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Effect of Physiological Parameters on Growth of *Aspergillus niger* and *Trichoderma harzianum*

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ABSTRACT

The study was aimed to carry out experiments to determine the optimal physiological parameters like pH, temperature and light regimes for the biomass production of Aspergillus niger and Trichoderma harzianum. The growth of fungi was recorded at 3rd, 5th and 7th day after incubation. A significant difference in the biomass production was recorded at tested pH levels i.e. 6.0, 7.0, 7.5 and 8.0. Both fungi showed highest biomass at pH 6.0. The plates were incubated at four different temperatures (20, 25, 30 and 35^{0}C) to evaluate the colony diameter (mm), both organisms showed best growth at 30^{0}C. Different light regimes i.e. 24h dark, 24h light, 8h dark & 8h light and 12h dark & 12h light on radial mycelial growth (mm) of two fungi was evaluated. The best growth of two fungi was recorded maximum at 12h dark & 12h light.

Key words: *A.niger, T.harzianum, pH, temperature and light regimes*

INTRODUCTION

In common with all microorganisms, fungi are profoundly affected by physical factors, such as temperature, aeration, pH, and light. These factors not only affect the growth rate of fungi but also can act as triggers in developmental pathways. Temperature, pH and light are key factors in the growth and development of the fungal mycelium. Microorganisms such as filamentous fungi *Trichoderma* and *Aspergillus* regulate their metabolism in response to changing environmental conditions. Agnihotri[1] revealed that among these factors, pH and temperature are probably most important environmental parameters affecting the mycoparasitic activities. A specific value of pH is required to note the maximum growth where these agents can be multiplied. Chavan[3] studies on the variation of pH by the *Trichoderma* isolates showed optimum growth and sporulation rate at different pH values ranging from 2 to 7 and maximum good growth at p^H 5.96. Fungal growth depends on temperature levels. Temperature directly affects the many integrated metabololic activities of fungi such as digestion, assimilation, respiration, relocation and synthesis.

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The metabolic reaction rate increases with increase in temperature until the heat denatures enzymes required for growth. Every fungus possesses three cardinal growth temperatures: a minimum, an optimum and a maximum. These levels indicate respectively, the beginning of growth, the best growth and ceasing of growth. Affect of the temperature investigated by the Belli *et al*[2] for the growth of *Aspergillus* on agar media and Soesanto *et al*[8] studied dry weight of *Trichoderma harizianum* on PDA showed best at 25^0C to 30^0C range. Konstantinova[5] observed light stimulates asexual and sexual reproduction in most fungi and its effect is closely related to nutrition and temperature. Most of the fungi show growth when exposed to different light regimes such as continuous light, continuous dark and altering cycles of dark and light. The present study investigated effects of abiotic factors (pH, temperature and light) on the radial growth and biomass yield of *Trichoderma harizianum* and *Aspergillus niger.*

MATERIALS AND METHODS

Isolation and characterization of fungi

Isolates were isolated from soil samples collected from rhizosphere composite soil sample i.e. sesame, mustard, linseed and soybean fields of Agronomy Section, College of Agriculture, Nagpur. All the isolates were isolated on PDA medium by following serial dilution plate technique as described by Johnson and Curl and isolates were identified up to species level based on phenotypic characters like colony colour and growth; size and shape of conidiophore, phialides and conidia. The cultures were identified using the available literature and confirmed by morphological characters i.e. isolate-1 is identified as *Aspergillus niger* and isolate-2 is *Trichoderma harzianum.*.

Physiological parameterspH

Growth of *Aspergillus niger* and *Trichoderma harzianum* at various pH levels was determined *In Vitro*. pH levels was adjusted from pH 6, 7, 7.5 and 8 by using NaOH and dilute HCl and added in potato dextrose broth. Observations were noted on mycelial dry weight basis on 3, 5 and 7 days after inoculation at 272^0C. Whatman paper No.40 was used to filter the cultured broth. The mycelial filtrate was air dried and then kept in oven at 60^0C for 1hr. This was done successively for 2-3 times till the oven dry weight becomes constant. The oven dry mycelium weights of test isolates noted separately.

Temperature and light

The effect of varying temperature and light ranges was carried out on potato dextrose agar. Mycelial disc of 6 mm diameter was cut from 4 days old culture plates. Test isolates were placed at the centre of Petri plate containing potato dextrose agar. The plates were incubated at different temperature ranges viz. 20, 25, 30 and 35^0C in the incubator and light ranges viz. 24h dark, 24h light, 8h dark and 8h light and 12h dark and 12h light keeping the light source uniform. The observations were noted on radial mycelial growth on 3^{rd}, 5^{th} and 7^{th} day.

RESULTS AND DISCUSSION

Effect of pH levels on biomass of *Aspergillus niger* and *Trichoderma harzianum*

Growth of the both fungi were determined at pH levels 6.0, 6.5, 7.0 and 8.0 *In Vitro*. There was gradual increase in the mycelial dry weight from 3^{rd} to 7^{th} day. Particularly, maximum biomass production recorded at pH level 6.0 and it was significantly maximum over all other pH levels under study.It is revealed from the data effect of pH on *Aspergillus niger* and *Trichoderma harzianum* recorded on 3^{rd}, 5^{th}, and 7^{th} day after incubation. The two organisms *Aspergillus niger* and *Trichoderma harzianum* showed maximum mycelial dry weight in the treatment pH 6.0 i.e. 308.00 and 337.00 mg respectively. Next to this treatment pH 7.0 significantly better than the remaining treatments. However minimum mycelial dry weight was recorded in pH 8.0.

