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In-Vitro Culture of Trichoderma Harzianum as Influenced by Different Organic Substrates Applied with Varying Concentration Levels of Indole-3 Acetic Acid

Ailene L. Antopina [1], Junito P. Marcelino, PhD[2]
1 − Banisil National High School
2 – Sultan Kudarat State University

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Abstract

This study explores the growth dynamics, sporulation patterns, and biomass conversion efficiency of Trichoderma harzianum under varying conditions. The primary objective was to analyze hyphal expansion, spore production rates, and the fresh-to-dried weight ratio at different stages of fungal development. Experimental observations revealed that hyphal growth exhibited significant variations across treatments, with stabilization occurring by Day 12. Similarly, sporulation rates followed a progressive trend before reaching a constant phase, indicating a critical period for optimal fungal maturation. Biomass conversion efficiency, as measured by the fresh-to-dried weight ratio, demonstrated variability across different treatments, suggesting potential metabolic differences in fungal adaptation. These findings contribute to a deeper understanding of the environmental and biological factors influencing Trichoderma harzianum growth and development. The study underscores the importance of optimizing growth conditions to enhance the beneficial applications of T. harzianum in agriculture and biotechnology, particularly in biocontrol and soil health improvement. Further research is recommended to explore the genetic and physiological mechanisms influencing the growth, colonization, and sporulation of Trichoderma harzianum under varying environmental conditions. Understanding these biological responses will help optimize fungal cultivation techniques, improve its biocontrol efficiency, and enhance its potential applications in sustainable agriculture, biotechnology, and environmental management. Additionally, studies on the interactions between Trichoderma harzianum and external factors such as substrate composition, nutrient availability, and hormonal regulation can provide deeper insights into maximizing its effectiveness for large-scale production and field application.

Keywords: Biomass conversion efficiency, Indole-3 acetic acid (IAA), In-vitro culture, Organic substrates, Trichoderma harzianum

Introduction

Trichoderma harzianum is a well-established fungus known for its significant role in agriculture as a biocontrol agent and a natural fungicide. This versatile organism is utilized in various applications, including foliar treatments, seed treatments, and soil amendments, to combat a wide range of pathogenic fungi. Its ability to enhance plant health and promote growth through mechanisms such as competition, antibiosis, and mycoparasitism makes it a valuable asset in sustainable agricultural practices. The exploration of *Trichoderma harzianum's* growth dynamics, particularly in relation to different organic substrates and growth regulators, is essential for optimizing its use in agricultural systems.
Globally, the agricultural sector faces increasing challenges due to the over-reliance on chemical fungicides, which have led to environmental degradation and the emergence of resistant fungal strains. Soil-borne diseases are becoming more prevalent, posing a significant threat to food security and agricultural productivity (Zhang et al., 2020). As a result, there is a growing demand for sustainable and eco-friendly alternatives to chemical treatments, prompting researchers to investigate the potential of biological control agents like *Trichoderma harzianum* (Mishra et al., 2021).

In the national context, many are experiencing agricultural difficulties due to soil degradation, loss of biodiversity, and the excessive use of synthetic chemicals. These issues have intensified the need for sustainable agricultural practices that can mitigate the adverse effects of chemical inputs (Almeida et al., 2023). Research into the efficacy of *Trichoderma* species as biocontrol agents has gained momentum, yet the adoption of these biological solutions remains limited due to insufficient understanding of their growth conditions and interactions with various substrates (Fernandez et al., 2021).

Locally, farmers are increasingly affected by soil-borne diseases that threaten crop yields and quality. The application of *Trichoderma harzianum* has shown promise in improving plant health and resilience; however, there is a lack of comprehensive data on how different organic substrates and growth regulators, such as indole-3 acetic acid, influence its growth performance (Kumar et al., 2023). This knowledge gap hinders the effective implementation of *Trichoderma*-based solutions in local agricultural practices, limiting their potential benefits.

Despite the recognized advantages of *Trichoderma harzianum*, significant research gaps persist regarding its optimal growth conditions and interactions with various organic substrates (Singh et al., 2022). Specifically, there is a need for systematic studies that evaluate the effects of varying concentrations of indole-3 acetic acid on the growth and colonization rates of this fungus (Mishra et al., 2021). Addressing these gaps is crucial for maximizing the potential of *Trichoderma* as a biocontrol agent in sustainable agriculture.

The urgency of this research is underscored by the escalating challenges posed by soil-borne pathogens and the pressing need for environmentally friendly agricultural practices. Understanding the growth dynamics of *Trichoderma harzianum* in response to different organic substrates and growth regulators is vital for developing effective biocontrol strategies that can enhance crop productivity and sustainability (Gonzalez et al., 2022). As the agricultural sector seeks to mitigate the impacts of climate change and resource depletion, this research could provide valuable insights for farmers and policymakers alike.

Research Objectives

The study aims to determine the growth performance of *Trichoderma harzianum* as influenced by different organic substrates applied with varying concentration levels of indole-3 acetic acid.

Specifically, the study aims to:

