

# PHYSIOCHEMICAL, BIOCHEMICAL AND PHYTOCHEMICAL PROFILING OF SODIUM ALGINATE AND ITS ROLE AS A SEED COATING POLYMER TO ENHANCING SEED QUALITY IN OKRA (*ABELMOSCHUS ESCULENTUS*)

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## ABSTRACT

Sodium alginate, a naturally occurring polysaccharide extracted from brown marine algae, has emerged as a promising biopolymer for sustainable agricultural applications. This study investigated the extraction, characterization, and functional evaluation of sodium alginate derived from *Sargassum myricostum* and assessed its potential as a biodegradable seed-coating polymer for improving seed quality traits in okra (*Abelmoschus esculentus* L.). The extraction yielded 25% sodium alginate. Physicochemical characterization revealed medium viscosity (90 cP), an alkaline pH (7.20), a blackish-brown colour, and high water-absorption capacity. Biochemical analysis indicated a high carbohydrate content (70%) with negligible protein and lipid fractions, while phytochemical screening detected only saponins, indicating high purity. ICP-MS analysis revealed a high sodium content (200,000 ppm), confirming the sodium alginate form, and FTIR analysis verified the structural integrity and purity of the extracted alginate through characteristic O-H and C=O absorption bands with high transmittance (>95%). The extracted alginate was infused with selected biomolecules and applied as a seed-coating polymer. Among the treatments, T<sub>14</sub> exhibited the best performance, recording 96% germination, 40.58 cm seedling growth, 0.463 g dry matter production per 10 seedlings, higher vigour indices I and II, and only 2% abnormal seedlings. In contrast, the uncoated control (T<sub>0</sub>) showed the lowest germination (86%), seedling growth (30.34 cm), dry matter production (0.343 g per 10 seedlings), vigour indices, and the highest proportion of abnormal seedlings (9%). These findings demonstrate that sodium alginate derived from *S. myricostum* is an effective, eco-friendly alternative to synthetic polymer and microplastic-based seed coatings. As a natural hydrocolloid, it significantly enhances seed quality attributes and holds considerable promise for sustainable and environmentally responsible agricultural practices.

**KEYWORDS:** Okra, sodium alginate, seed coating, biopolymer, biomolecules, organic agriculture

## 1. INTRODUCTION

Okra (*Abelmoschus esculentus*), also known as bhendi or lady's finger, is a widely cultivated vegetable across tropical, subtropical, and warm temperate regions. It serves as both an important food source and a source of income for smallholder farmers. The crop's rich composition of fiber, vitamins, minerals, and antioxidants contributes significantly to nutrition and food security (Gemede et al., 2014). In recent years, the industrial demand for okra has increased, primarily due to the valuable mucilage extracted from its pods, which is used in food and pharmaceutical applications (Leng, 2018). Despite its economic and nutritional importance, okra productivity is often constrained by factors such as the limited use of improved varieties, poor seed management, low availability of high-quality seeds, and the presence of hard seeds that hinder uniform germination (Demir, 1997). Moreover, high temperature and humidity conditions during storage adversely affect seed viability and field performance. The relatively high oil content in okra seeds (20-40%) further accelerates lipid peroxidation, damaging cell membranes and resulting in slow or uneven germination (Jarret et al., 2011). The low adoption of high-yielding varieties also reduces the implementation of improved cultivation practices (Abayneh, 2019). To overcome these

challenges, seed invigoration and coating technologies have been developed to enhance germination, emergence, and early plant vigor. Conventional polymer-based coatings improve seed handling, ensure uniform pesticide or nutrient delivery, and facilitate better adhesion of treatment chemicals to seed surfaces (Ekebafé et al. 2011). Such coatings form a thin, uniform layer that create micro environment around the seed and diffuses easily and is non-toxic to seeds. They act as physical barriers, reducing the leaching of inhibitory substances from seed coats and limiting excess oxygen diffusion to the embryo (Kumar et al. 2014). This controlled microenvironment around the seed improves stress tolerance, delays ageing, and enhances seedling establishment. Additionally, polymers act as temperature-sensitive coatings that regulate water uptake during imbibition, thereby improving germination uniformity under fluctuating environmental conditions (Johnson et al, 1999). However, growing awareness of the environmental impacts associated with synthetic polymers particularly their persistence in soil, disruption of microbial diversity (Mustapha et al., 2025), and contribution to agricultural microplastic pollution (Accinelli et al. 2019) has prompted a shift toward eco-friendly alternatives. Among the biodegradable options, natural polymers such as sodium alginate, derived from brown seaweeds, have gained significant attention. Sodium alginate is a non-toxic, renewable biopolymer composed of  $\beta$ -D-mannuronic and  $\alpha$ -L-guluronic acid units, which confer excellent gel-forming ability, adjustable viscosity, and high water-retention capacity, making it particularly suitable for seed-coating applications (Ahmad et al., 1994). Research has shown that alginate-based coatings can enhance germination rate, seedling vigor, and stress tolerance while minimizing ecological risks compared to synthetic coatings (Skrzypczak et al., 2021). Brown seaweeds, especially *Sargassum* species, are rich sources of alginate, although extraction yield and quality vary depending on species, growth stage, and environmental conditions (Kalimuthu, 1980; Dobrincic et al., 2020). In addition to alginate extraction, seaweeds have long been used as organic soil amendments and natural biostimulants to improve seed germination, plant growth, and resilience to environmental stresses (Baweja et al., 2019; Norrie and Keathley, 2005). Sodium alginate is particularly advantageous in seed-coating due to its high water-uptake capacity, which supports proper seed imbibition and promotes germination (McHugh, 1987). It is widely used in agriculture for coating seeds, fruits, and stem tips (Cisneros-Zevallos and Krochta, 2006). Although most research has focused on its application in synthetic seed production, limited studies have explored its direct effects on germination indices and seedling traits in various crops. For instance, sodium alginate concentrations of 2% and 3% in *Ferulago angulata* L. produced synthetic seeds with maximum germination (Sorbi & Moradi, 2018), while 3.5% sodium alginate improved germination percentage in synthetic seeds of Persian oak. (Fayzi, 2016). Similarly, plant-derived polymers in general offer advantages such as biodegradability, low cost, light weight, and wide availability, further supporting their use in sustainable agricultural practices (Kamath & Park, 1993). When enriched with bioactive substances like humic and fulvic acids, these coatings can stimulate root growth, enhance nutrient uptake, and improve seedling establishment (Chen et al., 2021). Humic substances are commonly applied to mitigate the harmful impacts of chemical fertilizers on soils, and numerous researchers have demonstrated their significant positive influence on plant growth (Ghabbour & Davies, 2001). Fulvic acids, in particular, are effective due to their small molecular size and chelating ability, enabling them to penetrate plant cell membranes and enhance nutrient transport (Bocanegra et al., 2006; Yu et al., 2023). Antioxidants such as ascorbic acid and  $\alpha$ -tocopherol also improve seed physiological performance by reducing oxidative stress during germination (Sivasakthi and Renganayaki 2022). Moreover, the incorporation of plant growth regulators like brassinosteroids (BRs) further strengthens the benefits of biopolymer coatings. BRs interact synergistically with auxins to promote root growth, increase germination rate, and enhance biomass accumulation in both roots and shoots (Zhang et al., 2008; Pravina and Renganayaki 2025). They also regulate gene expression, enzyme activity, and nitrogen fixation, contributing to improved yield potential (Khan et al., 2015). Similarly, salicylic acid (SA) functions as an important signaling molecule that improves water potential, maintains pigment stability, and confers tolerance to abiotic stresses such as drought and salinity (Raskin, 1992; Hayat et al., 2010; Bakry et al., 2012). Glutathione and ascorbic acid are key antioxidants that support seed germination by managing reactive oxygen species (ROS) and maintaining cellular redox balance. During the early stages of germination, glutathione (GSH) typically rises temporarily, contributing to dormancy release and modulating abscisic acid (ABA) signalling both essential for successful germination and seedling establishment. Higher GSH levels are also linked with stronger antioxidant protection and better stress tolerance during this phase (Koramtla et al., 2021). Ascorbic acid is a widespread non-enzymatic antioxidant that not only neutralizes reactive oxygen species (ROS) but also influences several essential physiological processes in plants (Akram et al., 2017). Overall, seed coatings enriched with bioactive compounds, antioxidants, and plant growth regulators can create a beneficial microenvironment around seeds, promoting rapid germination, uniform emergence, and improved seedling establishment. The integration of biodegradable polymers like sodium alginate with bioactive additives represents a sustainable approach to seed enhancement, ensuring high physiological performance while safeguarding soil health and ecological balance (Skrzypczak et al., 2021). The objectives of the present study were: i) To extract and characterize sodium alginate from *Sargassum myriocystum*, examining its physicochemical, biochemical, and phytochemical properties and ii) to evaluate its efficacy as a biodegradable seed-coating polymer, supplemented with biostimulants, growth regulators, and antioxidants, for improving germination, vigor and seedling establishment in okra. By combining seaweed-derived alginate with functional additives, this study aims to develop sustainable seed-coating approaches that enhance productivity while minimizing environmental impact.

