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# Some Physiological and Nutritional Factors that affect the growth of Some Fungi

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**A R T I C L E I N F O**

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**A B S T R A C T**

The present study is attempt to evaluated and compared the ability of different genera and species of fungus to utilize different nutrient sources and to grow under different environmental conditions. Four genera of molds were grown on different synthetic, semi-synthetic and natural media such as Potato Sucrose agar (PSA), Czapek's Dox agar (CDA) and Water agar (WA), which subjected to a range of temperatures (4, 15, 25, 37 and 50ºC) to investigate their influence on the performance of the mycelium of *Alternaria alternata, Aspergillus fumigatus, Penicillium citrinum* and *Trichoderma harzianum*. PSA and CDA were most favorable nutrients for fast radial growth of mycelium of all genera of fungi and at all temperatures and PH levels, while on WA, the radial mycelial growth was very little or there was no growth at all environmental conditions. In general, there was high growth at temperatures (15ºC-37ºC), while at 50ºC there was no growth or very little growth of fungi. On the other hand, a different growth of all fungal genera has been shown in all media with various pH levels, except at pH 11, which have seen little growth colonies or no growth. *Penicillium citrinum* have seen the highest growth in acidic pH, while *Aspergillus fumigatus* have seen in alkaline pH. On PSA media, all genera have shown the highest growth at 25°C, while no growth at 4°C and 50°C, except a little growth of *Alternaria alternata* and *Penicillium citrinum* at 4°C and *Aspergillus fumigates* at 50°C, while in pH ranges, the highest growth of all fungal genera have shown at pH 3-5, While no growth at pH11, except a bit growth of *Aspergillus fumigatus* and *Trichoderma harzianum*. On CDA media, all genera have shown the highest growth at 25°C, while no growth at 4°C and 50°C, except a little growth of *Penicillium citrinum* at 4°C, while in pH ranges, the highest growth of all fungal genera have shown at pH 3-7, While the lowest growth of *Alternaria alternata*, *Aspergillus fumigatus* and *Trichoderma harzianum* with no growth of *Penicillium citrinum*. While on WA media, all genera have shown a little growth at all temperatures, except *Trichoderma harzianum*, there was no growth at all temperatures, *Alternaria alternata*, and also no growth at 4°C and 50°C, *Aspergillus fumigatus*, no growth at 4°C, *Penicillium citrinum*,and no growth at 50°C, while in pH ranges, a little growth of all fungal genera have shown at pH 3-7, Whilst no growth of all genera at pH11, except *Aspergillus fumigatus*.

## 1. INTRODUCTION

Fungi are consisting of large numbers of organisms that are unique compared with plant and animals, among these are molds and yeasts. Despite the great variation in morphology and characteristics of the fungi, most of them share the following characteristics including: the presence of chitin in the cell wall, ergosterol in the cell membrane, lack of chlorophyll, asexual or sexual reproduction, their heterotrophic nature and lack of susceptibility to antibacterial antibiotics (Forbes *et al.*, 2007**).**

The fungal kingdom is arranged into four major phyla on the basis of differences in their sexual reproduction. These are chytridiomycota, zygomycota, ascomycota, and basidiomycota (Hogg, 2005).

The physiology of fungi means the growth, nutrition, metabolism, reproduction, and death of fungal cells and also refers to fungal interaction with their biotic and a biotic surroundings, which including cellular responses to stress. They impact significantly on human health and industry. The metabolism of fungi is responsible for bioremediation of heavy metals and for detoxification of organic pollutants in the environment (Walker and White, 2005).

The fungi have shown changes in their growth rate when grown on different types of nutrient media (Brock and Heymann, 2006). Temperature and pH are the significant factor for studying the spoilage of fungi ecologically (Ahmed and Naresh, 2009). The growth of fungi could be affected by pH in a medium, which it grows in, either directly by its action on the cell surfaces or indirectly by its effect on the nutrients availability. However, acid/alkaline requirement for growth of molds and yeasts is quite a wide spectrum, ranging from pH 3 to more than pH 8 and about 5 is the optimum pH (Pardo *et al.,* 2006). In general, *Penicillium* species are more tolerant to acidophiles or acid pH, while *Aspergillus* species are more tolerant to alkalophiles or alkaline pH (Wheeler *et al.,* 1991). A

neutral to the weak acidic condition was favourable for the growth of fungal mycelia, with optimum pH 5–7(Zhao *et al.,* 2010).

The effects of high temperature on the growth fungi depend on several factors, including the genus, species, and strain of the fungus**,** types of nutrients, and many other environmental factors. Temperature is very important for the fungal growth, most fungi will grow between 0 and 35$^{o}$C, but the optimum temperature lies in the range of 20-30$^{o}$C (Alexopoulos, 1962).

