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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 to7. 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). Jitendra 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 cellulolytic 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±2ºC. 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 10^5cfu, and incubated at 28±2ºC. Fungal mates were harvested on previously weighted filter paper, washed by D. water, dried at 70ºC 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±2ºC 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 Fp$_{ase}$ 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 secretary 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 71and 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 7days. 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.

Fungi / Days	Linear growth (mm)		Dry weight (mg/100ml)		Cellulolytic activity (clear zone method (mm))					
					Avicelase		CMCase		Fpase	
	F. oxysporum	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*
3	38C	70B	965EF	980DEF	14.40DE	13.50E	13.00GH	12.50H	12.50E	12.50E
6	65B	90A	980DEF	995CD	15.50CD	15.50CD	14.80FG	14.10G	13.80D	13.60DE
9	90A	90A	1000BC	1013AB	17.60AB	16.70BC	17.30CD	15.50EF	14.50CD	14.00CD
12	90A	90A	1025A	963F	18.00AB	17.50AB	17.50CD	15.60EF	14.60CD	14.50CD
15	90A	90A	982DE	-	18.50A	18.00AB	18.30BC	16.10DE	15.00C	15.00C
18	-	-	-	-	19.70A	19.40A	21.00A	19.80AB	19.50A	18.50AB
21	-	-	-	-	19.20A	19.00A	19.75AB	19.65AC	19.40AB	18.23B

-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.

Fungi / Carbon source	Linear growth (mm)				Dry weight (mg)	
	Fusarium oxysporum		*Trichoderma viride*		*Fusarium oxysporum*	*Trichoderma viride*
	Incubation period (days)					
	7	15	7	15	15	15
Arabinose	90A	90A	80BC	90A	694E	414J
Avicel	0.0F	71D	0.0F	80BC	890C	508HI
CMC	0.0F	85AB	0.0F	82B	N.D	N.D
Glucose	90A	90A	90A	90A	498I	600G
Maltose	70D	90A	90A	90A	504I	707E
Pectin	90A	90A	90A	90A	635FG	723DE
Raffinose	70D	90A	50E	90A	473I	542H
Starch	90A	90A	90A	90A	1426A	1308B
Sucrose	90A	90A	90A	90A	653F	746D
Saw dust	0.0F	90A	0.0F	75CD	N.D	N.D

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

TABLE 3. Effect of different cellulosic substrates on dry weight and cellulolytic activity of *Fusarium oxysporum* and *Trichoderma viride* isolates.

Substrate \ Fungi	Dry weight (mg/100ml)		Cellulolytic activity, clear zone method (mm)					
			Avicelase		CMC_{ase}		Filter $paper_{ase}$	
	Fusarium oxysporum	*Trichoderma viride*	*Fusarium oxysporum*	*Trichoderma viride*	*Fusarium oxysporum*	*Trichoderma viride*	*Fusarium oxysporum*	*Trichoderma viride*
Cotton pulp	1011BC	994BC	22.0A	17.50BCD	19.75BC	19.0BCD	13.33D	13.92CD
Mixed pulp	998BC	1000BC	19.13AB	15.0DE	17.50D	17.0D	16A	15.25AB
Japanese paper	833EF	945CD	19.25AB	15.50CDE	20.50AB	18.66BCD	13.75D	13.50D
Linen pulp	810EF	767F	18.17BC	14.0E	18.88BCD	19.13BCD	14.50BCD	13.75D
Journal paper	1089A	1060AB	19.38AB	17.0BCDE	22.38A	19.13BCD	15.63AB	15.88AB
Wood pulp	881DE	1002BC	18.83AC	16.0BCE	20.25AB	18.0CD	13.75D	14.0CD

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

Highest level of CMC_{ase} activity was produced on journal paper by *F. oxysporum* and *T. viride* at 22.38 and 19.13mm, respectively. Also, the best substrate for Fp_{ase} activity was mixed pulp recorded at 16mm and 15.25mm for *F. oxysporum* and *T. viride,* respectively. On the other hand, the lowest value of Avicelase (18.17mm) and CMCase (17.50mm) and FP_{ase} came from *F. oxysporum* on linen pulp, mixed pulp and cotton pulp, respectively. The lowest value of Avicelase (14mm), CMC_{ase} (17mm) and Fp_{ase} (13.50mm) were measured for *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 *F. oxysporum* increased as temperature increased to give maximum linear growth at 30°C, and for dry weight at 25°C. The growth of *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 *T. viride* and *F. oxysporum* isolates are mesophilic strains which prefer moderate temperature for their growth (20-30°C). Similar results for *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 4. Effect of different temperatures on linear growth, dry weight and cellulolytic activity of *Fusarium oxysporum* and *Trichoderma viride* isolates.

