



RESEARCH ARTICLE

Role of pH and temperature in modulating the growth of *Trichoderma asperellum*

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Abstract

The genus *Trichoderma* is asexually and sexually reproducing filamentous fungi that are widely distributed. These *Trichoderma* species are used extensively in agriculture as biocontrol agents to manage plant diseases. In view of this present study was conducted at College of Agriculture, Central Agricultural University, Imphal during the 2021–22 cropping season. In this study, various levels of pH and temperature were evaluated to investigate variations in mycelial growth among the isolates. The pH and temperature are significant factors influencing fungal growth. The study investigated how pH and temperature interact to influence the radial growth, fresh weight and dry weight of *Trichoderma asperellum* under *in vitro* conditions. The radial growth of the isolates improved as the temperature rise from 30–34 °C, within a pH range of 4–8. The highest fresh and dry weights of the mycelium were recorded at pH 4 when the temperature was either 26 °C or 30 °C. Radial growth, fresh weight and dry weight of the isolates peaked at 30 °C. Maximum fresh and dry mycelial weights occurred within the pH range of 4–6. This study demonstrated that the interaction between pH and temperature has a significant impact on the growth of *T. asperellum* isolates. The optimum pH (4–6) and temperature (30 °C) identified in this study can be used to optimise the mass multiplication and commercial production of *T. asperellum*. These findings will aid in developing efficient bioformulations with improved quality, consistency and biocontrol efficacy.

Keywords: interaction; mycelial growth; pH; temperature; *Trichoderma asperellum*

Introduction

Fungal plant pathogens play an important role in causing diseases which lead to tremendous losses in cultivated crops. Intensified use of systemic fungicides has resulted in an enormous accumulation of toxic metabolites, that are unsafe to humans, animals and the environment (1). In order to avoid these harmful effects, effective alternative methods should be implemented. *Trichoderma* can produce plant growth stimulators, including indole-3-acetic acid (IAA) and harzianolide. Moreover, this genus enhances crop photosynthetic efficiency, decomposes soil organic matter, boosts the availability of soil nutrients, promotes greater plant height and stem diameter along with other agronomic characteristics and raises overall productivity (2–4). Most *Trichoderma* species can

produce bioactive compounds and have antagonistic effects on plant-pathogenic nematodes and fungi, according to numerous studies (5). Bioactive substances that can effectively boost crop resistance, reduce plant diseases and promote plant growth include secondary metabolites and cell wall breaking enzyme (6–9).

Today, the main approach to managing plant diseases involves chemical methods, such as using insecticides and fungicides. The misuse of chemical pesticides has severely polluted the environment and contributed to the rise of pathogen resistance to these chemicals, even though chemical control remains beneficial and supports increased agricultural output (10, 11). Many studies have demonstrated that *Trichoderma* offers effective biological control and can reduce the need for chemical pesticides. More

efficient and appropriate strains must be discovered to be identified as prospective biocontrol agents because there are currently few biocontrol agents available against *Trichoderma* spp. (12–15). Biological control agents offer an attractive method for managing plant pathogens and have been studied for many years. The increased use of biological control of plant diseases requires genetic development of biocontrol agents as well as enhanced production and delivery methods (16). *Trichoderma* is a uniquely effective mycoparasite. *Trichoderma* with reduced doses of chemical has the potential to control plant pathogens with minimal impact on the environment (17). Therefore, considering the cost of chemical pesticides and the hazardous risk involved, biological control of plant diseases appears to be an effective and ecofriendly approach being practiced globally (18).

Trichoderma can improve the solubility of soil nutrients, boost plant development and improve plant rhizosphere microecology (19–21). Resistance to abiotic stressors like UV, salt and high temperatures can be enhanced and promoted by plant interactions with *Trichoderma*. Indole-3-acetic acid (IAA), abscisic acid (ABA), ethylene (ET), gibberellic acid (GA) and cytokinins (CK) are among the plant growth hormones that *Trichoderma* primarily produces (22–25). Even though *Trichoderma* exhibits significant potential for use in agriculture, there are still certain issues with its development and application (26–30). Field test performance is inconsistent and the biological control effect is reduced because *Trichoderma* spore preparations are typically living fungal formulations that are easily affected by various environmental factors, including humidity, temperature, soil pH and the existing soil microbial community, when used outdoors. Moreover, certain microorganisms need to be stored at low temperatures to maintain a high concentration of live organisms upon application and biological control agents generally have a limited shelf life. To develop *Trichoderma* biocontrol strains engineered for resistance to chemical pesticides and low temperatures, the initial step involves identifying and producing effective strains that can be screened through genetic engineering methods. To enhance the effectiveness of control and extend the shelf life of biological control agents, it is necessary to develop efficient *Trichoderma* strains compatible with various application methods. Second, it's important to investigate how *Trichoderma* spp. and other microbes interact (31, 32).