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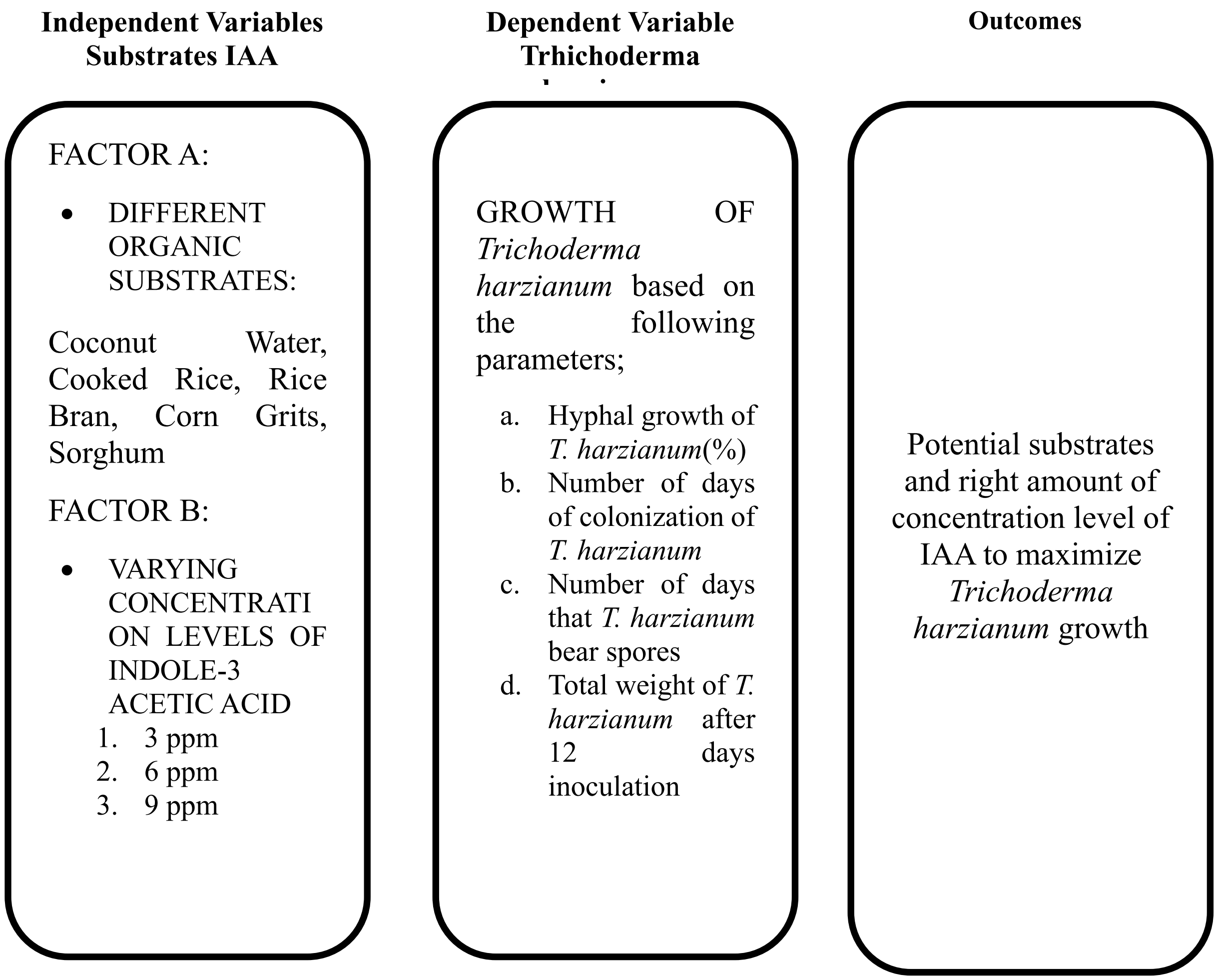
1. Determine the percentage growth performance of *Trichoderma harzianum* in varying concentration levels of indole-3 acetic acid (IAA);
2. Determine the growth performance of *Trichoderma harzianum* as influence of different culture media;
3. Determine the interaction effects of IAA and different culture media on the growth of *Trichoderma harzianum* in terms of:
    a. Days to colonization;
    b. Days to produce spores
    c. Fresh weight and;
    d. Dry weight.

Conceptual Framework

The study comprised the input, process, and output. The input consisted of different organic substrates (FACTOR A) and different concentration levels of IAA (FACTOR B).

As shown in Figure 1, the process primary dealt with the growth of *Trichoderma harzianum* based on the hyphal growth of *T. harzianum* (%), number of days of colonization of *T. harzianum*, number of days that *T. harzianum* bears spores, total weight of fresh *harzianum* in different substrates, and total weight of *harzianum* after 12 days inoculation in different substrates.



Research Methodology

Materials and Methods

Various materials used for this study includes 60 pcs sterilized petri dish (20ml), Indole-3 Acetic Acid (IAA), organic subsrates such as coconut water, cooked rice, rice bran, corn grits and sorghum. A digital weighing scale, test tubes, , rubber bands, cotton, autoclavea, pH kit, alcohol lamp, calculator, apron, and alcohol are used during the study.

Experimental Design and Treatments

The study was conducted using the two-factorial experiments in a Completely Randomized Design (CRD) with Different Organic Substrates as Factor A and Varying Concentration Levels of Indole-3 Acetic Acid as a Factor B.

The treatments were as follows:

Factor A – Different Organic Substrates

T1 – Corn grits (30mg;10ml IAA)
T2 – Cooked rice (30mg;3ml IAA)
T3 – Rice bran (30mg;20ml IAA)
T4 – Sorghum (30mg;5ml IAA)
T5 – Cocout water (20ml;1ml IAA)

Factor B – Varying Concentration Levels of Indole-3 Acetic Acid (IAA)

A1- 3ppm
A2 – 6ppm
A3 – 9ppm

Time and Place of the Study

This study was conducted at the **Soils Laboratory of the City Agriculturist's Office, Lagao, General Santos City**, as it provides the necessary **controlled environment, specialized equipment, and technical expertise** essential for accurate soil analysis and experimentation. The facility is well-equipped to support **scientific investigations related to soil composition, nutrient content, and microbial interactions**, ensuring **reliable and precise data collection**. Additionally, conducting the experiment in this location allows access to **local soil samples and agricultural resources**, which are crucial for assessing the study's relevance to the region's farming conditions and practices. This study was conducted last May 2022.

