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Favourable Traits for Growth of *Trichoderma* Species at Varying pH, Temperature and Agitation

KEYWORDS

Agitation, Biomass production, Temperature Effect, *Trichoderma*.

Kalpana Kanchan

Department of Botany, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra- 282005, India.

G. P. Satsangi

Department of Botany, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra- 282005, India

ABSTRACT *Present experiment was carried out to determine the optimal parameters for the biomass production of Trichoderma. It is quite essential to determine the physical conditions that are favourable for the growth of Trichoderma species. A significant difference in the biomass production was recorded among the species at tested pH levels i.e. 7, 6 and 5. The most favourable pH range 7 in which total dry weight of mycelium varies between 0.7 and 0.97 g. Trichoderma isolates were evaluated for their ability to tolerate high temp (52±1°C) and compared with their respective performance at optimum temp. (28±1°C). It was observed that at optimum temperature (28±1°C) colony growth of these isolates ranged between 85-90 mm and at high temp. (52±2°C) it ranged between 18.9-55.5 mm. Aeration by agitation was also checked at different speeds and greatest biomass was recorded at 150 rpm.*

INTRODUCTION

The ecological importance of this genus, particularly of its mycelium, is to take part in the decomposition of plant residues in soil. Mycoparasitic Trichoderma strains are able to recognize the host hyphae, to coilaround them, develop haustoria and penetrate the cell wall of the host [1]. Commercial preparations of Trichoderma sp. for biological control consist of bulk produced conidia, which are the asexual reproductive units of this fungus. The studies have suggested that the carbon and nitrogen (C:N) ratio, in addition to the ambient pH, are the main atmospheric factors influencing conidiation in Trichoderma [11, 8, 3, 10, 7]. As known Trichoderma strains have unique importance as biocontrol and should have better stress tolerance levels than the plant pathogens against which they are going to be used for biological control [2]. The abiotic factors deteriorated the antagonistic properties of pH that also influence the mycelia growth of phytopathogenic fungi as well as biocontrol agents. In all microorganisms included Trichoderma, the external factors modify its morphological characteristics as well as physiological functions. Among these factors, pH is probably the most important environmental parameters affecting the mycoparasitic activities of Trichoderma strains. A particular value of pH is required to note the maximum growth where these biocontrol agents can be multiplied and pathogen can be controlled. Many workers revealed that Trichoderma isolates showed optimum growth and sporulation rate at different pH values ranging from 2 to 7 [4, 16].

The residual toxicity due to fungicides used for the control of soil borne pathogens is an important environmental concern. Therefore, the improvement of stress tolerance in Trichoderma strains could result in increasing their efficacy against plant pathogenic fungi even under unfavourable environmental conditions. So, for exploiting the optimal antagonistic potential of Trichoderma which is to be applied as biocontrol agent (BCA) the effect of pH on their mycelial growth should be tested. Hence, an investigation was undertaken to study, compare, and assess the effect of pH on biomass production of Trichoderma sp. at different days of incubation. In the present study, comparision and assess the effect of pH, temperature and agitation on biomass production of Trichoderma sp. at different days of incubation.

MATERIALS AND METHODS

Culture preparation

Four species of Trichoderma were assessed for biomass production on Potato Dextrose Agar (PDA) for the optimization study on 5th, 8th, 11th, 14th, 17th days at 5, 6 and 7 pH. The pH of the medium was adjusted at 5 to 7 with HCL or NaOH prior to sterilization. The medium was sterilized at 121°C for 15 min in an autoclave.

Preparation of standardized inoculums

Spore suspension was prepared by adding 10 ml of sterile distilled water to mature (4-5 days) fungal colonies on PDA plates to dislodge the spores from the mycelium. The spores were counted using haemocytometer to evaluate spore concentration of about 105 spores/ml. These suspensions were used to inoculate 100 ml PD broth in 250 ml [9]. The cultures were incubated at 28°C in an incubator shaker operating at 150 rpm for 48 hours. The active growing cultures were aseptically washed three times with sterilized distilled water to remove remaining media. Three times washed then incubated for growth at 5th, 8th, 11th, 14th and 17th days the biomass was calculated by obtaining the dry weight of mycelium using oven dry method.

Physical Parameters

pH: The influence of initial medium pH on fungal growth was investigated at pH 5, 6 and 7. 10% standard inoculum was inoculated in a 250 ml Erlenmeyer flask containing 100 mL broth (PDB) incubated at 25^{oC} in orbital shaker at 150 rpm for 6 days. The pH which promoted the highest biomass production was considered for subsequent steps of the investigation.

Comparative tolerance of *Trichoderma* isolates towards optimum high (52±2°C) temperature

The studies on colony growth of four isolates was determined at optimum (28±1°C) and high (52±2°C) temp regimes. Petri plates were poured with PDA medium in its three replicates. Mycelial discs of 5 mm in diameter from 3 days old cultures were placed in the centre of each PDA plate. Observations were recorded at 72 hours.

