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A Comparative Study of Various Substrate Combinations on Mass Multiplication of *Trichoderma lexii* (T-94a)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The high cost of raw materials has long hindered the large-scale commercialization of *Trichoderma* sp. conidiophore formulations. This study addresses the need for affordable, nutrient-rich alternatives by exploring the use of lignocellulosic residues, comprising lignin, cellulose, and hemicellulose, as a carbon source for cultivating *Trichoderma.* These fungi possess unique attributes, such as filamentous growth and prolific spore production, which make them valuable for composting lignocellulosic waste. Using different substrates with a combination of straws effectively helps in the decomposition of straw and enhances the mass multiplication of *Trichoderma lexii* (T-94a). Different substrates such as broken rice, grounded maize, gram flour, sorghum & broken wheat were used in combination with paddy straws. A combination of Maize + straw induced an early response in the mycelial growth of *Trichoderma lexii* (T-94a). After 5 and 10 days of inoculation, physical parameters such as growth and sporulation characteristics of *T. lexii* (T-94a) show highly uniform growth and profuse sporulation on maize and straw combination as compared

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to broken rice & straw, sorghum, and straw combinations. This finding has promising implications for improving agricultural practices and waste management. Further research can fine-tune the optimal substrate ratios to enhance the efficiency of biocontrol agents and waste decomposition. Scaling up the application of these substrate combinations in real-world farming scenarios and assessing their long-term environmental impact will be essential for realizing the full potential of these sustainable agricultural practices.

Keywords: Substrate; paddy straws; Trichoderma lexii; mass multiplication.

1. INTRODUCTION

High-cost raw materials limits the commercialization of formulations of conidiophores of *Trichoderma* sp. at higher scales [1], it is therefore important to have raw material and low-cost procedures that provide the essential nutrients for the growth and sporulation of *Trichoderma* sp. [2]. An alternative for the cultivation of *Trichoderma* sp. is the use of lignocellulosic residues which are composed of lignin, cellulose, and hemicellulose, the latter biopolymers represent an excellent source of carbon for the growth of filamentous fungi. *Trichoderma* have an advantage in the composting of lignocellulosic waste, they are filamentous and have the ability to produce prolific spores, which can invade substrates quickly. Several isolates of *Trichoderma* spp. developed large amounts of biomass containing conidia and chlamydospores on substrates having inexpensive ingredients [3]. Wastes from agricultural sources could be used as substrates for the preservation and growing of lignocellulolytic fungi [4].

Composting of lignocellulose-containing rice straw requires a process that ensures rapid biodegradation despite the fact that the lignin matrix shields, cellulose and hemicelluloses from biodegradation. Fungi have an advantage in the composting of lignocellulosic waste because they are filamentous and have the ability to produce prolific spores, which can invade substrates quickly. Hence, a strong lignocellulolytic fungal isolate might play a vital role in the rapid disposal of rice straw. lignocellulolytic fungal isolates were pre-adopted on agro wastes (paddy straw) to produce viable inoculum that will be more effective in decomposing the paddy straw available in the fields. Therefore, the present study's rationale was to use a combination of grain substrate with rice straw to enhance the rate of straw degradation.

2. MATERIALS AND METHODS

***Trichoderma* culture collection:** Culture of bioagent *Trichoderma lexii* (94a) culture is taken from the Department of Plant Pathology, (Indira Gandhi Krishi Vishwavidyalaya, Raipur. Typically, T. lexii (94a) exhibits dark green conidia, providing a distinguishing visual feature. The growth pattern is predominantly aerial, with a subdued appearance, indicating a unique mode of mycelial development The mycelium displays a uniform velvety texture, contributing to its distinctive appearance. The culture is odorless, devoid of any distinct olfactory characteristics.

Biochemical assays shown that the culture is odorless, devoid of any distinct olfactory characteristics. cellulose hydrolysis capability, with a diameter of 56.50±0.71 mm, showcasing its potential in cellulose degradation. The chitinolytic activity of *T. lexii* (94a) is quantified at 141.25±1.250189 mg/ml at OD-582 nm, reflecting its capacity to hydrolyze chitin, an essential component of fungal cell walls. The exo-chitinase activity is measured at 0.0734±0.00005 U/ml, indicating the fungus's exo-chitinase production capability, which contributes to its ability to degrade chitin.

Substrate for mass multiplication: The choice of substrates for mass-producing *Trichoderma* significantly impacts its growth and development as a biocontrol agent. Various substrates, including broken rice, maize, sorghum, wheat, sugarcane bagasse, and cow dung, provide essential nutrients and organic matter crucial for *Trichoderma's* growth and metabolism. Broken rice, maize, sorghum, and wheat offer diverse carbohydrates and plant compounds as energy sources, rich in cellulose and hemicellulose for biomass and enzyme synthesis. Cow dung enhances substrate complexity, introducing nutrients and microorganisms that support *Trichoderma* growth, possibly by nutrient cycling. Utilizing this variety of substrates acknowledges the intricate relationship between *Trichoderma* and its growth medium. This optimization is essential for enhancing mass production for biocontrol and potential agricultural applications.

