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EFFECT OF DIFFERENT CARBON AND NITROGEN SOURCES ON *SCLEROTIUM ROLFSII* SACC. MYCELIAL GROWTH AND SCLEROTIAL DEVELOPMENT

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ABSTRACT

In vitro studies were conducted on Potato Dextrose Agar using different carbon (C) and nitrogen (N) sources to evaluate their effects on the mycelial growth, and the sclerotial development of three Tunisian *Sclerotium rolfsii* Sacc. isolates. Radial growth was optimum on basal medium supplemented with ammonium chloride (0.48 gram of nitrogen per liter (g of $N.L^{-1}$)) as N source but was restricted on L-Arginine and completely inhibited on ammonium acetate amended media (0.48 g $N.L^{-1}$). Sclerotial initiation occurred from the 3rd to the 12th day of incubation for all tested isolates. Potassium nitrate was the most suitable N source for sclerotial formation whereas sclerotial development was completely inhibited on ammonium acetate amended medium. Optimal sclerotial germination was recorded using L-Arginine (78-80%) followed by L-Asparagine (46-94%) and ammonium chloride (46-88%) as N sources. Nevertheless, the lowest sclerotial germination rate was noted on sodium nitrate and ammonium acetate amended media. As for C sources (16 gram of carbon per liter (g of $C.L^{-1}$)), optimal radial growth occurred using D-mannitol for Sr1 and Sr2 isolates and maltose for Sr3, but no mycelial growth was recorded using sodium citrate for all isolates. All C sources tested, except sodium citrate, were suitable for sclerotial formation, production, and germination. Mature sclerotia became brownish after 6 to 12 days of incubation and sclerotial production was highest using D-mannitol, maltose, and D-glucose, depending on isolates used, as C sources. Optimal germination of sclerotia was noted using D-glucose, D-mannitol and maltose for Sr1 isolate, maltose for Sr2 and D-glucose and maltose for Sr3. It was concluded that N and C sources are both important factors for the growth of *S. rolfsii* and its survival.

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INTRODUCTION

Sclerotium rolfsii Sacc. is a serious ubiquitous soilborne phytopathogenic fungus, causing Southern blight of a wide range of agricultural and horticultural crops (Aycock, 1966 and Anahosur, 2001 and Galdames and Diaz, 2010 and Kwon *et al.*, 2013 and Shen *et al.*, 2014 and Mahadevakumar *et al.*, 2015). The fungus is notorious for its ability to induce dark stem rot, during any plant growth stages, followed by drooping and wilting of leaves and gradually wilting of the whole plant. Such wilted plants show white cottony fungal thread girdling the basal part of the stem and moving below the stem to roots (Kator *et al.*, 2015 and Sun *et al.*, 2020 and Punja *et al.*, 1985). This pathogen survives as

sclerotia on decayed plant material in the soil which germinate later and attack surrounding host plants (Sachslehner *et al.*, 1997 and Ludwig and Haltrich, 2002). During its pathogenesis, *S. rolfsii* secretes pectinolytic enzymes and oxalic acid (Ferrar and Walker, 1993 and Ansari and Agnihotri, 2000 and Punja *et al.*, 1985). These compounds affect cell walls, hydrolyze pectin and alter host defensive responses (Bateman and Beer, 1965 and Kritzman *et al.*, 1977 and Punja, 1985 and Ferrar and Walker, 1993).

This fungus is widely distributed and causes heavy economic losses on many crops (Aycock, 1966 and Gurha and Dubey, 1983 and Fery and Dukes, 2002 and Billah, 2017 and Sun *et al.*, 2020). Yield losses are estimated as 53.4% on cowpea (Fery and Dukes, 2002), 5-20% on peppermint (Singh and Singh, 2004), 10-45% on tomato and artichoke (Banyal *et al.*, 2008), 25-90% on potato (Anahosur, 2001), and 60% as plant losses (McCarter and Kays, 1984).

