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REVIEWER'S REPORT

Manuscript No.: IJAR- 58673

Title: ***Eichhornia crassipes-Based Hydroponic Growth Medium: A Sustainable Approach to Plant Cultivation and Resource Valorization*****.**

Recommendation:
Accept as it is .....
Accept after Minor revision.....
Accept after major revision
Do not accept (*Reasons below*)

Rating	Excel.	Good	Fair	Poor
Originality		✓		
Techn. Quality		✓		
Clarity			✓	
Significance			✓	

Reviewer Name: **ANAPANA GOPAL**

Reviewer's Comment for Publication.

General Comments

The manuscript presents an interesting and potentially useful approach for valorizing *Eichhornia crassipes* biomass into a CMC-based hydrogel for cultivation of *Vigna radiata*. The topic is relevant to sustainable agriculture, invasive aquatic weed management, biomass valorization and soilless cultivation. The authors compare Eichhornia-CMC hydrogel with CMC hydrogel and soil and evaluate several plant-growth and biochemical parameters.
However, several important methodological, statistical, calculation and interpretation problems need to be addressed before the manuscript can be considered for publication. In its current form, I recommend Minor Revision.

Minor Comments

1. The experimental design does not clearly establish this as a hydroponic system

The manuscript describes the experiment as hydroponic cultivation, but the Materials and Methods do not describe a hydroponic nutrient solution, nutrient formulation, electrical conductivity, pH management, or nutrient supply regime. The plants were placed directly on growth media in paper cups and later harvested at the microgreen stage.
The authors should clarify:

- What exactly constitutes the hydroponic system?
- Was a nutrient solution supplied?
- What was its composition?
- How frequently was it supplied?
- What were the pH and EC values?
- How much water/nutrient solution was supplied to each treatment?

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If no nutrient solution was used, the terminology **"hydroponic growth medium"** should be reconsidered. The experiment may be better described as a **soilless/substrate-based cultivation experiment**.

2. Panchakavya is a major confounding factor

The Eichhornia-CMC hydrogel was prepared using **3% CMC + 5% Eichhornia polymer + 1% Panchakavya**, whereas the manuscript describes CMC hydrogel and soil as comparison treatments.

This is a critical experimental-design problem.

If Panchakavya was included only in the Eichhornia-CMC treatment, the improved plant performance cannot be attributed specifically to *Eichhornia crassipes*. Panchakavya itself may contain nutrients and biologically active compounds that influence plant growth.

The authors should either:

- include a Panchakavya-containing control; or
- explain clearly that the effect represents the **combined Eichhornia + CMC + Panchakavya formulation**.

The present conclusion attributing the growth enhancement to the Eichhornia-CMC hydrogel is therefore too strong.

3. The statistical analysis is incomplete

The manuscript states:

"All experiments were performed in triplicate"

and proposes one-way ANOVA followed by an appropriate post-hoc test at $p < 0.05$.

However, the Results tables do **not provide**:

- mean ± SD/SE for most parameters;
- F-values;
- degrees of freedom;
- *p*-values;
- post-hoc test results;
- statistical superscripts.

Therefore, statements such as **"significantly enhanced"** in the Conclusion are not supported by the presented results.

The authors should actually perform and report the statistical analysis rather than merely stating that ANOVA "should be evaluated."

4. "Triplicate" needs clarification

The manuscript should specify whether the three replicates represent:

- three independent biological experiments;
- three containers;
- three plants;
- or three technical measurements from the same sample.

If three measurements were taken from the same experimental unit, they should not automatically be treated as independent biological replicates.

The experimental unit and sample size (**n**) should be explicitly reported for every parameter.

5. The water-content values in Table 4 appear mathematically incorrect

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Table 4 reports:

- Eichhornia hydrogel: fresh weight 60 g, dry weight 4 g, water content **53.33%**
- CMC hydrogel: 55 g, 3.5 g, **48.63%**
- Soil: 57 g, 3.7 g, **50.50%**.

However, if water content is calculated conventionally as:
Water content (%) = [(Fresh weight − Dry weight) / Fresh weight] × 100
the values would be approximately:

- Eichhornia: **93.33%**
- CMC: **93.64%**
- Soil: **93.51%**

Therefore, the reported values require immediate verification.
The authors must provide the exact formula used and correct Table 4 if necessary.

6. Table 9 contains unclear and apparently incorrect presentation of shoot/root measurements

Table 9 reports:

- Eichhornia: **8 cm ± 29.2 cm** shoot length and **3 cm ± 9 cm** root length
- CMC: **5 cm ± 22.2 cm** and **1 cm ± 6.5 cm**
- Soil: **7 cm ± 28.8 cm** and **2.8 ± 8.2 cm**.

