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Morphological and Biological Characterization of *Monosporascus cannonballus* isolates, responsible of watermelon decline in Kairouan's area

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Watermelon grown in Kairouan's region is infected by vine decline disease caused by *M. cannonballus*. *In vitro* tests showed that this pathogen produces perithecia with a diameter of 495 µm releasing ascospores with a diameter of 44 µm. The mycelium is very fine, rarely visible in the media KOMADA and TANAKA. The colony of different isolates on substrate culture such as Malt, MS and S, appears very dense. On PDA, the grayish brown color characteristic of this ascomycete was observed. The perithecia production *in vitro* test for this fungus requires relatively long period of incubation (45 days). The effect of culture media showed that the PDA, MS and Malt are the best for the mycelia development of *M. cannonballus*. The most favorable culture media for fructification are in descending order MS, S, PDA, Malt. *M. cannonballus* isolates tested in this study showed an optimum temperature of mycelial growth and reproduction of 30°C. MT15 and MT12 isolates originated from Chebika (Kairouan government) showed a significant mycelial growth at all temperatures of incubation. MT7 and MT14 isolates from the same region were the most fertile. The results of pH effect on *M. cannonballus* development have revealed that the maximum mycelial growth occurred at pH 6 for most of isolates and for the fructification occurred at pH 4 and pH 8. For the osmotic pression with the addition of NaCl and KCl, the optimal development for majority of isolates is registered at -0.5 MPa and -2 MPa, with a minimum at -4MPa. The inhibition of the fructification of the *M. cannonballus* isolates occurred beyond -2MPa.

Key Words: *M. cannonballus*, *in vitro*, culture media, temperature, pH, osmotic pressures

Monosporascus root rot and vine decline caused by *Monosporascus cannonballus* (Pollack & Uecker) has become a major problem for watermelon production in many growing areas of the cucurbits in Tunisia (Martyn *et al.* 1994; Boughalleb *et al.* 2006; Ben Salem, 2009; Boughalleb *et al.*, 2010). Symptoms caused by *M. cannonballus* include a yellowing, death of crown leaves and a gradual decline of the plants approaching maturity, and consequently, a general collapse of the plant (Martyn and Miller, 1996).

Since the mid-1980s, this disease has been prevalent in cucurbit-production areas located in hot semi-arid to arid regions, as well as subtropical environments (Martyn & Miller 1996; Aegerter *et al.*, 2000, Cohen *et al.*, 2000). In recent years, a re-emergence of *Monosporascus* root rot and vine decline has been noticed. Severe outbreaks of this disease have been reported in new areas such as Egypt (El-Desouky & El-Wakil 2003), Brazil, (Sales *et al.*, 2004) and Italy (Chilosi *et al.*, 2008), and expanded its geographical and host ranges in regions

where it was already present (Sarpeleh 2008; Boughalleb *et al*., 2010; Sales *et al*., 2010). *M. cannonballus* produces ascospores in perithecia formed on affected roots at the end of the cropping season (Martyn & Miller 1996). Ascospores probably function as the primary survival structure, as well as the primary inoculums of the fungus for root infection (Stanghellini *et al*., 1996, 2000). *M. cannonballus* have shown a high optimum growth temperature (Martyn and Miller, 1996; Martyn *et al*., 1996; Pivonia *et al*., 1997; Pivonia *et al*., 2002). *In vitro* vegetative growth of this pathogen is between 15 and 40°C with an optimum at 30°C (Martyn and Miller, 1996; Bruton *et al*., 1999; Stangellini *et al*., 2000; Pivonia *et al*., 2002 ; Waugh *et al*., 2003).

Little information is available on the adaptability of *M. cannonballus* to different environmental variables such as water potential or pH, and how they may influence the phenotypic variability and the reproductive potential of this fungus. *M. cannonballus* appears to be adapted to hot, semi-arid climates with saline and alkaline soils (Martyn & Miller 1996). Information about the environmental requirements of *M. cannonballus* has been inferred from areas where the fungus has been found and by *in vitro* studies. *M. cannonballus* grows optimally at pH 6 and 7, can tolerate relatively high levels of sodium and calcium chloride salts (8 and 10 %) and maximum mycelial growth occurs at osmotic water potential of -0.6 to -0.8 MPa (Martyn & Miller 1996; Ferrin & Stanghellini 2006). Perithecial production is also affected by soil temperature, reaching its optimum between 25 and 30°C (Waugh *et al*., 2003). Recently, an evaluation of soil factors associated with ascospore density conducted in watermelon fields in Tunisia, revealed that the pH of the soil had a strongly significant negative linear relationship with ascospore density indicating that pHs closer to the optimal one in vitro (pH 6 and 7) are more conducive for ascospore production (Boughalleb *et al*., 2010).