## 2. MATERIALS AND METHODS

## 2.1 Collection and authentication

The Mandapam coastal region, where the *Sargassum myriocystum* was collected, is located at approximately 9.2886° N latitude and 79.1329° E longitude. This area is part of the Gulf of Mannar, a coastal region known for its rich seaweed diversity. The freshly harvested seeds of Okra var. ArkaAnamika (Latitude: 10.12° N or 10° 07' 13" N and Longitude: 77.55° E or 77° 32' 50" E) were procured from Horticultural college and Research Institute, Periyakulam, Theni district. The variety was developed by the Indian Institute of Horticultural Research (IIHR), Bangalore and the laboratory experiment was conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India during 2024-2025.

## 2.2 Design of the experiment

With four replications, the experiment was done in a completely randomized block design and statistical tool is ANOVA

## 2.3 Extraction of sodium alginate

Sodium alginate is extracted according to Calumpong et al., 1999 with some modifications. 25 g of dried seaweed (*Sargassum myriocystum*) powder was dissolved in 800 ml of 2 % formaldehyde for 24 hours to remove pigmentation. After such period supernatant was decanted and seaweed was collected and washed twice by distilled water. Then seaweed was dissolved in 800 ml of 0.2 M HCl and leaved it for 24 hours to convert alginate into alginic acid, after such period again supernatant was discarded and seaweed was collected, then washed by distilled water to remove acid. Collected seaweed was dissolved in 2 % Sodium carbonate solution and heated under water bath for 3 hours at 90-100°C to dissolve the alginic acid into soluble sodium alginate. After that the solution was cooled and centrifuged under 5000 rpm for 30 min. Then supernatant was collected carefully and added three volume of ethanol and it precipitate the sodium alginate. It was purified twice with 100 ml of acetone and dried @ 65°C. Dried sodium alginate powder was dissolved in distilled water and again precipitate with three volume of ethanol and dried @ 65°C and grind it as fine powder and used for further studies.

## 2.4. Sodium alginate characteristics

### 2.4.1 Physiochemical Properties of Sodium Alginate

#### 2.4.1.1 Yield (g)

. Extracted sodium alginate yield was calculated as follows: Weight of alginate/ Weight of seaweed dried biomass \*100

#### 2.4.1.2 Estimation of pH and Color

About one per cent w/v of sodium alginate polysaccharide powder was prepared in distilled water and the pH of the solution was measured using pH meter. The color was evaluated by visual inspection

#### 2.4.1.3 Moisture Content

The moisture of the alginates was determined according to AOAC (1995) through the loss in mass of sodium alginate after heating at 105°C for 24 h.

#### 2.4.1.4 Viscosity

One gram of dried sodium alginate was dissolved in 100 ml of distilled water and the viscosity was measured by using Brookfield viscometer.

#### 2.4.1.5 Water absorbancy

To 25 ml of distilled water 0.5 g of sodium alginate powder was immersed and kept for 24 hours under room condition to reach absorption equilibrium. After specified time, the unabsorbed water was decanted and the swollen sodium alginate was weighed. The relative water absorbency was calculated (Chen et al., 2004),

$$\text{Water absorbency (g/g)} = (M_2 - M_1) / M_1$$

Where,

M<sub>1</sub> – initial weight of sodium alginate

M<sub>2</sub> – final weight of swollen sodium alginate after 24 hrs

#### 2.4.1.5 Solubility behaviour

As per Sivasakthi (2022) the solubility behaviour of the polysaccharide was tested with different inorganic and organic solvents viz., warm water, cold water, ethanol (99.9%), methanol (99.85%), acetone (99.5%) and petroleum ether (99.0%). One gram of sample was taken and added to 25 ml of solvent and stirred with the help of glass rod for 1 min and then solubility behaviour was observed and recorded.

### 2.4.2 Biochemical analysis of sodium alginate

#### 2.4.2.1 Carbohydrates analysis

The carbohydrate content of the sodium alginate was determined by the phenol sulphuric acid method described by Dubois et al., (1956)

#### 2.4.2.2 Proteins analysis

The proteins were tested following Lowry et al., (1951). The protein was calculated using bovine albumin serum as the standard.

#### 2.4.2.3 Lipid's analysis

According to the AOAC methodologies (1950) the total lipids were estimated gravimetrically using chloroform–methanol

#### 2.4.2.4 Ash analysis

The ash content was measured by a gravimetric method using AOAC (1950) by burning. 1 g of the product taken in a silica crucible and kept in a muffle furnace at 575°C for 24 h. After burning, the content was cooled, weighed, and expressed in terms of percentage.

#### **2.4.3 Phytochemical analysis**

To assess the purity of the extracted alginate, qualitative phytochemical screening was performed for compounds such as flavonoids, alkaloids, tannins, terpenoids, steroids, saponins, glycosides, phenols, and phlorotannins, following the methods of Trease and Evans (1989) and Sofowora (1993). These assays serve as rapid tests to detect the presence of residual secondary metabolites from seaweed, which, if present, indicate possible co-extraction of non-target compounds along with alginate.

##### **2.4.3.1 Test for flavonoids**

To determine the presence of flavonoids, a qualitative assay was carried out. For this, 0.5 g of sodium alginate was dissolved in diluted NaOH, followed by the addition of HCl. The formation of a yellow coloration that subsequently disappeared upon acidification indicated the presence of flavonoids.

