (ROI) to localize the abdominal region, block visual noise, and aid in consistent placement of pupae. Both an external and a smaller internal ROI were used to minimize light scattering and speed up image processing. Kamtongdee *et al.* (2013b) presented a feasibility study on sex identification using pattern matching with normalized cross-correlation (NCC) in Cartesian and polar coordinates. The approach achieved moderate accuracy with fast processing times, and it emphasized the use of low-complexity, adaptable, and easy-to-implement techniques.

Sumriddetchkajorn *et al.* (2013) examined spectral imaging for silkworm gender classification. Using inexpensive LEDs that emit red, green, and white light, they found that the female-specific chitin gland was highlighted most effectively under red light, enhancing gender classification accuracy. Cai *et al.* (2014) explored the use of soft X-ray imaging and multidimensional data processing for gender determination in cocoons. Principal component analysis (PCA) was applied to 11 morphological features to identify clustering trends. Classifiers based on SVM, back-propagation neural networks, KNN, and LDA were evaluated and improved through cross-validation.

Sumriddetchkajorn *et al.* (2015) proposed a fault-tolerant optical penetration technique using dual-wavelength lighting with red and white light. Gender identification was achieved by analyzing visual zones in images captured under white light, allowing for faster identification of female pupae. Kamtongdee *et al.* (2015) developed and validated a highly accurate optical penetration-based system for silkworm gender classification. Key components included optical noise reduction, polarization filtering, automated ROI selection, and image magnification. The optical ROI formed automatically in white light conditions. Celik *et al.* (2017) introduced a non-destructive vision-based method to automatically classify silkworm cocoons by gender to improve egg production. Discriminative learning was applied, and experiments showed effective performance by both SVM and neural network classifiers.

Katsuma *et al.* (2018) demonstrated that W-chromosomes carry feminizing genes and that piRNA-mediated interactions are key in sex determination in *Bombyx mori*. Zhu *et al.* (2018) proposed a fast-automated sorting system using near-infrared spectroscopy. Static spectral differences and noise levels were analyzed as factors affecting gender discrimination accuracy. Tao *et al.* (2018a) studied gender and species identification using hyperspectral imaging. Spectra from the posterior region were analyzed using the successive

projection algorithm for wavelength selection and PCA for feature extraction. Tao *et al.* (2019) developed a novel model using near-infrared and visible hyperspectral imaging. Mean spectral data were computed from ROI, and the optimal five wavelengths were selected using SPA. Liu and Wang (2019) proposed a computer vision-based MLGBP feature extraction method using neural networks for classification.

Joseph Raj *et al.* (2019) proposed a multisensory gender classification system using weight and digital images of cocoons. Shape-based features were extracted and combined with weight data to train an SVM classifier. A conveyor system and air blower sorted cocoons into appropriate containers, tested on Pure Mysore and CSR2 breeds. Lin *et al.* (2019) proposed a spectral noise reduction method followed by the development of an SVM classification model. The pre-labeled data enabled gender classification of previously unmarked pupae. Tao *et al.* (2018b) used hyperspectral imaging to simultaneously identify species and sex. PCA was applied for dimensionality reduction. CNN outperformed conventional classifiers like SVM and KNN, although its performance declined slightly (to 95.09%) with pre-processed data. Chinnakotr *et al.* (2020) used LED lighting near sexual markings, followed by image processing to classify gender. Results showed 92% accuracy for males and 98% for females, with an overall accuracy of 95%. Gulzar *et al.* (2020) used CNN with transfer learning for seed classification, applying techniques like hybrid weight adjustment and learning rate decay. Symmetry-based data augmentation improved labeling and feature extraction, achieving 99% accuracy on both training and test sets.

Thomas and Thomas (2021) noted that gender identification is traditionally done by trained experts, which is time-consuming and requires expertise. Their review of classification techniques (X-ray, MRI, hyperspectral imaging, NIR, optical penetration) found that SVM outperformed other algorithms in most cases. Ma *et al.* (2021) combined multivariate spectral assessment with a low-cost, short-wavelength NIR detector to build a calibration model for gender classification across eight silkworm species. Techniques like PCA, LDA, and PLSDA yielded 98.44% accuracy. Dai *et al.* (2021) measured near-IR diffuse transmission spectra from cocoons, evaluating LDA, CNN, and SVM classifiers. Kanjanawanishkul (2021) proposed an image-based grading system for Eri silkworm pupae, aiming to improve sorting efficiency and quality. Thomas and Thomas (2022) developed an X-ray imaging technique for non-invasive gender classification using hybrid silkworm cocoons. Features extracted from the pupae shape were fed into an AdaBoost classifier with logistic regression as the base learner.