Speed of agitation

The effects of agitation during incubation on growth were

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carried out in Trichoderma specific medium at optimum pH using an orbital shaker at 100, 150, 200 and 250 rpm. The agitation speed that promoted the highest biomass production was used for the subsequent steps of the investigation.

RESULTS AND DISCUSSSION

Effect of pH on the biomass of *Trichoderma* sp.:- The mycelia growth was observed among all isolated of Trichoderma species. It has been found that among 5, 6 and 7 pH values maximum number of isolates showed high biomass production at 7 pH followed by 6 pH and minimum at 5 pH respectively. The biomass production after 17th days i.e at the end of the experiment ranged from 0.7-0.97 g in all treatments with increasing time all isolates showed a significant increase in biomass at different pH levels (Fig.1). The biomass production of T. harzanium, T. hamatum and T. longibrachiatum were significantly higher than any other species at all pH levels. Whereas T. virens showed moderate biomass production and was observed with the increase of incubation period from 5 to 17 days. All Trichoderma species initially produced high biomass at pH 7 but slowly the preference was shifted towards pH 5 when incubation time was increased. Our study showed none of the strains showed higher biomass production at 5 pH. Verdin et al. [16] reported that most fungi do not grow at very low pH values.

Effect of optimum and high temperature on colony growth of *Trichoderma* sp.:- Good colony growth was produced by all the species of Trichoderma. Maximum growth was observed in T. harzianum (90 mm) when incubated at optimum temp. (28˚C) as compared to incubation at high which resulted in the growth of (55.5 mm). Followed by colony growth produce (90 mm) by T. hamatum at optimum and at high temp. (25 mm). By T. longibrachiatum 85 mm at optimum and at high temp. 21 mm. The colony growth of T. virens was 79 mm at optimum and 18.9 mm at high temp. (Fig. 2) There are significant difference between optimum and high temperature. The common incubation temperature for the growth of fungi such as A. niger [6], Trichoderma sp., Fusarium sp., Penicillium sp. and Graphium sp. [13] is taken to be 30°C. Sharma et al. [14] reported that media, temperature and pH had profound effect on growth of fungi. They also reported that none of the Trichoderma species grew high at or above 40°C. Singh et al. [15].

Effect of agitation: As for the effects of aeration T. harzianum showed an increase in biomass when agitation increased up to 100 rpm, then reduced when the speed of agitation increased up to 250 rpm (Fig. 3) There is no significant difference between speed of agitation of 150 and 200 rpm. Although species of Trichoderma produced higher biomass at 100 rpm. Agitation influenced the microbe to absorb more nutrients and the amount of dissolved oxygen in the cultivation medium [12]. Agitation speed has also been proven to be a critical factor

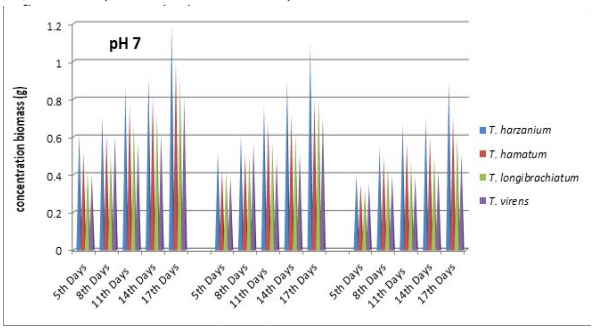

Fig.1 Biomass production of *Trichoderma* species at pH 7, pH 6, pH 5.

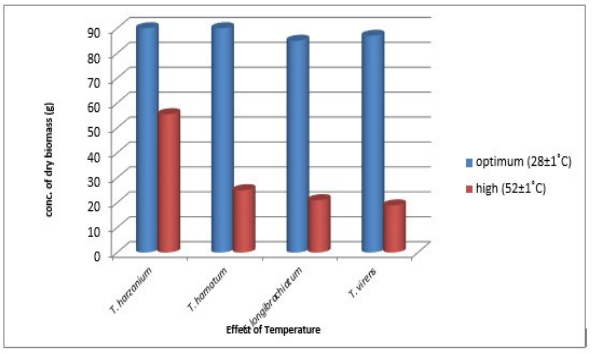

Fig.2. Effect of temperature at optimum (28±1˚C) and high (52±1˚C).