To determine the optimal environmental and nutritional conditions for *S. rolfsii* growth and survival, Ayed *et al.* (2018a) and Ayed *et al.* (2018b) demonstrated under laboratory conditions that temperatures, pH, aeration and culture media have significant effects on these parameters. However, there is little information on pathogen nutritional requirements in semi-synthetic media. Therefore, this study was undertaken to examine the effect of different nitrogen and carbon sources on the mycelial growth, sclerotial production and germination of three Tunisian *S. rolfsii* isolates.

MATERIALS AND METHODS

Pathogen culture and sclerotial production

Three *S. rolfsii* isolates, originally isolated from artichoke stem rot (Sr1) and rotted potato tubers (Sr2 and Sr3), were used in the current investigation. They were identified in a previous study (Ayed *et al.*, 2018a). They were maintained in the laboratory of Phytopathology at the Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia. Pathogen cultures used in the current study were previously grown for one week on Potato Dextrose Agar (PDA) at 30 °C and in the dark.

Mature sclerotia were harvested from 21-day-old cultures, placed into fine nylon bags and washed frequently in sterile distilled water to remove excess agar. They were then filtered through Whatman n°1 sterile filter paper, placed in sterile plastic Petri plates and dried in a laminar flow cabinet for 4 hours. After drying, sclerotia were sized by dry sieving (425-1000 µm) and collected before being used in the trials (Ritchie *et al.*, 2009).

Nitrogen and Carbon sources

The effects of nitrogen and carbon sources were carried out on basal medium consisting of 1.75 g KH_2PO_4, 0.75 g $MgSO_4.7H_2O$, 15 g agar and 1 L of distilled water (Townsend, 1957). For nitrogen sources, inorganic (Ammonium chloride, Potassium nitrate, Sodium nitrate, Ammonium acetate) and organic (L-Arginine, L-Asparagine) sources were autoclaved separately and added to batches of sterile medium, with D-glucose (40 $g.L^{-1}$: 16 g of $C.L^{-1}$) as carbon source, to give a nitrogen level of 0.48 $g.L^{-1}$.

Carbon sources (D-glucose, Glycerol, D-Mannitol, Maltose, and Sodium citrate) were tested on the basal medium supplemented with potassium nitrate (KNO_3: 3.5 $g.L^{-1}$ (0.48 g of $N.L^{-1}$)) as a nitrogen source. All carbon sources were autoclaved separately and added to the batches of sterile medium, except for disaccharides which were added to the medium after filter sterilization. The total level of the carbon added in each case was 16 $g.L^{-1}$. The tested media was adjusted to pH 6 and poured into Petri plates before pathogen inoculation.

Mycelial growth and sclerotial production

To examine the effect of different N and C sources on *S. rolfsii* mycelial growth, mycelial plugs (6 mm in diameter), cut from the margin of 7-day-old cultures, were placed in the centre of Petri plates containing agar media adjusted with the tested N and C sources and supplemented with streptomycin sulfate (300 $mg.L^{-1}$). Inoculated plates were incubated at 30 °C in darkness (Ayed *et al*., 2018a, 2018b). The diameters of the developing colonies were measured after 24, 48 and 72 h of inoculation and the radial growth rate (mm/day) was calculated.

The same cultures were further incubated for additional 21 days. During this period, sclerotial production was monitored and determined at 3-day intervals (Maurya *et al.*, 2010).

For the monitoring of sclerotial production, brown sclerotia were removed with a sharp scalpel, placed in fine mesh nylon bags and washed with sterile distilled water to remove adhering agar. They were counted and

the average number of mature sclerotia produced per plate was determined. After counting, sclerotia were placed on pre-dried and weighed Whatman n°1 filter papers and incubation at 70 °C for 48 h. The dry weight of 100 sclerotia formed per plate was determined.

For all parameters noted, ten replicate plates were used per individual treatment (per isolate and per treatment (tested C or N sources).

