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Growth Responses of Thermophilic fungi with Reference to their Physiological Conditions

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Abstract:
Thermophilic fungi exist in most of the environments,being thermophilic, they require elevated temperature for their growth. Temperature is one of the most important ecological key factor which affect the survival and growth of microorganisms.These fungi were isolated from the coal mine soil dumping area. Their temperature and pH requirementswere studied by measuring the radial mycelial growth and mycelial dry weight. *Humicolainsolens*grows at 50-55^{0}C, shown good growth at p^H 5-6.*Malbrancheapulchella* was slow growing at high temperatures, only 35-40^{0}C temperature was suitable for good growth. Two distinct patterns of growth were found on mycelial dry weight. pH 7-8 was suitable for its growth. *Melanocarpusalbomyces*, showed fast growth at 45^{0}C. Wide range of pH from 5-8 was found to be suitable for its growth. *Sporotrichum thermophile* was found to be growing faster at 40^{0}C. Maximum growth was observed at 6th day of incubation. Neutral to slight alkaline pH was resulted maximum radial mycelial growth within 4 days of incubation. These fungi are unique in their nature of growth with respect to temperature and pH requirements. The present study will be helpful to understand the physiology of the thermophilic fungi.

Key words: Thermophilic fungi, temperature, pH, mycelial dry weight, radial mycelial growth.

Introduction

Temperature is one of the most important ecological key factor which affect the survival and growth of microorganisms.Depending on the temperature tolerance fungi can be categorize as psychrophiles, mesophiles and thermophiles. However, about thermophilic fungi, Cooney & Emerson(1964) formulated less elaborate definition as "the thermophilic species can conveniently be defined as those with minima for growth at or above 20^{O}C and maxima for growth at 50^{O}C or above" whereas thermotolerant fungi are ones that have a thermal maximum near 50^{O}C and a minimum below 20^{0}C (Mouchacca 1997).

Thermophilic fungi have worldwide distribution. It seems more likely that a generally worldwide distribution is a result of the worldwide occurrence of self-heating masses (Maheshwari et al., 1987). All of which may self heat to spontaneous ignition. These fungi are believed to be significant contributors to self-heating and biodeterioration in each case. These fungi have usually been sought in hot habitats; these habitats have therefore, provided the preponderance of concerning occurrence and possible growth. These fungi can grow at high temperature, in man made habitats such as cooling towers, effluents from nuclear power reactors, ducts used for thermal insulations (Johri and Satyanarayana 1986) and that the ability to do so is a dominant characteristic of these species.

The present work deals with the temperature and pH requirement studies of four thermophilic fungi isolated from coal mine soil.

Materials and Methods:

Thermophilic fungi are known to be present ubiquitously. Some of the soil habitats considering their self heating were considered for isolating the Thermophilic fungi such as coal mine soil. The isolations were made on Emersons YpSs agar medium (Thakre and Johari, 1976), after isolations fungal cultures were made pure by single spore inoculation technique. The isolated cultures were identified with the help of available literature from Mycology Laboratory Dept of Botany, RTM Nagpur University, Nagpur. All the isolates were subjected to common temperature to grow at 45^0C. Then the identified cultures of *Humicolainsolens, Malbrancheapulchella, Melanocarpusalbomyces*and*Sporotrichum thermophile,*were tested for themperature (30-55^0C and pH (4-10) requirements. Radial Mycelial Growth (RMG), Mycelial Dry weight (MDW) was measured (Thakre 1984).

Result and Discussion

1. *Humicolainsolens*:

The RMG had similar pattern of growth at all the temperatures tested. However, the growth was rapid at 50 and 55^OC. The growth of *Humicolainsolens* at these temperatures was completed on 4^{th} day of incubation. At lower temperatures i.e. 30 and 35^OC the growth was slower (Fig 1A).

In case of MDW the maximum weight was found on 9^{th} day of incubation at all the temperatures. The growth was minimum at 25 and 60^OC. At rest of the temperatures the growth was more of less equal (Fig 1B).

