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Comparative Study on the Growth of Trichoderma Harzianum on Various Grains an Experimental Approach to Enhance Biocontrol Agent Production

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Abstract: The growing environmental and health issues due to excessive use of chemical pesticides have increased the need for environmentally friendly alternatives in agriculture. Integrated Pest Management (IPM) offers a green solution by combining biological, cultural, and chemical approaches to minimize pest damage without impacting beneficial organisms. In biological alternatives, biopesticides have become widely popular because they are specific, environmentally benign, and less toxic compared to traditional pesticides. Trichoderma species, particularly Trichoderma harzianum, are widely recognized for their applications in plant disease management, plant growth promotion, and soil health improvement. This study investigates bulk cultivation of T. harzianum on different grains to determine optimal substrate materials and develop a talc-based biopesticide. Findings showed that maize and mixed grains supported maximum growth of the fungus, while rice and wheat were suboptimal. The developed product exhibited maximum viability and shelf-life stability, validating its field efficacy in green agriculture. Statistical analysis confirmed uniform growth patterns on substrates. The study indicates the potential of T. harzianum as a very effective and eco-friendly biocontrol agent, a good substitute for chemical pesticides, and a means of promoting environmentally friendly crop management practices.

Keywords: *Integrated Pest Management. Biopesticide, Trichoderma Harzianum, Sustainable Agriculture, Talc-Based Formulation.*

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I. INTRODUCTION

An ecological strategy for dealing with possible pest issues, integrated pest management (IPM) uses a combination of chemical, cultural, and biological treatments. In 1967, R.F. Smith and R. van den Bosch presented the idea of integrated pest management (IPM). After years of research into biologically based control methods, integrated pest management (IPM) came up with the idea of creating production systems that are both environmentally friendly and commercially viable. These systems place an emphasis on the “management” of pests below economic damage levels rather than their complete eradication. Minimizing insect damage, protecting human health, conserving beneficial species, and reducing environmental impact are the major aims of Integrated insect Management (IPM). Pest management that does not compromise aesthetics or the economy is the goal of integrated pest management (IPM), while also preventing pest borne diseases and reducing exposure to pesticides. Additionally, IPM seeks to conserve beneficial insects, pollinators, and other non-target organisms, maintaining ecosystem balance and promoting biodiversity. By adopting IPM strategies, individuals can reduce their reliance on chemical pesticides, protecting the environment and promoting sustainable agriculture practices. (Stern et al., 1959).

To maximize efficiency, IPM programs integrate many methods of management. Combining approaches that complement one another is the most successful long-term strategy for pest management.

Pest monitoring and identification constitute the subsequent elements of an IPM program, since it is critical to identify species that pose dangers and to choose between integrated management strategies and targeted pesticide applications.

Preventive measures plan to use various agronomic methods to lessen infestations. Rotating crops, introducing pest-resistant species, or using pre-treated seeds are all ways to prevent pests in integrated pest control.

Application of the most suitable IPM methods given that preventative measures failed. Alternatives for integrated pest management (IPM) range from less intrusive to more drastic

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measures. When manual methods of eradication or trapping fail, it may be necessary to resort to chemical spraying, either targeted or broadcast.

- *The Components of Integrated Management Shed Light on the Process of Developing and Launching an IPM Program:*

- Monitor your crops regularly.
- Take prevention measures.
- Identify pests timely and assess the risks.
- Decide on the necessity of action and how IPM will work.
- Consider and apply all appropriate integrated management options.
- Analyze the results.

II. INTEGRATED PEST MANAGEMENT METHODS

- Biological control
- Cultural control
- Mechanical control
- Chemical control

- *Benefits of IPM*

Integrated pest control has enormous direct and indirect societal advantages for agriculture.

- By reducing or eliminating the need for pesticides, Integrated Pest Management (IPM) helps to keep the environment safe. When all other options for pest control have been exhausted, integrated pest management (IPM) makes use of pesticides at the lowest effective dose. They are also useful for controlling pest organisms to an appropriate level while causing as little harm to the environment as possible.
- IPM increases profits. The profitability of the grower or farmer is guaranteed by the IPM program since it employs the most cost-effective pest management strategies.
- Loss of crop due to pests is less likely. Reducing crop loss or damage caused by pests is another goal of pest control and monitoring strategies.
- Employment, public health, and the welfare of those connected to agriculture would all see long-term societal advantages from IPM.

- *Pesticides*

To protect plants against insects, weeds, and other pests that might stunt their development, farmers use pesticides, which can be either natural or manufactured compounds. The chemicals were categorised based on their chemical make-up, potential dangers, and uses (B.A. Khan et al. 2023, A.). Pesticide residues can be found in a variety of foods, including fresh produce, processed foods, water, air, and dirt. Agricultural pesticides and nutritional exposure pose significant acute and chronic health risks, particularly in underdeveloped nations. Chemical pesticides provide risks to human health due to their carcinogenic, cytotoxic, and mutagenic properties (L. Fang, et al. 2020).