Sclerotial germination

Sclerotia of a similar size (21-day-old) were used in this study. Ten sclerotia were placed onto Petri plates containing culture media supplemented with the different N and C sources tested and incubated at 30 °C in darkness (Ayed *et al.*, 2018a and Ayed *et al.*, 2018b). Germination was determined after 24, 48 and 72 h of incubation by examining each sclerotia for any outgrowing hyphae observed under a binocular microscope. A sclerotium was considered as germinated when outgrowing hyphae were equal to or greater than its diameter. Ten replicate plates, containing 10 sclerotia, were used per individual treatment and the percentage of germinated sclerotia per plate was calculated (Ayed *et al.*, 2018a and Ayed *et al.*, 2018b).

Statistical analysis

Data analyses were performed following a completely randomized factorial design where fungal treatment (*S. rolfsii* isolates) and the tested factors (N or C sources) were the two fixed factors. Mean values were evaluated and separated using Fisher's protected LSD and/or Duncan's Multiple Range tests (at $P \leq 0.05$). Statistical analyses were carried out using SPSS software version 20. All the experiments were repeated twice and for each test, the mean data was presented in the current study.

RESULTS

Effect of nitrogen sources on *S. rolfsii* mycelial growth and survival

Effect on radial mycelial growth

The mean diameter of *S. rolfsii* colonies, noted after 3 days of incubation at 30 °C, varied significantly (at $P \leq 0.05$) depending on N sources only but no significant differences were noted between the three tested isolates. Furthermore, as no significant interaction was noted between both factors, results were presented and commented considering the mean radial growth per N source only as given in Figure 1.

All *S. rolfsii* isolates showed optimum mycelial growth on the basal medium amended with ammonium chloride (20.08 - 20.45 mm/day) but complete growth inhibition occurred on ammonium acetate treated medium. Sodium nitrate and potassium nitrate supported also good radial growth of all isolates with 18.7-18.94 mm/day and 17.8-18.52 mm/day, respectively. The poorest mycelial growth was recorded on basal medium amended with L-arginine (8.67-10.34 mm/day) (Figure 1).

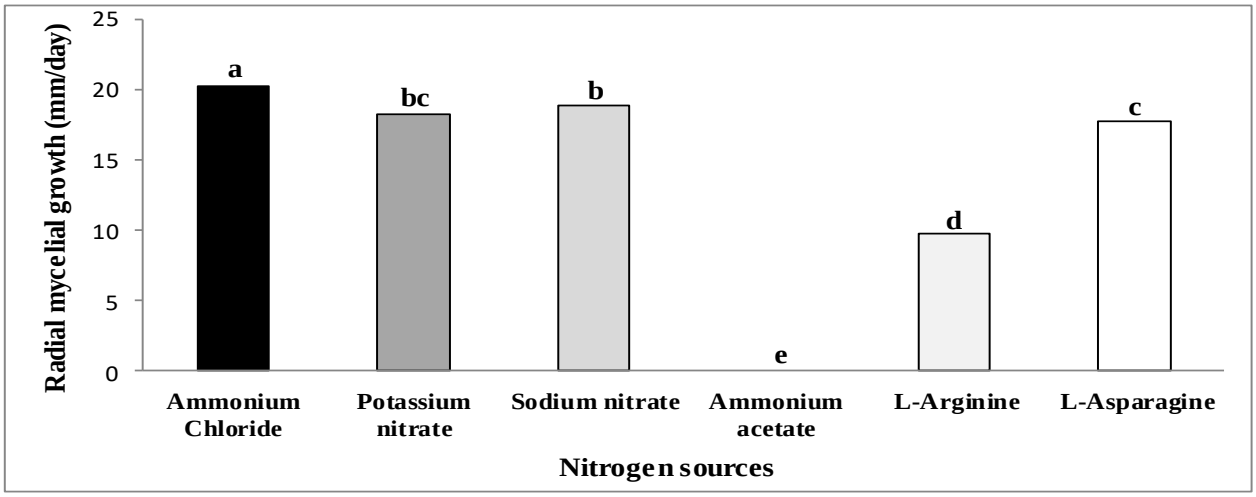

Figure 1. Effect of various nitrogen sources (0.48 g of $N.L^{-1}$) on the mycelial growth of *Sclerotium rolfsii* (combined data of three isolates) recorded on basal medium, amended with D-glucose (16 g of $C.L^{-1}$), after 3 days of incubation at 30°C in the dark.