##### **2.4.3.2 Test for alkaloids**

To test for the presence of alkaloids, 0.5 g of sodium alginate was dissolved in 10 mL of 0.1 N HCl and filtered. The filtrate was then used for qualitative analysis. For the assay, 1 mL of 1% HCl was added to 3 mL of the filtrate, followed by a few drops of Mayer's reagent. The formation of a creamy white precipitate confirms the presence of alkaloids.

##### **2.4.3.3 Test for tannins**

To analyze the presence of tannins, a qualitative method was used. 0.5 g of sodium alginate was boiled in 10 mL of distilled water and then filtered. A few drops of FeCl<sub>3</sub> 0.1% were added and observed for a blue-black or brownish green color, indicates the presence of tannins.

##### **2.4.3.4 Test for terpenoids and Steroids**

To analyze the presence of terpenoids and steroids, a qualitative method was used. Sodium alginate 0.5 g was added to 2 mL of chloroform. Then, 3 mL of conc. sulfuric acid was added carefully to a layer form. A reddish-brown color for the interface indicated terpenoids presence.

##### **2.4.3.5 Test for saponins**

To analyze the presence of saponins, a qualitative method was used. First, 0.5 g of sodium alginate was dissolved in 5 mL of distilled water. The solution was shaken speedily and observed for a stable persistent foam. The foaming was mixed with three drops of olive oil and shaken strongly and the formation of a milky mass was observed, which indicates the existence of saponins.

##### **2.4.3.6 Test for glycosides**

The analysis of the presence of glycosides was done with a qualitative method using 0.5 g of sodium alginate dissolved in 5 mL of distilled water. Then, 10 mL of 50% sulphuric acid was added to 1 mL of this extract. The mixture was heated in boiling water for 5 min. Then, 10 mL of Fehling's solution (5 mL of each solution A and B) was added and boiled. A brick red precipitate indicated the presence of glycosides.

##### **2.4.3.7 Test for phenolic compounds**

To analyze the presence of phenolic compounds, a qualitative method was used. 100 mg of sodium alginate was boiled with 1 mL of distilled water then filtered. Then, 2 mL of filtrate was taken and 2 mL of 1% FeCl<sub>3</sub> solution was added. The establishment of bluish black color indicated the existence of phenol.

##### **2.4.3.8 Test for phlorotannins**

To analyze the presence of phlorotannins, a qualitative method was used. Sodium alginate 0.5 g was dissolved in 5 mL of distilled water and filtered. Then the filtrate was boiled with 2% HCl solution. A red precipitate indicated the presence of phlorotannins.

#### **2.4.4 ICPMS analysis**

One g of sodium alginate sample was induced into Inductively Coupled Plasma Mass Spectrometry (ICP-MS) quantifies trace elements by ionizing samples in Department of Forage crops, Tamil Nadu Agricultural University.

#### **2.4.5 Identification of functional groups by Fourier Transforms Infra-Red (FTIR)**

The functional groups of the isolated sodium alginate were identified using an FTIR spectrophotometer (FT/IR-4600 Type A) equipped with an Attenuated Total Reflectance (ATR Pro One) sensor at the Central Instrumentation Centre, The American College, Madurai, Tamil Nadu, India. Spectral data were recorded in the range of 650–4000 cm<sup>-1</sup>.

#### **2.5 Preparation of seed coating polymer**

After studying all the physiochemical, biochemical and phytochemical properties. Sodium alginate 2 g was taken and dissolves in 100 ml of distilled water (by magnetic stirrer) and 0.5 ml of glycerol was added to the polymer and different biostimulant, plant growth regulators and antioxidant were added at respective concentration to the polymer. For 1 kg of seed 250 ml of polymer is required for uniform seed coating.

##### **Treatment details:**

The Okrai seeds var. Arka Anamika were coated with different treatments such as

T<sub>0</sub> - Control (Uncoated seed)

T<sub>1</sub> - Sodium alginate polymer (2%) 250 ml/kg of seed

T<sub>2</sub> - 0.2 g Humic acid infused to 100 ml of Sodium alginate polymer (2%)

T<sub>3</sub> - 0.3 g Humic acid infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>4</sub> - 0.4 g Fulvic acid infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>5</sub> - 0.5 g Fulvic acid infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>6</sub> - Salicylic acid 200 ppm to 100 ml of infused Sodium alginate polymer (2%)  
 T<sub>7</sub> - Salicylic acid 250 ppm infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>8</sub> - Brassinolide 0.5 ppm infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>9</sub> - Brassinolide 1.5 ppm infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>10</sub> - 0.05 g Glutathione infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>11</sub> - 0.10 g Glutathione infused to 100 ml of sodium alginate polymer (2%)  
 T<sub>12</sub> - 0.2 g Ascorbic acid infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>13</sub> - 0.4 g Ascorbic acid infused to 100 ml of Sodium alginate polymer (2%)  
 T<sub>14</sub> - 0.3 g Humic acid + 250 ppm Salicylic acid + 0.4 g Ascorbic acid infused to 100 ml of Sodium alginate polymer (2%)

Seeds were coated with respective treatments and cross linked with 5 % CaCl<sub>2</sub> for 20 min. After that seeds were dried at room temperature for 24 hours.

## 2.6 Observations on seed quality parameters of okra

### 2.6.1 Seed germination (%)

The germination test was carried out in roll towel method according to ISTA, 2013 protocol. After 21 days of germination, the seedlings were evaluated and the normal seedlings were counted and germination percentage (GP) was calculated, according to the formula:

**Germination percentage (GP)** = (Total number of normal seedlings germinated / Total number of seeds evaluated) × 100

### 2.6.2 Abnormal seedlings (%)

The germination test was carried out in roll towel method according to ISTA, 2013 protocol. After 21 days of germination, the seedlings were evaluated and the abnormal seedlings were counted and expressed in percentage

$$\text{Abnormal seedlings (\%)} = \left( \frac{\text{Total number of abnormal seedlings}}{\text{Total number of seeds evaluated}} \right) \times 100$$

### 2.6.3 Root length (cm)

After the germination count, ten normal seedlings were randomly chosen from each replication and the length of the roots from the collar area to the tip of the primary root was measured.

### 2.6.4 Shoot length (cm)

Shoot length was measured on the same seedlings which were used for measuring the length of the roots. The distance along the shoot from the collar to the tip of the primary leaf was measured.

### 2.6.5 Seedling Length (cm)

Seedling length generally refers to the total length of a young plant from the tip of the shoot to the tip of the root. It is usually measured in centimeters (cm) and is obtained by summing:

Seedling length = Shoot length (cm) + Root length (cm)

### 2.6.6 Dry matter production (g seedlings<sup>-10</sup>)

The seedlings which are used for seedling length measurement same seedlings were placed in a butter paper cover separately and dried in shade for 24 h and then kept in an oven maintained at 85 ± 2 °C for 24 h. Dry weight was recorded and the mean values were expressed in gram. (ISTA, 2013).

### 2.6.7 Vigour index (Abdul-Baki and Anderson, 1973)

Vigour index values were computed using the following formula and the mean values were expressed in whole number.