The objective of this study was to evaluate the effect of temperature, pH, and water potential on mycelial growth and perithecial production of *M. cannonballus* isolates collected from Kairouan region in Tunisia.

Material and Methods

Fungal isolates

Seven *M. cannonballus* isolates obtained from roots of watermelon crops exhibiting symptoms of *Monosporascus* root rot and vine decline in different cucurbit growing regions in Tunisia were arbitrarily selected and used in the present study (Table 1). All isolates were hyphal-tipped and stored at 25°C in darkness in plastic vials containing sterilized peat. Prior to use, a small portion of the colonized peat from each plastic vial was transferred to potato dextrose agar (PDA) plates and allowed to grow at 25°C in darkness for 10 days.

Effect of different culture media on mycelial growth and perithecia formation of *Monosporascus cannonballus*

Six culture media were used to determine the most appropriate for the mycelial development of *M. cannonballus* isolates. Those culture media were the S, MS, KOMADA, Malt, TANAKA and PDA. For each isolate and each medium was carried out four replicates. The test was performed for 5 *M. cannonballus* isolates (MT2, MT4, MT8, MT12 and MT15) (Table 1). Mycelial growth was estimated when it reached at least two thirds of of the first Petri dish by measuring the average of perpendicular diameters of the most dissimilar from each colony. Colonies were further incubated until 45 days after the mycelial plugs were placed on the agar for perithecia formation and quantification as described by Cluck *et al.* (2009). Two plugs (8mm diameter) were randomly selected

and removed from each colony and each plug was considered a replicate (four plugs/isolate). Each plug was placed between two clear glass slides and flattened in order to observe and count the perithecia. Perithecia were observed under a low power stereoscope. Each plug consisted of a volume of 0.151 cm3 and the number of perithecia was expressed in cm3.

Effect of temperature on mycelial growth and perithecia formation of *Monosporascus cannonballus*

The effect of temperature on mycelial growth and perithecia formation of *M. cannonballus* in culture was determined on PDA. Mycelial plugs (8 mm in diameter) obtained from the growing edge of colonies were transferred to the centre of PDA plates which were kept in the dark at the experimental temperatures: 15, 20, 25, 30, 35, and 40°C. There were two replicates for each isolate and temperature combination. The diameter of each colony was measured twice perpendicularly when it reached at least two thirds of the plate and used to calculate the mean growth rate as cm per day. The test was performed for 7 isolates of *M. cannonballus* governorate of Kairouan (Table 1). The numbers of perithecia were evaluated as described before. The experiment was conducted twice.

Table 1: Origin and date of collection of *Monosporascus cannonballus* isolates

Regions	Localities	Isolats collected	Sampling date
Chebika	Achraoui	**MT2**	24/07/2007
	Ouled Elaiibidi	**MT7** **MT8**	18/08/2007
	Sidi Ali Ben Salem	**MT12** **MT14** **MT15**	18/08/2007
Sbika	Ain Boumerra	**MT4**	06/08/2007

Effect of pH on mycelial growth and perithecia formation of *Monosporascus cannonballus*

The effect of pH on mycelial growth and perithecia formation of *M. cannonballus* in culture was determined on PDA. Mycelial plugs (8mm in diameter) obtained from the growing edge of colonies were transferred to the centre of PDA plates which were adjusted to pH 4, 5, 6, 7, and 8 with the addition of 50 mM citrate phosphate buffer (pH 4 and 7) or 50mM TriseHCl buffer (pH 8), respectively (Gomori, 1955). Plates were incubated in the dark at 25°C. There were two replicates for each isolate and pH combination. Mean growth rates and the number of perithecia were evaluated as described before. The experiment was conducted twice.

