Extraction and Purification of Scleroprotein Obtained from Spiderweb

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Abstract. Spider silk belonged to the scleroprotein family; a group of structural proteins found in living organisms. Other members of this group include collagen, present in ligaments, and keratin, which forms fingernails, feathers, horns, and hoofs. Specific scleroproteins such as keratin, collagen, elastin, and fibroin were analysed in this study. The scleroprotein was extracted from spiderwebs, dialyzed, and purified using column chromatography. The total protein concentrations measured were 0.08 mg/ml in crude samples and 0.10 mg/ml after dialysis. Following column chromatography, the protein concentration increased to 0.15 mg/ml. The respective concentrations of keratin, collagen, and fibroin were 0.12 mg/ml, 0.11 mg/ml, and 0.08 mg/ml. Protein profiles were identified using SDS-PAGE with Bovine Serum Albumin as a standard. Keratin exhibited notable stimulatory effects on plant growth, specifically in *Vigna radiata* and *Macrotyloma uniflorum*. Treated *Vigna radiata* samples grew to an average height of 5.7 cm, compared to 4.1 cm in untreated controls. Similarly, treated *Macrotyloma uniflorum* plants reached a height of 6.3 cm, while untreated plants measured 4.4 cm after seven days of germination. The number of leaves also increased, with treated *Macrotyloma uniflorum* showing five leaves versus three in untreated samples, and treated *Vigna radiata* producing four leaves compared to two in untreated plants.

Keywords: Spider silk, scleroprotein, total protein, Bovine Serum Albumin, Keratin.

1 Introduction

Spider silk was a primary source of scleroprotein, secreted from the spinneret portion of spiders (Vollrath & Knight, 2001). Silk fibroins, produced by both insects and spiders, were often classified as keratins, although their phylogenetic relationship to vertebrate keratins remained uncertain (Kaplan et al., 1994). Spider silk, a natural polypeptide polymeric protein, consisted of long chains of amino acids. It belonged to the scleroprotein group, which provided structural support to living organisms. Scleroproteins, including collagen and keratin, varied chemically based on amino acid composition and sequence. Fibroin, the main

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component of spider silk, was further divided into spidroin 1 and spidroin 2, both categorized under the keratin family (Lazaris et al., 2002).
Scleroproteins, known for their fibrous and insoluble nature, played vital roles in supporting tissues, muscles, skin, and other structures. Spider silk exhibited a unique combination of crystalline and amorphous regions, contributing to its strength and elasticity. This structure was formed by β-pleated sheets organized into supermolecular aggregates (Hinman & Lewis, 1992). Spinneret and gland structures enabled spiders to control silk extrusion efficiently. Silk's commercial value lay in its remarkable mechanical properties, making it suitable for various applications. In 2000, Nexia, a Canadian biotechnology firm, produced spider silk proteins in transgenic goats, yielding 1–2 grams per litre of milk (Lazaris et al., 2002).
Among scleroproteins, keratin, collagen, elastin, and fibroin held significant importance (Kaplan et al., 1994). Keratin, a structural protein in skin, hair, and nails, existed in two forms: alpha-keratin in softer tissues and beta-keratin in harder structures. Collagen was abundant in connective tissues, providing resilience and structural integrity, while elastin contributed elasticity to tissues and blood vessels. Fibroin, derived from spiders and silk-producing insects, found applications in biomedical and biotechnology fields. Scleroproteins had diverse industrial uses, including cosmetic formulations like anti-wrinkle creams and hair conditioners. Increasing interest in renewable and eco-friendly materials further highlighted their significance. This study focused on isolating scleroprotein from spider webs, an abundant and inexpensive source often regarded as waste, to explore its potential uses.

2 Materials and Methods

2.1 Collection and pretreatment of Spiderweb

Fresh spider webs were gathered from various indoor and outdoor environments. Small debris such as sticks and stones were carefully removed using a scalpel. The cleaned webs were then used for extraction.

2.1.1 Extraction of Scleroprotein from Spider Web (Simmons et al., 1996)

One gram of spider web was mixed with 30 ml of 8M urea – 25mM Tris-HCl buffer (pH 9.0) containing 25mM 2-mercaptoethanol. The mixture was stirred at 37°C for 4 hours and centrifuged at 30,000g for 30 minutes at 4°C. The supernatant was collected and subjected to further centrifugation at 250,000g for 1 hour at 4°C. The final supernatant, containing crude protein, was retained, and the pellet was discarded.

