

## Physiological Studies on Ten Thermophilic and Thermotolerant Fungi Isolated From Different Locations in the Western Region of Saudi Arabia

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**ABSTRACT.** Physiological studies were carried out on ten thermophilic and thermotolerant fungi isolated from the soil of different locations in Saudi Arabia. There were strong relationship between soil temperature, pH and salinity and the growth of the examined fungi. In order to obtain more information about the possible role played by these fungi in the soil fertility, their ability to produce amylase, casease and cellulase ( $C_x$ ), *in vitro* were studied. All the fungi except *Thermomyces lanuginosus*, were able to produce enzymes. *Thermomyces lanuginosus* was unable to produce cellulase enzyme.

Species of fungi capable of growing at temperatures above 45°C are few and can be divided into two categories: thermophilic and thermotolerant (Cooney and Emerson 1964) Thermophilic fungi are those having a minimum growth temperature above 25°C (Graveri *et al.* 1964) Evans (1971) studied the critical temperature for the growth of 35 fungal species and divided them into (1) strong thermophiles, with a minimum growth temperature of 25°C or above, (2) weak thermophiles, with a minimum growth temperatures of 20°C or just above, and a maximum of 50°C or above, (3) strong thermotolerants, which grow sparsely below 20°C and well at or above 50°C, and (4) thermotolerants in which growth can take place below 20°C and becomes very slow at or above 50°C.

Very few reports are recorded about the effect of both pH and salinity on the growth of thermophilic and thermotolerant fungi. Bokhary *et al.* (1984) indicated that a pH value between 7.5 and 8.4, which is a characteristic of the soils in Saudi Arabia, was particularly favourable for growth both thermophilic and thermotolerant fungi. They also added that the relative range of pH seems to have little or no effect on the growth rate of these fungi. Khodair *et al.* (1991) found that the density of alkalophilic

fungi was significantly lower than alkalophilic bacteria in the saline soil of Makkah district, Saudi Arabia. The same authors also indicated that the population of alkalophilic fungi in the studied soils was not affected so much by the salinity of these soils in the presence of low organic matter.

The enzymatic activity of both thermophilic and thermotolerant fungi was reported by several investigators (Garrett 1963, Chang 1967, Chang and Hudson 1967, Hudson 1968, Webster 1970, Hedger and Hudson 1974, Deacon 1979, Mishera *et al.* 1981 and Deacon 1985).

The aim of the present investigation was to obtain more information about the effect of temperature, pH and salinity on the growth of both thermophilic and thermotolerant fungi in the western region of Saudi Arabia, besides the activity of these fungi, to produce certain enzymes as a step towards the possible role played by these fungi in soil fertility.

### Materials and Methods

Ten fungal isolates from soil samples were isolated from 18 locations in western Saudi Arabia. The fungi used were identified according to the proved keys given by Thom and Raper (1945), Gilmann (1956), Brown and Smith (1957), Cooney and Emerson (1964), Ames (1969) and Booth (1971). These fungi were *Paecilomyces variotii* Bainier, *Thermomyces lanuginosus* Tsiklinsky, *Acremonium alabamense* Morgan-Jones, *Chaetomium olivaceum* Ames, *Mucor pusillus* Lindt, *Aspergillus flavus* Link, *A. niger* Van Tieghem, *A. ochraceus* Wilhelm, *A. fumigatus* Fresenius and *Penicillium dupontii* Tansey.

### Physiological Studies

#### 1. Temperature Relationship

The effect of different incubation temperatures ranging from 10 to 60°C, with 5 increment, was tested against the fungal growth represented as mycelial dry weight, for all ten fungi in liquid glucose Czapek's medium (Crisan 1962). Portions of 50 cm<sup>3</sup> of the medium were distributed into 100 ml conical flasks. One disc (0.5 cm in diameter) from 4 days old culture of each fungus was used as inocula. All the fungi were allowed to grow under static conditions in a water jacketed incubator (Wali *et al.* 1979). The growth in mycelia were harvested by filtration after 3, 6, 9, 12 and 15 days of incubation, then washed thoroughly with distilled water, dried in an oven at 105°C for 48 hours till constant weight and finally weighed. There were 3 replicates for each combination of fungus and treatment.