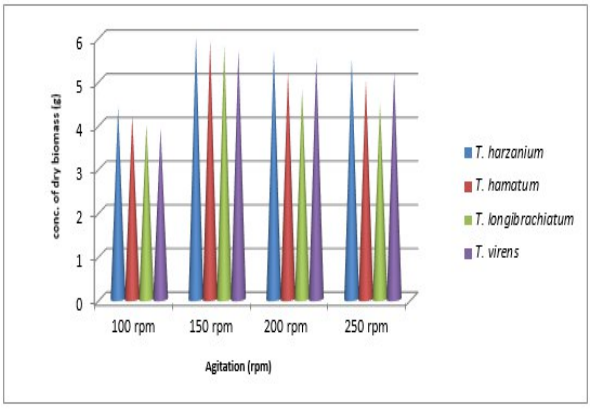

Fig.3. Effect of Agitation speed.

CONCLUSION

To conclude that growth of different species of Trichoderma at varied with the change in pH, temperature and agitation speeds in order to reveal all the relevant and favourable parameters. As Trichoderma is an ecofriendly biological control agent other soil borne plant pathogens, it is necessary to grow it at suitable conditions before it is used for commercial purposes.

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REFERENCES

1. Abdullah, F., Ilias, G. N. M. and Nelson, M. (2007). Hyperparasitic mechanisms employed by the fungal biocontrol agent in a Trichoderma-Ganoderma interaction. In Exploring Life as a Catalyst for Technological Advancement. Proceedings of the 9th Symposium of the Malaysian Society of Applied Biology, UniversitiSains Malaysia, 107-130.
2. Bandyopadhyay, S., Subhendu, J., Dutta, S. (2003). Effect of different pH and temperature levels on growth and sporulation of Trichoderma. Environmental Ecological 21: 770-773.
3. Bastos, C. N. (2001). Effect of temperature, pH and nutrition on growth and sporulation of Trichoderma stromaticum sp. nov., an antagonist of cocoa witches' broom pathogen. Summa Phytopathologica 27: 73-76.
4. Begoude, B. A., Lahlali, R., Friel, D., Tondje, P. R., Jijakli, M. H. (2007). Response surface methodology study of the combined effects of temperature, pH, and aw on the growth rate of Trichoderma asperellum. Journal of Applied Microbiology 103: 845-854.
5. Bitton G, Boylan RA (1985) Effect of acid precipitation on soil microbial activity: I. Soil core studies. Journal of environmental quality 14: 66-69.
6. Delille, D., Coulan, F., Pelletier, E. (2004). Effects of temperature warming during a bioremediation study of natural and nutrient-amended hydro carbon contaminated sub-Antarctic soils. Cold Regions Science and Technology, 40: 61-70.
7. Gao, L., Sun, M. H., Liu, X.Z., Che, Y. S. (2007). Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. Mycological Research, 111: 87-92.
8. Jackson, A. M., Whipps, J. M., Lynch, J. M. (1991) Effects of temperature, pH and water potential on growth of four fungi with disease biocontrol potential. World Journal of Microbiology and Biotechnoogylogy 7: 494-501
9. Kendrick A, Ratledge C(1996) Cessation of polyunsaturated fatty acid

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formation in four selected filamentous fungi when grown on plant oil. Journal of the American Oil Chemists' Society 73: 431-435.
10. Kredics, L., Manczinger, L., Antal, Z., Pénzes, Z., Szekeres, A., et al. (2004). In vitro water activity and pH dependence of mycelial growth and extracellular enzyme activities of Trichoderma strains with biocontrol potential. Journal of Applied Microbiololgy 96: 491-498.
11. Lewis, J. A., Papavizas, G. C. (1983). Production of Chlamydospores and conidia byTrichoderma spp. in liquid and solid growth media. Soil Biology & Biochemistry 15: 351-357.
12. Purwanto, L. A., Ibrahim, D., Sudrajat, H. (2009). Effect of Agitation Speed on Morphological Changes in Aspergillus niger Hyphae During Production of Tannase. World Journal of Chemistry, 4: 34-38.
13. Santos, V. L., Linardi, V. R. (2004). Biodegradation of phenol by a filamentous fungi isolated from industrial effluents identification and degradation potential. Process Biochemistry 39: 1001-1006.
14. Sharma, R. L., Singh, B. P., Thakur, M. P., Thapak, S. K. (2005). Effect of Media, Temperature, pH and Light on the Growth and Sporulation of Fusarium oxysporum f. sp. Lini (Bolley) Snyder and Hensan. Annals of Plant Protection Sciences 13:172-174.
15. Singh, A., Shahid, M., Pandey, N. K., Kumar, S., Srivastava, M., Biswas, S. K. (2011) Influence of Temperature, pH and media for growth and sporulation of Trichoderma atroviride and its shelf life study in different carrier based formulation. Journal of Plant Diseases and Protection 6: 32-34.
16. Verdin, A., Sahraoui, A. L., Durand, R. (2004). Degradation of benzo[a] pyrene by mitosporic fungi and extracellular oxidative enzymes. International Biodeterioration & Biodegradation 53:65-70.