- *Pesticide Behaviour in the Environment*

It is possible for pesticides to reach the environment through processes like transfer (or mobility) and degradation, which occur when they are either disposed of or applied to a target plant (Singh D.K., 2012, Scholtz M *et al.*, 2007, Liu Y *et al.*, 2015). The many chemical classes expose their distinct environmental behaviours. Organochlorine chemicals, like DDT, have minimal acute toxicity but a remarkable capacity to accumulate in tissues, where they continue to cause long-term harm. Because of their persistent nature, even after being outlawed in most nations, their traces persist in the environment. Pesticides with organophosphate groups have a short half-life but significant acute toxicity in animals (Kim K.-H *et al.*, 2016, Damalas C.A. *et al*., 2011).

- *Biopesticides*

Biopesticides are a type of pesticide that mostly consists of substances that are either found in nature or are generated from live organisms or their metabolites. When compared to chemical insecticides, their harmful effects are less. A biopesticide, in its simplest form, is a pesticide formulation that mimics the action of an agent found in nature. They could eliminate unwanted pests. Based on the components they contain, the EPA classifies biopesticides into one of three broad groups (Seiber et.al., 2014): (1) “items that actively contain biocontrol agents, such as (e.g. Bacillus thuringienis), fungus, viruses, or protozoa; (2) Transgenes called Plant-Incorporated-Protectants (PIPS) are embedded in plant materials and are responsible for the production of pest control chemicals. PIPS also include the genetic material and protein that make up these compounds; (3) biochemical pesticides (e.g. based on naturally occurring substances having same structure and execute their duties and manage pests using non-toxic methods. The term biopesticide is not universally understood, as shown in studies by Hicks et al. (2018) and Essiedu et al. (2020). As a result, organisations like the International Organisation for Biological Control and the International Biocontrol Manufacturer's Association (IBMA) have advocated for the use of the term biocontrol agents (BCAs) rather than biopesticides (Guillon., 2008). Based on the bioactive components or agents utilised for pest management, the US EPA has classified biopesticides into three main categories:

- Biochemical biopesticides
- Microbial biopesticides
- GMO based biopesticide
- Mode of Action of Biopesticides

Biopesticides have different effects on different types of microbes. (Liu X., et al. 2021).

- *Trichoderma*

Plant diseases, insect pest problems, crop pesticide residues, water and soil pollution, and an overabundance of chemical fertilisers and pesticides are all results of conventional farming practices, such as planting the same crop on a large scale for an extended period (Bardin et al., 2015). *Trichoderma* is a biological fungus that is highly successful in controlling a range of plant diseases. It is found mostly in soil, air, and on plant surfaces, among other

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ecological settings. (Haouhach *et al.*, 2020; Zheng *et al.*, 2021; Wang R. *et al.*, 2022).

Several plant diseases, including soil-borne illnesses, as well as diseases affecting the leaves and spikes, can be controlled with the use of *Trichoderma* (Samuels et al., 2006; Vicente et al., 2020; Abbas et al., 2022). Disease prevention, increased plant development, better nutrient utilisation, increased plant resilience, and remediation of agrochemical contamination are all possible thanks to *Trichoderma.* (Tilocca *et al.*, 2020; Fontana *et al.*, 2021; Sánchez-Montesinos *et al.*, 2021; Al-Surhanee, 2022; Tyśkiewicz *et al*., 2022).

To biocontrol, many Trichoderma strains have been engineered to ward against bacterial, nematode, and insect-borne plant diseases (Abdullah N.S. et al., 2021; Ferreira et al., 2021). Of the almost two hundred species of bacteria that may harm plants, the ones responsible for the worst crop failures on a global scale include *Agrobacterium, Pseudomonas, Erwinia, Ralstonia*, and *Xanthomonas*. Secondary metabolites secreted by Trichoderma are thought to be responsible for its antibacterial action.

- *Trichoderma Harzianum*

For the control of plant diseases, *Trichoderma harzianum* is a commonly utilised *Trichoderma* species. (Meher *et al.,* 2020; Rush *et al.*, 2021) In addition to its mycoparasite actions, *T. harzianum* may regulate hormone levels and function as a biofertilizer, increasing plant absorption of carbon dioxide and mineral ions, all of which contribute to plant growth promotion. (Stewart and Hill, 2014; Marra *et al.*, 2021). According to Rush et al. (2021), out of 27 species of Trichoderma, T. harzianum/T. afroharzianum produces the most biopesticides and chemicals that promote plant development. The molecular processes of biocontrol have been extensively studied (Daguerre *et al*., 2014; Sood *et al.*, 2020; Abbas et al., 2022; Chen *et al.*, 2022), and a practical technique for enhancing T. harzianum strains has emerged through rational genetic engineering (Chen et al., 2021). However, there is no mention of genetic enhancement, and many commercial strains are said to be wild-type isolates.

III. MATERIAL AND METHOD

- *Experimental Site and Duration of Study*

The study was carried out in Plant Pathology Laboratory, Regional Central Integrated Pest Management Centre, Lucknow a sub-office of Directorate of Plant Protection, Quarantine & Storage, Ministry of Agriculture and Farmers Welfare, Government of India. This independent lab work was conduct during period ranging from 1st January to 12th February 2025 during my M.Sc. Botany specialization in Plant Pathology dissertation special paperwork in final year of 2024-25 of two years of M.Sc. (Botany) program.

- *Sterilization of Glassware*

All the glassware used for study were cleaned with detergent and then dipped in Chronic acid for 20 minutes. Then again washed in running tap water and then wiped to remove moisture and left to dry.