Pavitra and Raghavendra (2022) discussed various algorithms for detecting and classifying silk seeds, including fertility assessment. Their review evaluated benefits, limitations, and potential research directions.

**Table 1:** Summarised literature review and related findings

Authors and Year	Methodology	Objective	Findings
Liu <i>et al.</i> (2008)	MRI and T2-weighted Imaging	Gender identification using MRI-based tissue relaxation model	MRI can accurately differentiate male and female silkworms based on tissue contrast
Sumriddetchkajorn and Kamtongdee (2012)	Optical Penetration Using Near-IR Light	Safe gender identification of pupae	High accuracy in gender recognition by highlighting the gender gland using near-IR light
Kamtongdee <i>et al.</i> (2013a)	Light Penetration with Confined ROI	Minimization of noise in light penetration	Small rectangular ROI improves placement and minimizes noise interference
Kamtongdee <i>et al.</i> (2013b)	Pattern Matching (NCC in Cartesian and Polar Coordinates)	Feasibility of pattern matching for sex identification	Achieved moderate precision and faster turnaround time for identifying male pupae
Sumriddetchkajorn <i>et al.</i> (2013)	Spectral Imaging Analysis	Influence of different wavelengths on gender determination	High recognition accuracy by highlighting female-specific chitin glands with red light
Cai <i>et al.</i> (2014)	X-ray Imaging and PCA	Non-destructive gender identification using X-rays	Successful identification of different ROI in cocoons, enhanced performance with SVM and PCA
Sumriddetchkajorn <i>et al.</i> (2015)	Optical Penetration with Dual Wavelength	Fault-tolerant gender identification	Utilized red and white light to identify female pupae faster
Kamtongdee <i>et al.</i> (2015)	Optical Noise Reduction and Polarization Filtering	Enhanced accuracy in gender identification	High accuracy achieved through optical ROI formation and noise reduction
Mahesh <i>et al.</i> (2017)	Vision-Based Non-destructive Technology	Automated classification of cocoons based on gender	SVM and NN classifiers provided good performance in classifying male and female cocoons
Katsuma <i>et al.</i> (2018)	Gene Analysis	Role of W-chromosomes in sex determination	Identified key genes involved in feminizing character and pi-RNA interactions in <i>Bombyx mori</i>
Zhu <i>et al.</i> (2018)	NIR Spectroscopy	High-speed sex identification and sorting of pupae	Near-IR spectroscopy with chemometrics achieved high accuracy in gender sorting
Tao <i>et al.</i> (2018a)	Hyperspectral Imaging	Feasibility of using HSI for sex determination	HSI with PCA for feature extraction achieved high accuracy in identifying gender
Tao <i>et al.</i> (2019)	NIR and Visible Spectrum HSI	Analysis of sex discrimination using HSI	Developed models using ROI-based mean spectral data with selected wavelengths
Liu and Wang (2019)	Computer Vision-Based MLGBP	Machine learning-based gender classification	Successfully used ANN for automatic identification of silkworm cocoons
Joseph Raj <i>et al.</i> (2019)	Multi-Sensor System	Gender classification using weight and image processing	High accuracy achieved in gender classification with a conveyor and air blower system
Lin <i>et al.</i> (2019)	Semi-Supervised Learning with Pre-Labeling	Noise minimization in NIR spectroscopy	Improved accuracy in gender identification of silkworms using modified SVM models
Tao <i>et al.</i> (2018b)	Hyperspectral Imaging and PCA	Simultaneous identification of sex and species	CNN outperformed SVM and KNN in gender determination
Chinnakotr <i>et al.</i> (2020)	LED-Based Light Penetration	Separation of male and female pupae	Achieved 95% overall accuracy in sex classification using LEDs
Gulzar <i>et al.</i> (2020)	CNN and Transfer Learning	Gender classification of silkworm seeds	Achieved 99% accuracy in classification using CNN and hybrid weight adjustment techniques
Thomas <i>et al.</i> (2023)	X-ray, MRI, HSI, Optical Penetration	Review of various methods for silkworm gender classification	SVM found to be superior in most cases for gender classification
Ma <i>et al.</i> (2021)	Short-Wavelength NIR Spectroscopy	Creation of calibrating model for sex identification	Achieved 98.44% accuracy using PCA, LDA and PLSDA for calibration models
Dai <i>et al.</i> (2021)	CNN and Feature Detection	Gender and variety detection in silkworms	CNN successfully identified both gender and variety of silkworm cocoons
Kanjanawanishkul (2021)	Image-Based Grading System	Grading of Eri silkworm pupae	High sorting precision achieved with image-based grading
Thomas and Thomas (2022)	X-ray Imaging and Ensemble Learning	Non-destructive gender classification	Used AdaBoost with logistic regression for accurate gender classification
Pavitra and Raghavendra (2022)	Image Analysis	Counting and categorization of silkworm eggs	Reviewed benefits and drawbacks of existing methods, identified potential issues
Thomas and Thomas (2022)	CNN, VGG16, Efficient Net	Classification of silkworm cocoon hybrid races	Achieved high validation accuracy using Efficient Net
Raju <i>et al.</i> (2023)	Weight-Based Gender Segregation	AI and ML for gender segregation based on weight	95% accuracy achieved in weight-based gender segregation
Vasta <i>et al.</i> (2023)	Machine Prototype for Sorting	Automated cocoon sorting for quality improvement	Successfully developed a prototype for automated sorting of silk cocoons using cameras and algorithms