Temperature °C \ Fungi	Linear growth (mm)		Dry weight (mg/100ml)		Cellulolytic activity {clear zone method (mm)}					
					Avicelase		CMCase		Filter paperase	
	F. oxysporum	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*
10	0D	0D	940DE	98BCD	15.CE	15DE	13.E	14E	0F	0F
15	0D	0D	970CD	990ABC	16CDE	17.5C	16CD	15.20DE	10.1EF	1F
25	74B	90A	1014AB	1002AB	16CDE	14.30E	15.DE	17.13BC	13DE	12.DE
30	90A	9A	1006ABC	1025A	17.5C	17.70BC	20.50A	19.16AB	15.5AB	14BC
35	12D	19C	980BCD	1008ABC	19.8AB	21A	21A	19.75A	16A	14.8ABC
40	0D	0D	910E	1001ABC	10.95F	17CD	16.6CD	15.60CDE	12DE	13CD

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

Mishra & Khan (2015) emphasized that, the limit temperature for growth range of *Trichoderma viride* was between 10-35ºC and the optimal fall between 20 and 30ºC. 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 40ºC 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 (Grffin, 1994).

TABLE 5. Effect of different pH's on linear growth, dry weight and cellulolytic activity of *Fusarium oxysporum* and *Trichoderma viride* isolates.

pH's \ Fungi	Linear growth (Ømm)		Dry weight (mg/100ml)		Cellulolytic activity {clear zone method (Ømm)}					
					Avicelase		CMCase		Filter paperase	
	F. oxysporum	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*	*F. oxysporum*	*T. viride*
3.5	90A	90A	600BC	570C	18.5BC	15.75DE	22A	19.5AB	19ABC	13D
4.5	90A	90A	570C	560C	19.25B	18BCD	18BC	18BC	19.5AB	16.5C
5.5	90A	90A	670AB	640C	28.5A	18.25BCD	18BC	17.25BC	19ABC	20.5A
6.5	78B	85A	690A	720A	16.25CDE	18.25BCD	16.5C	17BC	17BC	17BC
7.5	55D	70C	585BC	665AB	16CDE	15E	17.5BC	17BC	17BC	16.5C
8.5	30F	45E	510C	560C	15E	14.5E	16.5C	16C	17BC	13.6D

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

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, CMCase 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.

Fungi / RH (%)	*Fusarium oxysporum*	*Trichoderma viride*
100	35F	90A
92.3	44E	85.8A
74.6	41E	76B
49	26.3G	62.5C
27	24.6G	57D

-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 18-22ºC.
- 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.

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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 *Fusarium oxysporum* و *Trichoderma viride* المعزولة من المخطوطات القديمة المتهالكة. وشملت الدراسة تأثير مصادر الكربون المختلفة وفترات التحضين ودرجات الحرارة ودرجة الحموضة والرطوبة النسبية على النمو الفطرى على البيئة الصلبة وعلى النمو الجاف على البيئة السائلة وكذلك قياس النشاط الأنزيمى لانزيمات السليوليز وقد تبين من النتائج أن :-

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

2- تبين زيادة نمو الفطرين على البيئة الصلبة والسائلة بزيادة درجة الحرارة وبلغت اقصاها بين درجة حرارة 25، 30م°.

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

4- امكن للفطرين النمو على جميع مصادر الكربون المستخدمة وكان أفضل نمو وانتاج انزيمات السليوليز بعد 7 أيام تحضين مع استخدام لب القطن كمصدر للكربون.

5- كما أدت زيادة الرطوبة النسبية إلى زيادة فى نمو الفطرين على البيئه الصلبة حيث بلغ اقصى نمو عند 92.3% رطوبة نسبية للفطر *Fusarium* ووصلت إلى 100% رطوبة نسبية للفطر *Trichoderma*.