## 2. Effect of pH

The fungal growth for the forementioned fungi on different pH levels *i.e.*, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0, was determined in liquid glucose Czapek's medium inoculated and incubated by the same technique mentioned before. Incubation period in this experiment was 12 days and temperature used was the optimum for each fungus, as it was 35°C for *Chaetomium olivaceum*, *Aspergillus niger* and *A. fumigatus*, 40°C for *Paecilomyces variotii*, *Thermomyces lanuginosus*, *Acremonium alabamense*, *Aspergillus flavus*, *A. ochraceus* and *Penicillium dupontii*, and 45°C for *Mucor pusillus*. Mycelial growth for the fungi at the end of the incubation period was collected and weighed by the same method mentioned in temperature relationship. Number of replicates was 3 for each fungus at any pH level.

## 3. Effect of Salinity

To study the effect of salinity on the fungal growth for the previously mentioned ten fungi, sodium chloride (NaCl) was added to liquid glucose Czapek's medium to get the following concentrations: 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0 and 16.0%, respectively (Zidan and Abdel-Mallek 1987). The tested fungi were inoculated in conical flasks (100 ml) containing the prepared liquid medium then, flasks were incubated in a water jacketed incubator under static conditions for 12 days at the optimum temperature for each fungus. Mycelial growth for the fungi at the end of the experiment, were collected and weighed by the same method mentioned before. Control of this experiment was liquid glucose Czapek's medium without sodium chloride. Number of replicates was 3 for each fungus at any level of sodium chloride concentration.

## Enzymatic Activity

During the course of the present investigation, enzymatic activities for *Paecilomyces variotii*, *Thermomyces lanuginosus*, *Acremonium alabamense*, *Chaetomium olivaceum*, *Mucor pusillus*, *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. fumigatus* and *Penicillium dupontii* were tested and determined *in vitro*.

## Starch-hydrolyzing Activity

Starch hydrolyzed enzyme activity for all the tested fungi was estimated using Czapek's agar medium containing 0.5% soluble starch as an only carbon source in medium (Collins 1969).

## Proteolytic Activity

The ability of fungi to produce casease capable to decompose protein in Czapek's agar medium containing Casein (0.2%) as a sole source of nitrogen in medium was determined according to the method described by Allen (1960).

### *Cellulase Enzyme Activity*

Fungi were allowed to grow for 14 days at the optimal temperature for each on a liquid Czapek's medium in which glucose was replaced by carboxymethyl-cellulose (Talboys and Busch 1970). Cellulase ( $C_x$ ) enzyme activity was assayed by the method described by the same authors (1970).

