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AGGRESSIVENESS AND MORPHOLOGICAL VARIABILITY OF SMALL SPORE *Alternaria* SPECIES ISOLATED FROM ALGERIA

Nabahat Bessadat*, Soumaya Benichou, Mebrouk Kihal and Djamel Eddine Henni

Applied Microbiology Laboratory, Depart of Live Science, Faculty of Nature and Live Sciences University of Oran, Es-Senia, Bp. 1524 El M'naouer 31000 Oran, Algeria.

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ABSTRACT

Natural epidemics of blight disease are strongly influenced by environmental conditions, even though several diseases appears every year in north western of Algeria which can cause complete loss of the crop when the infection is severe. The fungi which are frequently associated with leaf blight, stem blight and apical fruit rot of *Solanaceae* family are *Alternaria arborescens*, *A. alternata* and *A. tenuissima*. These fungi were identified on morphological characteristics. For that, eighty one small spore strains belonging to the *A. alternata* section were assessed for disease symptoms, percent disease incidence (PDI) and area under the diseases progress curve (AUDPC) on three different tomato cultivars. Among the tested fungal isolates, it revealed that PDI of the isolates were changed according to the cultivar and the highest blight disease incidence was found in *A. tenuissima* strain 164 (96% in Saint Pierre, 59% in Cherry tomato and 69% in Rio Grande), it was followed be *A. alternata* strain 156 (90% in Saint Pierre, 82% in cherry tomato and 46% in Rio Grande) and *A. arborescens* strain 65 (49% in Saint Pierre, 85% in cherry tomato and 63,15% in Rio Grande). According to the aggressiveness component of the isolates, the classical behavior of the cultivars was confirmed, and Saint Pierre and Rio Grande cultivars were found susceptible with a slightly higher mean of AUDPC (413.72 and 390.48 respectively)but Cherry tomato cultivar was found to be resistant with the lowest AUDPC mean 227.18 ± 166.10. Based upon results of present study, it was concluded that a complex of small spore *Alternaria* species and isolates found on *Solanaceae* lesions are not equally pathogenic but majority of *A. tenuissima* isolates are not the part of this complex.

* Corresponding author

E-mail: bessadatnabahat@hotmail.com (Nabahat Bessadat)

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1 Introduction

Vegetables belong to family *Solanaceae* are important due to their nutritional as well as economical values, and are widely cultivated in Algeria (Snoussi, 2009). Farmers face heavy yield losses in quality and quantity of these crops due to blight diseases caused by fungal pathogen *Alternaria spp*. Many species of the genus *Alternaria* causes diseases in tomato, potato and eggplant in all the continents of the world (Pryor et al., 2000). It becomes highly destructive in temperate humid climates, as it is the case in the northwestern growing areas of Algeria. This disease can infect the plant at all stages of growth and development and affect all the aboveground parts of the plant, in severe cases it can lead to complete defoliation (Peralta et al., 2005, Verma & Verma, 2010). The fruit is usually affected on the stem and depressed rot spots appear on either the green or ripe fruit (Morris et al., 2000; Blancard et al., 2012).

Phenotypic variation within fungal populations can generally be detected using morphological, cultural and pathogenic characteristics. Understanding the pathogenic nature and mechanisms by which such variation arises within a population is of paramount importance for devising a successful disease management strategy. In addition, reliable and repeatable techniques for large-scale screening are necessary to identify host plant resistance. Fungal inoculations (spores and mycelia) and fungal toxins have been developed for blight disease and collar rot resistance screening under field, glasshouse, and laboratory conditions (Chaerani & Voorrips,2006). Assays in a glasshouse or controlled environmental chamber with seedlings or small plants provide uniform, favorable, repeatable environmental conditions and permit several cycles of screening, thus offering more reliable results (Banerjee et al., 1998; Foolad et al., 2002). Disease severity can typically expressed as percent defoliation and area under the disease progress curve (AUDPC) and are the most common criteria used for evaluation of early blight resistance from data expressed over time, other indices used are percent of disease index (PDI) and cumulative disease index (CDI) for either stem or foliage infections (Thirthamallappa & Lohithaswa, 2000; Chaerani et al., 2007; Boedo et al., 2012).

Area under the disease progress curve (AUDPC) was used to determine disease progress for polycyclic foliar pathogens where resistance is governed by quantitative trait loci (Shaner & Finney, 1977; Jeger & Viljanen-Rollinson, 2001). Moreover, many workers demonstrated that AUDPC is the best criterion to compare early blight severity on different cultivars (Christ, 1991; Kumar & Srivastava, 2013; Mirkarimi et al., 2013). The importance of the *Alternaria* species in blight disease is seen differentially. Especially the impact of the small spore species, mainly *A. alternata* is controversially discussed. While some researchers have seen the effect of two other species i.e *A. solani* and *A. alternata* as causal agents or pathogen complex (Leiminger & Hausladen2013; Stammler et al., 2014), others are convinced that only *A. solani* is pathogenic (Turkensteenetal., 2010). Furthermore *A. alternata* would be survive assaprophyte, which colonizes leaf lesions wherever this lesion came from ozone damage, variation specific, caused by *A. solani* or other species of *Alterneria* and is therefore a secondary invader. The experiments were performed with the objective to contribute to the elucidation of the importance of *A. alternata* section causing blight disease in Algeria and to test the resistance of three cultivated tomato lines in Algeria.