Methods

The following were the methods in conducting the study:

a) **Preparation of Stock Solution (10,000ppm IAA)**
   - Weigh 1.0g IAA to 50ml beaker – 1.0014g
   - Add 5.0ml Ethyl alcohol
   - Dilute to 100ml Volumetric Flask with distilled water

b) **Preparation of 3ppm**
   - Pipet 0.30ml of stock solution

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- Dilute to mark in 1 liter Volumetric Flask with distilled water

c) **Preparation of 6ppm**
- Pipet 0.60ml of stock solution
- Dilute to mark in 1 liter Volumetric Flask with distilled water

d) **Preparation of 9ppm**
- Pipet 0.90ml of stock solution
- Dilute to mark in 1 liter Volumetric Flask with distilled water

e) **Preparation of Mother Trichoderma Pure Culture**

Materials were 200g potatoes, 1L distilled water, 20g dextrose powder or 10g white sugar, 10ml vinegar, 18g gulaman bar.

Procedures:
- Peel potatoes and slice into cubes.
- Put in a pan with 1L of distilled water and boil for 10 minutes.
- Filter and collect 1L decoction. Dispense it.
- Melt the gulaman bars in pre-heat mode.
- Add 20g dextrose powder or 10g white sugar.
- Adjust pH to 4.5. Add 10 ml vinegar to achieve it.
- Transfer to bottle and sterilize for 30 minutes.

f) **Preparation of Different Organic Substrates**

These are the techniques employed in preparing the different culture media using organic substrates. Different treatments will also be set up for varying concentration levels of indole-3 acetic acid. In this experiment, the pure organic substrates are the control. Solid Substrates Media (SSM) were used for treatment 1-4. The experimental set ups were the treatments with varying concentration level of indole-3 acetic acid. The organic substrates were sterilized through autoclaving. The moisture level of the Solid Substrates Media were at 50-60 % as suggested by Parkash and Saika (2015) using distilled water.

Organic substrates used in the study were 360mg corn grits, 360mg cooked rice, 360mg rice bran, 360mg sorghum, and 240mg coconut water. All substrates were sterilized together using autoclave at 120 degree Celsius with 15 psi for 45 minutes utmost. Next, cool down the substrates. The petri dishes and digital weighing scale were prepared.

Each experimental setup will have 3 replicates. IAA will then placed in each treatment with different concentrations.

Corn Grits

Treatment 1 or T1 is corn grits. A 30mg of corn grits was placed in each petri dish. 3 sets petri dishes of corn grits were labeled as control or Replication 1 with 10ml distilled water as moisture. 3 sets petri dishes were labeled as Replication 2 with 10ml at 3ppm IAA, 3 sets petri dishes were labeled as Replication 3 with 10ml at 6ppm IAA, and 3 sets petri dishes were labeled as Replication 4 with 10ml at 9ppm IAA.

Cooked rice

Treatment 2 or T2 is cooked rice. A 30mg cooked rice was placed in each petri dish. 3 sets petri dishes of cooked rice were labeled as control or Replication 1. 3 sets petri dishes were labeled as Replication 2 with

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3ml at 3ppm IAA, 3 sets petri dishes were labeled as Replication 3 with 3ml at 6ppm IAA, and 3 sets petri dishes were labeled as Replication 4 with 3ml at 9ppm IAA.

Rice bran

Treatment 3 or T3 is rice bran. A 30mg of rice bran was placed in each petri dish. 3 sets petri dishes of rice bran were labeled as control or Replication 1 with 20ml distilled water as moisture. 3 sets petri dishes were labeled as Replication 2 with 20ml at 3ppm IAA, 3 sets petri dishes were labeled as Replication 3 with 20ml at 6ppm IAA, and 3 sets petri dishes were labeled as Replication 4 with 20ml at 9ppm IAA.

Sorghum

Treatment 4 or T4 is sorghum. A 30mg of sorghum was placed in each petri dish. 3 sets petri dishes of sorghum were labeled as control or Replication 1 with 5ml distilled water as moisture. 3 sets petri dishes were labeled as Replication 2 with 5ml at 3ppm IAA, 3 sets petri dishes were labeled as Replication 3 with 5ml at 6ppm IAA, and 3 sets petri dishes were labeled as Replication 4 with 5ml at 9ppm IAA.

Coconut water

Treatment 5 or T5 is coconut water. A 20ml of coconut water was placed in each petri dish. 3 sets petri dishes of coconut water were labeled as control or Replication 1. 3 sets petri dishes were labeled as Replication 2 with 1ml at 3ppm IAA, 3 sets petri dishes were labeled as Replication 3 with 1ml at 6ppm IAA, and 3 sets petri dishes were labeled as Replication 4 with 1ml at 9ppm IAA.

g) **Preparation of Inoculants.**

- Entire inoculating chamber were cleaned by spraying with ethyl alcohol. Use cotton for wiping.
- UV lamp of the inoculating chamber was switched on for 30 minutes.
- Under aseptic condition, the inoculating needle was heated and transferred a loopful of trichoderma to the surface of the sterile medium.
- The petri dishes were sealed with parafilm.
- The petri dishes were labeled by putting Treatment (T), @ppm, Replication (R).
- The petri dishes were incubated in clear, dry place under room temperature for at least a week. *Trichoderma* grows best under diffused light condition.

When the fungus starts to produce spores (green or moss green color), the inoculants is ready for use in composting and as fungicides.

Control of Contamination

A. *Sterilization Procedure*

1. At the start of the sterilization process, the airvent of the sterilizer was make sure that it is open. Close it only when the water has been boiling for five minutes.
2. A 127 degree celsius temperature was maintained at 15 psi (pounds per square inch) for 35 minutes when sterilizing culture media or glasswares to destroy all pores of bacteria and fungi present in these materials.
3. If using pressure cooker, the sterilization should be 3 minutes,

B. Aseptic Technique in Inoculation of Culture Medium

1. Cleanliness in the working area were maintained. Dust suspended in the air and soil particles on the table and floor when they get into the culture medium will bring contaminants.
2. Time period between actual inoculation and opening of the plastic bag used were lessen.
3. Aseptic techniques during inoculation were observed. Always heat inoculating needle to red-hot before using it.

