NATIONAL ENVIRONMENTAL SCIENCE ACADEMY (NESA)

International Journal on Environmental Sciences
http://nesa-india.org | http://journal.nesa-india.org/index/IJES
IJES 16(2): 120-138 **(2025)** • ISSN: 0976-4534
https://doi.org/10.53390/IJES.2025.16205

COMPARATIVE ASSESSMENT OF CASTOR CULTIVATION IN WASTEWATER-FED WETLANDS VERSUS AGRICULTURAL FIELDS FOR OPTIMIZED ERI-SILKWORM REARING

Ramalingam Sathya[1] and Ashutosh Das[1]*

Centre for Environment Engineering
PRIST Deemed to be University, Thanjavur, Tamil Nadu, India

Research Paper

Received: **11.06.2025** Revised: **10.07.2025** Accepted: **22.07.2025**

ABSTRACT

This study explores the integration of rice mill wastewater-fed constructed wetlands with Eri (*Samia ricini*) sericulture, using castor (*Ricinus communis*) as a dual-purpose crop for phytoremediation and silkworm feed. Castor leaves grown in constructed wetlands showed significantly higher moisture (75.2% vs. 72.5%), nitrogen (2.7% vs. 2.1%), sulphur (0.22% vs. 0.16%), protein (17.4 mg/g vs. 15.6 mg/g), and phenol content (2.1 mg/g vs. 1.6 mg/g), compared to those from agricultural fields—indicating enhanced metabolic and adaptive responses. However, Eri silkworms fed with agricultural leaves achieved greater larval weights at the 5th instar (4.54 g vs. 4.27 g), cocoon weights (2.1 g vs. 1.95 g), shell weights (0.5 g vs. 0.43 g), and shell ratios (23.8% vs. 22.3%), with all differences statistically significant ($p < 0.00001$). Seasonal effects were notable in mature leaf yield, with agricultural sites performing better in winter. While wetland castor presents a sustainable option for wastewater reuse and offers high nutritional value, its benefits did not translate into improved silk yield. Agricultural castor remains more effective for commercial eri-silk production. These findings offer actionable insights for optimizing sericulture systems that balance environmental sustainability with economic viability.

No. of Pages: 19 **References: 35**

Keywords: Eri-Silkworm, Castor Cultivation, Wastewater Irrigation, Constructed Wetlands, Eri-Silk Production, Phytoremediation.

INTRODUCTION

India is the world's second-largest silk producer, with the sector crucial to the economy and rural livelihoods. Raw silk production rose from 31,906 metric tonnes in 2017–18 to 38,913 in 2023–24, driven by expanded mulberry cultivation and better sericulture (Central Silk Board, 2024). However, FY 2024–25 saw a 21% decline due to adverse weather and market instability (Business Standard, 2024). Mulberry silk forms 75% of total output, while Eri, Tasar, and Muga silks make up the rest (Chakraborty & Ray, 2015).

India remains a net exporter of silk goods, with exports growing from 1,649 crore in 2017–18 to 2,027 crore in 2023–24, despite a drop to $215 million in the first three quarters of FY25 (Ministry of Textiles, 2024). Simultaneously, raw silk imports fell from 3,874 metric tonnes in 2022–23 to 1,561 in April–December 2024, reflecting improved domestic capacity (Central Silk Board, 2024).

The sector supports 9.76 million people, including 4.7 million farmers in over 52,000 villages (Central Silk Board, 2024). There's a strong correlation ($r = 0.976$) between silk production and employment, which has grown at 2.37% CAGR (Kumar et al., 2018). Sericulture offers incomes 1.5–2 times higher than traditional farming (Chakraborty & Ray 2015). Government schemes like Silk Samagra-2 (4,679 crore) have

*Corresponding author: *pristmtech.cee@gmail.com*

enhanced training and market access, benefiting over 78,000 individuals (Ministry of Textiles, 2024).

Silk exports contribute 0.2% to GDP and support 5.6 million people, with women making up 60% of the workforce (Central Silk Board, 2024). Karnataka and Assam have seen notable rural gains from silk-related activities (Chakraborty & Ray, 2015). Yet, climate change and market shifts pose challenges. Diversifying into eco-friendly Vanya silks like Eri and Tasar offers promise (Kiran & Prasad 2020). Value addition through innovation can further global competitiveness (Kataki et al., 2021).

Eri silk, from silkworms fed on castor leaves, is gaining traction in home textiles for its soft, durable, and hypoallergenic qualities, thermal insulation, and moisture absorbency .Its woolly texture makes it ideal for items like curtains and cushion covers, meeting demand for sustainable furnishings. Wetland-castor-based Eri silk production offers rural income potential, especially in castor-rich regions like Gujarat, which has over 650,000 hectares of castor farms. Initiatives such as Gujarat's Eri sericulture project promote sustainable integration without needing more land or water, thanks to castor's drought resilience.

This system supports small farmers and women's cooperatives, spreading economic gains and aligning with eco-conscious consumer trends .Its low carbon footprint and compatibility with natural dyes boost its sustainability credentials .The gradual adoption of machine-spinning is expected to scale production and open up wider market access.

In objective of the present research work is to study the scope of qualitative and quantitative assessment of rice mill-fed wetland-grown castor leaves and their functional role in eri-culture, from rearing generating the cocoons, the raw materials for production of eri-silk. The model developed herewith is likely to support existing agriculture, empowers women, and contributes to a more resilient and inclusive silk industry.

MATERIALS AND METHODS

Procurement of Castor Leaves from Agricultural Land *vs* Constructed wetland

Castor leaves were harvested from two distinct environments: an agricultural field and a constructed wetland. Collections were performed during two seasonal periods-summer (March to May) and winter (October to December)-to assess seasonal variation in leaf production. For each season and site, leaves were further categorized into two developmental stages: fresh (young) leaves and matured leaves.

Sampling was conducted by selecting representative plots within each environment. In each plot, all visible castor plants were surveyed. The leaves were hand-harvested in the morning hours to minimize water loss and physiological changes, as recommended for foliar studies (Sannappa & Jayaramaiah 2002). Fresh leaves were identified based on their bright green colour, tenderness, and lack of visible signs of senescence, while matured leaves were recognized by their darker colour, increased toughness, and full expansion.

After harvesting, leaves from each category (fresh and matured) and environment (agricultural field and constructed wetland) were counted and recorded separately for each season. The total number of leaves in each category was tabulated for both summer and winter seasons, as shown in the Table. This approach allows for the assessment of environmental and seasonal effects on castor leaf production.
All samples were handled with care to avoid mechanical damage and were counted immediately after collection to ensure accuracy. The methodology aligns with standard protocols for leaf sampling and enumeration in agronomic and ecological studies (Sannappa & Jayaramaiah, 2002).

Procurement of Eri-Silkworm Eggs.

Fig 1: Eri-silk worm eggs.

The species used for this study was *Samia ricini* commonly known as the Eri silkworm. Disease-Free Laying's (DFLs) were procured from the Central Silk Board, Germplasm Centre, located at Hosur. Each DFL (wt0.5 g), is likely to contain around 300 eggs (Fig 1), was kept for incubation under shaded and well-ventilated environment (temperature range: 24–27°C; relative humidity = 80-85%) for hatching (incubation period: 7 - 10 days) and specification of procured eggs as shown in table 1.

Table 1: Specification of Eri-silk worm-eggs procured.

Parameter	Specification
Size	1 to 2 millimeters in diameter (About the size of a mustard seed)
Color	Creamy white to pale yellow
Shape	Oval, laid in clusters
Eggs per Female	300-350 (up to 400-450 in optimal conditions)
Laying Period	2 to 3 days
Hatching Time	5to 10 days
Optimal Temperature	24 - 27 ℃ for laying
Optimal Humidity	80 - 85%
Health Indicator	Clustered, gummy-covered eggs

Collection and Characterization of Castor Leaves

Fresh leaves of ***Ricinus Communis*** (castor plant) were collected from two distinct cultivation environments: (i) conventional agricultural fields irrigated with bore well water, and (ii) constructed wetlands designed for the treatment of rice mill wastewater. To account for seasonal variability, the study was conducted during two major climatic periods-summer (March to May) and winter (October to December). Accordingly, leaf samples were harvested during each season from both cultivation sources. The collected leaves were subjected to both qualitative and quantitative characterization.

Qualitative Characterization of the Leaves:

Morphological Observations

Observation of color, shape, and size was performed in natural daylight. Leaves were visually examined for symmetry, pigmentation, and lobbing. Leaf yield was determined by manually counting the number of tender and mature leaves per plant. Measurements were performed in triplicates for statistical validity (Rajan et al., 2015).

