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Growth promotion of native *Trichoderma asperellum* on pH, Temperature and Sugar concentration

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Abstract
Trichoderma spp. is the bio-control agents intensively used in the field of agriculture for diseases management. *Trichoderma* species are now well recognised as effective biocontrol agents for a number of industrial phytopathogens. For the current experiment, four native isolates of *Trichoderma asperellum* were collected from Plant Pathology Department, College of Agriculture, CAU, Imphal. In this research, pH, temperature and sugar concentrations at different levels were considered to study the isolates of mycelial growth variation of *T. asperellum*. Optimum pH, temperature and sugar concentrations were studied on potato dextrose agar media were prepared at different pH 4, pH 5, pH 6, pH 7 and pH 8, temperature 26 ˚C, temperature 28 ˚C, temperature 30 ˚C, temperature 32 ˚C and temperature 34 ˚C) and sugar concentrations (10 g/L, 15 g/L, 20 g/L, 25 g/L and 30 g/L). For optimization, at pH 5 the maximum radial growth was obtained whereas fresh weight and dry weight was obtained at pH 5 and pH 6 respectively. The maximum radial growth was observed at temperature 28 ˚C and 30 ˚C and fresh weight and dry weight was observed at temperature 34 ˚C. At 20 g/L of sugar concentration, the maximum radial growth as well as fresh weight and dry weight of the mycelium were found to be suitable. The results of this investigation demonstrated that the growth of *T. asperellum* isolates is greatly influenced by the pH, temperature and sugar concentration. The results of the current study showed that the growth of the isolates of *T. asperellum* was significantly influenced by environmental factors.

Keywords: *Trichoderma asperellum*, pH, temperature, sugar concentration

Introduction
The genus *Trichoderma* is ubiquitous in most types of soils, roots and environment. The fungus's modes of action of this fungus includes competition for nutrients and space, mycoparasitism, stress tolerance by promoting plant root growth and development, induced resistance and inactivation of pathogens enzymes (Samuels, 1996) [11]. *Trichoderma* species are free living and they provide favorable effects to plants. They are parasites of other fungus and aggressive, avirulent plant symbionts. The biological management of soil borne fungal plant pathogens by utilising fungi antagonist like *Trichoderma* spp. has received a lot of attention among several groups of plant diseases. (Harman *et al.*, 2004) [6]. The practice of employing *Trichodrma viride* for biological pathogen management is being promoted against the soil borne plant parasitic fungi (Bailey *et al.*, 2004) [2]. The employment of biological control agents, which employs microorganisms that interact with plant pathogens and lessen the frequency of plant disease, is a feasible and efficient eco-friendly strategy. The most prevalent biocontrol agents against plant pathogenic fungi are *Trichoderma* fungi (Anand and Reddy, 2009) [1]. For an efficient integrated pest management system, biological control agents can be used with cultural and physical control methods while chemical use is restricted. This leads to a more environmentally friendly approach to the treatment of plant diseases. *Trichoderma* are used in a variety of ways to mange plant diseases, including antibiosis, solubilization and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogen's enzymes (Monte, 2001) [10]. With the changing of temperature on the earth surface, environmental factors like temperature, humidity, soil pH has a major role in the development of the disease. Therefore climate change is expected to have effect on diseases and pathogens. *Trichoderma* species is one of the most convenient culturable fungi as well as avirulent plant symbionts. As a result, the goal of this study was to find out the optimization effect of pH, temperature and sugar concentration on the growth of isolates of *T. asperellum*.

Materials and Methods

Four identified fungus were collected from the Department of Plant Pathology, College of Agriculture, C.A.U, Imphal. Each isolates of *T. asperellum* was maintained as a pure culture on Potato Dextrose Agar (PDA). To assess the growth characteristics, native isolates of *T. asperellum* were subcultured on PDA medium in petri plates (90 mm in diameter).

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

Sl. No.	Biocontrol agents	Accession No.
1.	*Trichoderma asperellum* (TA1)	MH257327
2.	*Trichoderma asperellum* (TA2)	KU933475
3.	*Trichoderma asperellum* (TA3)	KT601340
4.	*Trichoderma asperellum* (TA4)	KU933476

Effect of different pH on radial growth of native isolates of *T. asperellum*

The effect of pH on radial growth was evaluated on PDA. PDA was prepared in five 250 ml conical flasks with 150 ml media in each conical flask. The pH of the medium was adjusted to 4, 5, 6, 7 and 8 with a help of a digital pH meter using 0.1 N Hydrochloric acid and 0.1 N Sodium hydroxide. In an autoclave, all conical flasks containing media were sterilized at 1.1 kg/cm^2 for 20 minutes. For each pH level, each sterilized petri plate was filled with 20ml of PDA medium and allowed to solidify. Five mm mycelial discs were taken with the use of cork borer from the actively growing cultures of the various isolates and placed in the middle of the petri plates. Five replications were maintained for each treatment. Plates with the inoculum were incubated at temperature 26 °C until it fully covered the plates. Each of the four isolates, the growth was measured radially (In cm) with the help of measuring scale for every 24 hours interval till the plates were fully covered.

Effect of different temperatures on radial growth of native isolates of *T. asperellum*

The effect of temperature on radial growth was evaluated on PDA. Five conical flasks (250 ml) of 150 ml PDA were prepared and the pH of the medium will be adjusted with a help of a digital pH meter using 0.1N Sodium chloride and 0.1N Hydrochloric acid. In an autoclave, all conical flasks containing media were sterilized at 1.1 kg/cm^2 for 20 minutes. For each temperature level, each sterilized petri plate was filled with 20ml of PDA medium and then it is allowed to solidify. Five mm mycelial discs were taken with the use of cork borer from the actively growing cultures of the various isolates and placed in the middle of the petri plates. Five replications were maintained for each treatment. Plates with the inoculum were incubated at five different temperatures i.e., 26 ℃, 28 ℃, 30 ℃, 32 ℃, 34 ℃ until it fully covered the plates. Each of the four isolates, the growth was measured radially (in cm) with the help of measuring scale for every 24 hours interval till the plates were fully covered.