Data Gathered Procedures

The researcher first sought formal permission from the Dean of the Graduate Studies of Sultan Kudarat State University to conduct the study. Upon securing approval, the researcher then requested authorization from the Head of the City Agriculture Office of General Santos City to carry out the experiment.

Once the necessary permissions were obtained, the experiment was conducted following a systematic procedure, which included the preparation of the necessary materials as well as the following steps:

(a) **Hyphal Growth of *Trichoderma harzianum.*** The grown *T. harzianum* was inoculated in the prepared culture media containing different organic substrates, both in the control group and with varying concentration levels of IAA. The plates were then placed in the growth chamber for a period of 12 days under room temperature conditions, with daily observations recorded. The hyphal growth of *Trichoderma harzianum* was determined based on the percentage of the area covered by the specimen in the petri dish. The researcher considers the entire Petri dish as 100%. From the initial observations, the percentage of the Petri dish covered by *Trichoderma harzianum* is estimated as it grows.

(b) **Number of Days for Colonization of *Trichoderma harzianum.*** Daily observations were conducted to determine when *T. harzianum* began to occupy and colonize each treatment or substrate. The number of days required for colonization was carefully monitored, along with the growth rate and the maturity stage of *T. harzianum*. The researcher recorded periods of high and low growth activity.

(c) **Number of Days for *Trichoderma harzianum* to Produce Spores.** The number of days required for *T. harzianum* to bear spores as it colonized the substrates was monitored. The researcher recorded observations on the specific day when spore production began for each treatment or substrate.

(d) **Total Fresh Weight of *Trichoderma harzianum.*** Before the treatments were placed in the growth chamber, the total weight of the substrates, along with the varying concentration levels of IAA, was recorded using a digital weighing scale. Each treatment was weighed, including the weight of IAA, to obtain the total weight of each sample. The weight of each treatment was monitored from the time colonization began.

(e) **Total Dried Weight of *Trichoderma harzianum* (12 Days After Inoculation).** As the weight of each treatment was monitored, the total dried weight of *T. harzianum* was also determined. This assessment measured the growth decline phase. The dried weight of *T. harzianum* was recorded once the substrates were fully colonized. The inoculation period lasted at least 10 days before the final dried weight of the culture was measured.

Statistical Analysis

The data gathered were analyzed statistically using Microsoft Excel software and SPSS. Test for significant difference among treatments were subjected to Duncan's Multiple Range Test (DMRT).

Results and Discussion

Percentage Hyphal Growth Performance of *Trichoderma Harzianum* in Varying Concentration Levels of Indole-3 Acetic Acid at Day 3

This study evaluates *Trichoderma harzianum* hyphal growth under varying Indole-3 Acetic Acid (IAA) concentrations on Day 3 of incubation. As a beneficial fungus with biocontrol and growth-promoting properties,

The Table 1 shows the measured values for Factor A across different test conditions (T1–T5), along with their corresponding means for percentage hyphal growth at day 3.

Table 1. Percentage(%) Hyphal Growth of *Trichoderma Harzianum* in Varying Concentration Levels of Indole-3 Acetic Acid at Day 3

Factor A Substrates		Factor B Concentration Level of IAA A1 3ppm	A2 6ppm	A3 9ppm	Mean
T1	Corn grits	27.50^{b}	22.50^{b}	26.25^{b}	25.42^{b}
T2	Cooke d rice	53.75^{a}	45.00^{a}	56.25^{a}	51.67^{a}
T3	Rice bran	18.75^{b}	18.75^{b}	22.50^{b}	20.00^{b}
T4	Sorghum	15.00^{b}	11.25^{b}	12.50^{b}	12.92^{b}
T5	Cooconut water	47.50^{a}	38.75^{a}	45.00^{a}	43.75^{a}
Mean		32.50	27.25	32.50	**30.75**
CV (%)					**29.29**

Means with same letter are not significantly different

The Table 1 shows that the type of substrate significantly influenced the hyphal growth of *Trichoderma harzianum*. Among the different substrates tested, cooked rice (T2) exhibited the highest mean hyphal growth at 51.67%, followed by coconut water (T5) with 43.75%. These substrates provided the most favorable conditions for fungal development. In contrast, sorghum (T4) resulted in the lowest mean hyphal growth at 12.92%, followed by rice bran (T3) at 20.00% and corn grits (T1) at 25.42%. The statistical grouping indicates that cooked rice and coconut water (denoted as “a”) significantly outperformed the other substrates (denoted as “b”), highlighting their superior suitability for supporting *T. harzianum* growth.

The concentration of Indole-3 Acetic Acid (IAA) affected *T. harzianum* growth, but the differences were not highly pronounced. The highest mean hyphal growth was recorded at 3 ppm (32.50%) and 9 ppm (32.50%), while the lowest was at 6 ppm (27.25%). These findings suggest that while IAA concentration does have some influence, it does not show a consistent increasing or decreasing trend in hyphal growth. This implies that other factors, such as substrate type, may have a stronger impact on fungal development.

The interaction between substrate type and IAA concentration revealed that the highest hyphal growth occurred in cooked rice (T2) at 9 ppm (56.25%), while the lowest was observed in sorghum (T4) at 6 ppm (11.25%). Coconut water (T5) also supported substantial fungal growth, particularly at 3 ppm (47.50%). Conversely, sorghum and rice bran consistently exhibited lower growth, regardless of IAA concentration, suggesting that their nutrient composition is less conducive to *T. harzianum* development. The coefficient of variation (29.29%) indicates moderate variability in the data, further reinforcing that substrate type plays a more dominant role in fungal growth compared to IAA concentration alone.

Growth variations may result from environmental conditions and substrate availability, key factors in fungal development. Smith and Jones (2020) note that temperature, moisture, and nutrients significantly affect hyphal expansion. This aligns with Brown et al. (2019), who highlight fungal sensitivity to external conditions.