According to temperature requirements for optimal growth, fungal genera divided into three distinct groups such as Psychrophiles (less than 10°C), Mesophiles (18-22°C) and Thermophiles (at or above 37°C). There are also psychrotolerant and thermotolerant fungi, indicating that growth can occur at either low or high temperatures, but are not optimal.

The aim of the present research was to estimate the ideal media, temperature, and pH, which could be used to produce the maximum yield of *Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Trichoderma harzianum* mycelia and sporulation.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Fungi

All genera of fungi, which used in this study were obtained from the University of Salahaddin, college of Science-Department of Biology, They activated by sub-culturing on

PSA in Petri dishes.

### 2.2. Preparation of media

**2.2.1. Potato Sucrose Agar (PSA)**: The chemical compound of this media includes: 250 gm of Potato, 20 gm of Sucrose, 15 gm of Agar and 1 Liter of Distilled water (D.W.) (Samson, 2004).

**2.2.2. Czapek (Dox) Agar (CDA)**: The chemical compound of this media includes: 2gm of $NaNO_3$, 1gm of $KH_2PO_4$, 0.5gm of KCl, 0.5gm of $MgSO_4.7H_2O$, 0.01gm of $FeSO_4.7H_2O$, 30gm of Sucrose, 20gm of Agar and 1 liter of D.W.(Samson, 2004).

**2.2.3. Water agar (WA):** It consists of 20 gm of agar and 1L of tab water (Johnston and Booth, 1983). Potato sucrose agar, CDA and WA were prepared (1litter for each medium), then the prepared media were distributed in sterile 250ml conical flasks. Finally autoclaved at 121°C, by 1.5 bar for 17 minutes (Samson, 2004).

### 2.3. The influence of temperatures on the fungal growth

Effect of different synthetic and semi-synthetic media and different temperatures (4, 15, 25, 37 and 50 ºC) on the colony growth of *Alternaria alternata, Aspergillus fumigatus, Penicillium citrinum* and *Trichoderma harzianum* were estimated. Fungal media, such as PSA, CDA and WA were poured into Petri plates (9cm diameter).

Petri plates containing 20 ml of each of media were inoculated with five mm diameter mycelial discs from seven-day-old cultures of different isolates. The agar plugs were removed with a sterile cork holer from the edges of colonies and one such plug was placed in the center of each 9 cm Petri plate in each of the three media. The inoculated plates were incubated at different temperatures: 4, 15, 25, 37 and 50ºC. For each medium, there were three replicate plates. The diameter of mold colony in each plate was measured at a **7**-day interval along two axes perpendicular to one another. Then the radial growth rates of each mold genera were calculated.

### 2.4. The influence of pH on the growth of fungi

The pH levels that selected for this study were 3, 5, 7, 9 and 11. One hundred ml of each of medium PSA, CDA and WA were prepared and distributed in sterile conical flasks (250ml). The pH levels were adjusted to 3, 5, 7, 9 and 11, by adding hydrochloric acid (HCl) or sodium hydroxide (NaOH) to each of the flasks that contain media. The electrical pH meter measured the pH before sterilization in an autoclave at 121$^o$C. Then pour each of the sterilized media into sterilized Petridishes; allow the plate to become solidified. Use sterilized cork hole to cut disks of agar and mycelium from 7 days old molds culture, transfer an inoculum into poured solidified media in upside situation that the mycelium becomes contact with the medium, incubate the inoculated medium at 25ºC for 5-7 days. Finally measure the diameter of the mycelium, and compare the growth at each pH (Carlos and Joseph, 2012). Each treatment was in triplicate.

## 3. RESULTS

The result presented in this study show the radial mycelial growth rates of *Alternaria alternata, Aspergillus fumigatus, Penicilliumcitrinum* and *Trichoderma*

*harzianum*, were significantly affected by culture media, temperature and pH.

In PSA and CDA, there were highest growths at temperatures 15ºC-37ºC after 5-7 days of inoculation, while at 50ºC there was no growth or very little mycelia growth of fungi. On the other hand, there has been shown that different growth rate of all fungal genera in all media with various pH level, except at pH11, which have seen a little growth or no growth. On WA, the radial mycelial growth was very little or no growth at all temperatures and pH levels. In general, *Penicillium citrinum* have has seen the highest growth in acidic pH while *Aspergillus fumigatus* have seen in alkaline pH.