Effect of different sugar concentrations on radial growth of native isolates of *T. asperellum*

The effect of sugar concentrations on radial growth was evaluated on PDA. Five conical flasks (250 ml) of 150 ml PDA media at different concentrations of sugar (10 g, 15 g, 20 g, 25 g and 30 g) per litre were prepared and the pH of the medium will be adjusted with a help of a digital pH meter using 0.1 N Sodium chloride and 0.1N Hydrochloric acid. In an autoclave, all conical flasks containing media were sterilized at 1.1kg/cm^2 for 20 minutes. For each sugar level, each sterilized petri plate was filled with 20ml of PDA medium and it is allowed to solidify. Five mm mycelial discs were taken with the use of corn borer from the actively growing cultures of the various isolates and placed in the middle of the petri plates. Five replications were maintained for each treatment. Plates with the inoculum were incubated at temperature 26 °C until it fully covered the plates Each of the four isolates, the growth was measured radially (in cm) with the help of measuring scale for every 24 hours interval till the plates were fully covered.

Statistical analysis

Statistical analysis of the data from each experiment was performed wherever required. ANOVA with factors in combinations. In the present study, the selected levels of the three factors along with the four isolates were then evaluated in a two-level factorial randomized complete block design, where the isolates were common for each of physical factors tested. The tested were pH, temperature and sugar concentration. The statistical analysis was done in R software.

Experimental Results and Discussion

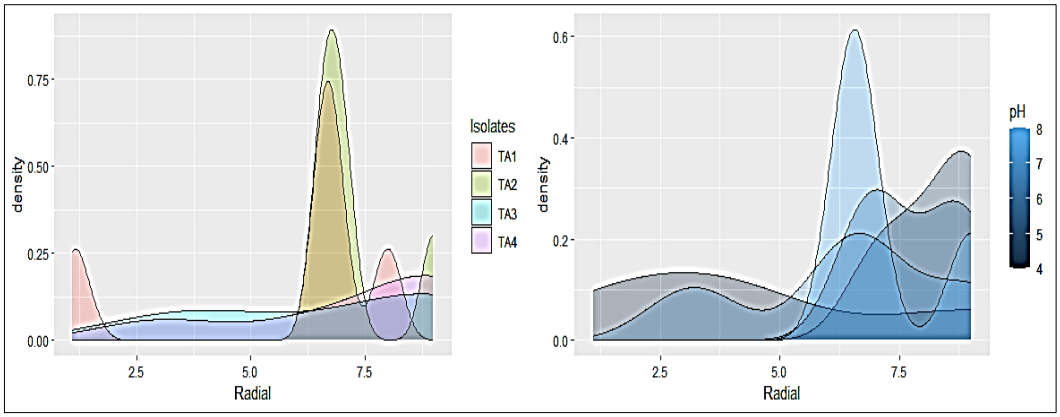
Effect of different pH on radial growth of native isolates of *T. asperellum*

Five pH levels for the growth of the different native isolates of *T. asperellum* were studied *in vitro* and the results are presented in Table 2, Plate1. Results showed that among different pH levels pH 5 was found suitable for the maximum radial growth of the isolates where mean radial growth was found to be 8.25cm. Followed by next best pH for the growth of the fungus was at pH 6 (7.83 cm), pH 7 (7.75 cm), pH 8 (4.62 cm) and the least was found at pH 4 (4.40 cm).The results also showed that the radial growth of the different isolates showed the significantly difference at different pH levels. Each isolates was found to be significantly at different pH level. Maximum radial growth was shown by isolates TA2 (9.00), TA3 (9.00), TA4 (9.00) at pH 5 and at pH 7, TA3 and TA4 also showed maximum radial growth (9.00). The least radial growth was shown by isolate TA1 (1.20 cm) at pH 4. These conclusions are supported by the research done by Shahid *et al.* (2011) [12] and he was observed that majority of *Trichoderma* spp. showed optimal growth at pH 5.0. Zehra *et al.* (2016) [14] was found that at pH 4.6 growth was very high. As per the density graph of the radial growth of isolates at different pH levels, the radial growth of all the isolates ranged between 1.20 cm to 8.75 cm (Graph 1).

Table 2: Radial growth of *T. asperellum* isolates at different pH

Isolates	Radial growth (cm)					Mean
	pH levels					
	4	5	6	7	8	
TA1	1.20 (0.34)	8.00 (0.95)	6.90 (0.90)	6.60 (0.88)	6.60 (0.88)	5.86 (0.79)
TA2	7.00 (0.90)	9.00 (1.00)	6.94 (0.90)	6.40(0.87)	2.20(0.50)	6.31(0.84)
TA3	4.40 (0.73)	9.00(1.00)	8.48(0.98)	9.00(1.00)	3.20(0.62)	6.82(0.87)
TA4	3.00(0.60)	9.00(1.00)	9.00(1.00)	9.00(1.00)	6.48(0.87)	7.30(0.90)
Mean	3.90(0.66)	8.75(0.99)	7.83(0.94)	7.75(0.75)	4.62(0.91)	
				S. Ed	CD (5%)	
Isolates				0.03	0.05	
pH				0.03	0.07	
Isolates * pH				0.07	0.14	

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



Graph 1: Density graph showing radial growth of isolates of *T. asperellum* at different pH