### *Statistical Analysis*

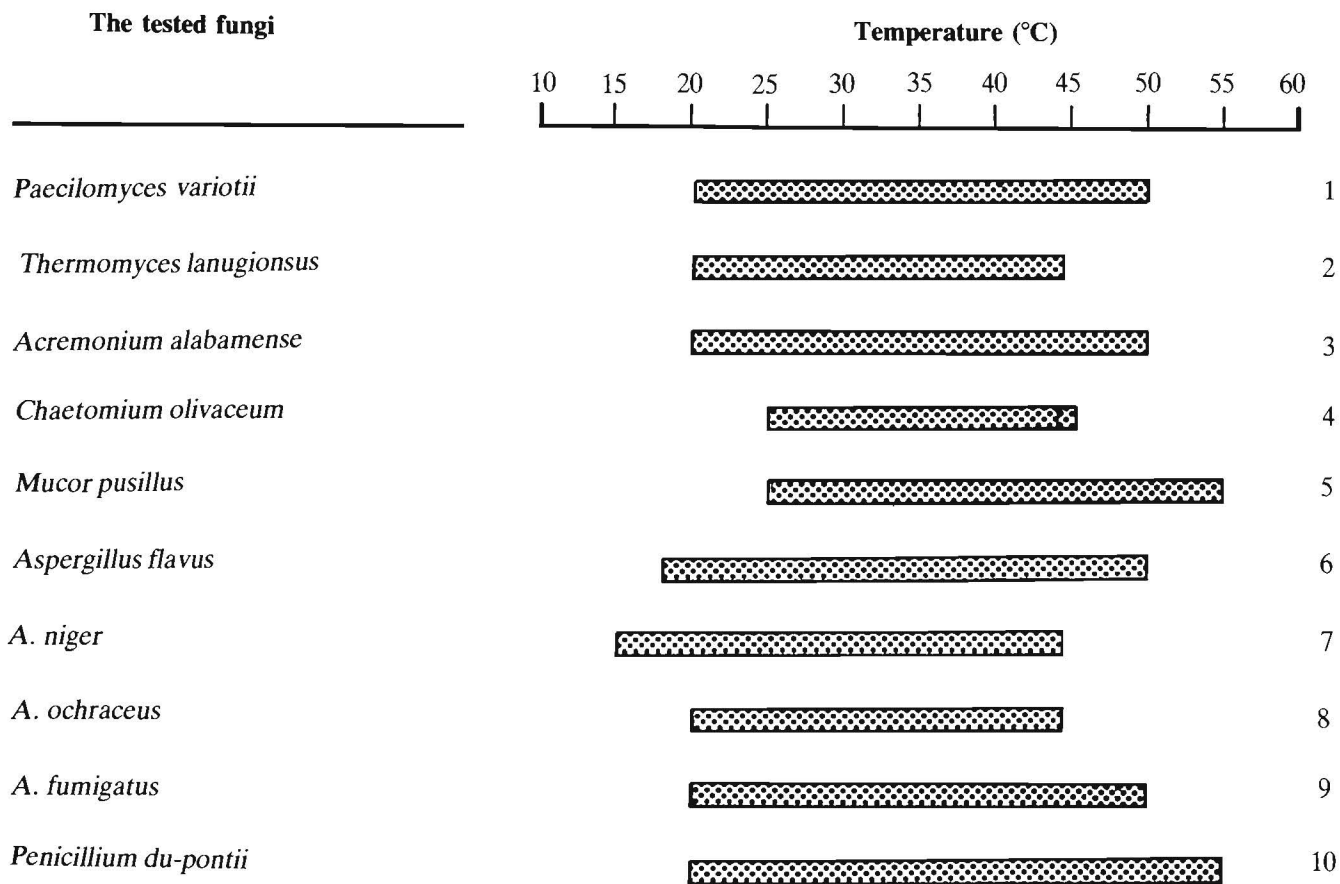
Data presented in this investigation were statistically analysed to calculate the least significant differences using the method suggested by Fisher (1948).

## **Results and Discussion**

### *1. Temperature Relationship*

Data illustrated in Fig. (1) and Table (1) clearly show that the critical temperatures for the growth of the tested fungi were ranged from 45 to 55°C. In all cases, mycelial dry weights increased with the increase of incubation periods till 12 days of incubation, then decreased with the increase of the incubation period (15 days). Maximum temperatures recorded for the fungal growth were 45°C, for *Thermomyces lanuginosus*, *Chaetomium olivaceum*, *Aspergillus ochraceus* and *A. niger*, 50°C for *Paecilomyces variotii*, *Acremonium alabamense*, *Aspergillus flavus* and *A. fumigatus*, and 55°C for *Mucor pusillus* and *Penicillium dupontii*. On the other hand, minimum temperatures recorded for fungal growth were 15°C for *Aspergillus niger*, 20°C for *Paecilomyces variotii*, *Thermomyces lanuginosus*, *Acremonium alabamense*, *Aspergillus flavus*, *A. ochraceus*, *A. niger*, *A. fumigatus* and *Penicillium dupontii*, and 25°C for *Chaetomium olivaceum* and *Mucor pusillus*. Data also indicate that the optimum temperatures for the growth of fungi were 35°C for *Chaetomium olivaceum*, *Aspergillus niger* and *A. fumigatus* and 40°C for *Paecilomyces variotii*, *Thermomyces lanuginosus*, *Acremonium alabamense*, *Aspergillus flavus*, *A. ochraceus*, *A. fumigatus*, *Mucor pusillus* and *Penicillium dupontii*.

Cooney and Emerson (1964) defined a thermophilic fungus as one in which growth can take place at a maximum temperature at or above 50°C and a minimum at or above 20°C while a thermotolerant fungus is one that has a thermal maximum near 50°C and a minimum below 20°C. On the other hand, Evans (1971) stated that fungi which can be grown near or above 50°C, could be divided into four groups. These groups were (1) strong thermophiles (with minimum growth temperature 25°C or above, and high maximum temperatures), (2) weak thermophiles (minimum growth temperature at 20°C or just below 20°C and a maximum at 50°C or above), (3) strong thermotolerant (which grow sparingly below 20°C and grow well at or above (50°C), and (4) thermotolerant in general (grow well below 20°C and very slowly near 50°C).



