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CHARACTERIZATION OF THE PATHOGEN CAUSING EARLY BLIGHT DISEASE ON POTATO

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ABSTRACT

Early blight of potato caused by *Solanum tuberosum* L., is one of the most significant foliar diseases in Algeria, ranking second only to late blight in terms of economic impact. This study aimed to characterize Alternaria isolates associated with early blight based on their cultural, morphological, physiological, and pathological features. The analysis revealed considerable variation among isolates, allowing the identification of two distinct species: *Alternaria solani* (six isolates) and *Alternaria alternata* (seven isolates). Pathogenicity tests indicated that neither of the two tested potato cultivars, Arizona and Rudolph, exhibited resistance to early blight. In contrast, biocontrol assays yielded promising results, demonstrating substantial inhibition of mycelial growth, ranging from 40% to 84%, and a marked reduction in disease severity on both cultivars. These findings highlight the potential of biological control strategies as an alternative to chemical fungicides. However, further validation under field conditions is necessary to confirm their effectiveness in natural environmental and agronomic settings.

Keywords: *Alternaria alternata, Alternaria solani,* Morphological, *Solanum tuberosum*, Screening

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Potato (*Solanum tuberosum* L.) is a major agricultural crop in Algeria, ranking second in importance after cereals. In 2022, over 130,396 hectares were cultivated, yielding more than 4,299,816 tons of potatoes (FAO, 2024). Early blight, primarily caused by *Alternaria solani* Sorauer and *Alternaria alternata* (Fr.) Keissler, poses a significant threat to potato production (Takataev *et al.*, 2024). Yield losses of 30-35% can occur when more than 40% of plants in a field are affected. The development and severity of early blight are closely linked to plant age and environmental factors such as high temperatures, elevated humidity, and abiotic stress conditions (Wolters *et al.*, 2021). These pathogens cause considerable damage by accelerating leaf senescence, thereby reducing photosynthetic capacity and ultimately impacting tuber yield. The progression of the disease is strongly influenced by both environmental conditions and host plant physiology (Leiminger and Hausladen, 2012).

Chemical control remains effective against early blight during the main cultivation season (spring), but its efficacy significantly declines during the off-season period in greenhouses (summer) (Wolters *et al.*, 2021). Currently, no potato cultivar with confirmed resistance to early blight are commercially available, and disease management relies heavily on fungicide application (Abuley and Nielsen, 2017). However, this strategy is not sustainable in the long term due to the emergence of fungicide-resistant *Alternaria* strains and the negative environmental impacts associated with chemical treatments (Landschoot *et al.*, 2017). The increasing resistance of *Alternaria* spp. to fungicides, combined with climatic conditions that favour disease development, highlights the urgent need for alternative control methods, particularly the development and deployment of resistant cultivars.

Genetic resistance through the cultivation of resistant potato varieties represents one of the most effective and sustainable strategies for managing early blight. Resistance screening is typically performed using artificial inoculation under controlled conditions, either in field trials or via detached leaf assays (Wolters *et al.*, 2021). To

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improve disease control, a thorough understanding of the physiological, cultural, and morphological characteristics of the two primary causal agents, *Alternaria solani* and *A. alternate*, is essential.

This study aimed to:

i) identify the *Alternaria* species responsible for early blight in potato based on morphological and pathogenic characteristics;

ii) characterize the cultural and morphological traits of *A. solani* and *A. alternata*, including responses to different pH levels and culture media; and

iii) evaluate the resistance of two widely cultivated potato varieties in Algeria to early blight through artificial inoculation under controlled conditions.

MATERIALS AND METHODS

Fungal strains

A total of thirteen *Alternaria* isolates were obtained from infected potato (*Solanum tuberosum* L.) leaves displaying typical early blight symptoms, collected from different fields (Table 1). The fungal isolates were purified through multiple sub-culturing steps, followed by single-spore (monospore) isolation to ensure genetic uniformity. All purified isolates were maintained on Potato Dextrose Agar (PDA) at 4°C for further morphological, cultural, physiological, and pathogenic characterization.

All *Alternaria* isolates were obtained from symptomatic potato leaves collected from fields in the Mostaganem region, Algeria. A total of thirteen isolates were purified and maintained for further analysis (Table 1). Specifically, ten isolates (03, 04, 14, 19, 20, 21, 23, 24, 27, 28) were obtained from the 'Spunta' variety in the Sirat area; two isolates (05, 18) were collected from the same variety in the Hassi Mameche area; and one isolate (30) was collected from the 'Fabula' variety in the Garden Valley area. Identification of the isolates to species level was based on morphological characteristics and pathogenicity, following the criteria established by Zheng *et al.* (2015).