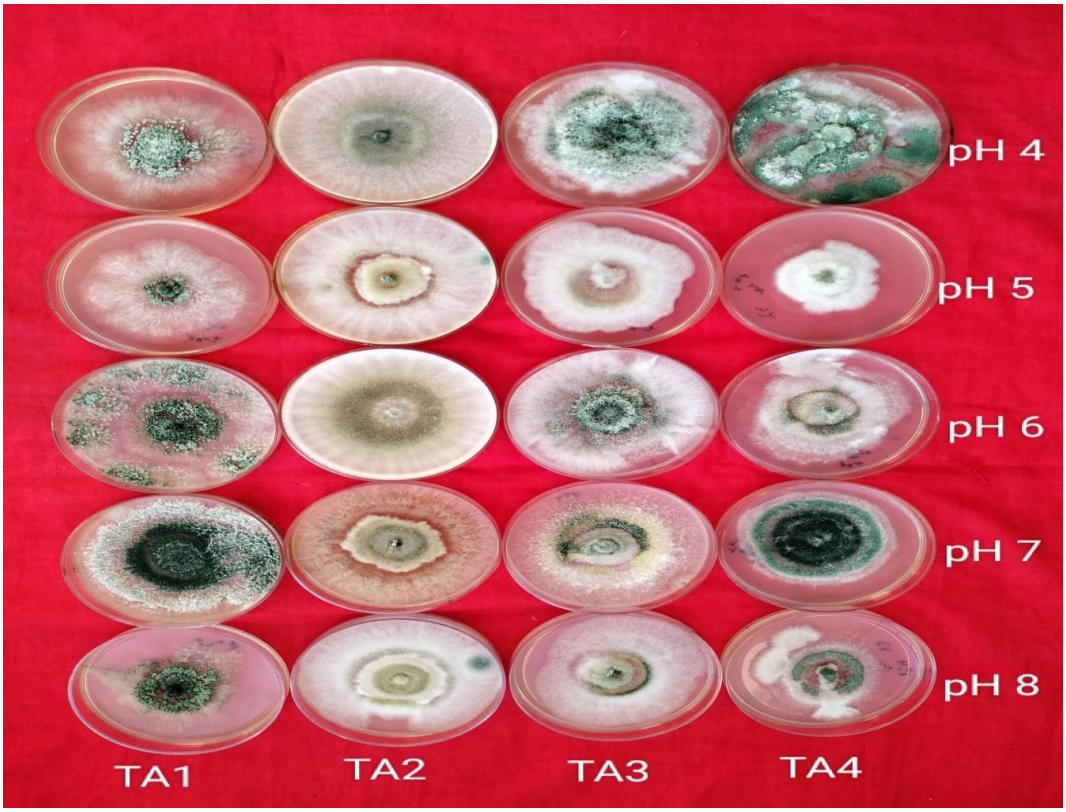


Plate 1: Radial growth of *T. asperellum* isolates at different pH

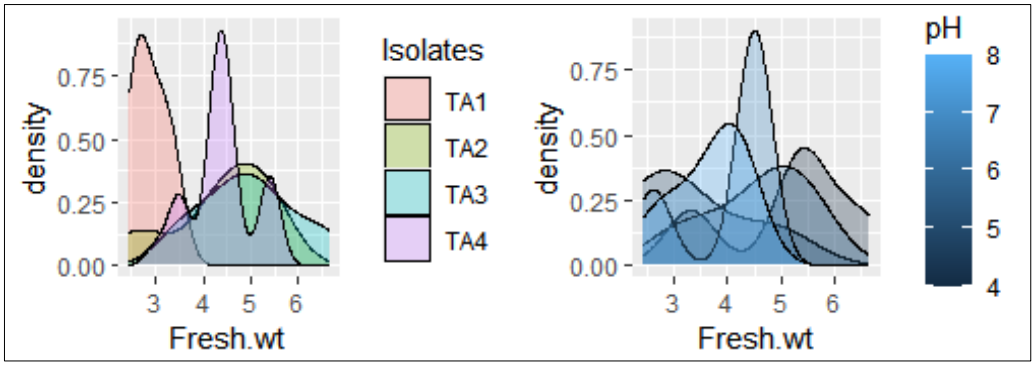
Effect of different pH on mycelial fresh weight of native isolates of *T. asperellum*: The mycelial fresh weight of four native isolates of *T. asperellum* at different pH levels are presented in Table 3, Plate2. Results showed that among different pH levels pH 5 was found suitable for the maximum growth of fresh weight of isolates where mean fresh weight was found to be 5.12 g. Followed by next best pH for the growth of the fungus was at pH 6 (4.56 g), pH 7 (4.04 g), pH

8 (3.67 g) and the least was found at pH 4 (3.43g).The results also showed that the mycelial fresh weight of the different isolates showed the significantly difference at different pH levels. Each isolates was found to be significantly at different pH level. Maximum growth was shown by isolates TA3 (6.40 g) at pH 5 and least growth was shown by isolate TA1 (2.60 g) at pH 4. The findings are in close agreement with the results of Hamzah *et al*. (2012) [5] who examined that the maximum biomass production was achieved at pH 5.5. Srivastava *et al*. (2014) [13] who reported that the maximum dry weight obtained at pH 6.5 and pH 7.0 in *Trichoderma harzianum.* As per the density graph of the mycelial fresh weight of isolates at different pH levels, the mycelial fresh weight of all the isolates ranged between 2.60 g to 6.40 g. Isolate TA4 have shown trimodal curve, whereas all the other isolates have shown unimodal curve and had wide diversity for mycelial fresh weight (Graph 2).

Table 3: Effect of mycelial fresh weight of *T. asperellum* isolates at different pH levels

Isolates	Mycelial fresh weight (g)					Mean
	pH levels					
	4	5	6	7	8	
TA1	2.60(0.56)	3.32(0.64)	3.20(0.62)	2.63(0.56)	2.73(0.57)	2.90(0.59)
TA2	2.80(0.58)	5.34(0.80)	5.20(0.79)	4.60(0.75)	4.16(0.71)	4.42(0.73)
TA3	4.90(0.77)	6.40(0.87)	5.38(0.80)	4.44(0.74)	3.58(0.66)	4.94(0.77)
TA4	3.40(0.64)	5.40(0.81)	4.44(0.74)	4.48(0.74)	4.22(0.72)	4.39(0.73)
Mean	3.43(0.64)	5.12(0.78)	4.56(0.74)	4.04(0.70)	3.67(0.67)	
				S. Ed	CD (5%)	
Isolates				0.04	0.07	
pH				0.04	0.08	
Isolates * pH				0.08	0.18	

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



Graph 2: Density graph showing mycelial fresh weight of isolates of *T. asperellum* at different pH

Plate 3: Mycelial Fresh weight growth of *T. asperellum* isolates at different pH