Bars sharing the same letter are not significantly different according to Duncan Multiple Range test (at $P \leq 0.05$).

Effect on sclerotial formation and production

As indicated in Table 1, sclerotial formation was affected by N sources and *S. rolfsii* isolates. Sclerotial initiation of all isolates started after 3 to 12 days of incubation depending on tested treatments. It started on the 3rd day after incubation on basal medium amended with potassium nitrate and L-asparagine, and on the 6-12th days using the remaining nitrogen sources. The network developed to so-called initial and grew to white immature sclerotium on the 6-15th day of incubation and brown mature sclerotia were observed only after 12-21 days. Potassium nitrate and sodium nitrate were the most suitable N sources for *S. rolfsii* sclerotial formation as sclerotia became brownish at the 9-15th and 12-18th day of incubation, respectively. However, a complete inhibition of any sclerotial development, till 21days of culture, was recorded on basal growth medium amended with ammonium acetate .

Table 1. Effect of various nitrogen sources (0.48 g of $N.L^{-1}$) on the sclerotial development of three *Sclerotium rolfsii* isolates on basal growth medium, amended with D-glucose (16 g of $C.L^{-1}$), noted during 21 days of incubation at 30°C in the dark.

N Sources	Isolates	Days after incubation		
		First initials	White sclerotia	Dark mature sclerotia
Ammonium chloride	Sr1	9	12	15
	Sr2	6	9	12
	Sr3	12	-	-
Potassium nitrate	Sr1	3	6	9
	Sr2	3	6	15
	Sr3	3	6	12
Sodium nitrate	Sr1	6	9	12
	Sr2	6	9	15
	Sr3	6	12	18
Ammonium acetate	Sr1	-	-	-
	Sr2	-	-	-
	Sr3	-	-	-
L-Arginine	Sr1	6	15	18
	Sr2	6	15	21
	Sr3	12	-	-
L-Asparagine	Sr1	3	9	12
	Sr2	3	6	9
	Sr3	3	9	-

The average number of mature sclerotia produced per plate, after 21 days of incubation, varied significantly (at $P \leq 0.05$) depending on N sources tested and isolates used. A significant interaction was also observed between these two factors. As indicated in Table 2, the highest sclerotial yields were noted on Sr2 and Sr3 cultures grown on potassium nitrate amended medium (102.6 and 33.2 sclerotia/plate, respectively), while Sr1 formed significantly more sclerotia on ammonium chloride (6.6 sclerotia/plate), potassium nitrate (21.4 sclerotia/plate) and sodium nitrate (22.6 sclerotia/plate) amended media followed by those modified using the other N sources (Table 2).

For all N sources tested (pooled data of all N sources), Sr2 isolate produced significantly (at $P \leq 0.05$) more sclerotia than Sr1 and Sr3.

Effect on sclerotial germination

After 24 h of incubation at 30°C, the germination of sclerotia of all of *S. rolfsii* isolates occurred on all N source-amended media and varied significantly (at $P \leq 0.05$) depending on tested N sources and isolates as indicated by the significant interaction noted between these two fixed factors. As shown in Table 3, optimal germination was recorded on ammonium chloride, L-Arginine and L-Asparagine amended basal media, for Sr1 and Sr2 isolates, while that of Sr3 was noted only using L-Arginine. However, the lowest sclerotial germination

rate was observed on sodium nitrate added to the basal medium for all tested isolates (Table 3).

For the data of all N sources combined, the highest number of germinated sclerotia was recorded for Sr1 isolate (76.8%) followed by Sr2 (68.4%) and Sr3 (48.4%).

Table 2. Effect of various nitrogen sources (0.48 g of $N.L^{-1}$) on the number of sclerotia produced by three *Sclerotium rolfsii* isolates after 21 days of incubation at 30°C on basal growth medium amended with D-glucose (16 g of $C.L^{-1}$).