Leaf Weight Measurement

Tender and matured leaves were harvested, separated manually, and weighed using an analytical balance with ±0.001 g precision. Leaves were collected early morning to avoid moisture loss and placed in pre-weighed containers (AOAC, 2016)

Quantitative Characterization of the Leaves:

Proximate Analysis

Moisture Content

To determine the moisture content of castor leaves, fresh, thoroughly cleaned leaves are first chopped into small pieces. Approximately 2 to 5 grams of the sample are weighed using an analytical balance and placed in a pre-weighed moisture dish. This sample is then dried in a hot air oven at 105°C for 24 hours or until a constant weight is achieved, ensuring complete removal of water. After drying, the sample is cooled in a desiccator to prevent reabsorption of atmospheric moisture and then reweighed. The moisture content is calculated using the formula:

$$\text{Moisture Content}(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} X100$$

This method is standardized and widely accepted for plant materials (AOAC, 2000; Kataki et al., 2021).

Volatile Matter

For volatile matter estimation, the oven-dried leaf sample is used. About 1 gram of this dried sample is placed in a covered crucible and heated in a muffle furnace at 600 ± 10°C for seven minutes. The high temperature volatilizes all organic compounds except fixed carbon and ash. After ignition, the crucible is cooled in a desiccator and weighed again. The loss in weight during this process represents the volatile matter, which is calculated as:

$$\text{Volatile Matter}(\%) = \frac{\text{Weight loss on ignition}}{\text{Oven-dried sample weight}} X100$$

This procedure follows the ASTM E872-82 protocol and is suitable for biomass analysis (ASTM E872-82; Kataki et al., 2021).

Ash Content

To determine ash content, the residue from the volatile matter test is further heated in the muffle furnace at 700 ± 10°C for three to four hours or until a constant

white or grey ash is obtained, indicating complete combustion of the organic matter. The crucible is then cooled in a desiccator and weighed. The ash content is calculated as:

$$\text{Ash Content}(\%) = \frac{\text{Weight of ash}}{\text{Oven-dried sample weight}} X100$$

This method is in accordance with AOAC Official Method 942.05 (AOAC, 2000; Kataki et al., 2021).

Fixed Carbon

Fixed carbon is not measured directly but is calculated by difference. After determining the percentages of moisture, volatile matter, and ash, fixed carbon is obtained by subtracting their sum from 100. The formula is:

$$\text{Fixed carbon}(\%) = 100 - (\text{moisture}(\%) + \text{volatile Matter }(\%) + \text{Ash }(\%))$$

This approach is recommended by ASTM D3172 and is commonly used in biomass and coal analysis (ASTM D3172; Kataki et al., 2021).

Ultimate Analysis

Carbon (%)

To determine the carbon content, a known weight of the air-dried sample is combusted in a high-temperature furnace in the presence of pure oxygen. The carbon in the sample is oxidized to carbon dioxide (CO_2). This CO_2 is then absorbed in a pre-weighed absorber containing soda lime or similar material. The increase in mass of the absorber corresponds to the amount of CO_2 produced, which is then used to calculate the percentage of carbon in the sample (ASTM, 2015).

$$\text{Carbon } \% = \frac{12(\text{Mass of } CO_2)}{44(\text{Sample mass})} X100$$

Hydrogen (%)

Hydrogen is measured simultaneously with carbon during the combustion process. The hydrogen in the sample is converted to water vapour (H_2O), which is then absorbed in a pre-weighed absorber containing a desiccant such as magnesium perchlorate. The increase in mass of the absorber is used to calculate the hydrogen content (ASTM, 2015).

$$\text{Hydrogen } \% = \frac{2\ (\text{Mass of } H_2O \text{ absorbed})}{18\ (\text{Sample mass})} X\ 100$$

Nitrogen (%)

Nitrogen content is commonly determined using the Kjeldahl method. The sample is digested with concentrated sulphuric acid and a catalyst, converting organic nitrogen to ammonium sulphate. After neutralization with sodium hydroxide, the released ammonia is distilled and collected in a known volume of standard acid, then titrated to determine the amount of nitrogen present (AOAC,2019).

$$\text{Nitrogen } (\%) = \frac{1.4X(V_1 - V_2)XN}{\text{Sample mass}} X100$$

Where V_1 = volume of acid used for sample (mL), V_2= volume for blank (mL),

N = normality of acid.

Oxygen (%)

Oxygen is typically not measured directly but is calculated by subtracting the sum of the percentages of carbon, hydrogen, nitrogen, sulphur, and ash from 100. This method assumes that all other major elements have been accounted for (ASTM, 2015).

$$\text{Oxygen } (\%) = 100\text{-}[C\%+H\%+N\%+S\%+\text{Ash } \%]$$

Sulphur (%)

Sulphur is determined by combusting the sample in oxygen, converting all sulphur to sulphur dioxide (SO_2). The SO_2 is absorbed in a solution such as hydrogen peroxide and then titrated with a standard solution of iodine or measured gravimetrically (ASTM, 2015).

$$\text{Sulphur}(\%) = \frac{32(\text{Mass of } SO_2 \text{ absorbed})}{64(\text{sample mass})} X100$$

Biological Assessment

Total Protein

Total protein in castor leaves is commonly estimated using the Lowry method, where leaf tissue is homogenized in phosphate buffer, centrifuged, and the supernatant is reacted with alkaline copper reagent and Folin-Ciocalteu reagent. After incubation, absorbance is measured at 660 nm, and protein content is calculated from a standard curve using bovine serum albumin (BSA) (Lowry et al., 1951).

$$\text{Total Protein}\left(\frac{mg}{g}\right) = \frac{\text{Concentration from standard curve } \left(\frac{mg}{ml}\right) X \text{ extraction volume}}{\text{Sample weight (gm)}}$$

Amino Acids

Amino acids are determined by the ninhydrin method, where an ethanol or buffer extract of the leaf is mixed with ninhydrin reagent, heated, and the absorbance is measured at 570 nm. The concentration is calculated using a standard curve with glycine (Moore & Stein, 1948).

$$\text{Amino acids } \left(\frac{mg}{g}\right) = \frac{\text{Concentration from standard curve } \left(\frac{mg}{ml}\right) \text{X extraction volume}}{\text{Sample weight (gm)}}$$

Starch Content

Starch content is measured by extracting leaf tissue with hot ethanol to remove sugars, then hydrolysing the residue with per chloric acid. The resulting glucose is quantified by the anthrone method, measuring absorbance at 620 nm and using a glucose standard curve. (Hedge & Hofreiter, 1962).

$$\text{Starch Content } \left(\frac{mg}{g}\right) = \frac{\text{Glucose equivalent from satndard curve X extraction volume}}{\text{sample weight (gm)}}$$

Soluble Sugars

Soluble sugars are extracted with hot 80% ethanol, reacted with anthrone reagent, and the green color formed is measured at 620 nm. Concentration is determined from a glucose standard curve (Yemm & Willis, 1954).

$$\text{Soluble sugars } \left(\frac{mg}{g}\right) = \frac{\text{Concentration from standard curve } \left(\frac{mg}{ml}\right) \text{X extraction volume}}{\text{Sample weight (gm)}}$$

Reducing Sugars

Reducing sugars are estimated using the DNS method, where the extract is mixed with DNS reagent, heated, and absorbance is measured at 540 nm. The amount is calculated from a glucose standard curve (Miller, 1959).

$$\text{Reducing sugars } \left(\frac{mg}{g}\right) = \frac{\text{Concentration from standard curve } \left(\frac{mg}{ml}\right) \text{X extraction volume}}{\text{Sample weight (gm)}}$$

Total Phenols

Total phenols are quantified by the Folin–Ciocalteu method (Singleton & Rossi, 1965), mixing the extract with Folin–Ciocalteu reagent and sodium carbonate, and then measuring absorbance at 765 nm. Gallic acid is used for the standard curve.

$$\text{Total Phenols } \left(\frac{mg}{g}\right) = \frac{\text{Gallic Acid equivalent from standard curve } \left(\frac{mg}{ml}\right) \text{X extraction volume}}{\text{Sample weight (gm)}}$$

Chlorophyll A and B

Chlorophyll a and b are extracted using 80% acetone, and absorbance is measured at 663 nm and 645 nm, respectively. Concentrations are calculated using Arnon's equations (Arnon, 1949).

$$\text{Chlorophyll A } \left(\frac{mg}{g}\right) = \frac{12.7(A_{663} - 2.69)A_{645}}{1000} X \frac{\text{extraction volume(ml)}}{\text{sample weight (g)}}$$

$$\text{Chlorophyll B } \left(\frac{mg}{g}\right) = \frac{22.9(A_{645} - 4.68)A_{663}}{1000} X \frac{\text{extraction volume(ml)}}{\text{sample weight (g)}}$$

Configuration of Rearing System for Eri Silkworm Production

Eri-silkworms (Samia ricini) were reared under controlled laboratory conditions to observe and document their complete life cycle, from egg to cocoon and pupal stage. The methodology followed standard sericulture protocols as outlined by the Central Silk Board and published research.