In order to enhance the quality of *Trichoderma* biopesticides, the yield of antagonistic substances at the level of cell metabolic flow, genome, proteome and metabolome must be monitored in addition to conventional indicators like pH, dissolved oxygen and temperature during the fermentation process (31). For *Trichoderma* products *in vivo*, however, new scientific quality requirements must be established, such as raising the amount of antagonistic compounds or activity indicators (33). Global temperatures are rising as a result of climate change. As climate change continues, there is a growing need to understand how temperature changes affect the fungus. The *Trichoderma asperellum* species exhibits varied behaviour across different pH and temperature conditions. Although the influence of pH and temperature on the growth of *Trichoderma* species has been

reported previously, the physiological responses of individual isolates often vary depending on their genetic makeup and ecological origin. The *T. asperellum* isolates used in the present study were recovered from diverse ecological niches and were previously identified as promising antagonists against important soil-borne plant pathogens. However, information regarding their isolate-specific growth responses under different pH and temperature regimes is limited. Therefore, this study aimed to determine how pH and temperature interact to influence the growth of *T. asperellum* isolates.

Material and Methods

Four strains of *T. asperellum* (Table 1) were collected from the Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal. On potato dextrose agar (PDA), each isolate of *T. asperellum* was maintained as a pure culture and kept at - 4 °C for future use. Subculturing of isolates was done on PDA medium on petri plates (90 mm in diameter) for the assessment of growth attributes.

Effect of pH and temperature on radial growth, fresh and dry weight of *Trichoderma asperellum*

The impact of pH and temperature on radial growth, fresh weight and dry weight was assessed on PDA and potato dextrose broth (PDB), respectively. Potato dextrose agar was prepared in five 250 mL conical flasks, each filled with 150 mL of media. The pH of the medium was set to 4, 5, 6, 7 and 8 using a digital pH meter, with adjustments made via 0.1N hydrochloric acid (HCl) and 0.1N sodium hydroxide (NaOH). The acid or alkali was added dropwise with continuous stirring and the pH was monitored using a calibrated digital pH meter until the desired value was attained. All conical flasks containing media were autoclaved at 1.1 kg/cm² for 20 min. For each pH level, 20 mL of PDA medium was dispensed into each sterilised Petri dish and allowed to solidify. From the actively growing cultures of the various isolates, 5 mm mycelial discs were obtained using a cork borer and placed at the centre of the Petri plates. Each treatment was replicated five times. The procedure was carried out five times, each at a different temperature: 26 °C, 28 °C, 30 °C, 32 °C and 34 °C. The inoculated plates were incubated at various temperatures until they completely covered the plates. The growth of all four isolates were measured radially (in cm) using a measuring scale at each 24 hr interval until the plates were completely covered.

The fresh and dry weights of the fungus were measured on PDB under varying pH and temperature conditions. Fifty millilitres of each broth, at different pH levels (4, 5, 6, 7 and 8), was evenly distributed into separate 100 mL conical flasks, which were then autoclaved at 1.1 kg/cm² for 20 min and subsequently cooled. From the actively growing cultures of the various isolates, 5 mm mycelial discs were obtained using a cork borer and then individually inoculated into each flask. For each treatment, 5 replicates were kept. The procedure was carried out five times, each at a different temperature: 26 °C, 28 °C, 30 °C, 32 °C and 34 °C. The conical flasks that had been inoculated were incubated at various temperatures for 5 days. Five days after incubation, the fungal mycelial mat was

Table 1. List of the isolates of *Trichoderma asperellum* were used for the study and their accession number

S. No.	Biocontrol agents	Isolation code	Accession number
1.	<i>Trichoderma asperellum</i> (TA1)	TKS-H	MH257327
2.	<i>Trichoderma asperellum</i> (TA2)	NCIPMCAU-7	KU933475
3.	<i>Trichoderma asperellum</i> (TA3)	NCIPMCAU-25	KT601340
4.	<i>Trichoderma asperellum</i> (TA4)	NCIPMCAU-123	KU933476

filtered separately through pre-weighed Whatman No. 1 filter paper and dried overnight in a hot air oven at 60 °C. The dry weight of the fungal mycelium in each treatment was determined by subtracting the weight of the filter paper. The dried and cooled filter papers containing mycelial mats were then reweighed to estimate fungal growth on a dry weight basis, using the following formula:

$$W = W_2 - W_1 \quad (\text{Eq. 1})$$

where, W = the weight of the mycelial mat,

W₁ = the weight of the filter paper

W₂ = the total weight of the fungal mycelial mat and filter paper

Statistical analysis

The experimental data were subjected to an analysis of variance (ANOVA). An ANOVA was carried out to determine the individual and interactive effects of the experimental factors. The study followed a three-factor factorial randomised complete block design (RCBD) to assess the influence of different levels of three variables in combination with four fungal isolates. The same set of isolates was included across all treatment combinations involving the physical parameters under investigation, namely temperature and pH. Data processing and statistical evaluations were performed using the R statistical software environment.

