

Original Research Article

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Assessment of a *Spirulina platensis*–Vegetable Extract Formulation as a Foliar Biofertilizer for Plant Growth

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ABSTRACT

The development of biologically derived agricultural inputs is of interest for improving plant performance while reducing reliance on conventional chemical inputs. This investigation evaluated a formulation containing *Spirulina platensis* biomass and extracts of selected cruciferous vegetables. *S. platensis* was grown in Zarrouk's medium, while aqueous extracts were prepared from radish (*Raphanus sativus*), broccoli (*Brassica oleracea* var. *italica*), cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), and turnip (*Brassica rapa*). Equal portions of the vegetable extracts were combined with *Spirulina* biomass and a commercial bioenzyme to obtain the test formulation. The preparation showed complete solubility after dilution and a reported pH of 6.4–6.8. Its antibacterial activity was examined by disc diffusion against the selected bacterial strains. The combined vegetable extract produced inhibition zones as large as 18 mm against *Staphylococcus aureus* and *Bacillus subtilis*. Plant-growth observations further showed greater reported shoot and root lengths in treated mustard, sunflower, and fenugreek plants than in the corresponding controls. Molecular docking generated reported binding-affinity values between –9.0 and –4.4 kcal/mol. These observations suggest that the *Spirulina*–vegetable formulation merits additional experimental assessment as a biofertilizer and foliar treatment.

Keywords

Spirulina platensis, Zarrouk's medium, glucosinolates, biofertilizer, seed treatment

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Introduction

Crop production is fundamental to food security and has an important role in the global economy. Growing demand for agricultural produce has contributed to extensive use of chemical fertilizers. Long-term or excessive application of such inputs, however, has been associated with deterioration of soil quality,

contamination of water resources, eutrophication, disturbance of beneficial microbial communities, and ecological imbalance. These concerns have encouraged investigation of alternative approaches based on biological resources. Biofertilizers are agricultural preparations that use beneficial microorganisms or biologically derived components to improve nutrient availability and support soil fertility. Such preparations

can provide an alternative means of supporting plant development while maintaining biological activity within the soil environment (Bhattacharyya and Jha, 2012). Spirulina, generally associated with *Arthrospira*, is of particular interest because its biomass contains proteins, vitamins, minerals, pigments, and several bioactive constituents. These characteristics make photosynthetic Spirulina biomass a potential resource for agricultural formulations (Kumar *et al.*, 2013).

Cruciferous vegetables contain nutritionally important minerals, vitamins, antioxidant compounds, and glucosinolates. Combining extracts from these vegetables with Spirulina biomass may therefore provide a formulation containing multiple biologically derived components. The present investigation was designed to prepare such a formulation and examine its suitability for foliar and soil application, its activity against selected bacterial pathogens, and its association with plant-growth measurements.

Specifically, *S. platensis* was cultivated under alkaline conditions and subsequently incorporated with extracts of selected cruciferous vegetables. The resulting preparation was examined for physicochemical characteristics, antimicrobial activity, and plant-growth effects. Molecular docking was also undertaken to investigate the reported interaction between a glucosinolate-related ligand and its target protein. The study therefore combines formulation development with preliminary biological and computational evaluation.

Materials and Methods

Cultivation of Spirulina

A culture of *Spirulina platensis* was procured from Mahathi Biotech Private Ltd., Ashok Nagar, Chennai. The organism was introduced into sterile Zarrouk's medium adjusted to pH 10.5 and maintained for 25 days under indirect sunlight.

Preparation of Vegetable Extracts

Five cruciferous vegetables described in the study as glucosinolate-containing materials were selected: radish (*Raphanus sativus*), broccoli (*Brassica oleracea* var. *italica*), cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), and turnip (*Brassica rapa*). The vegetables were obtained from a

local grocery supermarket in Egmore, Chennai. For each vegetable, approximately 35 g of material was weighed and cut into smaller pieces. The material was gently macerated with a mortar and pestle and transferred to sterile distilled water for a 45-day soaking period. During this period, the preparations were swirled three times per day.

Incorporation of Bioenzyme

A commercially available bioenzyme obtained from an agricultural supplier was incorporated into the formulation at a volume of approximately 5 mL. The source manuscript associates bioenzyme activity with possible changes in soil properties through processes including cation exchange, specific binding, and surfactant action, with possible consequences for nutrient availability.

