In-vitro Effect of Different Physiological and Biochemical Parameters on Two Fungal Isolates Involved Biodeterioration of Egyptian Ancient Old Documents

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Physiological and biochemical characteristics of Fusarium oxysporum and Trichoderma viride isolated from old manuscripts of the General Book Organization of Egypt including effects of incubation periods, carbon sources, temperature, pH and relative humidity on linear growth (mm) and dry weight (mg) were established. Cellulolytic activity (Avicelase, CMCase, and Fpase) were measured by the size of the clear zone on specific media. The results show that the best incubation period for F. oxysporum was 9 day on PDa solid medium and 12 day for liquid medium, while for T. viride was 6 and 9 day for solid and liquid media respectively. Cellulase production increased gradually over an incubation period up to a maximum of 18 day for both F. oxysporum and T. viride beyond which a slight decline in the enzyme activity could be observed.

The two isolates grow and attain maximum linear and dry weight at temperatures degree between 25 and 30°C. Both organisms can grow on PDa solid medium along a pH ranging from pH 3.5-8.5, with maximum growth at pH 3.5-6.5. F. oxysporum and T. viride grow effectively on all tested carbon sources (sucrose, maltose, lactose, glucose, arabinose, starch, pectin and raffinose) reaching maximum growth after 7 days of incubation. Cotton pulp was considered the best carbon source for inducing the highest avicelase enzyme activity for both F. oxysporum and T. viride. Also, maximum growth was attained over a range of relative humidity, as the growth of F. oxysporum was increased by increasing the RH up to 92.3% and up to 100% for T. viride.

Keywords: Fusarium oxysporum, Trichoderma viride, Avicelase, CMCase, and Fpase, Environmental conditions.

Introduction

Biodeterioration of archival and library materials is a worldwide problem, which causes great damage to unique manuscripts and book (Zyska, 1993 and Shamsian et al., 2006). Paper is primarily composed of cellulose and other substances which are susceptible to degradation by cellulolytic fungi especially by the fungi Fusarium oxysporum and Trichoderma viride (Kowalik, 1980 and Smith, 1980). Physiochemical requirements of fungi are known to vary significantly not only within genera but also across strain of isolates.

Effect on fungal growth

Temperature

The optimum growth temperatures for the majority of fungi has been to fall between 25°C to 30°C. Above 40°C the growth is poor and in some cases mortality may occur (Sharma & Rajak, 2003). In earlier reports F. oxysporum was found to reach its maximum growth rate at 30°C (Farooq et al., 2005), 25°C (Somu & Thammaiah, 2015), between 25-30°C (Mogensen et al., 2009). Trichoderma viride has been reported to reach its maximum growth at 30°C (Ramanathan & Vinodhkumar, 2013) and at 22.5°C (Lieckfeldt et al., 1999).

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**pH**

Moulds differ in their pH requirements and most of them will grow well over a pH range from 3 to 7. The growth of *F. oxysporum* was found to reach its maximum growth at pH 5-6 as reported by Gupta et al. (2010) and Naik et al. (2010). In addition, Anita et al. (2013) stated that, the most favorable pH range of *T. viride* was between 6.5 to 7.5 while, Ramanathan & Vinodhkumar (2013) reported that *T. viride* grow well between pH’s 4 and 6.

**Relative humidity**

All moulds require moisture for growth but the humidity required varies widely. As reported by Benaouali et al. (2014) the optimum humidity for growth of *F. oxysporum* ranged from 74 to 80%, while in another report the minimum relative humidity for growth and spore germination of *F. oxysporum* was 89% and maximum growth was seen at 100 % RH (Yadav et al., 2014). On the other hand, the minimum relative humidity for growth and spore germination for *T. viride* was 94% and the maximum at 100 % RH (Bello et al., 2012).

**Carbon source**

The utilization of various carbon compounds may depend on either the ability of the fungus to utilize certain simpler forms or its power to convert the complex carbon compounds into simpler easily used forms, which may be easily utilized (Thammaiah et al., 2014). Several investigators found that, glucose was the best carbon source for growth of *F. oxysporum* (Irzykowska et al., 2012 and Benaouali et al., 2014), while, Naik et al. (2010) reported that growth of isolates was best on sucrose followed by fructose and maltose.

