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EFFECT OF AERATION DYNAMICS ON BIOMASS PRODUCTION AND SPORULATION EFFICIENCY OF *Trichoderma harzianum* DURING MASS MULTIPLICATION

Polina Naga Ratnam[1], Pankaj B. Deore[1*], Shubham A. Pol[1], Dinesh R. Nandre[2], S. Kavithanjali[1] and Shaikh Humera Shanoor[1]

[1]Department of Plant Pathology, Mahatma Phule Krishi Vidyapeeth, Dhule - 424 004, Maharashtra, India.

[2]Krishi Vigyan Kendra, Dhule - 424 004, Maharashtra, India.

*Corresponding author E-mail : dr.pankaj.b.deore@gmail.com

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ABSTRACT

Optimizing cost-effective liquid media and gaseous exchange is crucial for the commercial mass multiplication of the biocontrol fungus *Trichoderma harzianum*. This investigation evaluated ten customized agro-industrial broth formulations integrating potato starch, corn flour, tapioca, jaggery, lentil powder, soybean flour, yeast extract and trace mineral salts against a Potato Dextrose Broth control (T_{11}) under hermetically sealed (airtight) and passively ventilated (aerated) regimes at 28 ± 1°C using a Completely Randomized Design. Morphological progression was assessed via a cumulative qualitative scoring index (scale 6–30) encompassing mycelial density, surface colonization and conidial pigmentation, alongside gravimetric dry biomass quantification. Airtight incubation severely constrained fungal morphogenesis and biomass yield due to headspace hypoxia, with qualitative scores and dry weights peaking at 20 and 0.50 g, respectively. Under sealed conditions, corn-flour-based T_7 (0.50 g; score 18), tapioca-based T_6 (0.47 g; score 20) and potato starch–jaggery formulation T_9 (0.44 g; score 12) nearly doubled the biomass of the control T_{11} (0.25 g; score 6). Conversely, passive aeration triggered a profound metabolic surge across all substrates. Six formulations (T_4, T_5, T_6, T_8, T_9 and T_{10}) attained the maximum qualitative score of 30, forming luxuriant, dark-green conidiating mats. Gravimetrically, T_9 generated the highest aerated dry biomass (1.87 g), performing at statistical parity with T_6 (1.81 g), T_8 (1.65 g), $_T$7 (1.36 g) and T_{10} (1.31 g) delivering up to a 2.8-fold yield increase over the aerated control T_{11} (0.66 g). Notably, jaggery-rich T_8 exhibited a 12.7-fold aeration-induced biomass recovery (0.13 g to 1.65 g), whereas T_6 and T_7 demonstrated exceptional resilience across both oxygen regimes.

Key words : Aeration dynamics, Agro-industrial substrates, Conidiogenesis, Liquid fermentation, Mycelial biomass.

Introduction

Biological control of soil-borne plant pathogens using antagonistic fungi such as *Trichoderma harzianum* offers an ecologically sustainable alternative to synthetic fungicides (Harman *et al.*, 2004). However, commercial bioformulation is frequently constrained by the high cost of standard semi-synthetic media like Potato Dextrose Broth (PDB), which lack the complex nutritional heterogeneity required for optimal biomass accumulation and conidiogenesis (Lewis and Papavizas, 1983).

Utilizing inexpensive agro-industrial by-products such as starches, flours and jaggery provides a viable economic alternative. In filamentous fungi, vegetative growth and sporulation depend heavily on substrate composition and the carbon-to-nitrogen (C:N) balance (Gao *et al.*, 2007). Furthermore, because *T. harzianum* is an obligate aerobe, physical aeration dynamics within the culture vessel serve as a critical physiological switch (Verma *et al.*, 2007). In hermetically sealed vessels, rapid oxygen depletion induces hypoxia, severely limiting bioenergetic efficiency and morphological progression. Conversely, passive aeration eliminates respiratory bottlenecks, unlocking the fungus's full biosynthetic potential for robust biomass

production and conidiogenesis.

Despite these principles, the interactive effects of customized agro-industrial substrate formulations under contrasting airtight and aerated fermentation regimes remain insufficiently integrated. Therefore, this study evaluated ten customized liquid media formulations alongside a standard PDB control to determine their efficacy on morphological development and mycelial biomass productivity of *T. harzianum* under airtight and passively aerated conditions.

