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Research Article

EFFECTS OF NUTRITIONAL AND ENVIRONMENTAL FACTORS ON *PHYLLOSTICTA CITRICARPA*, THE CAUSAL AGENT OF CITRUS BLACK SPOT

Sabrine Mannai, Najwa Benfradj, Naima Boughalleb-M'Hamdi

Higher Institute of Agronomy of Chott Mariem, Department of Biological Sciences and Plant Protection, 4042 Sousse, L21AGR05, University of Sousse, Tunisia.

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ABSTRACT

Citrus black spot, caused by *Phyllosticta citricarpa*, has been reported in Tunisia since 2020 and has negatively impacted yield and marketability. The environmental conditions required for the growth of *P. citricarpa* remain poorly understood, limiting further biological and epidemiological studies. In the present study, the effects of different pH levels, culture media, temperatures, and citrus juices on the development of *P. citricarpa* were evaluated both *in vitro* and *in vivo*. The results showed significant differences in mycelial growth based on pH values, culture media, and their interaction. Mycelial growth ranged from 0 cm on OA medium at pH 5 to 7.26 cm on the same medium at pH 9, one month after incubation. Furthermore, *P. citricarpa* was able to grow within a temperature range of 15°C to 35°C, with optimal colony growth observed at 25°C. Among the citrus juices tested, juice from mature fruits of *Citrus aurantium* exhibited the strongest inhibitory effect on the mycelial growth of *P. citricarpa in vitro* and reduced symptom development *in vivo assay*. These findings enhance our understanding of the optimal conditions for *P. citricarpa* growth and highlight the potential of *C. aurantium* juice as a natural inhibitor for managing citrus black spot.

Corresponding Author: Naima Boughalleb-M'Hamdi
Email: n.boughalleb2017@gmail.com
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INTRODUCTION

Citrus fruits are among the most widely cultivated fruits worldwide (Jaouad et al., 2020) and are affected by many diseases (Irshad et al., 2012; Fateh et al., 2017; Saeed et al., 2019). Citrus Black Spot (CBS) is a major disease that causes significant economic losses in citrus production (Ferreira et al., 2021). The causal agent of CBS is the fungus *Phyllosticta citricarpa*, which is typically identified through conventional techniques such as culturing and microscopy, methods that can take several weeks to complete. This makes the timely identification of infected plants and fruits more challenging (Stringari et al., 2009). Symptoms of CBS may appear only after a latent period of several months (Frare et al., 2019), and infected fruits may develop visible lesions after packing, leading to rejection in export markets and substantial financial losses.

There is limited information on the population structure, reproductive strategies, and introduction pathways of *P. citricarpa*. These aspects have been explored through genotyping studies of isolates from regions including South Africa, the United States, Australia, China, Brazil, and Tunisia (Carstens et al., 2017; Loos et al., 2024). However, there is no consensus in the literature

regarding the specific period during which citrus fruits are most susceptible to infection or the precise duration of the CBS incubation period (Frare et al., 2019).

The epidemiology of CBS is influenced by several factors, including inoculum availability, environmental conditions, the citrus growth cycle, and the age of fruits and leaves (Kotzé, 1981; 2000). Fungal growth and sporulation, whether in submerged, liquid surface, or solid-state culture, are affected by environmental conditions such as the cultivation medium, temperature (Campbell et al., 2001), photoperiod (Vogelgsang and Shamoun, 2002), carbon and nitrogen sources, and pH (Issaly, 2005). In field conditions, factors like humidity, solar radiation, temperature, leaf and fruit wetness, orchard architecture and orientation (Andrade et al., 2009), inoculum concentration, fruit diameter (Frare et al., 2019), and light intensity (Guarnaccia et al., 2019) significantly influence CBS incidence, severity, and latency. However, the specific conditions that support the development of *P. citricarpa* are still not well understood (Wang and Dewdney, 2019).

Darnell-Smith (1918) reported that orange peel extract stimulates conidial germination of freshly discharged *P. citricarpa* spores within 12 hours. Kiely (1984) attributed this effect to citrus compounds such as citric, tartaric, lactic, and oxalic acids. More recently, Wang and Dewdney (2019) observed enhanced spore germination and appressorium formation in the presence of citrus juice, with optimal conditions being pH 3.4, growth on potato dextrose agar (PDA), and incubation for 18-24 h at 24°C. Despite these findings, no selective medium has yet been developed for isolating this pathogen (Peres et al., 2007).

In 2019, CBS was first reported in Tunisia on *Citrus limon* and *C. sinensis* in orchards in the Nabeul governorate (Boughalleb-M'Hamdi et al., 2020). This finding confirmed that *P. citricarpa* can establish itself in dry, Mediterranean-type climates (Martínez-Minaya et al., 2015). To mitigate the impact of the disease, a better understanding of the basic biology and environmental requirements of this pathogen is essential (Buijs et al., 2021). The first critical step in the successful infection and colonization of host tissue by fungal pathogens is spore attachment (Gareth Jones, 1994). Accurate disease identification is therefore crucial for developing effective management strategies aimed at improving fruit quality and yield (Aguilera-Cogley and Vicent, 2019).

Therefore, the objective of the present study was to evaluate the effects of different culture media, pH levels, citrus juice, incubation periods, and temperatures on the growth and sporulation of *P. citricarpa* in pure cultures.

MATERIALS AND METHODS

Isolate of *Phyllosticta citricarpa*

An isolate of *P.citricarpa* (OM679448), obtained from infected *Citrus limon* fruits showing symptoms of CBS in orchards located in Bou Argoub, Tunisia, was used in this study. This isolate has been confirmed as the causal agent of the disease in Tunisia (Benfradj et al., 2024). The fungus was cultured on Potato Dextrose Agar (PDA) medium in 90 mm Petri dishes and incubated at 25°C in the dark for 14 days prior to use.