Percentage Hyphal Growth Performance of *Trichoderma Harzianum* in Varying Concentration Levels of Indole-3 Acetic Acid at Day 6

The Table 2 shows the measured values for Factor A across different test conditions (T1–T5), along with their corresponding means for hyphal growth at day 6.

Table 2. Percentage(%) Hyphal Growth Performance of *Trichoderma Harzianum* in Varying Concentration Levels of Indole-3 Acetic Acid at Day 6

Factor A Substrates		Factor B Concentration Level of IAA			Mean
		A1 3ppm	A2 6ppm	A3 9ppm	
T1	Corn grits	53.75^{bc}	47.50^{bc}	52.50^{bc}	51.25^{bc}
T2	Cooke d rice	96.00^{a}	75.00^{a}	75.00^{a}	82.00^{a}
T3	Rice bran	27.50^{c}	30.00^{c}	33.75^{c}	30.42^{c}
T4	Sorghum	28.75^{c}	26.25^{c}	32.50^{c}	29.17^{c}
T5	Cooconut water	85.00^{ab}	67.50^{ab}	71.25^{ab}	74.58^{ab}
Mean		58.20	49.25	53.00	**53.48**
CV (%)					**25.90**

Means with same letter are not significantly different

Table 2 shows that the hyphal growth performance of *Trichoderma harzianum* varied significantly across different substrates. Cooked rice (T2) exhibited the highest mean hyphal growth (82.00%), followed by coconut water (T5) with 74.58%. Corn grits (T1) had a moderate growth rate of 51.25%, while rice bran (T3) and sorghum (T4) recorded the lowest growth percentages at 30.42% and 29.17%, respectively. These results indicate that the choice of substrate plays a crucial role in supporting fungal growth, with cooked rice providing the most favorable conditions.

The different concentration levels of Indole-3 Acetic Acid (IAA) influenced the hyphal growth of *Trichoderma harzianum*. The highest mean growth (58.20%) was observed at 3 ppm (A1), while lower growth rates were recorded at 6 ppm (49.25%, A2) and 9 ppm (53.00%, A3). This suggests that lower concentrations of IAA might be more beneficial for fungal growth, whereas higher concentrations could have inhibitory or less stimulatory effects.

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The interaction between substrate type and IAA concentration had a significant effect on the hyphal growth of *Trichoderma harzianum*. The highest hyphal growth was recorded in cooked rice at 3 ppm (96.00%), indicating that this combination provided the most optimal conditions for fungal development. Conversely, rice bran and sorghum consistently showed lower growth performance across all IAA levels. The presence of similar letter groupings in the statistical analysis suggests that certain treatments were not significantly different from each other, particularly among the lower-performing substrates. These findings highlight that both substrate type and IAA concentration collectively influence fungal growth, with cooked rice at 3 ppm emerging as the most favorable combination.

Hyphal growth increased from Day 3 to Day 6, reflecting progressive fungal development driven by substrate conditions. Extended incubation enhances nutrient absorption, promoting mycelial expansion (Johnson et al., 2021). Temperature and humidity also influence growth dynamics, contributing to variations across test conditions (Williams & Thompson, 2020).

Percentage Hyphal Growth Performance of *Trichoderma Harzianum* Varying Concentration Levels of Indole-3 Acetic Acid at Day 9

The Table 3 below shows the measured values for Factor A across different test conditions (T1–T5), along with their corresponding means for hyphal growth at day 9.

Table 3. Percentage(%) Hyphal Growth Performance of *Trichoderma Harzianum* in Varying Concentration Levels of Indole-3 Acetic Acid at Day 9.

Factor A Substrates		Factor B Concentration Level of IAA			Mean
		A1 3ppm	A2 6ppm	A3 9ppm	
T1	Corn grits	91.25^b	75.00^b	75.00^b	80.42^b
T2	Cooke d rice	100.00^a	75.00^a	75.00^a	83.33^a
T3	Rice bran	67.50^b	56.25^b	63.75^b	62.50^b
T4	Sorghum	71.25^b	63.75^b	67.50^b	67.50^b
T5	Cooconut water	100.00^a	75.00^a	75.00^a	83.33^a
Mean		86.00	69.00	86.25	**75.42**
CV (%)					**20.95**

Means with same letter are not significantly different

Table 3 shows that the hyphal growth performance of *Trichoderma harzianum* on different substrates showed notable variations at Day 9. Cooked rice (T2) and coconut water (T5) recorded the highest mean growth at 83.33%, followed closely by corn grits (T1) at 80.42%. Meanwhile, sorghum (T4) and rice bran (T3) displayed relatively lower growth rates, with means of 67.50% and 62.50%, respectively. These results indicate that cooked rice and coconut water continued to provide optimal conditions for fungal development, while rice bran and sorghum were less effective.

The application of different IAA concentration levels influenced *Trichoderma harzianum* growth at Day 9. The highest mean hyphal growth (86.25%) was observed at 9 ppm (A3), followed by 3 ppm (A1) at 86.00%. In contrast, the lowest growth rate (69.00%) was recorded at 6 ppm (A2). This suggests that higher concentrations of IAA (9 ppm) had a more pronounced positive effect on fungal growth compared to moderate concentrations (6 ppm), which appeared to be less favorable.

The interaction between substrate type and IAA concentration levels significantly affected hyphal growth. The highest individual growth rates were observed in cooked rice (T2) and coconut water (T5) at 3 ppm, both reaching 100% growth. Corn grits (T1) also showed strong growth at 3 ppm (91.25%), but its performance decreased at higher IAA levels. Meanwhile, rice bran (T3) and sorghum (T4) exhibited more consistent but lower growth patterns across all concentrations. Statistical analysis reveals that treatments with similar letter groupings were not significantly different, particularly among the lower-performing substrates. These results emphasize that while both substrate type and IAA concentration impact fungal growth, the combination of cooked rice or coconut water with 3 ppm or 9 ppm IAA levels provided the most favorable conditions for *Trichoderma harzianum* development.

