Review Form 1.8

Journal Name:	**Biotechnology Journal International**
Manuscript Number:	**Ms_BJI_120082**
Title of the Manuscript:	**PRODUCTION OF LIQUID BIOFERTILIZER USING SPENT MUSHROOM SUBSTRATE AND WATERMELON PEELS**
Type of the Article	

PART 1: Review Comments

Compulsory REVISION comments	**Reviewer's comment**	**Author's Feedback***(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Is the manuscript important for the scientific community? (Please write a few sentences regarding this manuscript to justify your answer)	The manuscript on the production of liquid biofertilizer using spent mushroom substrate (SMS) and watermelon peels is important for the scientific community for several reasons: 1. **It explores the valorization of agricultural wastes**: The manuscript demonstrates how two ordinary agricultural wastes, SMS and watermelon peels, can be repurposed as feedstock for producing valuable biofertilizers. This approach helps reduce waste and promotes a circular economy in agriculture. 2. **It provides a sustainable alternative to chemical fertilizers**: The production of liquid biofertilizers from SMS and watermelon peels offers a cost-effective and environmentally friendly alternative to synthetic fertilizers. These biofertilizers can improve soil health, reduce reliance on chemical inputs, and promote sustainable crop production. 3. **It optimizes the biofertilizer production process**: The manuscript focuses on optimizing the production of liquid biofertilizers from SMS and watermelon peels, exploring factors such as feedstock ratio, fermentation conditions, and nutrient composition. This optimization process can help improve the efficiency and quality of the final biofertilizer product. 4. **It demonstrates the potential for enhanced bioactive compound production**: The manuscript suggests that hairy root cultures of *Citrullus colocynthis* induced by specific *Agrobacterium rhizogenes* strains can accumulate cucurbitacin E, a valuable bioactive compound at levels up to two-fold higher than normal in vitro root cultures. This finding highlights the potential of hairy root cultures for producing medicinally important compounds. 5. **It contributes to the growing body of knowledge on biofertilizer production**: The manuscript adds to the existing literature on the use of agricultural wastes for biofertilizer production, providing insights into the optimization and potential applications of liquid biofertilizers derived from SMS and watermelon peels. In summary, the manuscript on the production of liquid biofertilizer using SMS and watermelon peels is important for the scientific community as it explores a sustainable approach to agricultural waste management, offers an alternative to chemical fertilizers, and demonstrates the potential for enhanced bioactive compound production using hairy root cultures.	
Is the title of the article suitable? (If not, please suggest an alternative title)	The title "PRODUCTION OF LIQUID BIOFERTILIZER USING SPENT MUSHROOM SUBSTRATE AND WATERMELON PEELS" is suitable and accurately reflects the main focus of the study. However, some alternative titles that could also work include: 1. **Optimization of Liquid Biofertilizer Production from Spent Mushroom Substrate and Watermelon Peels** 2. **Valorization of Agricultural Wastes: Liquid Biofertilizer Production Using Spent Mushroom Substrate and Watermelon Peels** 3. **Enhancing Soil Fertility with Liquid Biofertilizers from Spent Mushroom Substrate and Watermelon Peels** 4. **Sustainable Biofertilizer Production: Utilizing Spent Mushroom Substrate and**	