2.2 Purification of Crude Protein by Dialysis

2.2.1 Dialysis

2.2.1.1 Pre-treatment of Dialysis Membrane

The dialysis membrane was soaked in 100 ml of sodium bicarbonate (2g) and 1mM EDTA solution. It was boiled in water for 10 minutes to enlarge the pores, rinsed thoroughly, and boiled again in double-distilled water for 10 minutes. Finally, the membrane was immersed in 50% ethanol/1mM EDTA solution and stored at 4°C.

2.2.1.2 Dialysis Setup

One end of the dialysis tube was securely tied, and the protein sample was filled to half its length. The tube was sealed and placed in a beaker containing 5mM Tris-HCl buffer (pH 8.0) with 25mM 2-mercaptoethanol at 4°C for 48 hours. The resulting precipitate was centrifuged at 250,000g for 1 hour, dissolved in the extraction buffer, and centrifuged again to remove impurities. The purified protein was desalted using distilled water and stored at -20°C. Protein activity was tested from the dialysate.

2.2 Separation of Protein Using Column Chromatography

The dialyzed protein sample was applied to a Sephadex G-200 column and eluted with 0.05M tris buffer (pH 8.5) containing 0.01M 2-mercaptoethanol at a flow rate of 20 ml/hour. Protein concentration was determined using Lowry's method.

2.4 Estimation of Protein (Lowry's et al., 1951) Method

2.4.1 Reagents

- **Reagent A:** 2% Na_2CO_3 in 0.1N NaOH
- **Reagent B:** 2% $CuSO_4 \cdot 5H_2O$
- **Reagent C:** 2% Sodium or Potassium Tartarate
- **Reagent D:** Prepared by mixing 0.5 ml of reagent B, 0.5 ml of reagent C, and 99 ml of reagent A (freshly made).
- **Reagent E:** Folin-Ciocalteu's reagent (1N solution).

2.4.2 Standard Preparation

BSA (Bovine Serum Albumin) was prepared at 1 mg/ml.

2.4.3 Protein Content Determination

BSA standards of increasing concentration were prepared in 1 ml volumes. Two ml of reagent D was added to each, mixed, and incubated for 10 minutes at room temperature. Next, 0.6 ml of reagent E was added, mixed, and incubated for 20 minutes. Optical density was measured at 660 nm, and a standard curve was plotted. Protein concentration in the keratin sample (diluted 1:50) was determined.

2.5 Isolation of Individual Proteins

2.5.1 Keratin

One gram of spider web was incubated with 0.5mM sodium sulfide at 30°C and pH 10-13 for 5 hours with occasional stirring. The protein was filtered and centrifuged at 10,000 rpm for 5 minutes. The supernatant was used as a crude extract.

2.5.1.1 Purification Using Ammonium Sulfate

A saturated ammonium sulfate solution was prepared by dissolving 35g in 50 ml of water. This solution was added gradually to the crude extract until a color change was observed. The precipitate was centrifuged at 10,000 rpm for 5 minutes, and the pellet was dialyzed against 0.5M Tris-HCl buffer (pH 7.5) for 24 hours at 4°C. The protein activity was measured.

2.5.2 Collagen

One gram of spider web was treated with 0.05M Na_2HPO_4 (pH 8.7-9.1) at 4°C to remove non-collagen compounds. The collagen was extracted using 0.5M acetic acid with 5mM

EDTA, adding pepsin (50 mg/g of spider web) over 24 hours at 4°C. This process was repeated 3-4 times, followed by centrifugation at 3,000 rpm for 15 minutes, and salted out with 4M NaCl.

2.5.3 Fibroin

Spider web was boiled in 0.02M Na_2CO_3 solution for 20 minutes. The residue was washed thoroughly with distilled water and dried. The dried silk fibroin was dissolved in 9.3M LiBr solution at 70°C for 2.5 hours, dialyzed against water for 48 hours, and centrifuged to obtain a crude extract.

2.6 Estimation of Keratin, Collagen, and Fibroin

Keratin concentration was estimated using Lowry's method, collagen was measured via hydroxyproline content (Neuman and Logan method, 1950), and fibroin was quantified using the Bradford method by detecting dye absorption at 595 nm.