Effect of pH on growth of *P. citricarpa*

The effect of pH on the growth of *P. citricarpa* was assessed using three media: PDA, Oat Agar (OA), and Malt Extract Agar (MEA). Four different pH levels (5, 6, 7, and 9) were prepared by adjusting the medium with N/10 HCl or NaOH using a pH meter prior to autoclaving. After sterilization, 20 ml of each medium was poured into 90 mm Petri dishes. Once solidified, a 5 mm mycelial plug from the fungal culture was placed at the center of each dish. Each treatment was replicated four times, and the entire experiment was repeated twice. The perpendicular diameters of the fungal colonies were measured weekly for one month. The average radial growth was calculated after subtracting the initial 5 mm diameter of the inoculum plug.

Effect of temperature on growth of *P. citricarpa*

For temperature effect assessment, *P. citricarpa* cultured on PDA at 25°C in darkness for 14 days was used. A 5 mm mycelial plug was transferred to the center of fresh PDA plates (90 mm). Each treatment was replicated four times, and the experiment was repeated twice. Plates were incubated at six different temperatures (15, 20, 25, 30, 35, and 40°C) in darkness for one month. The radial growth of the fungus was measured weekly in four directions from the center of the plug, and the average was recorded.

In vitro effect of citrus juice on mycelial growth of *P. citricarpa*

Fresh juice was extracted from four citrus cultivars at different maturity stages, including *Citrus limon* (mature and immature), *C. aurantium* (mature and immature), *C. sinensis* (mature), and *C. limetta* (mature). The juices were filtered using sterile filter paper, sterilized using 0.45 µm membrane filters, and stored at -20°C until use. Four juice concentrations (10, 10^{-1}, 10^{-2}, and 10^{-3}) were prepared

(Table 1). For each treatment, 100 µl of citrus juice at the desired concentration was aseptically added to 20 ml of PDA cooled to 45°C, mixed thoroughly, and poured into 90 mm Petri dishes. After solidification, a 5 mm agar plug containing the fungus was placed in the center of each plate. Plates were then incubated at 25°C in the dark. Each treatment was replicated four times.

Table 1. Types and concentrations of citrus juices used in the study.

Citrus juice	Doses			
	10	10^{-1}	10^{-2}	10^{-3}
Mature lemon	J1.1	J1.2	J1.3	J1.4
Immature lemon	J2.1	J2.2	J2.3	J2.4
Mature orange	J3.1	J3.2	J3.3	J3.4
Immature orange	J4.1	J4.2	J4.3	J4.4
Mandarin	J5.1	J5.2	J5.3	J5.4
Sweet lemon	J6.1	J6.2	J6.3	J6.4

***In vivo* effect of citrus juice on severity of CBS**

The most effective citrus juice identified in the previous *in vitro* assay was selected for *in vivo* evaluation to determine its effect on the development of *P. citricarpa* lesions. The juice was adjusted to pH levels ranging from 2 to 9 at intervals of 1 pH unit using 1 N HCl or 1 N NaOH, measured with a pH meter (Thermo Fisher Scientific). The solutions were then filter-sterilized using 0.45 µm membrane filters.

The efficacy of the citrus juice was tested on citrus fruits naturally infected with CBS, which exhibited typical hard spot symptoms. Six distinct dark lesions were selected on each fruit for evaluation. A volume of 10 µl of citrus juice at each pH level was applied to the center of each lesion, and the fruits were incubated at ambient temperature. Six fruits were used per treatment. The diameter of each dark spot was measured weekly for two months using a ruler. For the control treatment, the same volume of sterile distilled water was applied instead of citrus juice.

Statistical analyses

Statistical analyses were performed using analysis of variance (ANOVA). For the *in vitro* and *in vivo* assays, a completely randomized factorial design was used to analyze the effect of pH and citrus juice on *P. citricarpa* mycelial growth, with two fixed factors. The effect of temperature on *P. citricarpa* growth was analyzed using a completely randomized design, with a sole fixed factor. Data analysis was conducted using SPSS software version 20 (IBM SPSS, Armonk, NY, USA), and mean separations were performed using the Student-Newman-Keuls (S-N-K) test at $p < 0.05$.

RESULTS

Effect of pH on growth of *P. citricarpa*

The results indicated a significant effect ($P < 0.05$) of pH, culture media, and their interaction on the mycelial growth of *P. citricarpa*, starting from the second evaluation date (14 days after incubation). No fungal growth was observed during the first evaluation (7 days after incubation).

After 14 days of incubation, mycelial growth ranged from 0 cm on PDA and OA media at pH 5 to 3.19 cm on PDA at pH 9. By 21 days, colony diameters ranged from 1.68 cm on PDA at pH 5 to 5.77 cm on PDA at pH 9. After one month of incubation, growth ranged from 0 cm on OA at pH 5 to 7.26 cm on OA at pH 9. Thus, pH 9 supported the highest mycelial growth of *P. citricarpa*, particularly on OA medium at 25°C (Table 2, Figure 1, 2 and 3).

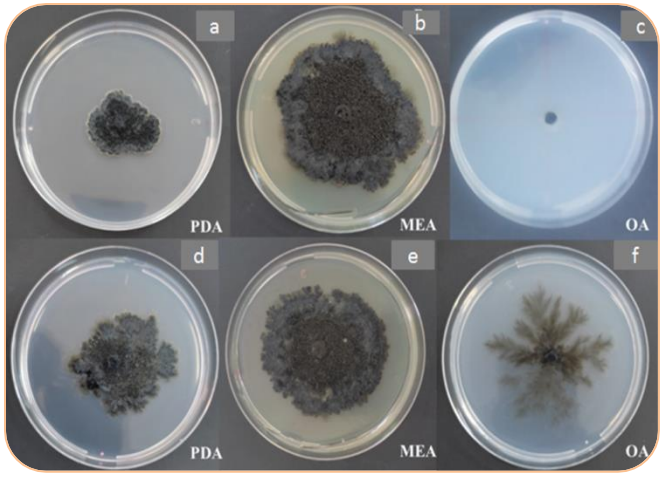


Figure 1. *P. citricarpa* cultured on PDA, OA, and MEA media at pH 5 (a, b, c) and pH 6 (d, e, f).