Vigour index I = Germination (%) × Root length (cm) + Shoot length (cm)

Vigour index II = Germination (%) × Drymatter production (g seedling<sup>-10</sup>)

## 3. RESULTS

### 3.1 Sodium alginate characteristics

#### 3.1.1 Physiochemical

Sodium alginate extracted from *Sargassum myriocystum* yielded 25%, demonstrating efficient recovery of the polysaccharide from the brown seaweed (Fig. 2). This yield falls within the range of 14.26–26.07% previously reported for *S. myriocystum* by Kalimuthu (1980), indicating that the extraction procedure employed in the present study was effective. Similar yields have also been reported in other *Sargassum* species (Dalal et al., 2021). Variations in alginate yield are mainly influenced by the seaweed species, maturity, seasonal growth stage, environmental conditions such as light intensity, water temperature, nutrient availability, and extraction methodology (Dobrincic et al., 2020). Therefore, the yield obtained in the present investigation confirms that *S. myriocystum* is a promising and sustainable source of sodium alginate for agricultural and industrial applications. The extracted sodium alginate exhibited a blackish-brown colour (Fig. 1) with a pH of 7.20, indicating its near-neutral nature. According to Coppen (1995), these characteristics are consistent with acceptable quality standards for sodium alginate. The darker colour observed in the present study may be attributed to the presence of residual natural pigments and saponins retained during extraction, whereas Rashedy et al., (2021) reported comparatively lighter alginate preparations obtained using different purification procedures. Although colour variation is

expected depending on the extraction process, it does not adversely affect the functional properties of sodium alginate intended for non-food applications such as seed coating. The viscosity of the 1% (w/v) sodium alginate solution was 90 cP, indicating a medium-viscosity grade suitable for seed-coating applications. According to the Food Chemicals Codex (FCC), the acceptable viscosity range for commercial sodium alginate varies from 10 to 5000 cP, depending on its intended application. The measured viscosity in the present study is therefore well within the recommended range. Viscosity is one of the most important rheological properties governing film formation, adhesion, gel strength, and coating uniformity. It is influenced by several factors, including alginate source, molecular weight, extraction procedure, polymer concentration, pH, temperature, and ionic composition of the solution. Similar observations regarding the importance of rheological behaviour in alginate applications have been reported by Fenoradosoa et al. (2018). The medium viscosity obtained in the present study is particularly advantageous for seed coating because it enables uniform polymer deposition without excessive solution thickness. The extracted sodium alginate contained 10% moisture, which is below the 15% maximum limit recommended by FAO standards (Coppen, 1995), indicating good storage stability and product quality. The polymer dissolved readily in both cold and hot water, whereas it remained insoluble in acetone, ethanol, methanol, and petroleum ether (Fig. 2). Similar solubility characteristics were reported by Zhou et al., (2020), who demonstrated that sodium alginate readily dissolves in water owing to its ionic and highly hydrophilic nature. Unlike several conventional polysaccharides that require heating for complete dissolution, sodium alginate forms a stable viscous colloidal solution under normal conditions, thereby facilitating its practical use in agricultural formulations, including seed-coating technologies. A remarkable property of the extracted alginate was its water absorption capacity of 5.5 g, with the polymer increasing from 1 g to 5.5 g after 24 h. This pronounced swelling behaviour reflects the hydrophilic nature of sodium alginate and its ability to retain large quantities of water through hydrogel formation. Ma et al., (2015) reported that sodium alginate hydrogels can absorb 200–300 times their own weight in water because of the abundance of hydroxyl and carboxyl functional groups within the polymer network. Such high water-retention capacity is particularly beneficial for seed-coating applications. Furthermore, Leandro et al., (2020) emphasized that comprehensive physicochemical characterization is essential to ensure the quality, safety and functionality of alginate intended for large-scale industrial applications, particularly in the food sector. The present findings satisfy these quality requirements and demonstrate the suitability of the extracted sodium alginate for further applications.

### 3.1.2 Biochemical

The biochemical composition of the extracted sodium alginate is presented in Table 1. Carbohydrates were the predominant component, accounting for 70% of the extract, whereas protein (0.95%) and lipid (0.90%) contents were comparatively low. The ash content was determined to be 18.15%, representing the total mineral residue present in the extracted alginate. The predominance of carbohydrates indicates that the extracted product was rich in polysaccharides, whereas proteins and lipids were present only in minor quantities, suggesting effective enrichment of the alginate fraction during extraction. Although the polysaccharide content obtained in the present study was slightly lower than that reported by Osman et al., (2020), it was within the range reported for alginate extracted from various alginophyte species by Rioux et al., (2007), indicating that the extraction efficiency achieved in this study was comparable with previously published reports. The ash content (18.15%) was within the acceptable range reported for commercial sodium alginate. According to the Food Chemicals Codex (1981), sodium alginate should possess ash levels within specified quality standards, while Chapman and Chapman (1980) reported ash values of up to 23% for commercially prepared alginate. Therefore, the ash content obtained in the present investigation is consistent with internationally accepted standards, indicating the satisfactory quality of the extracted sodium alginate.

### 3.1.3 Phytochemical

Furthermore, qualitative phytochemical screening revealed the absence of flavonoids, alkaloids, tannins, phlorotannins, terpenoids, steroids, glycosides and phenolic compounds (Table 1), indicating that most secondary metabolites present in the seaweed biomass were effectively removed during extraction. Only the saponins were detected in the extract. Since saponins are carbohydrate-based compounds, their presence is consistent with the structural composition of alginate. The absence of other phytochemicals indicates high purity of the extracted sodium alginate, which enhances its suitability for industrial use. Similar findings were reported by Sivagnanavelmurugan et al., (2018) and Kavitha et al., (2019), who also observed the absence of major metabolites in purified alginates, with saponins being the only detectable compound. The detection of only saponins suggests that the purified sodium alginate possesses a high degree of purity, making it suitable for subsequent applications where a clean polysaccharide matrix is required. Overall, the physiochemical, biochemical and phytochemical characteristics demonstrate that sodium alginate extracted from *Sargassum myriocystum* possesses desirable compositional properties for further utilization as a natural biopolymer, particularly as a seed-coating material.

### 3.1.4 ICP-MS elemental analysis

The elemental composition of the extracted sodium alginate, determined by ICP-MS and expressed in parts per million (ppm), is presented in Table 2. Sodium (Na) was the predominant element with a concentration of 200,390.405 ppm. The other major elements detected were calcium (Ca) (3,876.649 ppm), magnesium (Mg) (620.544 ppm), potassium (K) (200.573 ppm), phosphorus (P) (164.078 ppm), and iron (Fe) (158.246 ppm). Trace

elements, including aluminium (Al) (28.574 ppm), boron (B) (17.813 ppm), and titanium (Ti) (7.107 ppm), were present in comparatively lower concentrations. Heavy metals such as lead (Pb), arsenic (As), chromium (Cr), and cadmium (Cd) were detected at 8.685, 3.429, 2.075, and 0.158 ppm, respectively. Other elements including lithium (Li), beryllium (Be), vanadium (V), cobalt (Co), nickel (Ni), molybdenum (Mo), silver (Ag), antimony (Sb), cesium (Cs), and mercury (Hg) were detected only in trace quantities ( $\leq 1$  ppm in most cases) (Table 2). The ICP-MS analysis demonstrated that sodium was the dominant element, accounting for 200,390.405 ppm, whereas all other elements were present at considerably lower concentrations. The predominance of sodium confirms the successful conversion of alginic acid into sodium alginate during extraction and indicates a high degree of purity of the extracted polymer. Similar elemental compositions have been reported for sodium alginate extracted from brown seaweeds such as *Laminaria* and *Ascophyllum nodosum* using ICP-OES and ICP-MS techniques (Marín et al., 2010). The comparatively low concentrations of heavy metals further support the suitability of the extracted alginate for agricultural and industrial applications.