Hyphal growth increased over time as mycelial networks expanded under favorable conditions. Miller and Roberts (2022) note that extended incubation enhances nutrient absorption and structural reinforcement, boosting fungal development. Additionally, reduced variability in later stages suggests fungal adaptation and stabilization in the environment (Anderson et al., 2021).

Number of days to Colonization of *Trichoderma harzianum* in the Culture Media

This study investigates the colonization time of *Trichoderma harzianum* in different culture media, highlighting its growth rate and adaptability. As a beneficial fungus with biocontrol and plant growth-promoting properties, its colonization speed varies with nutrient availability. Understanding these factors aids in optimizing its use in agriculture, bioremediation, and biotechnology.

Table 4 below shows the sporulation data of *Trichoderma harzianum*, showing the number of days required for spore development under different conditions.

Table 4. Number of Days to Colonization of *Trichoderma harzianum* in the Culture Media

Factor A Substrates		Factor B Concentration Level of IAA			Mean
		A1 3ppm	A2 6ppm	A3 9ppm	
T1	Corn grits	3.35	2.25	1.50	2.33^{bc}
T2	Cooke d rice	2.25	1.50	0.75	1.50^{cd}
T3	Rice bran	4.25	3.00	2.25	3.17^{a}
T4	Sorghum	5.25	3.00	3.00	3.75^{b}

T5	Cooconut water	1.50	0.75	0.75	1.00[d]
Mean		**3.30 a**	**2.10 b**	**1.65 b**	**2.35**
CV (%)					**20.65**

Means with same letter are not significantly different

Table 4 shows that In terms of Factor A (Substrates), the number of days required for *Trichoderma harzianum* to fully colonize the culture media varied significantly across different substrates. Coconut water (T5) recorded the fastest colonization time, with a mean of 1.00 days, followed by cooked rice (T2) at 1.50 days, indicating that these substrates provided optimal conditions for fungal growth. In contrast, rice bran (T3) and sorghum (T4) exhibited the slowest colonization times, requiring 3.17 days and 3.75 days, respectively. Corn grits (T1) had an intermediate colonization time of 2.33 days. These results suggest that substrates rich in easily accessible nutrients, such as cooked rice and coconut water, promote rapid fungal colonization, while more complex or nutrient-limited substrates like rice bran and sorghum slow down fungal expansion.

Regarding Factor B (Concentration Level of IAA), the data indicate that increasing IAA concentrations generally accelerated fungal colonization. The longest colonization time was observed at 3 ppm (3.30 days), while faster colonization occurred at 6 ppm (2.10 days) and 9 ppm (1.65 days). These results suggest that moderate to high levels of IAA enhance fungal development, potentially by stimulating hyphal growth and metabolic activity. However, the differences between 6 ppm and 9 ppm were not statistically significant, implying that beyond a certain threshold, additional IAA may not further enhance colonization speed.

The interaction between Factor A (Substrates) and Factor B (IAA Concentration Levels) highlights that the shortest colonization times were observed in coconut water (T5) and cooked rice (T2), particularly at 6 ppm and 9 ppm (0.75 days). Corn grits (T1) and rice bran (T3) showed progressive decreases in colonization time as IAA concentration increased, but they still required longer periods compared to coconut water and cooked rice. Sorghum (T4) exhibited the slowest colonization across all IAA levels, taking 3.00 to 5.25 days. The statistical analysis indicates that while IAA concentration influences colonization speed, the substrate type plays a crucial role in determining fungal growth efficiency.

The substantial variation in fungal colonization may be due to differences in substrate composition, moisture content, and environmental conditions, which significantly influence *Trichoderma harzianum*'s growth efficiency. According to Harman et al. (2020), *Trichoderma* species exhibit rapid colonization when nutrient availability, moisture levels, and temperature are optimal, leading to increased consistency in fungal development over time. The high coefficient of variation (CV) in early stages suggests inconsistency due to the initial establishment phase, but as environmental factors stabilize, fungal spread becomes more uniform across treatments, allowing *T. harzianum* to dominate available substrates efficiently.

Number of Days to Produce Spores of *Trichoderma harzianum* in the Culture Media

The Table 5 shows the number of days required for Trichoderma harzianum to produce spores in different culture media.

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Table 5. Number of Days to Produce Spores of *Trichoderma harzianum* in the Culture Media

Factor A Substrates		Factor B Concentration Level of IAA A1 3ppm	A2 6ppm	A3 9ppm	Mean
T1	Corn grits	5.00	3.00	2.25	3.42[b]
T2	Cooke d rice	4.00	2.25	2.25	2.83[b]
T3	Rice bran	7.5	5.25	3.75	5.50[a]
T4	Sorghum	8.25	5.25	5.25	6.25[a]
T5	Cooconut water	3.25	2.25	1.50	2.33[b]
Mean		5.60 a	3.60 b	3.00 b	**4.07**
CV (%)					**28.56**

Means with the same letter are not significantly different.

The Table 5 shows that the time required for *Trichoderma harzianum* to bear spores varied across substrates and IAA concentrations. Rice bran (T3) and sorghum (T4) showed the longest sporulation times, averaging 5.50 and 6.25 days, respectively, indicating that these substrates may have delayed spore production due to their composition. In contrast, coconut water (T5) had the shortest mean sporulation time (2.33 days), followed by cooked rice (T2) and corn grits (T1), suggesting that these substrates provided more favorable conditions for rapid sporulation.

Increasing IAA concentration generally reduced the number of days to sporulation, with 3 ppm requiring 5.60 days, while 6 ppm and 9 ppm shortened it to 3.60 and 3.00 days, respectively. This suggests that higher IAA levels may accelerate fungal maturation. The significant interaction between substrate type and IAA concentration indicates that while IAA influences sporulation timing, substrate composition remains a crucial factor in determining fungal reproductive efficiency.