Effects of temperature on growth of *P. citricarpa*

The results indicated that *P. citricarpa* is capable of growing between 15°C and 35°C, with optimal colony growth observed at 25°C. No growth was recorded at 15°C and 40°C. Statistical analysis revealed a highly significant difference among the four evaluation dates at each tested temperature, based on the SNK test ($P < 0.05$). Mycelial growth ranged from 0.00 cm to 3.11 cm at 20°C, from 1.31 cm to 6.64 cm at 25°C, from 1.11 cm to 5.29 cm at 30°C, and from 0.87 cm to 2.10 cm at 35°C (Figure 4 and 5).

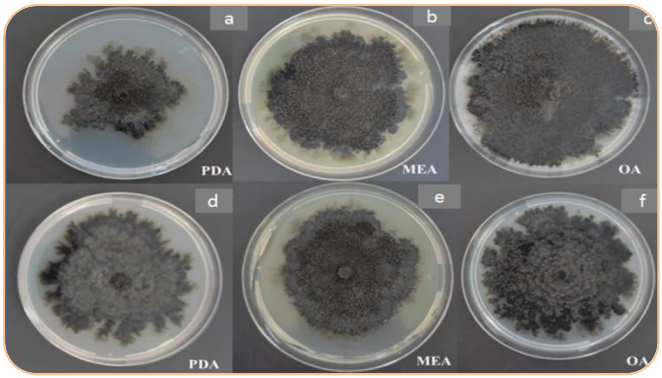


Figure 2. *P. citricarpa* cultured on PDA, OA, and MEA media at pH 7 (a, b, c) and pH 8 (d, e, f).

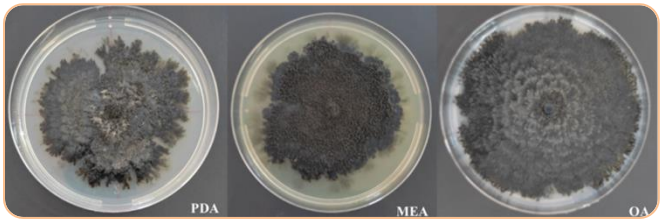


Figure 3. *P. citricarpa* cultured on PDA, OA, and MEA media at pH 9.

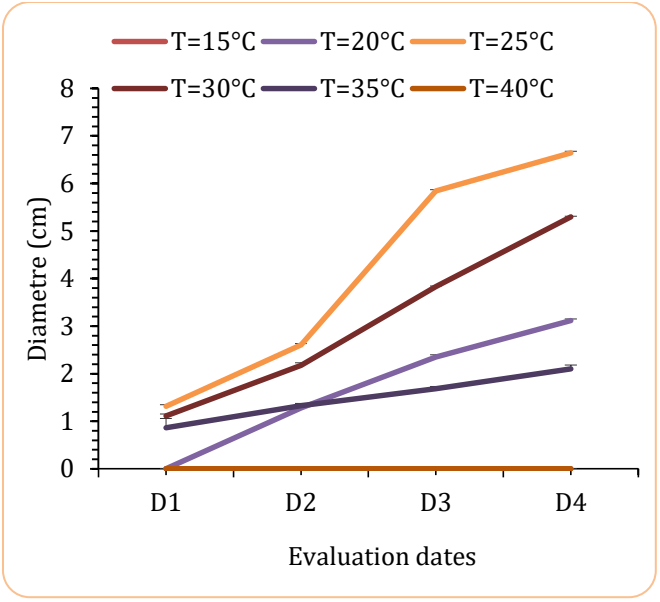


Figure 4. Mycelial development of the citrus black spot pathogen *P. citricarpa* at different temperatures (15, 20, 25, 30, 35, and 40 °C) after 7 (D1), 14 (D2), 21 (D3), and 30 days (D4) of incubation in darkness.

Table 2. Mycelial growth of *P. citricarpa* on PDA, OA, and MEA media at different pH levels, incubated at 25°C in darkness.

		Mycelial growth of *P. citricarpa* at pH levels of				
Medium	Evaluation date	5	6	7	8	9
PDA	D1	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
	D2	0.00±0.00 ^{c}	0.82±0.03^{b}	0.00±0.00 ^{c}	3.16±0.00 ^{a}	3.19±0.00 ^{c}
	D3	1.68±0.39 ^{b}	1.77±0.18 ^{b}	2.22±0.44 ^{b}	4.47±0.56 ^{a}	5.77±0.56 ^{a}
	D4	3.23±0.37 ^{c}	3.44±0.65 ^{bc}	3.73±0.58 ^{b}	5.98±0.32 ^{a}	6.03±0.61 ^{a}
MEA	D1	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}	0.00±0.00 ^{a}
	D2	2.00±0.32 ^{b}	2.47±0.43^{ab}	2.7±0.35 ^{ab}	2.35±0.73^{ab}	2.95±1.26 ^{a}
	D3	3.98±0.38^{bc}	3.85±0.27 ^{c}	4.08±0.46 ^{bc}	4.46±0.70 ^{ab}	4.81±0.66 ^{a}
	D4	5.35±0.53 ^{c}	5.31±0.30 ^{c}	5.73±0.49 ^{b}	5.96±0.61 ^{ab}	6.25±0.19 ^{a}
OA	D1	0.00±0.00 ^{b}	0.00±0.00 ^{b}	0.68±0.00 ^{a}	0.00±0.00 ^{b}	0.00±0.00 ^{b}
	D2	0.00±0.00 ^{b}	2.98±0.69 ^{a}	3.47±0.55 ^{a}	2.95±0.10 ^{a}	2.92±0.12 ^{a}
	D3	0.00±0.00 ^{b}	4.86±0.73 ^{ab}	5.35±0.44 ^{a}	4.3±0.00 ^{b}	4.43±0.80 ^{b}
	D4	0.00±0.00 ^{b}	6.82±0.15 ^{a}	7.12±0.12 ^{a}	7.20±0.11 ^{a}	7.26±0.25 ^{b}

D1: 7 days after incubation, D2: 14 days after incubation, D3: 21 days after incubation, D4: 30 days after incubation. Means followed by the same letter in the same row are not significantly different from each other according to the SNK test (P > 0.05).