Materials and Methods

The investigation was carried out at the Department of Plant Pathology, Govt. College of Agriculture, Dhule, Maharashtra, India. Axenic stock cultures of *T. harzianum* were procured from the departmental culture collection and maintained on Potato Dextrose Agar (PDA) slants at 4°C, with periodic subculturing to preserve physiological vigour. Prior to experimentation, the fungi were activated on fresh PDA plates and incubated at 28 ± 1°C for 5 days to obtain actively proliferating mycelial margins.

Preparation of Substrate–ingredient Media formulations

Based on preliminary *in vitro* screening, promising cost-effective substrate–ingredient formulations were selected to evaluate the influence of aeration dynamics during mass multiplication and benchmarked against a standard unamended control medium, Potato Dextrose Broth (T_{11}). The treatment details are as given in Table 1.

The customized liquid formulations were prepared

Table 1 : Treatment details.

Trt.	Ingredients
T_1	Potato Starch (10g) + Lentil Powder (5g) + Soybean Flour (5g) + Yeast (4g) + KH_2PO_4 (1g) + K_2HPO_4 (1g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (1g) + $ZnSO_4 \cdot 7H_2O$ (20mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (20mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (150mg) + Chloramphenicol (50mg)
T_2	Corn Flour (20g) + Lentil Powder (10g) + Soybean Flour (10g) + Yeast (3g) + KH_2PO_4 (1.5g) + K_2HPO_4 (0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (15mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (100mg) + Chloramphenicol (50mg)
T_3	Jaggery (40g) + Lentil Powder (10g) + Soybean Flour (10g) + Yeast (4g) + KH_2PO_4 (1.5g) + K_2HPO_4 (0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (15mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (100mg) + Chloramphenicol (50mg)
T_4	Potato Starch(15g) + Jaggery(20g) + Lentil Powder (10g) + Soybean Flour (10g) + Yeast (4g) + KH_2PO_4 (1.5g) + K_2HPO_4 (0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (15mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (100mg) + Chloramphenicol (50mg)
T_5	Potato Starch (15g) + Lentil Powder (10g) + Soybean Flour (10g) + Yeast (4g) + KH_2PO_4 (1.5g) + K_2HPO_4 (0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (15mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (100mg) + Chloramphenicol (50mg)
T_6	Tapioca (30g) + Lentil Powder (15g) + Soybean Flour (15g) + Yeast (3g) + KH_2PO_4 (1g) + K_2HPO_4 (0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (10mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (200mg) + Chloramphenicol (50mg)
T_7	Corn Flour (30g) + Lentil Powder (15g) + Soybean Flour (15g) + Yeast (3g) + KH_2PO_4 (1g) + K_2HPO_4 (0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (10mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (200mg) + Chloramphenicol (50mg)
T_8	Jaggery (40g) + Lentil Powder (15g) + Soybean Flour (15g) + Yeast (3g) + KH_2PO_4 (1g) + K_2HPO_4 (0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (10mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2$.2H, O (200mg) + Chloramphenicol (50mg)
T_9	Potato Starch(20g) + Jaggery(20g) + Lentil Powder (15g) + Soybean Flour (15g) + Yeast (3g) + KH_2PO_4 (1g) + K_2HPO_4 (0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (10mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (200mg) + Chloramphenicol (50mg)
T_{10}	Potato Starch(20g) + Lentil Powder (15g) + Soybean Flour (15g) + Yeast (3g) + KH_2PO_4 (1g) + K_2HPO_4(0.5g) + $MgSO_4 \cdot 7H_2O$ (0.5g) + KCl (0.5g) + $ZnSO_4 \cdot 7H_2O$ (10mg) + $MnSO_4 \cdot H_2O$ (10mg) + $FeSO_4 \cdot 7H_2O$ (15mg) + $CuSO_4 \cdot 5H_2O$ (1mg) + $CaCl_2.2H_2O$ (200mg) + Chloramphenicol (50mg)
T_{11} (Control)	Potato Dextrose Broth + Chloramphenicol (50mg)

by homogenizing graded combinations of agro-industrial carbon and organic nitrogen substrates comprising sago (tapioca pearl), lentil powder, soybean flour, corn flour, jaggery and potato starch in distilled water. Yeast extract was incorporated as a source of B-complex vitamins, amino acids and trace mineral cofactors, while chloramphenicol (50 mg/L) was amended across all treatments to prevent bacterial contamination. Measured aliquots (100 mL) of each broth formulation were dispensed into 250 mL conical flasks and sterilized by autoclaving at 121°C and 1.1 kg/cm² (15 psi) pressure for 20 min.