2 Materials and Methods

2.1 Sampling and isolation of the pathogen

Four hundred and five infected plant samples such as leaves, fruits, tubers, stems and peduncles of *Solanaceae* family with typical disease symptoms were collected from different localities of north western Algeria during 2011- 2013 cropping seasons. For fungus isolation, small segments of diseased tissue along with some healthy portion (5×5 mm^2) were cut by sterilized razor and surface sterilized in 2% Sodium hypochlorite (NaOCl) for 2 minutes. Surface sterilized plant tissue were rinsed by sterilized distilled water for removing the last trace of Sodium hypochlorite solution, dried on filter paper and placed Petri plates containing 15 ml of potato sucrose agar medium (PSA). Three to four pieces of sterilized tissue were placed in each Petri plate and incubated for 7 days at 25±2°C in incubator. The composition of potato sucrose agar medium used was as described in Samson et al. (2002).

2.2 Purification and Maintenance of isolated pathogens

The culture was purified by two methods viz hyphae tip method and single spore technique. Hyphae tip method was conducted as described in Ganie et al. (2013), hyphal tips growing out of tissue segments were cut off with sterilized inoculation needle and transferred to potato sucrose agar slants and incubated at 25±2°C for further growth.

The single spore technique was conducted as per the method described by Sofi et al. (2013). Spore suspension was prepared from 7 days old PSA culture by 2% sterile agar solution, in Petri plates which were incubated at 25±2°C for 24 h to 48h. Germinating spores were observed under a stereoscopic microscopic and were transferred by sterilized needle on potato sucrose agar suspension and incubated for 4 to 5 days. Fully developed pure cultures were stored at 4°C in 5cm PSA petridishes supplemented with 5 ml of 30% glycerol and maintained by repeated sub-culturing at an interval of 30 days for further studies. Fungus was repeatedly isolated from artificially infected leaves and purified by the method described above to retain its vigor.

2.3 Identification of the pathogen

Morphological characters of all the isolates strain were studied on PSA culture as described in Simmons (2007). The

characters which consider for identification were mycelium color, pigmentation, colony type, development of secondary conidiophores, degree of branching, conidial shape and size.

2.4 Spore production

All the isolated fungal isolates were maintained on PSA at 20°C for 7 days. To induce sporulation, cultures were transferred on 23-25°C for 6 days on PSA at natural day light with 16 h/day light. Conidial suspensions were prepared as described in Boedo et al. (2012). Spore density was counted by a haemocytometer and adjusted to 10^5 conidia per mL. Tween 20 was added to the suspension at a final concentration of 0.05%.

2.5 Cultural conditions

Tomato (*Solanum lycopercicum*) seeds were planted in plastic pots (4×4×4 cm diameter) containing sterilized peat soil and sand (3:1) and maintained under glasshouse conditions (25-30°C) of Es-Sénia University. Plants were irrigated regularly on requirement.

2.6 Glasshouse test

Four week old Plantlets of *S. lycopersicum* cvs 'Saint Pierre', 'Rio Grande' (susceptible) (ITCMI, 2010), and 'Cherry tomato' (resistant) were inoculated separately by spraying 10 ml conidia suspension of each isolate of *Alternaria sp*. at a concentration of 10^5 spores/mL or a sterile water control (Nadia et al.,2007; Kumar & Srivastava, 2013). The plants were covered with polyethylene bags for 2 days to increase humidity and accelerate infection and then grown under normal conditions in the glasshouse at 26°C (Pelletier &Fry, 1989; Shahbazi et al., 2011).

2.7 Assessment of *Alternaria sp*. Aggressiveness

The number of lesions per leaf was counted at 7, 15 and 21 day after inoculation (DAI). There may be possible that large lesions were actually a merged combination of several smaller lesions and a large lesion was considered equivalent to 10 small lesions. The whole study was carried out with three replications for each cultivar and each fungal isolate studied. Percent disease index (PDI) was calculated by using the formula given by James (1974)

$$\text{Percent disease Index (\%)} = \frac{\text{Nomber of damaged leaves}}{\text{Nombrer of total leaves examined}} \times 100$$

based on a 0–5 scale adapted from Boedo et al. (2012), according to the percentage of necrotic leaf area (0: no visible disease symptoms, 1: < 5% leaf area affected, 2: 5% ≤ leaf area affected < 20%, 3: 20% ≤ leaf area affected < 40%, 4: 40% ≤ leaf area affected < 60%, 5: 60% ≤leaf area affected).