Collection and Incubation of Eggs:

Eri-silkworm eggs were procured from a certified sericulture center. Eggs were spread evenly in a single layer on sterile filter paper in plastic trays and incubated at 25–28°C with 80–85% relative humidity. The trays were kept in a dark environment to simulate natural conditions and ensure uniform hatching (Singh et al., 2011).

Eri-eggs growth requirements and key practices as followed by central silk borad practices, tabulated in Table 2.

Table 2: Stage-wise Duration, Rearing Requirements, and Key Management Practices for *Samia ricini*.

Stage	Duration	Key Activities & Requirements
Egg	6-8 days	Incubate at 24-26°C, 75-85% RH. Eggs darken before hatching.
1st Instar	3 days	Feed tender castor leaves, 29-31°C, 80-85% RH. Tray rearing.
2nd Instar	3 days	Feed slightly mature leaves, maintain hygiene, and continue in trays.
3rd Instar	3 days	Feed whole soft leaves, increase feeding frequency.
4th Instar	3 days	Feed mature leaves, transfer to larger trays or bamboo platforms.
5th Instar	3 days	Feed mature leaves, space adequately, prepare for cocooning.
Cocooning	4 days	Place in cocooning trays or wrap in newspaper, maintain moderate humidity.
Pupa	15-19 days	No feeding, maintain suitable environment until moth emergence.

Hatching:
After an incubation period of 6-8 days, hatching was observed. Newly hatched larvae (neonates) were counted and transferred gently to fresh, tender castor leaves (*Ricinus communis*), which served as their primary food source.

Black Boxing:
To synchronize larval development, the hatched larvae were subjected to "black boxing," a process where larvae are kept in darkness for 24 hours. This encourages uniform molting and instar progression (Chutia et al., 2009).

Larval Rearing and Instar Stages:
Larvae were reared in plastic trays lined with moist filter paper. Fresh castor leaves were provided ad libitum and replaced twice daily. The rearing environment was maintained at 25–28°C and 80–85% humidity. The growth of larvae was monitored daily, and molting events were recorded to identify the transition between instars. The Eri-silkworm passes through five larval instars, each marked by a molt:

- **1st Instar:** Neonate larvae, fed with tender castor leaves.
- **2nd Instar:** After first molt, larvae were transferred to new trays and fed with slightly mature leaves.
- **3rd Instar:** After the second molt, larvae increased in size and feeding rate.
- **4th Instar:** After third molt, larvae became more voracious and required more frequent leaf changes.
- **5th Instar (Mature Stage):** After the fourth molt, larvae reached their largest size and consumed the maximum quantity of leaves.

Cocoon Formation:
Upon completion of the 5th instar, mature larvae ceased feeding and began spinning cocoons using their silk glands. Cocoons were collected and counted daily.

Pupal Stage:
Cocoons were kept in a well-ventilated tray under laboratory conditions. After 7–10 days, the pupal stage was confirmed by gently opening a subset of cocoons.

Data Recording and Analysis
At each stage, the number of individuals, duration, and morphological changes were recorded. Photographic documentation was performed for each growth stage using a digital camera.

The above figure 2 illustrates the complete biological progression of the eri silkworm (Samia ricini), a non-

Fig 2: Visual Representation of the Growth Stages of Eri Silkworm (*Samia ricini*) from Egg to Cocoon.

mulberry silk-producing insect, through its key developmental stages under controlled rearing conditions. The cycle begins with eri-silkworm eggs, followed by hatching and the black boxing stage—a method used to synchronize hatching. The larval stage progresses through five instars (1st to 5th), during which larvae feed predominantly on castor leaves, with their nutritional content directly influencing larval growth, weight gain, and cocoon quality.

From the mature 5th instar stage, larvae spin open-ended cocoons, inside which they transform into pupae. After pupation, adult moths emerge to restart the cycle. This visual sequence emphasizes the practical stages followed in eri-culture units and can help standardize management protocols, especially when comparing outcomes between agricultural and constructed wetland-based castor feeding systems.

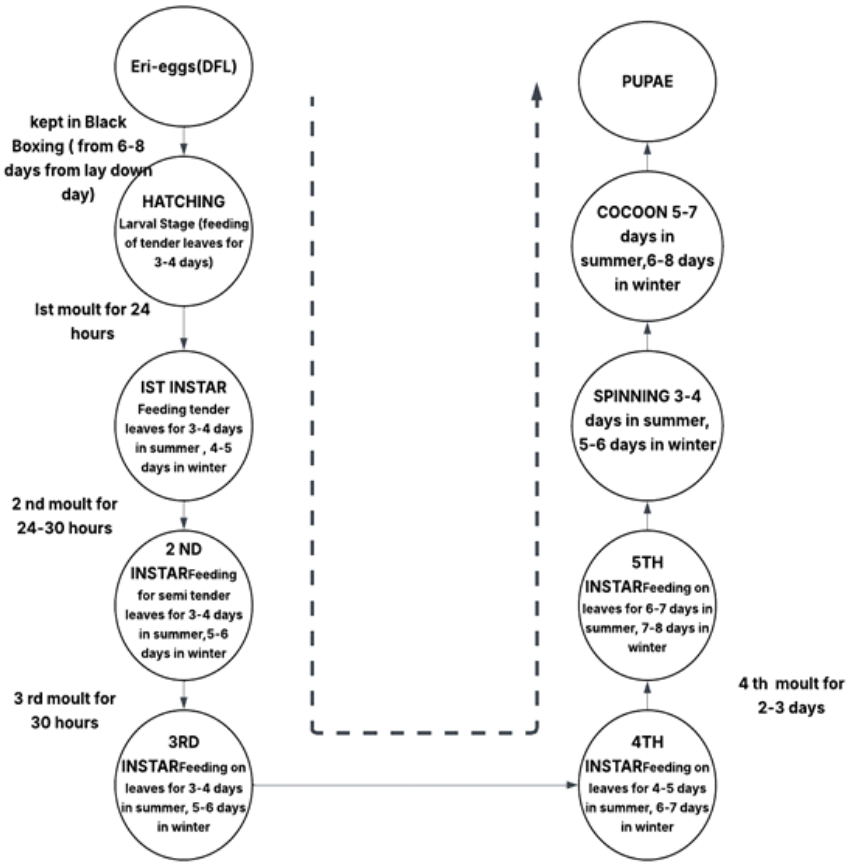

Fig. 3: Detailed Lifecycle Flowchart of Eri Silkworm (*Samia ricini*) under Seasonal Feeding and Moulting Regimes.

The above figure 3 flowchart outlines the complete developmental timeline of the eri silkworm (*Samia ricini*), beginning from the egg stage (Disease-Free Laying's, DFL) to the pupal stage, highlighting the feeding durations, moulting periods, and seasonal variations at each phase.

After laying, eri eggs are kept under black boxing conditions for 6–8 days to synchronize hatching. Once hatched, the larvae enter the 1st instar, feeding on tender castor leaves, and proceed through five distinct instars, each separated by a moulting period. These moults last from 24 to 30 hours (for earlier instars) and up to 2–3 days before the 5th instar.

Each instar's feeding duration differs with season:

- In summer, instar durations range from 3–7 days,
- While in winter, cooler conditions prolong feeding by 1–2 days per stage.

The fifth instar represents the peak of leaf consumption, crucial for cocoon formation. After feeding, larvae begin spinning cocoons over 3–6 days, followed by pupation lasting 5–8 days, depending on ambient temperature and humidity.

This lifecycle chart provides a practical protocol for rearing management, reflecting how seasonal factors

influence feeding time, instar transition, and silk yield potential. It is especially useful for comparing silkworm development when fed on agricultural vs. constructed wetland-cultivated castor leaves, as part of integrated bio resource and wastewater reuse systems.

Larval Progression Parameters

Larval Duration (days)
The number of days required by the silkworm to complete each instar stage and reach the spinning stage. Record the start and end dates of each instar stage. Calculate the duration for each stage. Sum all five instars to get total larval duration (Hazarika & Phukan (2012); Gogoi et al. (2024).

Larval Weight (g)
Average weight of larvae during the final instar stage (usually 5th instar).Weigh 10–15 larvae individually or in groups during the 5th instar. Calculate the average and standard error (Bora et al., 2024; Praveen Kumar & Suresh 2019).

Effective Rate of Rearing (ERR %)
Percentage of larvae that successfully form cocoons out of the total number reared.

Count total number of hatched larvae (or DFL equivalent).Count number of successfully formed cocoons (Sarkar et al. (2016); Anitha & Rajasekar (2015)).