Effect of water potential on mycelial growth and perithecia formation of *Monosporascus cannonballus*

The effect of Js on mycelial growth and perithecia formation of *M. cannonballus* in culture was determined on PDA. Mycelial plugs (8mm in diameter) obtained from the growing edge of colonies were transferred to the centre of PDA plates amended with KCl or NaCl prior to sterilization to obtain seven values: -0.5, -1.0, -2.0, -3.0, -4.0, -5.0, and -6.0 MPa, according to Robinson & Stokes (1959). Plates were incubated in the dark at 25°C. There were two replicates for each isolate, type of solute and Js combination. Mean growth rates and the number of perithecia were evaluated as described before. The experiment was conducted twice.

Statistical Analysis

Analyses of variance (ANOVA) were conducted with data obtained from culture media, temperature, pH and osmotic pression to analyze potential trial and treatment interactions. In all cases, ANOVA analyses indicated that the data between the two replications were similar ($P > 0.05$), thus data of all variables from both experiments were combined.

Mycelial growth and perithecial formation data were analyzed by multivariate factorial analysis using the GLM (SPSS.12).

Results

Effect of culture media on mycelial growth and perithecia formation of *Monosporascus cannonballus*

The mycelial growth of *M. cannonballus* on different culture media showed significant differences on mycelia growth and fructification of *M. cannonballus* isolates ($p<0.05$). The mycelium is very fine, rarely visible in the petri dish on those two media KOMADA and TANAKA. For Malt, MS and S, the colony of the fungus appears very dense and whitish. On PDA medium it appears grayish-brown color characteristic of this pathogen. Statistical analyses indicated that the most favorable medium was PDA with a radial growth of 5.73 cm, followed by the Malt and MS media a value of 3.76 and 3.12 cm, respectively (Table 2).

Table 2: Effect of culture media on colony diameter (cm) and number of perithecia (per/cm³) of 5 *M. cannonballus* isolates

	Colony diameter (cm)					
Isolates	**KOMADA**	**MALT**	**TANAKA**	**MS**	**S**	**PDA**
MT2	1.45 ab[a]	3.6[c]	3a	5.04a	2.92b	8.5a
MT8	1.63 a	2.6	2.56b	2.66bc	3.9a	4.96b
MT4	1.74a	3.72	2.48b	5.32a	1.63c	5.52b
MT12	0.87 b	2.52	0.54c	1.88c	3.16b	8.39a
MT15	1.1ab	3.18	0.44c	3.89ab	1.67c	1.27c
Means	1.36	3.12	1.80	3.76	2.65	5.73
***P* Values[d]**	0.071	0.48	0	0.001	0	0
	Perithecia production (Number/cm³-					
MT2	0	2.26±0.62b[b]	0	31.96ab	0b	13.5ab
MT8	0	0±0b	0	0c	0b	0c
MT4	0	8.82±0.5ab	0	19.37b	3.52b	10.36ab
MT12	0	7.97±0.04ab	0	45.38a	11.12b	0c
MT15	0	17.65±1.2a	0	29b	53.83a	35.68a
Means	0	7.34	0	25.14	13.69	11.91
***P* Values[d]**	-	0.009**	-	0.000**	0.000**	0.000**
Isolates			0.000**			
Media			0.000**			
Isolates*Media			0.000**			

[a] *The average of two values (horizontal and vertical) for the four Petri dishes*
[b] *The average surface of the two tiles for the four Petri dishes*
[c] *No classification (Means are not statistically different)*
[d] *p Values (0,05) by GLM*
Means with the same letter are not statistically different (p <0.05 by Duncan test).

However, the TANAKA and KOMADA media were the poorest media, with the lowest values with 1.36 cm. Thus, PDA culture medium was used for further studies of mycelial growth. Obtained results showed that the isolate MT2 had the highest value of mycelia growth with 4.1 cm, followed by the isolate MT4 (3.4 cm) and MT8 (3.05 cm) on all substrates used. For the majority of the isolates tested, the mycelial development is optimal on PDA. However the MS medium could be appreciated by this pathogen (Table 2). The highest number of perithecia was found on culture medium MS about 25.14per/cm³. No fructification was observed on KOMADA and TANAKA. However, we have noticed a remarkable sporulation of *M. cannonballus* isolate MT15 on MS culture

medium (53.83 per/cm^3) when he showed the slowest development in all nutrient substrate tested (Table 2). The favorable environment for mycelia development is not necessarily the best for the fructification. This was applied, for the isolates MT2 and MT12, which have showed a maximum mycelial growth on PDA but a poor fructification on the same medium. However, the isolate MT4 has showed an optimal development and good fructification on MS medium (Table 2).