The area under the disease progress curve (AUDPC) value was calculated according to the formula used by Shaner & Finney (1977).

$$\sum_{i=1}^{n-1} \left[\left(\frac{Xi + 1 + Xi}{2}\right) \times (ti + 1 - ti)\right]$$

Whereas
Xi is the disease index expressed as a proportion at the i^{th} observation;
ti is the time (days after planting) at the i^{th} observations;
n is the total number of observations

Average and standard deviation were performed using MS EXCEL (2010). Koch's postulates were fulfilled by examinations of the diseased leaves first for associated fungus by the observation of the margins of the diseased spots (10x X 10x) under microscope and then by the isolation of the pathogen on PSA medium as previously described.

3 Results and discussion

3.1 Isolates from diseased leaf samples

During 2011 to 2013 cropping seasons occurrence of leaf blight disease from the members of *Solanceae* family (*S. lycopersicon, S. tuberosum, Capsicum annuum and S. melongena*) was observed in growing field of the north western of Algeria.

Appearing of Symptoms started from the leaf tips and along the margins of the leaf petiole. Infected portion is showing small brown lesions, yellowing the lower leaves and concentric circles with dark layers of spores were also observed and progressing upwards under high humidity conditions. At severe infection, lesions enlarged and causing death of the infected portion (Mirkarimi1 et al., 2013; Ganie et al., 2013). A total of eighty one isolates were obtained from the infected materials among them seventy four were small spore isolates which were isolated from the member of *Solanaceae,* rest seven isolates were isolated from the other crops showing typical disease symptoms belong to the *A. alternata* section.

Isolated pathogens were identified on basis of morphological characteristics as described by Simmons (2007) and are named in table 1. The most prevalent species on *Solanaceae* were *A. alternata* and *A. tenuissima* (Figure 1- A, B). Pathotype *A. arborescens* was also isolated from diseased tomato stems but with low incidence (Figure 1-C). However the fact that these three species could be isolated from typical lesions does not necessarily show that three species are virulent.

Table 1 Sources of *Alternaria spp*. isolates evaluated in this study in numerical order.

Isolate	Species	Town and region isolated from	Host- organ	Date
1	*A. tenuissima*	Mostaganem	Tomato- fuit	Mar. 2011
8	*A. alternata*	HassiBounif - Oran	Pea- leaf	May. 2011
11	*A. tenuissima*	Djebel Lekhar- Oran	Potato- leaf	May. 2011
20	*A. tenuissima*	HassiBounif ITCMI - Oran	Potato- leaf	Jun. 2011
24	*A. tenuissima*	HassiBounif ITCMI - Oran	Potato- leaf	Jun. 2011
28	*A. tenuissima*	Sidi Ben Okba - Oran	Eggplant- leaf	Jul. 2011
30	*A. tenuissima*	Djebel Lekhar - Oran	Tomato - leaf	Jul. 2011
32	*A. alternata*	Sidi Ben Okba - Oran	Tomato - leaf	Jul. 2011
36	*A. tenuissima*	Mostaganem	Pepper - fuit	Jul. 2011
38	*A. tenuissima*	Mostaganem	Tomato- fruit	Jul. 2011
41	*A. tenuissima*	Tatba - Alger	Cabbage- leaf	Jul. 2011
42	*A. alternata*	Tatba - Alger	Cabbage- leaf	Jul. 2011
45	*A. tenuissima*	Stidia - Mostaganem	Tomato- leaf	Sept. 2011
46	*A. tenuissima*	Stidia - Mostaganem	Tomato- leaf	Sept. 2011
47	*A. tenuissima*	Stidia - Mostaganem	Tomato- leaf	Sept. 2011
49	*A. tenuissima*	Ouriah - Mostaganem	Tomato- leaf	Sept. 2011
51	*A. tenuissima*	Ouriah - Mostaganem	Tomato- fruit	Sept. 2011
52	*A. tenuissima*	Ouriah - Mostaganem	Tomato- fruit	Sept. 2011
54	*A. tenuissima*	Mamache - Mostaganem	Potato- leaf	Sept. 2011
55	*A. tenuissima*	Mamache - Mostaganem	Potato- stem	Sept. 2011
56	*A. tenuissima*	Mamache - Mostaganem	Potato- stem	Sept. 2011
57	*A. alternata*	Mamache -Mostaganem	Potato- leaf	Sept. 2011
58	*A. tenuissima*	Mamache -Mostaganem	Potato- leaf	Sept. 2011
59	*A. tenuissima*	Mamache -Mostaganem	Potato- leaf	Sept. 2011
60	*A. tenuissima*	Stidia - Mostaganem	Bean - leaf	Sept. 2011
65	*A. arborescens*	Stidia - Mostaganem	Tomato- stem	Sept. 2011
69	*A. alternata*	Stidia - Mostaganem	Tomato- leaf	Sept. 2011
70	*A. tenuissima*	Mamache -Mostaganem	Potato- leaf	Sept. 2011
71	*A. alternata*	Mamache – Mostaganem	Potato- stem	Sept. 2011
72	*A. tenuissima*	Mamache - Mostaganem	Eggplant- leaf	Sept. 2011
73	*A. tenuissima*	Mamache - Mostaganem	Eggplant- leaf	Sept. 2011
75	*A. tenuissima*	Mamache - Mostaganem	Eggplant- leaf	Sept. 2011
77	*A. alternata*	Mamache – Mostaganem	Potato- leaf	Sept. 2011
79	*A. alternata*	Ouriah - Mostaganem	Tomato- leaf	Sept. 2011
81	*A. tenuissima*	Ouriah - Mostaganem	Tomato- leaf	Sept. 2011
82	*A. tenuissima*	Stidia - Mostaganem	Tomato- leaf	Sept. 2011
83	*A. tenuissima*	Stidia - Mostaganem	Tomato- leaf	Sept. 2011
84	*A. alternata*	Stidia - Mostaganem	Tomato- leaf	Sept. 2011
85	*A. alternata*	Stidia- Mostaganem	Tomato- leaf	Sept. 2011