In our findings, the faster RMG at 50-55^OC was recorded and 45-50^OC temperature look to be favourable for the production of maximum MDW. Apinis (1952) recorded 55^OC temperature suitable for the maximum growth.However, Cooney and Emerson (1964) reported 35-40^OC temperature for the maximum growth to occur. The RMG at pH 5-7 was

faster for this isolate. The RMG of *Humicolainsolens* seemed indifferent to pH of medium except for pH 4. The RMG at pH 4 was linear but significantly lesser than at other pH. At pH 5 and 6 rapid growth was recorded while, at rest of the pH the growth had a medium pace (Fig 1C).

The MDW was found to be increased from pH 3 to 7 and then it dropped steadily till pH 10 to reach the lowest value (Table 1).

2. *Malbrancheapulchella:*

The RMG was found to be slow as it could not attain the maximum growth at any of the temperature tested. A lag phase of two days was recorded in most of the cases. Still the growth was comparatively slower till 5th day subsequently a rapid growth was recorded at 35 and 40^{O}C. While at other temperatures the growth remained sluggish (Fig 2A).

Two distinct pattern of growth were seen in case of MDW. The moderate temperature (35- 50^{O}C) favoured the growth and maximum dry weight was attained within 6days. Whereas, the extreme temperature (25, 30, 55 and 60^{O}C) retarded the growth. The maximum growth in this case was achieved in 7 days (Fig 2B).

A lag phase of three days was seen in terms of RMG at almost all the pH. Even after 3days the growth was slow but steady in all the cases except at pH 7 and 8. At pH 7 the growth was complete within 6days while it took 8days to complete the growth at pH 8. On the other hand, the growth was not complete in 8days at rest of the pH (fig. 2C).

The MDW increased linearly from pH 3.0 to pH 7.0 to attain the maximum of 0.146g. It then further declined again linearly till pH 10 (Table 1).

RMG was faster for this isolate at 35-40^{O}C and maximum MDW was recorded at 45^{O}C similar findings were reported by Apinis(1952), Cooney and Emerson (1964), Salarand Aneja(2006) and Maheshwari *et al*., (2000). Whereas, neutral pH was favourable for the growth.

3. ***Melanocarpusalbomyces*:**

The RMG was found to be comparatively more at 45^{O}C than at the rest of the temperatures tested. The growth at 45^{O}C was completed within 6 days of incubation while at rest of the temperatures the growth completed in 7-8 days (Fig. 3A).

The MDW was comparatively less in the cultures incubated at 25, 55 and 60^{O}C. At other temperatures the growth was more or less same. Similarly, the maximum MDW was recorded on 6th day of incubation except for the culture incubated at 25 and 60^{O}C (Fig 3B).

The rapid RMG was observed at pH 5, 6, 7 and 8 attaining the 9cm growth after 4 days of incubation. However, maximum RMG was observed at pH 7 in initial days of (3

days) of incubation and later on it declined. Moreover, extremely different behavior of RMG was recorded with pH 10, (Fig 3C).

The pH 5 to 7 was found to be suitable for MDW production with maximum dry weight production at pH 6 (Table 1).

In present study the distinct RMG and MDW of *Melanocarpusalbomyces* at 45^{O}C was evident. Similar result is also reported by Maheshwari *et al*., (2000). However, Cooney and Emerson (1964) has depicted that, their isolate grows better at 40^{O}C whereas, the growth at slightly acidic to neutral pH was quite faster (Coutts and Smith, 1981).

4. ***Sporotrichum thermophile***

The *Sporotrichum thermophile* had completed its growth between 5 to 7 days at all the temperatures tested. Moreover, the growth was more or less equal in these cases. However, the rapid growth was found at 40^{O}C followed by 30, 35 and 45^{O}C (Fig 4A).

The good MDW production was achieved between 30 and 55^{O}C. Whereas, MDW production was distinctly low at 25 and 60^{O}C. Similarly, the maximum MDW was attained on 6th day of incubation in most of the cases (Fig 4B).

Neutral to slightly alkaline pH (7 & 8) resulted in maximum RMG within 4 days of incubation. This was followed by the growth at pH 4 and 9, where the growth was completed on 6th day. At rest of the pH the growth was slowest (Fig 4C).

The MDW production of *Sporotrichum thermophile* was optimum between the pH 5, 6 and 7. However, maximum dry weight was recorded at pH 6 followed by 5 and 7, respectively (Table 1).