- *Preparation of Cultural Media and Pouring in Petri Dishes*

- *Potato Dextrose Agar (PDA)*

Potato Dextrose Agar media was prepared for isolation, purification of fungus as well as for its maintenance.

Table 1 Composition of PDA

Potato	200g
Dextrose	20g
Agar Agar	20g
Distilled water	1000ml

- *Method of PDA Preparation*

- Wash 200g potato, peel off the skin, and slice them into small pieces.
- Boil the sliced potato in 500 ml distilled water for 30 minutes in an open vessel or pressure cooker for 20 minutes.
- Collect the potato extract by filler through muslin cloth or net filter.
- Add 20g Dextrose to the potato extract.
- Add 20g Agar to the potato extract.
- Mix thoroughly the potato- agar mixture and make the volume to 1000ml with distilled water.
- Autoclave for 20 min at 121°C at 15 psi
- Dispense 20-25 ml portion into sterile Petri dishes.
- Final pH, 5.6 ± 0.2.

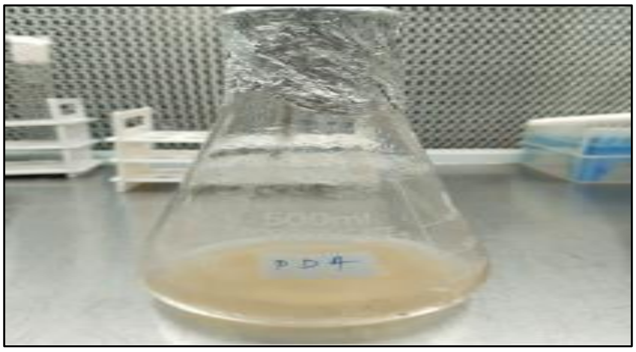

Fig 1 PDA

- *Inoculation of Trichoderma harzianum in Petri Dishes*

The PDA (Potato Dextrose Agar) flask was removed from the autoclave and placed in the Laminar Air Flow Chamber under for cooling. Then, the media will be poured into petri dishes and exposed to UV light and left undisturbed until the media solidifies.

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After the media solidifies, *Trichoderma harzianum* inoculation will be done from the mother culture into the petri dishes using an inoculation needle. For inoculation, a clean, sterilized inoculation needle will be taken, heated to red hot using the Bunsen burner, and then allowed to cool. After cooling, small portion of the *Trichoderma harzianum* mother culture was picked up using needle and transferred to the Petri dishes. After that, the Petri dishes will be sealed with parafilm tape and placed in BOD (Biochemical Oxygen Demand) incubator for 9 days at 25-30°C. Within 8-9 days, the full growth of *Trichoderma harzianum* will be visible in Petri dishes.

➢ *Mass Production of T. Harzianum on Different Grains*

- *Material Required*

✓ Maize/Bajra/rice/wheat/mixed grain (800g each)
✓ Mother culture of *Trichoderma harzianum* (provided by Plant Pathology lab, RCIPMC, Lucknow)
✓ Plastics bags (20)
✓ Plastics tubes (20)
✓ Cotton/cotton plugs
✓ Measuring cylinder (250 ml)
✓ Silver foil
✓ Rubber bands
✓ Bunson burner
✓ Distilled water
✓ Cork borer
✓ Streptomycin
✓ Talc powder
✓ Carboxy methyl cellulose (CMC)

➢ *Inoculation of T. Harzianum in Grains*

First, 800gm of grains was filled into 4 packets of 200g each in polybags. Then 200ml of distilled water will be measured using a measuring cylinder and added to each packet, followed by the addition of streptomycin. After that, a plastic tube will be attached to each packet and secured with rubber band to create space for inoculation.

After completing these steps, the packets will be sealed with cotton plugs and sliver foil and then placed in autoclave at 121° for 20 minutes at 15 psi for sterilization. The laminar air flow chamber will be cleaned with 90% alcohol, and UV for 45 minutes.

Autoclaved grain packets will be placed inside it. The UV light will be turned on for 1 hour to eliminate any potential contamination risks and let the packets cool down. After the packets have cooled down, the spirit lamp will be lit, and using cork borer, 4 colonies of *T. harzianum* will be carefully taken out from the petri dish and added to each packet. Then, the packets will be placed in the BOD (Biological oxygen demand) incubator for 13 days at temperature of 25-30°C.

From the third day onwards, *Trichoderma* started growing in the grain packets and data was recorded after 3rd, 7th, 10th and 13th day respectively.

➢ *Talc Based Formulation*

The fully grown colonies of *Trichoderma* on the grains will be taken out and placed in a tray to dry for 24 hours under shade at optimum room temperature of 25° to 30° C and covered the tray with sheet. After drying, the 300 g of *Trichoderma* was ground to powder. The *Trichoderma* powder was mixed with Talc powder (900 g) in a 1:3 ratio and then Carboxy Methyl Cellulose (5 g) was added as supplement food. After that powder was sealed in small packets weighing 200 g of each. The talc formulations of Trichoderma have shelf life of 3 to 4 months.