Results and Discussion

Effect of pH and temperature on radial growth, fresh and dry weight of *Trichoderma asperellum*

The radial development of *T. asperellum* mycelium responded differently across the tested pH-temperature combinations. Statistical analysis showed that radial growth of *T. asperellum* differed significantly for all the treatments. The maximum growth of all the isolates (9.00 cm), the combination of temperature 30 °C with pH (4 and 8) was found favourable for the growth of the fungus. The lowest growth rate was observed at 26 °C across all pH levels except pH 5 (Supplementary Fig. 1 and Table 2). The finding indicated that the growth of TA3 was high at all the pH and temperature combinations except pH combination with 26 °C. Among the combination of different level of pH and temperature, the results observed that the combination of temperature 30 °C with pH (4 and 8) was found suitable for the radial growth of *T. asperellum*. The maximum radial growth was obtained at temperature 30 °C (9.00 cm) with pH (4 and 8) and the lowest growth was recorded at 26 °C with pH 4 (4.40 cm) and at 30 °C with pH 7 (4.36 cm) (Table 2). The results also showed that the radial growth of the different isolates was significantly affected by all the combinations of pH and temperature. The maximum radial growth was found at TA3 at all the pH with temperature combination where the mean radial growth was found to be at temperature 28 °C that is 9.00 cm, 32 °C that is 9.00 cm, 34 °C that is 9.00 cm, 30 °C that is 9.00 cm except at pH 7 that is 5.38 cm followed by TA4 that is 7.54 cm and least was found at TA1 that is 7.10 cm. The findings indicated that TA2, TA3 and TA4 exhibited high radial growth of mycelium at all the temperature with pH combinations except TA2 at lower temperature 26 °C. The density plot illustrating the combined effects of pH and temperature on mycelial radial growth revealed that the radial growth of all isolates varied between 1.20 cm and 9.00 cm (Fig. 1).

The combination of different level of pH and temperature, the results showed that the combination of temperature 26 °C with pH 4 was found suitable for the mycelial fresh weight. The maximum fresh weight was obtained at 26 °C with pH 4 (5.06 g), followed by next best temperature for the mycelial fresh weight was at 26 °C with pH 6 (4.61 g) and least was observed at 28 °C with pH 8 (2.90 g) (Supplementary Fig. 2 and Table 3). The results also revealed that the mycelial fresh weight of the different isolates showed a significant effect at all the combinations of pH and temperature. The maximum mycelial fresh weight was found at TA3 followed by TA4 at temperature and least was found at TA1. The findings indicated that TA2, TA3 and TA4 exhibited high mycelial fresh weight at the lower temperature of 26 °C with all the pH combinations. As per density graph for the fresh weight of mycelium between pH and temperature, the density of for mycelial fresh weight of all the isolates were found ranging between 2.90–5.06 g (Fig. 2).

The interaction between temperature and pH significantly influenced the mycelial dry weight of *T. asperellum*. Among the tested combinations, 26 °C coupled with pH 4 produced the highest mycelial dry weight (0.65 g), followed by 26 °C with pH 6 (0.59 g). In contrast, the lowest dry weight (0.34 g) was recorded at 28 °C and pH 8 (Table 4). Statistical analysis revealed significant differences in mycelial dry weight among the fungal isolates across all temperature and pH combinations. With respect to isolate performance, TA3 exhibited the highest average mycelial dry weight (0.59 g), followed by TA4 (0.53 g), whereas TA1 recorded the lowest mean dry weight (0.37 g). The results further demonstrated that isolates TA2, TA3 and TA4 consistently produced greater mycelial biomass at the lower temperature of 26 °C irrespective of the pH level tested. Analysis of the density distribution graph showed that the mycelial dry weight of all isolates ranged from 0.20–0.85 g under the various temperature and pH treatments (Fig. 3).

The differential growth of *T. asperellum* under varying pH and temperature combinations may be attributed to their effects on enzyme activity, membrane stability, nutrient uptake and overall metabolism. The optimum growth observed at 30 °C with pH 4–8 for radial growth and at 26 °C with pH 4 for biomass production indicates that these conditions favour efficient metabolic activity and resource utilisation (34, 35). These findings are consistent with the natural ecology of *T. asperellum*, which commonly inhabits rhizosphere soils and decomposing organic matter under moderate temperatures and slightly acidic to neutral pH (36, 37). The identified optimum conditions are also valuable for commercial mass multiplication and formulation development by enhancing biomass production and propagule quality. Furthermore, the superior performance of isolate TA3 suggests isolate-specific genetic and physiological differences related to metabolic efficiency, nutrient utilisation and stress tolerance (38, 39).

