

Impact Factor: 3.4546 (UIF) DRJI Value: 5.9 (B+)

# Effect of some physical factors on growth of five fungal species

SADIQ R. M. ALI<sup>1</sup> The General Company for Food Products, Baghdad, Iraq AHMED J. FRADI The Ministry of Education, Baghdad, Iraq ALAA M. AL-AARAJI Department of Biology, College of Science Baghdad University, Baghdad, Iraq

#### Abstract:

Fungal growth responses to temperature, pH and incubation period were studied. This studied provides the influence of different temperature, pH and incubation period on the growth of fungi. A uniform pH gradient, ranging from pH 3 to 11, temperature ranging from 20°C to 40°C and incubation period ranging from 2 to 10 days. Effects of temperature, pH and incubation period on the growth of fungi were determined in vitro. The control isolates was incubated for 7 days at 29±2°C. Optimum temperature occurred between 25°C and 30°C, pH ranges between 5 and 6 and incubation period ranges between 6 and 8 days. Four isolates of fungi grew at 35°C and all isolates grew at 20, 25 and 30°C but no fungal isolates grew at 40°C. Reports have shown that growth of fungi could be affected by pH, at pH 5 and 7 all fungal isolates were grew, at pH 3 only Mucor spp. was little grew and at pH 9-11 no fungal isolates grew. The influence of incubation period on the growth of the fungi growing on sabouraud dextrose agar, the results were Rizopus spp. and Fusarium spp. grew after 2 days, while, after 4 days, all fungal isolates were showed initial growth and between 6-10 days all fungal isolates were showed Approached growth compared with control. The aim of this research is provide detailed information for five fungal species "researchers often

<sup>&</sup>lt;sup>1</sup> Corresponding author: Sadeqraheem7@yahoo.com

do their research" about the effect of some factors on the growth of fungus.

Key words: Fungi, pH, Temperature, Incubation period .

#### **INTRODUCTION**

Fungi can be divided into groups according to temperature requirements for optimal growth. These requirements are dependent on water availability and nutrients, and are measured under carefully controlled conditions. The groups are Psychrophiles, Mesophiles and Thermophiles. The effects of heat on fungi are related to the chemical reactions within the fungal cells [1]. For optimum growth, temperatures must be in a range that allows the most efficient progression of the chemical reactions necessary for growth. As temperatures progress above the optimum temperature, the chemical efficiently, and growth reactions occur less slows [2]. Eventually, the temperature can reach a point where growth stops, and cell components begin to be actually damaged by the heat. Enzymes are proteins that change structurally when heated to their limit of tolerance [3]. Likewise, membranes, which contain lipids, change in structure, and their function of protecting and regulating the internal environment of the cell becomes compromised [4]. The major factors affecting growth of fungi are nutrients, temperature, light, aeration, pH and water activity. Temperature, the majority of fungi are mesophilic, i.e., they can grow at temperatures within the range of 10-35°C. Optimum temperatures for growth may range between 15 and 30°C. However. some fungi such as Chaetomium thermophilum and Penicillium dupontii are thermophilic, i.e., they can grow at 45°C or higher and fail to grow below 20°C [5].

A few fungi are psychrophilic and are unable to grow above 20°C. A significant number are psychrotolerant and are able to grow both at freezing point and at room temperature.

pH, fungi differ in their pH requirements. Most will grow well Some the pН range 3-7. such as Aspergillus over niger and Penicillium funiculosum can grow at pH 2 and below The most influencing factors for fungal growth is [6]. temperature.  $\mathbf{As}$ fungi cannot control their internal temperature. Fungi can live in a relatively large range of temperatures, but their growth rate and metabolism are different at different temperatures even when other conditions, e.g. nutrient and water activity are constant [7]. Normally, the temperature at which a fungi have the highest biomass increase rate is accepted as the optimum temperature level of that fungi [8]. However, it is not well known whether this is also the temperature at which the fungi is growing most efficiently and under least stress. The understanding of the effect of temperature on fungal growth is an essential part of fungal physiology [9]. It has been shown that pH and temperature are important criteria for understanding the ecology of fungi, especially mycotoxigenic species [10]. However, acid/alkaline requirement for growth of fungi is quite broad, ranging from pH 3.0 to more than pH 8.0, with optimum around pH 5.0 if nutrient requirements are satisfied [11]. In general, Aspergillus spp. are more tolerant to alkaline pH while Penicillium species appear to be more tolerant to acidic pH. Studies on pH reveal that fungi grow at pH 3.0 - 8.0, with maximum production of dry mycelial weight and sporulation at pH 5.5 and pH 6.5 respectively, in liquid media [12].