Thomas *et al.* (2023) focused on image classification of the FC1 and FC2 Bivoltine hybrid races of silkworms. The study employed convolutional neural networks (CNN), VGG16, and EfficientNet models for classification tasks. Using the EfficientNet architecture, the validation accuracy achieved was 99.99% for FC1 and 99.9% for FC2, demonstrating excellent model performance in distinguishing between these hybrid races. Raju *et al.* (2023) presented a method for gender classification during the cocoon stage based primarily on weight. The study also incorporated the concept of mathematical modeling for silk moth sex identification through sensor-based mechanisms. The proposed system achieved an accuracy of approximately 95%.

Vasta *et al.* (2023) developed a prototype machine for the automatic sorting of silk cocoons, aimed at optimizing the reeling process. The system utilized cameras and image processing algorithms to classify cocoons based on parameters such as size, shape, staining, and viability (live vs. dead pupae). The prototype demonstrated a sorting speed of up to 80 cocoons per minute. The study also explored the integration of various sensing technologies-including MRI, hyperspectral imaging, X-ray imaging, and optical penetration-combined with machine learning techniques such as SVM, CNN, and PCA. The overarching goal was to enhance accuracy and efficiency in cocoon classification through a synergy of image processing, spectral analysis, and artificial intelligence. A summarized overview of related techniques is presented in Table 1.

Common trends in the literature reviewed suggest that there has been an inclination toward the merger of imaging techniques and machine learning approaches for quick and accurate gender determination of silkworm pupae. The primary goal is to reduce the dependence risks towards human classification while maintaining levels of accuracy. Even with these developments, there are still challenges yet to be addressed such as image noise, spectrally overlapping signals and even computationally intensive tasks. It is anticipated that further research efforts implementing multi modal analysis techniques along with best model design would be necessary for further real-world applications.

## Materials and Methods

The review is carried out when a review process has been established. The research problem is first formulated. Once the research goals are assigned and then appropriate studies are chosen using databases like "Google Scholar", "Scopus", "Science Direct", "Web of Science" and "Springer Link". Following the selection of pertinent research, a series of quality and exclusion criteria-based filters are applied for assessments. After extracting all the pertinent information from the chosen studies, the information was finally synthesized to address the research questions.

## Research Objective

The aim of our study is to know about the published work in the field of gender classification of silkworms. Studies have been examined from a variety of angles to get an understanding. The following research problems used in this study are:

- What types of algorithms have been employed in the literature to determine the silkworms' gender?
- What characters have been employed in the literature for gender classification in silkworm
- Which evaluation techniques and parameters have been chosen in the literature to identify the gender of silkworms
- What are the challenges in detecting a silkworm's gender

## Search Strategy

An automated search performs basic searching. The Search terms" gender" AND" classification" AND" Sericulture" were entered first. The Search terms were obtained from articles and abstracts were examined to determine synonyms. Five databases were used for the search. The following is a detailed explanation of the search strings for each database:

- Scopus: The search string is" Gender" AND" classification" AND" Sericulture" (all fields) AND" machine learning" AND" Silkworm" make up the search string
- ScienceDirect: Gender classification and sericulture is the search term
- SpringerLink" Silkworm gender classification" AND" machine learning" is the search query (anywhere)
- Google Scholar:" Silkworm Gender Classification" is the search string (anywhere)
- Web of Science:" Machine learning,"" Silkworm," and" gender classification" did not turn up any publications on Web of Science

Dispersal of various publications over various datasets is shown in Table (2).