The results are in partial agreement with the findings of a previous study (40), which reported that varied effects of pH and temperature on the growth of *Trichoderma viride* showed that pH 6 and temperature 25 °C are most suitable for the growth of *T. viride*. It was reported that between pH 4.5–5.5 and temperature between 20–37 °C are most suitable for the growth of *T. viride* (41). It was reported that the optimum pH range for the growth of *Trichoderma* species lied between pH 5–6 (42). Similarly, the influence of varying pH levels on the growth and sporulation of indigenous *Trichoderma* isolates and found that colony diameter, growth rate and sporulation were reduced at pH 8 as compared to pH 4 (43). It was

Table 2. Effect of interaction between different pH and temperature on radial growth of native isolates of *Trichoderma asperellum*

Radial growth (cm)		26					28					30					32					34					
Temperature (°C)		4	5	6	7	8	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8	Mean
Isolates	TA1	1.20 (0.34)	8.00 (0.95)	6.90 (0.90)	6.60 (0.88)	6.60 (0.88)	5.14 (0.79)	6.24 (0.86)	6.66 (0.88)	9.00 (1.00)	8.70 (0.99)	9.00 (1.00)	7.52 (0.93)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	5.30 (0.80)	5.30 (0.80)	7.20 (0.91)	3.38 (0.64)	9.00 (1.00)	7.20 (0.91)	8.18 (0.96)	9.00 (1.00)	6.18 (0.86)	8.26 (0.96)	7.10 (0.89)
	TA2	9.00 (1.00)	7.00 (0.90)	6.94 (0.90)	2.20 (0.50)	6.40 (0.87)	7.66 (0.85)	7.66 (0.94)	9.00 (1.00)	7.46 (0.93)	9.00 (1.00)	9.00 (1.00)	6.28 (0.86)	7.18 (0.91)	2.00 (0.48)	9.00 (1.00)	8.28 (0.97)	9.00 (1.00)	9.00 (1.00)	7.22 (0.91)	9.00 (1.00)	9.00 (1.00)	5.50 (0.81)	8.30 (0.97)	9.00 (1.00)	9.00 (1.00)	7.50 (0.91)
	TA3	4.40 (0.73)	9.00 (1.00)	8.48 (0.98)	3.20 (0.62)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	5.38 (0.80)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	8.60 (0.98)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	8.40 (0.96)
	TA4	3.00 (0.60)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	8.82 (0.99)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	1.40 (0.31)	9.00 (1.00)	5.30 (0.80)	9.00 (1.00)	9.00 (1.00)	5.62 (0.82)	5.42 (0.81)	5.68 (0.82)	5.38 (0.80)	9.00 (1.00)	7.18 (0.91)	8.70 (0.99)	7.54 (0.91)
Mean		4.40 (0.67)	8.25 (0.96)	7.83 (0.94)	5.25 (0.75)	7.12 (0.91)	7.32 (0.91)	7.98 (0.95)	8.42 (0.97)	8.57 (0.98)	8.93 (1.00)	9.00 (1.00)	7.95 (0.95)	8.55 (0.98)	4.36 (0.65)	9.00 (1.00)	6.97 (0.89)	8.08 (0.95)	8.55 (0.98)	6.31 (0.84)	8.11 (0.95)	7.72 (0.93)	6.92 (0.89)	8.83 (0.99)	7.84 (0.94)	8.74 (0.99)	
		S. Ed					CD 5%																				
Isolates		0.026471					0.052041																				
Temperature		0.029595					0.058183																				
pH		0.029595					0.058183																				
Isolates * Temperature		0.05919					0.116366																				
Isolates * pH		0.05919					0.116366																				
Temperature * pH		0.066177					0.130101																				
		0.132353					0.260203																				

*(The values in the parenthesis are log (x+1) transformed)

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Table 3. Effect of interaction between different pH and temperature on mycelial fresh weight of native isolates of *Trichoderma asperellum*