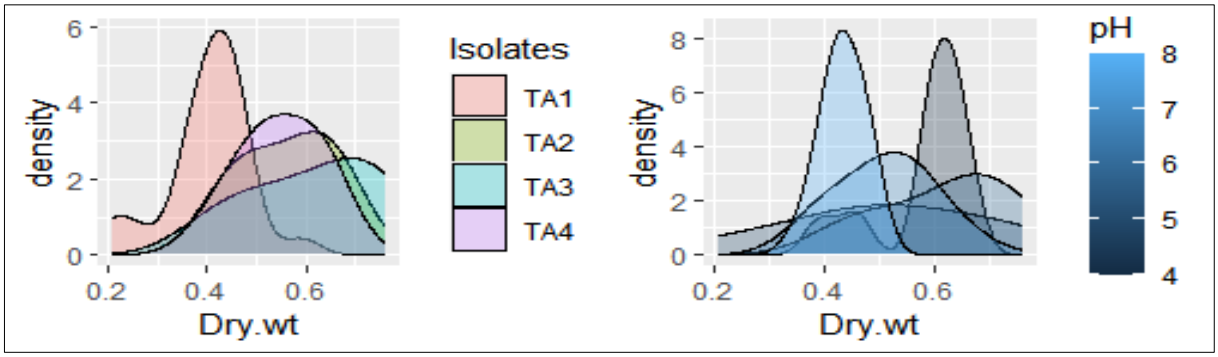
Effect of different pH on mycelial dry weight of native isolates of *T. asperellum*

The mycelial dry weight of four native isolates of *T. asperellum* at different pH levels are presented in Table 4. The results showed that among the different pH levels pH 6 was found suitable for the maximum dry weight of the isolates where mean dry weight was found to be 0.62 g. The next best pH for the growth of fungus was at pH 4 (0.58 g), pH 5 (0.51 g), pH 7 (0.51 g) and the least was found at pH 8 (0.44 g). The results also showed that the mycelial dry weight of the different isolates showed the significantly difference at different pH levels. Each isolates was found to be significantly at different pH level. Maximum dry weight was shown by isolates TA4 (0.74 g) at pH 6 and least growth was shown by isolate TA1 (0.30 g) at pH 5. The conclusions are in close agreement with the results of Hamzah *et al. (*2012) [5] who examined that the maximum biomass production was achieved at pH 5.5. The findings are in partial agreement with the results of Bhattriprolu (2008) [3] who studied the varied effects of pH on the growth of *Trichoderma viride.* The findings of Bhattriprolu (2008) [3] showed that pH 6 are most suitable for the growth of *Trichoderma viride* and Jayaswal *et al.* (2003) [9] who reported that between pH 4.5 to 5.5 are most suitable for the growth of *Trichoderma viride.* As per the density graph of the mycelial dry weight of isolates at different pH levels, the mycelial dry weight of all the isolates ranged between 0.30 g to 0.74 g (Graph 3).

Table 4: Effect of mycelial dry weight of *T. asperellum* isolates at different pH levels

Isolates	Mycelial dry weight (g)					Mean
	pH levels					
	4	5	6	7	8	
TA1	0.46(0.16)	0.30(0.11)	0.46(0.16)	0.40(0.15)	0.40(0.15)	0.40(0.15)
TA2	0.62(0.21)	0.46(0.16)	0.66(0.22)	0.60(0.20)	0.47(0.17)	0.56(0.19)
TA3	0.63(0.21)	0.73(0.24)	0.74(0.24)	0.54(0.19)	0.43(0.16)	0.61(0.21)
TA4	0.62(0.21)	0.56(0.19)	0.62(0.21)	0.50(0.18)	0.44(0.16)	0.55(0.19)
Mean	0.58(0.20)	0.51(0.18)	0.62(0.21)	0.51(0.18)	0.44(0.16)	
				S. Ed	CD (5%)	
Isolates				0.01	0.02	
pH				0.01	0.03	
Isolates * pH				0.03	0.06	

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



Graph 3: Density graph showing mycelial fresh weight of isolates of *T. asperellum* at different pH

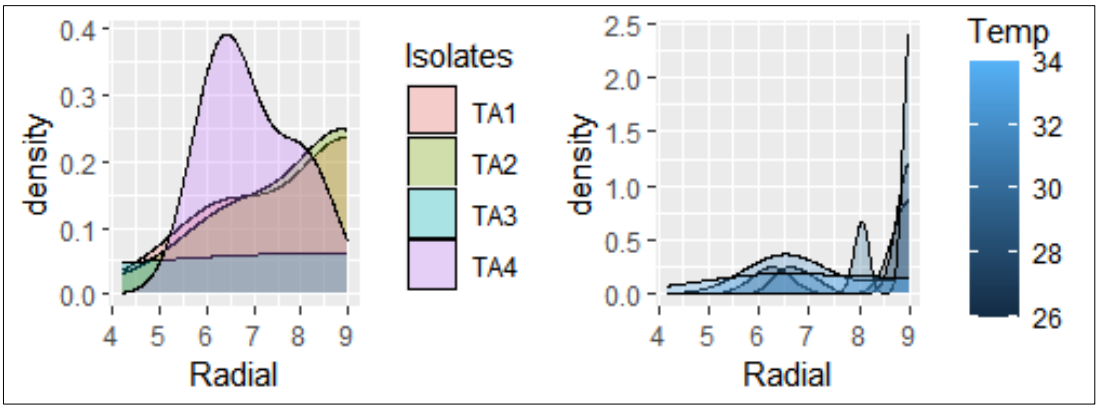
Effect of different temperatures on radial growth of native isolates of *T. asperellum*

Five different temperatures for the growth of the different native isolates of *T. asperellum* were studied *in vitro* and the results are presented in Table 5, Plate 3. Results showed that among different temperature 28 ˚C and 30 ˚C was found suitable for the maximum radial growth of the isolates where mean radial growth was found to be 8.76 cm. The next best temperature for the growth of the fungus was at temperature 26 ˚C (8.31 cm), 32 ˚C (7.16 cm) and the least was found at 34 ˚C (7.04 cm). The results also showed that the radial growth of the different isolates showed significantly difference at different temperature. Each isolates was found to be significantly at different temperature. Maximum radial growth was shown by isolates TA3 where the mean radial growth was 9.00 cm at all the temperature. TA1 and TA2 were also found maximum growth at temperature 26 ˚C, 28 ˚C and 30 ˚C. The least growth was shown by isolate TA2 (5.66 cm) at 34 ˚C. The results agree with the findings of Shahid *et al.* (2011) [12] who found that *Trichoderma longibrachiatum* growth was maximum at 30 °C. The results are in partial agreement with the findings reported by Bhattriprolu (2008) [3] showed temperature 25 ºC are most suitable for the growth of *Trichoderma viride.* As per the density graph of the radial growth of isolates at different temperature, the radial growth of all the isolates ranged between 4.50 cm to 9.00 cm (Graph 4).