**Table 2:** Distribution of publication in different databases

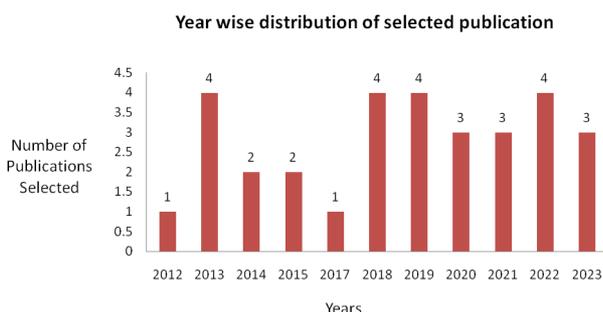
Database	Articles Retrieved	Articles After Exclusion
Scopus	37	14
ScienceDirect	17	01
SpringerLink	380	02
Google Scholar	3,360	15
Total	3,794	32

## Exclusion Criteria

The papers were examined and ranked according to the desired and undesired criteria to determine the parameters for the review and exclude irrelevant studies. These are the exclusion criteria displayed:

1. Publications are not related to the gender detection in silkworm and machine learning
2. Publications are not written in English
3. A publication that has already been obtained from another database or is a duplicate
4. The publication's entire text is not accessible
5. The publication has been published before 2012 except 01 paper for the year 2008

32 research paper and 02 books were chosen for further study after all five exclusion criteria were applied, as shown in the Table (1). Year wise selection of selected publication is shown in Figure (4).



**Fig. 4:** Year wise selection of selected publication

**Table 3:** Methods, classifiers and parameters used in gender detection

Methodology Used	Sample Size	Classifier	Parameter Used
Fault Tolerance optical Penetration (Sumriddetchkajorn <i>et al.</i> , 2015)	Total 96 Female 40, Male 56	Image Processing	Chitin Gland
Computer Vision (Mahesh <i>et al.</i> , 2017)	Total 167 (CSR2) Female 44, Male 47 Pure Mysore Female 35, Male 41)	KNN, LDA, NN, SVM	Weight, Volume Geometric, etc.
VIS-NIR (Tao <i>et al.</i> , 2019)	Total 520 (Male 260 Female 260)	SVM, RBF-NN	Full spectra, Feature wavelength, etc.
NIR With Chemo metrics (Zhu <i>et al.</i> , 2018)	Total 1600 for Static Spectra and Dynamic Spectra	SIMCA, PCA	Static Spectra, Dynamic Spectra
Multi-Sensor (Joseph Raj <i>et al.</i> , 2019)	Total 167 (CSR2 91 Pure Mysore 76)	SVM	The Area, Perimeter, Major Axis, Eccentricity, Circularity, Rectangularity, Solidity, Convex Area
X-Ray (Thomas and Thomas, 2022)	FC1 1156 (Male 589 Female 567) FC2 1222 (Male 623 Female 599)	X-ray images, image processing	Width, Height Length, Area, Weight Perimeter, Volume, Convexity

## Results

Gender differentiation in the egg stage is practically difficult although sex can be distinguished in the fifth stage of larvae but this is difficult because larvae are constantly moving. The ideal time to determine sex at grainage centres is during the pupal stage. A tiny spot on the ninth abdominal segment identifies males, while a vertical line on the eighth abdominal segment identifies females. Various classifiers, parameters for gender classification used in different studies are shown Table (3). Trained professionals use physical observation method manually for gender separations, but there are chances of error.