Mycelial fresh weight (g)		26					28					30					32					34					Mean		
Temperature (°C)		4	5	6	7	8	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8			
pH	TA1	3.29 (0.63)	2.63 (0.56)	3.18 (0.62)	2.69 (0.57)	2.78 (0.58)	2.32 (0.52)	2.64 (0.56)	2.60 (0.56)	2.60 (0.56)	2.50 (0.54)	3.10 (0.61)	2.87 (0.59)	2.86 (0.59)	2.78 (0.58)	2.79 (0.58)	1.47 (0.39)	1.47 (0.39)	2.76 (0.58)	2.27 (0.51)	2.43 (0.54)	2.94 (0.60)	3.71 (0.67)	1.96 (0.47)	2.84 (0.58)	2.78 (0.57)			
	TA2	5.24 (0.80)	2.70 (0.57)	4.85 (0.77)	4.57 (0.75)	3.74 (0.68)	3.36 (0.64)	2.80 (0.58)	3.38 (0.64)	3.20 (0.62)	3.48 (0.65)	4.52 (0.74)	3.07 (0.61)	4.38 (0.73)	4.44 (0.74)	4.10 (0.71)	3.38 (0.64)	3.18 (0.62)	3.36 (0.64)	3.85 (0.69)	2.94 (0.60)	3.71 (0.67)	3.03 (0.61)	3.74 (0.68)	4.09 (0.71)	3.72 (0.67)			
	TA3	6.45 (0.87)	4.85 (0.77)	5.25 (0.80)	4.56 (0.75)	3.33 (0.64)	5.23 (0.79)	4.20 (0.72)	5.18 (0.79)	4.20 (0.72)	2.32 (0.52)	4.34 (0.73)	4.13 (0.71)	4.83 (0.77)	4.12 (0.71)	4.14 (0.71)	4.15 (0.71)	3.43 (0.65)	4.12 (0.71)	3.48 (0.65)	3.07 (0.61)	4.81 (0.76)	5.03 (0.78)	4.09 (0.71)	4.77 (0.76)	3.70 (0.67)	4.31 (0.72)		
	TA4	5.26 (0.80)	3.54 (0.66)	5.16 (0.79)	5.14 (0.79)	3.83 (0.68)	3.29 (0.63)	3.40 (0.64)	2.60 (0.56)	4.30 (0.72)	3.30 (0.63)	4.54 (0.74)	4.20 (0.72)	3.71 (0.67)	4.54 (0.74)	3.24 (0.63)	4.24 (0.72)	3.95 (0.69)	3.16 (0.62)	3.89 (0.69)	3.37 (0.64)	4.73 (0.76)	4.91 (0.77)	4.06 (0.70)	4.43 (0.73)	4.21 (0.72)	4.04 (0.70)		
Mean		5.06 (0.77)	3.43 (0.64)	4.61 (0.74)	4.24 (0.71)	3.42 (0.64)	3.55 (0.65)	3.26 (0.62)	3.44 (0.64)	3.58 (0.65)	2.90 (0.59)	4.13 (0.71)	3.57 (0.66)	3.95 (0.69)	3.97 (0.69)	3.57 (0.66)	3.31 (0.62)	3.39 (0.64)	3.35 (0.64)	3.37 (0.64)	2.95 (0.60)	4.05 (0.70)	4.17 (0.71)	3.46 (0.64)	3.96 (0.69)	3.70 (0.67)			
Isolates		CD 5 %																											
Temperature		S. Ed																											
pH		0.013296																											
Isolates * Temperature		0.014865																											
Isolates * pH		0.029224																											
Temperature * pH		0.014865																											
Isolates * Temperature * pH		0.02973																											
Isolates * Temperature * pH		0.058448																											
Isolates * Temperature * pH		0.02973																											
Isolates * Temperature * pH		0.033239																											
Isolates * Temperature * pH		0.065347																											
Isolates * Temperature * pH		0.066478																											
Isolates * Temperature * pH		0.130694																											

*(The values in the parenthesis are log (x+1) transformed)

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Table 4. Effect of interaction between different pH and temperature on mycelial dry weight of native isolates of *Trichoderma asperellum*

Mycelial dry weight (g)																										
Temperature (°C)		26				28				30				32				34				Mean				
pH		4	5	6	7	8	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8					
TA1		0.50 (0.18)	0.30 (0.11)	0.38 (0.14)	0.37 (0.14)	0.36 (0.13)	0.38 (0.14)	0.44 (0.16)	0.32 (0.12)	0.31 (0.12)	0.31 (0.12)	0.33 (0.12)	0.37 (0.14)	0.33 (0.12)	0.35 (0.13)	0.41 (0.15)	0.25 (0.10)	0.40 (0.15)	0.39 (0.14)	0.36 (0.13)	0.39 (0.14)	0.42 (0.15)	0.28 (0.11)	0.48 (0.17)	0.44 (0.16)	0.37 (0.14)
		0.74 (0.24)	0.46 (0.16)	0.69 (0.23)	0.55 (0.19)	0.43 (0.16)	0.55 (0.19)	0.41 (0.15)	0.46 (0.16)	0.35 (0.13)	0.37 (0.14)	0.54 (0.19)	0.42 (0.15)	0.51 (0.18)	0.61 (0.21)	0.51 (0.18)	0.37 (0.14)	0.42 (0.15)	0.51 (0.18)	0.46 (0.16)	0.49 (0.17)	0.38 (0.14)	0.52 (0.18)	0.48 (0.17)	0.51 (0.18)	0.49 (0.17)
TA3		0.79 (0.25)	0.73 (0.24)	0.70 (0.23)	0.65 (0.22)	0.56 (0.19)	0.64 (0.21)	0.65 (0.22)	0.64 (0.21)	0.56 (0.19)	0.26 (0.10)	0.56 (0.19)	0.53 (0.18)	0.65 (0.22)	0.56 (0.19)	0.53 (0.18)	0.54 (0.19)	0.46 (0.16)	0.60 (0.20)	0.52 (0.18)	0.46 (0.16)	0.74 (0.24)	0.54 (0.19)	0.66 (0.22)	0.49 (0.17)	0.59 (0.20)
		0.57 (0.20)	0.55 (0.19)	0.59 (0.20)	0.67 (0.22)	0.57 (0.20)	0.51 (0.18)	0.50 (0.18)	0.30 (0.11)	0.54 (0.19)	0.42 (0.15)	0.53 (0.18)	0.56 (0.19)	0.41 (0.15)	0.64 (0.21)	0.43 (0.16)	0.64 (0.21)	0.48 (0.17)	0.46 (0.16)	0.54 (0.19)	0.52 (0.18)	0.70 (0.23)	0.50 (0.18)	0.59 (0.20)	0.52 (0.18)	0.53 (0.19)
Mean		0.65 (0.22)	0.51 (0.18)	0.59 (0.20)	0.56 (0.19)	0.48 (0.17)	0.52 (0.18)	0.50 (0.18)	0.43 (0.15)	0.44 (0.16)	0.34 (0.13)	0.49 (0.17)	0.47 (0.17)	0.48 (0.17)	0.54 (0.19)	0.47 (0.17)	0.45 (0.16)	0.44 (0.16)	0.49 (0.17)	0.47 (0.17)	0.47 (0.17)	0.56 (0.19)	0.46 (0.16)	0.55 (0.19)	0.49 (0.17)	
		*(The values in the parenthesis are log (x+1) transformed)																								
Isolates		S. Ed				CD 5 %																				
Temperature		0.004322				0.008496																				
pH		0.004832				0.009499																				
Isolates * Temperature		0.004832				0.009499																				
Isolates * pH		0.009663				0.018998																				
Temperature * pH		0.009663				0.018998																				
Isolates * Temperature * pH		0.010804				0.02124																				
Isolates * Temperature * pH		0.021608				0.04248																				