### 3.1.5 FTIR spectral analysis

The FTIR spectrum of the extracted sodium alginate showed characteristic absorption bands corresponding to the functional groups of alginate (Table 3; Fig. 4). Broad peaks at 3699.764, 3584.056, 3295.750 and 3281.286  $\text{cm}^{-1}$  were assigned to O–H stretching vibrations of hydroxyl groups associated with alcohols, phenols and carboxylic acids. The absorption band at 3100.011  $\text{cm}^{-1}$  represented aromatic C–H stretching. Strong absorption bands between 1818.544 and 1717.300  $\text{cm}^{-1}$  corresponded to C=O stretching vibrations of carboxylic acids, esters and anhydrides, confirming the presence of uronic acid residues that constitute the alginate backbone. In addition, the peaks observed at 1270.860, 1087.655 and 1050.050  $\text{cm}^{-1}$  were attributed to C–O stretching vibrations of ester and alcohol groups, confirming the polysaccharide structure of the extracted sodium alginate. Fourier Transform Infrared Spectroscopy (FTIR) is a well-established analytical technique for identifying functional groups and characterizing biomaterials (Li et al., 2011). It has been widely employed for the identification of pure compounds (Stefanowicz et al., 2009), analysis of mixtures (Basiuk, 2001), detection of impurities (Pandey et al., 2016) and determination of material composition (Hakim and Shanks, 2011). The characteristic O–H and C=O absorption bands observed in the present study are in agreement with previously reported spectra of *Sargassum*-derived sodium alginate (Latifi et al., 2015), confirming the presence of mannuronic and guluronic acid residues that form the alginate polymer. The hydroxyl and carboxyl functional groups contribute to the excellent water absorption, gel-forming ability and calcium-mediated cross-linking properties of sodium alginate, making it an ideal hydrogel-forming biopolymer. These characteristics are particularly advantageous for seed-coating applications, where improved moisture retention and enhanced seed imbibition can promote better germination and seedling establishment under moisture-deficit conditions (Belatmanian et al., 2020).

Overall, the biochemical, phytochemical, ICP-MS and FTIR analyses confirmed that the extraction procedure successfully produced high-quality sodium alginate from *Sargassum myriocystum*. The biochemical analysis revealed a carbohydrate-rich composition, phytochemical screening indicated minimal interference from secondary metabolites, ICP-MS confirmed sodium as the predominant element with only trace amounts of other elements, and FTIR verified the characteristic functional groups of sodium alginate. Collectively, these results demonstrate the purity and desirable physicochemical properties of the extracted alginate, supporting its suitability as a natural biopolymer for seed-coating applications. Accordingly, the purified sodium alginate was selected as the coating polymer for evaluating seed quality parameters in okra under moisture stress conditions.

After confirming with these parameters sodium alginate further used for seed coating and studied the seed quality parameters on okra.

### 3.2 Seed coating effect

The effect of different sodium alginate-based seed coating treatments on the physiological quality of okra seeds is presented in Table 4 and Fig 6. Significant differences were observed among the treatments for germination, abnormal seedlings, seedling growth, dry matter production and vigour indices. Germination percentage ranged from 86 to 93%, with the highest germination (93%) recorded in T<sub>14</sub>. Treatments T<sub>2</sub>, T<sub>3</sub>, T<sub>9</sub> and T<sub>13</sub> also exhibited relatively higher germination (89%), whereas the lowest germination (86%) was observed in T<sub>0</sub>, T<sub>1</sub>, T<sub>10</sub> and T<sub>12</sub> (Fig 3). The improved germination observed in T<sub>14</sub> indicates the beneficial effect of sodium alginate incorporated with humic acid, salicylic acid and ascorbic acid on seed physiological quality. During the seed-coating process, sodium alginate was ionically crosslinked with calcium chloride, in which calcium ions ( $\text{Ca}^{2+}$ ) interact with the guluronic acid residues of alginate to form a three-dimensional calcium alginate hydrogel through the well-established "egg-box" model (Lee and Mooney, 2012). This cross linked hydrogel enhances coating stability, increases water-holding capacity and enables the gradual release of the incorporated bioactive compounds, thereby creating a favourable microenvironment for seed imbibition and uniform germination (McHugh, 1987). In addition, seed coating has been reported to enhance nutrient availability, activate antioxidant enzymes and stimulate the accumulation of beneficial metabolites, resulting in improved seed metabolism and germination (Rocha et al., 2019).

The percentage of abnormal seedlings ranged from 2 to 9%, with T<sub>14</sub> recording the lowest abnormal seedlings (2%), while T<sub>0</sub>, T<sub>1</sub>, T<sub>10</sub> and T<sub>12</sub> recorded the highest abnormality (9%). The reduction in abnormal seedlings under T<sub>14</sub> suggests that the cross linked sodium alginate matrix provided a protective environment for normal embryo development while ensuring the sustained release of the incorporated bioactive compounds throughout germination. Significant differences were also observed in seedling growth among the treatments. Root length

ranged from 17.23 cm in the control (T<sub>0</sub>) to 22.36 cm in T<sub>14</sub>, while shoot length increased from 13.11 cm in T<sub>0</sub> to 18.22 cm in T<sub>14</sub>. Consequently, the highest total seedling length (40.58 cm) was recorded in T<sub>14</sub> compared with 30.34 cm in the control. The enhanced seedling growth can be attributed to the synergistic action of humic acid, salicylic acid and ascorbic acid delivered through the sodium alginate coating. Besides serving as a biodegradable carrier, sodium alginate maintains adequate moisture around the seed and supports early metabolic activity owing to its high polysaccharide content. Humic acid further promotes root elongation by improving nutrient availability and exhibiting auxin-like activity (Chen et al., 2004; Mackowiak, 2001). Similar improvements in germination, seedling length and vigour following humic acid-infused polymer coatings were reported by Sivasakthi and Renganayaki (2022). Moreover, humic substances enhance membrane permeability, ion transport, respiration and ATP synthesis through increased enzymatic activity in the Krebs cycle, thereby promoting vigorous seedling growth (Zandonadi et al., 2007).