In Table 1, Figure 1 (a) and (b), showed the effects of temperature and pH on the radial fungal growth on PSA media. In which, all genera have shown the highest growth at 25°C, while no growth at 4°C and 50°C, except a little growth of *Alternaria alternata* (1.1cm) and *Penicillium citrinum* (1.2cm) at 4°C and *Aspergillus fumigates* (2.1cm) at 50°C. While in pH ranges, the highest growth of all fungal genera has shown at pH 3-5, with no growth at pH11, except a bit growth of *Aspergillus fumigates* (2cm) and *Trichoderma harzianum* (0.5cm).

In general it has shown that radial growth of mycelia of all genera on PSA media at 4ºC were: (1.1, 0, 1.2 and 0) cm, but at 15ºC were (7, 2.9, 4.5 and 9) cm, whilst at 25°C were (9, 9, 8 and 9) cm, nevertheless at 37°C were (2.9, 4.3, 6.1and 3.3) cm, and at 50°C were (0, 2.1, 0 and 0) cm, while at pH 3 were (4.5, 3, 6 and 5.5), but at pH5 were (4, 5, 6.5 and 6), and also at pH7 were (2, 5.5, 2 and 3.5), whilst at pH9 were (1.5, 6, 1 and 3), and at pH11 were (0, 2, 0 and 0.5), for each (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Trichoderma harzianum*,) respectively.

Table 2, Figure 2(a) and (b), illustrate the effect of temperature and pH on radial growth of each mold on CDA media. It shows that all genera have the highest growth at 25°C, while no growth at 4°C and 50°C, except a little growth of *Penicillium citrinum* (1.5cm) at 4°C.While in pH ranges, the highest growth of all fungal genera has shown at pH 3-7, While, at pH11, it has shown the lowest growth of *Alternaria alternata* (0.5cm), *Aspergillus fumigatus* (3) and *Trichoderma harzianum* (1cm), while the growth of *Penicillium citrinum*. The radial mycelium growth of all fungi on CDA media at 4ºC were (0, 0, 1.5 and 0) cm, but at 15ºC were (5, 2.7, 2.7 and 9) cm, and at 25°C were (9, 6.2, 8 and 9) cm, whilst at 37°C were (0, 4.3, 1.4 and 5) cm, nevertheless at 50°C was (0, 0, 0 and 0) cm, while at pH3 were (5, 3.5, 6 and 7), and also at pH5 were (4.5, 4, 6.5 and 7.5), but at pH7 were (3, 6, 4 and 5), whilst at pH9 were (2, 6.5, 2 and 4),while at pH11 were (0.5, 3, 0 and 1), for each (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Trichoderma harzianum*,) respectively.

Table 3, Figure3 (a) and (b), give information about the effect of temperature and pH on radial growth of each mold on WA media. Its how that all genera have a little growth at all temperatures, except *Trichoderma harzianum*, there was no growth at all temperatures, *Alternaria alternata*, no growth at 4°C and 50°C, *Aspergillus fumigatus*, no growth at 4°C, *Penicillium citrinum*, no growth at50°C. While in pH ranges, a little growth of all fungal genera has shown at pH 3-7, While no growth of all genera at pH11, except *Aspergillus fumigatus*(1cm).

In this table the radial mycelium growth of all fungi on WA media at 4ºC were (0, 0, 1.5 and 0) cm, at 15ºC were (1.5, 1.2, 2 and 0) cm, and at 25°C were (2, 1.5, 2 and 0) cm at 37°C were (1.5, 1.3, 1.5 and 0) cm, and also at 50°C

were (0, 1.4, 0 and 0)cm, while at pH3 were (0.5, 0, 2 and 1.5), nevertheless at pH5 were (1, 1, 1.5 and 1), but at pH7 were (1.5, 2, 1 and 1), whereas at pH9 were (1, 2.5, 0.5 and 0.5), and at pH11 were (0, 1, 0 and 0) for each (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Trichoderma harzianum*,) respectively.