N Sources	Number of sclerotia per plate			Mean no. of sclerotia per N source[1]
	Sr1	Sr2	Sr3	
Ammonium chloride	6.6 ab	31.8 b	0 b	12.8 b
Potassium nitrate	21.4 a	102.6 a	33.2 a	52.4 a
Sodium nitrate	22.6 a	22.6 b	12.4 b	19.2 b
Ammonium acetate	0 b	0 c	0 b	0 c
L-Arginine	1.2 b	1.2 c	0 b	0.8 c
L-Asparagine	0.2 b	1.6 c	0 b	0.6 c
Mean no. of sclerotia per isolate[2]	8.7 b	26.6 a	7.6 b	-

[1]Mean number of sclerotia per N source for the three isolates combined.

[2]Mean number of sclerotia per isolate for all N sources combined.

*LSD (N sources × *S. rolfsii* isolates) = 11.29 sclerotia at $P \leq 0.05$.

*For each isolate and each mean number of sclerotia (per N source or per isolate), values followed by the same letter are not significantly different according to Duncan Multiple Range test (at $P \leq 0.05$).

Table 3. Effect of various nitrogen sources (0.48 g of $N.L^{-1}$) on the sclerotial germination of three *Sclerotium rolfsii* isolates noted after 24 h of incubation at 30°C on basal medium with amended with D-glucose (16 g of $C.L^{-1}$).

N Sources	Sclerotial germination (%)			Mean sclerotial germination per N source (%)[1]
	Sr1	Sr2	Sr3	
Ammonium chloride	80 ab	88 a	46 c	71.3 b
Potassium nitrate	76 b	70 b	62 b	69.3 b
Sodium nitrate	54 c	26 c	8 d	29.3 c
L-Arginine	80 ab	78 ab	80 a	79.3 a
L-Asparagine	94 a	80 ab	46 c	73.3 b
Mean sclerotial germination per isolate (%)[2]	76.8 a	68.4 b	48.4 c	-

[1]Mean sclerotial germination per N source for the three isolates combined.

[2]Mean sclerotial germination per isolate for all N sources combined.

* Mycelial growth and sclerotial formation were completely inhibited on ammonium acetate amended basal medium. Therefore, sclerotial germination was not recorded in this study.

*LSD (N sources × *S. rolfsii* isolates) = 8.43% at $P \leq 0.05$.

*For each isolate and each mean of sclerotial germination (per N source or per isolate), values followed by the same letter are not significantly different according to Duncan Multiple Range test (at $P \leq 0.05$).

Effect of carbon source on *S. rolfsii* growth and survival

Effect on radial mycelial growth

The different *S. rolfsii* isolates responded differently to C sources as illustrated by the significant (at $P \leq 0.05$) interaction recorded between isolates and C sources tested for their effects on pathogen mycelial growth. Optimal radial growth occurred on D-mannitol amended basal medium for Sr1 and Sr2 isolates with an average rate of 22.5 and 22.8 mm/day, respectively, whereas Sr3 isolate grew faster (20.6 mm/day) on maltose-amended medium. D-glucose supported also an important radial growth of all isolates (19-20.7 mm/day), but the mycelial growth was restricted on glycerol-amended medium with 15.7-18.1 mm/day). Nevertheless, no mycelial growth was observed after 3 days of incubation at 30°C on culture medium modified using sodium citrate as C source (Table 4).

For all C sources combined, the highest mycelial growth was recorded on Sr1 and Sr2 cultures.

Effect on sclerotial formation and production

As for the mycelial growth, all C sources tested, except sodium citrate, were suitable for the sclerotial formation in *S. rolfsii* as sclerotial initiation started on the 3rd day after incubation and mature sclerotia became brownish at the 6th to 12th day of incubation. However, sclerotial development was completely inhibited on sodium citrate even after 21 days of incubation (Table 5).

Table 4. Effect of various carbon sources (16 g of $C.L^{-1}$) on the mycelial growth of three *Sclerotium rolfsii* isolates recorded after 3 days of incubation at 30 °C on basal medium with amended with potassium nitrate (0.48 g of $N.L^{-1}$).