Concerning *T. viride* previous workers reported that, sucrose and starch recorded good growth (Arotupin & Ogunmola, 2012). Jitiendra et al. (2012) obtained the best growth on dextrose.

**Incubation period**

Duration of time required for growth and metabolite production by different fungi varies significantly. The best incubation period to attain maximum growth on solid medium for *F. oxysporum* was 7 days (Al-Mahareeq, 2005), or 8 days (Srivastava et al., 2011). While, the best incubation period for growth of *T. viride* was 5 days on solid medium to attain maximum growth as detected by Dwivedi & Agrahari (2014).

**Factors affecting cellulases activity**

Culture conditions including constituents of the medium, pH and temperature have a great influence on their cellulases production. Odeniyi et al. (2009) stated that, optimization of various environmental factors causes an increase of enzymatic activities.

**Incubation period**

The Incubation period for maximum cellulases activity differs from one organism to another, 6 days of incubation was most suitable for production of cellulosytic enzymes by *F. oxysporum* on different culture media (Mehta & Mehta, 1985). In addition, maximum production of CMCase and Fpase by *T. viride* was observed after a fermentation period of 72h (Malik et al., 2010), or after 2 days of fermentation for Fpase (Andrade et al., 2011).

**pH**

Several investigators reported that cellulases from fungi show the highest activity at acidic pH and are unstable at alkaline condition (Hanumasri & Sudhakar, 2011). The CMCase of *F. oxysporum* shows maximum activity between pH 5.5 and 6.0 as reported by Dar et al. (2013). The optimum conditions for the production of cellulases by *T. viride* were between pH 5.0 –7.0 (Kandari et al., 2013). While, CMCase, Avicelase and FPase of *T. viride* have been reported to exhibit optimum production on media set to approximately pH 4 (Lee et al., 2002). On the other hand, Iqbal et al. (2011) reported that, cellulase enzymes of *T. viride* were completely active between a pH range 5-8 with optimum activity at pH value of 8. This little variation in pH optima may be due to the genetic variability among different species.

**Temperature**

The CMCase of *F. oxysporum*, displayed significant activity within a temperature range of 25 - 37°C with maximum activity at 28°C as reported by Dar et al. (2013). The optimum production of cellulases by *T. viride* has been also reported at 35°C (Kandari et al., 2013), at 55°C (Iqbal et al., 2011), and at 40°C (Mandels & Reese, 1964).

**Substrate**

Dar et al. (2013) used different carbon sources to test their effect on cellulase production from *F. oxysporum* including wheat straw, wheat bran, crystalline cellulose and carboxy methyl cellulose (CMC). The best cellulase activity was found on CMC; but much reduction or no activity was detected if grown on Avicel and cellobiose. Akinyele et al. (2013) found that CMC was the
best carbon source, for the production of cellulases by *T. viride*.

The present study aims to evaluate the *in-vitro* effect of some environmental factors on growth and cellulolytic enzyme activity pattern of two deteriorated fungi isolated from old documents.

**Materials and Methods**

*Fungal culture*

Through studies on fungal isolation involved on biodeterioration of ancient manuscripts two isolates of fungi namely, *Fusarium oxysporum* and *Trichoderma viride* were isolated and identified (Sahab et al., 2014) in the laboratory and were further confirmed by the Department of Plant Pathology, NRC of Cairo. The two fungal isolates were maintained on Czapek’s agar medium supplemented with 5% Avicel.

*Growth on solid media*

Equal amounts (10ml) of tested media were poured in Petri-dishes. After solidification, a disc (5mm diam.) from the fungal culture was set in the middle of each plate and then incubated at 28±2°C. Two colony diameters of each plate were measured daily and the average was calculated for each treatments. A set of three replicates were used for each particular treatment.

*Amount of mycelial growth*

One hundred ml of liquid sterilized medium were placed in each 250ml conical flask. A set of three replicates were used for each treatment and flasks were inoculated with a spore suspension of 1 X 10⁵ cfu, and incubated at 28±2°C. Fungal mates were harvested on previously weighted filter paper, washed by D. water, dried at 70°C for 24h and weighed.