Dry matter production varied from 0.343 g in T<sub>0</sub> to 0.463 g in T<sub>14</sub>, whereas T<sub>2</sub>, T<sub>3</sub>, T<sub>7</sub> and T<sub>13</sub> recorded intermediate values of approximately 0.398 g. The greater dry matter accumulation in T<sub>14</sub> indicates enhanced biomass production resulting from improved metabolic activity and nutrient utilization. Salicylic acid contributes to these responses by regulating photosynthesis, nitrogen metabolism, osmotic balance and antioxidant defence mechanisms (Miura and Tada, 2014). It also stimulates antioxidant enzymes, including superoxide dismutase, catalase and peroxidase, which protect germinating seeds against oxidative stress (Sedghi, 2010). Similar improvements in germination and early seedling growth following salicylic acid application have been reported in *Arabidopsis*, alfalfa and ginger (Alonso-Ramirez et al., 2009; Wang et al., 2009; Ghasemzadeh and Jaafar, 2013). Vigour Index I followed a trend similar to seedling growth, with T<sub>14</sub> recording the highest value among all treatments. Likewise, Vigour Index II reached a maximum value of 43 in T<sub>14</sub>, whereas the control recorded the lowest value of 29 (fig 3). The improved vigour indices reflect enhanced physiological activity and seedling robustness under the combined treatment. These responses are further supported by the role of ascorbic acid as a non-enzymatic antioxidant, which scavenges reactive oxygen species, protects cellular structures from oxidative damage and regulates physiological processes associated with seed germination and seedling development (Akram et al., 2017). Similar enhancement in seedling vigour following ascorbic acid treatment has been reported in alfalfa (Chen et al., 2021).

Overall, T<sub>14</sub> consistently outperformed all other treatments by producing the highest germination percentage, longest seedlings, greatest dry matter production and vigour indices, together with the lowest percentage of abnormal seedlings. The superior performance of this treatment can be attributed to the synergistic effects of humic acid, salicylic acid and ascorbic acid encapsulated within the calcium-crosslinked sodium alginate matrix. This biodegradable hydrogel not only enhances water availability and regulates the release of bioactive compounds but also promotes nutrient uptake, metabolic activity and antioxidant defence, thereby improving seedling establishment and early growth. Such integrated seed-coating systems represent an environmentally sustainable approach for enhancing seed physiological quality and crop establishment (Skrzypczak et al., 2021).

#### 4. Future Thrust

Future research should focus on optimizing the extraction and purification methods to improve the yield and functional quality of sodium alginate from *Sargassum myriocystum*. The integration of this biopolymer with different bioactive compounds, micronutrients, and beneficial microorganisms can be explored to enhance its efficiency as a multifunctional seed-coating material. Further studies under field conditions are essential to validate its performance across various crops and environments. Long-term investigations on soil health, microbial dynamics, and biodegradability will provide insights into its environmental safety and sustainability. Additionally, developing low-cost and scalable production techniques will support its industrial application and promote the replacement of synthetic polymers with natural, eco-friendly alternatives in sustainable agriculture.

#### 5. CONCLUSION

The present investigation demonstrated that sodium alginate extracted from *Sargassum myriocystum* possesses desirable physicochemical and biochemical properties suitable for agricultural use. The moderate viscosity, high carbohydrate content, and excellent water absorption capacity, combined with its biodegradability, make it an efficient and sustainable seed-coating polymer. The biopolymer and additives had a significant effect on the physiological seed performance in okra. Among the treatments, T<sub>14</sub> (sodium alginate biopolymer 2 % + humic acid 0.3 g + 250 ppm Salicylic acid + ascorbic acid 0.4 g) showed superior performance by improving stand establishment parameters such as germination, root length, shoot length, dry biomass production, vigour index I and II, and by reducing abnormal seedlings compared to the control. These findings highlight that seed coating with *S. myriocystum*-derived sodium alginate infused with biomolecules effectively enhances seed quality attributes and seedling growth. Thus, sodium alginate biopolymer represents an eco-friendly and sustainable alternative to synthetic polymers, supporting greener practices in modern seed technology.

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## Declarations

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## Competing interests

The authors declare that they have no competing interests.

## Availability of data and material

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Code availability

Not applicable.

## Authors' contributions

Pravina K. conducted the experiments, analyzed the data, and prepared the manuscript. All authors reviewed and approved the final manuscript.

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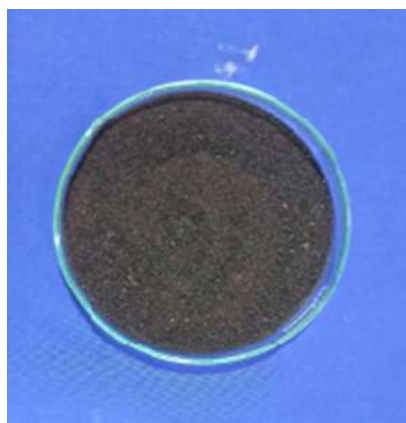
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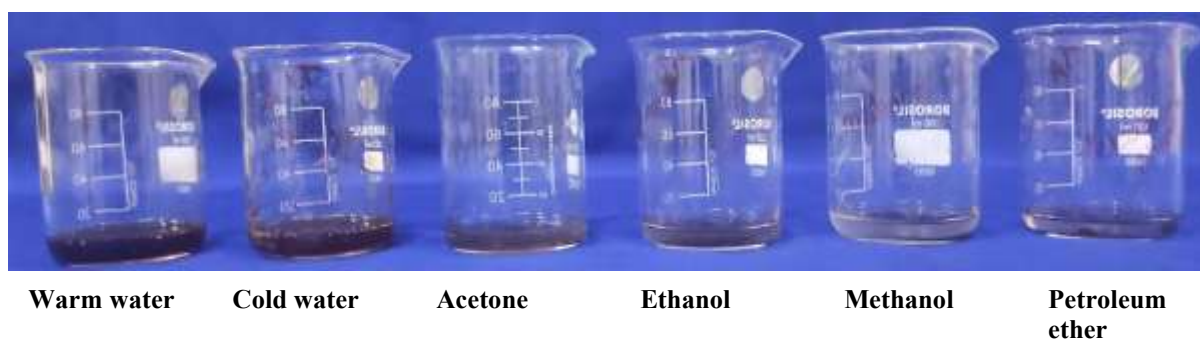
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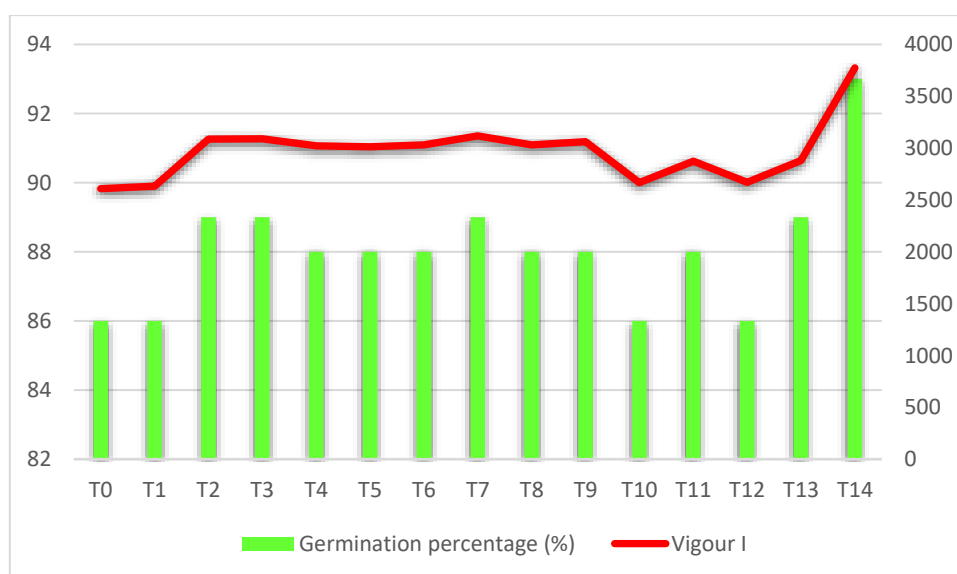
## Figures