Table 5: Radial growth of *T. asperellum* isolates at different temperatures

Isolates	Radial growth (cm)					Mean
	Temperature levels (˚C)					
	26	28	30	32	34	
TA1	9.00(1.00)	9.00(1.00)	9.00(1.00)	6.48(0.87)	5.84(0.83)	7.86(0.94)
TA2	9.00(1.00)	9.00(1.00)	9.00(1.00)	7.02(0.90)	5.66(0.82)	7.94(0.94)
TA3	9.00(1.00)	9.00(1.00)	9.00(1.00)	9.00(1.00)	9.00(1.00)	9.00(1.00)
TA4	6.24(0.86)	8.04(0.96)	8.04(0.96)	6.14(0.85)	7.66(0.94)	6.96(0.91)
Mean	8.31(0.96)	8.76(0.99)	8.76(0.99)	7.16(0.91)	7.04(0.90)	
				S. Ed	CD (5%)	
Isolates				0.10	0.19	
Temperature				0.11	0.21	
Isolates * Temperature				0.21	0.43	

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



Graph 4: Density graph showing radial growth of isolates of *T. asperellum* at different temperature

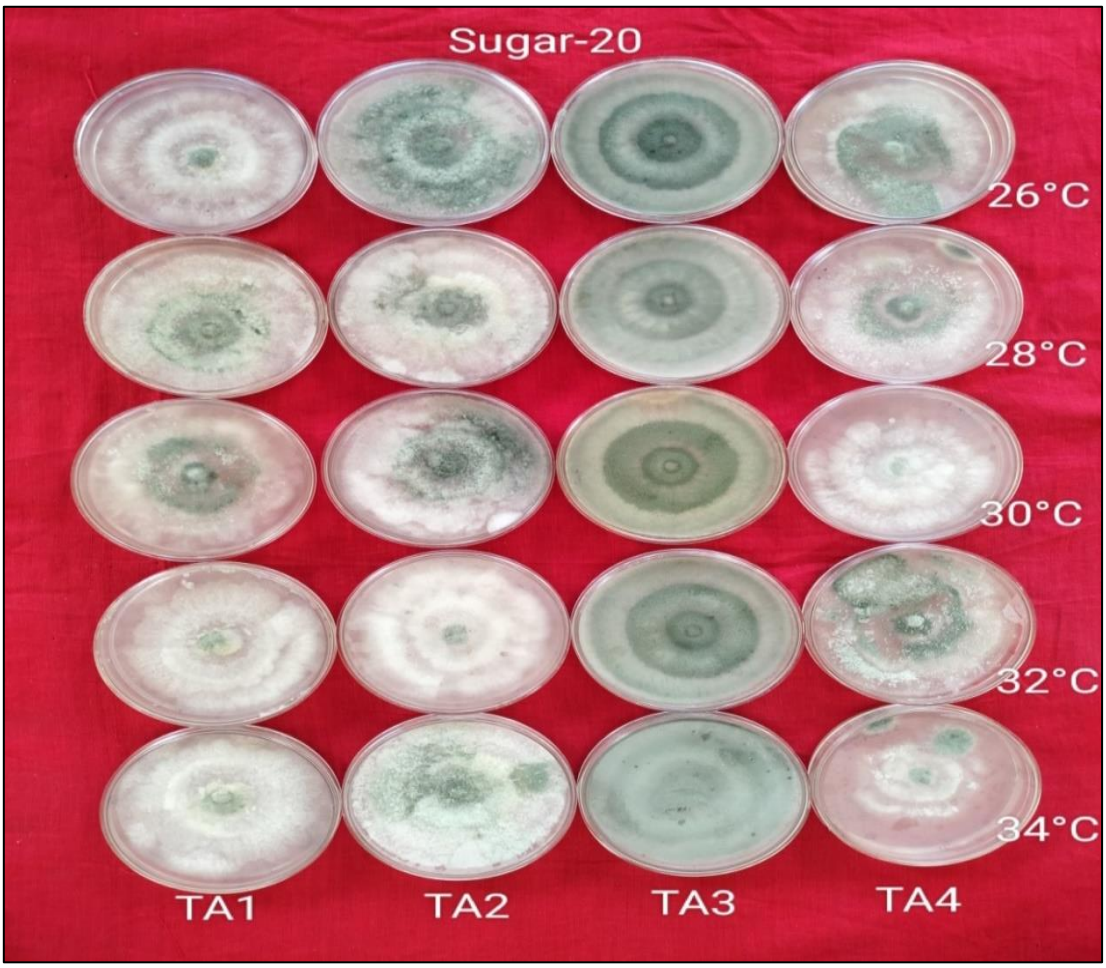


Plate 4: Radial growth of *T. asperellum* isolates at different temperatures

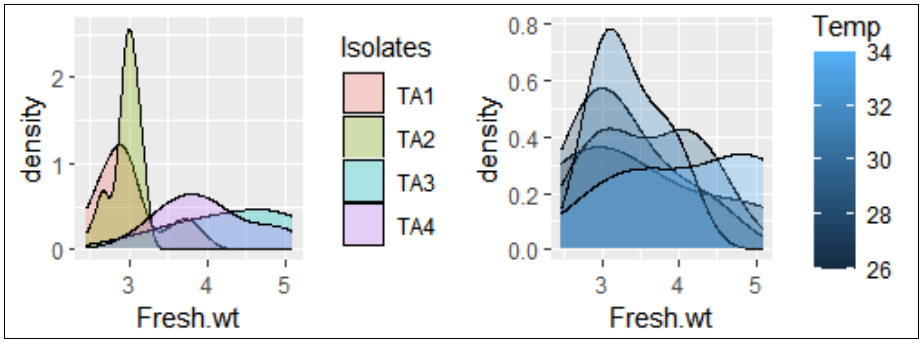
Effect of different temperature on mycelial fresh weight growth of native isolates of *T. asperellum*