This format is scientifically unclear.
If these represent **mean ± SD**, the values appear reversed or incorrectly formatted. If they represent minimum–maximum ranges, the ± symbol should not be used.
The authors should recheck the raw data and report the results as, for example:
Shoot length: 18.5 ± 2.3 cm
or:
Range: 8.0–29.2 cm
but not a combination of both.

7. The Seed Vigour Index requires verification

The manuscript defines:
SVI = Germination Percentage × Mean Seedling Length.
All three treatments have **100% germination**, while the reported SVI values are:

- Eichhornia = 382
- CMC = 287
- Soil = 370.

The manuscript needs to show the actual **mean seedling length used to calculate each SVI** and the units used.
Because germination was 100% in all treatments, the differences in SVI must arise entirely from seedling length. The reported values therefore need to be reconciled with the growth data in Table 9.

8. Leaf Area Index is incorrectly expressed in cm²

Table 10 is labelled **"Leaf Area Index (LAI)"**, but the values are reported as:

- 4 cm²
- 2 cm²
- 3.5 cm².

LAI is calculated as leaf area divided by ground area and is therefore **dimensionless**.

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If the authors actually measured leaf area, it should be reported as **cm²** and the table should be titled **Leaf Area**, not Leaf Area Index.
If LAI was genuinely calculated, the ground area must be provided and the results should have no cm^2 unit.

9. Ash content should be reported more appropriately
Table 3 reports ash contents of 3.65, 3.50 and 3.55 g.
Ash content is generally expressed as a percentage of the original dry sample mass unless there is a specific reason for reporting absolute ash mass.
The authors should provide:

- initial dry sample weight;
- final ash weight;
- calculation formula;
- number of replicates;
- units.

Without the initial sample mass, the reported ash values cannot be meaningfully compared.

10. Auxin identification by Salkowski assay needs cautious interpretation
The manuscript reports auxin concentrations of approximately **4.2–4.6 µg/mL** and interprets this as endogenous auxin in *Eichhornia* extract.
However, the Salkowski reaction is a **colorimetric assay and is not sufficiently specific to establish IAA identity by itself**.
The authors should therefore avoid saying that the experiment definitively "confirmed" IAA unless supported by an appropriate analytical confirmation method.
Ideally, IAA should be confirmed using HPLC, LC-MS/MS, or another validated analytical technique.
At minimum, the manuscript should clearly describe the result as **Salkowski-reactive indolic compounds/IAA-equivalent concentration**, depending on the actual standard used.

11. The nutrient contribution of the Eichhornia polymer was not characterized
The manuscript repeatedly suggests that the Eichhornia biomass supplied nutrients and enhanced mineral uptake. However, the authors themselves acknowledge that the elemental composition of the extracted polymer was not directly analyzed.
This makes statements about enhanced nutrient availability speculative.
The authors should ideally determine:

- N;
- P;
- K;
- Ca;
- Mg;
- Fe;
- other relevant minerals.

This would substantially strengthen the proposed mechanism.

12. Water-retention capacity was inferred but not directly measured

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The manuscript repeatedly attributes improved plant performance to the hydrogel's superior water-retention properties.
However, there is no clearly described direct measurement of:

- swelling ratio;
- water-holding capacity;
- water-release kinetics;
- water absorption;
- moisture retention over time.

The higher plant water content does **not by itself demonstrate that the hydrogel has superior water-retention capacity**.
The authors should either experimentally measure hydrogel water-retention properties or moderate these mechanistic claims.

13. Hydrogel characterization is insufficient

For a study proposing a new hydrogel substrate, basic material characterization is expected.
The manuscript should ideally include:

- swelling ratio;
- water absorption capacity;
- moisture retention;
- biodegradation;
- pH;
- electrical conductivity;
- mechanical stability;
- structural characterization;
- polymer composition.

The current characterization is essentially limited to extraction yield and pH.
Therefore, the claim that a stable and functional hydrogel has been developed requires stronger evidence.

14. The claim of "cost-effective" is not demonstrated

The Abstract describes the approach as a cost-effective alternative, and the Conclusion suggests that the material has potential to reduce production costs.
However, no economic analysis is provided.
There is no information on:

- biomass collection cost;
- transportation;
- drying;
- extraction;
- energy consumption;
- CMC cost;
- Panchakavya cost;
- processing cost;
- cost per unit hydrogel;
- comparison with cocopeat, rockwool or other commercial substrates.

The wording should therefore be changed from **"cost-effective"** to **"potentially cost-effective"** unless an economic analysis is conducted.

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15. Sustainability claims require further evidence

The study presents the hydrogel as biodegradable and sustainable. However, biodegradability itself has not been experimentally measured.