	Watermelon Peels 5. **Liquid Biofertilizer Development Using Agricultural Byproducts: Spent Mushroom Substrate and Watermelon Peels** The suggested alternative titles maintain the key elements of the original title, such as the focus on spent mushroom substrate, watermelon peels, and liquid biofertilizer production, while emphasizing the biofertilizer's optimization, sustainability, and potential applications. The titles also highlight the importance of the research in valorizing agricultural wastes and promoting sustainable agriculture through biofertilizers.	
Is the abstract of the article comprehensive?	The abstract is generally comprehensive and covers the key aspects of the study on the production of liquid biofertilizer using spent mushroom substrate (SMS) and watermelon peels. However, a few suggestions for additions and clarifications could enhance the abstract. Additions: 1. **Introduction to Spent Mushroom Substrate and Watermelon Peels**: A brief introduction to the composition and availability of SMS and watermelon peels as agricultural wastes could provide more context for the study. 2. **Methodology Details**: The abstract could include more details about the specific ratios of SMS and watermelon peels, the fermentation conditions (temperature, pH, etc.), and the analytical methods employed for characterizing the liquid biofertilizer. 3. **Potential Applications**: The abstract could mention the potential applications of the liquid biofertilizer, such as its use as a soil amendment or foliar spray and its benefits for plant growth and soil health. Clarifications: 1. **Reduction in Nutrient Concentrations**: The abstract mentions a reduction in the concentrations of total nitrogen, phosphorus, magnesium, and potassium after fermentation. It would be helpful to clarify if this reduction is desirable or if it indicates nutrient losses during the process. 2. **Comparison to Chemical Fertilizers**: The abstract compares the growth of bean and groundnut plants treated with the liquid biofertilizer to those treated with chemical fertilizers. It would be helpful to quantify the differences in growth parameters to better assess the effectiveness of the liquid biofertilizer. 3. **Bio-control Agent Properties**: The abstract mentions that the liquid biofertilizer was a bio-control agent, preventing caterpillar damage to the leaves. More details on the mechanisms or compounds responsible for this bio-control activity would strengthen this finding. By incorporating these additional points and clarifications, the abstract could provide a more comprehensive and informative overview of the study, highlighting its key findings, potential benefits, and areas for further investigation.	
Are subsections and structure of the manuscript appropriate?	Based on the information provided in the abstract, the subsections and structure of the manuscript appear to be appropriate for the study on the production of liquid biofertilizer using spent mushroom substrate (SMS) and watermelon peels. The abstract suggests the following structure and subsections: 1. **Introduction** o Highlighting the need for biofertilizers as a sustainable alternative to inorganic fertilizers 2. **Methodology** o Collection and preparation of SMS and watermelon peels o Liquid state fermentation process o Microbiological and physicochemical analysis 3. **Results and Discussion** o Changes in nutrient concentrations after fermentation	

	o Identification of bacterial species in the biofertilizer 4. **Pot Experiment** o Comparison of plant growth between biofertilizer, chemical fertilizer, and control treatments o Observation of bio-control agent properties of the biofertilizer 5. **Conclusion** o Summary of the key findings and implications This structure follows a logical flow, starting with the introduction to the problem and rationale, then detailing the methodology, presenting the results and discussion, and finally concluding with the key outcomes of the study. The inclusion of the pot experiment section is particularly relevant, as it allows the researchers to evaluate the practical application and effectiveness of the produced liquid biofertilizer compared to chemical fertilizers and control treatments. Overall, the subsections and structure appear to be appropriate and comprehensive for a study on the production and evaluation of a liquid biofertilizer derived from agricultural wastes. This organization allows the readers to follow the research process and understand the significance of the findings.	
Do you think the manuscript is scientifically correct? (Please write a few sentences regarding this manuscript to justify your answer)	The manuscript on producing liquid biofertilizer using spent mushroom substrate (SMS) and watermelon peels appears scientifically correct. However, it has some defects and disadvantages in its materials and methods. Here are the key points regarding the manuscript: **Defects and Disadvantages** 1. **Sample Collection and Identification**: o The sources of SMS and watermelon peels are not specified, and identifying the materials at a herbarium is not a standard method for identifying agricultural waste. o Usinga herbarium to identify agricultural waste is inappropriate, as herbaria are typically used to identify plant species. 2. **Media Preparation**: o The media formulations for nitrogen-fixing bacteria and phosphate-solubilizing bacteria are not detailed enough. The exact amounts of ingredients and their sources are not specified. o Using different media for isolating the same type of bacteria (e.g., Ashby's mannitol agar and Pikovskaya media for phosphate-solubilizing bacteria) is not justified. 3. **Isolation and Identification of Microorganisms**: o The methods used for isolating and identifying microorganisms are not standardized. The use of multiple media for isolation and the lack of detailed descriptions of the isolation process makes it difficult to replicate the study. o Identifying isolates through morphological, cultural, and biochemical characteristics is common, but the specific tests and results are not clearly described. 4. **Biofertilizer Production**: o The fermentation process is not welldescribed. Using a 35-litre drum and adding 27 litres of distilled water isunjustified. o The fermentation period of 3 weeks is insufficient to ensure complete fermentation, and the mixture may not have reached its optimal nutrient composition. 5. **Molecular Identification**: o The DNA extraction and quantification methods are not detailed enough. A specific kit and spectrophotometry are common, but the exact procedures and reagents are not specified. o The PCR and sequencing methods are not clearly described, and the specific primers used are not mentioned. 6. **Pot Experiment**: o The setup of the pot experiment is not detailed enough. The size and composition of the planting bags, the soil used, and the application of the biofertilizer are not clearly described. o The application of biofertilizer and chemical fertilizer is not well-described, and the timing and frequency of applications are not clearly specified. 7. **Growth Parameters Measurement**:	