The mycelial fresh weight of four native isolates of *T. asperellum* at different temperature levels are presented in Table 6, Plate 4. The results showed that among different temperature levels, temperature 34˚C was found maximum growth of fresh weight of isolates where mean fresh weight was found to be 4.17 g. The next best temperature for the growth of the isolate was at temperature 30˚C (3.57 g), 26˚C (3.46 g), 32 ˚C (3.39 g) and the least was found at 28 ˚C (3.29 g). The results also showed that the mycelial fresh weight of the different isolates showed the significantly difference at different temperature levels. Each isolates was found to be significantly at different temperature levels. Maximum growth was shown by isolates TA3 (5.03 g) and the least was shown by TA1 (2.64 g) at 26 ˚C. The findings are in close agreement with the results of Hole *et al.* (2016b) [7] reported highest mycelial weight at 30 °C. Jayaswal *et al.*, (2003) [9] who reported that between 20 ºC to 37 ºC are most suitable for the growth of *Trichoderma viride*. As per the density graph of the mycelial fresh weight of isolates at different temperature levels, the mycelial fresh weight of TA2 varied only between 2.40 g to 3.40 g, whereas for other isolates ranged between 2.40 g to 5.10 g (Graph 5).

Table 6: Effect of mycelial fresh weight of *T. asperellum* isolates at different temperature levels

Isolates	Mycelial fresh weight (g) Temperature levels(˚C)					Mean
	26	28	30	32	34	
TA1	2.64(0.56)	2.78(0.58)	2.87(0.59)	3.00(0.60)	3.71(0.67)	3.00(0.60)
TA2	2.74(0.57)	2.94(0.59)	3.07(0.61)	3.18(0.62)	3.03(0.61)	2.99(0.60)
TA3	4.90(0.77)	4.00(0.69)	4.13(0.71)	3.43(0.65)	5.03(0.78)	4.30(0.72)
TA4	3.54(0.66)	3.41(0.64)	4.20(0.72)	3.95(0.69)	4.91(0.77)	4.00(0.70)
Mean	3.46(0.64)	3.29(0.63)	3.57(0.66)	3.39(0.64)	4.17(0.71)	
				S. Ed	CD (5%)	
Isolates				0.06	0.12	
Temperature				0.07	0.14	
Isolates * Temperature				0.14	0.28	

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



Graph 5: Density graph showing mycelial fresh weight of isolates of *T. asperellum* at different temperature

Plate 5: Mycelial fresh weight growth of *T. asperellum* isolates at different temperatures

Effect of different temperature on mycelial dry weight of native isolates of *T. asperellum*

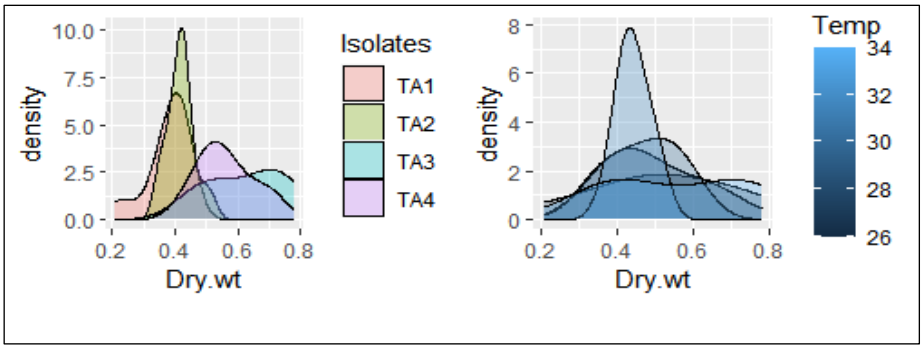
The mycelial dry weight of four native isolates of *T. asperellum* at different temperature levels are presented in Table 7. The results showed that among the different temperature levels, temperature 34 ˚C was found suitable for the maximum growth of dry weight of the isolates where the mean dry weight was found to be 0.56 g, followed by next best temperature for growth of fungus was at temperature 26 ˚C (0.51 g), 28 ˚C (0.49 g), 30 ˚C (0.47 g) and the least was found at 32 ˚C (0.44 g). The results also showed that the mycelial dry weight of the different isolates showed the significantly difference at different temperature levels. Each isolates was found to be significantly at different temperature levels. Maximum growth was shown by isolates TA3 (0.74 g) at 34 ˚C and the least was shown by TA1 (0.30 g) at 26 ˚C. The findings are in close agreement with the results of Hole *et al.* (2016b) [7] reported highest mycelial weight at 30 °C. The results are in partial agreement with the findings of Gangta *et al.* (2009) [4] who found that *Trichoderma* spp. growth was maximum at 25 °C As per the density graph of the mycelial dry weight of isolates at different temperature levels, the

mycelial dry weight of TA2 ranged between 0.30 g to 0.53 g, whereas for other isolates ranged between 0.20 g to 0.75 g (Graph 6).

Table 7: Effect of mycelial dry weight of *T. asperellum* isolates at different temperature levels

Isolates	Mycelial dry weight (g) Temperature (˚C)					Mean
	26	28	30	32	34	
TA1	0.30(0.11)	0.40(0.15)	0.37(0.14)	0.40(0.15)	0.42(0.15)	0.38(0.14)
TA2	0.46(0.16)	0.41(0.15)	0.42(0.15)	0.42(0.15)	0.38(0.14)	0.42(.15)
TA3	0.73(0.24)	0.65(0.22)	0.53(0.18)	0.47(0.17)	0.74(0.24)	0.62(0.21)
TA4	0.56(0.19)	0.51(0.18)	0.56(0.19)	0.48(0.17)	0.70(0.23)	0.56(0.19)
Mean	0.51(0.18)	0.49(0.17)	0.47(0.17)	0.44(0.16)	0.56(0.19)	
				S. Ed	CD (5%)	
Isolates				0.01	0.02	
Temperature				0.01	0.03	
Isolates * Temperature				0.03	0.05	

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



Graph 6: Density graph showing mycelial dry weight of isolates of *T. asperellum* at different temperature

Effect of different sugar concentrations on radial growth of native isolates of *T. asperellum*