Determination of Glucosinolate-Related Absorbance

Freeze-dried vegetable powder (100 mg) was extracted using 10 mL of preheated 70% ethanol. The extraction mixture was maintained at 70 °C for 30 min to inactivate myrosinase and then centrifuged at 5000 rpm for 10 min. The resulting supernatant was treated with sodium tetrachloropalladate (II). Absorbance was measured with a digital single-beam spectrophotometer. The recorded observations are presented for wavelengths of 220–240 nm; the source methodology additionally describes absorbance measurement at 420–425 nm (Bench Chem Report, 2025).

Formulation of the Biofertilizer

The five vegetable extracts were mixed in equal proportions. Spirulina biomass corresponding to 10 mg pellet per 100 mL of vegetable extract was added together with 5 mL of the commercial bioenzyme. The components were thoroughly mixed and maintained for a 3-day biocuring period. For the solubility assessment, 10 mL of the completed preparation was diluted with 100 mL of water.

Antimicrobial Activity Assay

Antibacterial activity was investigated using agar disc diffusion against *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and

Klebsiella pneumoniae. The strains were reported as isolates from stored vegetables and were obtained from HetroGene Biotech Laboratory.

Bacterial suspensions were adjusted to 0.5 McFarland turbidity and spread on Mueller–Hinton agar. Whatman No. 1 filter-paper discs, 6 mm in diameter, were loaded with 50 μ L of the test material and positioned on the inoculated plates.

Individual vegetable extracts and *Spirulina* extract were tested in the same manner, while sterile distilled water was used as the negative control. Following incubation at 37 °C for 24 h, the diameter of each inhibition zone was measured in millimetres.

Biofertilizer Application

Seed Material

Uniform, healthy, disease-free seeds of mustard, sunflower, and fenugreek were obtained from a horticultural seed supplier. Surface sterilization was carried out using 1% sodium hypochlorite for 2–3 min, followed by thorough washing with distilled water.

Experimental Design and Growth Measurements

The plant experiment followed a Completely Randomized Design (CRD) and included an untreated control. As reported in the source study, fenugreek seeds were immersed in the *Spirulina*–vegetable formulation for 12 h, planted in soil receiving a pretreatment dose of 10 mL/kg soil, and subsequently given 10 mL foliar applications on alternate days. Germination, shoot length, and root length were monitored for 20 days. Growth measurements were reported for mustard, sunflower, and fenugreek.

Molecular Docking Analysis

A PDB-format three-dimensional protein structure associated with glucosinolate degradation was obtained from the RCSB Protein Data Bank. The corresponding glucosinolate ligand was obtained from PubChem. PyMOL and MzDOCK were used to assess ligand–protein interaction and obtain binding-affinity values. The reported docking procedure was referenced to Trott and Olson (2010).

Results and Discussion

Growth of *Spirulina* in Zarrouk’s Medium

Visible development of *Spirulina platensis* occurred in the Zarrouk’s medium maintained at pH 10.5.

Visual Observation

- During cultivation, the medium changed from a clear or light-green appearance to a darker green/bluish-green colour (Figure- 1). This change was recorded as evidence of increased visible *Spirulina* biomass.
- The progressive darkening of the culture was therefore used as a qualitative indication of biomass development during the 25-day cultivation period.

Microscopic Observation

- Microscopic examination revealed filamentous trichomes with a spiral morphology.
- This observed morphology was considered consistent with *S. platensis* and provided a qualitative indication of the identity of the cultivated material (Figure- 2).

Glucosinolate-Related Measurements

The spectrophotometric observations obtained from the individual vegetable extracts are summarized in Tables 1–5. The values are reproduced as recorded in the study for each specified wavelength.

Recovery of *Spirulina* Biomass

Following cultivation in Zarrouk’s medium for 25 days shown in Figure-3, the *Spirulina* biomass was recovered from the culture medium for subsequent formulation of the biofertilizer. The harvested biomass was used as the *Spirulina* component in combination with the vegetable extracts for preparation of the foliar biofertilizer.