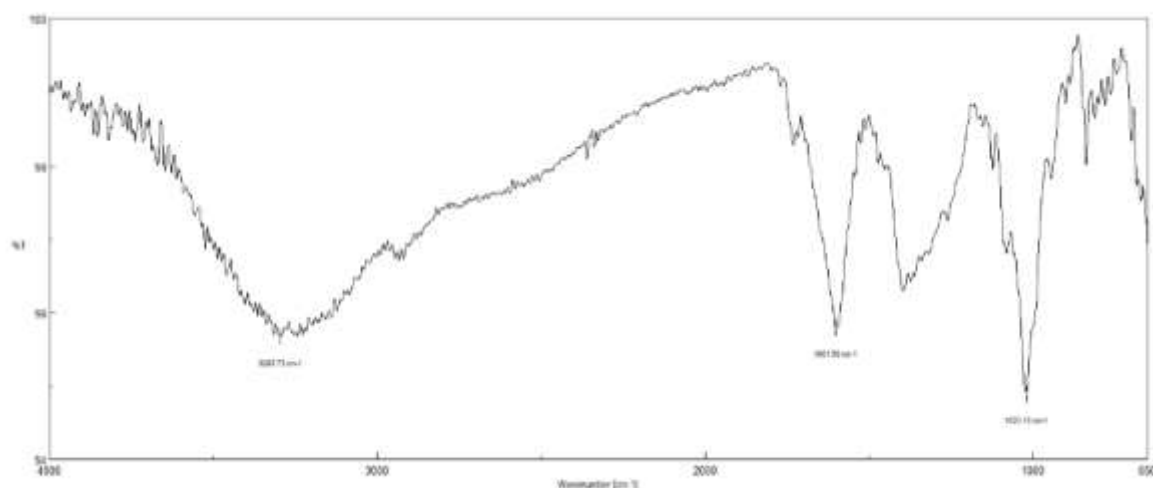
(Fig.1 Color of sodium alginate)



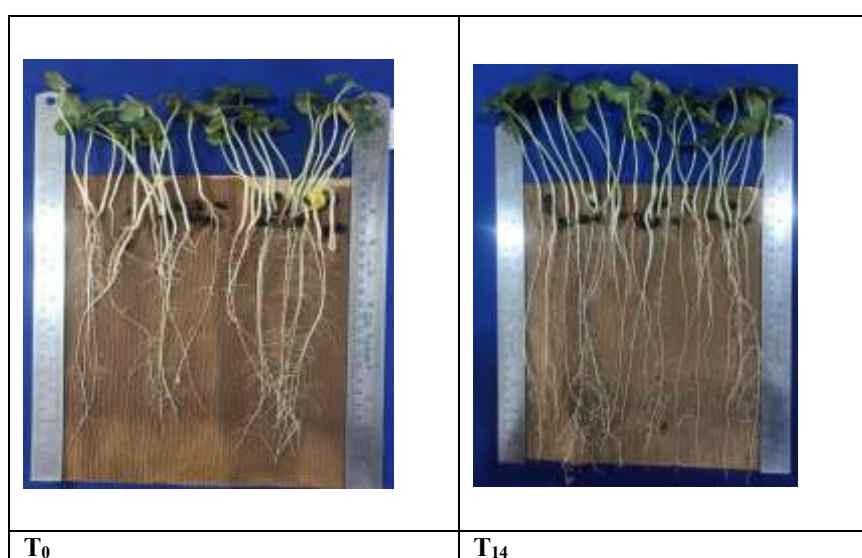
(Fig. 2 Solubility behaviour of sodium alginate)



**Fig. 3 Impact of biomolecules infused sodium alginate coating on germination percentage and vigour index I on Okra**



**Fig. 4** FTIR spectrum of sodium alginate isolated from *Sargassum myricocystum*



**Fig.6** Visualized difference in seedling

## Tables

**Table. 1** Represent the physiochemical, biochemical and phytochemical analysis of sodium alginate from seaweeds *sargassum myricocystum*

| PHYSIOCHEMICAL ANALYSIS     |                                       | BIOCHEMICAL ANALYSIS |         | PHYTOCHEMICAL ANALYSIS         |             |
|-----------------------------|---------------------------------------|----------------------|---------|--------------------------------|-------------|
| <b>Yield</b>                | 25 g                                  | <b>Carbohydrates</b> | 70 %    | <b>Flavonoids</b>              | –           |
| <b>pH</b>                   | 7.20                                  | <b>Proteins</b>      | 0.95 %  | <b>Alkaloids</b>               | –           |
| <b>Viscosity</b>            | 90 cp                                 | <b>Lipid's</b>       | 0.90 %  | <b>Tannins</b>                 | – (Absent)  |
| <b>Moisture content</b>     | 10 %                                  | <b>Ash content</b>   | 18.15 % | <b>Phlorotannins</b>           | –           |
| <b>Water absorbancy</b>     | 5.5 g                                 |                      |         | <b>Terpenoids and Steroids</b> | –           |
| <b>Solubility Behaviour</b> | Dissolves in both warm and cold water |                      |         | <b>Saponins</b>                | + (Present) |
|                             |                                       |                      |         |                                |             |
|                             |                                       |                      |         | <b>Glycosides</b>              | –           |
|                             |                                       |                      |         | <b>Phenolic Compounds</b>      | –           |

**Table. 2 Represent the IC-PMS analysis**

| <b>Element (Isotope)</b> | <b>Element Name</b> | <b>Sodium Alginate Sample (ppm)</b> |
|--------------------------|---------------------|-------------------------------------|
| 7 Li                     | Lithium             | 0.022                               |
| 9 Be                     | Beryllium           | 0.009                               |
| 11 B                     | Boron               | 17.813                              |
| 23 Na                    | Sodium              | 200390.405                          |
| 24 Mg                    | Magnesium           | 620.544                             |
| 27 Al                    | Aluminium           | 28.574                              |
| 31 P                     | Phosphorus          | 164.078                             |
| 39 K                     | Potassium           | 200.573                             |
| 44 Ca                    | Calcium             | 3876.649                            |
| 48 Ti                    | Titanium            | 7.107                               |
| 51 V                     | Vanadium            | 0.184                               |
| 52 Cr                    | Chromium            | 2.075                               |
| 55 Mn                    | Manganese           | 4.100                               |
| 57 Fe                    | Iron                | 158.246                             |
| 59 Co                    | Cobalt              | 0.125                               |
| 60 Ni                    | Nickel              | 0.876                               |
| 63 Cu                    | Copper              | 2.013                               |
| 66 Zn                    | Zinc                | 3.237                               |
| 75 As                    | Arsenic             | 3.429                               |
| 95 Mo                    | Molybdenum          | 0.084                               |
| 107 Ag                   | Silver              | 0.021                               |

| Element (Isotope) | Element Name | Sodium Alginate Sample (ppm) |
|-------------------|--------------|------------------------------|
| 111 Cd            | Cadmium      | 0.158                        |
| 118 Sn            | Tin          | 3.413                        |
| 121 Sb            | Antimony     | 0.010                        |
| 133 Cs            | Cesium       | 0.001                        |
| 137 Ba            | Barium       | 9.444                        |
| 202 Hg            | Mercury      | 0.007                        |
| 208 Pb            | Lead         | 8.685                        |