*Factors affecting fungal growth*

**Effect of different carbon sources**

Different carbon sources, (arabinose, glucose, maltose, pectin, raffinose, starch, and sucrose) were supplemented to Czapek’s agar medium separately according to their equivalent quantities of carbon as present in 3%, while Avicel and CMC were added at 1% (w.v). The linear growth and dry weights of the two tested isolates of *F. oxysporum* and *T. viride* were detected as mentioned before.

**Effect of incubation period**

Czapek’s liquid and solid media were used in this study. Petri-dishes containing Czapek’s agar medium and conical flasks (250ml) contained 100ml of Czapek’s liquid medium were inoculated with discs of 5mm diameter from the tested fungus as mentioned before. The inoculated dishes and flasks were incubated for different incubation periods, i.e., 3, 6, 9, 12, 15 and 18 days. The linear growth on solid medium and the amount of growth on liquid medium were recorded for three replicates were performed for each treatment.

**Effect of temperature**

Both solid and liquid Czapek’s medium were inoculated with the tested fungal isolates, then incubated at different temperatures (10-40°C at intervals of 5°C). The linear growth on solid medium and dry weight on liquid media were determined taking 3 replicates for each temperature condition.

**Effect of different pH values**

The pH of Czapek’s liquid and solid media used in this study was adjusted before sterilization by using 1N HCl or 1N NaOH to get different pH values, viz., 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5. Following inoculation with the tested fungus the plates and flasks were incubated at 28±2°C for 7 days on solid medium or 15 days for liquid medium. The rate of linear growth and mycelium dry weight were recorded taking 3 replicates for each treatment condition.

**Effect of Air relative humidity (RH)**

Czapek’s agar medium was adjusted to different RH values of 100, 92.3, 74.6, 49.0 and 27.0% by using different volumes of sulfuric acid and distilled water according to the method devised by (Stevens, 1916). Inoculated Petri-dishes with discs of 5mm diameter were turned upside down and 10ml of the prepared solution were poured in the lid of every dish and incubated at 28±2°C. The linear growth was measured until any plate was completely covered by fungal growth. Three replicates were performed for each particular treatment.

**Assay of cellulase (Cx) enzyme activity (Clear zone method)**

The previously selected fungal isolates were grown in 250ml Erlenmeyer flask containing 100ml of modified Czapek’s broth medium in which the carbon source was replaced by 1% paper pulp (Sidkey et al., 1997). The flasks were sterilized and inoculated with 1ml of the standard inoculums of fungal spores as mentioned before and then incubated at 28±2°C for 15 days. After the incubation period the contents of the flasks were centrifuged and the supernatant (cell free filtrate, CFF) was used as crude enzyme. Culture
filtrates from three flasks of each fungus were pooled together.

For determining the cellulolytic activity, Erlenmeyer flasks (250ml) each containing 100ml buffer (Citrate phosphate buffer pH5.5), plus 1% Avicel, CMC or filter paper, plus 1.5% agar were boiled until the ingredients were completely homogenized. An aliquot of 20ml of the above homogenized substrate were poured in each petri dish. After solidification of the plates make 2 holes were made on each side of the agar plate and 0.1ml (100μl) of the above cell free filtrate (CFF) were filled the well. After an incubation period of 20h at 28±2°C, the plates were flooded with iodine solution (1% iodine: 2% potassium iodide). The clear zone around the well was measured in mm, as the diameter of the zone increases as the cellulase activity increase.

**Results and Discussion**

Factors affecting fungal growth and cellulases activity: Effect of different factors, i. e., incubation period, carbon sources, temperature, pH and relative humidity on linear growth, dry weight and cellulolytic activity were studied.

**Influence of incubation period**

Results shown in Table 1 revealed that linear growth significantly increased as the incubation period increased to give maximum growth (90mm) after 9 days of incubation for Fusarium oxysporum and 6 days for Trichoderma viride. The dry weight also followed the same trend, as the maximum dry weight (mg) was obtained after 12 days (1025mg) for F. oxysporum and after 9 days (1013mg) for T. viride. On the other hand, lengthening the incubation period caused significant decrease in dry weight for both fungi. Similarly, Farooq et al. (2005) and Sharma & Pandey (2010) found the same trends. Moreover, Yadav et al. (2014) and Hossain et al. (2015) stated that, the widest colony diameter of F. oxysporum was attained after 7 days and after 8 days incubation for dry weight. T. viride exhibit it’s maximum growth diameter at 6 days of incubation for linear growth and for dry weight. These results also are in agreement with Arfarita et al. (2016) for linear growth and with Mishra & Khan (2015) for dry weight.