IV. RESULT

➢ *Comparative Growth of T. Harzianum on Different Grains*

Table 3 Shows the Growth Percentage of T. Harzianum on Maize

Day	R1	R2	R3	R4	Avg.
3rd	10%	15%	10%	15%	12.5%
7th	25%	25%	35%	40%	35%
10th	40%	60%	70%	75%	70%
13th	75%	80%	95%	100%	93.75%

Table 4 Shows the Growth Percentage of T. Harzianum on Bajra

Day	R1	R2	R3	R4	Avg.
3rd	10%	10%	5%	15%	10%
7th	25%	25%	15%	25%	22.5%
10th	40%	60%	55%	70%	62.5%
13th	75%	80%	65%	85%	78%

Table 5 Shows the Growth Percentage of T. Harzianum on Wheat

Day	R1	R2	R3	R4	Avg.
3rd	5%	0	5%	5%	3.75%
7th	10%	5%	15%	10%	10%
10th	25%	15%	25%	25%	22.5%
13th	40%	40%	50%	35%	41.25%

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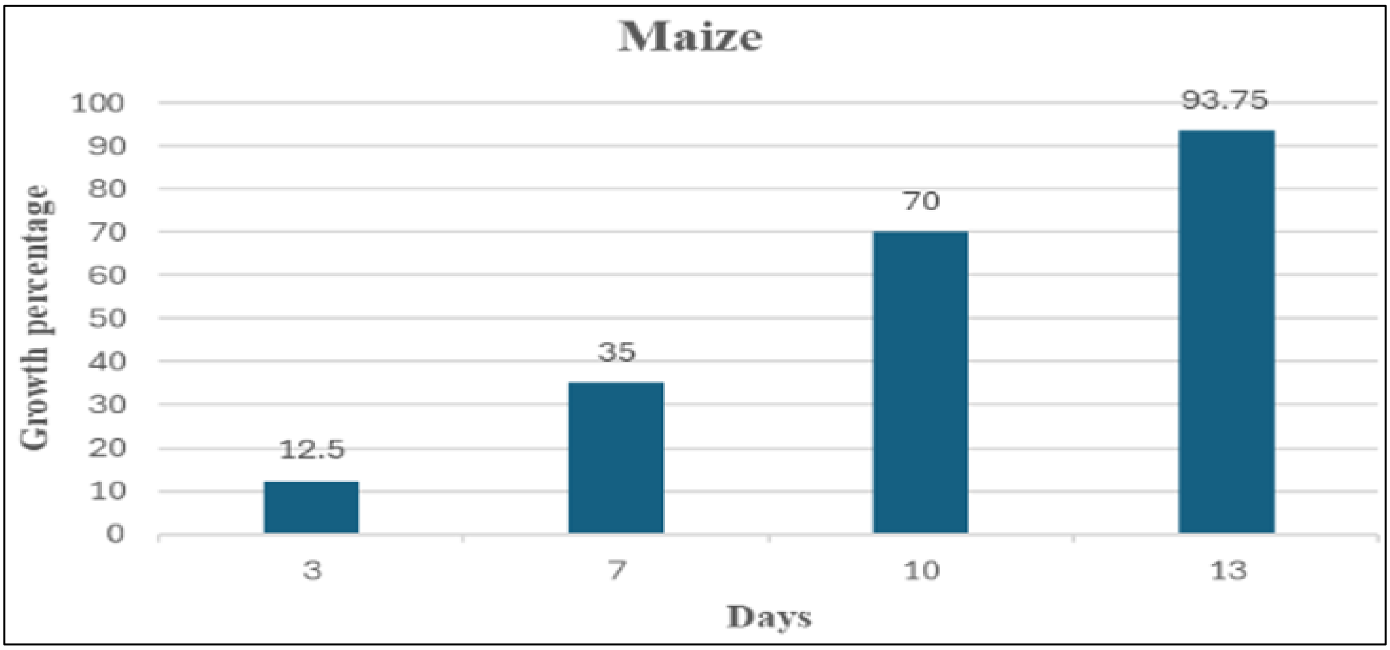
Table 6 Shows the Growth Percentage of T. Harzianum on Rice