2.7 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (Laemmli et al., 1970)

2.7.1 Gel Preparation

Table 1. Stacking Gel (4%) and Separating Gel (15%) Composition

Reagents	Stacking Gel (ml)	Separating Gel (ml)
Acrylamide	1.66	5.0
Buffer	1.33	2.5
Distilled Water	6.86	2.3
10% SDS	0.1	0.1
10% APS	0.2	0.2
TEMED	0.008	0.008

2.7.1.1 Procedure

Acrylamide solution for separating gel was poured between glass plates and polymerized. Stacking gel was added above it, a comb inserted to form wells, and the gel allowed to set. Samples mixed with loading buffer were boiled, loaded, and run at 100-200V. The gel was stained with Coomassie Brilliant Blue R-250 and destained.

2.8 Bio stimulant Activity (Du Jardin, 2015)

The bio stimulant effect of keratin was tested on *Vigna radiata* and *Macrotyloma uniflorum*. Three seeds of each were planted in paper cups filled with 100g of sterilized sandy-loamy soil. One ml of keratin was added to treatment groups, while control groups received no keratin. Plant height and leaf count were measured weekly.

3 Results and Discussion

3.1 Isolation of Scleroprotein

The supernatant containing the crude protein was successfully extracted from spider web samples using the prepared extraction medium, which included urea, Tris-HCl buffer, and 2-mercaptoethanol. This step yielded a protein-rich solution (Fig.1) that was subsequently processed for further purification and analysis.

Fig.1. Supernatant obtained from the spider web extraction medium

3.2 Estimation of Scleroprotein

The initial concentration of scleroprotein in the crude extract was determined to be 0.08 mg/ml. Following purification through dialysis and column chromatography, the concentration of the protein increased to 0.10 mg/ml and 0.15 mg/ml, respectively, as shown in Fig. 2. These results demonstrate the effectiveness of the purification process in enhancing protein yield.

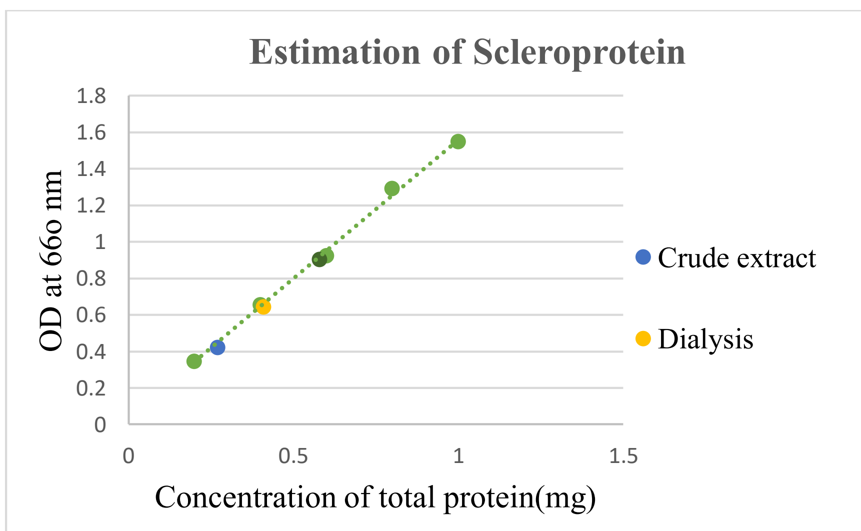


Fig. 2. Scleroprotein concentration at various purification stages

3.3 Isolation of individual Proteins

3.3.1 Isolation of Keratin

The isolation of keratin from spider web samples was performed using a separation medium, yielding a supernatant with a characteristic brown color (Fig. 3). This color change indicated the presence of keratin in the solution.

Fig. 3. Supernatant containing keratin from the separation medium

3.3.1.1 Precipitation using Ammonium Sulfate

Further purification of the keratin extract was achieved through ammonium sulfate precipitation at 30% saturation. The endpoint of the precipitation process was marked by a visible color change from brown to yellow, as depicted in Fig. 4. The precipitate was subsequently centrifuged and dialyzed to remove residual salts and impurities, significantly improving the purity of the keratin extract.

Fig. 4. Ammonium sulfate precipitation showing color change during keratin isolation

3.3.1.2 Estimation of Keratin

The concentration of keratin in the crude extract was found to be 0.12 mg/ml (Figure 5). This value closely aligns with findings reported by (Fujii et al., 2013), who isolated keratin from human hair. The consistency between these results underscores the reliability of the applied method.