**Fig. 1.** Minimum and maximum temperature for the growth of ten thermophilic and thermotolerant fungi.

In the light of our finding, we can divide the tested fungi into the following categories, (1) strong thermophilic fungus: *M. pusillus*, (2) weak thermophilic fungi: *P. dupontii*, *Paecilomyces variotii*, *Acremonium alabamense*, *Aspergillus flavus* and *A. fumigatus*, (3) thermotolerant in general: *Thermomyces lanuginosus*, *Aspergillus ochraceus*, *A. niger* and *Chaetomium olivaceum*. Bokhary *et al.* 1984 studied the thermophilic and thermotolerant fungi in the soil of the arid region of Saudi Arabia. The authors pointed out that *Acremonium alabanense* and *Thermomyces lanuginosus* were strong thermophilic fungi capable to grow between 30 and 55°C. On the other hand, they also indicated that *Mucor pusillus* was classified as a weak thermophilic fungus because growth can take place at a maximum temperature at 55°C and a minimum at 20°C These reflexible data could be explained by these fungi have different strains differentiated from each other in their own cardinal temperatures.

### 2. Effect of pH

Regarding the effect of medium pH on the growth of the tested soil fungi (Table 2), the results clearly indicate that all the fungi were able to grow at all pH values used. It was also obvious that a sharp decrease on mycelial dry weight for all the tested fungi occurred at a range of 9-10 pH. Optimum pH value of the culturing medium giving maximum mycelium dry weight for all the tested fungi was 8.5. Incubation of the tested fungi for 12 days at the optimum temperature of each on medium adjusted at pH 8.5 revealed that, *Acremonium alabamense* occupied the first position in the growth density whereas, the growth of *Aspergillus ochraceus*, *Penicillium dupontii* and *Mucor pusillus* was highly delayed.

These results could be explained by that these fungi were isolated from different locations of the western region of Saudi Arabia, known as alkaline soils (Anon 1985), so these fungi were adapted to grow at high pH values. Khodair *et al.* (1991) confirmed our results as they proved that the saline soils in Makkah district characterized by its alkalinity, contained high population of fungi and bacteria. The authors also identified the fungi and bacteria which they isolated from these locations as alkalophilic microorganisms.

### 3. Effect of Salinity

Data presented in Table (3) show that the mycelial dry weights determined for the tested fungi were greatly decreased with the increase of NaCl concentration in medium except the fungi namely *Aspergillus niger*, *A. fumigatus*, *Mucor pusillus* and *Penicillium dupontii*, whose growth increased with the increase of NaCl concentration in medium from 0.0 to 2.0%. Data also indicate that the last mentioned fungi were able to grow under high concentration of NaCl used (16%), indicating that these fungi are halotolerant, where no other tested fungi were able to grow under this concentration of NaCl. As to 14% concentration of NaCl in medium, it also appeared that the growth of fungi namely *Chaetomium olivaceum* and *Aspergillus ochraceus* was obviously less than that recorded for the other ones.

**Table 1.** Effect of different incubation temperatures on the growth of the tested fungi (mg mycelium dry weight) incubated for 3, 6, 9, 12 and 15 days

Fungi	Incubation period (days)	Mycelial dry weight (mg) for the fungi at temperature (°C)								
		15°C	20°C	25°C	30°C	35°C	40°C	45°C	50°C	55°C
<i>Paecilomyces variotii</i>	3	0.0	5.0	7.9	32.1	52.4	80.2	88.1	40.2	0.0
	6	0.0	7.4	14.6	41.4	88.6	118.4	120.6	51.6	0.0
	9	0.0	15.6	24.5	100.0	162.8	196.4	136.4	87.3	0.0
	12	0.0	17.9	43.8	186.2	265.1	282.4	143.4	72.2	0.0
	15	0.0	16.4	30.2	156.2	180.2	190.6	125.2	60.8	0.0
<i>Thermomyces lanuginosus</i>	3	0.0	20.8	47.6	65.6	100.2	119.2	29.4	0.0	0.0
	6	0.0	50.0	89.4	93.4	160.9	152.4	39.2	0.0	0.0
	9	0.0	84.6	155.1	156.7	200.1	180.2	39.4	0.0	0.0
	12	0.0	100.2	171.3	176.4	226.2	250.1	42.6	0.0	0.0
	15	0.0	80.0	142.0	140.0	198.0	180.0	34.2	0.0	0.0
<i>Acremonium alabamense</i>	3	0.0	20.2	56.2	60.2	80.2	80.1	75.6	35.2	0.0
	6	0.0	41.6	62.1	72.8	129.3	100.4	100.1	40.4	0.0
	9	0.0	75.1	100.3	120.5	180.4	190.6	162.4	41.2	0.0
	12	0.0	80.0	123.8	179.4	224.7	252.2	222.1	38.2	0.0
	15	0.0	70.2	90.4	150.2	180.6	200.0	180.2	32.4	0.0

Continued...