Table 3: Effect of temperature on mycelial growth and perithecial production of 7 *Monosporascus cannonballus* isolates

Isolates	Radial growth (cm/day)						Optimum growth temp. (°C)	Perithecia/cm3		
	15°C	20°C	25°C	30°C	35°C	40°C		25°C	30°C	35°C
MT2	0.005d[a]	0.261ab	0.742a	0.941a	0.754a	0.134d	29.0[b]	90.6d[c]	354.4abc	33.1a
MT4	0.006d	0.216b	0.614b	0.905a	0.581b	0.168d	28.9	88.8d	145.6de	0b
MT7	0.027bc	0.265a	0.541a	0.856b	0.848a	0.485b	31.3	155.6ab	474.4a	7.5b
MT8	0.004cd	0.346ab	0.488b	0.856a	0.773a	0.41a	30.4	108.8cd	245.6cd	0b
MT12	0.03cd	0.297a	0.797a	0.8b	0.831a	0.316a	29.8	103.8cd	330.0bc	0b
MT14	0.018a	0.249c	0.625c	0.919d	0.823c	0.399cd	30.4	161.9a	438.1ab	6.9b
MT15	0.018b	0.323a	0.745b	0.809c	0.76b	0.459bc	30.4	125bc	123.1e	0.6b
Means	0.0154	0.279	0.650	0.869	0.767	0.338		119.2	301.6	6.8
Values[d]	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**		0.000**	0.000**	0.000**

Effect	*p* Values[d]	*p* Values[d]
Isolates	0.000**	0.000**
Temperature	0.000**	0.000**
Isolates*t°C	0.000**	0.000**

[a] *Values of radial growth are the means of eight replicates (four/experiment)*
[b] *For each M. cannonballus isolate, temperature average growth rates were adjusted to a regression curve to estimate the optimum growth temperature*
[c] *Values of perithecia production are the means of eight replicates (four/experiment);* [d] *p Values (0,05) by GLM*

Effect of temperature on mycelial growth and perithecia formation of *Monosporascus cannonballus*

The effect of the temperature on mycelial growth and perithecia formation of the 7 *M. cannonballus* isolates is shown in Table 3. All isolates were able to grow on PDA over a range of temperatures from 20 to 40°C. Isolates MT2, MT4 and MT8, showed no growth at 15°C, while the growth rates of the other isolates were almost negligible at this temperature. Optimum growth temperatures for all isolates ranged between 28.9°C (isolate MT4) and 31.3°C (isolate MT2). The *M. cannonballus* isolate MT7, MT8 and MT12 have shown the highest value of radial growth in all temperature ($p<0.05$). Perithecia formation was obtained mainly at 25 and 30°C and was, in general, most abundant at 30°C. Only some isolates were able to produce perithecia at 35°C (MT2, MT14 and MT15). However MT15 produced very few perithecia at 30°C with 123.1per/cm^3 (Table 2). The optimum number of perithecia was recorded at 30°C for most isolates. The isolate producing the lowest number of perithecia is MT4 (145.6 per/cm^3). The most fertile isolate (474.4 per cm^3) was MT7 at 30°C. No fructification was observed at temperatures below 20°C and beyond 35°C (Table 3)

Effect of pH on mycelial growth and perithecia formation of *Monosporascus cannonballus*

The effect of the pH on mycelial growth and perithecia formation of the 7 *M. cannonballus* isolates is shown in Table 4.