In vitro* effect of citrus juice on growth of *P. citricarpa

The results showed significant variation in the mycelial growth of *P. citricarpa* in response to different citrus juices (P ≤ 0.05). On the first evaluation date, the percentage of inhibition ranged from 0% (J2.2, J2.3, and J6.4) to 30.94% (J3.1). On the second date, inhibition ranged from 0% (J1.1, J1.2, J2.2, J2.4, J3.2, J3.3, J3.4, J4.4, J6.2, and J6.4) to 17.08% (J4.2). On the third date, inhibition ranged from 0% (J1.2, J2.1, J2.3, J3.1, J3.3, J3.4, J4.1, and J4.4) to 13.78% (J5.4). On the final evaluation date, inhibition ranged from 0% (J1.1, J1.2, J1.4, J2.2, J2.4, J3.2, J3.3, J3.4, and J4.3) to 12.40% (J5.3). Among the tested juices, mature orange juice at concentration 10 (J3.1) showed the highest inhibitory effect on the mycelial growth of *P. citricarpa* (Figure 6, 7, 8, and 9).

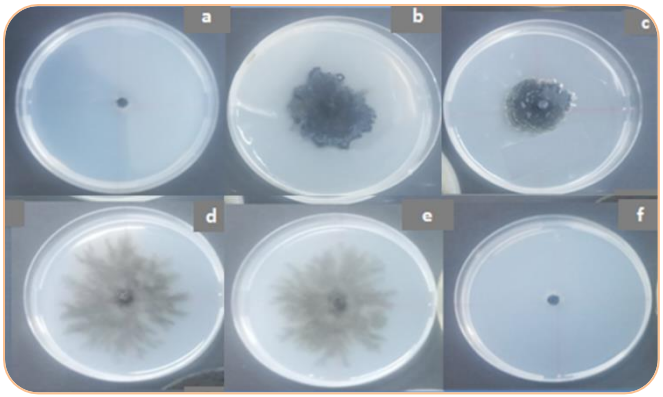


Figure 5. Morphological characteristics of *P. citricarpa* at different temperatures (a: 15°C, b: 20°C, c: 25°C, d: 30°C, e: 35°C, f: 40°C) after 30 days of incubation on PDA in darkness.

In vivo* effect of citrus juice on growth of *P. citricarpa

The mature orange juice (J3.1) at concentration 10, which demonstrated the strongest inhibitory effect *in vitro*, was selected for the *in vivo* experiment. The results revealed a highly significant difference between treatments and among evaluation dates. However, no significant interaction was observed between treatment and evaluation date (treatment × date). After one week of incubation (first evaluation), lesion diameters ranged from 0.10 cm (at pH 7 and pH 8) to 0.18 cm (at pH 10). After two and three weeks of incubation (second and third evaluations), lesion diameters ranged from 0.10 cm (at pH 8) to 0.19 cm (at pH 10). Overall, mature orange juice adjusted to pH 8 was the most effective in reducing lesions caused by *P. citricarpa* (Table 3).

Table 3. Lesion diameters caused by *P. citricarpa* under different pH levels of mature orange juice (J3.1) after 3 weeks of incubation at 25°C in darkness.

pH	Week		
	W1	W2	W3
2	0.13±0.07^{b}	0.15±0.08^{b}	0.15±0.08^{b}
3	0.12±0.03^{b}	0.13±0.04^{b}	0.13±0.04^{b}
4	0.11±0.02^{b}	0.11±0.04^{b}	0.11±0.04^{b}
5	0.11±0.02^{b}	0.12±0.04^{b}	0.12±0.04^{b}
6	0.11±0.02^{b}	0.11±0.03^{b}	0.11±0.03^{b}
7	0.10±0.02^{b}	0.12±0.04^{b}	0.12±0.04^{b}
8	0.10±0.02^{b}	0.10±0.02^{b}	0.10±0.02^{b}
9	0.11±0.02^{b}	0.12±0.04^{b}	0.12±0.04^{b}
10	0.18±0.09^{a}	0.19±0.09^{a}	0.19±0.09^{a}

Values are presented as means ± standard deviation. Treatments in the same column followed by the same letter are not significantly different according to the SNK test at P ≤ 0.05.

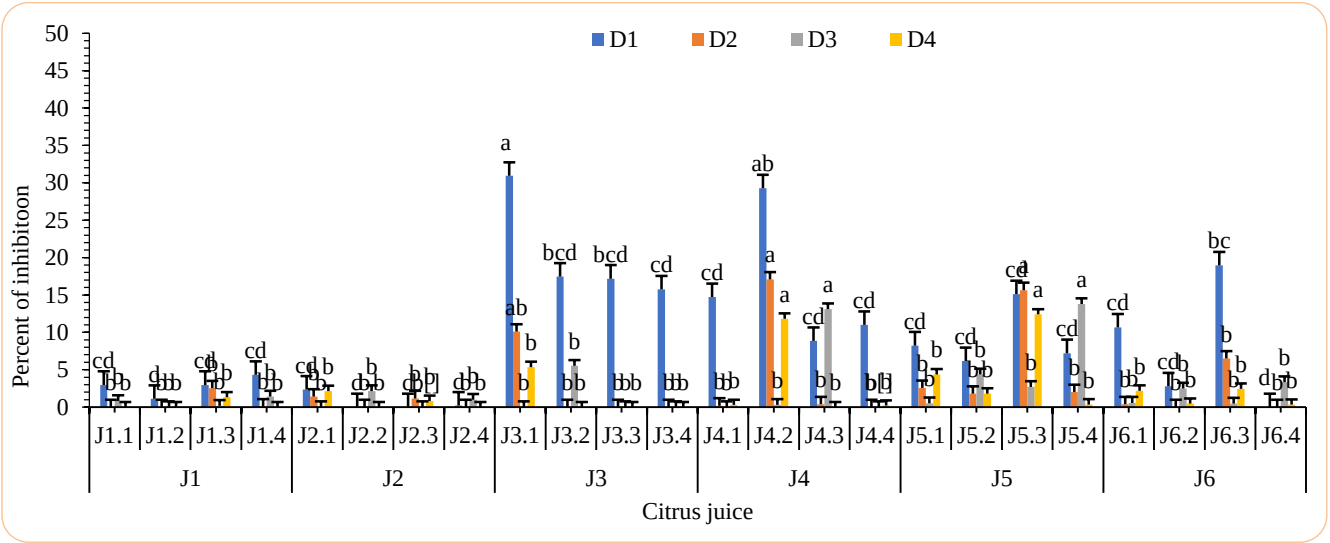


Figure 6. Percentage inhibition of mycelial growth of *P. citricarpa* by six types of citrus juices (J1: mature lemon juice; J2: immature lemon juice; J3: mature orange juice; J4: immature orange juice; J5: mandarin juice; J6: sweet lemon juice) evaluated at four time points (D1: 7 days after incubation; D2: 14 days after incubation; D3: 21 days after incubation; D4: 40 days after incubation). Values represent means ± standard deviation. Treatments within the same evaluation date followed by the same letter are not significantly different according to the SNK test at P ≤ 0.05.