The manuscript's own conclusion acknowledges that **biodegradation behaviour, nutrient-release kinetics, mechanical properties and economic feasibility remain to be investigated**.

Therefore, the current manuscript should describe the material as **potentially biodegradable/sustainable**, rather than experimentally demonstrating biodegradability.

16. Possible safety issue associated with water hyacinth biomass should be addressed

Eichhornia crassipes is known to accumulate contaminants from aquatic environments. The plants were collected from a freshwater lake at Porur, Chennai.

Before proposing the biomass as an agricultural substrate, the authors should consider testing the collected biomass/extract for potentially accumulated contaminants, particularly:

- heavy metals;
- other relevant pollutants depending on the collection site.

This is especially important if the eventual application is intended for edible microgreens.

17. The study duration is not sufficiently reported

The manuscript states that seedlings were initially maintained under dark conditions for four days and then transferred to natural daylight, with plants harvested at the microgreen stage.

However, the total cultivation period until harvest is not clearly stated.

The authors should provide:

- total experimental duration;
- exact harvest day;
- photoperiod/light conditions;
- temperature;
- humidity;
- watering/nutrient schedule.

These factors can substantially influence microgreen growth.

18. Soil control is not adequately characterized

The soil treatment is used as a major comparator, but the manuscript does not adequately describe the soil.

The authors should report:

- soil type;
- source;
- pH;
- EC;
- organic matter;
- moisture content;
- nutrient composition;
- sterilization/pre-treatment, if any.

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Without this information, reproducibility is limited.

19. The biochemical concentrations appear unusually low and require verification

The reported values are:

- carbohydrate: 0.035–0.045 mg/g;
- protein: 0.040–0.050 mg/g;
- lipid: 0.001–0.003 mg/g.

These values should be checked carefully for:

- unit correctness;
- dilution factors;
- extraction volume;
- sample mass;
- calibration curve;
- whether values should be mg/g fresh weight or dry weight.

The Methods section should provide enough calculation detail to reproduce these results.

20. Some mechanistic interpretations are stronger than the experimental evidence

The Discussion frequently attributes increased growth to:

- enhanced nutrient diffusion;
- improved root-zone aeration;
- improved nutrient uptake;
- endogenous auxin;
- increased photosynthesis;
- bioactive compounds.

However, most of these mechanisms were not directly measured.

For example, the manuscript itself appropriately acknowledges that the direct contribution of auxin was not established.

Similar caution should be applied throughout the Discussion.

Minor Comments

21. Scientific nomenclature and formatting

Ensure consistent formatting of:

- *Eichhornia crassipes*
- *Vigna radiata*

Scientific names should be italicized consistently.

22. Figure captions require language editing

For example, the caption:

"Growing of *Vigna radiata* L. plant in of *Eichhornia crassipes* Hydrogel"

should be rewritten grammatically.

Suggested:

Figure 3. Growth of *Vigna radiata* L. seedlings in Eichhornia-CMC hydrogel.

The photographs on page 18 provide useful visual evidence, but the experimental stages should be clearly labelled.

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23. Table titles and units require substantial standardization
Examples:

- "gms" → **g**
- "mg/g" → preferably **mg g^{-1}**
- "cm2" → **cm^2**
- "µg/ml" → **µg mL^{-1}**
- consistent capitalization of *Vigna radiata*.

24. Table 1 should not be called "phytochemicals" unless justified
Carbohydrates, proteins and lipids are biochemical constituents/macronutrients rather than a conventional phytochemical profile.
A more appropriate title would be:
Biochemical composition of *Vigna radiata* seedlings grown under different media.

25. The Results and Discussion should be separated or more clearly structured
At present, Results and Discussion are combined. This is acceptable for some journals, but the manuscript frequently repeats numerical findings followed by speculative mechanisms.
A more concise structure would improve readability.

26. References require careful verification
The reference list contains inconsistent formatting and some entries appear incomplete. For example, author names, journal titles, volume/issue information, DOI details and page ranges should be checked against the original publications.
The reference style should also be made completely uniform.

Strengths of the Manuscript

1. **Novel and environmentally relevant concept** using invasive *Eichhornia crassipes* biomass.
2. Direct comparison among **Eichhornia-CMC hydrogel, CMC hydrogel and soil**.
3. Multiple growth and biochemical parameters were evaluated.
4. The Eichhornia treatment showed higher reported biomass, leaf development, chlorophyll and seedling vigour than the other treatments.
5. The study provides photographic documentation of biomass preparation and plant cultivation.
6. The manuscript appropriately recognizes several areas requiring future research, including biodegradation, nutrient release, mechanical properties and economic feasibility.
7. The concept has potential relevance to circular bioeconomy and invasive-weed biomass utilization.

Recommendation
Minor Revision