Regarding the effect of incubation period on cellulolytic activity results of the present study tabulated in Table 1 clearly showed that, the two fungal isolates produced extracellular enzymes (Avicelase, CMCase, and Filter paper ase) during different incubation periods and the level of cellulases production significantly increased with increasing the incubation period up to a maximum of 18 days beyond this maximum value a slight decline in the enzyme activity could be observed. Data also showed that, F. oxysporum and T. viride produced the highest extracellular Avicelase activity (19.70 and 19.40mm) respectively after 18 days of incubation. For CMCase the highest value of the culture filtrate was also attained after 18 days of incubation and the same trend was also observed for Fpase production.

The different behavior of the two fungal isolates during cellulase biosynthesis could be related to their biological potential or to depletion of the nutrients and production of certain toxic metabolites which inhibit the further microbial growth and physiology resulting in the inactivation of secretory machinery of the enzymes (Bhatti et al., 2007).

**Influence of carbon source**

Data present in Table 2 revealed that, the tested organisms were able to grow and utilize different carbon sources during 7 days of incubation. On the other hand, both organisms were unable to use Avicel and CMC after 7 days (0.0). This may mean that cellulosic substrates required more incubation time with the selected organism to be hydrolyzed, since linear growth of F. oxysporum significantly increased after 15 days reaching 71 and 85 on Avicel and CMC, respectively. Arabinose, glucose, starch, and sucrose were the best carbon sources for growth of F. oxysporum which reached maximum growth (90mm) after 7 days. These results can be discussed in light that these substrates are easily metabolized by most of fungal strains. Overall, glucose, maltose, pectin and starch were the best carbon sources for the growth of T. viride. This result agrees with Farooq et al. (2005), while in contrast Raval & Parmar (1973) stated that poor growth and sporulation of F. oxysporum was recorded on lactose. However, Rashid (2012) found that each of glucose, fructose and cellulose as carbon sources gave the optimum growth of F. oxysporum. These results also agree with those recorded by Aanuoluwa et al. (2015) who stated that sucrose, maltose, lactose, glucose and mannitol supported the growth of T. viride at all concentrations.

Concerning the cellulolytic activity, data provided in the same Table 3 exhibit that cotton pulp is the best carbon source of those tested for inducing the highest Avicelase activity as seen by clear zones of 22 and 17.5mm for F. oxysporum and T. viride, respectively.

### TABLE 1. Effect of different incubation periods on linear growth, dry weight and cellulolytic activity of *Fusarium oxysporum* and *Trichoderma viride* isolates.

<table>
<thead>
<tr>
<th>Days</th>
<th>Linear growth (mm)</th>
<th>Dry weight (mg/100ml)</th>
<th>Cellulolytic activity (clear zone method (mm))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F. oxysporum</em></td>
<td><em>T. viride</em></td>
<td><em>F. oxysporum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Avicelase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>38C</td>
<td>70B</td>
<td>965EF</td>
</tr>
<tr>
<td>6</td>
<td>65B</td>
<td>90A</td>
<td>980DEF</td>
</tr>
<tr>
<td>9</td>
<td>90A</td>
<td>90A</td>
<td>1000BC</td>
</tr>
<tr>
<td>12</td>
<td>90A</td>
<td>90A</td>
<td>1025A</td>
</tr>
<tr>
<td>15</td>
<td>90A</td>
<td>90A</td>
<td>982DE</td>
</tr>
<tr>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*In each column, values followed by the same letters do not differ significantly (P ≥ 0.05).*