Preparation and Solubility of the Combined Formulation

Figures 4 and 5 illustrate the preparation and water-solubility of the combined *Spirulina*–vegetable extract formulation. As shown in Figure 4.1, the *Spirulina* biomass was combined with the mixed vegetable extract and 5 mL of bioenzyme, followed by a three-day biocuring period. The resulting formulation exhibited a

uniform dark appearance and a slightly fermented odour, indicating successful integration of the components. Figure 4.2 demonstrates the solubility of the prepared formulation, in which 10 mL of the formulation was diluted with 100 mL of water. Complete solubilization was observed after dilution, with no visible undissolved particles or sedimentation. The resulting homogeneous aqueous preparation indicates that the formulation can be readily dispersed in water, supporting its use as a foliar application in the subsequent plant-growth experiments.

Antimicrobial Activity

The antimicrobial activity of the Spirulina extract, individual vegetable extracts, and the combined vegetable extract formulation was evaluated against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae* using the agar disc diffusion method. The inhibition zones varied among the tested extracts and bacterial strains, indicating differences in antimicrobial response. The Spirulina extract produced inhibition zones of 14, 12, 15, 14, and 15 mm against *S. typhi*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *K. pneumoniae*, respectively. Among the individual vegetable extracts, turnip showed comparatively higher activity against *S. aureus* (16 mm), while broccoli produced inhibition zones of 11, 10, 12, 10, and 12 mm against the five tested organisms, respectively. The combined vegetable extract exhibited inhibition zones of 12 mm against *S. typhi*, 16 mm against *P. aeruginosa*, 18 mm against *S. aureus*, 18 mm against *B. subtilis*, and 14 mm against *K. pneumoniae*. The maximum inhibition observed was 18 mm against both *S. aureus* and *B. subtilis*, demonstrating that the combined vegetable extract showed appreciable antibacterial activity against these organisms. Overall, the observed inhibition zones indicate that both Spirulina and the vegetable-derived components contributed to the antimicrobial properties of the formulation, with the combined extract displaying enhanced activity against selected bacterial strains. These findings support the potential of the formulated preparation as a biofertilizer with additional antimicrobial characteristics.

Effect of the Formulation on Plant Growth using Bio-Fertilizer

The effect of the formulated Spirulina–vegetable extract preparation on plant growth was evaluated using mustard, sunflower, and fenugreek as test plants. Shoot

and root lengths were recorded after 20 days and compared with untreated control plants. The treated plants generally exhibited greater shoot and root development than the corresponding controls, indicating a positive growth response to the formulation.

Growth Response of Mustard (*Brassica juncea*)

Treatment with the formulated preparation resulted in increased growth of mustard plants compared with the untreated control. The treated plants attained a shoot length of 43 cm and a root length of 12 cm, whereas the control plants recorded shoot and root lengths of 28 cm and 10 cm, respectively.

Thus, the treated plants showed an increase in both shoot and root development relative to the control. The greater shoot elongation observed in the treated plants suggests that the formulation supported vegetative growth under the experimental conditions.

Growth Response of Sunflower (*Helianthus annuus*)

A difference in growth was also observed between treated and untreated sunflower plants. The treated plants recorded a shoot length of 194 cm and a root length of 40 cm, compared with 12 cm and 25 cm, respectively, in the control plants.

The reported values therefore indicate substantially greater shoot development in the treated group, accompanied by an increase in root length.

These observations suggest that application of the formulation was associated with enhanced sunflower growth during the experimental period.

Growth Response of Fenugreek (*Trigonella foenum-graecum*)

Fenugreek plants treated with the formulation showed greater growth than the untreated controls. The treated plants attained a shoot length of 78 cm and a root length of 11 cm, while the control plants recorded 23 cm and 4.5 cm, respectively. The increase in both shoot and root length indicates a positive growth response following treatment. The results further demonstrate that the formulation influenced vegetative development across the three plant species evaluated.