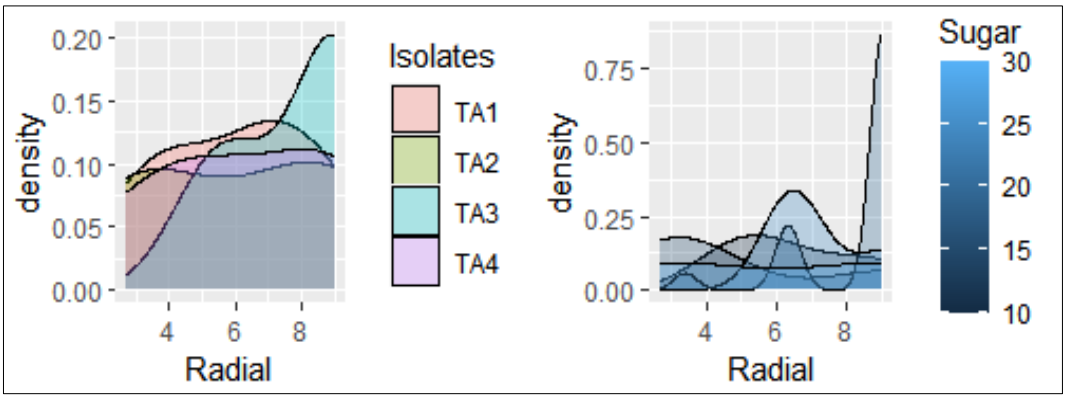
Five different sugar concentrations for growth of the different native isolates of *T. asperellum* were studied *in vitro* and the results are presented in Table 8, Plate 5. Results showed that among the different sugar concentration 20 g/L was found suitable for maximum radial growth of the isolates where mean radial growth was found to be 8.18cm. The next best sugar concentration for the growth of the fungus was at sugar concentration of 25 g/L (7.13 cm), 10 g/L (7.00 cm), 30 g/L (6.05 cm) and the least was found at 15 g/L (4.70 cm).The results also showed that the radial growth of the different isolates showed the significantly difference at different sugar concentration. Each isolates was found to be significantly at different sugar concentration. Maximum radial growth was shown by isolates TA1 at 20 g/L, TA2 at 10 g/L and 20 g/L, TA3 at 15 g/L, 20 g/L, 30 g/L and TA4 at 25 g/L and 30 g/L of sugar concentration and least was shown by TA2 at 15 g/L. The results are in close agreement with the findings of Islam *et al.* (2012) [8]. He observed that the mycelial growth was highest at 15% and 25%. As per the density graph of the radial growth of isolates at different temperature, the radial growth of all the isolates ranged between 2.80 cm to 9.00 cm (Graph 7).

Table 8: Radial growth of *T. asperellum* isolates at different sugar concentration

Isolates	Radial growth (cm) Sugar levels (g/L)					Mean
	10	15	20	25	30	
TA1	7.00(0.90)	4.20(0.72)	9.00(1.00)	7.00(0.90)	3.10(0.61)	6.06(0.83)
TA2	9.00(1.00)	2.80(0.58)	9.00(1.00)	6.20(0.86)	3.10(0.61)	6.02(0.81)
TA3	5.00(0.78)	9.00(1.00)	9.00(1.00)	6.30(0.86)	9.00(1.00)	7.66(0.93)
TA4	7.00(0.90)	2.80(0.58)	5.74(0.82)	9.00(1.00)	9.00(1.00)	6.71(0.86)
Mean	7.00(90)	4.70(0.72)	8.18(0.96)	7.13(0.91)	6.05(0.81)	
				S. Ed	CD (5%)	
Isolates				0.10	0.20	
Temperature				0.11	0.23	
Isolates * Temperature				0.23	0.46	

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

Plate 6: Radial growth of *T. asperellum* isolates at different sugar concentration



Graph 7: Density graph showing radial growth of isolates of *T. asperellum* at different sugar concentrations

Effect of different sugar concentrations on mycelial fresh weight growth of native isolates of *T. asperellum*

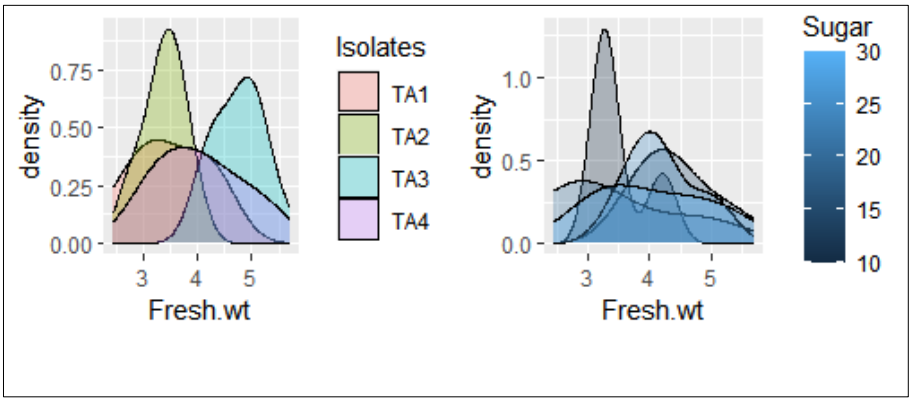
Five different sugar concentrations for the growth of different native isolates of *T. asperellum* were studied *in vitro* and the results are presented in Table9, Plate 6. Results showed that among the different sugar concentration 20 g/L was found suitable for the maximum radial growth of the isolates (4.43 g). The next best sugar concentration for the growth of the fungus was at sugar concentration 25 g/L (4.20 g), 30 g/L (4.05 g), 10 g/L (3.50 g) and the least was found at 15 g/L (3.43 g).The results also showed that the mycelial fresh weight of the different isolates showed the significantly difference at different sugar concentration. Each isolates was found to be significantly at different sugar concentration. Maximum mycelial fresh weight was shown by isolates TA3 (5.30 g) and the least was shown by TA1 (2.60 g). The results

are in close agreement with the findings of Islam *et al.* (2012) [8] that the mycelial growth was highest at 15% and 25%. As per the density graph of the mycelial fresh weight of isolates at different sugar concentration, the mycelial fresh weight of all the isolates ranged between 2.40 g to 5.80 g (Graph 8).