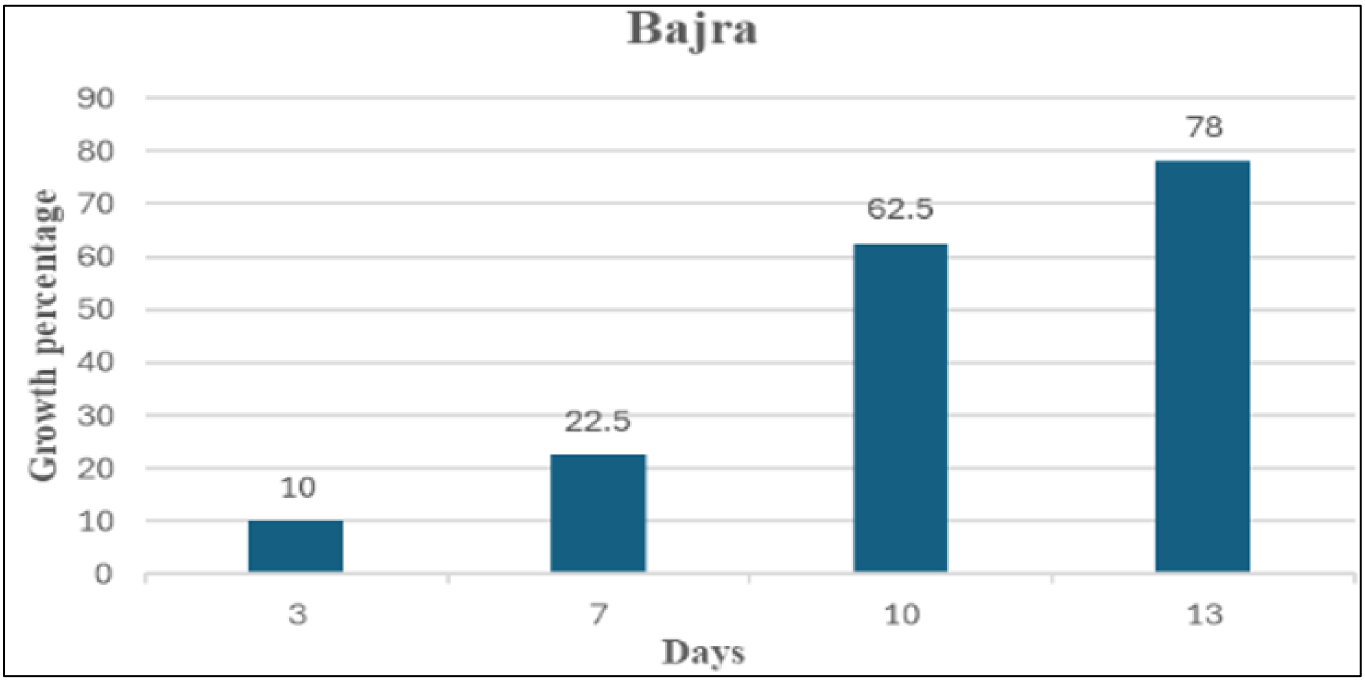
Day	R1	R2	R3	R4	Avg.
3rd	0%	2%	0%	2%	1%
7th	5%	10%	5%	5%	6.25%
10th	10%	25%	10%	10%	13.75%
13th	15%	50%	20%	15%	25%

Table 7 Shows He Growth Percentage of T. Harzianum on Mixed Grains

Day	R1	R2	R3	R4	Avg.
3rd	10%	5%	10%	5%	6.25%
7th	25%	20%	25%	15%	21.25%
10th	50%	45%	55%	50%	50%
13th	85%	70%	90%	75%	80%