Radial growth on pH adjusted PDA demonstrated a big pH tolerance by *M. cannonballus*, but pH 6 (0.53 cm / day) and 7 (0.37 cm/day) exhibited the highest growth rates for all isolates except for MT14, which grew best at pH 4 (0.41cm/day). It was found that the isolate MT4 have the largest mycelia growth with 0.41 cm/day, followed by the isolate MT2 (0.37 cm / day) and the isolate MT7 (0.34 cm / day). But, it was the lowest for MT8 with 0.27 cm / day for all pH values. Most of the isolates were able to produce perithecia at all pH values. Some exception was isolate MT-8 which was unable to produce perithecia at pH 7 (Table 4). The isolate MT12 seemed the most productive with 129.5 per/cm^3 and for the lowest fertile was MT15 with 72.25 per/cm^3. All isolates showed a significant sporulation at pH 8 with 208.3 per/cm^3 and pH4 (110.89 per/cm^3). However, the number of perithecia produced was low at pH7.

Table 4: Effect of pH on mycelial growth and perithecial production of 7 *Monosporascus cannonballus* isolates

Isolates	Radial growth (cm/d)				
	pH 4	**pH 5**	**pH 6**	**pH 7**	**pH 8**
MT2	0.35 b[a]	0.23 b	0.58a	0.41 bc	0.27 a
MT4	0.35 b	0.33 a	0.58 a	0.53 a	0.25 a
MT7	0.3 c	0.22 bc	0.51 b	0.47 ab	0.21 a
MT8	0.22 d	0.23bc	0.55 a	0.22 d	0.09 b
MT12	0.26 d	0.19 c	0.48 b	0.42 abc	0.12 b
MT14	0.41 a	0.34 a	0.39 c	0.34 c	0.12 b
MT15	0.30 c	0.20 bc	0.59 a	0.17 d	0.10 b
Means	0.31	0.25	0.53	0.37	0.17
***p* Values[d]**	0.000**	0.000**	0.000**	0.000**	0.000**
	Perithecia/cm^3				
MT2	100.60bc[b]	78.8[c]	105[c]	90.6a	159.4bc
MT4	60.6c	77.5	54.4	73.8a	228.8ab
MT7	155ab	87.5	108.8	58.1a	172.5bc
MT8	70.6c	88.1	98.1	0b	276.3a
MT12	181.3a	56.9	64.4	56.9a	288.1a
MT14	111.9bc	103.1	105.6	69.4a	207.5abc
MT15	96.3bc	66.9	54.4	18.1b	125.6c
Means	110.9	79.83	84.39	52.41	208.31
***p*Values[d]**	0.002	0.666	0.156	0.000**	0.002

Effect	***p*Values[d]**
Isolates	0.000**
pH	0.000**
Isolates*pH	0.000**

[a] *The average of two values (horizontal and vertical) for the four Petri dishes*

[b] *The average surface of the two tiles for the four Petri dishes*

[c] *No classification between means;* [d] *p Values (0,05) by GLM*

Means with the same letter are not statistically different (p <0.05 by Duncan test)

Effect of water potential on mycelial growth and perithecia formation of *Monosporascus cannonballus*

The effect of water potential on mycelial growth and perithecia formation of the 7 *M. cannonballus* isolates is shown in Tables 5 and 6 for NaCl and KCl, respectively. Similar results were obtained with both salt types, but, in general, *M. cannonballus* was more tolerant to KCl than NaCl, resulting in lower radial growth values when NaCl was used to adjust water potential. Radial growth decreased progressively as water potential decreased

and was severely limited at -5.0 to -6.0 MPa. For both salt types, perithecia formation was highest at -0.5, decreased at -1.0 MPa and occurred just in some isolates at -2.0 MPa. No perithecia formation was observed beyond -2MPa for both NaCl and KCl salts.