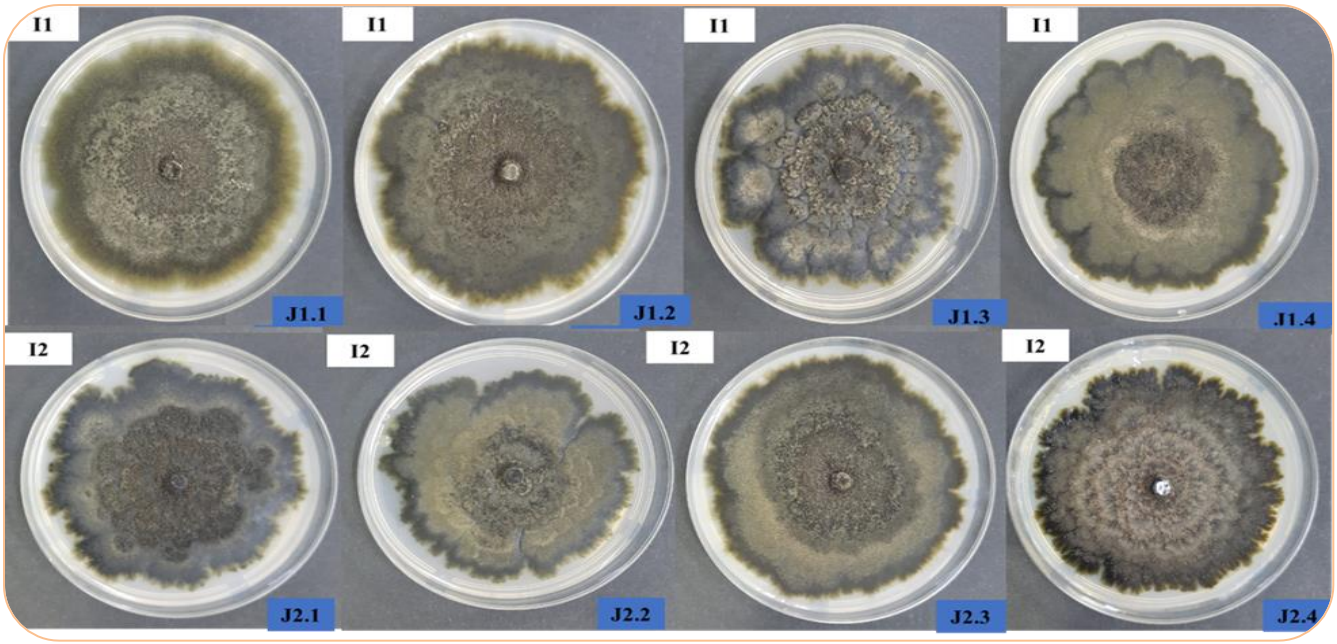


Figure 7. Effect of mature (J1) and immature (J2) *C. limon* juices on the mycelial growth of *P. citricarpa* after 1 month of incubation at 25°C in the dark.

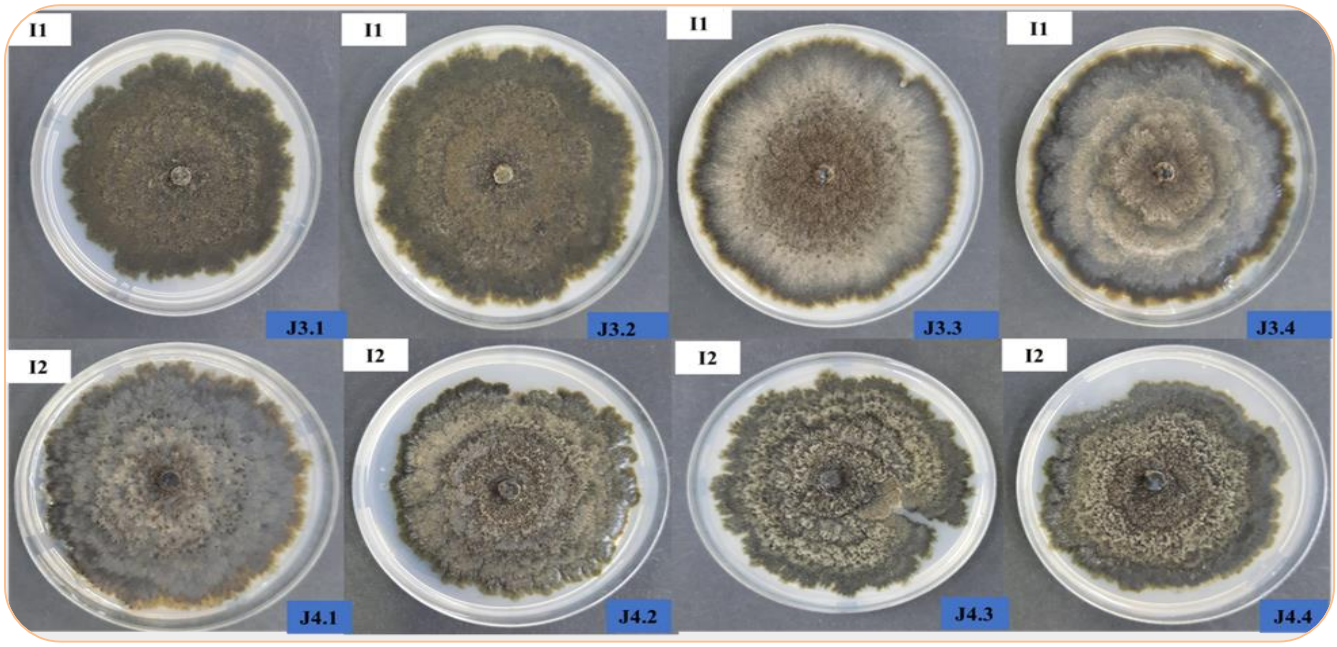


Figure 8. Effect of mature (J3) and immature (J4) *C. aurantium* juice on the mycelial growth of *P. citricarpa* after 1 month of incubation at 25°C in darkness.