Table.1 Spectrophotometric Readings for the Five Vegetable Extracts

S. No	OD (nm)	RECORDED VALUE				
		Radish	Cabbage	Turnip	Cauliflower	Broccoli
1	220	3.71	3.81	2.81	2.71	1.71
2	225	3.78	3.78	3.78	3.58	2.8
3	230	3.83	3.73	3.73	3.73	3.70
4	235	3.15	3.15	3.15	3.15	3.65
5	240	2.78	2.78	3.88	3.80	3.89

Table.2 Antibacterial Inhibition Zones of Spirulina and Vegetable Extracts

Bacterial pathogen	Spirulina platensis extract	Radish	Cabbage	Turnip	Cauliflower	Broccoli	Total vegetable extract
<i>Salmonella typhi</i>	14mm	10mm	8mm	10mm	9mm	11mm	12mm
<i>Pseudomonas aeruginosa</i>	12mm	6mm	8mm	6mm	8mm	10mm	16mm
<i>Staphylococcus aureus</i>	15mm	10mm	8mm	16mm	9mm	12mm	18mm
<i>Bacillus subtilis</i>	14mm	8mm	7mm	9mm	9mm	10mm	18mm
<i>Klebsiella pneumoniae</i>	15mm	10mm	8mm	8mm	10mm	12mm	14mm

Table.3 Effect of the Spirulina–vegetable extract formulation on shoot and root growth of test plants after 20 days

Test plant	Treatment	Shoot length (cm)	Root length (cm)
<i>Brassica juncea</i> (Mustard)	Formulation-treated	43	12
	Untreated control	28	10
<i>Helianthus annuus</i> (Sunflower)	Formulation-treated	194	40
	Untreated control	12	25
<i>Trigonella foenum-graecum</i> (Fenugreek)	Formulation-treated	78	11
	Untreated control	23	4.5