Inoculation and Aeration regimes

Upon cooling to ambient temperature under a laminar airflow cabinet, each flask was aseptically inoculated with a uniform 5 mm mycelial disc excised from the actively growing periphery of a 5-day-old *T. harzianum* culture using a sterile cork borer. To evaluate the role of gaseous exchange on vegetative proliferation and sporulation, the inoculated conical flasks were subjected to two distinct aeration regimes:

a) **Airtight (Sealed) Regime :** Culture flasks were hermetically sealed using airtight, non-permeable closures wrapped securely with Parafilm to prevent external gaseous exchange and restrict available oxygen to the initial headspace volume.

b) **Aerated (Ventilated) Regime :** Culture vessels were plugged with sterile, non-absorbent cotton plugs to permit continuous passive diffusion of molecular oxygen into the flask and the dissipation of respiratory carbon dioxide.

All cultures were arranged in a Completely Randomized Design (CRD), replicated thrice, and incubated under stationary conditions in a Biological Oxygen Demand (BOD) incubator maintained at 28 ± 1°C.

Qualitative assessment of Growth and sporulation

At the end of the incubation period, the qualitative developmental response of the fungus across each substrate–ingredient formulation was evaluated visually using three morphological criteria: **Growth Type** (mycelial mat density and thickness), **Cover/Spread** (extent of surface colonization across the liquid medium), and **Colour** (intensity of conidial pigmentation). Each parameter was scored independently on a standardized 1 to 5 ordinal rating scale (Table 2).

For each treatment, mean scores (ranging from 1.0 to 5.0) were calculated for each of the three individual parameters across replications and the individual ratings were summed to derive a Cumulative Total Score to rank overall phenotypic vigour.

Table 2 : Qualitative rating scale for evaluating morphological growth and sporulation of *Trichoderma* spp.

Score	Description
1	Very poor growth; very sparse, pure white mycelium with limited spread
2	Poor growth; thin white mycelium with slight green tint and limited spread
3	Moderate growth; intermediate density with light green and moderate spread
4	Good growth; dense green mycelium with extensive spread
5	Excellent growth; very dense, dark green mycelium with maximum spread

Quantitative estimation of mycelial biomass (Dry weight)

Following qualitative scoring, the quantitative mycelial biomass produced in each culture flask was determined gravimetrically. The fungal mycelial mats were harvested by filtering the culture contents through pre-dried, pre-weighed Whatman No. 1 filter paper cones. The retained biomass was rinsed thoroughly with sterile distilled water to remove residual medium constituents and soluble salts. The filter papers containing the washed mycelial mats were subsequently dried in a hot-air oven at 60°C until a constant weight was achieved, cooled in a desiccator and weighed on a precision analytical balance. Net dry mycelial biomass was calculated by subtracting the initial dry weight of the filter paper and expressed in grams (g).

Results and Discussion

The physiological performance of *T. harzianum* during mass multiplication is governed by the interaction between the nutritional composition of the basal substrate and the gaseous microenvironment within the culture vessel (Papavizas, 1985; Harman *et al*., 2004). As an obligate aerobic deuteromycete, *T. harzianum* depends on molecular oxygen to drive bioenergetic efficiency, vegetative hyphal branching and the transition to conidiogenesis (Verma *et al*., 2007). Evaluating diverse substrate–ingredient media formulations across airtight and passively aerated regimes in the present study demonstrated that headspace gas exchange acts as a primary physiological switch determining how effectively the fungus converts medium nutrients into structural biomass and mature phialoconidia.