Table 5: Effect of water potential, established using NaCl, on mycelial growth, and perithecial production of 7 *Monosporascus cannonballus* isolates

Isolates	Radial growth (cm/d)						
	-0.5MPa	-1MPa	-2MPa	-3MPa	-4MPa	-5MPa	-6MPa
MT2	0.583[c]	0.579 ab[a]	0.501 ab	0.214 cd	0.104 b	0.105 a	0.033
MT4	0.591	0.506 b	0.549 a	0.113 e	0.09 b	0.051 bc	0.019
MT7	0.635	0.635 a	0.543 a	0.493 a	0.186 a	0.098 ab	0.036
MT8	0.635	0.634a	0.502 ab	0.188 d	0.07 b	0.023 c	0.020
MT12	0.598	0.598 a	0.544 a	0.365 b	0.180 a	0.044 c	0.03
MT14	0.620	0.628 a	0.506 ab	0.284 c	0.081 b	0.051 bc	0.02
MT15	0.598	0.585 ab	0.432 b	0.371 b	0.103 b	0.063 abc	0.029
Means	0.61	0.59	0.51	0.29	0.12	0.06	0.03
***P* Values**	0.711	0.032	0.123	0.000**	0.000**	0.1	0.913
	Perithecia /cm³						
MT2	128.04a[b]	55.1a	15a	0	0	0	0
MT4	46.13d	30.4 ab	8.5abc	0	0	0	0
MT7	60ab	30.8ab	10.5ab	0	0	0	0
MT8	104.5d	18.4b	1.3bc	0	0	0	0
MT12	70.30cd	27.1b	2.8bc	0	0	0	0
MT14	102.9ab	13.2b	0.4c	0	0	0	0
MT15	87.6bc	13.7b	1.1bc	0	0	0	0
Means	85.638	26.957	5.657	0	0	0	0
***p* Values[d]**	0.000**	0.02	0.07				
Effect	***p* Values[d]**						
Isolates	0.000**						
NaCl	0.000**						
Isolates*NaCl	0.000**						

[a] *The average of two values (horizontal and vertical) for the four Petri dishes*
[b] *The average surface of the two tiles for the four Petri dishes*
[c] *No classification between means;* [d] *p Values (0,05) by GLM*
Means with the same letter are not statistically different (p <0.05 by Duncan test).

Effect of water potential for NaCl

The maximum radial growth of all *M. cannonballus* isolates is obtained only at water potential of -0.5MPa, -1 MPa and -2 MPa with respectively 0.61, 0.59 and 0.51 cm/day. For the two osmotic pressions -3 MPa and -4MPa, there is a little reduction of radial growth of all isolates studied. No development was obtained above -6MPa. The isolate MT7 was the lowest sensitive to the increase of the potential water osmotic with 0.36 cm / day, followed by MT12 (0.32 cm / day) and MT4 with 0.28 cm / day (Table5). Production of perithecia was significantly more sensitive to potential water than the radial growth of all isolates at all osmotiques pressions. Indeed, all isolates were found productive on PDA amended by NaCl -0.5 MPa with 85.64 per/cm3, followed by -1MPa with 26.97 per/cm3. Generally, the production of perithecia was reduced when the osmotic pression is reduced (Table 5). Only the isolate MT2 showed a significant sporulation at the different treatments with 38.35 per/cm3.

Effect of water potential for KCl

Optimal radial growth of all *M. cannonballus* isolates was recorded at the three first

osmotic pressions tested -0.5 MPa, -1 MPa and -2 MPa with respective values of 0.85, 0.84and 0.72cm/day ($p < 0.05$). About the reaction of the isolates studied, the isolate MT12 was the most resistant when the potential water increase with a radial growth about 0.5 cm / day, followed by MIT15 (0.48/day) and MT8 (0.35 cm/day) (Table 6). Most of *M. cannonbalus* isolates were found productive at -0.5 MPa about 94.52 per/cm^3 then at-1MPa with 62.52per/cm3. MT2 was the most fertile for all osmotic pressions tested with 39.92 per/cm^3 followed by MT12 with a production of perithecia about 37.88 per/cm^3 and the isolate MT7 with 33.59 per/cm^3 ($p < 0.05$).