DISCUSSION

A successful parasitic relationship between fungal pathogens and host cells is established when several prerequisites involved in the infection process are met, including surface wettability of the substrate, the presence of exogenous nutrients, and favorable environmental conditions (Emmett and Parbery, 1975; Kuo and Hoch, 1996; Blank and Murray, 1998; Liu and Xiao, 2005; Wang et al., 2019). Given the recent emergence of *P. citricarpa* in Tunisia (Boughalleb-M'Hamdi et al., 2020), elucidating the factors that influence its conidial germination is not only fundamental to understanding its biology but also crucial

for developing effective disease control strategies.

In the present study, we evaluated the effects of pH, temperature, and different citrus juices on the development of *P. citricarpa*. The results indicated that pH 9 promoted the highest mycelial growth. Zimmermannová-Pastirčáková (2002) investigated the effects of various nutrient media and pH levels on mycelial growth and sporulation of *P. sphaeropsoidea*, a pathogen causing leaf blotch in *Aesculus* species, and found that optimal colony growth and sporulation occurred at pH 6 to 8.

Furthermore, our study demonstrated that *P. citricarpa* can grow between 15°C and 35°C, with optimal colony growth observed at 25°C. Similarly, Korf (1998) reported an optimal germination temperature of 22°C. However, this fungus is capable of growing across a broader temperature range, from 5°C to 38°C, with an optimum of 26°C (Er et al., 2014). These findings are consistent with those of Brodrick and Rabie (1970) and Guarnaccia et al. (2017), who reported optimal mycelial growth and symptom development at 27°C.

Another study highlighted a rapid decline in the viability and reproductive capacity of *P. citricarpa* in CBS lesions on whole fruit, peel segments, and pulp-exposed peel pieces when subjected to sunlight. During these exposure periods, temperatures around 28°C and relative humidity levels of approximately 75% were recorded (Lanfranchi, 2012; EFSA, 2014). Under such conditions, the survival and reproduction of *P. citricarpa* are expected to be significantly diminished, if not completely halted, as high humidity is essential for lesion and pycnidium formation (Kiely, 1948; Wang and Dewdney, 2015). Indeed, Wang and Dewdney (2015) observed no pycnidium formation below 72% RH, and Kiely (1948) reported spermagonia development only above 73% RH. Conversely, under milder and more humid conditions, longer survival and reproduction periods may be possible and warrant further investigation.

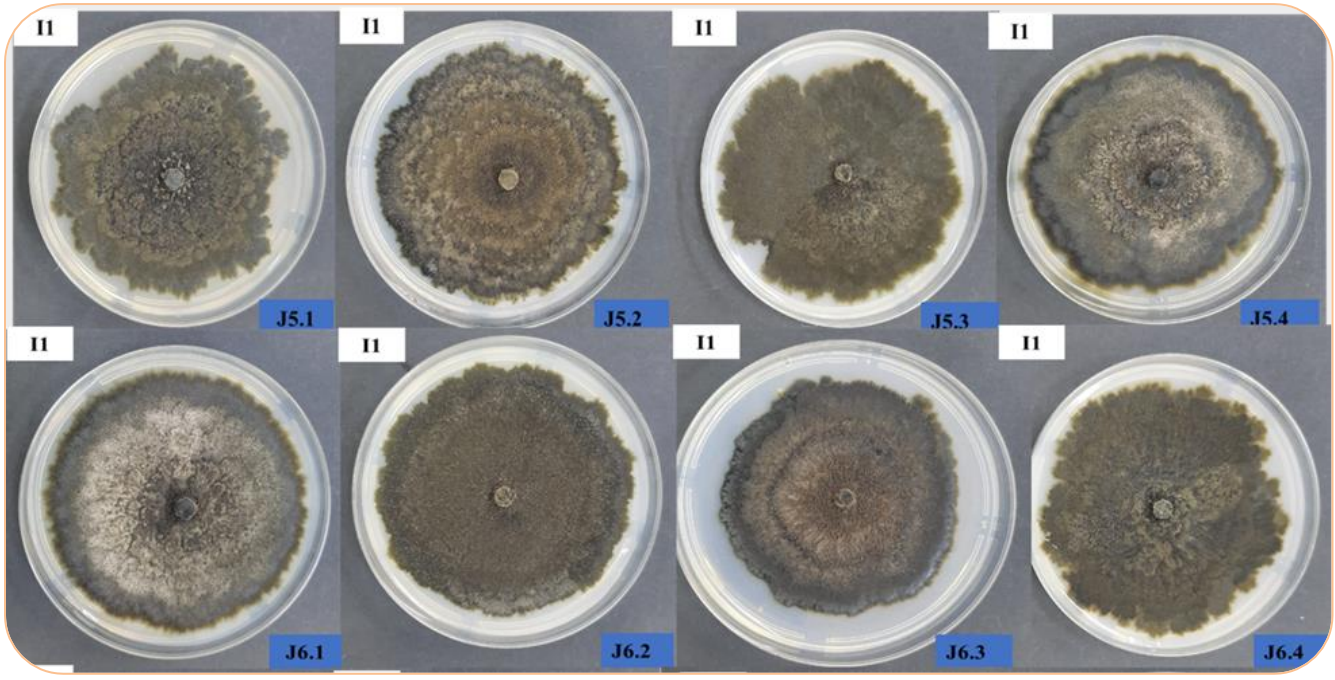


Figure 9. Effect of mature juice from *C. sinensis* (J5) and *C. limetta* (J5) on the mycelial growth of *P. citricarpa* after 1 month of incubation at 25°C in darkness.