#### MATERIALS AND METHODS

Seven different pH levels of Sabouraud dextrose agar (SDA) were prepared by addition of 65g agar and made up to one liter with distilled water. The pH of each medium was adjusted by adding either hydrochloric acid (0.1M HCl) or sodium hydroxide (0.1M NaOH) to get the required pH values [13]. The pH level was measured using electrical pH meter before sterilization in

autoclave at 121°C. The plates were incubated for seven days at temperature (approximately  $29 \pm 2$  °C). Temperature test was the plate with SDA have been incubated at different temperatures. While, incubation period test was by incubated the plate for different date. Two millimetre discs of the culture of fungi were obtained from the growing edges of cultured SDA colonies using sterilized cork borer. The agar plugs were transferred to the centre of SDA plates (one plug per plate) at different temperature levels [4]. Data obtained were analysed using one way Analysis of Variance (ANOVA) and the group means compared by Duncan Multiple Range Test (DMRT) using the Statistical Package for Social Science (SPSS) version 16.0. (2007).

## **RESULTS AND DISCUSSIONS**

#### Effect of the temperature

The study of effect of different temperature (20°C, 25°C, 30°C, 35°C and 40°C) on growth of five fungi was shown in figure 1. The results showed that the best studied growth temperature was 30°C, 25°C, 20°C, 35°C then 40°C respectively. The statical analysis shows a significant differences (P<0.05) exist between fungal growth curve under different incubation temperatures as shown in table 1. These results were similar to another study which is observed the optimal growth occurring at 25-30°C [14]. The effects of heat on fungi depend on many factors, including the genus, species and strain of the fungus, the amount of available water, kinds of nutrients, and many other environmental factors.



Figure 1: Effect of temperature on fungal growth

In addition to previous results, we noted the stability of the fungal count at 25-30°C, these results compatible with [15] whom mentioned that most fungi are mesophilic, and have growth optima within the temperature range that people find comfortable. This is why so many fungi appear when moisture enters homes, schools, hospitals and work environments. Because of air conditioning and heating, mesophilic fungi flourish in occupied environments in all climates.

Table 1: Effect of temperature on fungal growth compared with control

Fungi	Colony control diameter (mm) at 29 $^\circ\mathrm{C}$	$20~^{\circ}C$	$25~^{\circ}\mathrm{C}$	30 °C	$35~^{\circ}\mathrm{C}$	40 °C
		Growth rate diameter (mm)				
Rizopus spp.	37	33	36	36	3	0
Penecillium spp.	9	8	8	9	0	0
Alternaria spp	11	9	10	10	5	0
Fusarium spp.	19	15	16	16	4	0
Mucor spp.	12	10	10	9	3	0

#### Effect of the pH

Fungi generally grow well in acidic conditions [16], but some species favor neutral to slightly alkaline conditions. The results of effect of different pH values (3-5-7-9 and 11) on the growth of five fungi are shown in figure 2. The results show that the best pH is 5-6. Table 2 exhibit a significant differences (P<0.05) between fungal growth curve fungi isolates under different pH values.



Figure 2: Effect of pH on fungal growth

Some species possess cellulolytic enzymes having optimal pH, between 6.8–9.0, and the cellulolytic enzymes of species show an optimal pH range between 5.5–6.8 [17]. The obtained results were similar to results of another study carried out by [18], who observed that the optimum pH of fungi spp. growth was 5-6. [16] pointed out that environmental H<sup>+</sup> concentration has little direct effect on fungal metabolism due to the buffering system in hyphae but may influence the ionization of salts in solution and the permeability of the plasmalemma of the hyphae. Furthermore, enzyme activity is affected by H<sup>+</sup> concentration. The fungal inhibitory effect seems to lessen with increasing pH as shown by the increasing amount of fungal growth at the higher pH values [19]. The growth of fungi could be affected by pH in a medium in which it grows, either directly by its action on the cell surfaces or indirectly by its effect on the availability of nutrients [20].