The maximum RMG and MDW was found at 45^{O}C. Thakre (1975) reported the similar results of *Sporotrichum*sp. from coal mine. pH 7-8 was favorable for this fungus. In contrast to this Coutts and Smith (1981) reported good growth at pH 4 for this fungus.

Conclusion

These fungi are known to produce thermostable enzymes which are industrially very important. *Humicolainsolens, Malbrancheapulchella, Melanocarpusalbomyces* and*Sportrichum thermophile* were tested for their temperature and pH requirements, three of them grows well onhigh temperature, among these fungi *Humicolainsolens* was able to grow at 55^{O}C, they are true thermophiles. Whereas, *Malbrancheapulchella* was growing slowly at these temperatures. The pH requirement for these fungi is raging from 5-8. These can be utilized for biotechnological as well as agricultural processes.

Acknowledgement

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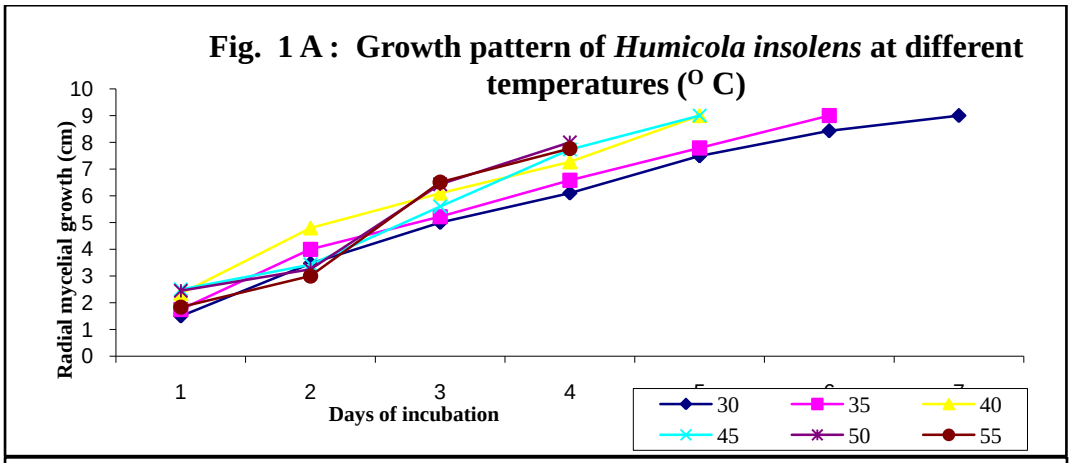
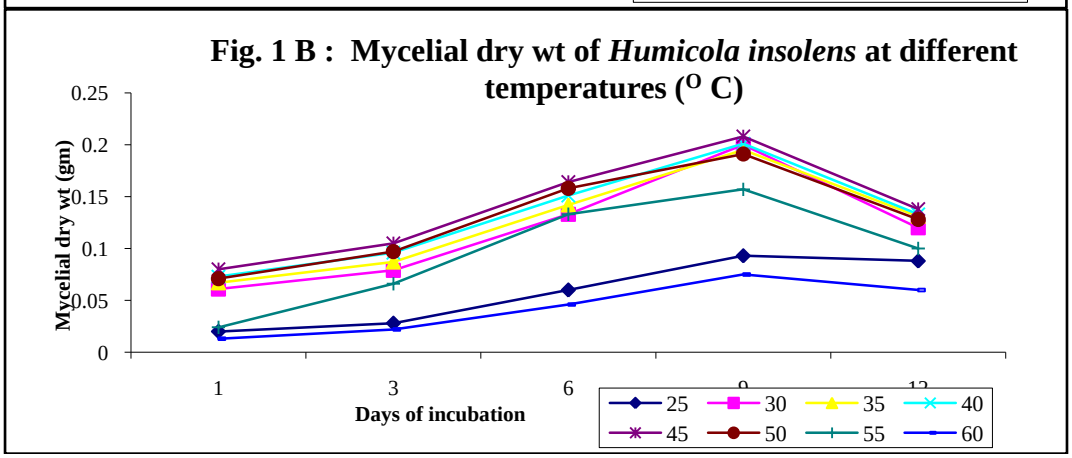
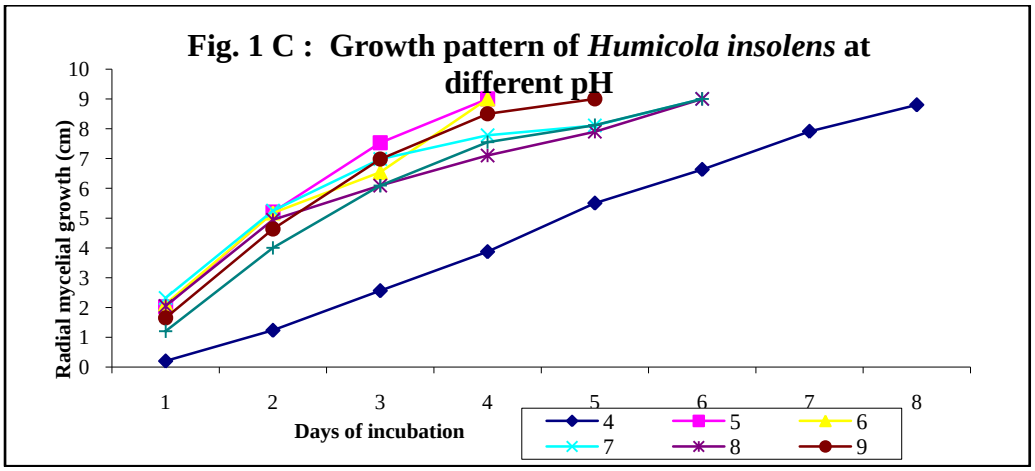
Table 1: Effect of Different pH levels on Mycelial dry wt production