Moreover, mature juice of *C. aurantium* exhibited the strongest inhibitory effect on mycelial growth of *P. citricarpa* isolates *in vitro* and reduced symptom development *in vivo*. In fact, mature orange juice adjusted to pH 8 was most effective in minimizing lesions caused by *P. citricarpa*. Our findings partially align with those of Wang and Dewdney (2019), who tested juices from seven citrus cultivars and found that all significantly enhanced conidial germination and appressorium formation, with the highest rates (over 80%) observed in 1.5% 'Valencia' juice. They also revealed that the effects of juice were concentration- and pH-dependent, noting that no conidial

germination occurred in 1.5% 'Valencia' juice at pH levels below 2 or above 7.

Given that natural citrus juice is a complex solution whose composition is influenced by cultivar and fruit maturity, the specific components responsible for antifungal activity remain unidentified. Therefore, Wang and Dewdney (2019) tested a synthetic citrus juice based on the formulation by Cánovas et al. (1997), which proved as effective as natural 'Valencia' juice in promoting conidial germination and appressorium formation. Their analysis of critical components in the synthetic juice identified sugars, salts, citric acid, and thiamine as key contributors to germination and appressorium formation.

These findings enhance our understanding of biology of *P. citricarpa* and offer a reliable, practical system for further applications. Future research should focus on evaluating the effectiveness of the most promising citrus juice extracts in field conditions to reduce the need for chemical treatments, thereby lowering both economic and environmental costs of disease control.

CONCLUSION

This study demonstrated that *P. citricarpa* exhibited the highest mycelial growth on PDA medium adjusted to pH 9 and incubated at 25°C. Furthermore, the development of the fungus was significantly inhibited on media amended with mature *C. aurantium* juice *in vitro*, and the same juice effectively reduced lesion size on *Citrus limon* fruits *in vivo*. Consequently, future trials should assess the efficacy of this juice in managing CBS in infested citrus orchards.

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AUTHORS' CONTRIBUTIONS

NB, SM and NBM conceptualized the studies; designed and developed the methodology; SM and NB carried out the investigations and performed data curation; NB analyzed the data and wrote the original draft; NBM supervised the studies; and NB, SM and NBM proofread and approved the manuscript.

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This research did not receive any grant from funding agencies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

REFERENCES

Aguilera-Cogley, V., Vicent, A. 2019. Etiology and distribution of foliar fungal diseases of citrus in Panama. Tropical Plant Pathology 44(6), 519-532.

Andrade, A. G. D., Pimenta, A. A., Goes, A. D. 2009. Influência do alinhamento de plantio na severidade da mancha-preta-dos-citros, produção e qualidade do suco. Revista Brasileira de Fruticultura 31, 1033-1041.

Benfradj, N., Mannai, S., Jeandel, C., Boughalleb-M'Hamdi, N., 2024. Geographic distribution, prevalence, and incidence of citrus black spot caused by *Phyllosticta citricarpa* in Tunisia. Journal of Phytopathology 172(2), e13292.

Blank, C.A., Murray, T.D., 1998. Influence of pH and matric potential on germination of *Cephalosporium gramineum* conidia. Plant Disease 82, 975-978.

Boughalleb-M'Hamdi, N., Fathallah, A., Benfradj, N., Ben Mahmoud, S., Ali, B. H., Medhioub, A., Jaouadi, I., Huber, J., Jeandel, C., Ioos, R. 2020. First report of citrus black spot disease caused by *Phyllosticta citricarpa* on *Citrus limon* and *C. sinensis* in Tunisia. New Disease Reports, 41(1), 8.

Brodrick, H.T., Rabie, C.J., 1970. Light and temperature effects on symptom development and sporulation of *Guignardia citricarpa* Kiely on *Citrus sinensis* (Linn) Osbeck. Phytophylactica 2, 157-164.

Buijs, V. A., Zuijdgeest, X. C., Groenewald, J. Z., Crous, P. W., De Vries, R. P. 2021. Carbon utilization and growth-inhibition of citrus-colonizing *Phyllosticta* species. Fungal Biology 125(10), 815-825.

Campbell, R. G., Wagner, M. M., Teegarden, G. J., Boudreau, C. A., Durbin, E. G. 2001. Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. Marine Ecology Progress Series 221, 161-183.

Cánovas, M., García-Cases, L., Iborra, J.L., 1997. Shifts in metabolism and morphology of *Rhodococcus fascians* when debittering synthetic citrus juices in

the absence of aeration. Biotechnology Letters 19, 1181-1184.

Carstens, E., Linde, C.C., Slabbert, R., Miles, A.K., Donovan, N.J., Li, H., Zhang, K., Dewdney, M.M., Rollins, J.A., Glienke, C., Schutte, G.C., Fourie, P.H., McLeod, A., 2017. A global perspective on the population structure and reproductive system of *Phyllosticta citricarpa*. Phytopathology 107, 758-768.

Darnell-Smith, G.P., 1918. An account of some observations upon the life-history of Phoma citricarpa McAlp., the cause of the "black spot" disease in citrus fruit in New South Wales. Proceedings of the Linnean Society of New South Wales, 43, 868-881.

EFSA., 2014. PLH Panel (EFSA Panel on Plant Health), Scientific Opinion on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory with identification and evaluation of risk reduction options. EFSA Journal 12, 243.

Emmett, R.W., Parbery, D.G., 1975. Appressoria. Annual Review of Phytopathology 13, 147-165.

Er, H.L., Hendricks, K., Goss, E.M., Smith, M., Schubert, T.S., Roberts, P.D., van Bruggen, A.H.C., 2014. Isolation and biological characterization of *Guignardia* species from citrus in Florida. Journal of Plant Pathology 96, 1-13.

Fateh, F.S., Mukhtar, T., Kazmi, M.R., Abbassi, N.A., Arif, A.M., 2017. Prevalence of citrus decline in district Sargodha. Pakistan Journal of Agricultural Sciences 54, 9-13.