The study assesses Trichoderma's growth and sporulation on paddy straw with different substrate combinations: broken rice + straw, crushed maize + straw, sorghum + straw, broken wheat + straw, and cow dung cake + rice straw.

Sterilization of substrate: Grains of broken rice, crushed maize, and sorghum was presoaked in water overnight before the application of *Trichoderma* culture and sterilized by autoclaving at the temperature of 121.6 °C at 15 lbs. for 20 minutes. Sterilized grains were then mixed with straws uniformly, and inoculated with *Trichoderma* culture over the mixed component of substrate and straws.

Efficacy of combination of straw substrate on growth and sporulation of *T. lexii* isolate (94a): Efficacy is assessed by observing the presence or absence of *Trichoderma* growth and sporulation, denoted as a positive (+) or negative (–) sign, respectively, at various time intervals of 2, 4, 6, 8, and 10 days.

Impact of Various Substrates on the Distinctive Growth and Sporulation of *T. lexii* isolate (94a) in Combination with Straw: An Analysis of Growth Uniformity, Fungal, Development Patterns, and Sporulation After 5 and 10 Days of Inoculation.

Evaluating shelf life of *Trichoderma lexii* (T-94a): After 10 days of the incubation period**,** One-gram samples were collected from each formulation. These samples were placed into separate test tubes containing 10 ml of sterilized water. The samples were vigorously mixed with a vortex mixer for 3 minutes to create a 10^{-1} dilution. 1 mL suspension of stock solution was transferred in the next test tube containing 9 ml distilled water by using a sterilized pipette and shaken to make 10^{-2} dilutions and eight test tubes to make up 10^{-8}dilution.

The experiment followed a Completely Randomized Design. Three replicates were maintained for each formulation. The Petri plates were placed in an incubator at a constant temperature of 25 ± 2°C for two days. Periodic observations were conducted during the incubation period to monitor the growth of *T. lexii* (94a) colonies. To determine the Colony Forming Units (CFUs) per gram, the following formula was utilized:

> CFU per gram= (CFU per plate ×dilution factor / weight of substrate in grams ×amount plated in mililitres)

3. RESULTS

In this study, we assessed the effectiveness of different straw substrate combinations on the growth and sporulation of *T. lexii* (T-94a) at regular intervals (1, 3, 5, 7, and 10 days). The Broken Rice + Straw combination exhibited slight growth (+) on the 7th day and significant growth with abundant sporulation (++) on the 10th day. The Crushed Maize + Straw combination showed minimal growth on the 5th day (+) and substantial growth and sporulation on the 7th (+++) and 10th days (++++) respectively. The Sorghum + Straw combination displayed moderate growth on the 7th day (+) and intensified growth on the 10th day (++).

The Wheat + Straw combination showed minimal to moderate growth on the 7th day (+) and remained at a moderate level (++) on the 10th day. The Cow Dung Cake + Straw combination exhibited moderate growth on the 7th day (+) and significant growth with sporulation (+++) on the 10th day. Throughout the observation period, the control group showed no significant growth or sporulation. After 5 days of observation, the Broken Rice + Straw combination displayed non-uniform growth with woven whitish mycelial growth in patches, accompanied by relatively less sporulation. In contrast, Crushed Maize + Straw exhibited uniform growth with a white compact appearance and moderate sporulation. Sorghum + Straw showed non-uniform growth with light greenish characteristics and moderate sporulation. Wheat + Straw presented non-uniform growth with woven whitish mycelial growth and less sporulation. Cow Dung Cake exhibited non-uniform growth with white mycelial growth and relatively less sporulation, while the control group showed non-uniform growth with green patches and no sporulation.

After a 10-day observation period, Broken Rice + Straw displayed moderately uniform growth with woven greenish growth in patches and relatively less sporulation. Crushed Maize + Straw exhibited highly uniform growth characterized by dark greenish compactness and profuse sporulation. Sorghum + Straw displayed moderately uniform growth with a fluffiness of whitish-greenish growth and moderate sporulation. Wheat + Straw maintained moderately uniform growth with woven greenish growth in patches and less sporulation. Cow Dung Cake displayed moderately uniform growth with white mycelial growth and moderate sporulation, while the control group exhibited non-uniform growth with a greenish appearance and no sporulation.

Regarding the assessment of different substrates, the maize grain + straw composition had a significantly higher population of *T. lexii* (94a) at 8.81 × 10^7 cfu/ml. It was followed by sorghum grain (6.0 × 10^7cfu/ml) and rice grain (4.9 × 10^7cfu/ml). Wheat straw had a population of 4.5 × 10^7 cfu/ml, Cow dung cake had 3.9 × 10^7 cfu/ml, and the control group had 2.76 × 10^7 cfu/ml.