86	*A. tenuissima*	Stidia - Mostaganem	Tomato- leaf	Sept. 2011
87	*A. tenuissima*	Stidia - Mostaganem	Tomato- stem	Sept. 2011
89	*A. tenuissima*	Biskra	Eggplant- peduncle	Oct. 2011
91	*A. tenuissima*	Biskra	Eggplant- peduncle	Oct. 2011
97	*A. alternata*	Canastel - Oran	Tomato- leaf	Nov. 2011
98	*A. tenuissima*	Kouir - Mascara	Lettuce- leaf	Nov. 2011
99	*A. tenuissima*	Kouir - Mascara	Potato- leaf	Nov. 2011
100	*A. alternata*	Kouir - Mascara	Potato- stem	Nov. 2011
102	*A. tenuissima*	Kouir - Mascara	Potato- leaf	Nov. 2011
104	*A. tenuissima*	Kouir - Mascara	Potato- leaf	Nov. 2011
105	*A. alternata*	Kouir - Mascara	Potato- stem	Nov. 2011
107	*A. alternata*	Mostaganem	Tomato- fruit	Nov. 2011
109	*A. tenuissima*	Mascara	Potato- tuber	Nov. 2011
120	*A. tenuissima*	Mostaganem	Tomato-fruit	Nov. 2011
121	*A. tenuissima*	Mostaganem	Tomato-fruit	Nov. 2012
126	*A. tenuissima*	Mostaganem	Tomato-fruit	Dec. 2011
129	*A. alternata*	Kouir - Mascara	Potato- leaf	Dec. 2011
130	*A. tenuissima*	Kouir - Mascara	Pepper - leaf	Dec. 2011
131	*A. alternata*	Kouir - Mascara	Pepper - stem	Dec. 2011
132	*A. tenuissima*	Kouir - Mascara	Pepper - fruit	Dec. 2011
135	*A. alternata*	Khesibia - Mascara	Tomato- leaf	Dec. 2011
137	*A. tenuissima*	kouir - Mascara	Potato- leaf	Dec. 2011
138	*A. tenuissima*	kouir - Mascara	Potato- leaf	Dec. 2011
139	*A. tenuissima*	Khesibia - Mascara	Potato- leaf	Dec. 2011
140	*A. tenuissima*	Khesibia - Mascara	Potato- leaf	Dec. 2011
141	*A. alternata*	Khesibia - Mascara	Potato- leaf	Dec. 2011
142	*A. tenuissima*	Khesibia - Mascara	Potato- leaf	Dec. 2011
143	*A. alternata*	Khesibia - Mascara	Potato- leaf	Dec. 2011
150	*A. tenuissima*	Kouir - Mascara	Potato- stem	Dec. 2011
151	*A. tenuissima*	Mostaganem	Pea - leaf	Dec. 2011
153	*A. tenuissima*	Ain Témouchent	Potato- leaf	Dec. 2011
155	*A. tenuissima*	Ain Témouchent	Potato- leaf	Dec. 2011
156	*A. alternata*	Ain Témouchent	Potato- leaf	Dec. 2011
157	*A. tenuissima*	Ain Témouchent	Potato- leaf	Dec. 2011
161	*A. alternata*	Ain Témouchent	Concomber- leaf	Dec. 2011
164	*A. tenuissima*	Ain Témouchent	Tomato- leaf	Dec. 2011
165	*A. alternata*	Ain Témouchent	Tomato- leaf	Dec. 2011
167	*A. alternata*	Mostaganem	Tomato- fruit	Dec. 2011
168	*A. alternata*	Mostaganem	Tomato- fruit	Dec. 2011
174	*A. tenuissima*	Mostaganem	Tomato- fruit	Sept. 2012
194	*A. alternata*	HassiAmeur - Oran	Tomato- leaf	Oct. 2012
229	*A. alternata*	Walhassa- Tlemcen	Tomato- leaf	May. 2013