C Sources	Radial growth (mm/day)			Mean radial growth per C source (mm/day)[1]
	Sr1	Sr2	Sr3	
D-glucose	19.9 b	20.7 b	19 b	.199 c
Glycerol	18.1 c	15.7 c	16.8 c	.169 d
D-Mannitol	22.5 a	22.8 a	19.5 b	21.6 a
Maltose	20.5 b	21.1 b	20.6 a	20.7 b
Sodium citrate	0 d	0 d	0 d	0 e
Mean radial growth per isolate (mm/day)[2]	.162 a	.161 a	.152 b	-

[1]Mean radial growth per C source for the three isolates combined.

[2]Mean radial growth per isolate for all C sources combined.

*LSD (C sources × *S. rolfsii* isolates) = 0.69 mm/day at $P \leq 0.05$.

*For each isolate and each mean radial growth (per C source or per isolate), values followed by the same letter are not significantly different according to Duncan Multiple Range test (at $P \leq 0.05$).

Table 5. Effect of various carbon sources (16 g of $C.L^{-1}$) on sclerotial development of three *Sclerotium rolfsii* isolates on basal medium, amended with potassium nitrate (0.48 g of $N.L^{-1}$), noted during 21 days of incubation at 30 °C in the dark.

C Sources	Isolates	Days after incubation		
		First initials	White sclerotia	Dark mature sclerotia
D-glucose	Sr1	3	6	9
	Sr2	3	6	12
	Sr3	3	6	12
Glycerol	Sr1	-	3	6
	Sr2	3	6	12
	Sr3	3	6	12
D-Mannitol	Sr1	-	3	6
	Sr2	3	6	9
	Sr3	3	6	9
Maltose	Sr1	3	6	9
	Sr2	3	6	12
	Sr3	3	6	9
Sodium citrate	Sr1	-	-	-
	Sr2	-	-	-
	Sr3	-	-	-

The average number of brown sclerotia per plate, produced after 21 days of incubation at 30 °C on basal medium, varied significantly (at $P \leq 0.05$) depending on tested C sources and *S. rolfsii* isolates and their interactions. As given in Table 6, Sr1 isolate showed optimal sclerotial production on D-mannitol or maltose amended basal media, estimated at 11.2 and 8.5 sclerotia/plate, respectively, but was significantly reduced using the other C sources. For Sr2 and Sr3, sclerotial yield was high using D-glucose with an average

of 35 and 70 mature sclerotia/plate, respectively. Nevertheless, when grown on sodium citrate, sclerotial production was very restricted and inhibited. For all the C sources pooled, the number of sclerotia produced by Sr3 (31.3 sclerotia/plate), 21 days after incubation on amended basal medium, was significantly higher than that of the Sr2 (16.5 sclerotia/plate) and Sr1 (5.3 sclerotia/plate) isolates (Table 6).

Effect on sclerotial germination

The germination of *S. rolfsii* sclerotia, after 24 h of incubation on C source amended PDA media at 30 °C, varied significantly (at $P \leq 0.05$) depending on tested C sources and pathogen isolates and on their interactions (Table 7).

Table 6. Effect of various carbon sources (16 g of $C.L^{-1}$) on the number of sclerotia produced by three *Sclerotium rolfsii* isolates after 21 days of incubation at 30 °C on basal medium amended with potassium nitrate (0.48 g of $N.L^{-1}$).

C sources	Number of sclerotia per plate			Mean number of sclerotia per C source[1]
	Sr1	Sr2	Sr3	
D-glucose	2 c	35 a	70 a	35.7 a
Glycerol	5 bc	16.6 b	18.6 c	13.4 c
D-Mannitol	11.2 a	16 b	14 c	13.7 c
Maltose	8.5 ab	14.8 b	53.8 b	25.7 b
Sodium citrate	0 c	0 c	0 d	0 d
Mean number of sclerotia per isolate[2]	5.3 c	16.5 b	.313 a	-

[1]Mean number of sclerotia per C source for the three isolates combined.

[2]Mean number of sclerotia per isolate for all C sources combined.

*LSD (C sources × *S. rolfsii* isolates) = 7.59 sclerotia at $P \leq 0.05$.