Sr.No.	Fungi	Mycelial dry wt(gm) at varying pH levels							
		3	4	5	6	7	8	9	10
	Humicolainsolens	0.050	0.061	0.078	0.097	0.102	0.075	0.048	0.036
	Malbrancheapulchella	0.030	0.102	0.110	0.137	0.146	0.125	0.100	0.071
	Melanocarpusalbomyces	0.040	0.043	0.201	0.220	0.105	0.090	0.078	0.060
	Sporotrichum thermophile	0.030	0.043	0.061	0.076	0.058	0.041	0.035	0.030







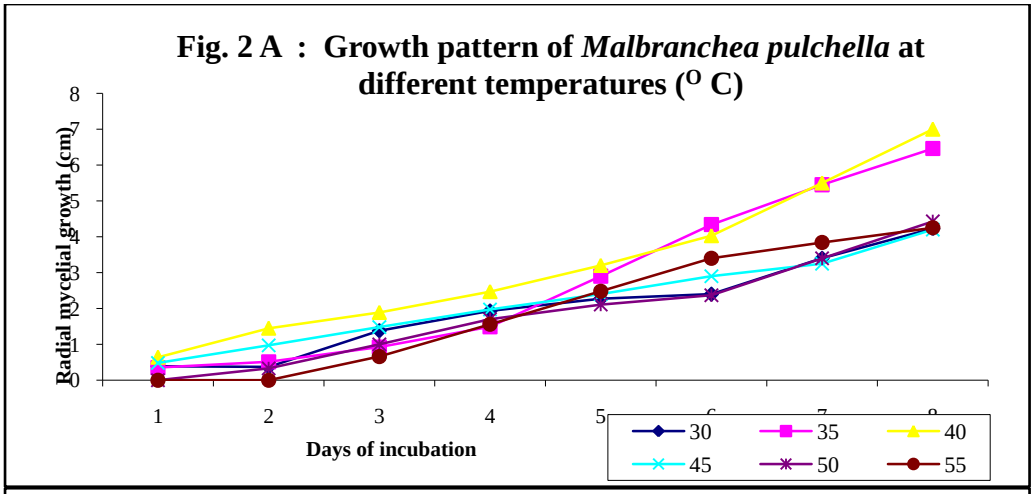


Fig. 2 A : Growth pattern of *Malbranchea pulchella* at different temperatures (O C)
Radial mycelial growth (cm)
Days of incubation
30
35
40
45
50
55