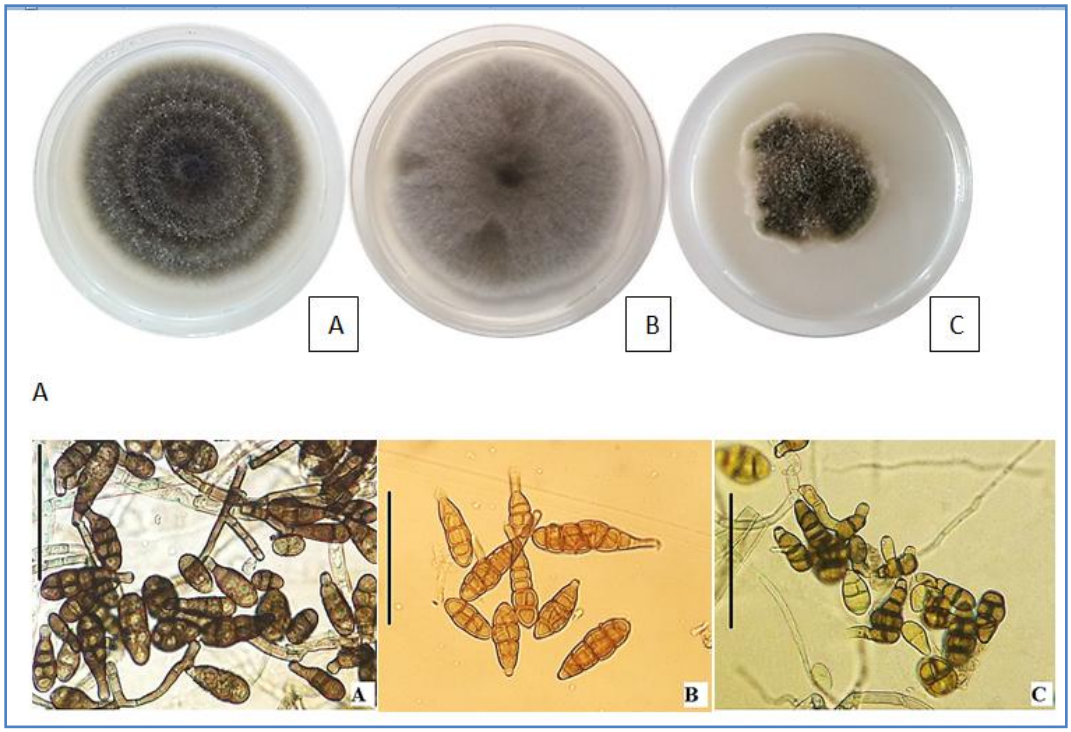

Figure 1 Morphology of small- spore *Alternaria* species causing blight disease on member of *Solanaceae* in Algeria on PSA culture. A: *A. alternata*, B: *A. tenuissima*, C: *A. arborescens*. Bars= 50µm.

3.2 Morphology and identification of the fungus

Morphological characters of fungus isolated from diseased tissues on potato sucrose agar medium are presented in Table 2.The various morphological characters of pathogen observed on culture medium were as the follow.

3.2.1 Macroscopic characters

All the isolated fungal strain grown well on PSA and formed grey to black colonies with tints olive or brown mycelium of about 90 mm in diameter in 7 days, when incubated at 25±2°C. Colonies are spreading cottony, velvety or appressed (possessing a texture similar to cotton, felt or velvet) with or without zonation. The underside of the colonies color varied from grey brown to black.

3.2.2 Microscopic characters

Three distinct species-groups were identified based on sporulation pattern and morphology, namely *A. arborescens*, *A. alternata* and *A. tenuissima* species groups. In general, conidial shape were obclavate and muriform, with short beaks, septet, dark colored or pale as showed in figure 1, conidia were formed in long or moderate chains; each species has its own sporulation pattern. The conidiophores were also septate, short or long, simple or branched, flexuous pale to olive brown in color. According to Simmons & Roberts (1993) within *A. alternata* group more species may be segregated. The data in this study support this opinion.