······································							
Fungi	Colony control diameter (mm) at pH = 5. 7	pH 3	pH 5	pH 7	pH 9	pH 11	
		Growth rate diameter (mm)					
Rizopus spp.	39	0	30	9	0	0	
Penecillium spp.	10	0	7	2	0	0	
Alternaria spp	11	0	9	2	0	0	
Fusarium spp.	20	0	15	5	0	0	
Mucor spp.	10	1	9	2	0	0	

Table 2: Effect of pH on fungal growth compared with control

#### Effect of incubation period

Tested the effect of period of five fungi at 2, 4, 6, 8 and 10 days, it was shown in figure 3. The results showed that the best

studied period was 6-8 days, the growth was gradually per days. The statical analysis shows a significant differences (P<0.05) exist between fungal growth curve under different incubation temperatures as shown in table 3.



Figure 3: Effect of incubation period on fungal growth

Fungi require different conditions for optimal growth [21], fungi derive all of their energy and growth materials from their growth medium, through biochemical decomposition processes [22]. However, all the materials for growth must already be present in the growth medium [23]. Specific time spans required during 3-7 days vary respective to species and variety [24].

Table 3: Effect of incubation period on fungal growth compared with control

Fungi	Colony control diameter (mm) at 7 days	2 days	4 days	6 days	8 days	10 days
		Growth rate diameter (mm)				
Rizopus spp.	40	3	6	23	39	41
Penecillium spp.	10	0	1	5	10	11
Alternaria spp	11	0	1	5	12	13
Fusarium spp.	20	2	4	12	21	22
Mucor spp.	12	0	1	6	12	14

## CONCLUSION

Temperature is a factor affecting fungi, most of fungi were fungicidal effectiveness at acute increase or decrease the temperature. The number of culturable fungi decreased sharply under acidic and alkaline cultivation conditions, while the number of culturable fungi remained relatively constant over the pH range 5–6. The cultural period was very affecting on fungal growth, Therefore, it is must selective the appropriate period for the best fungal growth.

## REFERENCES

[1] Rosfarizan, M.; Ariff, A.B.; Hassan, M.A. and Karim, M.I. (2000). Influence of pH on kojic acid fermentation by *Aspergillus flavus*. Pakistan Journal of Biological Sciences. 3:977–982.

[2] El-Aasar, S.A. (2006). Cultural Condition Studies on Kojic Acid Production by *Aspergillus parasiticus*. International Journal of Agriculture & Biology. 4:468–473.

[3] Carlos, A. and Josep, A. (2012). Effects of Temperature, pH and Water Potential on Mycelial Growth, Sporulation and Chlamydospore Production in Culture of Cylindrocarpon species Associated with Black Foot of Grape vines. Phytopathologia Mediterranea. 51(1): 37–50.

[4] Nielsen, K.F. (2001). Mould growth on building materials, secondary metabolites, mycotoxins and biomarkers. PhD thesis. Technical University of Denmark. Lyngby, Denmark.

[5] Sibounnavoung, P.; Kalaw, S.P.; Divina, C.C. and Soytong,
K. (2009). Mycelial Growth and Sporulation of *Emericella nidulans*, A New Fungal Antagonist On Two Culture Media.
Journal of Agricultural Technology. 5(2): 317-324.

[6] Wadso, L.; Li, Y. and Bjurman, J. (2004). Measurements on two mould fungi with a calorespirometric method. Thermochimica Acta. 422: 63–68.

[7] Gock, M.A.; Hocking, A.D.; Pitt, J.I. and Poulos, P.G. (2003). Influence of temperature, water activity and pH on growth of some xerophilic fungi. Int. J. Food Microbiol. 81: 11–19.