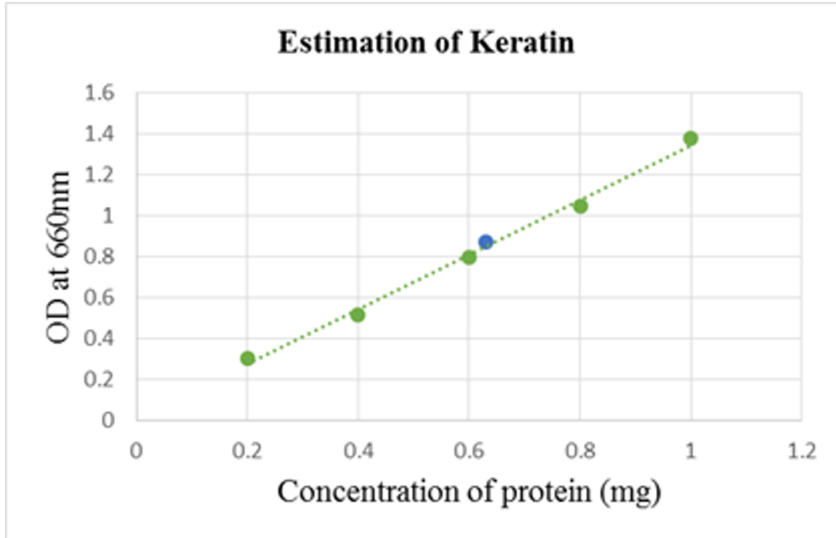


Fig. 5. Keratin concentration in the crude sample

3.3.2 Estimation of Collagen and Fibroin

The concentration of collagen in the crude extract was determined to be 0.11 mg/ml (Fig.6), while fibroin exhibited a concentration of 0.08 mg/ml (Fig. 7). These concentrations were consistent with previous studies on protein extraction methods from similar biomaterials.