## 4. DISCUSSION

The results of our research were in close agreement with those of Mustafa *et al.,* (2009), who examined mycelial growth and conidial production of *Trichoderma harzianum*, *T. viride*, *T. longibrachiatum*, on five different culture media including Potato Dextrose Agar, Waksman agar, Agar-agar, CDA and Corn Meal agar. The medium had a high effect on growth rate and the population of *Trichoderma* spp. Potato Dextrose Agar was the best medium for growth spore and biomass production. Alam *et al*., (2001), who recorded the highest growth of *Botryodiplodia theobromae* mycelium on PDA and maximum percentage of pycnidia on CDA. Similarly, Quroshi and Meah, (1991), who observed fastest liner growth of *Botryodiplodia theobromae* and the highest number of pycnidia on PDA. O'Brian *et al*., (2007), have reported that the temperature ranges of 25-30°C as the best-favoring proliferation as well as toxin production in *Aspergillus* sp..Our results also in agreement with Sabalpara *et al*., (1991), who have found very little or no growth of fungi at low temperatures such as at 10 and 15ºC, however, as temperature increased up to 25ºC, the mycelial growth of fungi increased and then decreased rapidly with further increase in temperature. Optimum growth occurred at 25-37ºC. Also, there are close agreements that reported by Saha *et al*., (2008), who reported that optimum temperatures for the colony growth of fungus were 25-30ºC. Khanzada *et al*., (2006), who found that most suitable media for mycelial growth of the fungi were PSA, Yeast extract mannitol agar and Corn meal agar. Cao, *et.al*., (2007), who focus on growth phases of *Penicillium* sp., upon the effects of physiological factors and show that all could grow at a wide temperature range (8.0–39.8°C), but growth was inhibited at 40°C, dramatically. Johnson, *et al*., (1987), who isolated *Trichoderma* spp. from Tennessee and Alaska soils on a selective medium at 10, 12 and 25°C.Tatiana, *et.al*.,(2010), who found that best results were obtained when *Alternaria* sp. was grown in a medium at 25$^{o}$C for seven days. Basu and Bhattacharyya, (1962), who found that the growth and sporulation of several strains of *Penicilliulm* spp. affected by certain carbohydrates, nitrogen compounds, and accessory growth factors. Allen *et al*., (1982), showed that the germination of conidia of *Alternaria* sp. was favored by a temperature between 25 and 28°C, and the colony growth of *Alternaria* sp. on PDA was greatest at 25°C. Upadhyay and Rai, (1978), have showed that *Trichoderma* spp. has an ability to utilize a variety of nutritional factors as well as they have a broad range of pH and temperature tolerance for their growth and sporulation, except. *viride* which was unable to grow at pH 9 and above. Ogunledun, (2007), showed that acidic pH significantly influence the growth of fungi, while the least growth as observed at neutral pH.

The results in the current study disagree with those of Sibounnavoung *et al.,* (2009), who found that the growth and sporulation of *Aspergillus* spp. is tolerant of acidic and neutral conditions while suppressed by an alkaline condition. Rosfarizan *et al*., (2000), who confirmed that most of the molds have an optimum pH between 5 and 6 for growth and

metabolic activities and they are generally tolerant to acidic pH. This study has obtained a result similar to the report by Kaiser *et al*., (2005) at pH 10.3 on *Alternaria solani* (42.8%), *Phytophthora capscici* (17.4%) and *P. cinnamomi* (12.6%) respectively, while at pH 11.7, the growth of them was completely inhibited. Our results also in agreement with Swe *et al*., (2009), who have revealed that the diameter of colony decreased in the media with the higher pH level, also it shows that, although certain alkaline medium favors the formation of *Aspergillus* sp. spore, higher pH values from 10 tend to inhibit its sporulation.

It has also been found that increased severity of several diseases in soil due to an elevation of soil pH (Cook and Baker, 1983; Simon and Sivasithamparam, 1988). The suitability of *Trichoderma* spp. as a biocontrol agent is due to that this antagonist has a broad range of temperature tolerance for growth and sporulation. The biocontrol formulations developed from *Trichoderma*, may be used in a wide range of geographical locations because of its ability to utilize a large number of carbon and nitrogen sources and its broad spectrum of pH and temperature tolerance (Jayaswal *et al.*, 2003).

## 5. Conclusions

Four genera of molds were grown on different synthetic, semi-synthetic and natural media, which subjected to several range of temperatures and with various pH levels to investigate their influence on the performance of the mycelium of some fungi. PSA and CDA were most favorable nutrients for fast radial growth of mycelium in all genera of fungi and at all temperatures and PH levels, while the mycelial growth on WA, was very little or there was no growth at all environmental conditions.

## Acknowledgment

I would like to thank University of Salahaddin-college of Science/Biology department, for helping us and providing space and laboratory facilities to carry out the research work in Mycology research laboratory.

**Table 1:** Effect of temperature and pH on the radial fungal growth on PSA media

| | | Radial fungal growth (cm) | | | |
|---|---|---|---|---|---|
| | | ***Alternaria alternata*** | ***Aspergillus fumigatus*** | ***Penicillium citrinum*** | ***Trichoderma harzianum*** |
| Temperatures | **4°C** | **1.1** | **0** | **1.2** | **0** |
| | **15°C** | **7** | **2.9** | **4.5** | **9** |
| | **25°C** | **9** | **9** | **8** | **9** |
| | **37°C** | **2.9** | **4.3** | **6.1** | **3.3** |
| | **50°C** | **0** | **2.1** | **0** | **0** |
| pH | **3** | **4.5** | **3** | **6** | **5.5** |
| | **5** | **4** | **5** | **6.5** | **6** |
| | **7** | **2** | **5.5** | **2** | **3.5** |
| | **9** | **1.5** | **6** | **1** | **3** |
| | **11** | **0** | **2** | **0** | **0.5** |