Table 6: Effect of water potential, established using KCl, on mycelial growth, and perithecial production of 7 *Monosporascus cannonballus* isolates

Isolates	Radial growth (cm/d)						
	-0.5MPa	-1MPa	-2MPa	-3MPa	-4MPa	-5MPa	-6MPa
MT2	0.905 a[a]	0.9 a	0.797 ab	0.349 bc	0.171 bc	0.154 a	0.04[c]
MT4	0.914 a	0.855 ab	0.848 a	0.172 d	0.152 bcd	0.08 bc	0.02
MT7	0.752 b	0.752 b	0.645 bc	0.585 a	0.221 ab	0.116 ab	0.04
MT8	0.752 b	0.752 b	0.594 c	0.224 cd	0.083 d	0.03 c	0.02
MT12	0.923 a	0.923a	0.843 a	0.575 a	0.254 a	0.05 c	0.04
MT14	0.8 b	0.813 ab	0.654 bc	0.379 b	0.102 cd	0.06 bc	0.02
MT15	0.923 a	0.907 a	0.685 bc	0.596 a	0.149 bcd	0.08 bc	0.03
Means	0.852	0.843	0.724	0.411	0.162	0.082	0.032
p Values[d]	0.000**	0.16	0.03	0.000**	0.000**	0.001	0.842
	Perithecia/cm^3						
MT2	118.4a[b]	55.8b	38.2a	0	0	0	0
MT4	71.5b	53.9 b	0.2b	0	0	0	0
MT7	97.8b	80.3 ab	0.1b	0	0	0	0
MT8	96.8c	24.9b	0.3b	0	0	0	0
MT12	107ab	96 a	37.2a	0	0	0	0
MT14	96.2b	63ab	15.8ab	0	0	0	0
MT15	73.9c	63.6ab	16.9ab	0	0	0	0
Means	94.51	62.5	15.53	0	0	0	0
p Values[d]	0.000**	0.000**	0.001				

Effect	*p* Values[d]
Isolates	0.000**
KCl	0.000**
Isolates*KCl	0.000**

[a] *The average of two values (horizontal and vertical) for the four Petri dishes*
[b] *The average surface of the two tiles for the four Petri dishes*
[c] *No classification between means;* [d] *p Values (0,05) by GLM*
Means with the same letter are not statistically different ($p < 0.05$ by Duncan test).

Discussion

This work has identified differences in the effect of different culture conditions, and their interactions, on the mycelial growth and the reproductive potential among 7 isolates of *Monosporascus cannonballus* collected in watermelon fields of Kairouan's government in Tunisia. For media studies, our results confirmed those of Park *et al.*, 1994; Tsay and Tung , 1995; Martyn and Miller, 1996; Heo *et al.* , 2001 and Hamza *et al.* , 2007) have reported that PDA was the culture medium the most favorable for mycelia growth and production of perithecia.

The isolates showed optimal growth temperatures over the range of 27 and 34°C. This result is in agreement with the well known thermophilic nature of *M. cannonballus* (Martyn & Miller 1996). In fact, all the isolates were collected from Mediterranean areas like Tunisia characterized by the hot summer temperatures, when cucurbits crops are grown in the fields. These environmental conditions, together with the use of plastic mulches, may raise soil temperatures to a level conductive to growth and infection by *M. cannonballus*, which also favours production of perithecia. Waugh *et al.* (2003) demonstrated that optimal temperatures for perithecia development are between 25 and 30°C. Our study also confirmed that perithecial production occurred mainly at these temperatures, although some isolates were able to produce perithecia even at 35°C.

All isolates showed a broad pH tolerance for mycelia growth and perithecia formation. This is in agreement with Martyn & Miller (1996), who indicated that *M. cannonballus* grows optimally at pH 6 and 7, but will grow at pH values up to at least 9.0; the growth is reduced below pH 5 and almost completely inhibited at pH 4. Although, Hamza *et al.* (2007) have reported that the isolates of *M. cannonballus* present an optimum of mycelial growth at a pH between 7 and 8. Related to the effect of water potential, the mycelial growth rate of *M. cannonballus* isolates was reduced as water potential decreased. Perithecial production was more sensitive to reduced water potential than was mycelial growth and was restricted to water potential values from -0.5 to -2.0 MPa. Ferrin & Stanghellini (2006) evaluated the effect of water potential on mycelial growth and perithecial production on four *M. cannonballus* isolates collected from diseased cantaloupe roots in California. These authors also observed a pattern of reduction in mycelial growth as water potential decreased, but perithecia were produced only until water potential values of -0.71 and -0.88 MPa for KCl or NaCl, respectively. Few studies have addressed the study of salinity on development and production of perithecia of *M.cannonballus*. However, the effect of NaCl and KCl salts on the growth of other pathogens has been reported by several authors.

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