Alternaria taxonomy has long been mainly based on conidial morphology and sporulation pattern. Nishimura & Kohmoto (1983) analyzed by using a statistical method based on the size of conidia and concluded that *Alternaria* isolates producing small spores and they are known as "collective species" or *alternata*. Similar types of findings were reported by various researchers (Kusaba & Tsuge, 1995; Johnson et al., 2000; Tsuge, 2003). Furthermore, Simmons & Roberts (1993) introduced 3-dimensional sporulation pattern as a mean of scoring small-spore species to facilitate their segregation and identification. More recently, small-spore forming *Alternaria* species were grouped into the *alternata* section and it comprises almost 60 *Alternaria* species (Woudenberg et al., 2013; Lawrence et al., 2013). The molecular variation within *alternata* section is low and these species were mainly differentiated on the behalf of phenotypic variation. This it is also well recognized that pathogenic populations (pathotypes) with narrow host range exist within the *alternata* section. *A. arborescens* responsible for the tomato stem canker constitutes a typical example (Grogan et al., 1975, Mesbahet al., 2000, Simmons, 2000).

Table 2 Various morphological characteristics of small spore forming *Alternaria* species causing blight disease in *Solanaceae*.

Structure	Species Characters		
	A. alternata	***A. tenuissima***	***A. arborescens***
Color	Olivaceous to dark green with and without zonation	Gray-brown to olisvaceous in color mainly without zonation	Dark green with greyish surface without zonation
Colony	Appressed to velvety sometimes with a cottony center	Spreading, cottony to velvety	cottony to Velvety, slightly furrowed with appressed center
Conidia shape	Obpyriform to ovate to obclavate, yellowish-brown to brown, with 1-8 transverse and 0-3 longitudinal or oblique septa.	Obclavate or ellipsoidal, brown to golden brown, some conidia with minutely verrucose walls. Mature conidia with 4-9 transverse septa and 0-4 longitudinal or oblique septa.	Ovate, obpyriform to ellipsoidal, mature conidia had 1-6 transverse septa and 0-3 longitudinal or oblique septa.
Conidia size	6,5-59,8×4,2-16,5 µm	9,8-60,20 × 8,6-15,5 µm	8,6-38,4 × 3,2-12,8 µm
Sporulation type	**3:** conidial chains of 2-6 units long and typically produce branches (1 to 5 conidia) having a long defined primary conidiophore with few terminal and sub terminal branches.	**5:** moderately long to long chains of more than 9 conidia, branching of chains usually was minor (1 to 2 conidia) or lacking.	**4:** conidia chain appeared as low bushy clumps of well-branched chains.

3.3 Assessment of *Alternaria sp*. Aggressiveness

In order to confirm the differences in virulence, pathogenicity of *Alternaria* small spore isolates, a glasshouse study at Es-Senia University campus were conducted on four weeks old tomato plant cultivars i.e. 'Saint Pierre', 'Cherry tomato' and 'Rio Grande'. Control plants which were sprayed with sterilized distilled water didn't showed any symptoms even after 21 days of inoculation. The inoculated plants with small spore *Alternaria* strains showed leaf blight symptoms with different aggressiveness levels, the data are presented in Table 3 and 4 revealed that mean disease incidence and the AUDPC varied among the isolates and tomato cultivars.

Significant differences were found in cultivars on the basis of area under disease progress curve (AUDPC). Mean AUDPC for cultivar Saint Pierre was 413.72. Similar type of differences was also reported in cultivar Rio Grande (AUDPC mean of 390.48). However, Cherry tomato cv. was reported resistant to fungus and the mean AUDPC was 227.18. In present study highest susceptibility of early blight disease was reported in tomato cv. Saint Pierre and these observations are in agreement with the findings of Fontem (1993) who found highest susceptibility of *Alternaria* blight in Saint Pierre, Heinz 1370 and Marmande. Furthermore, Fontem (2003) also reported that tomato cultivars Rio Grande and Roma VF were less susceptible to early blight and in this aspect these observation are contradictory to the present findings.

Amongst the various tested *A. alternata* strains, maximum disease incidence was recorded in the isolate 165 with 90% in Saint Pierre, 82% in cherry tomato and 46% in Rio Grande which was in category '5' at 0-5 point scale, this isolates was followed by isolate 161 which recorded 87% in Saint Pierre 9,7% in Cherry tomato and 81% on Rio Grande which was in category '4'. Isolate 97 had the minimum disease incidence with 5% on Saint Pierre, 2.5% in Cherry tomato, 24% in Rio Grande and was in category '2'. However, strain 164 had the maximum disease incidence among the *A. tenuissima* isolates with 96% in Saint Pierre, 59% in Cherry tomato and 69% in Rio Grande followed by strain 45 which recorded 86% in Saint Pierre, 33% in Cherry tomato and 92% in Rio Grande, both stains were in '5' category. The minimum disease incidence was recorded on the *A. tenuissima* strain 51with 14% in Saint Pierre, 2.9% in Cherry tomato and 3% in Rio Grande. While the only *A. arborescens* isolate (65) was categorized in group '5' with a high disease incidence on the three tomato cultivars Saint Pierre, Cherry tomato and Rio Grande with 49%, 85% and 63.15% respectively. The *A. tenuissima* isolate 164 was highly virulent as compared to all tested isolates with the highest mean of area under disease progress curve (AUDPC) 1026 followed by the *A. arborescens* strain 65 with a mean of 839.66.