Figure.1 Culture of Spirulina platensis in Zarrouk's Medium after 25 Days



Figure.2 Microscopic View of *S. platensis*

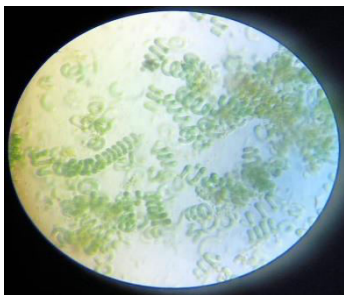


Figure.3 Spirulina Pellet

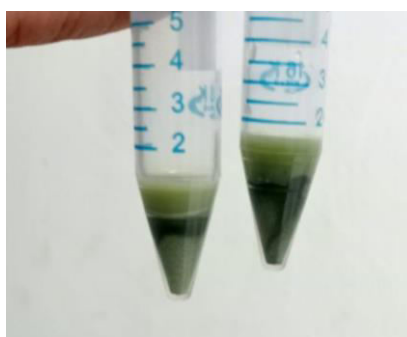


Figure.4 Combined *Spirulina platensis*–vegetable formulation

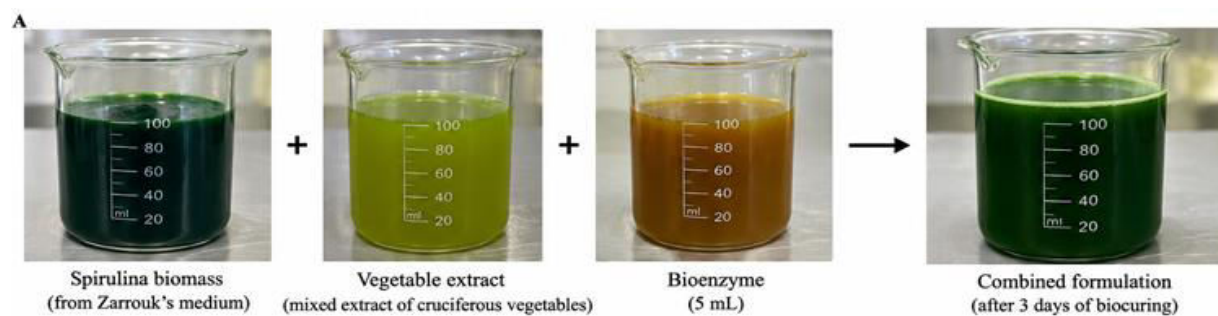


Figure.5 Solubility test

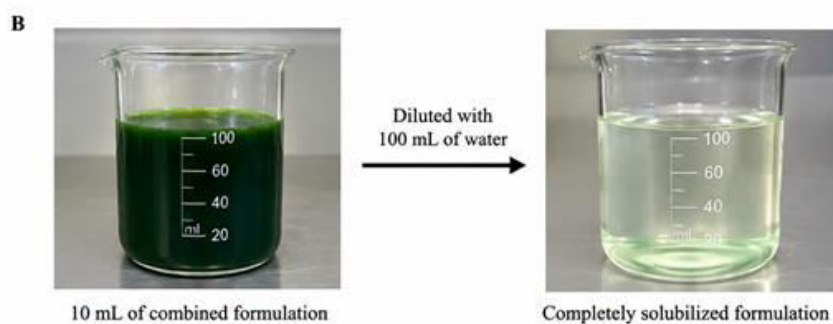


Figure.6 Antimicrobial activity of *Spirulina* extract, individual vegetable extracts, and the combined vegetable extract against selected bacterial pathogens, expressed as inhibition zones (mm).

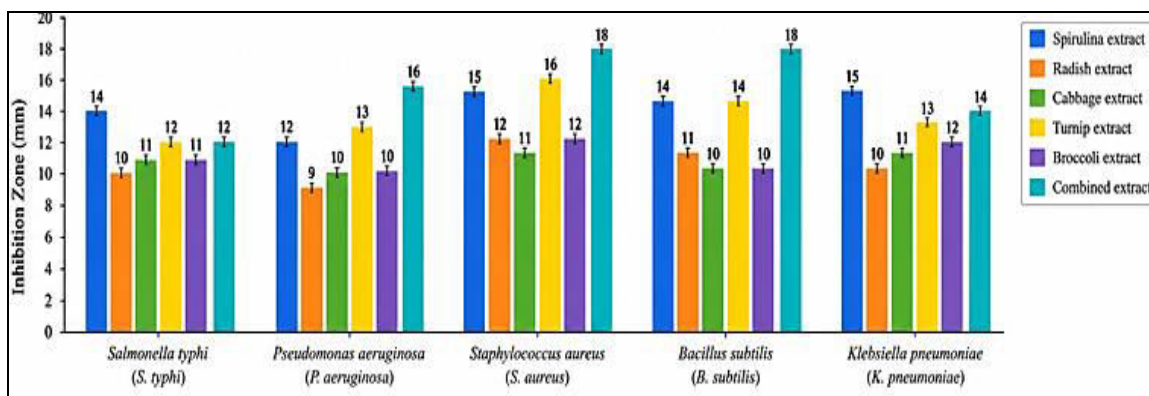


Figure.7 Agar disc diffusion assay showing the inhibition zones produced by the combined vegetable extract against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae*.