Effect of different substrate–ingredient media formulations on the growth of *T. harzianum* under airtight conditions

Cultivation of *T. harzianum* under hermetically sealed (airtight) conditions revealed substantial formulation-dependent variability in colony growth type, surface coverage and conidial pigmentation, while imposing an overarching constraint on morphological progression across the experimental series (Table 3). Among the sealed cultures, formulation T_6 supported the highest qualitative development, securing a cumulative score of 20 (mean scores of 3.5 for both growth type and substrate coverage and 3.0 for colour), indicative of intermediate-to-dense mycelia and extensive radial spread. Formulation T_1 ranked second with an aggregate score of 19 (mean ratings of 2.5 for growth type and 3.5 for both coverage and pigmentation), followed by moderate colonization in T_7 (cumulative score of 18, with a uniform mean of 3.0 across all three criteria) and T_2 (cumulative score of 17, with means of 2.5 for growth type and 3.0 for both spread and colour). Excluding the basal control, formulation T_8 proved least supportive under sealed conditions, registering a minimal cumulative score of 7 (mean scores of 1.0 for growth type and coverage and 1.5 for colour), which reflected sparse, predominantly white mycelial growth with negligible spread. Suboptimal qualitative development was similarly observed in treatments T_3 (9), T_5 (10) and T_{10} (10). Nevertheless, when benchmarked against the unamended control T_{11} which suffered severe morphological arrest under airtight confinement (total score of 6, recording the baseline mean of 1.0 across all parameters) every evaluated formulation, specifically T_6 (20), T_1 (19), T_7 (18), T_2 (17), T_4 (12), T_9 (12), T_5 (10), T_{10} (10), T_3 (9) and T_8 (7), achieved higher cumulative scores, confirming visibly improved hyphal density, colonization and colouration over the control.

Gravimetric quantification of dry mycelial biomass under airtight incubation corroborated these formulation-specific differences (Table 2). Formulation T_7 produced the maximum dry mycelial weight (0.50 g) and remained statistically at par with all other treatments except the two lowest-yielding media, T_8 (0.13 g) and T_5 (0.12 g). Conversely, treatment T_5 recorded the minimum mycelial biomass (0.12 g), though it was statistically comparable to all formulations other than the top two performers, T_7 (0.50 g) and T_6 (0.47 g). Relative to the unamended control T_{11} (0.25 g), enhanced biomass accumulation was achieved in T_7 (0.50 g), T_6 (0.47 g), T_9 (0.44 g), T_4 (0.35 g) and T_{10} (0.32 g). Formulation T_2 (0.25 g) equaled the control yield, whereas T_1 (0.24 g), T_3 (0.17 g), T_8 (0.13 g) and T_5 (0.12 g) accumulated less dry weight than T_{11}.

The general restriction of colony development and biomass yield across sealed container peaking at a score of 20 and a dry weight of 0.50 g stems from rapid oxygen exhaustion within the closed headspace. As germinating propagules and extending hyphal tips consume dissolved and gaseous oxygen via mitochondrial respiration, the sealed microenvironment becomes progressively hypoxic and hypercapnic (Smits *et al.*, 1996; Rahardjo *et al.*, 2006). Oxygen depletion impairs cytochrome-mediated oxidative phosphorylation, curbing intracellular adenosine triphosphate (ATP) generation and limiting the energy-intensive biosynthesis of cell-wall chitin, β-1,3-glucans, and membrane sterols (Lejeune and Baron, 1995; Kredics *et al.*, 2003). However, the ability of formulations T_7, T_6 and T_9 to nearly double the biomass of the control T_{11} demonstrates that targeted nutritional fortification partially buffers *T. harzianum* against hypoxic stress. When molecular oxygen is scarce, filamentous fungi shift central carbon metabolism toward glycolytic substrate-level phosphorylation to sustain cellular maintenance and basal growth (Verma *et al.*, 2007). Formulations T_7, T_6 and T_9 evidently supplied readily assimilable simple carbohydrates alongside bioavailable organic nitrogen and vitamin cofactors that entered glycolysis directly without requiring oxygen-intensive extracellular enzymatic cleavage (Jackson *et al.*, 1991; Gao *et al.*, 2007).