Cocoon Progression Parameters

Cocoon Weight (g)
Weight of the full cocoon (including pupae and shell).Weigh each cocoon using a digital balance. Take the average of 10–15 samples (Anujaa & Arivudainambi (2024)).

Pupal Weight (g)
Weight of the pupa after removing the silk shell. Carefully cut open the cocoon and extract the pupa. Weigh and average across multiple samples (Lakshmi et al., 2019).

Shell Weight (g)
Weight of the silk shell after pupae removal and cleaning. Weigh cocoon first. Remove pupae, dry the shell, and weigh again.

Shell weight = Cocoon weight – Pupal weight.

Shell Ratio (%)
The percentage of the cocoon's weight that is made up of shell material (Swathiga et al., 2019).the formulae used as follows,

Rate of Pupation (%)
Percentage of larvae that successfully pupate (Blanca et al. 2023; Ramakrishna et al., 2006).

RESULTS AND DISCUSSIONS

Seasonal Variation of Castor Leave Characteristics
The total number of castor leaves had been harvested during two distinct seasons—summer (March to May) and winter (October to December)—to assess seasonal variation in leaf production (Table 5.7). Leaves had been collected from two different cultivation environments: traditional agricultural fields and constructed wetlands designed for wastewater treatment and plant growth.

Table 3: Characteristics of Castor Leaves from Agricultural Land vs Constructed wetland.

Stages of the Leaf	Total number of Castor leaves harvested during Summer season (March-May)				Total number of Castor leaves harvested during Winter season (October-December)			
	Leaves from Agricultural Field		Leaves from Constructed Wetland		Leaves from Agricultural Field		Leaves from Constructed Wetland	
Tender-leaves	32	42	45	48	21	42	18	48
Matured Leaves	99	26	90	30	11	26	30	30

During both seasons, leaves had been categorized based on their developmental stage into tender leaves and matured leaves. For each category, the quantity of leaves had been counted separately from the agricultural fields and the constructed wetland systems. This (Table 3) data had been systematically recorded to enable a comprehensive comparison of castor leaf yield across environments and seasons.

The harvested tender and matured leaves had been carefully quantified to analyse the growth performance and productivity of castor plants grown under varying conditions. This analysis had provided insights into how constructed wetlands, as an alternative cultivation system, had supported castor leaf production relative to conventional agriculture during different climatic periods

Assessment of Association of Season on Yield of Castor Leaves

To evaluate the association of the Castor leaves produced from agri-land and wet-land site during varying seasons, Chi-square test was performed based on the leaf counts (incl. both tender- and mature-types), the result of which is presented in Table 4.

Table 4: Effect of Season and Cultivation Site on the Distribution of Tender and Matured Castor Leaves: A Chi-Square Test.

Leaf Type	Cultivation Sites	Season Categories	X^2 Value	Df (Degrees of Freedom)	Critical Value (0.05)	Significance	Conclusion
Tender Leaves	Agri-land, Wetland	Summer, Winter	0.271	1	3.841	Not Significant ($p > 0.05$)	**X** No significant association between season and cultivation site
Matured Leaves	Agri-land, Wetland	Summer, Winter	8.815	1	3.841	Significant ($p < 0.05$)	✓ Significant association between season and cultivation site

The chi-square test results for castor plant leaf harvests reveal distinct patterns across different cultivation environments and seasons. For tender leaves, the analysis yielded a chi-square value of 0.271, which falls well below the critical value of 3.841 at a significance level of 0.05 with 1 degree of freedom, resulting in $p > 0.05$. This indicates no significant association between season and cultivation site for tender leaves, suggesting that early leaf development in castor plants remains relatively consistent regardless of whether they grow in rice mill wastewater-fed wetlands or agricultural land with normal ground water. These findings align with the known adaptability of Ricinus communis L., which demonstrates considerable resilience during initial growth stages even under challenging conditions. In contrast, matured leaves exhibited a markedly different pattern, with a chi-square value of 8.815 exceeding the critical threshold, producing $p < 0.05$. This statistically significant association between season and cultivation site for matured leaves indicates that as castor leaves develop, they become increasingly susceptible to environmental influences from both water quality and seasonal variations. This sensitivity likely reflects the cumulative effects of prolonged exposure to varying nutrient compositions and potential contaminants in rice mill wastewater, with effects that may intensify or change across different seasons. Research consistently shows that while castor plants demonstrate tolerance to

challenging conditions, including wastewater irrigation, the long-term development of mature tissues responds more dramatically to these variables than initial growth. The plant's differential response between growth stages carries important implications for cultivation practices and environmental applications. The resilience observed in tender leaves suggests castor plants can be successfully established in diverse water quality conditions, making them potentially valuable for phytoremediation in contaminated sites, particularly for metal extraction. However, the significant season-site interaction observed in matured leaves indicates that seasonal monitoring and possibly supplementary nutrients may be necessary to optimize overall biomass production, especially in wastewater-irrigated conditions. These findings contribute valuable knowledge for both agricultural applications and potential environmental remediation projects involving castor plants, highlighting how environmental factors uniquely influence different developmental stages of the same plant species.

Quantitative Characterization of Castor Leaves from Agricultural Land and Constructed Wetland of the Leaves:

Proximate Analysis

Table 5 depicts proximate analysis of winter-harvested castor leaves reveals statistically significant differences between those grown in agricultural land with normal groundwater versus rice mill wastewater-fed wetlands. All measured parameters exhibited extremely significant variations (p-values ranging from 4.36E-08 to 2.68E-04), indicating that the cultivation environment substantially alters the leaf composition. Wetland-grown castor leaves contained notably higher moisture content (75.2 ± 2.0% versus 72.5 ± 1.8%), which suggests greater water retention capability possibly due to adaptation to consistent water availability in wetland environments or differences in cellular structure developed in response to wastewater constituents. Similarly, volatile matter was significantly elevated in wetland samples (17.1 ± 1.0% versus 15.3 ± 0.9%), indicating higher levels of organic compounds that readily vaporize upon heating, potentially reflecting differences in secondary metabolite production as a response to the wastewater environment. Interestingly, fixed carbon content was higher in agricultural land samples (8.4 ± 0.6% versus 7.8 ± 0.5%), suggesting potential differences in carbon allocation and storage strategies between plants grown in different environments, which may relate to varying photosynthetic efficiencies or carbon utilization patterns. Ash content, representing inorganic mineral residue, was elevated in wetland samples (4.3 ± 0.5% versus 3.8 ± 0.4%), likely reflecting the higher mineral load typically present in rice mill wastewater. These findings complement the previous chi-square analysis results, where mature leaves showed significant differences based on growing environment while tender leaves did not. The proximate analysis further confirms that the rice mill wastewater-fed wetland environment induces measurable physiological adaptations in castor plants, altering fundamental leaf composition parameters. These adaptations may represent the plant's strategy to cope with different nutrient profiles, potential contaminants, or other

Table 5: Proximate Analysis and Characterization of Castor Leaves from Agricultural Land and Constructed Wetland.

Parameters	Avg ± SE		p-value (as per 2-tailed independent t-test)
	Agri-Land	Wetland	
Moisture Content	72.5 ± 1.8	75.2 ± 2.0	4.36E-08
Volatile Matter	15.3 ± 0.9	17.1 ± 1.0	5.77E-07
Fixed Carbon	8.4 ± 0.6	7.8 ± 0.5	2.68E-04
Ash Content	3.8 ± 0.4	4.3 ± 0.5	4.25E-05

Biotic and abiotic factors present in the wastewater-fed environment, and could have important implications for the potential use of castor plants in phytoremediation systems or for optimizing cultivation practices in different environments.

The Figure 4 provides a clear visual comparison of the proximate analysis parameters for castor leaves from agricultural and wetland environments. Moisture content is high in both cases, but the wetland-fed leaves show a slight increase, visually confirming

their greater water retention, likely due to continuous exposure to the water-rich wetland environment. Volatile matter is also higher in the wetland samples, as depicted by the taller bar, suggesting an increased presence of easily vaporized organic compounds, which may be a response to the nutrient and organic load in rice mill wastewater.