### TABLE 2. Effect of different carbon sources on the linear growth (mm) of *Fusarium oxysporum* and *Trichoderma viride* isolates.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Linear growth (mm)</th>
<th>Dry weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td><em>Fusarium oxysporum</em></td>
<td><em>Trichoderma viride</em></td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Arabinose</td>
<td>90A</td>
<td>90A</td>
</tr>
<tr>
<td>Avicel</td>
<td>0.0F</td>
<td>71D</td>
</tr>
<tr>
<td>CMC</td>
<td>0.0F</td>
<td>85AB</td>
</tr>
<tr>
<td>Glucose</td>
<td>90A</td>
<td>90A</td>
</tr>
<tr>
<td>Maltose</td>
<td>70D</td>
<td>90A</td>
</tr>
<tr>
<td>Pectin</td>
<td>90A</td>
<td>90A</td>
</tr>
<tr>
<td>Raffinose</td>
<td>70D</td>
<td>90A</td>
</tr>
<tr>
<td>Starch</td>
<td>90A</td>
<td>90A</td>
</tr>
<tr>
<td>Sucrose</td>
<td>90A</td>
<td>90A</td>
</tr>
<tr>
<td>Saw dust</td>
<td>0.0F</td>
<td>90A</td>
</tr>
</tbody>
</table>

*In each column, values followed by the same letters do not differ significantly (P ≥ 0.05).*
Highest level of CMC\textsubscript{ase} activity was produced on journal paper by \textit{F. oxysporum} and \textit{T. viride} at 22.38 and 19.13 mm, respectively. Also, the best substrate for FP\textsubscript{ase} activity was mixed pulp recorded at 16 mm and 15.25 mm for \textit{F. oxysporum} and \textit{T. viride}, respectively. On the other hand, the lowest value of Avicelase (18.17 mm) and CMCase (17.50 mm) and FP\textsubscript{ase} came from \textit{F. oxysporum} on linen pulp, mixed pulp and cotton pulp, respectively. The lowest value of Avicelase (14 mm), CMCase (17 mm) and FP\textsubscript{ase} (13.50 mm) were measured for \textit{T. viride} on linen pulp, mixed pulp and Japanese paper, respectively.

The variation in cellulases activity by the two fungi may be due to the influence of carbon sources on the growth of cellulolytic organisms as reported by Mandels et al. (1974). Almost, similar results for the induction of cellulases enzyme on pure cellulosic substrate were reported by Krogh et al. (2010).

**Temperature**

The influence of temperature on fungal growth and cellulolytic activity were determined by using Czapek’s agar solid and liquid media at pH 6.5 for both isolates.

Results presented in Table 4 revealed that, the growth of \textit{F. oxysporum} increased as temperature increased to give maximum linear growth at 30°C, and for dry weight at 25°C. The growth of \textit{T. viride} was increased also as temperature increased to give maximum linear growth at 25°C, and for dry weight at 30°C.

Raising the temperature to 35°C caused a decrease in the linear growth and mycelium dry weight of both fungi. In addition to that, both isolates failed to grow on solid medium at 10, 15 and 40°C, in spite of their ability to grow on liquid medium with variant quantities.

These results show the \textit{T. viride} and \textit{F. oxysporum} isolates are mesophilic strains which prefer moderate temperature for their growth (20–30°C). Similar results for \textit{F. oxysporum} were also recorded by Farooq et al. (2005) and Stelica et al. (2015). Moreover, Ali & Vidhale (2013) reported that decrease or increase in incubation temperature is an essential factor because of its importance in microorganism growth, metabolite production and suppression of cell viability. Another possible reason could be due to the breaking down of enzyme at higher temperature.

### Table 3. Effect of different cellulosic substrates on dry weight and cellulolytic activity of \textit{Fusarium oxysporum} and \textit{Trichoderma viride} isolates.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Substrate</th>
<th>Dry weight (mg/100ml)</th>
<th>Cellulolytic activity, clear zone method (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Fusarium oxysporum}</td>
<td>Avicelase</td>
<td>22.0a</td>
<td>19.75BC</td>
</tr>
<tr>
<td></td>
<td>CMC\textsubscript{ase}</td>
<td>17.50BC</td>
<td>15.0D wym</td>
</tr>
<tr>
<td></td>
<td>Filter paper \textsubscript{ase}</td>
<td>19.75BC</td>
<td>15.0D wym</td>
</tr>
<tr>
<td>\textit{Trichoderma viride}</td>
<td>Avicelase</td>
<td>19.0BCD</td>
<td>17.50BD</td>
</tr>
<tr>
<td></td>
<td>CMC\textsubscript{ase}</td>
<td>15.0DDE</td>
<td>14.50BD</td>
</tr>
<tr>
<td></td>
<td>Filter paper \textsubscript{ase}</td>
<td>17.50BD</td>
<td>14.50BD</td>
</tr>
</tbody>
</table>