Crucially, the airtight dataset revealed a distinct physiological uncoupling between visual qualitative scores and gravimetric dry weight across several intermediate formulations. While, T_1 and T_2 achieved high qualitative scores (19 and 17 respectively) driven by extensive surface spread and greenish pigmentation, they yielded only 0.24 g and 0.25 g of dry biomass. Conversely, T_9 and T_4 registered modest visual scores (12 each), but accumulated significantly heavier dry weights of 0.44 g and 0.35 g, performing at statistical parity with T_7 (0.50 g). This divergence reflects contrasting adaptive growth strategies under combined nutritional and respiratory stress (Munoz *et al.*, 1995; Steyaert *et al.*, 2010). In T_1 and T_2, localized nutrient stoichiometry triggered an exploratory foraging response characterized by thin, effuse surface hyphae (growth-type score of 2.5) that rapidly covered the medium surface and underwent premature stress-induced conidiation, resulting in visually expansive colonies with minimal structural mass. By contrast, the nutrient matrices of T_9 and T_4 promoted dense, compact vegetative hyphal aggregation and submerged colonization while delaying energy-costly pigment synthesis under low oxygen tension, yielding lower visual scores but substantial dry biomass (Agosin *et al.*, 1997). Meanwhile, the severe growth arrest

observed in T_8 (0.13 g), T_5 (0.12 g) and T_3 (0.17 g) under sealed conditions despite their strong subsequent performance under aeration indicates acute self-asphyxiation. Substrates rich in complex polymeric starches and proteins demand inducible extracellular hydrolytic enzyme secretion (amylases, cellulases, and proteases), which entails a high initial specific oxygen uptake rate that rapidly exhausts headspace oxygen in sealed containers and blocks morphogenesis (Rahardjo *et al*., 2006; Kubicek *et al*., 2014).

Effect of different substrate–ingredient media formulations on the growth of *T. harzianum* under aerated conditions

Provision of passive gaseous exchange under aerated incubation markedly elevated qualitative growth attributes and biomass productivity across the entire spectrum of substrate ingredient formulations (Table 4). Six formulations T_4, T_5, T_6, T_8, T_9 and T_{10} achieved optimal phenotypic expression, each attaining the maximum cumulative score of 30 with uniform ceiling mean scores of 5.0 across growth type, surface coverage and colouration, manifesting as thick, dark green mycelial mats spanning the entire medium surface. Near-maximal development was also recorded in T_7 (total score of 29; mean scores of 5.0 for growth type and colour, and 4.5 for cover/spread) and T_2 (total score of 28; mean scores of 4.5 for growth type and coverage and 5.0 for colour). Even the lowest-ranking amended formulation under aerobic incubation, T_3 (cumulative score of 24), maintained a uniform mean score of 4.0 across all three morphological parameters, denoting dense green mycelial growth and extensive coverage that surpassed the highest score recorded under airtight confinement. Compared with the aerated control T_{11}, which exhibited commendable growth (total score of 25, with averages of 4.5 for growth type and coverage and 3.5 for pigmentation) nine formulations, namely T_4 (30), T_5 (30), T_6 (30), T_8 (30), T_9 (30), T_{10} (30), T_7 (29), T_2 (28) and T_1 (26), demonstrated superior qualitative vigour and deeper conidial colouration, whereas only T_3 (24) scored marginally lower.

Quantitative gravimetric determinations under aerated conditions (Table 3) confirmed substantial variations in biomass productivity once respiratory limitations were removed. Treatment T_9 emerged as the premier formulation, yielding the highest dry mycelial biomass (1.87 g) and performing at statistical parity with T_6 (1.81 g), T_8 (1.65 g), T_7 (1.36 g) and T_{10} (1.31 g). Similarly, the second-highest biomass producer, T_6 (1.81 g), was statistically at par with T_8 (1.65 g), T_7 (1.36 g), T_{10} (1.31 g) and T_2 (1.10 g). At the lower end of the spectrum, formulation T_1 generated the minimum mycelial dry weight (0.66 g) and shared statistical parity with the control T_{11} (0.66 g) as well as treatments T_5 (0.78 g), T_4 (0.88 g), T_3 (1.03 g) and T_2 (1.10 g). Evaluated against the aerated control T_{11} (0.66 g), every amended formulation except T_1 (0.66 g) produced higher dry mycelial biomass, with the leading formulations (T_9, T_6 and T_8) delivering a 2.5- to 2.8-fold yield enhancement.