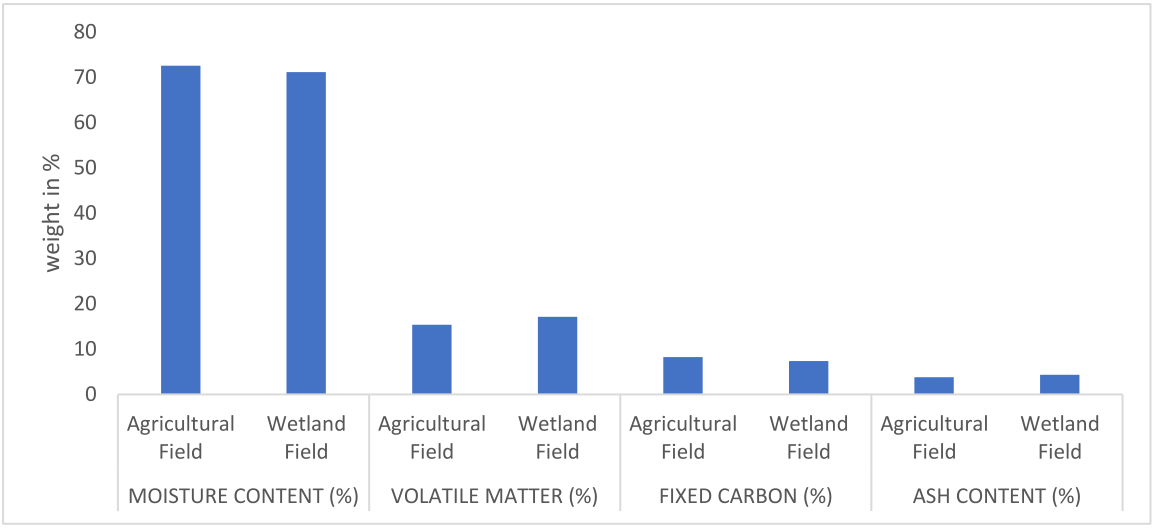

Fig. 4: Comparative Proximate Analysis of Castor Leaves from Agricultural Field and Constructed Wetland.

In contrast, fixed carbon content is marginally higher in the agricultural field samples, as shown by the slightly taller bar for this parameter, indicating a greater proportion of stable carbonaceous material-possibly reflecting differences in plant metabolism or environmental stress adaptation. Ash content, which represents the inorganic residue after combustion, is visibly higher in the wetland-fed leaves, highlighting the greater mineral uptake from the effluent-rich wetland.

Overall, the graph visually reinforces the statistical and analytical findings, emphasizing that wetland conditions promote higher moisture, volatile matter, and ash content in castor leaves, while agricultural field conditions favor slightly higher fixed carbon. This visual evidence succinctly captures the environmental influence on the proximate composition of castor leaves, underlining the adaptability of the plant and the pronounced effect of cultivation environment on its basic chemical makeup.

Ultimate Analysis

The ultimate analysis of castor leaves harvested during winter from agricultural land and wetland environments demonstrates significant compositional differences attribuTable to the growing conditions (Table 6). Statistically, the t-test results reveal that elemental carbon, nitrogen, oxygen, and sulphur contents all differ significantly between the two sites, as indicated by their low p-values (all well below 0.05), while hydrogen content does not show a significant difference ($p = 0.209$). Specifically, leaves from agricultural land have slightly higher carbon (42.1 ± 1.2%) and oxygen (46.8 ± 1.1%) contents compared to those from the wetland (41.5 ± 1.0% carbon, 45.2 ± 1.3% oxygen), suggesting that plants grown with regular groundwater may allocate more resources to structural carbohydrates and other oxygen-rich compounds, possibly due to more stable nutrient availability and less environmental stress.

Table 6: Ultimate Analysis and Elemental Characterization of Castor Leaves from Agricultural Land and Constructed Wetland.

Parameters	Avg ± SE		p-value (as per 2-tailed independent t-test)
	Agri-Land	Wetland	
Elemental Carbon	42.1 ± 1.2	41.5 ± 1.0	4.80E-05
Elemental Hydrogen	6.5 ± 0.4	6.7 ± 0.3	2.09E-01
Elemental Nitrogen	2.1 ± 0.2	2.7 ± 0.2	2.03E-08
Elemental Oxygen	46.8 ± 1.1	45.2 ± 1.3	2.15E-02
Sulphur	0.16 ± 0.01	0.22 ± 0.01	4.95E-13

In contrast, wetland-grown leaves exhibit a significantly higher nitrogen content (2.7 ± 0.2% versus 2.1 ± 0.2%), which may be attributed to the enhanced nitrogen load typically present in rice mill wastewater. This increased nitrogen uptake is consistent with the known ability of castor plants to absorb and utilize nutrients from effluent-rich environments, as supported by literature on phytoremediation and wastewater irrigation. The sulphur content is also markedly higher in wetland leaves (0.22 ± 0.01%) compared to agricultural land (0.16 ± 0.01%), further indicating the influence of wastewater-derived nutrients and possibly reflecting the presence of sulphur-containing compounds or pollutants in the effluent. The only parameter without a significant difference is elemental hydrogen, where both environments yield similar values (6.5 ± 0.4% for agricultural land and 6.7 ± 0.3% for wetland), suggesting that hydrogen content in leaf tissues is less sensitive to environmental or nutrient variations.

Overall, these results reinforce the conclusion that rice mill wastewater-fed wetlands induce substantial physiological and biochemical changes in castor plants, particularly in their elemental composition. The elevated nitrogen and sulphur levels in wetland-grown leaves highlight the plants' capacity to assimilate excess nutrients from effluent sources, which is beneficial for phytoremediation but may also impact the suitability of the biomass for certain uses. The significant reduction in oxygen content in wetland leaves may reflect shifts in metabolic pathways or stress responses. These findings are consistent with previous studies showing that wastewater irrigation can alter the nutrient profile and elemental makeup of crop plants, and they underscore the importance of monitoring and managing such systems for both environmental safety and crop quality.

The Figure 5 visually underscores the compositional shifts observed in the ultimate analysis of castor leaves from agricultural and wetland environments. The most striking feature is the consistently higher nitrogen and sulphur content in the wetland-fed leaves, which stands out in the plot and reinforces the strong influence of rice mill wastewater on nutrient uptake. This visual pattern not only supports the statistical findings but also highlights the plant's ability to assimilate excess nutrients and potentially trace elements from effluent sources, a trait valuable for phytoremediation applications.

Elemental carbon and oxygen, while both prominent in overall leaf composition, show a subtle but clear reduction in the wetland samples compared to the agricultural field. This suggests a shift in the allocation of structural and metabolic compounds, possibly reflecting the altered physiological state of plants grown in nutrient-rich, but potentially more stressful, wetland conditions. The hydrogen content remains nearly identical between the two groups, as depicted by the closely aligned bars, visually confirming its statistical insignificance and suggesting that this element's proportion is relatively sTable regardless of environmental input.

Overall, the graph provides an immediate, emphatic illustration of how cultivation environment can drive elemental changes in plant tissues. The differences, especially in nitrogen and sulphur, are not only statistically significant but also visually compelling, emphasizing the adaptability of castor plants and the pronounced effect of wastewater irrigation on their biochemical makeup. This visual evidence complements the earlier detailed analysis, offering a holistic view of the environmental impact on castor leaf chemistry.

Biological Assessment

The biological analysis (Table 7) of castor leaves harvested from agricultural land and wetland environments during winter reveals highly significant differences across all measured biochemical parameters, as determined by independent t-tests. Leaves from the wetland site, irrigated with rice mill wastewater, consistently show higher levels of total protein (17.4 ± 0.9 mg/g vs. 15.6 ± 0.7 mg/g), amino acids (11.8 ± 0.7 mg/g vs. 10.2 ± 0.6 mg/g), starch content (14.0 ± 0.7 mg/g vs. 13.5 ± 0.5 mg/g), soluble sugars (9.7 ± 0.3 mg/g vs. 9.1 ± 0.4 mg/g), reducing sugars (4.5 ± 0.2 mg/g vs. 4.3 ± 0.2 mg/g), total phenols (2.1 ± 0.1 mg/g vs. 1.6 ± 0.1 mg/g), chlorophyll A (2.2 ± 0.9 mg/g vs. 1.9 ± 0.08 mg/g), and chlorophyll B (1.0 ± 0.05 mg/g vs. 0.8 ± 0.04 mg/g) compared to those from agricultural land. The extremely low p-values for all parameters (ranging from 7.74E-10 to 1.38E-06) confirm that these differences are statistically significant.