*(The values in the parenthesis are log (x+1) transformed)

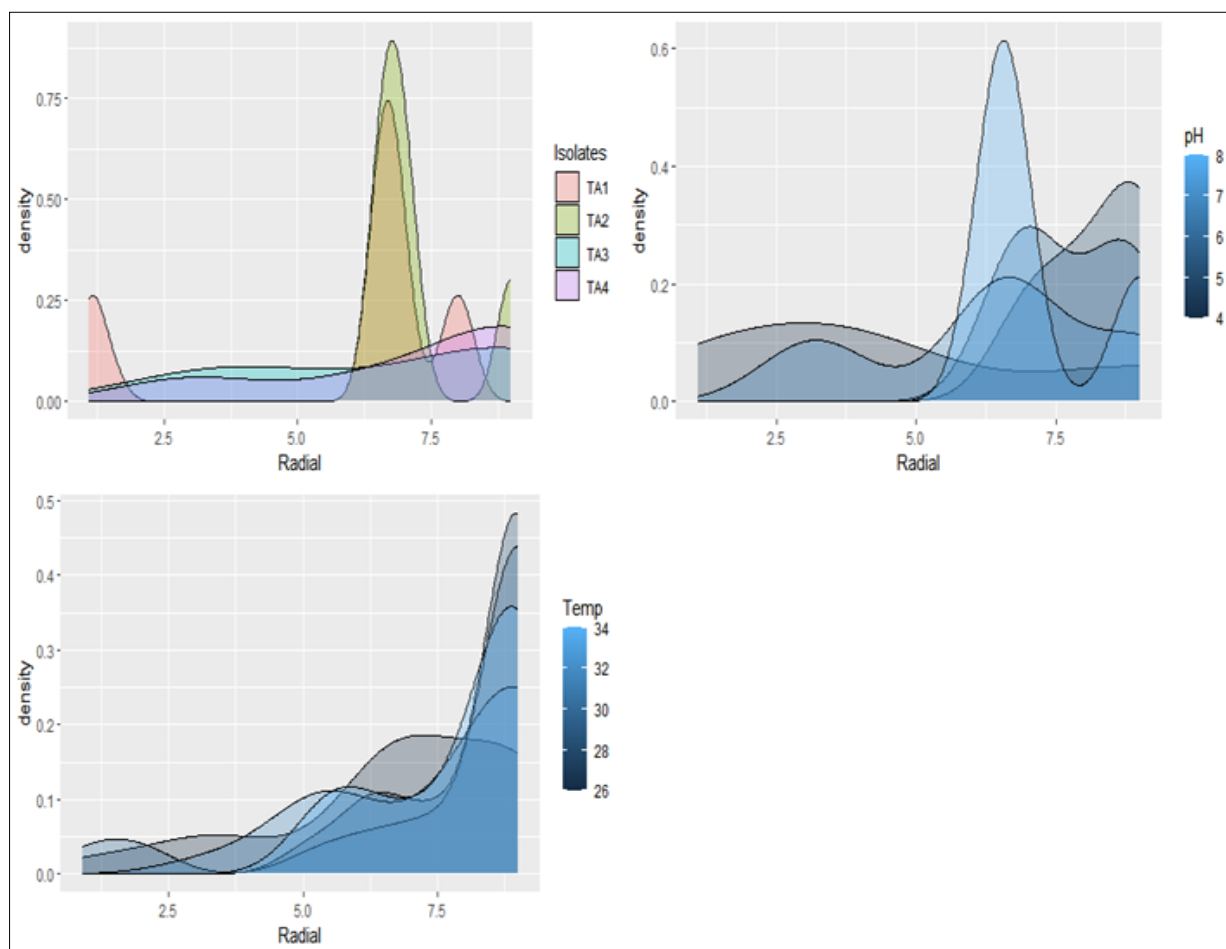


Fig. 1. Density graph showing radial growth of isolates of *Trichoderma asperellum* at different pH and different temperatures.