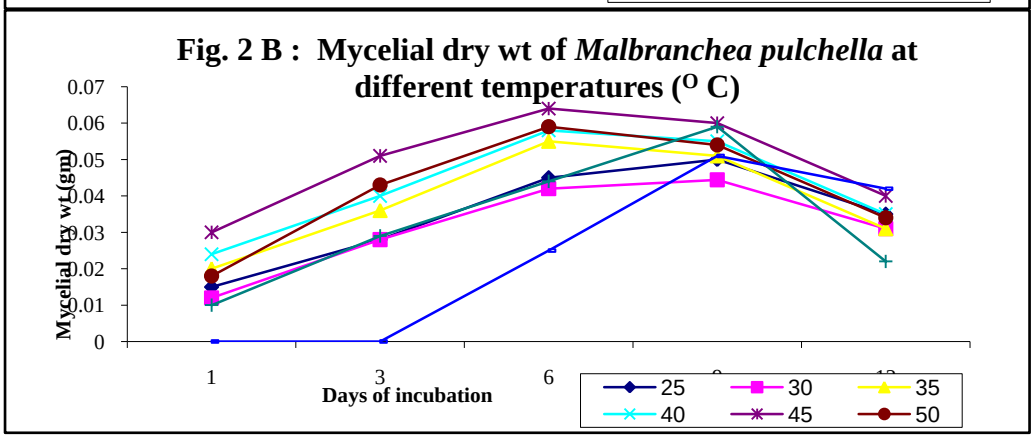


Fig. 2 B : Mycelial dry wt of *Malbranchea pulchella* at different temperatures (O C)
Mycelial dry wt (gm)
Days of incubation
25
30
35
40
45
50

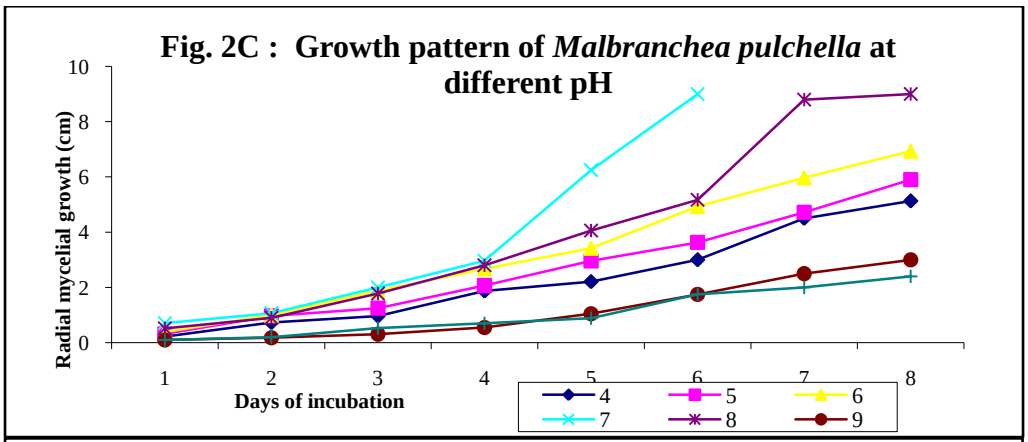


Fig. 2C : Growth pattern of *Malbranchea pulchella* at different pH
Radial mycelial growth (cm)
Days of incubation
4
5
6
7
8
9

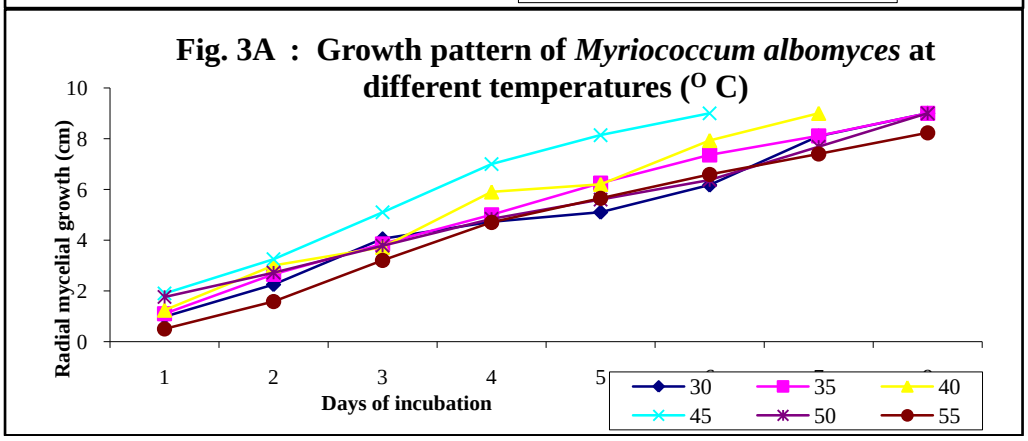


Fig. 3A : Growth pattern of *Myriococcum albomyces* at different temperatures (O C)
Radial mycelial growth (cm)
Days of incubation
30
35
40
45
50
55