As per review, it is revealed that spectral data can precisely classify the gender of silkworm. Some of the studied methods such as NIR, HIS technology, etc., require the cutting of cocoons to obtain the pupae due to which cocoon silk filament may get damaged. Whereas other methods where cocoon cutting is not required such as in the case of X-ray, MRI, etc. are much costlier and not as per suitability to the seed production centres and may also face the problem of exact image capturing of the pupae. The most expensive DNA or chemical methods are those that require a controlled environment under laboratory conditions this needs a lot of time and is typically carried out with the help of trained professionals. These types of methods are much costlier and usually Unsuitable for large-scale productions. The multisensory technique includes the study of parameters such as rectangularity, solidity, the convex area and weight, etc. The SVM algorithm is used in this study and its accuracy ranges from 86.48-93.54%. The image was taken from above. In Hyper Spectral Imaging CNN, SVM and KNN algorithms are employed; their accuracy ranges from 95 to 98%. Cut is made on the cocoon for the removal of pupae; the cost of computation is expensive and challenging to use. Regarding the computer vision method, the parameters used include entropy, contrast, correlation, eccentricity, energy, area, perimeter, circularity and about 90% accuracy was achieved using a neural network technique. There are chances of silk waste during the removal of pupae when a cut is made on the cocoon. The SIMCA algorithm is utilized in near-infrared spectroscopy. In this cocoon is cut for obtaining the pupae, so there are chances of silk waste. In the Optical penetration technique accuracy of about 98% was achieved when the techniques of image processing were employed. In this technique, the pupae's rear end needs to be positioned with extreme caution. Erroneous placement leads to misclassification. In the X-ray technique, the parameters that are used are the area, the perimeter, eccentricity, etc. LDA, KNN, SVM, etc., are the algorithms employed in this and the estimated accuracy of all of these algorithms is about 93%. Equipment is expensive and challenging to operate. Where as in non-destructive x-ray technique gives an accuracy of 95-96%. Fluorescence spectroscopy is also

used. But it is a type of destructive approach. There might be challenges regarding the improvement of a working model, especially concerning its accuracy when more data is gathered to test and train. One of the additional challenges may include the implementation of models at the commercial level in a non-destructive way. Various techniques along with limitations are shown in Table (4).

**Table 4:** Comparison of silkworm gender identification techniques limitations and best use cases

Technique	Limitations	Best Use Case
MRI and T2-weighted Imaging	Expensive equipment, complex setup	Detailed gender identification in laboratory settings
Optical Penetration and Near-IR	Affected by noise, light scattering	Quick identification for pupae classification
Pattern Matching using NCC	Lower accuracy compared to spectral methods	Real-time identification where speed is crucial
Spectral Imaging Analysis	Limited to specific wavelengths	Laboratory and precise classification
X-ray Imaging with PCA	Requires expensive X-ray equipment	Non-invasive classification inside cocoons
Dual Wavelength Lighting	Lower male detection accuracy	Quick and simplified female identification
Zernike Moments and SVM	Complex shape feature extraction	Automated systems for cocoon classification
Near-IR Spectroscopy	Sensitive to noise and environmental factors	High-throughput automated classification
Hyperspectral Imaging	High computational cost	Simultaneous species and gender classification
Multi-Sensor System	High complexity and hardware requirements	Gender classification using weight and image data
Short-Wavelength NIR with PCA	Sensitive to noise, requires fine-tuning	Gender classification in controlled environments
Convolutional Neural Network	Requires large training data	Simultaneous classification of gender and variety
X-ray Imaging with AdaBoost	Requires X-ray imaging and advanced processing	Non-invasive identification for complex environments
Automated Sorting with ML	Limited to cocoon size and shape constraint	High-volume industrial sorting

## Discussion

An evaluation of the different methods for silkworm pupal and cocoon gendering shows recent developments in imaging as well as machine learning methods. Manual inspection of the gender by trained personnel is very much time-consuming as well as error prone making an automated and non-invasive strategy necessary. X-ray imaging, MRI Imaging and, spectral imaging has been found to be effective in separating male and female pupae using some features of their structure and

anatomy. Nevertheless, these imaging techniques are relatively expensive due to the sophisticated equipment and facilities needed as well as the controlled environments required for them rendering their uptake in extensive scenarios difficult. More recent papers highlight how integrating imaging methods with machine-learning models like SVM, LDA or CNN can aid in overcoming such limitations. It is very clear that machine learning algorithms can facilitate very complex feature sets such as texture, shape and spectral data for potential faster and accurate determining of gender. Feature extraction and pattern recognition have been successfully carried out using CNN thereby catering for gender and variety detection simultaneously. However, such approaches involve high computing loads and effort and often have to depend heavily on mass annotated datasets.

As evident in the above literature, it would appear that no one approach will be fool-proof; however, imaging and machine learning approaches are likely to provide a breakthrough in the determination of sex in silkworm pupae that is reliable, accurate and low-cost. It will be necessary to further develop these hybrid models in the future and minimize computational resources and also investigate real-world limitations such as different environments and execution in real time.