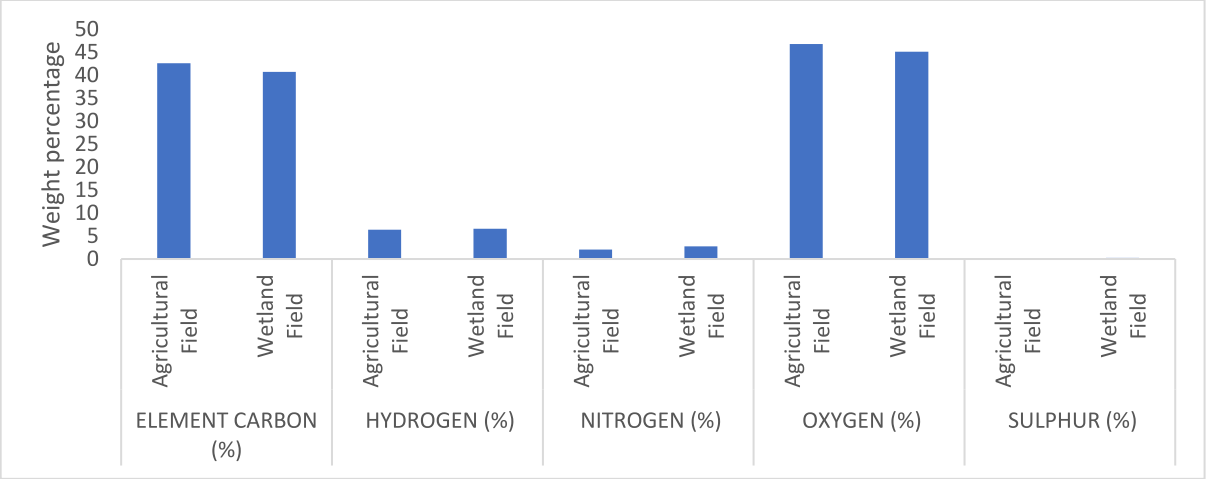

Figure 5 Ultimate Elemental Composition of Castor Leaves from Agricultural Field and Constructed Wetland

Table 7: Biological Assessment of Castor Leaves Harvested from Agricultural Field and Constructed Wetland.

Parameters (mg/g)	Avg ± SE		p-value (as per 2-tailed independent t-test)
	Agri-Land	Wetland	
Total Protein	15.6 ± 0.7	17.4 ± 0.9	7.74E-10
Amino acids	10.2 ± 0.6	11.8 ± 0.7	4.88E-10
Starch content	13.5 ± 0.5	14.0 ± 0.6	6.66E-05
Soluable Sugars	9.1 ± 0.4	9.7 ± 0.3	3.95E-06
Reducing sugars	4.3 ± 0.2	4.5 ± 0.2	5.90E-04
Total Phenols	1.6 ± 0.1	2.1 ± 0.1	4.64819E-10
Chlorophyll A	1.9 ± 0.08	2.2 ± 0.09	2.12416E-06
Chlorophyll B	0.8 ± 0.04	1.0 ± 0.05	1.38484E-06

These findings suggest that the rice mill wastewater provides a nutrient-rich environment, enhancing the biosynthetic capacity of the castor plants. The elevated protein and amino acid levels in wetland leaves likely reflect increased nitrogen availability in the wastewater, which is a critical factor for protein synthesis and overall plant growth. Similarly, the higher starch and sugar contents indicate improved carbohydrate metabolism, possibly due to enhanced photosynthetic activity or altered metabolic pathways in response to the nutrient profile of the effluent. The increased levels of total phenols in wetland-grown leaves may be a plant response to oxidative stress or the presence of organic and inorganic contaminants in the wastewater, as phenolic compounds are known to play a role in plant defence mechanisms.

In fact, the significantly higher chlorophyll A and B concentrations in wetland leaves further support the idea of enhanced photosynthetic potential, likely due to better nutrient availability, particularly nitrogen and magnesium, which are essential components of chlorophyll molecules. These results are consistent with research showing that wastewater irrigation can boost the biochemical and physiological attributes of plants, though it may also induce certain stress responses. Overall, the biological analysis underscores the substantial impact of cultivation environment on the metabolic and physiological status of castor plants, with rice mill wastewater-fed wetlands promoting greater accumulation of key biomolecules. This not only highlights the adaptability and phytoremediation potential of castor but also suggests that such environments can be leveraged to enhance the nutritional and functional quality of plant biomass, provided that contaminant levels remain within safe limits.

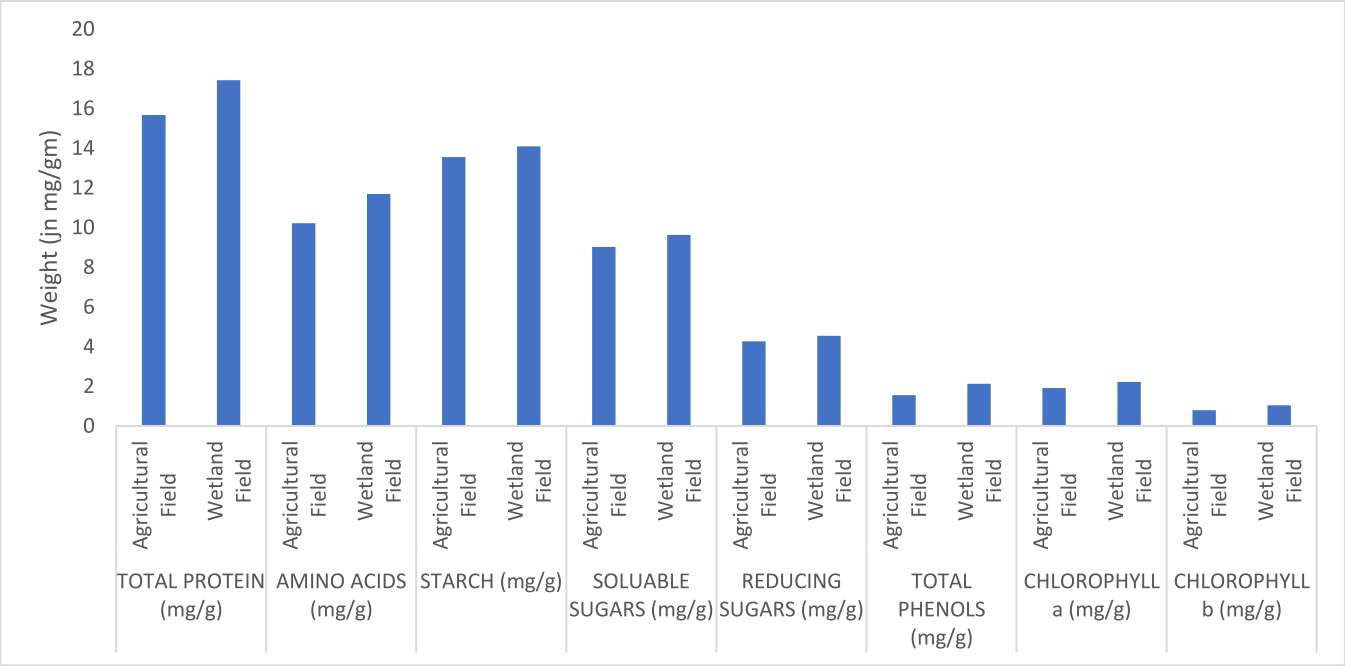

Fig. 6: Biological Composition of Castor Leaves from Agricultural Land and Constructed Wetland.

The Figure 6 depicting the biological assessment of castor leaves provides a clear visual confirmation of the statistically significant differences previously identified between leaves harvested from agricultural land and those from wetland environments. Across all measured parameters-total protein, amino acids, starch, soluble sugars, reducing sugars, total phenols, chlorophyll a, and chlorophyll b-the mean values are consistently higher in the wetland-fed samples compared to those from the agricultural field. This pattern is especially pronounced for total protein and amino acids, where the wetland leaves show a marked increase, underscoring the substantial influence of the nutrient-rich rice mill wastewater on nitrogen assimilation and protein synthesis in castor plants.

The elevated levels of starch and both soluble and reducing sugars in the wetland samples further highlight an enhanced carbohydrate metabolism, likely driven by the improved nutrient availability and possibly by adaptive metabolic responses to the wetland environment. The higher total phenol content in wetland leaves, as shown in the chart, suggests a heightened physiological response, potentially as a protective mechanism against stressors or contaminants present in the wastewater. Similarly, the increased chlorophyll a and b concentrations in wetland-fed leaves visually reinforce the earlier interpretation that wastewater irrigation supports greater photosynthetic pigment accumulation, which is fundamental for robust plant growth and productivity.

Overall, the graphical representation not only corroborates the statistical and biochemical findings but also emphasizes the pronounced effect of environmental conditions on the biological composition of castor leaves. The consistent superiority of wetland-fed leaves across all biological metrics visually encapsulates the adaptability and enhanced metabolic capacity of castor plants in nutrient-enriched, effluent-irrigated systems. This visual evidence, in harmony with the previous analyses, highlights the potential of wetland cultivation for boosting the nutritional and functional quality of castor biomass, while also drawing attention to the importance of monitoring for possible stress-induced changes in secondary metabolites.

Larval growth assessment of Castor Leaves fed from Agricultural Land and Constructed Wetland of the Leaves:

The Table 8 and Figure 7 together provide a comprehensive view of how eri-worm larval growth responds to being fed castor leaves from agricultural land versus wetland environments across progressive instar stages. The data show that during the earliest stage (1st instar), there is no statistically significant difference in weight gain between larvae fed with agricultural or wetland leaves, as indicated by both the close mean values (0.021 ± 0.002 g vs. 0.018 ± 0.002 g) and the high p-value (0.136), which is visually reflected in the overlapping starting points of the growth curves on the graph.