Table 1. Continued...

Fungi	Incubation period (days)	Mycelial dry weight (mg) for the fungi at temperature (°C)								
		15°C	20°C	25°C	30°C	35°C	40°C	45°C	50°C	55°C
<i>Chaetomium olivaceum</i>	3	0.0	0.0	11.3	18.0	22.2	40.6	39.6	0.0	0.0
	6	0.0	0.0	22.4	36.3	45.6	50.4	45.6	0.0	0.0
	9	0.0	0.0	46.9	66.4	78.8	60.6	66.6	0.0	0.0
	12	0.0	0.0	52.0	102.6	120.4	88.2	67.2	0.0	0.0
	15	0.0	0.0	30.4	80.6	82.5	70.2	42.6	0.0	0.0
<i>Mucor pusillus</i>	3	0.0	18.4	16.9	32.4	34.8	56.4	24.7	13.6	0.0
	6	0.0	12.4	28.7	76.7	76.8	87.8	42.9	17.8	0.0
	9	0.0	28.3	46.1	98.4	114.2	124.3	76.3	36.1	0.0
	12	0.0	32.8	82.0	120.8	152.9	180.2	76.2	35.5	0.0
	15	0.0	30.4	76.5	100.5	120.4	160.5	55.5	22.5	0.0
<i>Aspergillus flavus</i>	3	0.0	18.1	36.4	50.4	80.6	106.2	71.8	22.2	0.0
	6	0.0	24.3	52.6	72.6	122.0	161.4	75.3	32.4	0.0
	9	0.0	56.4	81.7	120.3	170.0	180.2	80.2	50.1	0.0
	12	0.0	82.1	113.2	180.2	226.6	236.4	106.0	51.0	0.0
	15	0.0	66.0	85.0	140.5	188.5	190.2	91.0	36.2	0.0

Continued...



Table 1. Continued...

Fungi	Incubation period (days)	Mycelial dry weight (mg) for the fungi at temperature (°C)								
		15°C	20°C	25°C	30°C	35°C	40°C	45°C	50°C	55°C
<i>A. ochroceus</i>	3	0.0	35.7	48.4	60.7	80.2	112.2	55.2	0.0	0.0
	6	0.0	56.2	60.4	82.9	125.0	173.4	68.8	0.0	0.0
	9	0.0	90.4	100.4	130.4	166.4	200.2	50.2	0.0	0.0
	12	0.0	120.3	160.0	180.4	214.2	278.2	42.4	0.0	0.0
	15	0.0	82.5	120.4	150.2	186.5	200.0	40.0	0.0	0.0
<i>A. niger</i>	3	0.0	24.6	44.1	56.3	66.2	80.22	15.4	0.0	0.0
	6	0.0	42.7	66.6	72.6	89.4	105.4	17.6	0.0	0.0
	9	8.6	50.8	87.4	100.7	123.6	150.2	18.4	0.0	0.0
	12	22.8	61.9	140.8	160.9	221.4	200.0	24.0	0.0	0.0
	15	20.2	56.5	100.0	120.5	170.2	160.5	17.5	0.0	0.0
<i>A. fumigatus</i>	3	0.0	38.9	58.7	60.6	90.4	122.2	39.2	22.1	0.0
	6	0.0	56.8	87.9	92.1	117.2	175.4	43.6	34.6	0.0
	9	0.0	81.4	106.7	113.8	178.3	187.4	51.7	40.1	0.0
	12	0.0	84.6	156.3	180.4	250.3	241.4	66.0	40.1	0.0
	15	0.0	72.5	100.5	140.0	200.2	200.0	55.0	35.5	0.0
<i>Penicillium dupontii</i>	3	0.0	7.1	26.2	28.6	36.1	52.6	37.1	21.2	8.1
	6	0.0	11.3	34.9	41.6	62.7	76.1	62.9	46.1	16.4
	9	0.0	22.1	62.4	73.1	96.4	96.1	87.1	72.3	28.3
	12	0.0	28.9	86.7	100.1	139.2	140.7	92.9	75.0	29.4
	15	0.0	25.0	74.5	85.2	100.5	102.0	80.0	60.5	18.0