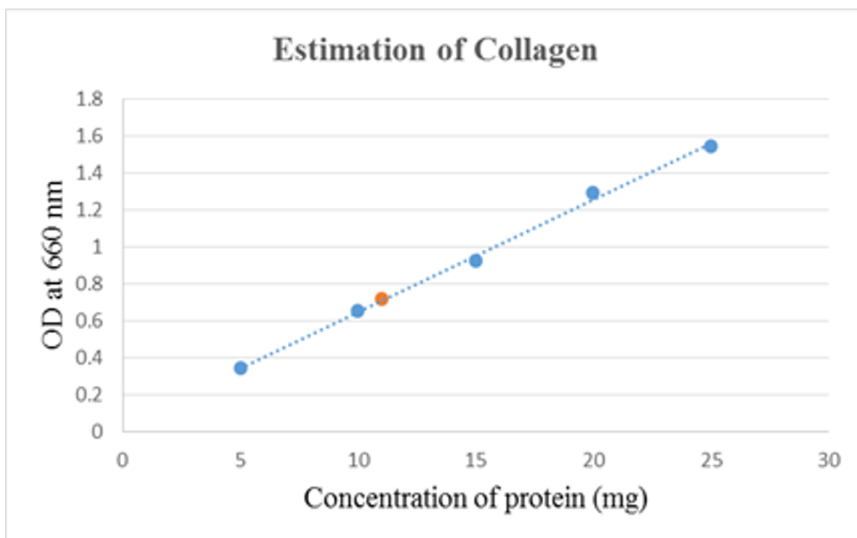


Fig. 6. Collagen concentration in the crude sample

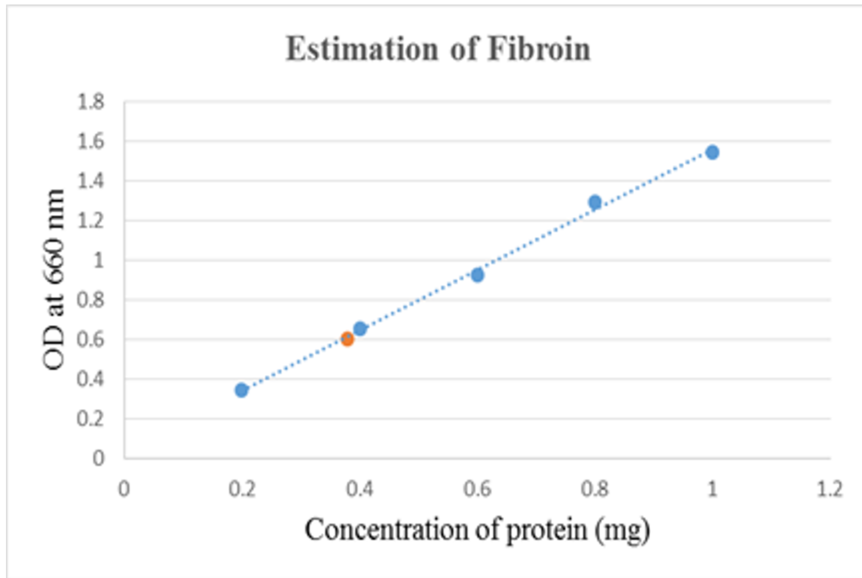


Fig. 7. Fibroin concentration in the crude sample

3.4 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The electrophoretic profiles of keratin, collagen, and fibroin were analyzed using SDS-PAGE (Fig. 8). The standard BSA protein profile (lane 1) displayed four distinct bands, which served as a reference. Keratin, collagen, and fibroin samples (lanes 2, 3, and 4, respectively) exhibited similar banding patterns, confirming the successful isolation of these proteins. These findings are comparable to the work of (Ku et al., 2001), who studied spider silk proteins in transgenic tobacco and potato plants.

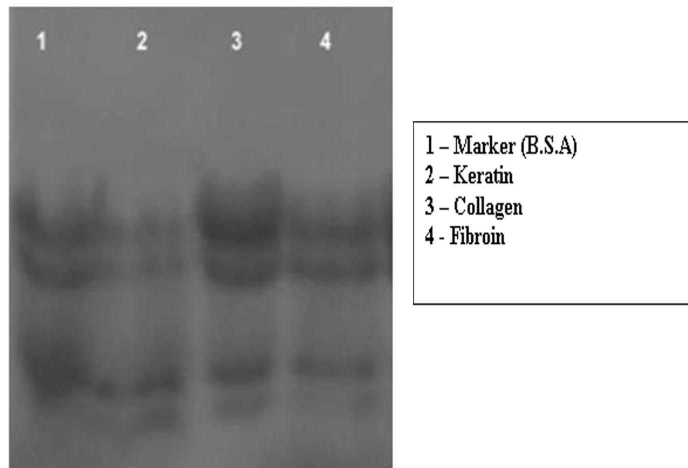
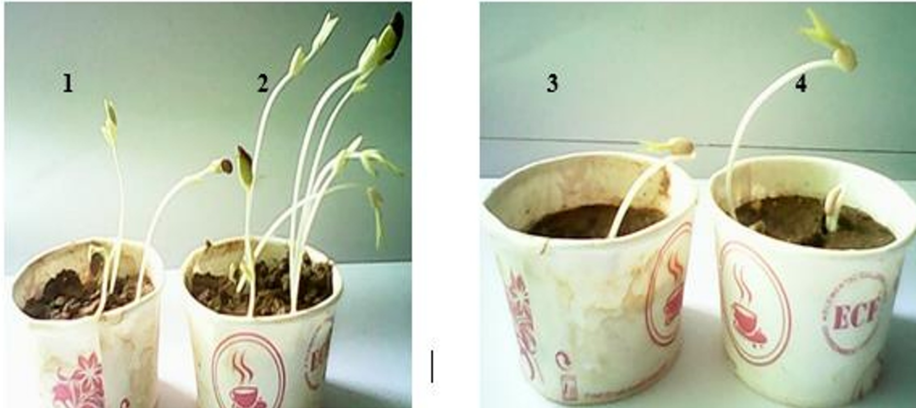


Fig. 8. SDS-PAGE profiles of keratin, collagen, and fibroin

3.5 Biostimulant Activity of Keratin

The biostimulant properties of keratin were evaluated by assessing plant growth in soil treated with keratin extract compared to untreated controls Fig. 9. Macrotyloma uniflorum and Vigna radiata were selected as test plants. In treated soil, Macrotyloma uniflorum reached an average height of 6.3 cm, while untreated samples attained only 4.4 cm. Similarly, Vigna radiata grew to an average height of 5.7 cm in treated soil compared to 4.1 cm in the control (Fig. 10 and 11).