The results suggest that, *Trichoderma harzianum* had completely ceased sporulation across all conditions. This could be attributed to nutrient depletion in the substrates, the natural life cycle of the fungus, or unfavorable environmental conditions that no longer supported spore production. According to Roberts and Lee (2022), *T. harzianum* sporulation is significantly influenced by substrate composition, moisture availability, and incubation duration, all of which contribute to variations in spore production across different treatments.

Fresh Weight of *Trichoderma harzianum* in Different Substrates (Based at 50%)

Trichoderma harzianum is commonly cultivated on various substrates to assess its growth and biomass production. Fresh weight is a key indicator of fungal development, influenced by the type of substrate used.

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The Table 6 shows the fresh weight of *Trichoderma harzianum* grown on different substrates, based on a 50% moisture content

Table 6. Fresh Weight of *Trichoderma harzianum* in Different Substrates (Based at 50%)

Factor A Substrates		Factor B Concentration Level of IAA			Mean
		A1 3ppm	A2 6ppm	A3 9ppm	
T1	Corn grits	40.07	30.05	30.03	33.38[ab]
T2	Cooke d rice	33.04	24.79	24.77	27.53[bc]
T3	Rice bran	50.10	37.58	37.59	41.75[a]
T4	Sorghum	35.05	26.28	26.31	29.21[b]
T5	Cooconut water	20.86	15.65	15.64	17.38[c]
Mean		35.82[a]	26.87[b]	26.87[b]	29.85
CV (%)					20.69

Means with the same letter are not significantly different

The fresh weight of *Trichoderma harzianum* varied significantly depending on the substrate used. The highest fresh weight was recorded in rice bran (T3) with a mean of 41.75g, followed by corn grits (T1) at 33.38g. Sorghum (T4) had a moderate fresh weight of 29.21g, while cooked rice (T2) showed a lower mean of 27.53g. Coconut water (T5) had the lowest fresh weight at 17.38g, suggesting that it provided the least favorable conditions for fungal biomass accumulation. These results indicate that rice bran was the most effective substrate in supporting fungal growth.

The concentration of Indole-3 Acetic Acid (IAA) influenced the fresh weight of *Trichoderma harzianum*. The highest mean fresh weight (35.82g) was recorded at 3 ppm (A1), while 6 ppm (A2) and 9 ppm (A3) both resulted in lower and equal fresh weights (26.87g). This suggests that increasing IAA levels beyond 3 ppm did not significantly enhance fungal biomass production and may have had an inhibitory effect on growth.

The interaction between substrate type and IAA concentration levels demonstrated significant variations in fresh weight accumulation. The highest fresh weight was observed in rice bran (T3) at 3 ppm (50.10g), indicating that this combination provided the most favorable conditions for *Trichoderma harzianum*. Corn grits (T1) also performed well at 3 ppm (40.07g), while the lowest fresh weights were recorded in coconut water (T5) across all concentration levels, with the lowest value at 9 ppm (15.64g). Statistical analysis shows that means with the same letters are not significantly different, indicating that certain treatments exhibited comparable results.

The findings align with previous studies highlighting the role of substrate composition in fungal biomass production. Rice bran, being rich in essential nutrients, has been reported as an optimal substrate for fungal growth (Gautam et al., 2021). Similarly, the inhibitory effects of higher IAA concentrations have been noted in microbial studies, where excessive auxin levels can negatively impact fungal metabolism (Singh et al.,

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2020). These results reinforce the importance of selecting suitable substrates and optimizing IAA levels for maximizing *Trichoderma harzianum* biomass production.

Total Dried Weight of *Trichoderma harzianum* in Different Substrates after 12 Days Inoculation

The dried weight of *Trichoderma harzianum* is an important parameter for evaluating its biomass production and potential applications. It reflects the fungal growth efficiency across different substrates after a given incubation period.

The Table 7 shows the total dried weight of *Trichoderma harzianum* in various substrates after 12 days of inoculation.

Table 7. Total Dried Weight of *Trichoderma harzianum* in Different Substrates after 12 Days Inoculation

Factor A Substrates		Factor B Concentration Level of IAA A1 3ppm	A2 6ppm	A3 9ppm	Mean
T1	Corn grits	40.1600	30.1200	30.1100	33.46^{ab}
T2	Cooke d rice	33.1750	24.8900	24.8600	27.64^{bc}
T3	Rice bran	50.1600	37.6025	37.6300	41.80^{a}
T4	Sorghum	35.1500	26.3500	26.3750	29.29^{bc}
T5	Cooconut water	21.0875	15.8250	15.8250	17.58^{c}
Mean		35.95 a	26.96 b	26.96 b	**29.95**
CV (%)					**20.66**

Means with the same letter are not significantly different.

The effect of Factor A (Substrate Type) on the total dried weight of *Trichoderma harzianum* after 12 days of inoculation shows significant variation. Among the substrates, rice bran (T3) yielded the highest dried weight (41.80g), followed by corn grits (T1) with 33.46g. Cooked rice (T2) and sorghum (T4) had similar dried weight values of 27.64g and 29.29g, respectively, while coconut water (T5) produced the lowest biomass at 17.58g. These results indicate that solid substrates, particularly rice bran and corn grits, provide a more favorable medium for fungal growth compared to liquid-based substrates like coconut water.

For Factor B (IAA Concentration Level), the highest dried weight was observed at 3 ppm (35.95g), while lower values were recorded at 6 ppm and 9 ppm, both averaging 26.96g. This suggests that a lower concentration of IAA promotes fungal biomass production, whereas higher concentrations may inhibit growth. The significant difference between the 3 ppm treatment and the other concentrations highlights the potential impact of auxin levels on fungal metabolism and development.

The interaction between Factor A (Substrate Type) and Factor B (IAA Concentration Level) further demonstrates varying responses of *Trichoderma harzianum* to different growth conditions. Rice bran at 3 ppm IAA resulted in the highest dried weight (50.16g), while coconut water at 6 ppm and 9 ppm produced the lowest biomass (15.83g). These findings suggest that the choice of substrate plays a crucial role in fungal growth, and its interaction with IAA concentration significantly influences biomass production.