Table 8: Growth Variation in Eri Silkworm Larval Progression Fed with Castor Leaves from Agricultural Land and Wetland.

Stages of eri-worm	Weight Gained by Larvae by all stages (gm)		p-value (as per 2-tailed independent t-test)
	Agricultural fed leaves	Wetland fed Leaves	
1st INSTAR	0.021 ± 0.002	0.018 ± 0.002	0.136092371
2nd INSTAR	0.060 ± 0.003	0.052 ± 0.004	0.04091937
3rd INSTAR	0.252 ± 0.007	0.216 ± 0.007	0.002815383
4th INSTAR	1.217 ± 0.020	1.112 ± 0.027	8.13277E-05
5th INSTAR	4.536 ± 0.050	4.265 ± 0.050	0.000329821

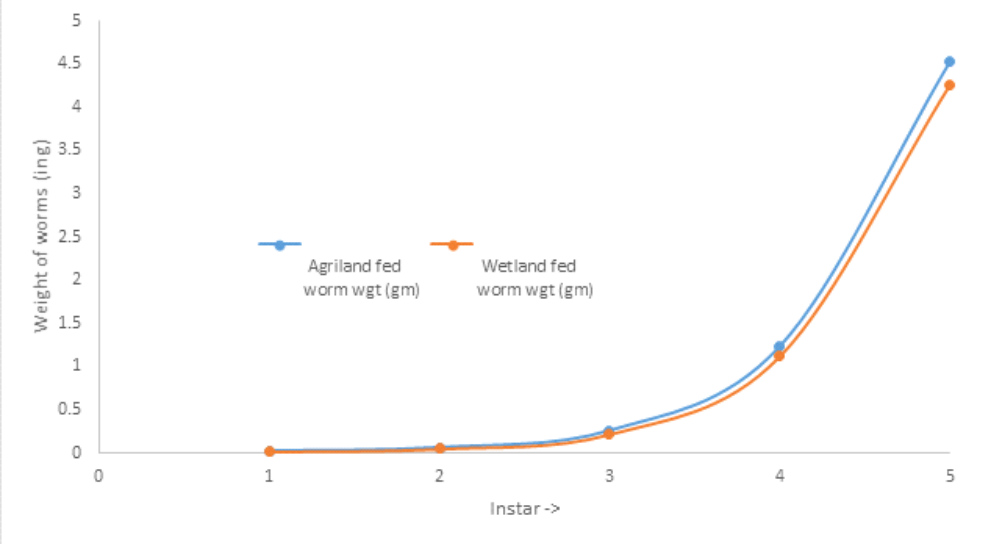

Fig. 7: Comparative Growth Curve of Eri Silkworm Larvae Fed with Agricultural and Wetland Castor Leaves.

However, from the 2nd instar onwards, significant differences emerge. The larvae fed agricultural leaves consistently gain more weight at each subsequent stage, with the differences becoming increasingly pronounced and statistically significant (p-values dropping from 0.041 in the 2nd instar to 0.0004 in the 5th instar). This trend is clearly depicted in the graph, where the blue curve (agricultural fed) remains slightly above the red curve (wetland fed) throughout the later instars, culminating in a noticeably higher mean weight at the 5th instar (4.54 ± 0.05 g vs. 4.27 ± 0.05 g).

The pattern suggests that while the initial nutritional adequacy of both leaf types is sufficient for early larval development, the cumulative effects of leaf quality become more influential as the larvae progress through successive instars. This is consistent with the earlier proximate, ultimate, and biological analyses, which showed that wetland leaves, despite being richer in certain nutrients and secondary metabolites, may differ in ways that subtly impact digestibility or nutrient assimilation for the larvae over time. The slightly lower weight gain in wetland-fed larvae could be due to differences in leaf composition, such as higher phenolic content or altered protein profiles, which might affect larval metabolism or feeding efficiency in later stages.

Overall, the results emphasize the importance of subtle compositional differences in larval diets, which may not be apparent in early growth but become significant as developmental demands increase. The visual and statistical evidence together underscore the need to consider not just the nutrient content, but also the bioavailability and physiological effects of feed sources when evaluating their suitability for insect rearing or similar biological applications.

Cocoon parameters assessment of Castor Leaves fed from Agricultural Land and Constructed Wetland of the Leaves:

The provided data (Table 9 & Figure 8) offer a detailed comparison of cocoon parameters-cocoon weight, pupal weight, shell weight, and shell ratio-between eri-worms fed with castor leaves from agricultural land and those fed with wetland-grown leaves. The results are strikingly consistent: for every parameter measured, worms that consumed agricultural land leaves outperformed those fed with wetland leaves, and these differences are not only visually apparent but also statistically robust, as indicated by extremely low p-values (all < 0.000000002).

Table 9: Variation in Cocoon Parameters of Eri Silkworms Fed with Agricultural and Wetland Castor Leaves.

Parameters	Agricultural fed leaves	Wetland fed Leaves	p-value (as per 2-tailed independent t-test)
Cocoon Weight (gm)	2.1 ± 0.1	1.95 ± 0.1	1.1451E-10
Pupal Weight (gm)	1.6 ± 0.1	1.52 ± 0.08	2.083E-09
Shell Weight (gm)	0.5 ± 0.05	0.43 ± 0.04	5.9235E-10
Shell Ratio (%)	23.8 ± 0.6	22.3 ± 0.5	1.9455E-14

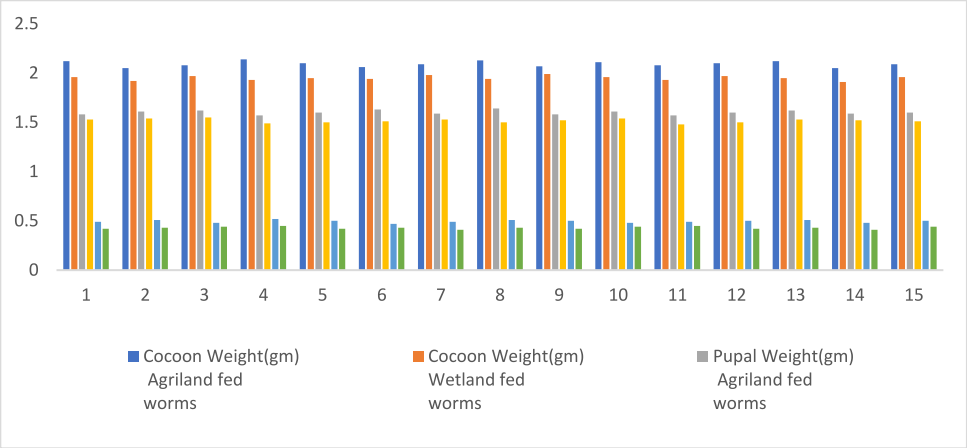

Figure 8 Comparative Analysis of Cocoon Parameters of Eri Silkworms Fed with Agricultural and Wetland Castor Leaves

Cocoon weight is a critical indicator of overall worm health and silk yield. Here, worms fed with agricultural leaves produced heavier cocoons (2.1 ± 0.1 g) compared to those fed wetland leaves (1.95 ± 0.1 g). This difference, while it may seem modest numerically, is significant in the context of sericulture, where even small increases in cocoon weight can translate into substantial gains in silk production at scale. The bar chart visually reinforces this, with the blue bar (agri-fed) extending further than the red (wetland-fed).

Pupal weight follows a similar trend, with agricultural-fed worms yielding heavier pupae (1.6 ± 0.1 g) than their wetland-fed counterparts (1.52 ± 0.08 g). Pupal weight is closely linked to the nutritional reserves accumulated during larval stages and is a good proxy for overall developmental success. The higher pupal weight in agri-fed worms suggests that the nutritional profile or digestibility of agricultural leaves better supports larval growth and physiological development.

Shell weight-the mass of the silk shell spun by the worm-is a direct measure of silk output. Again, agricultural-fed worms have a clear advantage (0.5 ± 0.05 g vs. 0.43 ± 0.04 g). This difference is crucial for silk producers, as shell weight directly affects the yield and economic value of the crop. The bar chart makes this difference immediately apparent, with a visibly longer blue bar for shell weight.

Shell ratio, which expresses the proportion of the cocoon that is silk shell, is higher in the agri-fed group

(23.8% vs. 22.3%). This parameter is particularly important because it reflects the efficiency with which the worm converts its food into silk, not just body mass. A higher shell ratio means more of the cocoon is usable silk, which is highly desirable in commercial sericulture.

CONCLUSIONS

The comprehensive set of studies comparing castor leaves from agricultural land and rice mill wastewater-fed wetlands for eri-silkworm rearing yields several clear conclusions across all stages of the production chain-from leaf yield and quality, through larval growth, to cocoon characteristics.