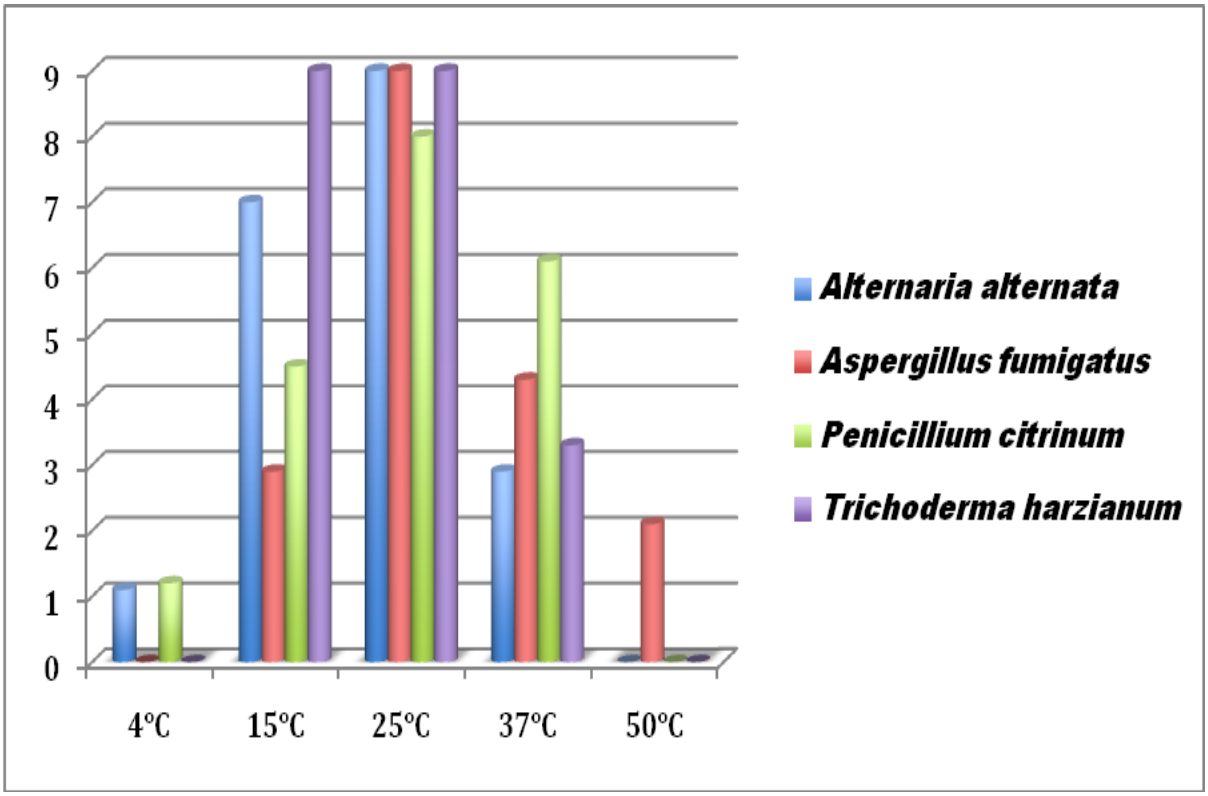


**Figure 1 (a):** Effect of temperature on the radial fungal growth on PSA media

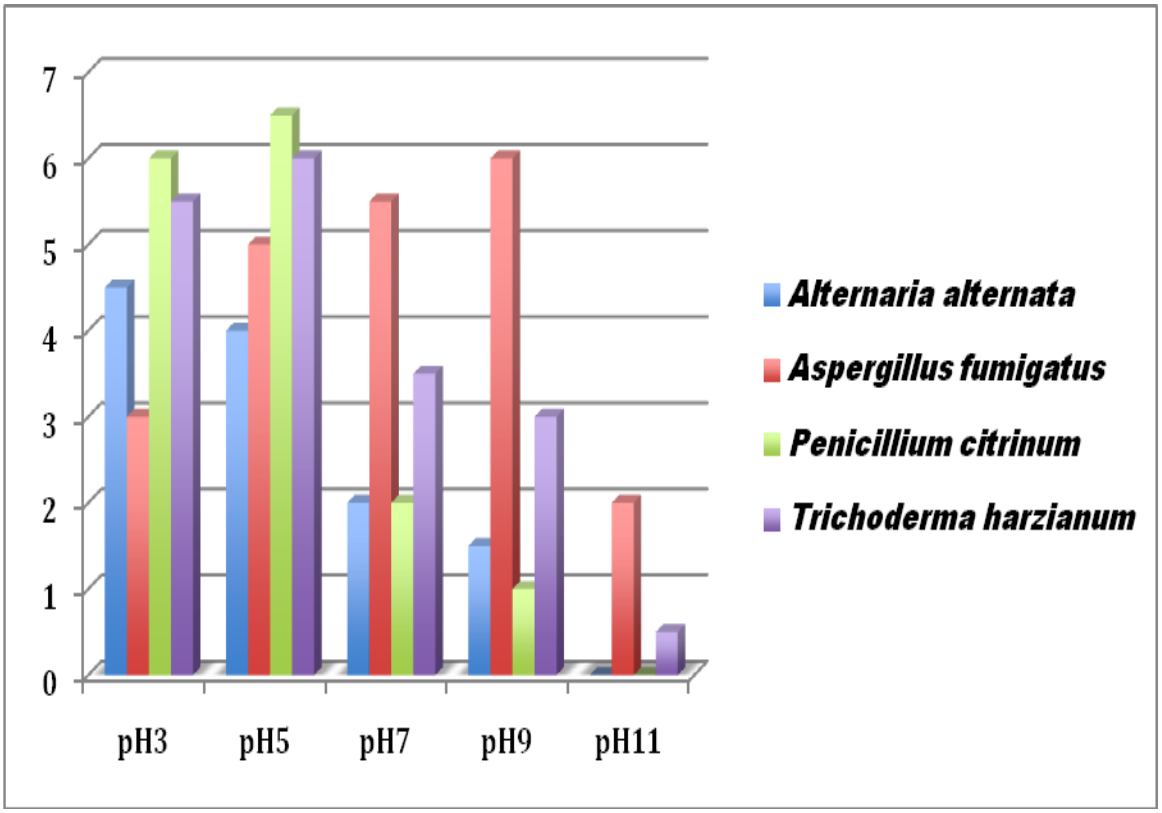


**Figure 1 (b):** Effect of pH on the radial fungal growth on PSA media

**Table 2:** Effect of temperature and pH on radial fungal growth on CDA media

| | | Radial fungal growth (cm) | | | |
|---|---|---|---|---|---|
| | | ***Alternaria alternate*** | ***Aspergillus fumigatus*** | ***Penicillium citrinum*** | ***Trichoderma harzianum*** |
| Temperature | **4°C** | **0** | **0** | **1.5** | **0** |
| | **15°C** | **5** | **2.7** | **2.7** | **9** |
| | **25°C** | **9** | **6.2** | **8** | **9** |
| | **37°C** | **0** | **4.3** | **1.4** | **5** |
| | **50°C** | **0** | **0** | **0** | **0** |
| pH | **3** | **5** | **3.5** | **6** | **7** |
| | **5** | **4.5** | **4** | **6.5** | **7.5** |
| | **7** | **3** | **6** | **4** | **5** |
| | **9** | **2** | **6.5** | **2** | **4** |
| | **11** | **0.5** | **3** | **0** | **1** |