Plant material

Two potato (*Solanum tuberosum* L.) varieties, Arizona and Rudolph, were used for resistance screening against early blight (Fig. 1). The Arizona variety originates from the United Kingdom and was developed through the breeding program designated UK 150-19D22. The Rudolph variety, also of British origin, is the result of a cross between the cultivars Chieftain and Stirling. Both varieties are widely cultivated and represent common commercial genotypes in Algerian potato production systems.

Culture medium

All *Alternaria* isolates and the actinomycete strain

Table 1. Origin and cultural/morphological characteristics of *Alternaria* spp. isolates collected from potato leaves

Fungal species	Isolate code	Origin of sampling	Cultural colour on PDA	Conidial morphology		Beak size (µm)
				Number of conidial septation	Conidial size (µm)	
Alternaria solani	AS03	Sirat, Mostaganem	Brown	2-3	13,2 × 34,5	5,5
	AS04	Sirat, Mostaganem	Brown	3-4	13 × 44,4	6,5
	AS05	Hassi Mamech, Mostaganem	Brown	2-3	13 × 54,2	4,6
	AS19	Sirat, Mostaganem	Brown	4-5	12 × 51,3	6,7
	AS24	Sirat, Mostaganem	Brown	3-4	12 × 33,2	6,1
	AS30	Garden valley, Mostaganem	Brown	2-3	11 × 32,2	5,4
Alternaria alternata	AA14	Sirat, Mostaganem	Brown to green	0-1	6,2 × 22	3,2
	AA18	Hassi Mamech, Mostaganem	Brown greyish	0-1	9,1 × 16,7	3,1
	AA20	Sirat, Mostaganem	Brown	0-1	7,5 × 14,2	2,7
	AS21	Sirat, Mostaganem	Brown	0-1	7,2 × 12,5	1,8
	AA23	Sirat, Mostaganem	Brown to green	0-1	5,5 × 11,4	2,1
	AA27	Sirat, Mostaganem	Brown	0-1	6,7 × 10,9	2,4
	AA28	Sirat, Mostaganem	Brown	0-1	7,8 × 12,3	2,8

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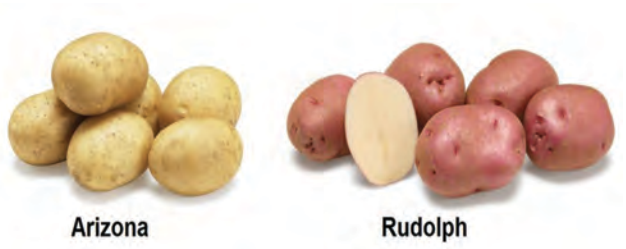


Fig. 1. The tubers shape of two potato varieties, Arizona and Rudolph

Streptomyces avermitilis, provided by Bouslama *et al.* (2025) from the Laboratory of Microbiology at Abdelhamid Ibn Badis University of Mostaganem, Algeria, were maintained and cultivated on Potato Dextrose Agar (PDA). The strain of *S. avermitilis* belongs to the mesophilic group of actinomycetes. The PDA medium was prepared with the following composition per liter: 200 g potato extract (potato juice), 20 g glucose, and 15 g agar, with the final pH adjusted to 5.5. All components were supplied by Sigma Co., France (Beliaev *et al.*, 2021).

Physiological study

Physiological parameters such as pH and culture medium composition play a critical role in the growth and development of phytopathogenic fungi, including *Alternaria* species. In this study, the effects of different pH levels and types of culture media on the mycelial growth of *Alternaria* isolates were investigated, following the approach described by Vijaya-Lakshmi *et al.* (2013). Mycelial development was evaluated under three pH conditions: 7.0 (neutral), 9.0 (alkaline), and 11.0 (highly alkaline). Simultaneously, the isolates were cultivated on three different types of culture media: Potato Dextrose Agar (PDA), Oxytetracycline-Glucose-Yeast Extract Agar (OGA), and Plate Count Agar (PCA). The interaction between culture medium and pH was assessed by measuring radial mycelial growth under standardized incubation conditions.

The composition of these three culture media is as follow:

- PDA per 1l & pH=5,7

Difco™ Potato Dextrose Agar Approximate Formula*
Per Liter Potato Starch (from infusion)**..... 4.0 g
Dextrose ..... 20.0 g
Agar ..... 15.0 g
*Adjusted and/or supplemented as required to meet performance criteria.
**Approximates 200 g of infusion from potatoes.
Difco™ Potato Dextrose Agar = 39g

- OGA per 1l & pH=5,5

Yeast extract ..... 5,00 g
Glucose ..... 20,00 g
Agar ..... 12,00 g
This medium can be adjusted and/or supplemented according to the performance criteria imposed.