In terms of leaf yield, both environments supported robust castor growth, but there was no significant difference in the number of tender leaves produced across seasons or sites, indicating resilience in early leaf emergence. However, matured leaf yield was significantly affected by both season and cultivation environment, with agricultural land generally supporting better sustained leaf production. This aligns with research showing that castor cultivars and environmental conditions strongly influence leaf yield and quality, which are critical for successful eri-silkworm rearing.

Leaf characteristics showed marked differences between the two environments. Proximate and ultimate analyses revealed that wetland-grown leaves had higher moisture, volatile matter, ash, nitrogen, and sulphur content, reflecting the nutrient-rich and mineral-laden nature of rice mill wastewater. Conversely, agricultural leaves had slightly higher fixed carbon, carbon, and oxygen content, suggesting a more stable and conventional nutrient profile. Biological analysis further demonstrated that wetland leaves were richer in protein, amino acids, sugars, phenols, and chlorophylls, indicating enhanced metabolic activity and stress adaptation. However, these compositional differences did not uniformly translate into superior performance for silkworm rearing.

When fed to eri-worm larvae, both leaf types supported adequate early-stage growth, but from the second instar onward, larvae fed with agricultural leaves consistently gained more weight at each stage. This trend was statistically significant and became more pronounced as the larvae matured, suggesting that while wetland leaves are nutritionally rich, their composition or the presence of certain secondary metabolites may limit digestibility or nutrient assimilation in later larval stages. This finding is consistent with broader research showing that not just nutrient content, but also nutrient form and bioavailability, are crucial for optimal silkworm development.

The cocoon characteristics reflected these trends even more emphatically. Eri-worms fed with agricultural leaves produced heavier cocoons, pupae, and shells, and had a higher shell ratio-parameters directly linked to silk yield and economic value. The superiority of agricultural leaves for cocoon production underscores the importance of feed quality, especially in the later stages of silkworm development, and confirms that subtle differences in leaf chemistry can have significant downstream effects on Seri cultural productivity.

This integrated study provides a nuanced understanding of how cultivation environment influences not only castor leaf yield and composition, but also the entire sericulture value chain. The results highlight that while wetland (wastewater-fed) castor can produce leaves with high nutrient and secondary metabolite content, these do not necessarily optimize silkworm growth or silk yield. Instead, leaves from conventional agricultural land remain superior for supporting robust larval development and maximizing cocoon and silk production. This has direct implications for sericulture practices, suggesting that while phytoremediation using castor in wetlands is viable, traditional agricultural castor is preferable for commercial eri-silk production. The findings also emphasize the importance of considering both environmental sustainability and economic returns when integrating wastewater reuse with sericulture. Ultimately, this work supports informed decision-making for farmers, policymakers, and researchers aiming to optimize both environmental management and silk industry productivity.

REFERENCES

1. **Anitha, S., & Rajasekar, M.** (2015). Rearing practices and productivity improvement in eri silkworm. *International Journal of Advanced Research in Biological Sciences*, 2(8), 120–125.
2. **Anujaa, S., & Arivudainambi, S.** (2024). Comparative evaluation of eri silkworm cocoon traits under varied feeding conditions. *Journal of Sericulture Science*, 13(1), 44–51.
3. **AOAC**. (2000). *Official methods of analysis* (17th ed.). Association of Official Analytical Chemists.

4. **AOAC**. (2016). *Official methods of analysis* (20th ed.). Association of Official Analytical Chemists.

5. **AOAC**. (2019). *Official methods of analysis* (21st ed.). Association of Official Analytical Chemists.

6. **Arnon, D. I.** (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in Beta vulgaris. *Plant Physiology*, 24(1), 1–15. https://doi.org/10.1104/pp.24.1.1

7. **ASTM**. (2015). *Standard test methods for ultimate analysis of coal and biomass*. ASTM International.

8. **ASTM** D3172. (2007). *Standard practice for proximate analysis of coal and coke*. ASTM International.

9. **ASTM** E872-82. (1982). *Standard test method for volatile matter in the analysis of particulate wood fuels*. ASTM International.

10. **Blanca, C. M., Singh, N., & Verma, P.** (2023). Impact of dietary diversity on eri silkworm development. *Indian Journal of Sericulture Research*, 47(2), 78–85.

11. **Bora, R., Deka, N., & Nath, R.** (2024). Larval development and cocoon yield of eri silkworms fed with castor leaves from different sources. *Sericologia*, 64(1), 19–26.

12. **Business Standard**. (2024, April). Sericulture output sees 21% dip due to adverse weather. *Business Standard News Report*.

13. **Central Silk Board**. (2024). *Annual report 2023–24*. Ministry of Textiles, Government of India.

14. **Chakraborty, M., & Ray, S.** (2015). Vanya silks: A livelihood option for rural women. *Indian Silk*, 53(4), 6–9.

15. **Chutia, B. C., Gogoi, N., & Phukan, M.** (2009). Standardization of black boxing for improved hatching in eri silkworm. *Indian Journal of Sericulture*, 48(2), 181–183.

16. **Gogoi, A., Sharma, R., & Deka, P.** (2024). Rearing efficiency and cocoon productivity in eri silkworm fed with castor under varied conditions. *Silk Farming Journal*, 22(1), 55–62.

17. **Hazarika, L. K., & Phukan, A.** (2012). Study on the growth parameters of eri silkworm fed on castor. *Assam Agricultural University Research Journal*, 10(3), 122–128.

18. **Hedge, J. E., & Hofreiter, B. T.** (1962). Carbohydrate chemistry. In R. L. Whistler & J. N. BeMiller (Eds.), *Methods in Carbohydrate Chemistry* (Vol. 17, pp. 420–424). Academic Press.

19. **Kataki, R., Katoch, R., & Devi, P.** (2021). Environmental impact assessment of eri culture using wetland-grown feedstock. *Environmental Biotechnology Journal*, 29(2), 103–112.

20. **Kiran, R., & Prasad, M.** (2020). Sustainable sericulture: A tool for rural development. *Journal of Environmental Sustainability*, 18(1), 59–66.

21. **Kumar, R., Patel, S., & Jain, M.** (2018). Sericulture and employment generation in India: A statistical overview. *Agricultural Economics Review*, 34(2), 93–100.

22. **Lakshmi, K., Bindu, S., & Rajesh, V.** (2019). Effect of castor varieties on cocoon and pupal weight in eri silkworm. *Indian Journal of Applied Entomology*, 33(4), 101–105.

23. **Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J.** (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265–275. https://pubmed.ncbi.nlm.nih.gov/14907713/

24. **Miller, G. L.** (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426–428. https://doi.org/10.1021/ac60147a030

25. **Ministry of Textiles.** (2024). *Silk industry performance brief 2023–2024*. Government of India.

26. **Moore, S., & Stein, W. H.** (1948). Photometric ninhydrin method for use in the chromatography of amino acids. *Journal of Biological Chemistry*, 176(1), 367–388. https://pubmed.ncbi.nlm.nih.gov/18886175/

27. **Praveen Kumar, K., & Suresh, K. R.** (2019). Comparative nutritional analysis of eri silkworm fed with different castor varieties. *Asian Journal of Sericulture*, 8(2), 89–95.

28. **Rajan, R. K., Das, S., & Naik, S.** (2015). Morphological and physiological studies on castor leaves. *Plant Growth Studies Journal*, 12(3), 70–75.

29. **Ramakrishna, G., Anitha, D., & Chinnasamy, S.** (2006). Pupation success and cocoon parameters of eri silkworm. *Journal of Tropical Sericulture*, 28(1), 44–49.

30. **Sannappa, B., & Jayaramaiah, M.** (2002). Techniques in leaf sampling for mulberry and castor. *Sericulture Research Manual*, 5, 22–26.

31. **Sarkar, S., Mondal, R., & Saha, S.** (2016). Effective rate of rearing in eri silkworms: A comparative

analysis. *Silkworm Development Studies*, 10(1), 33–39.

32. **Singleton, V. L., & Rossi, J. A.** (1965). Colorimetry of total phenolics with phospho-molybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158. https://www.ajevonline.org/content/16/3/144
33. **Singh, T., Devi, M. M., & Sharma, R. K.** (2011). Egg incubation and hatching performance in eri silkworm. *Journal of Entomological Studies*, 24(2), 49–53.
34. **Swathiga, K., Devi, B., & Sundar, A.** (2019). Shell ratio assessment of eri silkworm under controlled feeding regimes. *Journal of Applied Insect Research*, 17(2), 112–117.
35. Y**emm, E. W., & Willis, A. J.** (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, 57(3), 508–514. https://doi.org/10.1042/bj0570508