Ferreira, J. R., Sierra-Garcia, I. N., Guieu, S., Silva, A., da Silva, R. N., Cunha, Â. 2021. Photodynamic control of citrus crop diseases. World Journal of Microbiology and Biotechnology 37(12), 1-11.

Frare, G.F., Silva-Junior, G.J., Bassanezi, R.B., Ramires, T.G., Amorim, L., 2019. Sweet orange fruit age and inoculum concentration affect expression of citrus black spot symptoms. Plant Disease 103, 913-921.

Gareth Jones, E. B. 1994. Fungal adhesion. Mycological Research, 98961-981.

Guarnaccia, V., Groenewald, J. Z., Li, H., Glienke, C., Carstens, E., Hattingh, V., Fourie, P.H., Crous, P. 2017. First report of *Phyllosticta citricarpa* and description of two new species, *P. paracapitalensis* and *P. paracitricarpa*, from citrus in Europe. Studies in mycology, 87(1), 161-185.

Guarnaccia, V., Gehrmann, T., Silva-Junior, G. J., Fourie, P. H., Haridas, S., Vu, D., Spatafora, J., Martin, M., Robert, V., Grigoriev, I. V., Groenewald, J. Z., Crous, P. W. 2019. *Phyllosticta citricarpa* and sister species of global importance to Citrus. Molecular Plant Pathology, 20(12), 1619-1635.

Irshad, U., Mukhtar, T., Ashfaq, M., Kayani, M.Z., Kayani, S.B., Hanif, M., Aslam, S., 2012. Pathogenicity of citrus nematode (*Tylenchulus semipenetrans*) on *Citrus jambhiri*. Journal of Animal and Plant Sciences 22, 1014-1018.

Issaly, N., Chauveau, H., Aglevor, F., Fargues, J., Durand, A. 2005. Influence of nutrient, pH and dissolved oxygen on the production of *Metarhizium flavoviride* Mf189 blastospores in submerged batch culture. Process Biochemistry 40(3-4), 1425-1431.

Jaouad, M., Moinina, A., Ezrari, S., Lahlali, R. 2020. Key pests and diseases of citrus trees with emphasis on root rot diseases: An overview. Moroccan Journal of Agricultural Sciences 1(3), 149-160.

Kiely, T.B., 1948. Preliminary studies on *Guignardia citricarpa* (n. sp.), the ascigerous stage of *Phoma citricarpa* McAlp., and its relation to black spot of citrus. Proceedings of the Linnean Society of New South Wales 73, 249-292.

Korf, H.J.G., 1998. Survival of *Phyllosticta citricarpa*, anamorph of the citrus black spot pathogen. University of Pretoria, Pretoria, South Africa, p. 72.

Kotzé, J.M., 1981. Epidemiology and control of citrus black spot in South Africa. Plant Disease 65, 945-950.

Kotzé, J. M. 2000. Black spot. In L. W. Timmer, S. M. Garnsey, & J. H. Graham (Eds.), Compendium of citrus diseases (2nd ed., pp. 23–25). The American Phytopathological Society.

Kuo, K., Hoch, H.C., 1996. Germination of *Phyllosticta ampelicida* pycnidiospores: prerequisite of adhesion to the substratum and the relationship of substratum wettability. Fungal Genetics and Biology 20, 18-29.

Lanfranchi, M., 2012. Economic analysis on the enhancement of citrus waste for energy production. Journal of Essential Oil Research 24, 583-591.

Liu, Q., Xiao, C.L., 2005. Influence of nutrient and environmental factors on conidial germination of *Potebniamyces pyri*. Phytopathology 95, 572-580.

Loos, R., Mannai, S., Jeandel, C., Benfradj, N., Vicent, A., Boughalleb-M'hamdi, N., Aguayo, J., 2024. Mating

type and microsatellite genotyping indicate that the Tunisian population of *Phyllosticta citricarpa* is clonal and thrives only asexually. Fungal Biology 128, 1806-1813.

Martínez-Minaya, J., Conesa, D., López-Quílez, A., Vicent, A., 2015. Climatic distribution of citrus black spot caused by *Phyllosticta citricarpa*: a historical analysis of disease spread in South Africa. European Journal of Plant Pathology 143, 69-83.

Peres, N. A., Harakava, R., Carroll, G. C., Adaskaveg, J. E., Timmer, L. W. 2007. Comparison of molecular procedures for detection and identification of *Guignardia citricarpa* and *G. mangiferae*. Plant Disease 91(5), 525-531.

Saeed, M., Mukhtar, T., Rehman, M.A., 2019. Temporal fluctuations in the population of citrus nematode (*Tylenchulus semipenetrans*) in the Pothowar region of Pakistan. Pakistan Journal of Zoology 51, 2257-2263.

Stringari, D., Glienke, C., Christo, D. D., Maccheroni Jr, W., Azevedo, J. L. D. 2009. High molecular diversity of the fungus *Guignardia citricarpa* and *Guignardia mangiferae* and new primers for the diagnosis of the citrus black spot. Brazilian archives of biology and technology 52, 1063-1073.

Vogelgsang, S., Shamoun, S.F., 2002. Growth, sporulation, and conidia discharge of *Valdensinia heterodoxa*, a foliar pathogen of salal, as influenced by temperature and photoperiod *in vitro*. Mycological Research 106, 480-490.

Wang, N.Y., Dewdney, M.M., 2015. The effect of relative humidity on colonization of citrus twigs by *Phyllosticta citricarpa*, the citrus black spot pathogen. Phytopathology 105, 145-146.

Wang, N.Y., Dewdney, M.M., 2019. The effects of nutrition and environmental factors on conidial germination and appressorium formation of *Phyllosticta citricarpa*, the causal agent of citrus black spot. Phytopathology 109, 650-658.

Zimmermannová-Pastirčáková, K., 2002. Occurrence of Horsechestnut leaf blotch and cultural characteristics of its causal agent-fungus *Phyllosticta sphaeropsoidea*, an anamorph of *Guignardia aesculi*. Folia Oecologica 30, 245-250.