- PCA per 1l & pH=7

Composition (g/l):
Casein peptone..... 5,00
Yeast extract..... 2,50
Dextrose..... 1,00
Agar..... 15,0

Mycelial growth evaluation

The mycelial growth of isolates was assessed by measuring the radial diameter of mycelial growth of Alternaria isolates for 6 days of incubation, with four repetitions for each essay (Howell, 2003).

$L = (D - d)/2$

With: L: Mycelial growth (mm); D: Colony diameter (mm); and d: Explant diameter (5mm).

The mycelial growth averages are calculated by the following formula:

$V(mm/j) = \Sigma (Ln - Ln\text{-}1)/ n$

With: V: Mycelial growth per day (mm/j); and Ln, Ln-1 … Mycelial growth during the day n

Detached leaves screening test

Leaflets were excised from two potato varieties, Arizona and Rudolph, then surface-sterilized in 2% sodium hypochlorite solution for 3 minutes, followed by three rinses with sterile distilled water (Chaerani *et al.*, 2007). For each replication, three leaflets were used in the inoculation assay. To prepare the fungal inoculum, colonies of each *Alternaria* isolate were gently scraped from the culture medium into sterile distilled water using a sterile glass spatula. The resulting spore suspension was adjusted to a concentration of 2×10^7 conidia per milliliter, as determined using a Malassez counting chamber (Mmbaga *et al.*, 2011).

Rating scale of symptoms

Disease symptoms were evaluated 12 days post-inoculation on the potato leaflets. Severity was scored using the scale developed by Boedo *et al.* (2011), which assigns values from 0 to 5 corresponding to the percentage of necrotic leaf area: 0 (0%), 1 (10%), 2 (11-25%), 3 (26-50%), 4 (51-75%), and 5 (>75%) (Table 2). This scale quantifies the extent of infection relative to the total leaflet surface area. Based on the severity scores, leaflets

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Table 2. Symptom severity rating scale for early blight on potato leaflets

Score	Symptoms severity of percentage
0	No lesion development
1	Lesion less than 10% of leaflet surface (1 mm lesion diameter)
2	Lesion between 11 and 25% of leaflet surface (1-5 mm of lesion diameter)
3	Lesion between 26-50% of leaflet surface (5-9 mm of lesion diameter)
4	Lesion less than 51-75% of leaflet surface (10-14mm of lesion diameter)
5	Lesion >75% of leaflet surface (>15mm of lesion diameter)

(Boedo et al., 2011)

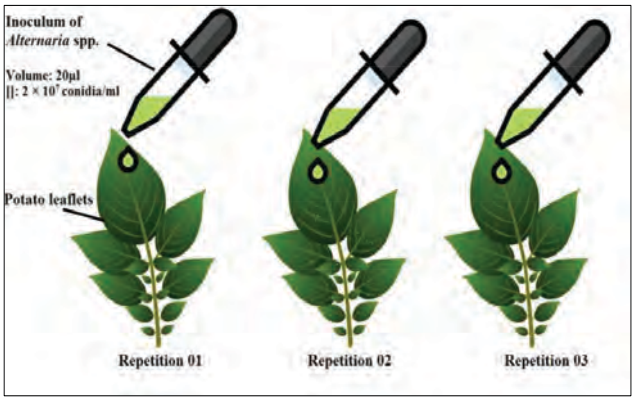


Fig. 2. Screening test protocol for early blight resistance using detached potato leaves

were classified into resistance categories following Bouhassan *et al.* (2004): scores ranging from 0.0 to 1.9 indicate resistance, while those from 2.0 to 5.0 denote susceptibility.

Statistical analysis

The variances (σ^2), means ($\bar{x}$), and standard deviations (σ) of four replicates in the *in vitro* tests and three replicates in the *in situ* experiments were calculated using XLStat software (AddinSoft, USA). Data were analyzed using a parametric one-way analysis of variance (ANOVA) with independent samples at a significance level of 5% ($p \leq 0.05$).

RESULTS AND DISCUSSION

The results demonstrated a significant effect across all conducted tests. Distinct differences in cultural and morphological characteristics were observed between the two *Alternaria* species responsible for early blight of potato, *Alternaria solani* and *A. alternata* (Table 1). Among the isolates, six were identified as *A. solani* and seven as *A. alternata*.