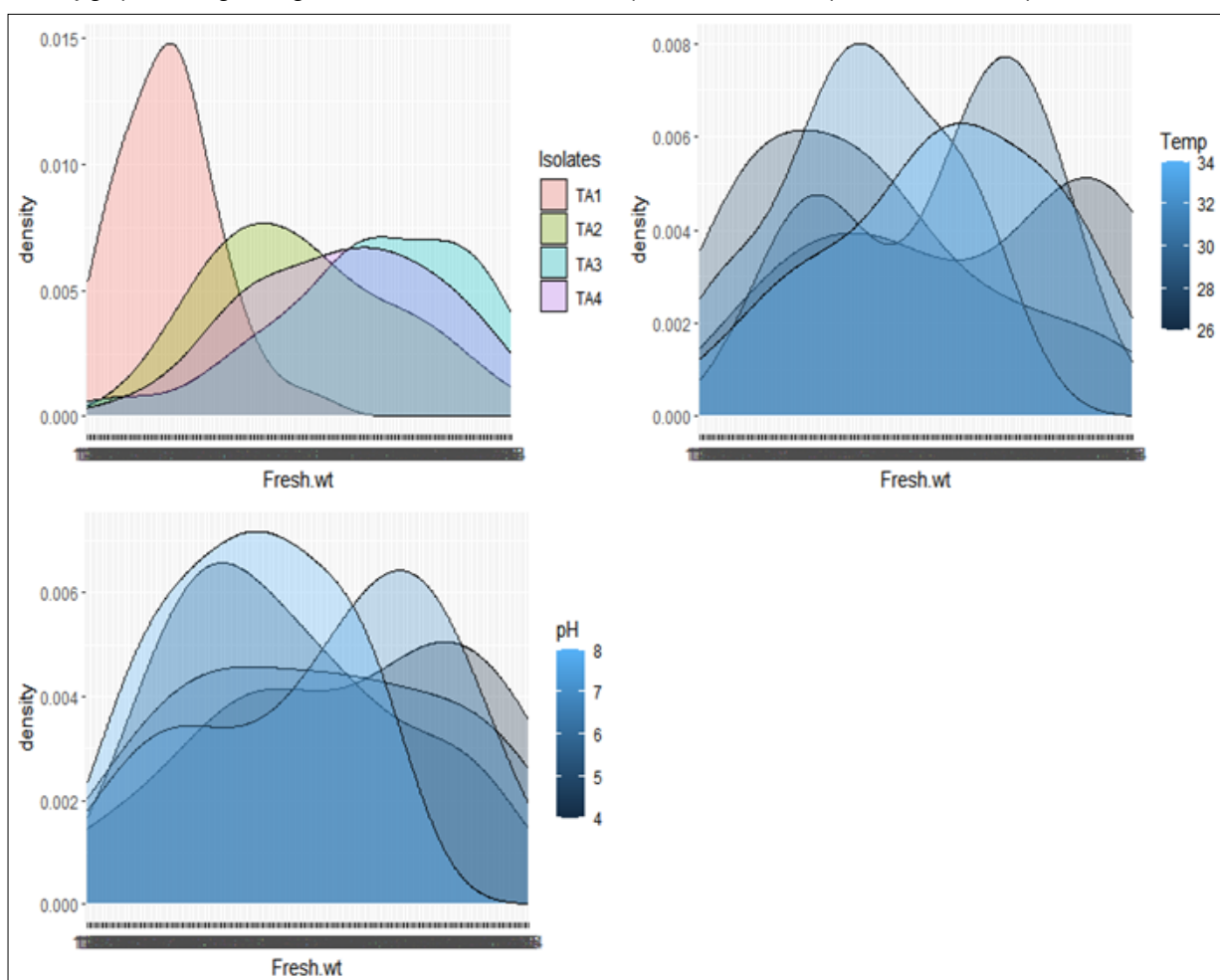


Fig. 2. Density graph showing mycelial fresh weight of isolates of *Trichoderma asperellum* at different pH and different temperatures.