The data presented in Table 3 and 4 revealed that 3.17% of the *A. alternata* isolates were in '5' category (lesions on more than 60% of inoculated sites) and 32.14% were in category '4' and 50% were in category 3 and 7.14% in category 2. Data also indicates that *A. tenuissima* isolates differed in their degree of virulence and 7.54% were in category '5' while 9,43% isolates were grouped categories '4' and low virulent category '3' and 41.5% of isolates has rating '2' at 0-5 point scales.

Table 3 Percent disease incidence and AUDPC of *A. alternata* and *A. arborescens* strains on tomato cultivars under glasshouse conditions.

Isolates	Different Tomato Cultivars												[a] Mean Score 21 DAI (at 0-5 scale)
	Saint Pierre				Cherry tomato				Rio Grande				
	% Disease incidence (PDI)			AUDPC	% Disease incidence (PDI)			AUDPC	% Disease incidence (PDI)			AUDPC	
	7 DAI	14 DAI	21 DAI		7 DAI	14 DAI	21 DAI		7 DAI	14 DAI	21 DAI		
8	5	12.5	43	365	3	12	79.1	533	6.5	11.5	49	401	4
32	4.5	10.5	13.5	209	1	8.5	22.5	217	5	11	24	273	3
42	13.5	24	52	605	0.5	1	1.5	40	30	49	70	1025	4
57	25	30.5	75	871	4.5	11.5	26.65	294	8	29	52.5	589	4
65	23	44	49	829	3.5	17.5	85	610	43.5	48.5	63.15	1080	5
69	4	11	12	211	3	9.5	19.15	227	6	12	31.5	331	3
71	2.5	13	52	423	3.33	5	12.5	135	6.5	15	25	319	3
77	2.5	14	46.5	394	3.55	16.55	55	473	4.5	16	49	435	4
79	7.5	14.5	31	356	1	3.5	24.15	170	2	2.5	6.5	78	3
84	3.5	10	19	220	7.5	23	24	410	5.5	23	72	617	3
85	6	13.5	49	417	1	1.66	3.33	50	2.5	17	47	408	3
91	9.5	29	61	657	6.66	16.5	60	496	2	4,5	12	110	4
97	2	2.5	5	70	0.5	2	2.5	52	3.5	6.55	24.5	205	2
100	6	20.5	52	502	1.66	3	5	70	6.55	18.5	86	642	4
105	3.5	15.5	32	343	2.5	4	17.5	146	0.5	3	3.55	50	2
107	5	20	60.55	529	2.5	15	37	349	7.5	31	43	558	4
129	17	61	69	1051	7.05	12.5	24	299	5	18.5	66	515	4
131	4.5	12	30	302	2	4.55	19,5	169	0	0.5	1.5	250	2
135	7.5	23.5	65	604	2.5	8.5	21.66	222	20	21.5	32	492	3
141	8.5	15.5	32	371	8	9	10.5	199	21	32	41.55	666	3
143	8.5	13	18.5	282	7	16	18.75	301	3	18.5	25.5	313	3
156	12.55	25	90	778	5	33	82	754	8	17	46	424	5
161	2	13.55	87	566	0	2,5	9,5	81	2	10	81	465	4
165	5.5	11.5	16.5	232	4.55	17.5	27.5	340	6.55	40	59.5	707	3
167	3	12.55	35	325	2	6	17	172	4.5	16	23	308	3
168	4	6.5	32.55	259	2	9	15	174	6	21	46,55	466	3
194	1	5	15	148	12	16	27.5	384	10	21	59	569	3
229	2.5	16	53.55	502	5.45	13.5	20	314	14	27	30	579	3

DAI: days after inoculation;[a]Mean disease index at 21 days post-inoculation rated on a 0–5 scale.

Table 4 Percent disease incidence and AUDPC of *A. tenuissima* strains on various tomato cultivars under glasshouse conditions

Isolates	Different Tomato Cultivars												[a] Mean Score 21 DAI (at 0-5 scale)
	Saint Pierre				Cherry tomato				Rio Grande				
	% Disease incidence (PDI)			AUDPC	% Disease incidence (PDI)			AUDPC	% Disease incidence (PDI)			AUDPC	
	7DAI	14 DAI	21 DAI		7 DAI	14 DAI	21 DAI		7 DAI	14 DAI	21 DAI		
1	15	50	69	954	5	16	36	380	14.5	23	35	479	4
11	8	23.5	52	556	4.5	5	16.66	164	3	6	26	206	3
20	2.5	8.5	22	221	3.5	11	26.66	282	5	16.5	49	456	3
24	5	27	29	460	1.65	3	7.5	81	3.5	8	16	192	2
28	0.5	1.5	4.5	55	2	2	20	141	4	11	20.5	243	2
30	3.5	11.5	21	288	2	3	6.66	93	1.5	2.5	3.5	78	2
36	7.5	10	32	309	4	20	35,5	385	5.5	20.5	34.4	407	3
38	5	15	61	480	4	18.5	53.33	473	8.5	27	55	578	4
41	12.5	44.5	67	842	3.33	6	12.5	135	7.5	14	32	315	2
45	15	22.5	86	711	14.5	28	33	529	27	47	92	1059	5