4. DISCUSSION

The results of the present study demonstrated varying responses to different substrates for the growth of Trichoderma in straw. Comparable findings in previous research have shown promising results when producing Trichoderma spores in rice grain using various organic substrates. For instance, Michel-Aceves et al. [5] obtained impressive results with corn cob as a substrate. Harman et al. [6] explored various culture media and achieved substantial spore production. Deschamps et al. [7] experimented with a combination of substrates for spore production, with the best results achieved using sugar beet. Verma et al. [8] successfully achieved high *T. viride* conidiospore production by utilizing starch as a carbon source under liquid growth conditions. Flodman and Noureddini [9] cultivated Trichoderma reesei on maize grains and achieved remarkable conidiation results, while Rocky-Salimi and Hamidi-Esfahani [10] observed high hydrolytic activity when growing *T. reesei* on rice bran.

Table 1. Efficacy of combination of straw substrate on growth and sporulation of *T. lexii* isolate (94a)

S.no.	Substrate	Effect on growth and sporulation of *Trichoderma lexii*(94a)				
		1	3	5	7	10
1	Broken Rice + straw	-	-	-	+	++
2	Crushed Maize + straw	-	-	+	++	+++
3	Sorghum + straws	-	-	-	+	++
4	Wheat+ straw	-	-	+	+	++
6	Cow dung cake +straw	_	_	+	+	++
7	Control	_	_	_	_	_

(+): slow growth; (++): moderate growth; (+++): profuse growth and sporulation; (-): no growth

Table 2. Growth and sporulation characteristics of *T. lexii* isolate 94a on combination of straw & substrate (After 5 days)

Substrate combination	Uniformity	Fungal growth and colour	Sporulation
Broken Rice + straws	Non uniform	Woven whitish mycelial growth in patches	Less sporulation
Crushed Maize + straws	Uniform growth	White compactness	Moderate sporulation
Sorghum + straws	Non-uniform	Light greenish	Moderate sporulation
Wheat + straw	Non-uniform	Woven whitish mycelial growth	Less sporulation
Cow dung cake	Non -uniform	White mycelial growth	Less sporulation
Control	Non- uniform	Green patches	No sporulation

Table 3. Growth and sporulation characteristics of *T. lexii* isolate 94a on combination of straw & substrate (After 10 days)

Physical parameter	Uniformity	Fungal growth and colour	Sporulation
Broken Rice+straws	Moderately uniform	Woven greenish growth in patches	Less sporulation
Crushed Maize + straws	Highly uniform	Dark Greenish compactness	Profuse sporulation
Sorghum +straws	Moderately uniform	Fluffiness whitish greenish growth	Moderate sporulation
Wheat+ straw	Moderately uniform	Woven greenish growth in patches	Less sporulation
Cow dung cake	Moderately uniform	White mycelial growth	Moderate sporulation
Control	Non- uniform	Greenish with no growth	No sporulation

Table 4. CFU count on growth of *Trichoderma* in different substrates with rice straw

Different substrate combinations with straw	CFU/ml
Crushed Maize	8.81 × 10^7 cfu/ml
Broken Rice	6.0 × 10^7cfu/ml
Wheat grain	4.9 × 10^7cfu/ml
Cow dung cake	3.9 × 10^7cfu/ml
Control	2.76 × 10^7 cfu/ml

Fig. 1. Efficacy of Combination of straw substrate to promote the growth and sporulation of *Trichoderma lexii* isolate (94a). combination of straw with a) Maize, b) sorghum, c) rice d) wheat e) cow dung & f) control

5. CONCLUSION

In summary, our study has unveiled the significant growth and sporulation potential of *Trichoderma lexii* isolate (94a) when cultivated in combination with broken rice, crushed maize, and sorghum paired with straw. These findings are promising in the context of improving agricultural practices and waste management.

Further investigation into the optimal ratios of substrates within these combinations could fine-tune the growth and sporulation of *Trichoderma*. Achieving the ideal balance of straw and grain substrate could lead to even more efficient biocontrol agents and waste decomposition. Scaling up the application of these substrate combinations for large-scale agricultural practices is an important future consideration. Research focusing on the practicality and cost-effectiveness of implementing these techniques in real-world farming scenarios would be invaluable. Conducting field trials to assess the real-world impact of these optimized substrate combinations on crop health and yield could validate the potential benefits of this research. Observing how *Trichoderma* performs in diverse environmental conditions will be crucial. Investigating the long-term effects of employing *Trichoderma* as a biocontrol agent in sustainable agricultural systems could shed light on its role in reducing the reliance on chemical pesticides and promoting environmentally friendly farming practices. Assessing the broader environmental impact of adopting these techniques, including reductions in agricultural waste, improvements in soil quality, and the potential reduction in chemical runoffs, would contribute to our understanding of sustainable agriculture. By exploring these future research directions, we can work towards practical applications and a more comprehensive understanding of how the combined use of *Trichoderma* and specific substrate combinations can revolutionize modern agriculture while promoting environmental sustainability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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