A. Macrotylomauniflorum B. Vignaradiata
(1&3-Keratin untreated, 2&4-Keratin treated)

Fig. 9. Biostimulant effect of keratin on *Macrotyloma uniflorum* and *Vigna radiate*

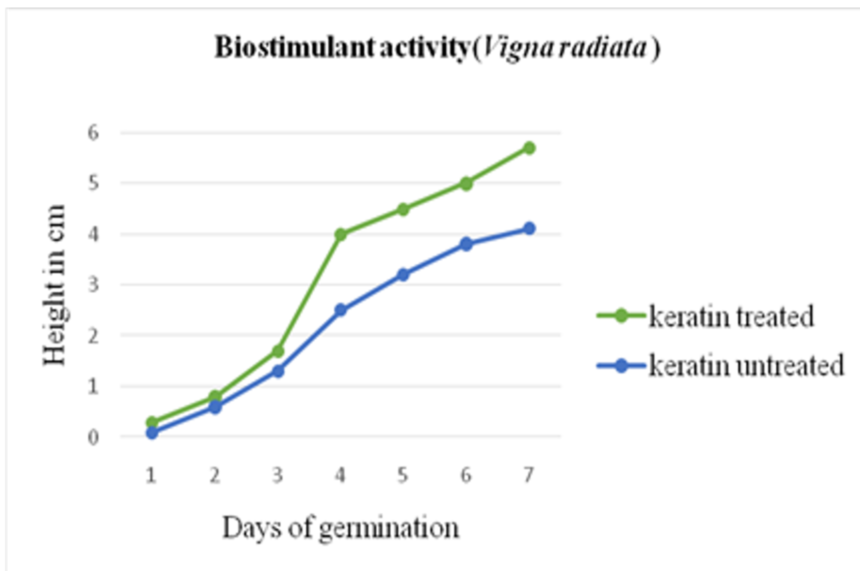


Fig. 10. Plant height of keratin-treated and untreated *Vigna radiata*

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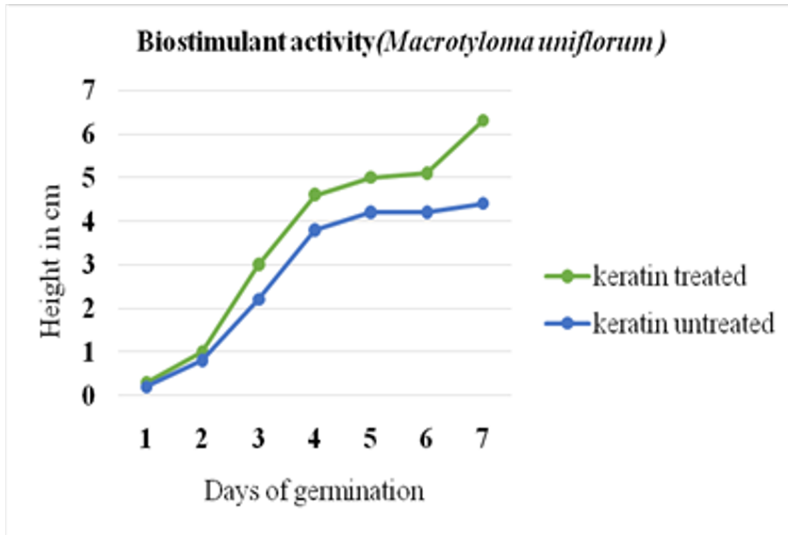

Fig. 11. Plant height of keratin-treated and untreated *Macrotyloma uniflorum*

Leaf count also demonstrated a positive effect of keratin treatment. Macrotyloma uniflorum exhibited an average of five leaves in treated samples versus three in controls. Vigna radiata produced four leaves with treatment and only two leaves without (Fig. 12 and 13). These findings align with the results of (Adetunji et al., 2013), who observed improved growth in cowpea plants fertilized with keratin-rich organic fertilizer derived from microbially hydrolysed feathers.