Graph 1 Graph Showing Growth of T. Harzianum on Maize



Graph 2 Graph Showing Growth of T. Harzianum on Bajra

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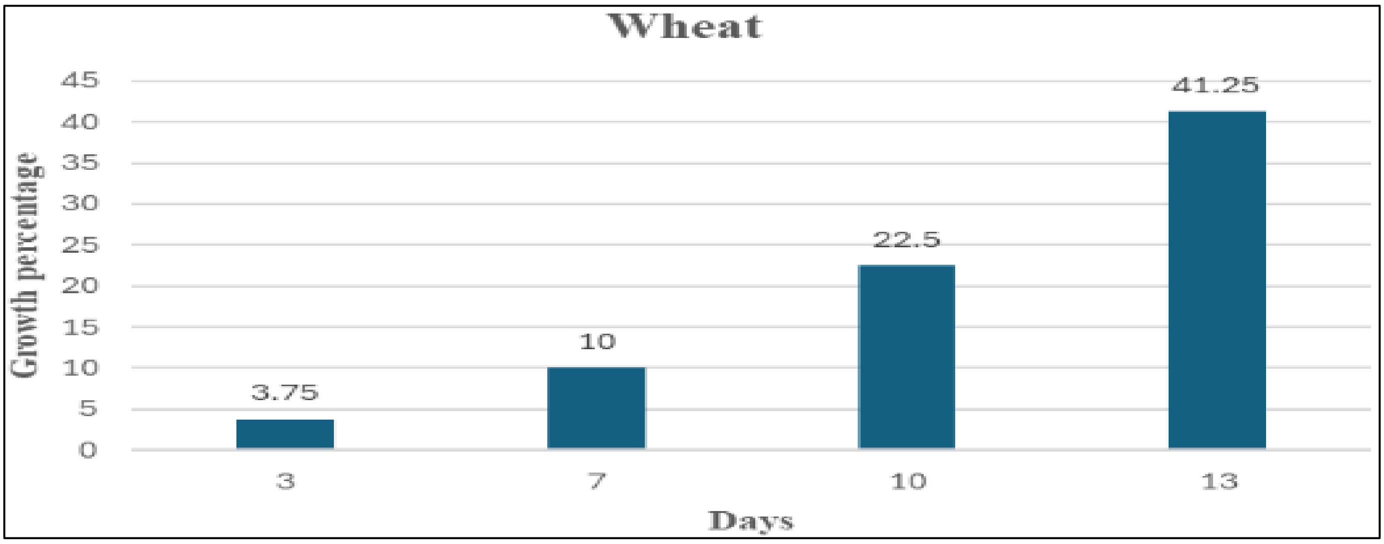
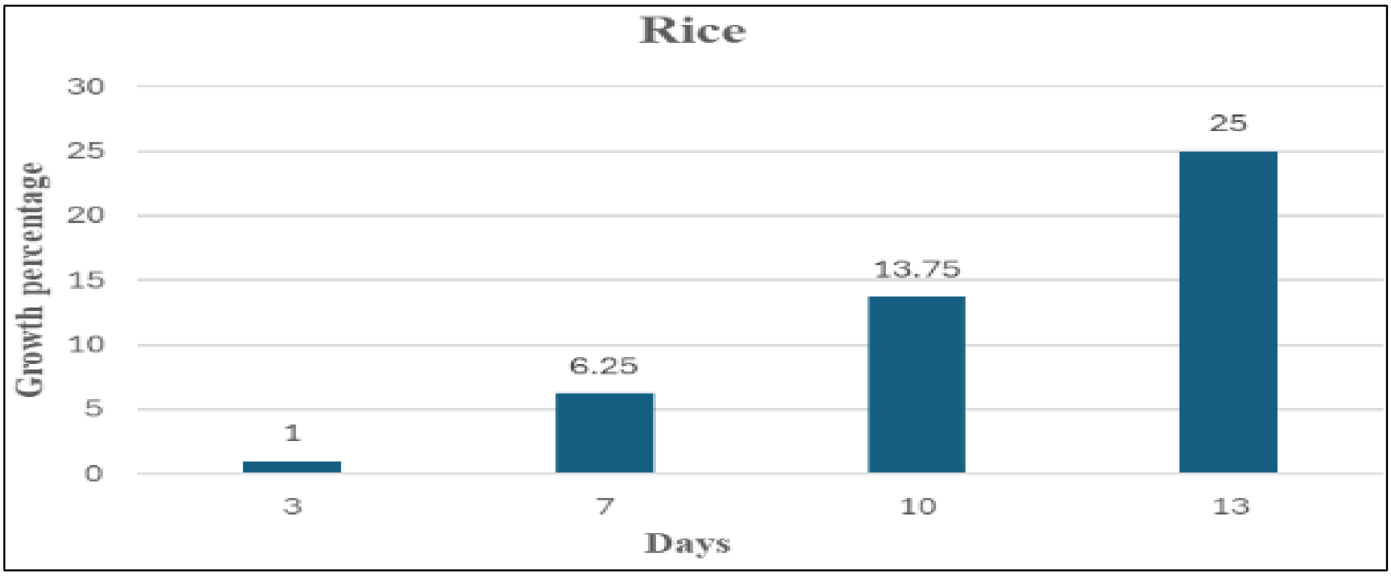
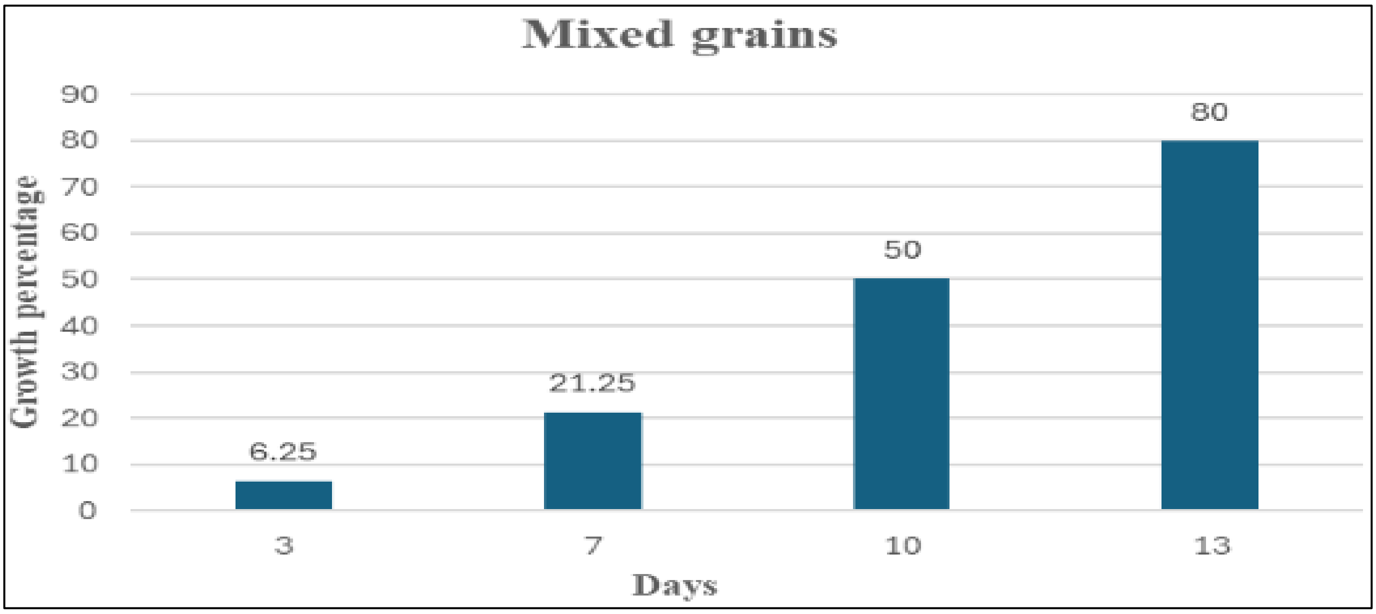