The diverse approaches pertaining to sex classification of silkworm pupae have greatly changed over time, from simply looking at them to having complex systems that do imaging and amputated parts and shape analysis. Each method has particular benefits and challenges related to precision, expense, automation potential and practicality in real-world situations. The manual observation method, commonly utilized by most seed production centers, is deeply shallow and individualized. Though lacking in resources, this method tends to be subject to a great deal of inaccuracies because of human blunders and exhaustion. Also, its lack of scalability renders it useless for high volume classification that require speed and exactness. Pupae feature identification techniques that use Near-Infrared (NIR) and other optical forms of imaging offer targeting internal as well as exterior surface aspects of the pupae. They are less safe, economical and more efficient. More advanced imaging entails the use of X-ray and MRI technologies. These innovations provide comprehensive contour outline, making them more effective at distinguishing different forms.

## Conclusion

Studies include both destructive as well as non-destructive methods for gender detection, but we should rely more on the non-destructive nature of methods due to the living nature of pupae which further undergo metamorphosis, otherwise destructive methods may interfere metamorphosis which will ultimately lead to poor silkworm seed production which is the base of

sericulture industry. Moreover, the process involved at the grainage centre is much more time-consuming and requires skilled labor, a lack of which may increase the chances of error. We should prefer only those kinds of methods that perform error-free gender detection without any harm to living material and no kind of silk loss. When the seed cocoon is cut at one end to obtain the pupa the silk gets damaged with the chances of cutting to the pupa also increasing, so to avoid such kind of damage the non-destructive methods with less time-consuming capability and of low cost should be incorporated. Most of the methods such as DNA-based gender separation, spectroscopic, chemo metrics, fluorescent techniques and techniques related to optics are either much costly or require complex infrastructural support along with damaging impacts to live material and are not in use as per practicability for commercial seed production which is the base for any sericulture industry. Physical methods based on mass and shape are also not reliable because in many cases many different silkworm species have a similar size and form for both the male and female so the techniques for identifying the sex of silkworms using mass and shape analyses also do not provide error-free results. X-ray and MRI techniques are also costly along with difficulty in exact image capturing. Present studies show that the accuracy of work for gender classification can be increased significantly if techniques for gender classification are assembled with computer vision, image processing and machine learning using different classifiers resulting in error-free less time-consuming and more work efficiency which may act as a strong platform towards non-destructive methods of gender classification, which is an extremely essential requirement in dealing with living material.

### *Future Scope*

Development of cost-effective non-destructive methods is the demand of small-scale industries in developing countries so a kind of work may be executed in this direction. Artificial Intelligence, Machine Learning, Deep Learning, etc. are modern technologies that can significantly improve sericulture. More research needs to be done in this particular field:

- Integration of several approaches: The combination of several non-destructive testing techniques and adequate image processing that guarantees good results can however produce an integrated system for silkworm sex determination
- Design of affordable and portable devices: MRI and X-ray imaging are effective in current practice but are not economical and thus cannot be recommended for broader applications. Further assessment should focus on designing and construction of cheaper handheld devices which will incorporate image recognition and sensing

intelligence technologies for commercialization within sericulture

- Machine learning facilitation: It will become efficient and less time-consuming to classify a specimen in different environments under the use of deep models and other advanced machine learning techniques such as transfer learning and reinforcement learning
- Extension to early-stage gender identification: The employment of these techniques to identify gender in the early life cycle stages such, larva and egg with the use non-invasive procedures will revolutionize the silk production industry by allowing gender determination at an early stage
- Fusion with robotic systems: Future studies may look at using these methods in combination with robotic systems for automation of the process of pupa handling, scanning and gender classification to minimize human work and errors

### *Challenges*

- Dependence on environment: Most approaches employing imaging and structural evaluations are overly sensitive to variations in light, background and imaging perspective which affects their precision and reliability.
- Restrained processing: Complete automated systems employing high-resolution imaging often integrate time-consuming and meticulous scrutiny with integrated granular analysis.
- Variability of data: Differences in the orientation of specimens, their shapes and prevailing conditions greatly affect classification.
- Convergence gaps: Some of the highly accurate methods are still not readily applicable in remote rural grainage centers because of equipment, training and financial costs
- Integrity of living material: Implementation of developing models at commercial level and treating biological material in a nondestructive way is a big challenge in this field.

### **Acknowledgment**

The authors receive technical support in basic understanding of the various aspects of silk seed production process including gender detection from silk seed production centre Palampur, District Kangra, Himachal Pradesh, India.

### **Funding Information**

The authors have not received any financial support or funding to report.

### **Author's Contributions**

All authors equally contributed to this study.

## Ethics

This manuscript is an original work. The authors declare that no ethical concerns are associated with this submission.

## Conflict of Interest

The authors declare no conflict of interest.

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