**Table. 3 FTIR frequency peak (cm<sup>-1</sup>) and functional groups of the extracted sodium alginate from seaweeds *sargassum myriocystum***

| S. No. | Wave number (cm <sup>-1</sup> ) | % Transmittance | Functional Group (Vibration)     | Bond Type / Assignment           |
|--------|---------------------------------|-----------------|----------------------------------|----------------------------------|
| 1.     | 3699.764                        | 98.5061         | O-H stretching (free)            | phenol, alcohol                  |
| 2.     | 3584.056                        | 97.6994         | O-H stretching (free)            | alcohol, phenol                  |
| 3.     | 3295.75                         | 95.6635         | O-H stretching (hydrogen-bonded) | alcohol                          |
| 4.     | 3281.286                        | 95.7942         | O-H stretching                   | carboxylic acid                  |
| 5.     | 3100.011                        | 96.2868         | C-H stretching (aromatic)        | aromatic hydrocarbon             |
| 6.     | 2906.2                          | 96.9026         | O-H stretching                   | alcohol                          |
| 7.     | 1818.544                        | 99.3767         | C=O stretching                   | anhydride                        |
| 8.     | 1780.939                        | 99.2866         | C-H bending                      | aromatic compound                |
| 9.     | 1775.154                        | 99.1976         | C=O stretching                   | conjugated anhydride             |
| 10.    | 1760.69                         | 99.156          | C=O stretching                   | carboxylic acid                  |
| 11.    | 1742.37                         | 98.6035         | C=O stretching                   | esters                           |
| 12.    | 1717.3                          | 98.3983         | C=O stretching                   | Carboxylic acids                 |
| 13.    | 1601.592                        | 95.7818         | C=C stretching                   | Aromatic Compounds               |
| 14.    | 1395.246                        | 96.3114         | O-H bending                      | carboxylic acid                  |
| 15.    | 1310.393                        | 96.8924         | O-H bending                      | phenol                           |
| 16.    | 1270.86                         | 97.3679         | C-O stretching                   | aromatic ester                   |
| 17.    | 1087.655                        | 96.9291         | C-O stretching                   | secondary alcohol                |
| 18.    | 1050.05                         | 96.5043         | C-O stretching                   | primary alcohol                  |
| 19.    | 1020.159                        | 94.885          | CO-O-CO stretching               | anhydride                        |
| 20.    | 900.5941                        | 98.8529         | C-H out-of-plane bending         | aromatic substitution            |
| 21.    | 830.205                         | 98.8992         | C-H bending                      | 1,2,3,4-tetrasubstituted benzene |

|     |          |         |                                   |                          |
|-----|----------|---------|-----------------------------------|--------------------------|
| 22. | 720.2824 | 99.4072 | aromatic out-of-plane C–H bending | benzene derivative       |
| 23. | 680.7488 | 97.7695 | C-H out-of-plane bending          | aromatic substitution    |
| 24. | 600.7175 | 96.2547 | M–O stretching                    | metal–oxygen (inorganic) |

**Table. 4 Impact of biomolecules infused Sodium alginate polymer on seed quality parameters of Okra**

| Treatments            | Abnormal seedlings (%) | Root length (cm) | Shoot length (cm) | Seedling length (cm) | Dry matter production (g/ 10 seedlings) | Vigour index II |
|-----------------------|------------------------|------------------|-------------------|----------------------|-----------------------------------------|-----------------|
| <b>T<sub>0</sub></b>  | 9                      | 17.23            | 13.11             | 30.34                | 0.343                                   | 29              |
| <b>T<sub>1</sub></b>  | 9                      | 17.59            | 13.29             | 30.88                | 0.351                                   | 30              |
| <b>T<sub>2</sub></b>  | 6                      | 19.46            | 15.21             | 34.67                | 0.398                                   | 36              |
| <b>T<sub>3</sub></b>  | 6                      | 19.46            | 15.23             | 34.69                | 0.393                                   | 36              |
| <b>T<sub>4</sub></b>  | 7                      | 19.21            | 15.12             | 34.33                | 0.394                                   | 35              |
| <b>T<sub>5</sub></b>  | 7                      | 19.17            | 15.09             | 34.26                | 0.392                                   | 34              |
| <b>T<sub>6</sub></b>  | 7                      | 19.37            | 15.26             | 34.43                | 0.391                                   | 34              |
| <b>T<sub>7</sub></b>  | 6                      | 19.68            | 15.37             | 35.05                | 0.398                                   | 35              |
| <b>T<sub>8</sub></b>  | 7                      | 19.29            | 15.18             | 34.47                | 0.394                                   | 35              |
| <b>T<sub>9</sub></b>  | 7                      | 19.49            | 15.32             | 34.81                | 0.396                                   | 34              |
| <b>T<sub>10</sub></b> | 9                      | 17.49            | 13.53             | 31.02                | 0.353                                   | 30              |
| <b>T<sub>11</sub></b> | 7                      | 19.39            | 15.27             | 34.66                | 0.397                                   | 32              |
| <b>T<sub>12</sub></b> | 9                      | 17.78            | 13.25             | 31.03                | 0.351                                   | 30              |
| <b>T<sub>13</sub></b> | 6                      | 19.42            | 15.31             | 34.73                | 0.398                                   | 32              |
| <b>T<sub>14</sub></b> | 2                      | 22.36            | 18.22             | 40.58                | 0.463                                   | 43              |
| <b>Mean</b>           | 7                      | 20.05            | 15.88             | 35.93                | 0.410                                   | 35              |
| <b>S.Ed</b>           | 0.088                  | 0.265            | 0.203             | 0.415                | 0.006                                   | 0.388           |
| <b>CD (0.05%)</b>     | 0.178*                 | 0.535*           | 0.409*            | 0.839*               | 0.013*                                  | 0.784*          |

(T<sub>0</sub> – control, T<sub>1</sub>– Sodium alginate Polymer 2 % 250 ml, T<sub>2</sub> - 0.2 g Humic acid, T<sub>3</sub>- 0.3 g Humic acid, T<sub>4</sub> - 0.4 g Fulvic acid, T<sub>5</sub>- 0.5 g Fulvic acid, T<sub>6</sub> - Salicylic acid 200 ppm, T<sub>7</sub> - Salicylic acid 250 ppm, T<sub>8</sub>– Brassinolide 0.5 ppm, T<sub>9</sub> - Brassinolide 1.0 ppm, T<sub>10</sub>- 0.05 g Glutathione, T<sub>11</sub> - 0.20 g Glutathione, T<sub>12</sub> - 0.2 g Ascorbic acid, and T<sub>13</sub> - 0.4 g Ascorbic acid T<sub>14</sub> - 0.3 g Humic acid + 250 ppm Salicylic acid + 0.4 g Ascorbic acid)