**Table 2.** Effect of different degrees of pH on the growth of the tested fungi (mg mycelial dry weight) after 12 days of incubation at the optimum temperature for each fungus

Fungi	Incubation temperature °C	Mycelial dry weight (mg) at pH						
		7.0	7.5	8.0	8.5	9.0	9.5	10.0
<i>Paecilomyces variotii</i>	40	192.3	221.6	236.7	240.5	200.2	160.2	121.1
<i>Thermomyces lanuginosus</i>	40	171.4	181.7	200.4	218.9	180.4	171.7	116.2
<i>Acremonium alabamense</i>	40	170.2	200.4	242.7	250.6	180.2	150.1	110.0
<i>Chaetomium olivaceum</i>	35	110.7	122.9	131.2	141.9	110.1	90.4	78.1
<i>Mucor pusillus</i>	45	121.1	131.2	151.4	151.8	130.1	110.2	88.1
<i>Aspergillus flavus</i>	40	180.2	191.7	200.4	222.6	190.2	160.2	118.6
<i>A. ochraceus</i>	40	174.8	182.4	200.3	200.4	170.1	163.2	128.4
<i>A. niger</i>	35	190.2	200.9	214.7	216.2	170.4	128.2	100.4
<i>A. fumigatus</i>	35	180.7	200.2	232.4	234.7	230.2	180.1	150.3
<i>Penicillium dupontii</i>	40	132.8	140.1	162.4	180.7	178.4	151.3	111.2

**Table 3.** Effect of different concentrations of NaCl (from 0.0% to 16.0%) on the mycelial growth (mg dry weight) of the tested fungi after 12 days of incubation at the optimum temperature for each fungus

Fungi	Incubation temperature (°C)	Mycelial dry weight (mg) determined for the fungi at NaCl Concentration (%)								
		0.0	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0
<i>Paecilomyces variotii</i>	40	171.2	140.7	100.3	70.2	43.1	15.6	2.6	0.0	0.0
<i>Thermomyces lanuginosus</i>	40	160.3	132.1	80.6	50.9	36.1	20.4	9.6	0.0	0.0
<i>Acremonium alabamense</i>	40	175.1	150.2	100.4	80.2	55.7	33.1	15.1	0.0	0.0
<i>Chaetomium olivaceum</i>	35	100.1	110.7	90.1	70.3	52.1	35.1	20.0	8.2	0.0
<i>Mucor pusillus</i>	45	90.1	100.2	74.3	60.4	54.1	30.1	15.0	5.0	2.2
<i>Aspergillus flavus</i>	40	180.1	150.1	120.2	83.7	50.1	22.9	5.1	0.0	0.0
<i>A. ochraceus</i>	40	161.6	150.2	100.1	80.3	60.1	30.2	15.7	3.2	0.0
<i>A. niger</i>	35	160.1	170.2	140.1	100.2	70.3	42.4	30.2	10.2	5.0
<i>A. fumigatus</i>	35	220.7	230.4	200.0	120.2	90.4	70.2	44.2	20.2	10.1
<i>Penicillium dupontii</i>	40	120.3	124.2	110.1	90.2	60.4	35.2	10.4	4.1	1.1

These results are partially in accordance with those obtained by Zidan and Abdel Mallek (1987), who indicated that the ability of *Aspergillus niger*, *A. ochraceus* and *A. tamarii* to grow in saline medium containing 16% of NaCl, was due to internal glycerol which accumulated as a metabolite in these fungi. This glycerol was then excreted to the external medium and after that used by the fungi as a carbon source necessary for continuous growth. Glycerol has also been reported as a metabolic substance accumulated in yeasts during growth in saline medium (Gustafsson and Norkans 1976, Brown 1978, Alder and Gustafsson 1980 and Alder *et al.* 1985).

#### *Enzymatic Activity*

In order to obtain more information about the possible role played by the tested thermophilic and thermotolerant fungi in the soil fertility of the western region of Saudi Arabia, the ability of these fungi to produce certain enzymes *in vitro* was studied.