Fig. 3 B : Mycelial dry wt of *Myriococcum albomyces*at different temperatures (^{O} C)

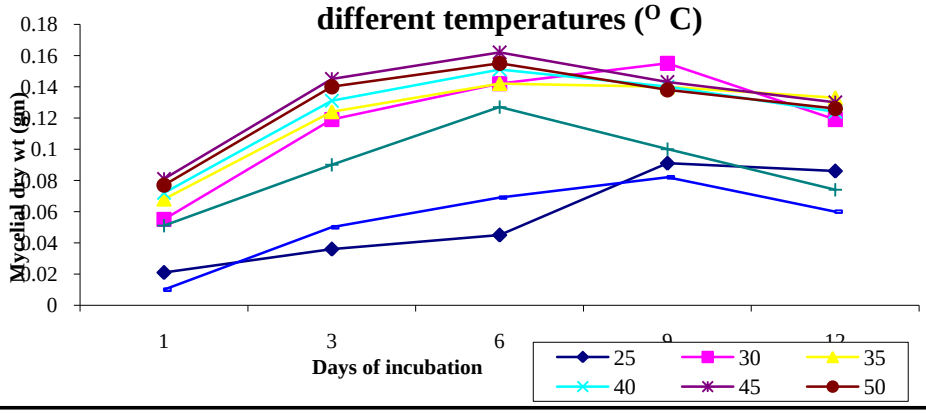


Fig. 3 C : Growth pattern of *Myriococcum albomyces* at different pH

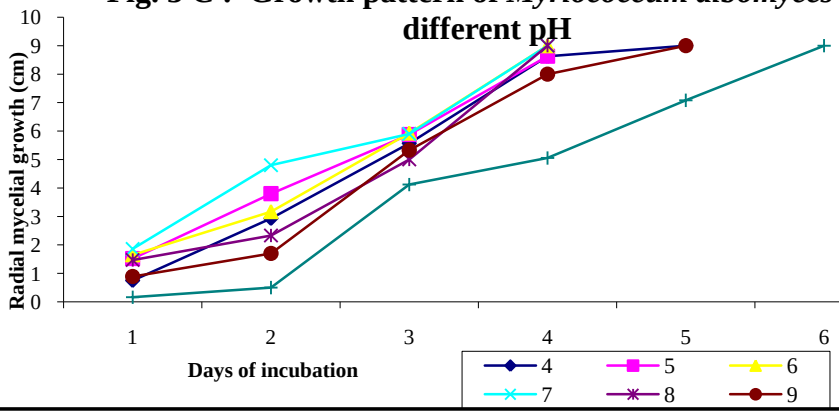
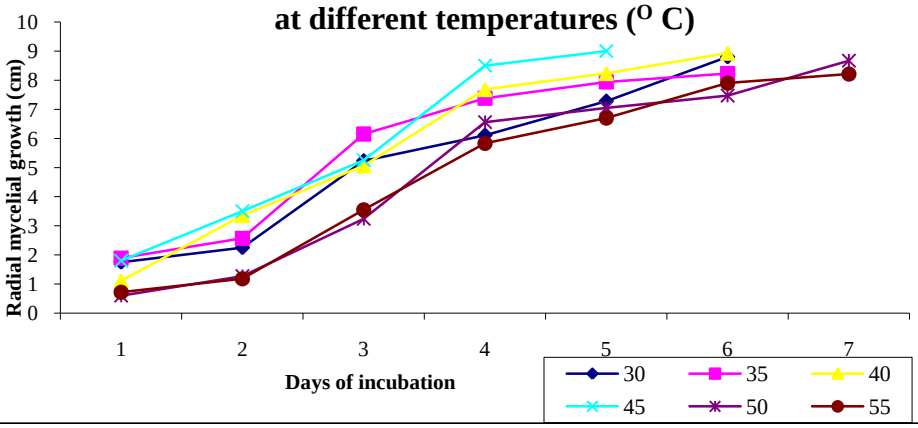