[8] Ahmed, A. and Naresh, M. (2009). Influence of physiological factors on growth, sporulation and ochratoxin A/B production of

new Aspergillus ochraceus grouping. World Mycotoxin Journal. 2(4): 429 – 434.

[9] Li, Y.; Wadso, L.; Larsson, L. and Bjurman, J. (2007). Correlating two methods of quantifying fungal activity: Heat production by isothermal calorimetry and ergosterol amount by gas chromatography-tandem mass spectrometry. Thermochimica Acta. 458: 77–83.

[10] Li, Y.; Wadso, L. and Larsson, L. (2009). Impact of temperature on growth and metabolic efficiency of *Penicillium* roqueforti – correlations between produced heat, ergosterol content and biomass. Journal of Applied Microbiology. 106: 1494 - 1501.

[11] Pardo, E.; Marin, S.; Ramos, A.J. and Sanchis, V. (2006). Ecophysiology of ochratoxigenic *Aspergillus ochraceus* and *Penicillium verrucosum* isolates. Predictive models for fungal spoilage prevention - a review. 23(4): 398-410.

[12] Deshmukh, A.J.; Mehta, B.P.; Sabalpara, A.N. and Patil, V.A. (2012). In vitro effect of various nitrogen, carbon sources and pH regimes on the growth and sporulation of Colletotrichum gloeosporioides Penz. And Sacc causing anthracnose of Indian bean. Journal of Biopest. 5: 46-49.

[13] Saha, A.; Mandal, P.; Dasgupta, S. and Saha, D. (2008). Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. Journal of Environmental Biology. 29(3): 407-410.

[14] Duarte, S.; Fernandes, I.; Nogueira, M.J.; Cassio, F. and Pascoal, C. (2012). Temperature alters interspecific relationships among aquatic fungi. Campus de Gualtar. Braga, Portugal.

[15] Elmsly, T.A. and Dixon, J. (2008). Growth rates of ripe rot fungi at different temperatures. New Zealand Avocado Growers' Association Annual Research Report. 8: 77 - 84.

[16] Dix, N.J. and Webster, J. (1995). Fungal ecology. London: Chapman & Hall.

[17] Enokibara, S.; Suzuki, A.; Fujita, C.; Kashiwagi, M.; Mori, N. and Kitamoto, Y. (1993). Diversity of pH spectra of cellulolytic enzymes in Basidiomycetes. Trans Mycol. Soc. Japan. 34: 221–228.

[18] Yamanaka, T. (2003). The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi *in vitro*. The Mycological Society of America, Lawrence. 95(4): 584–589.

[19] Abubakar, A.; Suberu, H.A.; Bello, I.M.; Abdulkadir, R.; Daudu, O.A. and Lateef, A.A. (2013). Effect of pH on mycelial growth and sporulation of *Aspergillus parasiticus*. Journal of Plant Sciences. 1(4): 64-67.

[20] Cheung, Y.Y.; Lee, S.H.C.; Hui, M. and Luk, T.N.M. (2014). Effect of pH on fungal growth: problems with using vinegar (5% acetic acid) in treating superficial fungal infections. Hong Kong J. Dermatol. Venereol. 22: 57-64.

[21] Auer, C.G. (2007). Growth and germination of some thermophilic fungi isolated from eucalypt wood chips. Pesq. Flor. bras., Colombo. 54: 149-152.

[22] El-Said, A.H.M. and El-Hady, G. (2014). Effect of moisture contents on the biodiversity of fungi contaminating *Cuminum cyminum* and *Pimpinella anisum* seeds under storage periods and Amylolytic Activity of fungal isolates. *Int.J.Curr.Microbiol.App.Sci.* 3(3): 969-991.

[23] Marusenko, Y.; Huber, D.; Ortiz, P. and Hall, S.J. (2010). How important are soil fungi in the nitrogen cycle of arid and semi-arid ecosystems. Journal of Ecology. 96: 413-420.

[24] Ulfig, K. (2005). Effect of Sewage Sludge Alkalization and Acidification on Keratinolytic and Keratinophilic Fungi. Polish Journal of Environmental Studies. 14(5): 647-653.