Table 9: Effect of mycelial fresh weight of *T. asperellum* isolates at different sugar concentration levels

Isolates	Mycelial fresh weight (g)				
	Sugar levels (g/L)				
	10	**15**	**20**	**25**	**30**
TA1	3.30(0.63)	2.60(0.56)	4.20(0.72)	4.20(0.72)	3.20(0.62)
TA2	3.30(0.63)	2.80(0.58)	3.80(0.68)	3.60(0.66)	3.40(0.64)
TA3	4.20(0.72)	4.90(0.77)	5.30(0.80)	5.00(0.78)	4.40(0.73)
TA4	3.20(0.62)	3.40(0.64)	4.40(0.73)	4.00(0.70)	5.20(0.79)
Mean	3.50(0.65)	3.43(0.64)	4.43(0.73)	4.20(0.71)	4.05(0.70)
				S. Ed	CD (5%)
Isolates				0.05	0.09
Sugar concentrations				0.05	0.11
Isolates * Sugar concentrations				0.11	0.21

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



Graph 8: Density graph showing mycelial fresh weight of isolates of *T. asperellum* at different sugar concentration

Plate 7: Mycelial fresh weight of *T. asperellum* isolates at different sugar concentration

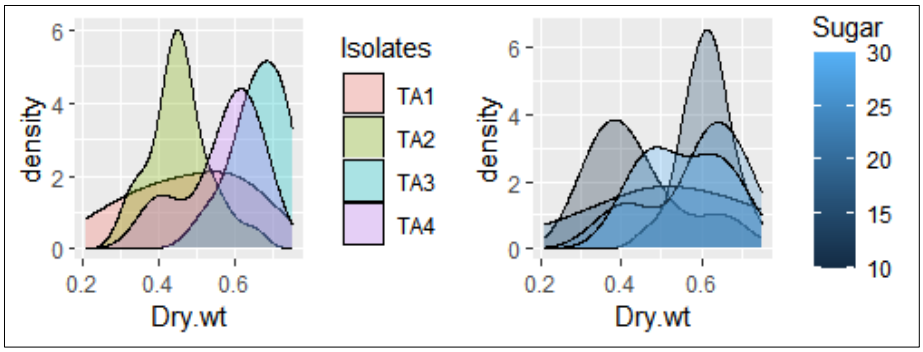
Effect of different sugar concentrations on mycelial dry weight growth of native isolates of *T. asperellum*

Five different sugar concentrations for the growth of the different native isolates of *T. asperellum* were studied *in vitro* and the results are presented in Table 10. Results showed that among the different sugar concentration 20 g/L was found suitable for the maximum radial growth of the isolates where the mean mycelial dry weight was found to be 0.61 g. The next best sugar concentration for the growth of the fungus was at sugar concentration 25 g/L (0.59 g), 30 g/L (0.56 g), 15 g/L (0.51 g) and the least was found at 10 g/L (0.33 g). The results also showed that the mycelial dry weight of the different isolates showed the significantly difference at different sugar concentration. Each isolates was found to be significantly at different sugar concentration. Maximum mycelial dry weight was shown by isolates TA3 (0.73 g) and the least was found at TA1 (0.30 g). The results are in close agreement with the findings of Islam *et al.* (2012) [8]. He observed that the mycelial growth was highest at 15% and 25%. As per the density graph of the mycelial dry weight of isolates at different sugar concentration, the mycelial dry weight of TA2 varied only from 0.40 g to 0.75 g, whereas for other isolates ranged between 0.20 g to 0.75 g (Graph 9).

Table 10: Effect of mycelial dry weight of *T. asperellum* isolates at different sugar concentration levels

Isolates	Mycelial dry weight (g)					Mean
	Sugar levels (g/L)					
	10	15	20	25	30	
TA1	0.36(0.13)	0.30(0.11)	0.60(0.20)	0.64(0.21)	0.48(0.17)	0.48(0.17)
TA2	0.38(0.14)	0.46(0.16)	0.56(0.19)	0.41(0.15)	0.46(0.16)	0.45(0.16)
TA3	0.60(0.20)	0.73(0.24)	0.65(0.22)	0.70(0.23)	0.63(0.21)	0.66(0.22)
TA4	0.40(0.15)	0.56(0.19)	0.63(0.21)	0.60(0.20)	0.65(0.22)	0.57(0.19)
Mean	0.44(0.16)	0.51(0.18)	0.61(0.21)	0.59(0.20)	0.56(0.19)	
				S. Ed	CD (5%)	
Isolates				0.01	0.03	
Temperature				0.02	0.03	
Isolates * Temperature				0.03	0.06	

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



Graph 9: Density graph showing mycelial dry weight of isolates of *T. asperellum* at different sugar concentration

Conclusion

In this present experiment showed that pH, temperatures and sugar concentrations significantly affect the growth of *T. asperellum.* These findings also found that the fungus growth was more on higher temperature with acidic pH and at particular sugar concentration under *in vitro* conditions. Present study showed that the pH, temperatures and sugar concentrations significantly affected the growth of *T. asperellum.* The pH values of nutritional medium are among the most crucial variables impacting the growth of *T. asperellum* because they are known to control mineral availability, which in turn affects enzymes and metabolic rates. Temperature played a vital part in expressing the activity of any biological system; it has enormous effect on the growth of *T. asperellum.* Temperature significantly affected on the radial growth of *T. asperellum.* It grew well between temperatures 26 ˚C to 30 ˚C. High temperature tolerance of *T. asperellum* is due to increase in accumulation of trehalose, mannose and raffinose. For survival of the fungus, sugar concentration was also greatly influence on the growth of fungus. Results from the current study showed that the fungus *T. asperellum* grew well at slightly acidic pH 5 and higher temperatures 28 ˚C and 30 ˚C and 20 g/L of sugar concentration were found suitable for the growth of the fungus. The soil of Manipur was acidic in nature due to heavy rainfall year round. The soil that was collected for the extraction of *T. asperellum* was from acidic soil of Manipur. Due to this reason in *in vitro* conditions, *T. asperellum* showed vigorous growth in acidic pH. The results will also help in the mass production of *T. asperellum* at optimum pH, temperature and sugar concentration. It was concluded that the isolates of *T. asperellum* exhibited growth at a wide range of pH, temperature and sugar concentrations.

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