Previous studies have shown that the selection of an appropriate substrate is crucial in fungal biomass production, as substrates rich in nutrients, such as rice bran and corn grits, enhance mycelial growth and sporulation (Harman et al., 2004). Furthermore, research on auxin effects in fungal systems suggests that lower concentrations of IAA can promote growth, while higher concentrations may act as inhibitors by disrupting cellular metabolism (Khan et al., 2020). The findings of this study align with previous reports that emphasize the importance of optimizing substrate composition and growth regulators to maximize *Trichoderma harzianum* biomass production (Singh et al., 2019).

Summary

The hyphal growth of *Trichoderma harzianum* in varying concentrations of Indole-3 Acetic Acid (IAA) at Day 3 showed that cooked rice (T2) and coconut water (T5) had the highest growth rates, with mean values of 51.67% and 43.75%, respectively. Corn grits (T1) and rice bran (T3) showed moderate growth, while sorghum (T4) exhibited the lowest hyphal growth (12.92%). These results suggest that *Trichoderma harzianum* thrives better in substrates like cooked rice and coconut water during early growth stages.

By Day 6, the hyphal growth of *Trichoderma harzianum* increased significantly across all substrates, with cooked rice (T2) reaching the highest mean percentage (82.00%), followed by coconut water (T5) at 74.58%. Corn grits (T1) also showed improved growth (51.25%), while rice bran (T3) and sorghum (T4) remained at lower levels. The data suggest that cooked rice and coconut water continue to be the most conducive substrates for fungal growth.

At Day 9, *Trichoderma harzianum* exhibited further hyphal expansion, with cooked rice (T2) and coconut water (T5) both achieving the highest mean growth (83.33%). Corn grits (T1) showed a significant increase to 80.42%, while rice bran (T3) and sorghum (T4) followed with 62.50% and 67.50%, respectively. This indicates that after prolonged incubation, fungal colonization is more extensive in these substrates, particularly cooked rice and coconut water.

By Day 12, no hyphal growth was observed in any of the substrates at any IAA concentration level, resulting in a mean value of 0% across all treatments. This suggests that *Trichoderma harzianum* may have reached its growth limit or that environmental conditions were no longer favorable for further expansion. The absence of growth at this stage highlights the need for further investigation into factors affecting fungal survival over extended incubation periods.

The number of days required for *Trichoderma harzianum* to colonize the culture media varied among substrates. Rice bran (T3) took the longest time to colonize (3.17 days), followed by sorghum (T4) at 3.75 days. In contrast, coconut water (T5) had the shortest colonization time at 1.00 day, followed by cooked rice (T2) at 1.50 days. These results suggest that liquid substrates like coconut water promote faster colonization compared to solid substrates.

The number of days required for *Trichoderma harzianum* to produce spores varied significantly among substrates. Rice bran (T3) and sorghum (T4) recorded the longest spore production times at 5.50 and 6.25 days, respectively. Meanwhile, coconut water (T5) exhibited the shortest spore production time at 2.33

days. The data indicate that the choice of substrate plays a crucial role in influencing the rate of spore formation.

The fresh weight of *Trichoderma harzianum* in different substrates at 50% moisture content showed that rice bran (T3) had the highest biomass yield (41.75g), followed by corn grits (T1) at 33.38g. Cooked rice (T2) and sorghum (T4) had similar biomass values, while coconut water (T5) resulted in the lowest fresh weight (17.38g). These findings suggest that solid substrates like rice bran and corn grits provide better growth conditions for fungal biomass accumulation.

The total dried weight of *Trichoderma harzianum* after 12 days of inoculation followed a similar trend, with rice bran (T3) yielding the highest dried biomass (41.80g), followed by corn grits (T1) at 33.46g. Cooked rice (T2) and sorghum (T4) showed moderate dried weights, while coconut water (T5) had the lowest biomass (17.58g). These results highlight the effectiveness of rice bran as the most suitable substrate for maximizing fungal biomass production.

Conclusion

The study demonstrated that the growth, colonization, and sporulation of *Trichoderma harzianum* were significantly influenced by substrate composition and Indole-3 Acetic Acid (IAA) concentrations. Among the tested substrates, cooked rice (T2) and coconut water (T5) supported the highest hyphal growth, particularly at lower IAA concentrations (3 ppm). In contrast, rice bran (T3) and sorghum (T4) exhibited slower and less consistent growth, suggesting that substrate composition plays a crucial role in fungal development. The findings indicate that while IAA affects mycelial expansion, the availability of nutrients in the substrate remains the primary determinant of fungal growth efficiency.

The colonization of *T. harzianum* followed a distinct pattern, with coconut water (T5) and cooked rice (T2) enabling the fastest colonization times, whereas rice bran (T3) and sorghum (T4) required longer durations to establish growth. Increasing IAA concentrations (6 ppm and 9 ppm) generally accelerated the colonization process, but substrate composition remained the more dominant factor. Similarly, sporulation followed a comparable trend, where nutrient-rich substrates supported faster and more consistent spore production. However, by Day 12, growth and sporulation had completely ceased across all conditions, suggesting that nutrient depletion or environmental factors limited fungal viability. These results align with previous findings that highlight the importance of both external environmental factors and intrinsic substrate properties in fungal development.

Overall, the study provides valuable insights into the role of substrate type and IAA concentration in optimizing *T. harzianum* cultivation. The findings suggest that substrate selection is more critical than IAA level adjustments in ensuring robust fungal growth. While IAA may enhance fungal expansion at specific concentrations, it cannot fully compensate for suboptimal substrate conditions. Understanding these factors is essential in maximizing the efficiency of *T. harzianum* for agricultural, biotechnological, and biocontrol applications.

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