Physiological studies revealed variable growth responses of these isolates across the three culture media tested: PDA, OGA, and PCA (Table 3). The highest mycelial growth rate was observed on PDA, with an average of 1.47 cm/day, compared to 1.30 cm/day on PCA and 0.88 cm/day on OGA. Similarly, pH had a significant effect on fungal growth, with the optimal mycelial expansion occurring at pH 7.0 (3.31 cm/day), while growth was markedly reduced at alkaline pH levels of 9.0 and 11.0, which did not exceed 1.3 cm/day (Table 3).

Table 3. Effect of culture medium type and pH level on mycelial growth of *Alternaria* spp.

Culture medium	Mycelial growth (cm/day) (Mean±SD	pH level	Mycelial growth (cm/day) (Mean±SD)
PDA	1,47a ± 0,3	7	3,31a ± 0,4
PCA	1,3a ± 0,22	9	1,29b ± 0,3
OGA	0,88b ± 0,18	11	1,27b ± 0,2
LSD	87,2*		67,3*
ddl	15		15

LSD: Least significant difference; ddl: Degree of liberty;
*: Significant at p≤0.05

Artificial inoculation of *Alternaria* isolates was performed on detached leaves of two potato varieties, Arizona and Rudolph, to screen for resistance. Both varieties exhibited hypersensitivity to early blight infection (Fig. 4). However, the Arizona variety showed greater susceptibility, with disease severity exceeding 75%, whereas Rudolph displayed less severe symptoms, with disease severity below 50% (Fig. 5).

This study aimed to characterize the cultural, morphological, and physiological features of two fungal species, *Alternaria solani* and *A. alternata*, the primary causal agents of early blight in potato. Cultural and morphological traits are essential for accurate identification and differentiation between these species, while physiological parameters help determine the optimal environmental conditions that promote disease development (Zheng *et al.*, 2015).

Although *A. solani* and *A. alternata* are the most commonly reported species associated with early blight, several other *Alternaria* species have also been identified as potential pathogens in potato,

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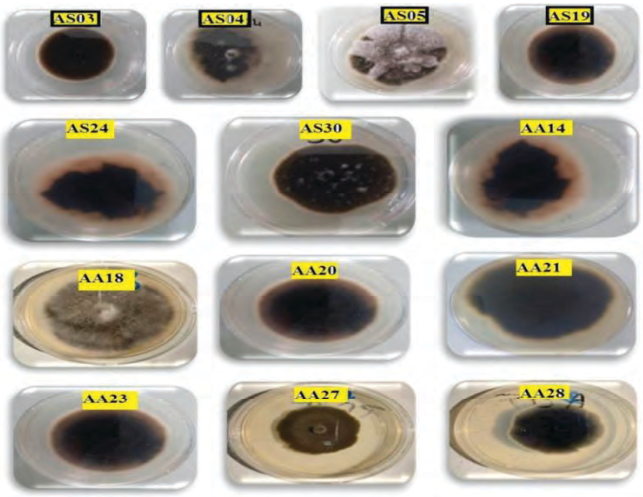


Fig. 2. Cultural aspects of *Alternaria solani* and *A. alternata* on PDA medium

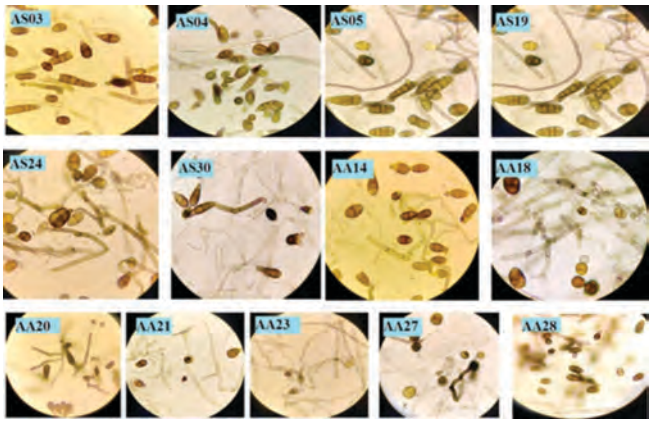


Fig. 3. Morphological aspects of *Alternaria solani* and *A. alternata* on PDA medium