*For each isolate and each mean number of sclerotia (per C source or per isolate), values followed by the same letter are not significantly different according to Duncan Multiple Range test (at $P \leq 0.05$).

Table 7. Effect of various C sources (16 g of $C.L^{-1}$) on the sclerotial germination of three *Sclerotium rolfsii* isolates on basal medium, with potassium nitrate (0.48 g of $N.L^{-1}$), noted after 24 h of incubation at 30°C.

C Sources	Sclerotial germination (%)			Mean sclerotial germination per C source (%)[1]
	Sr1	Sr2	Sr3	
D-glucose	96 a	84 b	54 a	78 a
Glycerol	84 b	66 c	34 b	61.3 c
D-Mannitol	96 a	86 b	34 b	72 b
Maltose	92 ab	96 a	48 a	78.7 a
Mean sclerotial germination per isolate (%)[2]	92 a	83 b	42.5 c	-

[1]Mean sclerotial germination per C source for the three isolates combined.

[2]Mean sclerotial germination per isolate for all C sources combined.

*Mycelial growth and sclerotial formation were completely inhibited on sodium citrate amended basal medium. Therefore, sclerotial germination was not recorded in this study. *LSD (C sources × *S. rolfsii* isolates) = 7.81% at $P \leq 0.05$

*For each isolate and each mean of sclerotial germination (per C source or per isolate), values followed by the same letter are not significantly different according to Duncan Multiple Range test (at $P \leq 0.05$).

The sclerotial germination was highest on D-glucose, D-mannitol and maltose modified media for Sr1 isolate (96, 96 and 92%, respectively), on maltose supplemented medium for Sr2 isolate (96%) and on media modified using D-glucose and maltose for Sr3 isolate (54 and 48%, respectively). For all C sources combined, the highest sclerotial germination (92%) was recorded for Sr1, followed by Sr2 (83%) and Sr3 (42.5%) (Table 7).

DISCUSSION

Sclerotium rolfsii, the causal agent of the Southern blight disease, is a soilborne fungus attacking a wide host plants (Galdames and Diaz, 2010 and Kwon *et al.*, 2013 and Shen *et al.*, 2014 and Mahadevakumar *et al.*, 2015).

It occurs worldwide and has a great survival ability under varied environmental and host conditions (Punja, 1985). Previous studies have concentrated on its behavior and on the effect of abiotic factors on its growth and survival (Ayed, 2019 and Ayed *et al.*, 2018a and Ayed *et al.*, 2018b). In the present study, our investigation focused on the effect of nitrogen and carbon sources on the mycelial growth and the sclerotial production and germination of three Tunisian *S. rolfsii* isolates.

All tested isolates showed optimum mycelial growth on ammonium chloride-amended medium. However, their growth was significantly restricted using L-Arginine as N source and was completely inhibited with ammonium acetate. Potassium nitrate, Sodium nitrate, and L-Asparagine were found to be suitable for pathogen growth. These results are in agreement with those of Divya and Narayanba (2017) for the suitability of ammonium chloride, and those of Khattabi *et al.* (2004) for potassium nitrate. Hussain *et al.* (2003) found that potassium nitrate had the ability to enhance *S. rolfsii* mycelial growth. Concerning sodium nitrate, Mostafa and Mohamed (2018) demonstrated that this N source allowed faster growth of *Rhizoctonia solani*. However, L-Arginine was unfavorable unsuitable nitrogen source for the mycelial growth of some fungal pathogens (Shim *et al.*, 2005 and Jayasinghe *et al.*, 2008). Nevertheless, this finding did not confirm a previous study (Muthukumar and Venkatesh, 2013) reporting the lowered mycelial growth of *S. rolfsii* using ammonium chloride as N source. Furthermore, Fariña *et al.* (1999) and Liu and Guo (2009) indicated that when nitrate was used as nitrogen source, fungal growth led to biomass concentrations higher than those obtained with ammonium.