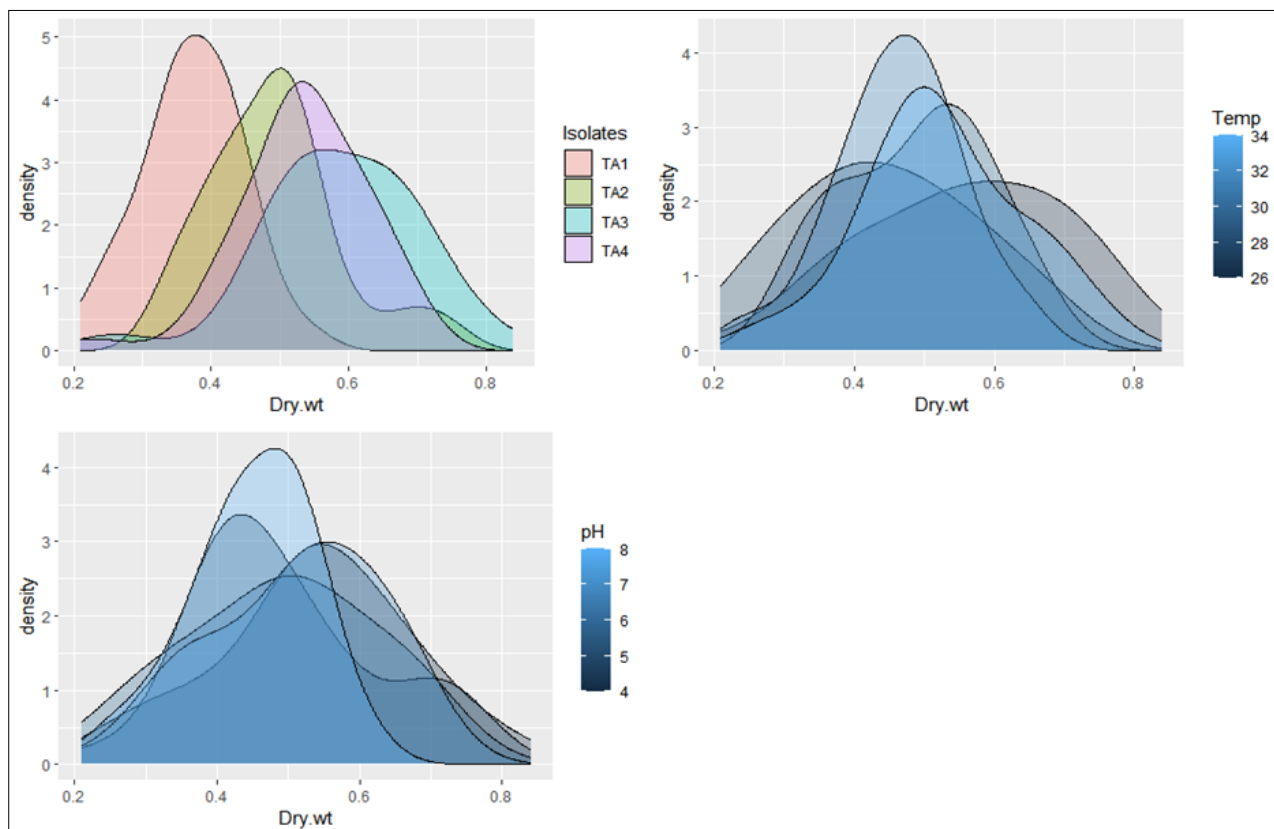


Fig. 3. Density graph showing mycelial dry weight of isolates of *Trichoderma asperellum* at different pH and different temperatures.

investigated how different temperature, pH and agitation influence the growth of *Trichoderma* species and reported that *Trichoderma harzianum* exhibited the optimal growth within the temperature range of 25–40 °C (44). The effect of varying temperature and pH on the growth of *Trichoderma* spp. and found that *T. harzianum* and *T. viride* were found to be in between temperature 25–40 °C and *T. asperellum* and *T. hamatum* were found to be in between temperature 25–35 °C and pH in between 4.6–7.6 for all the *Trichoderma* spp (45). It was also reported that *T. asperellum* exhibited optimal growth under slightly acidic conditions pH 5 at elevated temperatures of 28 °C and 30 °C were found to be favourable conditions for its growth development (46). The optimum pH and temperature identified in this study can be utilised to optimise fermentation and mass multiplication of *T. asperellum*, improve biomass production and formulation quality, enhance shelf life and product consistency and support the effective field application of *Trichoderma* based bio fungicides. Hence these *Trichoderma* spp. can be a noteworthy candidate in the development of formulation of region-specific biopesticides at proper optimising temperature and pH (47).

Conclusion

The present study demonstrated that pH and temperature significantly influenced the growth of *Trichoderma asperellum*, with maximum radial growth recorded at 30 °C under pH 4 and 8, while the highest fresh and dry biomass was obtained at 26 °C with pH 4. Among the four isolates, TA3 consistently exhibited superior growth performance, indicating its greater adaptability under varying environmental conditions. These findings provide valuable information for optimising culture conditions for the mass multiplication and formulation of *T. asperellum* based biocontrol agents, thereby improving their production efficiency and field application. However, the study was conducted under controlled

laboratory conditions, which may not fully represent field environments. Therefore, further research should evaluate the performance of these isolates under greenhouse and field conditions, investigate their biocontrol efficacy against target pathogens and explore the molecular mechanisms underlying isolate-specific responses to environmental factors.

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Authors' contributions

MMRA carried out the experiment, took observations and analysed the data. BS and SD guided the research by formulating the research concept and LNS approved the final manuscript. PK and KJS participated in the design of the study and performed the statistical analysis. IG, RJ, GG, SMI and KC contributed by imposing the experiment and helped edit, summarise and revised the manuscript. II, AAK, BT, NM and SKS helped summarise and revised the manuscript. FAK and RH read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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