This universal surge in vegetative vigour and dark

Table 3 : Effect of different substrate–ingredient media formulations on the growth of *T. harzianum* under airtight conditions.

S. no.	Treatment	Mycelial growth										Mycelial Biomass/dry weight(g)
		Growth type			Cover/Spread			Colour			Total Score	
		R1	R2	Av	R1	R2	Av	R1	R2	Av		
1	T_1	3	2	2.50	4	3	3.5	4	3	3.5	19	0.24 (0.48)
2	T_2	2	3	2.50	3	3	3.0	3	3	3.0	17	0.25 (0.50)
3	T_3	2	1	1.50	2	1	1.5	2	1	1.5	9	0.17 (0.41)
4	T_4	2	2	2.00	2	2	2.0	2	2	2.0	12	0.35 (0.59)
5	T_5	2	1	1.50	2	1	1.5	2	2	2.0	10	0.12 (0.35)
6	T_6	4	3	3.50	4	3	3.5	4	2	3.0	20	0.47 (0.69)
7	T_7	3	3	3.00	3	3	3.0	3	3	3.0	18	0.50 (0.71)
8	T_8	1	1	1.00	1	1	1.0	1	2	1.5	7	0.13 (0.36)
9	T_9	3	1	2.00	3	1	2.0	3	1	2.0	12	0.44 (0.66)
10	T_{10}	2	1	1.50	2	1	1.5	2	2	2.0	10	0.32 (0.57)
11	T_{11} (Control)	1	1	1.00	1	1	1.0	1	1	1.0	6	0.25 (0.50)
											SEd	0.10
											CD 1%	0.32

Note: *Figures in parenthesis are square root transformed ($\sqrt{n+1}$) values

green pigmentation confirms that continuous oxygen diffusion and the dissipation of respiratory carbon dioxide are essential for unlocking the full biosynthetic capacity of *T. harzianum* (Flodman and Noureddini, 2013; De la Cruz-Quiroz *et al*., 2017). In *Trichoderma*, aerial conidiophore differentiation, phialide formation and spore maturation are regulated by blue-light photoreceptors, cyclic adenosine monophosphate (cAMP) signaling and intracellular reactive oxygen species (ROS) generated via NADPH oxidases (Aguirre *et al*., 2005; Carreras-Villaseñor *et al*., 2012). Abundant molecular oxygen not only fuels tricarboxylic acid (TCA) cycle flux to supply carbon skeletons for structural wall synthesis, but also acts as a direct co-substrate for developmentally regulated multicopper laccases and polyketide synthases that cross-link dark green melano-pigments in the mature conidial wall (Steyaert *et al*., 2010). The superior gravimetric yields in T_9, T_6 and T_8 align with earlier findings demonstrating that composite media integrating slow-release polysaccharides, organic leguminous nitrogen and trace mineral cofactors support substantially higher mycelial mat thickness and spore competence in *T. harzianum* than standard semi-synthetic media (Prasad and Rangeshwaran, 2000; Rajput *et al*., 2014; Daryaei *et al*., 2016).

Comparing treatment performance across the two aeration regimes distinguishes two functional classes among the top formulations:

a) Strictly Aerobic, Nutrient-dense Formulations (T_8, T_9, T_{10} and T_5) : Formulation T_8 exhibited the most dramatic aeration-induced recovery, surging from a qualitative score of 7 and 0.13 g biomass under sealed conditions to a ceiling score of 30 and 1.65 g biomass under aeration a 12.7-fold increase in dry weight. Similarly, aeration increased biomass 4.25 fold in T_9 (0.44 g to 1.87 g; score 12 to 30), more than fourfold in T_{10} (0.32 g to 1.31 g; score 10 to 30), and 6.5 fold in T_5 (0.12 g to 0.78 g; score 10 to 30). This confirms that these matrices contain rich polymeric carbon and protein reserves requiring unrestricted aerobic respiration and active hydrolytic enzyme secretion for full assimilation (Smits *et al*., 1996; Kubicek *et al*., 2014).

b) Metabolically Versatile Formulations (T_6 and T_7) : Formulations T_6 and T_7 ranked in the top statistical tier under both airtight (0.47 g and 0.50 g) and aerated (1.81 g and 1.36 g) conditions. Their resilience across contrasting oxygen tensions points to a balanced dual-phase nutrient profile combining immediately metabolizable sugars (sustaining glycolytic survival under hypoxia) with complex slow-release nutrients (fueling heavy aerobic biomass synthesis when oxygen is abundant) (Danielson and Davey, 1973; Gao *et al*., 2007).