In each column, values followed by the same letters do not differ significantly (P >0.05).
Mishra & Khan (2015) emphasized that, the limit temperature for growth range of *Trichoderma viride* was between 10-35°C and the optimal fall between 20 and 30°C. Arfarita et al. (2016) reported that, the optimum temperature for growth of *T. viride* was found to be 25–27°C.

Concerning the effect of temperature on cellulolytic activity, data in Table 4 reveals that the two fungi were able to grow and produce cellulolytic enzymes at temperature of 40°C under this cultivation conditions. Data in the same table also showed that, both isolates were able to grow and produce cellulases at a wide range temperature of 10-40°C. High level of cellulase activity was detected when the incubation temperature adjusted at 35°C for both isolates below and above this temperature there is a decline in all cellulase activity. These results are in line the work of Gautam et al. (2010) who found that the growth of the fungus did not correlate with the production of the enzymes, as high temperature promotes the production of cellulase enzymes activity.

Effect of pH values

Buffered Czapek’s agar solid and liquid media were used in this experiment. It can be observed from Table 5 that, both *F. oxysporum* and *T. viride* isolates can grow on solid medium in a wide range of pH values from 3.5-8.5, with an optimal growth at pH 3.5-6.5, above which the linear growth decreased beyond this pH (towards alkalinity). On the other hand, on liquid medium the maximum dry weight was obtained at pH 6.5 for both isolates and decreased in more alkaline or more acidic media. Present data revealed that *F. oxysporum* and *T. viride* preferred acidic medium for optimal growth, although they could grow under natural or alkaline conditions. It seems that the pH of growth of *F. oxysporum* and *T. viride* was correlated with the pH of cellulases production, as it favored by acidic conditions.

Recently, many studies are in agreement with the present results (Rashid, 2012, Hossain et al., 2015 and Stelica et al., 2015). Maximum growth and sporulation of fungi at the optimum pH could be due to the permeability of the cell wall reaching its optimum allowing ready diffusion of nutrients needed for growth into the cell (Griffin, 1994).
The maximum avicelase, cMCase and Fpase activity of *F. oxysporum* was detected at pH 5.5, 3.5 and 4.5 detected 28.5, 22.0 and 19.5mm, respectively. Maximum Avicelase, CMCCase and Fpase activity of *T. viride* was detected at 6.5, 3.5 and 5.5, respectively. The effect of pH on the growth of *T. viride*, was reported by Mishra & Khan (2015) and Arfarita et al. (2016) who reported that *T. viride* was favored on slight acidic condition and best grown at pH 6.

Concerning the effect of pH values on cellulolytic activity of *F. oxysporum* and *T. viride*, data in Table 5 revealed that the activity of all cellulases for both fungi have a wide range of pH, ranged between pH 3.5-8.5, with maximum activity ranging between pH 3.5-5.5. Moreover, any increase in the pH led to decrease in enzyme activity.

The increase in the pH led to decrease in all cellulases activity for both fungi, as pH of 8.5 led to decreasing the activity of Avicelase, of *F. oxysporum* and *T. viride* by 45.61 and 26.83%, respectively compared with pH 5.5. These results are in harmony with the finding of Haltrich et al. (1996) and Dar et al. (2013), who reported that high acidic and high alkalinity shows negative effects on enzyme activity, but a medium with low acidic value of 5.5 was ideal for enzyme production. This might be due to the fact that fungal cultures require slightly acidic for their growth and enzyme biosynthesis. It is well known that the pH of the surrounding medium affects the availability of certain metallic ions, permeability of membranes, internal pH of the mycelia and enzymatic activities (Sharaf et al., 2012).