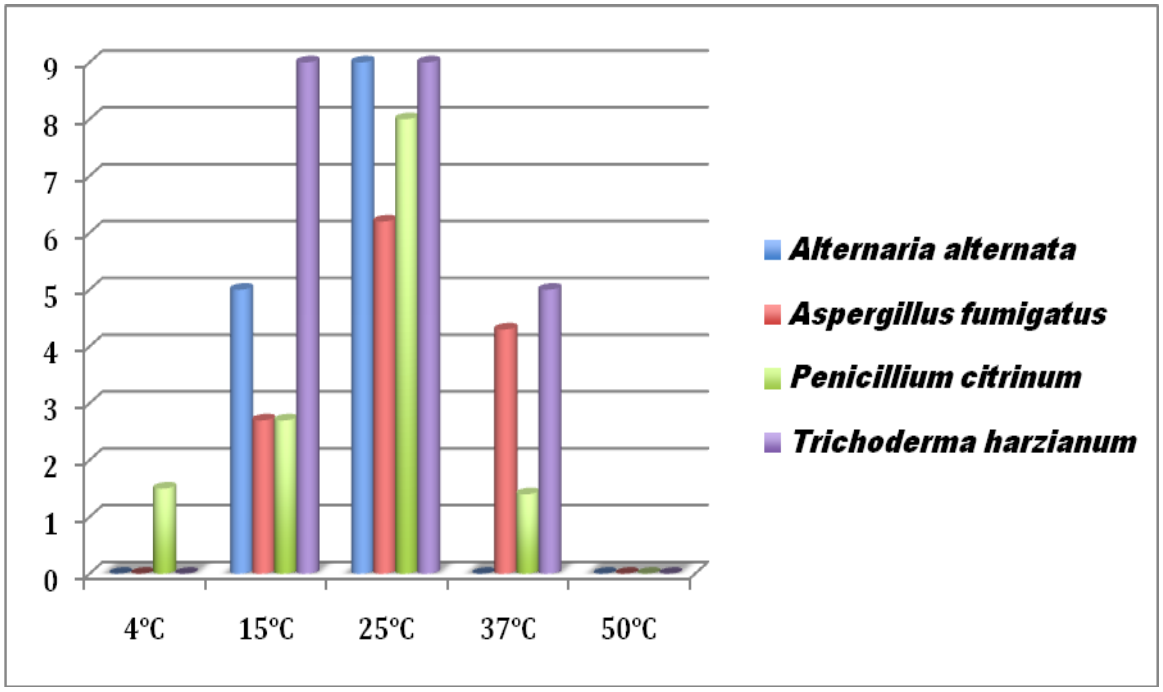


**Figure 2(a):** Effect of temperature on radial fungal growth on CDA media

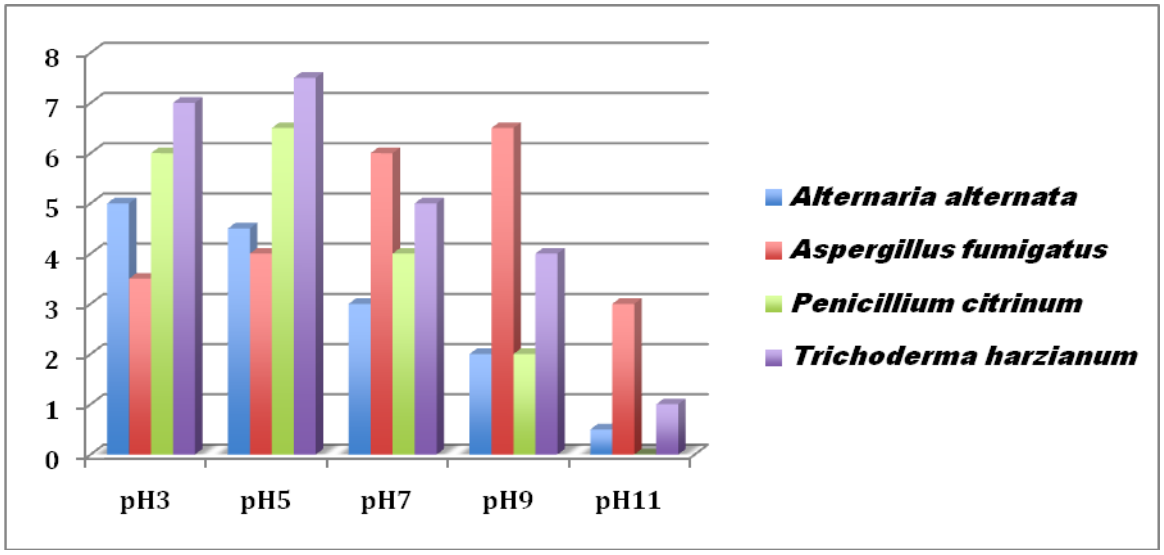


**Figure 2(b):** Effect of pH on radial fungal growth on CDA media

**Table 3:** The influence of temperatures and pH on radial fungal growth on WA media

| | Radial fungal growth (cm) | | | | |
|---|---|---|---|---|---|
| | **Fungi** | ***Alternaria alternate*** | ***Aspergillus fumigatus*** | ***Penicillium citrinum*** | ***Trichoderma harzianum*** |
| Temperatures | **4°C** | **0** | **0** | **1.5** | **0** |
| | **15°C** | **1.5** | **1.2** | **2** | **0** |
| | **25°C** | **2** | **1.5** | **2** | **0** |
| | **37°C** | **1.5** | **1.3** | **1.5** | **0** |
| | **50°C** | **0** | **1.4** | **0** | **0** |
| pH | **3** | **0.5** | **0** | **2** | **1.5** |
| | **5** | **1** | **1** | **1.5** | **1** |
| | **7** | **1.5** | **2** | **1** | **1** |
| | **9** | **1** | **2.5** | **0.5** | **0.5** |
| | **11** | **0** | **1** | **0** | **0** |