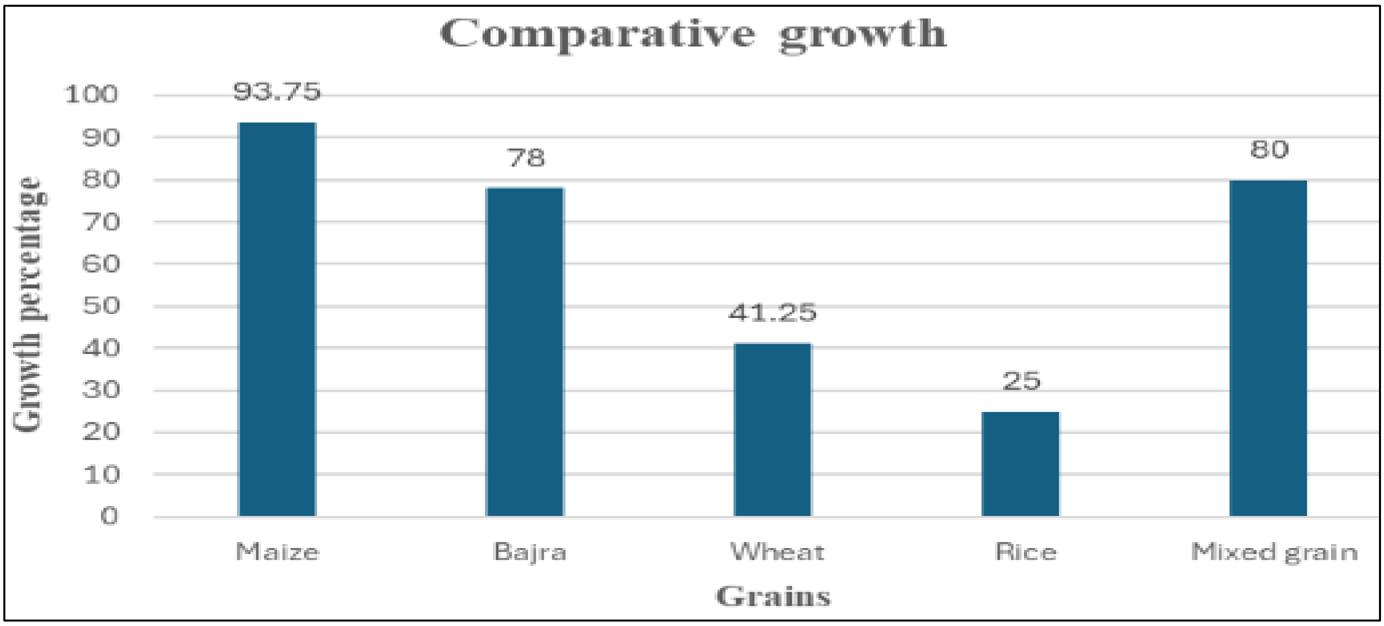
Graph 3 Graph Showing Growth of T. Harzianum on Wheat



Graph 4 Graph Showing Growth of T. Harzianum on Rice



Graph 5 Graph Showing Growth of T. Harzianum on Mixed Grain



Graph 6 Graph Showing Average Growth of All Grains

Fig 2 Maize

Fig 4 Wheat

Fig 3 Bajra

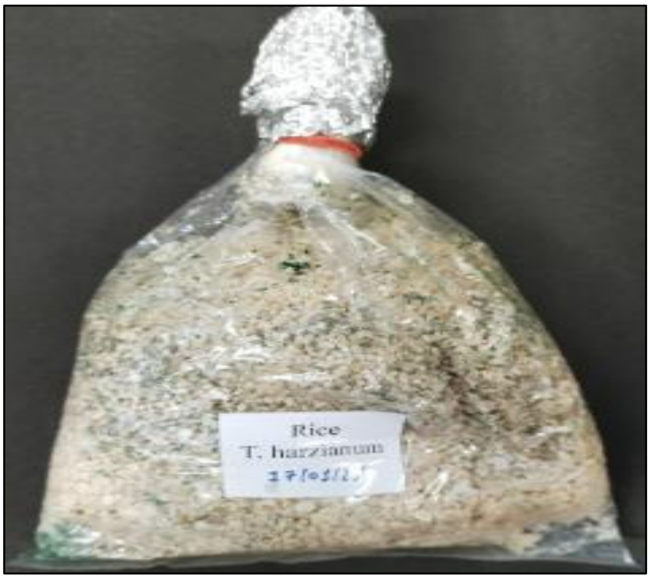


Fig 5 Rice

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Fig 6 Mixed grain

Fig 7 Biofertilizer

V. OBSERVATION

Mass production of *Trichoderma harzianum* was studied on different grains, including maize, bajra, wheat, rice, and mixed grains. The data was collected on the 3^{rd}, 7^{th}, 10^{th} and $13^{th\ day}$, and growth percentage were recorded.

➢ *Growth on Maize:*

- Initially, the growth was slow, with an average of 12.5% on the 3^{rd} day.
- By the 7^{th} day, growth increased to 35%, showing a significant rise.
- On the 10^{th} day, the growth reached 75%, and by the 13^{th} day, it peaked at 93.75%.
- Maize proved to be the most suitable substrate for *T. harzianum,* showing the highest growth rate among all grains.

➢ *Growth on Bajra:*

- The growth started at 10% on the 3^{rd} day, with a moderate increase to 22.5% by the 7^{th} day.
- A significance rise was observed on the 10^{th} day 62.5%, reaching 78% by the 13^{th} day.
- Bajra showed good support for fungal growth, through slightly less than maize.