##### *a) Starch Hydrolyzing Activity*

Tabulated data (Table 4), clearly show that all the tested fungi were active in starch-hydrolysis. It is obvious that *Mucro pusillus* was the most active fungus among the tested fungi, followed by *Aspergillus flavus*, *Penicillium dupontii*, *A. fumigatus*, *A. niger*, *Acremonium alabamense*, *Thermomyces lanuginosus*, *Aspergillus ochraceus*, *Paecilomyces variotii* and *Chaetomium olivaceum*, respectively. It was also observed that the hydrolyzed area significantly increased with the increase of incubation period. These results are partially in line with those obtained by several investigators, *i.e.*, Goodman (1950), Garrett (1963), Chang and Hudson (1967) Alexander (1982), Fahim *et al.* (1982) and Satyanarayana *et al.* (1985), who indicated that some thermophilic, thermotolerant and mould fungi have the ability to produce starch hydrolyzing enzymes, degrade starch into glucose units.

##### *b) Protein Hydrolyzing Activity*

It is clear from the data presented in Table (5) that all the tested fungi were active protein decomposers. Data also indicate that *Mucor pusillus*, *Penicillium dupontii* and *A. flavus* possessed high ability to decompose casein, the nitrogen source in the tested medium, compared with the other tested fungi. On the other hand, noticeable significant differences were obtained between the periods of incubation for each fungus. These findings are partially in accordance with those reported by Grajek (1988), who indicated that *Thermomyces lanuginosus*, *Humicola lanuginosa* and *Penicillium dupontii* were fungi possessed high ability to produce proteolytic enzymes which degraded protein components in plant debris of sugar-beet pulp in soil. Grajek (1988) also mentioned that these fungi have the ability to synthesize protein from sugarbeet pulp in solid state fermentation. Khan Mova and Pavlovska (1978) and Fahim *et al.* (1982) found that *A. niger* and *A. flavus*, the main fungi causing

deterioration in sorghum grains during storage, produced maximum amount of proteolytic enzymes *in vitro*. As to *Chaetomium olivaceum*, *Paecilomyces variotii*, *Mucor pusillus* and *Acremonium alabamense*, there were no published reports on the activity of these fungi to produce proteolytic enzymes, hence our results are considered a new record for these fungi in this field.

### c) Cellulotic Enzyme

Table (6) shows the average percentage of cellulase  $C_x$  activity of the ten tested fungi after 14 days of incubation at the optimum temperature for each. Data given in Table (6) indicate that all the tested fungi except *Thermomyces lanuginosus*, possess the ability to produce  $C_x$  enzyme *in vitro*. *Paecilomyces variotii* appeared to possess higher activity to produce that enzyme if compared with the other tested fungi, as the culture filtrate for the forementioned fungus exhibited the highest percentage of cellulolytic activity, being 78.57% (loss in viscosity). *Chaetomium olivaceum* became in the second position regarding its activity to produce  $C_x$  enzyme, followed by *A. flavus*, *A. niger*, *A. fumigatus*, *Mucor pusillus*, *Acremonium alabamense*, *A. ochraceus* and *Penicillium dupontii*, respectively. In the light of these findings, we can conclude that these fungi may play an important role in cellulose degradation, occur in plant debris in soil, hence it enriches soil with some necessary components and elements for best plant growth. Our results were also in agreement with those found by Garrett (1963), Chang (1967), Tansey (1971), Hedger and Hudson (1974), Mishera *et al.* (1981) and finally Deacon (1985), who indicated that most of these fungi have the ability to produce  $C_x$  enzyme *in vitro* and *in vivo*, in soils containing wheat straw and some other plant debris. As to *Thermomyces lanuginosus*, the exception in this study, which was not able to produce  $C_x$  enzyme, similar results were obtained by Deacon (1985) who indicated that this fungus is considered non-cellulolytic fungus and its existence in soil is usually in association with some cellulolytic ones, from which it utilizes glucose released by the action of cellulase enzymes produced by its partner.