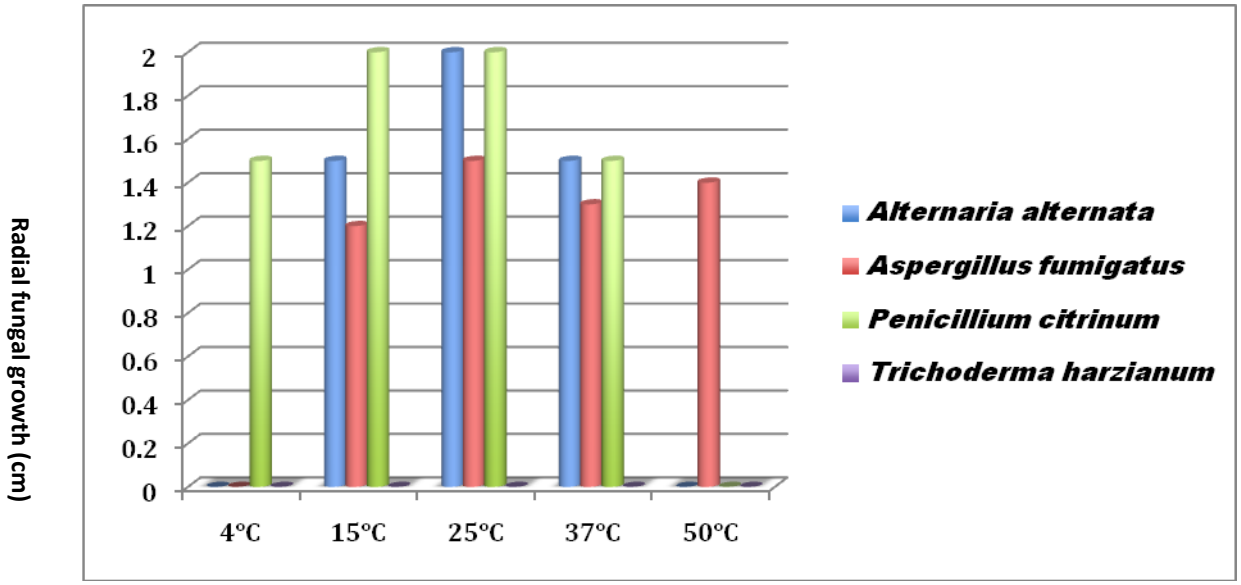


**Figure 3(a):** Effect of temperature on radial fungal growth on WA media

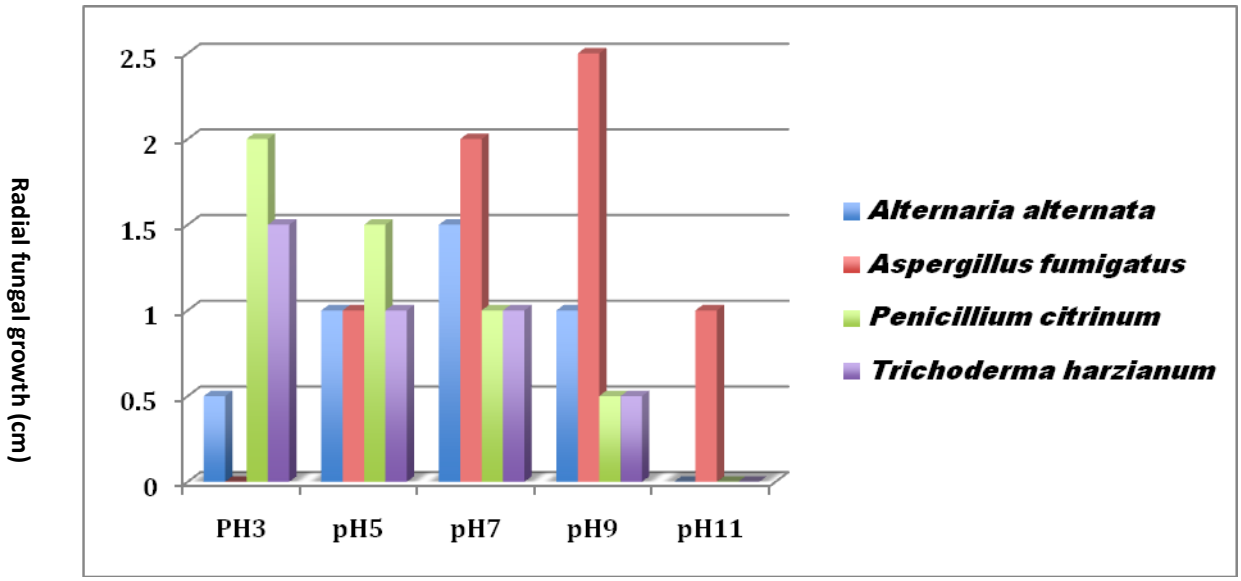


**Figure 3(b):** Effect of pH on radial fungal growth on WA media

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