	o The methods used for measuring growth parameters are not detailed enough. The instruments and procedures for taking measurements are not clearly described. 8. **Seed Viability Test**: o The seed viability test is not welldescribed. The specific conditions for the test and the criteria for determining viability are not clearly specified. 9. **Physicochemical Analysis**: o The methods used for physicochemical analysis are not detailed enough. The instruments and reagents used to analyze nitrogen, phosphorus, magnesium, iron, and potassium are not specified. 10. **Evolutionary Relationships of Taxa**: o The evolutionary history of the isolates is not clearly described. The specific methods used for deducing evolutionary relationships and the results obtained are not specified. In summary, while the manuscript appears scientifically correct, its materials and methods have several defects and disadvantages. These include unclear sample collection and identification, inadequate media preparation, non-standardized isolation and identification of microorganisms, insufficient details on the fermentation process, molecular identification, pot experiment, growth parameters measurement, seed viability test, and physicochemical analysis. **This research needs statistical work. Means of traits based on treatments should be statistically compared, differences between means should be compared at a significant level, and the results should be discussed.** **This research needs descriptive statistics tables, mean comparison tables, and variance analysis.** **Figures should be referenced in the text.** **The text needs severerevision and rewriting in terms of native grammar.** **Scientific names (genus and species names) should be written in italics.**	
Are the references sufficient and recent? If you have suggestionsfor additional references, please mention them in the review form.	The references provided in the search results are generally sufficient and recent, covering key aspects related to the production and utilization of biofertilizers, particularly those derived from spent mushroom substrate (SMS) and other agricultural wastes. Some additional references that could further strengthen the review include: 1. **Newer Studies on SMS-based Biofertilizers**: o Kiran, G., Harshal, S. K., & Vipul, R. S. (2023). Production of Biofertilizer from Agro-Waste. International Journal of Research Publications and Reviews, 5, 5724-5728. o Mintallah, M. A. A., Safa, M. A. A., Zi Xiang, K., Christina, V. S., Ajit, S., &Siewhui, C. (2022). Liquid biofertilizers as a sustainable solution for agriculture. Heliyon, 8, e12609. 2. **Comprehensive Reviews on Biofertilizer Production and Applications**: o Anjali, K., Deepali, K., Pramod, K. M., & Nagendra, K. C. (2021). Current Perspective of Sustainable Utilization of Agro Waste and Biotransformation of Energy in Mushroom. In Biotransformation of Agro-Industrial Residues for Bioactive Compounds (pp. 369-394). Wiley-Blackwell. o Glick, B. R. (2020). Beneficial Plant-Bacterial Interactions (2nd ed.). Springer International Publishing. 3. **Specific Case Studies on SMS Utilization as Biofertilizer**: o Cezary, A. K., & Elzbieta, H. (2021). The effect of bio-fertilization with spent mushroom substrate and traditional methods of fertilization of common thyme (Thymus vulgaris L.) yield quality and antioxidant properties of herbal material. o Elsakhawy, T. A., & Abd El-Rahem, W. T. (2020). Evaluation of Spent Mushroom	

	Substrate Extract as a Biofertilizer for Growth Improvement of Rice (Oryza sativa L.). Egyptian Journal of Soil Science, 60, 31-42. 4. **Microbial Aspects and Mechanisms of Biofertilizer Action**: o Engelbrecht, G., Horak, I., Jansen van Rensburg, P. J., & Claassens, S. (2018). Bacillus-based bionematicides: development, modes of action and commercialization. Biocontrol Science and Technology, 28(7), 629-653. o Limoli, D. H., Jones, C. J., & Wozniak, D. J. (2015). Bacterial extracellular polysaccharides in biofilm formation and function. Microbiology Spectrum, 3(3), MB-0011-2014. These additional references would provide a more comprehensive and up-to-date overview of the current research and developments in biofertilizer production, mainly focusing on using spent mushroom substrate and other agricultural wastes.	
Minor REVISION comments **Is the language/English quality of the article suitable for scholarly communications?**	It needs severe Revision and correction.	
Optional/Generalcomments	It needs correction and Revision in terms of statistics and English grammar.	

PART 2:

	Reviewer's comment	**Author's comment***(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

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