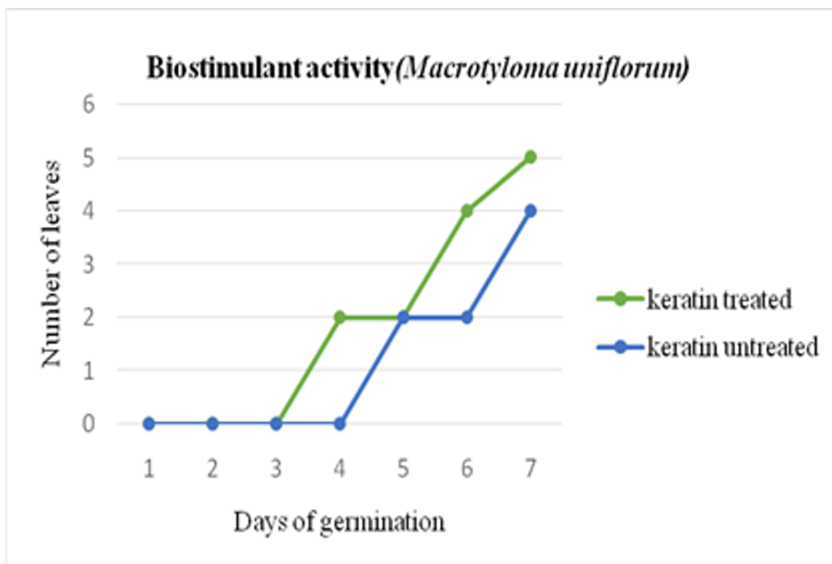

Fig. 12. Leaf count of keratin-treated and untreated *Macrotyloma uniflorum*

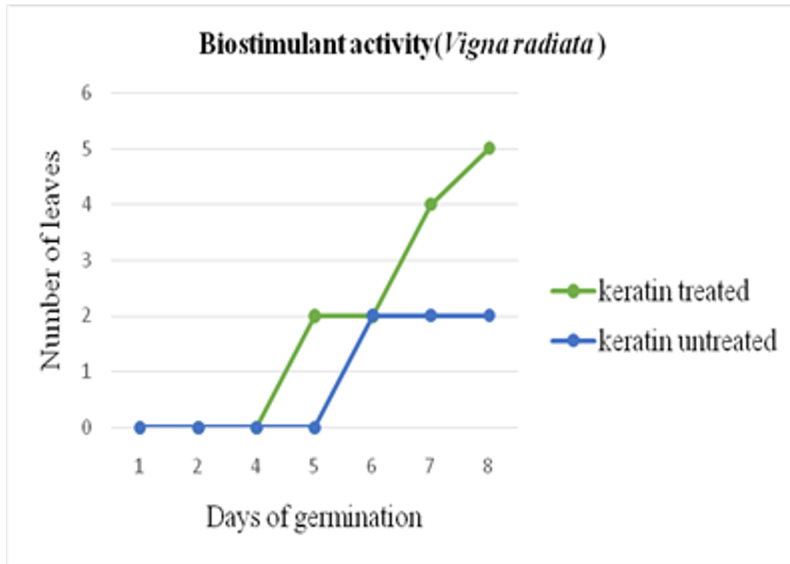

Fig. 13. Leaf count of keratin-treated and untreated *Vigna radiata*

4 Conclusion

The findings of the study demonstrated that keratin was successfully isolated from spider webs, which are widely regarded as a readily available and sustainable natural waste material. The isolation process yielded an adequate quantity of keratin, which holds significant potential for various applications. Keratin, a versatile biomolecule, is not only utilized in a variety of commercial industries but also has valuable uses in the agricultural sector.

The primary focus of this research was to evaluate the biostimulant properties of keratin when applied to different cereal crops. The results revealed that keratin had a positive influence on plant growth, manifesting in notable improvements in both plant height and leaf number. These findings suggest that keratin can serve as an effective and eco-friendly alternative to chemical fertilizers, offering a more sustainable solution for enhancing agricultural productivity.

Based on the evidence gathered, it is strongly recommended that keratin be explored further for its potential use in agricultural practices. Its natural origin, coupled with its beneficial effects on plant growth, positions keratin as a promising biostimulant that could help reduce reliance on chemical fertilizers, ultimately contributing to more sustainable and environmentally friendly farming practices.

Declarations

Ethics approval and consent to participate: Nil
Availability of data and materials: Data will be made available on request
Competing interests: The authors declare that there is no conflict of interest
Funding: Nil
Authors' contributions: R.V.Harunee and S.Yeswandhini : conceived and conducted the research; V.Nandhakumar: wrote the original draft; N.Ajith: data extraction and processing; V.Asothama Chakravarthi analysed the results; M.Nithya: reviewed the original draft.

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