**Influence of relative humidity on linear growth**

The influence of relative humidity on linear growth of *F. oxysporum* and *T. viride* was estimated on Czapek’s agar medium. It is clear from Table 6 that, *F. oxysporum* and *T. viride* were able to grow at a wide range of relative humidity. As, the linear growth of *F. oxysporum* was significantly increased to 24.6mm at 27% RH and reached to 44mm at 92.3% RH (78.86% increase). On the other hand, the maximum linear growth for *T. viride* was obtained at 100% RH, as the linear growth was significantly increased from 57mm to 90mm at 27% to 100%, respectively (57.89% increase).

These results are in good agreement with data reported by many authors (Al-Garni et al., 2007 and Bello et al., 2012). In addition, high relative humidity is widely recognized as a critical factor...
for fungi reproduction as reported with Stelica et al. (2015) who stated that, *F. oxysporum*, *T. viride* and *S. brevicaulus* grew optimally at very high RH (between 85 and 100%) with dense vegetative mass and abundant sporulation. However, Mishra & Khan (2015) stated that, the effect of different levels of relative humidity undoubtedly plays a very important role in sporulation on growth of *T. viride*. The present study suggested that *T. viride* was able to grow and sporulate at different levels of relative humidity, where it attained its best growth and sporulation at 80 and 90% RH.

**TABLE 6. Effect of relative humidity on the linear growth (mm) of *Fusarium oxysporum* & *Trichoderma viride* isolates.**

<table>
<thead>
<tr>
<th>RH (%)</th>
<th>Fungi</th>
<th>Fusarium oxysporum</th>
<th>Trichoderma viride</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>35F</td>
<td>90A</td>
<td></td>
</tr>
<tr>
<td>92.3</td>
<td>44E</td>
<td>85.8A</td>
<td></td>
</tr>
<tr>
<td>74.6</td>
<td>41E</td>
<td>76B</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>26.3G</td>
<td>62.5C</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>24.6G</td>
<td>57D</td>
<td></td>
</tr>
</tbody>
</table>

-In each column, values followed by the same letters do not differ significantly (P ≥ 0.05).

**Conclusion**

In conclusion, many the associated fungi had the enzymatic activity which may damage library materials during storage and also might pose health threats.
- Storage temperature must be between 18-22°C.
- Relative humidity of storage must be reduced to 50-55% RH.
- Avoid increasing the acidity of the paper of the manuscripts to be less than seven to stop the fungus activity during storage.
- Using CMC polymer in restoration processes of the paper for preservation of the old manuscripts instead of starch.

**References**


تأثير بعض الصفات الفسيولوجية والحيوية فى المعمل على عزلتين من الفطريات المسببة لتدهور المخطوطات المصرية القديمة

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الخصائص الفسيولوجية والكيميائية الحيوية لفطر المعزولة من المخطوطات القديمة المتهالك. وشملت الدراسة تأثير مصادر الكربون المختلفة وفترات التحضين ودرجات الحرارة ودرجة الرطوبة النسبية على النمو الفطرى على البيئة الصلبة وعلى النمو الجاف على البيئة السائلة وكذلك قياس النشاط الأنزيمي لانزيمات السليولوز. وقد تبين من النتائج أن:

1- وجد أن أفضل فترة لتحضين الفطر Fusarium oxysporum كانت عند 9 أيام من التحضين على بيئة البطاطس الدكسترودون الصلبة و 12 يوم على البيئة السائلة. كما زادت أنزيمات السليولوز ووصلت أقصاها بعد 18 يوم تحضين.

2- تبين زيادة نمو الفطرين على البيئة الصلبة والسائلة بزيادة درجة الحرارة وبلغت أقصاها بين درجة حرارة 23-30 م.

3- تبين أن الفطرين أمكنهم النمو على مدى واسع من الحموضة (3.5-8.5).

4- أمكن للفطرين النمو على جميع مصادر الكربون المستخدمة وكان أفضل نمو وانتاج أنزيمات السليولوز بعد 7 أيام تحضين مع استخدام نبات القطن كمصدر للكربون.

5- كما أدت زيادة الرطوبة النسبية إلى زيادة في نمو الفطرين على البيئة الصلبة حيث بلغ أقصى نمو عند 92.3% رطوبة نسبة للفطر Trichoderma ووصلت إلى 100% رطوبة نسبة للفطر Fusarium.

Trichoderma viride و Fusarium oxysporum