Fig. 4A : Growth pattern of *Sporotrichum thermophile* at different temperatures (^{O} C)



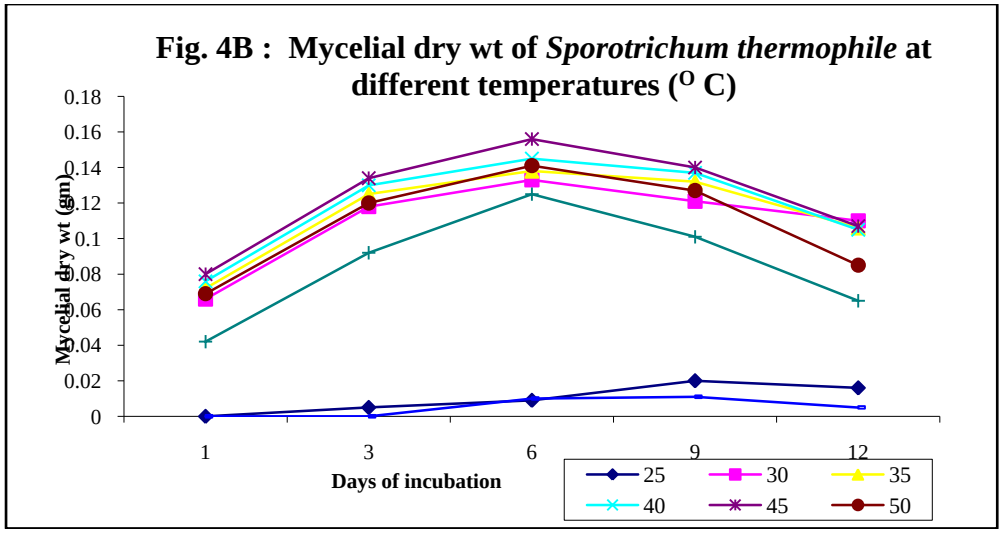


Fig. 4B : Mycelial dry wt of Sporotrichum thermophile at different temperatures (O C)
Mycelial dry wt (gm)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1
3
6
9
12
Days of incubation
25
30
35
40
45
50

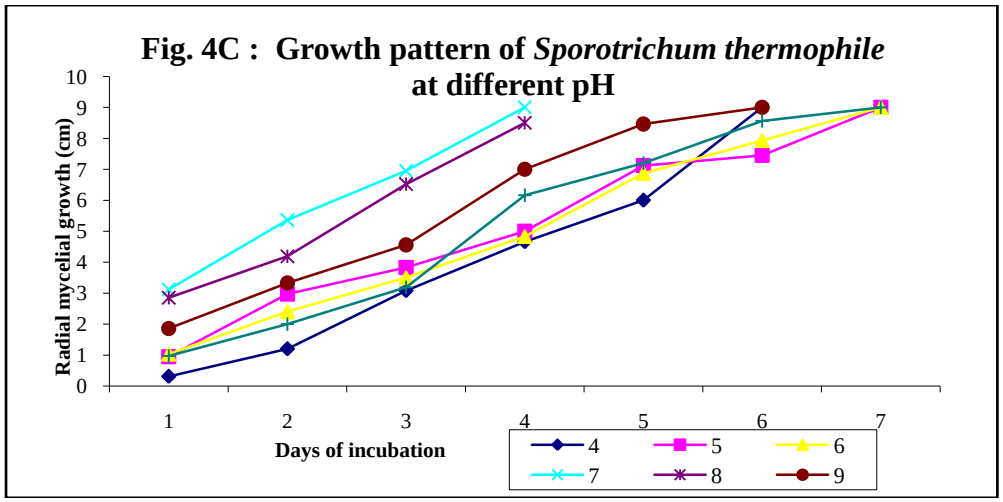


Fig. 4C : Growth pattern of Sporotrichum thermophile at different pH
Radial mycelial growth (cm)
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
Days of incubation
4
5
6
7
8
9