**Table 4.** Mean colony diameter of starch hydrolyzed area (mm) caused by each of the tested fungi after two incubation periods at the optimum temperature for each fungus

Fungi	Incubation temperature (°C)	Mean colony diameter of starch hydrolyzed area (mm) after incubation period extended to		L.S.D. (0.05) for incubation periods
		48 hours	96 hours	
<i>Paecilomyces variotii</i>	40	26.7	52.7	5.3
<i>Thermomyces lanuginosus</i>	40	21.3	61.7	9.8
<i>Acremonium alabamense</i>	40	23.7	61.7	8.1
<i>Chaetomium olivaceum</i>	35	30.7	51.3	3.5
<i>Mucor pusillus</i>	45	40.2	90.0	9.2
<i>Aspergillus flavus</i>	40	31.0	70.7	3.5
<i>A. ochraceus</i>	40	30.0	60.0	6.8
<i>A. niger</i>	35	21.3	66.7	7.7
<i>A. fumigatus</i>	35	35.3	69.3	8.6
<i>Penicillium dupontii</i>	40	35.0	70.4	3.5

**Table 5.** Mean colony diameter of protein hydrolyzed area (mm) caused by each of the tested fungi after two incubation periods at the optimum temperature for each fungus

Fungi	Incubation temperature (°C)	Mean colony diameter of protein hydrolyzed area (mm) after incubation period extended to		L.S.D. (0.05) for incubation periods
		48 hours	96 hours	
<i>Paecilomyces variotii</i>	40	22.7	44.3	7.0
<i>Thermomyces lanuginosus</i>	40	19.0	41.0	3.0
<i>Acremonium alabamense</i>	40	18.6	36.0	10.7
<i>Chaetomium olivaceum</i>	35	20.0	38.7	3.5
<i>Mucor pusillus</i>	45	40.0	82.3	11.1
<i>Aspergillus flavus</i>	40	30.7	50.6	6.1
<i>A. ochraceus</i>	40	33.3	69.7	4.7
<i>A. niger</i>	35	15.0	39.3	10.8
<i>A. fumigatus</i>	35	23.3	54.7	16.3
<i>Penicillium dupontii</i>	40	35.3	70.0	4.7

**Table 6.** Average percentage of cellulase (C<sub>x</sub>) activity of the tested fungi after 14 days incubation at the optimum temperature for each fungus

Fungi	Incubation temperature (°C)	Relative enzyme activity*
<i>Paecilomyces variotii</i>	40	78.57
<i>Thermomyces lanuginosus</i>	40	00.00
<i>Acremonium alabamense</i>	40	28.58
<i>Chaetomium olivaceum</i>	35	64.28
<i>Mucor pusillus</i>	45	35.71
<i>Aspergillus flavus</i>	40	50.00
<i>A. ochraceus</i>	40	21.43
<i>A. niger</i>	35	50.00
<i>A. fumigatus</i>	35	50.00
<i>Penicillium dupontii</i>	40	21.43

\* Average percentage of relative loss in viscosity of 0.2% CMC solution at pH 6.2 with the crude enzyme



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## دراسات فسيولوجية على عشر من الفطريات المحبة للحرارة العالية والقادرة على تحمل الحرارة المعزولة من مناطق مختلفة بالمملكة العربية السعودية

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أوضحت الدراسات الفسيولوجية على عشر من الفطريات المحبة للحرارة  
العالية والقادرة على تحمل الحرارة المعزولة من مناطق مختلفة من أراضي المنطقة  
الغربية وهي :

*Paecilomyces variotii*, *Thermomyces Lanuginosus*, *Acrimonium alabamense*,  
*Chaetomium olivaceum*, *Mucor pusillus*, *Aspergillus flavus*, *A. fumigatus* *A.*  
*ochraceus*, *A. niger* and *penicillium dupontii*,

ان هناك علاقة قوية بين درجة الحرارة، الملوحة ودرجة الحموضة وبين نمو هذه  
الفطريات حيث أثبتت الدراسات امتلاك هذه الفطريات قدرة عالية على تحمل  
درجات غير عادية من تلك العوامل .

ومن أجل الحصول على معلومات اضافية عن الدور الذي يمكن أن تلعبه  
هذه الفطريات في خصوبة التربة فلقد تمت دراسة مقدره هذه الفطريات على

انتاج بعض الانزيمات معملياً وهي انزيمات الاميليز Amylase hgh.d. Casease والسيلوليز Cellulase (C<sub>x</sub>) ، حيث أثبتت هذه الدراسات امتلاك الفطريات المستخدمة القدرة على انتاج الانزيمات المشار إليها باستثناء الفطر *Thermomyces lanuginosus* الذي لم يتمكن فقط من انتاج إنزيم السيلوليز.