Sclerotial initiation for all *S. rolfsii* isolates started after 3 to 12 days of incubation. Potassium nitrate and sodium nitrate were found to be the most suitable N sources for *S. rolfsii* sclerotial formation as sclerotia became brownish at the 9-15th and 12-18th day of incubation, respectively. However, a complete inhibition of any sclerotial development was recorded using ammonium acetate. The optimal sclerotial production was recorded on potassium nitrate-amended medium. These results confirmed Pany and Apparao (1963) findings reporting that *S. rolfsii* sclerotia were more abundant when potassium nitrate was used as N source. However, Elgorban *et al.* (2014) reported no differences in the number of sclerotia of *Sclerotinia sclerotiorum* grown on culture media supplemented with ammonium chloride and L-Arginine. Optimal germination occurred media amended with L-Arginine, L-Asparagine, and ammonium chloride. Nevertheless, the lowest sclerotial germination rate was recorded using sodium nitrate and ammonium acetate.

In the present study, we also evaluated the suitability of five carbon sources added to a basal medium for the growth of three *S. rolfsii* isolates. Optimal radial growth was noted using D-mannitol for Sr1 and Sr2 isolates and maltose for Sr3. D-glucose supported also an important radial growth of all isolates whereas no mycelial growth was noted using sodium citrate. These findings are in accordance with those of Al-Noimi and Kassim (2006) and Survase *et al.* (2006) who reported a significant mycelial growth of *S. rolfsii* using maltose and glucose. Furthermore, Bhagat (2013) mentioned that maltose and glucose supported a good growth of *S. rolfsii*. Divya and Narayanba (2017) recorded a maximum growth of the fungus when glucose was used as the main carbon source. Chandra and Purkayastha (1977) reported that most of the tropical edible macrofungi were in favor of utilizing glucose than other carbon sources. The preference of glucose over other carbon compounds may be due to its fast metabolization by fungi (Garraway and Evans, 1984). The utilization of various carbon compounds may depend on the activity of the fungus to utilize simpler forms or on its ability to convert the complex carbon compounds into simpler forms, which may be easily utilized (Muthukumar and Venkatesh, 2013). Moreover, the C source not only acts as a major constituent for building of cellular material, but is also used in synthesis of polysaccharide, and as energy source (Dunn, 1985 and Dube, 1983).

All C sources tested, except sodium citrate, were suitable for sclerotial formation, production, and germination. Mature sclerotia became brownish after 6 to 12 days of incubation with an optimal sclerotial production occurring on D-mannitol and maltose modified media for Sr1 isolate and on D-glucose for Sr2 and Sr3. These results are in agreement with other studies reporting that glucose and maltose were the best C sources supporting the formation of sclerotia (Zoberi, 1980). However, Liu and Guo (2009) showed that glucose and maltose, allowing optimal mycelial growth, could not induce sclerotial formation of *Polyporus umbellatus*. The optimum sclerotial germination, noted after 24 h of

incubation at 30°C on basal medium, was noted on growth media supplemented with D-glucose, D-mannitol and maltose for Sr1, with maltose for Sr2, and with D-glucose and maltose for Sr3. Punja *et al.* (1984) demonstrated that the addition of metabolizable carbon sources to substrates low in nutrients inhibits eruptive germination, possibly through catabolite repression. Moreover, Hyakumachi *et al.* (2014) reported that sclerotia become dependent on exogenous nutrients for germination when endogenous carbon loss reached 20% of available ^{14}C label. They become nearly completely dependent on exogenous supply of nutrients for germination, when carbon loss accounted for 40% of labeled carbon and death of sclerotia occurred when this reached about 50%.

CONCLUSION

In conclusion, considerable variations in mycelial growth, sclerotial development, and germination were observed using different N and C nutritional sources for *S. rolfsii* culture. Carbon sources such as ammonium chloride, potassium nitrate and L-Arginine were found to be suitable for mycelial growth, sclerotial production, and germination, respectively. For nitrogen sources, D-mannitol, maltose and D-glucose were found to be the most suitable for radial growth and sclerotial germination. However, further investigations into the effect on pathogen development under field conditions would provide a greater understanding of the biology of *S. rolfsii* isolates. Such studies would improve our understanding of the pathogen's population dynamics in soil and help to implement effective disease control methods.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.