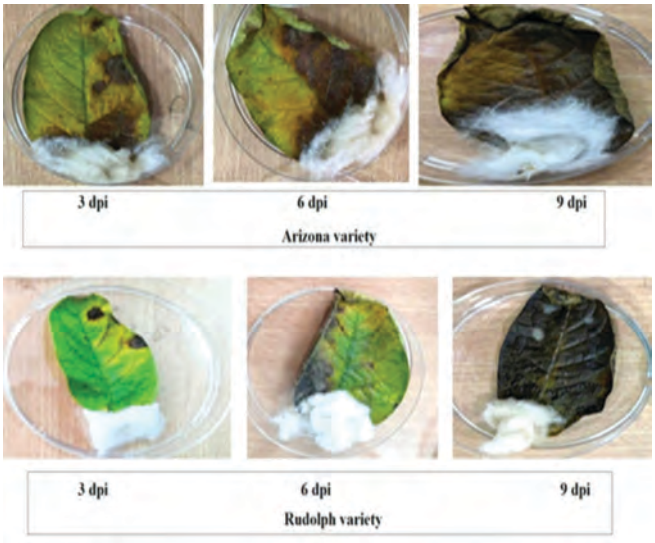


Fig 4. Reaction of two potato varieties (Arizona and Rudolph) to early blight disease

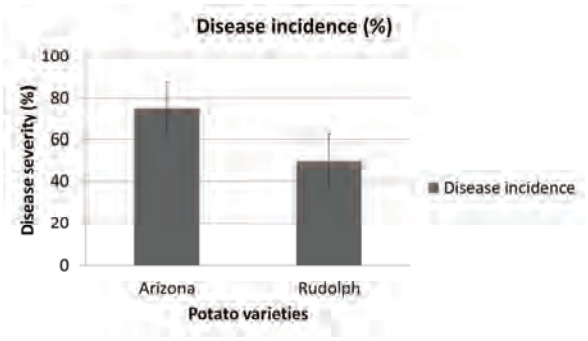


Fig. 5. Disease severity of early blight on two potato varieties inoculated with Alternaria spp.

including *A. tenuissima* (Zheng and Wu, 2013; Zheng *et al.*, 2015), *A. arborescens* (Tymon *et al.*, 2015), and *A. infectoria* (Sandoval *et al.*, 2014).

Our findings are consistent with earlier studies that reported variations in cultural, morphological, and physiological characteristics among *Alternaria* isolates (Mikarimi *et al.*, 2013; Marak *et al.*, 2014). In particular, the type of culture medium significantly influenced mycelial growth, as observed in other plant pathogens such as *Alternaria solani* on tomato (Shabana *et al.*, 2015), *Fusarium oxysporum* f. sp. *lycopersici* (Pandey *et al.*, 2024), *Phytophthora palmivora* in orchids (Khairum *et al.*, 2018), and edible mushrooms such as *Pleurotus ostreatus* (Poudel *et al.*, 2023) and *Agaricus bisporus* (Yadav and Chandra, 2014). pH levels also had a significant impact on fungal development. For example, optimal sclerotial production in *Sclerotinia rolfsii* occurs between pH 5 and 7 (Ayed *et al*., 2018), and pH plays a key role in regulating the growth-defense balance in several crops (Kesten *et al*., 2019). Similar to our observations with *Alternaria* spp., high pH levels were shown to support moderate fungal growth in other species such as *Sphaeropsis pyriputrescens*, the causal agent of Sphaeropsis rot in apple and pear (Kim *et al.*, 2005), and *Fusarium oxysporum* in soybean (Crus *et al.*, 2019). In the present study, screening tests revealed no evidence of resistance in the tested potato cultivars, Arizona and Rudolph. This finding aligns with previous research indicating the lack of resistant commercial varieties against early blight (Abbo *et al.*, 2018; Rodriguez *et al.*, 2004).

CONCLUSION

This study aimed to characterize *Alternaria* species responsible for early blight in potato cultivation, focusing on their cultural and

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morphological traits, and to evaluate the potential resistance of two commonly grown potato varieties, Arizona and Rudolph.

Based on morphological and pathogenic characteristics, two species were identified: *Alternaria solani* and *A. alternata*. Physiological assessments revealed that optimal fungal growth occurred at pH 7 and on Potato Dextrose Agar (PDA) medium. The resistance screening tests showed that both Arizona and Rudolph varieties were susceptible to early blight, with no observable resistance responses.

These findings contribute valuable information for understanding the pathogen's biology and its interaction with host plants. While no resistant cultivars were identified in this study, the results provide a foundation for future disease management strategies and emphasize the importance of confirming varietal susceptibility under natural field and environmental conditions. This knowledge is essential for guiding breeding programs and integrated control approaches aimed at reducing dependence on chemical fungicides.

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