➢ *Growth on Wheat:*

- The initial growth was the lowest among all grains, at 3.75% on the 3^{rd} day.
- Growth increased slowly to 10% on the 7^{th} day and 22.5% on the 10^{th} day.
- By the 13^{th} day, it reached 41.25%, making wheat the least favorable substrate for *T. harzianum.*

➢ *Growth on Rice:*

- On the 3^{rd} day, the fungal growth was 1% increasing slightly to 6.25% by the 7^{th} day.
- A steady rise was observed, reaching 13.75% on the 10^{th} day and 25% on the 13^{th} day.
- Rice supported the lowest overall growth making it the least effective substrate.

➢ *Growth on Mixed grains:*

- The 3^{rd} day growth was 6.25%, reaching 21.25% by the 7^{th} day.
- A sharp increase was seen on the 10^{th} day 50% and peaked at 80% by the 13^{th} day.
- Mixed grains provided a favorable environment for fungal growth, second only to maize.

➢ *Comparative Analysis:*

- The comparative growth analysis showed that maize (93.75%) and mixed grains (80%) were the best substrates.
- Bajra followed with 78% growth, indicating good fungal colonization.
- Wheat (41.25%) and rice (25%) exhibited significantly lower growth, making them less suitable for mass production.

➢ *Talc based formulation:*

The Talc based formulation of *Trichoderma harzianum* showed promising growth and remained stable over time. The spores retained their viability for up to 3-4 months, making it a reliable option for long-term use. However, since the formulation is in powder form, it needs to kept dry- exposure to moisture can degrade its quality and reduce its effectiveness.

One of the major advantages of this formulation is its ease of transportation and large-scale distribution. Since it is in a dry, powdered state, it can be supplied to farmers in bulk without significant loss of viability. This makes it a practical and scalable solution for agriculture applications. However, to maintain its potency, it must be stored properly in a dry environment.

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- *Statistical Analysis of Growth Data:*

To assess the variation in *T. harzianum* growth across different grains, statistical analysis was conducted, including mean growth percentage, standard deviation, and an ANOVA test.

- *Mean Growth Percentage*

The average growth percentage for each grain type was calculated as follow:

- Maize: 48.13%
- Bajra: 40.94%
- Wheat: 19.38%
- Rice: 12.44%
- Mixed Grain: 39.69%

The highest mean growth was observed on **Maize (48.13%)**, while the lowest was recorded on **Rice (12.44%)**.

- *Standard Deviation (Variability in Growth)*

The standard deviation values indicate how much the growth fluctuated across replicates:

- Maize: 31.03
- Bajra: 28.59
- Wheat: 15.37
- Rice: 12.93
- Mixed Grain: 29.24

Higher standard deviation in **Maize and Mixed Grains** suggests more variation in growth, whereas **Rice and Wheat** showed relatively stable growth patterns.

- *ANOVA Test for Statistical Significance:*

An ANOVA test was performed to determine whether the difference in growth among grains were statistically significant. The test resulted in a **p-value of 0.2147.**

- Since **p > 0.05,** the variation in growth across different grains is not statistically significant.
- This indicates that *T. harzianum* growth follows a similar trend across all tested grains, with only minor differences.

The statistical results suggest that while *T. harzianum* shows higher growth on some grains (e.g. Maize), the overall growth pattern is consistent across all tested grains. This implies that the biopesticide can be effectively cultivated on multiple substrates with minimal impact on its growth efficiency.

VI. DISCUSSION

With the increasing demand for eco-friendly farming solutions, the mass production of *Trichoderma harzianum* has become more important than ever. Unlike chemical pesticides, which can harm the environment and human health, *Trichoderma* offers a natural and sustainable way to protect crops. However, for large-scale production, choosing the right substrate is key.

In this study, different grains were tested to find the best medium for *Trichoderma* growth. The results clearly showed that maize and mixed grains performed the best, making them ideal for production. Surprisingly, rice did not support strong growth, which might be due to lower nutrient availability or poor moisture retention. This highlights the importance of selecting the right substrate to maximize efficiency.

Another crucial finding was the impact of temperature and humidity on *Trichoderma* growth. The results confirmed that maintaining optimal environmental conditions is essential for achieving higher yield and better quality biopesticides.

These insights can be highly beneficial for farmers and agricultural industries. By choosing the best-performing grains and maintaining proper conditions, farmers can produce their own *Trichoderma,* cut costs and reducing dependence on commercial biopesticides. In the future, more research can focus on improving production techniques and finding ways to further enhance *Trichoderma's* efficiency in real-world agriculture settings.

VII. CONCLUSION

This study explored different grains for the mass production of T. harzianum, a beneficial fungus widely used in sustainable farming. The results showed that maize and mixed grains provided the best growth, making them the most suitable choices for large-scale production. On the other hand, rice did not support the expected growth, which suggests that it may lack the ideal nutrients or moisture balance needed for Trichoderma development.

Additionally, the study highlighted how temperature and humidity play a crucial role in *Trichoderma's* growth. Maintaining the right conditions can significantly improve its yield, making the production process more efficient.

These findings are highly valuable for farmers and biopesticide producers, as they offer a cost-effective and practical way to grow *Trichoderma.* Farmers can use this knowledge to cultivate their reliance on chemical pesticides and promoting eco-friendly farming. Moving forward, future research can explore better fermentation techniques and environmental optimizations further enhance *Trichoderma* production.

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