Table 1: Effect of different pH ranges on the mycelial dry weight (mg) of *Aspergillus niger* and *Trichoderma harzianum* (days after incubation)

Sr.No.	Treatments	Mycelial dryweight (mg)					
		Aspergillus niger			*Trichoderma harzianum*		
		3rd day	5th day	7th day	3rd day	5th day	7th day
1.	T_1 - p^H- 6.0	173.33	286.33	308.00	178.33	298.00	337.00
2.	T_2 - p^H- 7.0	128.00	200.00	285.33	117.00	203.33	271.66
3.	T_3 - p^H- 7.5	118.66	185.66	227.33	111.33	193.66	242.00
4.	T_4 - p^H- 8.0	107.33	177.00	216.66	109.00	188.00	235.33
	F test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE ± (m)	1.00	2.09	1.95	0.68	1.81	1.49

The present study showed that growth of *Aspergillus niger* and *Trichoderma harzianum* were supportive in high acidic condition. *Trichoderma* spp. are known to favor by acidic environment[4].

Effect of temperature regimes on the radial mycelial growth of *Aspergillus niger* and *Trichoderma harzianum*

Temperature is undoubtedly one of the most important factors, which influence the occurrence and development of fungi. Radial mycelial growth of the both fungi were assessed at temperature levels 20, 25, 30 and 35^{0}C in BOD incubator. There was gradual increase in the mycelial growth from 3rd to 7th day. Particularly, maximum growth recorded at 30^{0}C temperature and it was significantly maximum over all other temperature regimes under study.It is revealed from the data effect of temperature on *Aspergillus niger* and *Trichoderma harzianum* recorded on 3rd, 5th, and 7th day after incubation. The two organisms *Aspergillus niger* and *Trichoderma harzianum* showed maximum radial mycelial growth in the treatment 30^{0}C temperature on 7th day was 90.00 mm. Next to this treatment 35^{0}C temperature regime significantly better than the remaining treatments. However minimum mycelial growth was recorded at temperature 20^{0}C.

Table 2: Effect of different temperature regimes on radial mycelial growth (mm) of *Aspergillus niger* and *Trichoderma harzianum* (days after inoculation)

Sr.No.	Treatments	Radial mycelia growth (mm)					
		Aspergillus niger			*Trichoderma harzianum*		
		3rd day	5th day	7th day	3rd day	5th day	7th day
1.	T_1 - 20^{0}C	15.60	22.53	38.00	14.33	22.30	37.36
2.	T_2 - 25^{0}C	19.70	29.83	65.33	18.50	24.90	44.90
3.	T_3 - 30^{0}C	23.20	38.00	90.00	21.00	34.90	90.00
4.	T_4 - 35^{0}C	21.00	33.76	83.00	20.20	31.40	70.53
	F test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE ± (m)	0.33	0.36	1.13	0.33	0.296	0.604
	CD (P=0.05)	1.08	1.17	3.68	1.09	0.966	1.977

The present study showed that the radial mycelial growth of *Aspergillus niger* and *Trichoderma harzianum* were recorded maximum at 30^{0}C. Manjulata Sood[7], concluded that *Aspergillus umbrosus* showed optimum growth occurred at 30^{0}C. This gives a range of 26-30^{0}C for its best growth. It tolerated temperature up to 35^{0}C. Almost all *Trichoderma* spp. showed better growth between 25^{0}C and 35^{0}C whereas, it get decreased at 35^{0}C [6].

Effect of different light regimes on the radial mycelial growth of *Aspergillus niger* and *Trichoderma harzianum*.

Provision of four light conditions was assessed and observed the effects on radial mycelial growth after 3, 5 and 7 days of inoculations. Alternate cycle of 12h light and 12h dark was preferred by *Aspergillus niger* and *Trichoderma harzianum* recorded maximum growth up to 90mm. Minimum growth was recorded at 24h darkness.

The data presented in Table 3 showed gradual enhancement in radial mycelial growth (mm) of *Aspergillus niger* and *Trichoderma harzianum* from 3rd to 7th day. The maximum radial mycelial growth 90.00 mm was recorded at treatment 12h dark & 12h light. Next to this treatment was 8h dark & 8h light and minimum growth was recorded at treatment 24h dark.

Table 3: Effect of different light regimes on radial mycelial growth (mm) of *Aspergillus niger* and *Trichoderma harzianum* (days after inoculation)

Sr.No.	Treatments	Radial mycelia growth (mm)					
		Aspergillus niger			*Trichoderma harzianum*		
		3rd day	5th day	7th day	3rd day	5th day	7th day
1.	T_1 - 24h dark	24.50	48.65	51.60	25.40	49.96	52.80
2.	T_2 - 24h light	30.83	51.00	84.03	31.80	52.10	85.85
3.	T_3 - 8h dark & 8h light	40.33	53.56	86.56	41.40	53.96	87.00
4.	T_4 - 12h dark & 12h light	45.90	55.00	90.00	45.33	54.50	90.00
	F test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE $\pm$ (m)	0.42	0.56	0.67	0.44	0.54	0.78
	CD (P=0.05)	1.39	1.86	2.21	1.44	1.76	2.56

Alternate cycle of 12h light and 12h dark favored the growth of *Aspergillus niger* and *Trichoderma harzianum*. However, subsequent treatments like continuous light and continuous darkness recorded differential response. Continuous darkness recorded comparatively lower growth. Konstantinova[5] stated that there is correlation between sporulation of *Trichoderma* spp. and light regimes.

CONCLUSION

An attempt has been made to grow *Aspergillus niger* and *Trichoderma harzianum* at varying pH, temperature and light regimes in order to reveal for a better growth. The present study shows that isolates were supportive to acidic environment, optimal temperature was 30^0C and alternate cycle of 12h light and 12h dark was favourable.

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