Finally, the aerated results highlight an important distinction between two-dimensional visual saturation and three-dimensional biomass density. Although, six formulations (T_4, T_5, T_6, T_8, T_9 and T_{10}) achieved the identical ceiling score of 30, their gravimetric dry weights varied more than twofold, from 0.78 g (T_5) and 0.88 g

Table 4 : Effect of different substrate–ingredient media formulations on the growth of *T. harzianum* under aerated conditions.

S. no.	Treatment	Mycelial growth										Mycelial Biomass/dry weight(g)
		Growth type			Cover/Spread			Colour			Total Score	
		R1	R2	Av	R1	R2	Av	R1	R2	Av		
1	T_1	4	4	4.0	4	5	4.5	4	5	4.5	26	0.66 (0.81)
2	T_2	4	5	4.5	4	5	4.5	5	5	5.0	28	1.10 (1.05)
3	T_3	4	4	4.0	4	4	4.0	4	4	4.0	24	1.03 (1.02)
4	T_4	5	5	5.0	5	5	5.0	5	5	5.0	30	0.88 (0.94)
5	T_5	5	5	5.0	5	5	5.0	5	5	5.0	30	0.78 (0.88)
6	T_6	5	5	5.0	5	5	5.0	5	5	5.0	30	1.81 (1.34)
7	T_7	5	5	5.0	5	4	4.5	5	5	5.0	29	1.36 (1.17)
8	T_8	5	5	5.0	5	5	5.0	5	5	5.0	30	1.65 (1.28)
9	T_9	5	5	5.0	5	5	5.0	5	5	5.0	30	1.87 (1.37)
10	T_{10}	5	5	5.0	5	5	5.0	5	5	5.0	30	1.31 (1.14)
11	T_{11} (Control)	5	4	4.5	5	4	4.5	4	3	3.5	25	0.66 (0.81)
											SEd	0.10
											CD 1%	0.31

Note: *Figures in parenthesis are square root transformed (√n+1) values.

(T_4) to 1.81 g (T_6) and 1.87 g (T_9). Likewise, T_1 scored higher qualitatively (26) than T_3 (24), yet T_1 yielded the lowest biomass (0.66 g), while T_3 accumulated 1.03 g. Once aerial hyphae and dark green conidiophores completely cover the medium surface, qualitative indices reach their upper limit of 5.0 (De la Cruz-Quiroz *et al*., 2017). However, media with lower total carbon concentrations or rapidly depleting nitrogen pools (such as T_1, T_4 and T_5) trigger early starvation signaling, shifting metabolic flux prematurely from vegetative hyphal thickening into superficial conidiation (Gao *et al*., 2007; Steyaert *et al*., 2010). Conversely, nutrient-dense, well-buffered formulations such as T_9, T_6, T_8 and T_7 sustain prolonged three-dimensional hyphal branching and deep mat formation alongside conidiogenesis, maximizing both visual vigour and true gravimetric biomass yield (Agosin *et al*., 1997; Daryaei *et al*., 2016).

Conclusion

Substrate composition and oxygen availability fundamentally dictate the mass multiplication efficacy of *Trichoderma harzianum*. Hermetically sealed environments severely limit growth through hypoxia, though formulations T_7, T_6 and T_9 successfully buffer this stress. Conversely, passive aeration unlocks the fungus's full biosynthetic potential, triggering luxuriant conidiogenesis and delivering up to a 2.8-fold biomass enhancement over controls, peaking at 1.87 g in T_9. Furthermore, media such as T_6 and T_7 demonstrate exceptional metabolic versatility across contrasting oxygen regimes, whereas others like T_8 exhibit strict aeration dependence. Conclusively, pairing balanced agro-industrial matrices with proper ventilation provides a robust, highly efficient strategy for commercial bio-inoculant production.

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