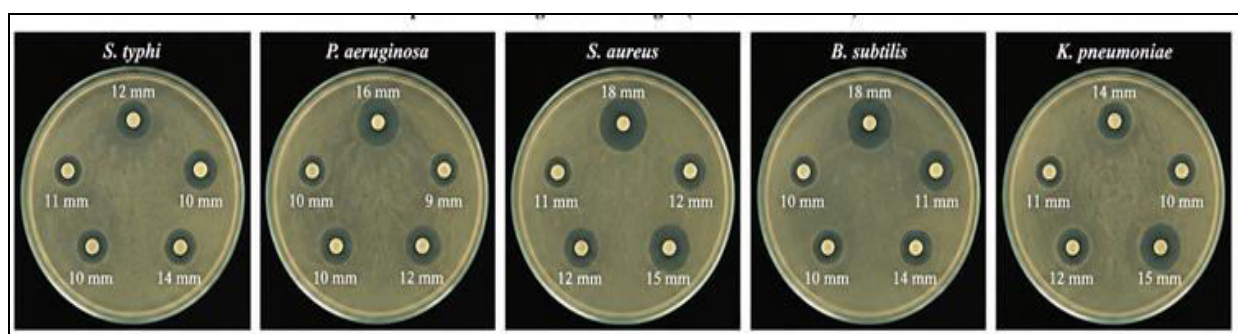
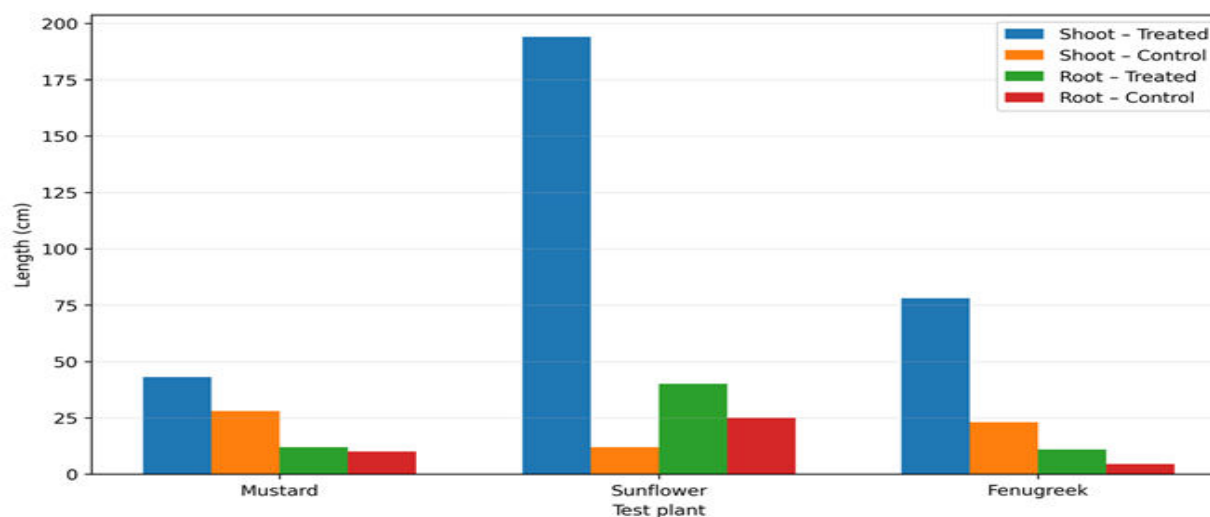


Figure.8 Effect of the *Spirulina*–vegetable extract formulation on shoot and root growth of mustard, sunflower, and fenugreek after 20 days.



In conclusion, the present study demonstrated the potential of the combined *Spirulina platensis*–vegetable extract formulation as a biofertilizer with plant-growth-promoting and antimicrobial properties. *Spirulina platensis* was successfully cultivated in Zarrouk's medium and incorporated with extracts of radish, cabbage, turnip, cauliflower, and broccoli to prepare the combined formulation. The formulation showed complete solubility after dilution with water, indicating its suitability for foliar application. The antimicrobial assay demonstrated inhibitory activity against all the bacterial pathogens tested. The total vegetable extract produced the maximum inhibition zone of 18 mm against both *Staphylococcus aureus* and *Bacillus subtilis*. Inhibition was also observed against *Salmonella typhi* (12 mm), *Pseudomonas aeruginosa* (16 mm), and *Klebsiella pneumoniae* (14 mm), demonstrating the antimicrobial potential of the vegetable-based formulation. The plant-growth experiment showed clear differences between formulation-treated and untreated plants. In mustard, shoot length increased from 28 to 43 cm (53.6% increase), while root length increased from 10 to 12 cm (20.0% increase). In sunflower, the reported shoot length increased from 12 to 194 cm (1,516.7% increase) and root length from 25 to 40 cm (60.0% increase). In fenugreek, shoot length increased from 23 to 78 cm (239.1% increase), whereas root length increased from 4.5 to 11 cm (144.4% increase). These comparisons indicate that the formulation was associated with increased shoot and root development across all three tested plant species under the experimental conditions.

Overall, the findings indicate that the combined *Spirulina*–vegetable extract formulation has potential as a sustainable agricultural input by integrating plant-growth enhancement with antimicrobial activity. Further replicated experiments, statistical validation, field trials, and optimization of formulation concentration and application frequency are required to establish its effectiveness under practical agricultural conditions.

Author Contributions

Ranal Catherin A: Methodology, Writing original draft. Sadhana R: Methodology, Writing original draft. Rajasri S: Software, Validation, Formal Analysis. Rishimithra K: Methodology, Writing original draft. Radhika S: Methodology, Writing original draft. Vidyadharani G: Concept, Preparation, Writing - Review & Editing

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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