46	4.5	12	30	300	1	3	38.33	218	3	15	49	410	3
47	4,5	13	22.5	275	1	2	2.95	58	8.5	25	40.5	486	3
49	3	4.5	28.5	223	0.5	1	3.33	48	2	4.55	6.5	95	2
51	4	9.5	14	203	1.5	2	2.9	60	0	1	3	37	2
52	2	9	18	195	1.25	5.5	12.5	130	4.5	7.5	12	162	2
54	6	12	13.5	225	2.5	11	26.6	253	7	13.5	29.05	322	3
55	2	7.5	15	172	1.5	2.5	3.75	69	1.5	3.33	13	118	2
56	7	10	20.5	272	0.5	1	1.5	40	12.5	22.5	34.5	481	2
58	2.5	3	6	89	4.5	17.5	21.65	309	2.5	6	9	129	2
59	1.55	2.45	5	71	4.5	16	41.66	410	1.55	9.55	20	214	2
60	4	9	11.5	164	3.5	15	70	521	4.5	10	35	318	3
70	1.5	2	3	54	2	3.5	5	71	2.5	9.5	24.5	225	2
72	23.5	47	63	955	3.5	6	9.15	133	0	0.5	1	13	3
73	1	2	8.5	73	0.55	1	5.88	56	1	1.5	6	61	2
75	13	27.5	54	498	2.5	6	15	320	4.5	9.5	15	560	3
81	4.5	29.5	38	512	6	13.33	59.5	465	3.5	4.5	12	124	3
82	3.5	8	29	258	1	2	2.95	56	2	3.5	10	92	2
83	1	4.5	23.5	158	0	0.55	1	20	0.55	2	4.55	51	2
86	4	15	38.5	393	2.95	6.5	10	156	8	12.5	32	350	3
87	2.5	4	8	85	2.95	7.5	19.15	163	7	11.5	24	361	2
89	9.5	20	41	486	3	9.5	35	299	6	14.5	33	357	3
98	24.5	50	86	1066	3	12.5	40	336	7.5	26.5	67	110	5
99	2.5	27.55	46	527	3.5	7	12.5	161	30	41	55	608	3
102	3.5	6	13.55	162	1	1.5	4.55	66	8.5	35	66	898	3
104	4	6.5	15	178	2	4.5	10	108	2	17	42	723	3
109	4.5	12.5	46	404	0.5	2.5	3.33	56	1	2.5	9	390	2
120	11	31	54	659	1.66	4.5	10	123	21	30	58	88	4
121	8	12	16	255	2	9.5	21.65	228	9	21	58	726	3
126	2.5	23	47.5	485	1.5	2.5	4	63	1.5	20	27	557	3
130	1.5	3	10	118	1	2.95	5	87	4	8.5	9	347	2
132	0	1	3	35	3	7.5	34.5	265	2.5	10	12	181	2
137	2	3	6	79	0	0	0.5	9	21	37	64	178	3
138	4.5	10.5	19	222	1	2.5	5	66	4.5	12.5	26	859	2
139	7	13	15	248	1.55	3.5	7.55	96	12	17	21	286	2
140	20	30.5	41.25	650	2.5	3.5	5	79	7.5	21	46	358	3
142	7.5	12	16.55	257	1.25	14.55	52.6	408	16.5	25.5	29	489	3
150	9	21	72	580	2.5	12	29.15	251	5	23.55	92	507	5
151	16	52	63	926	7	14.5	55	454	6	28	44	631	4
153	5	11.5	53	480	1.5	4.5	10.88	139	4	6.5	10.5	503	3
155	8	18.5	54.,5	522	6	14.5	27.5	321	3	7.5	13	176	3
157	14	32	86.66	852	2	3.5	9.15	101	4	15	27	143	4
164	35.5	63	96	1544	9.5	17	59	600	23	33	69	293	5
174	4.5	16	21.55	305	2.5	6.5	17.99	186	2.5	5	12.5	934	2

DAI: days after inoculation; [a]Mean disease index at 21 days post-inoculation rated on a 0–5 scale.

Table 5 Effect of small spore *Alternaria*species on the development of blight symptoms in a glasshouse on tomato cultivars.

S. No	Species	Symptoms [a]	Changes in lesion size [b]	S. No	Species	Symptoms [a]	Changes in lesion size [b]
1	*A. tenuissima*	5	+	**89**	*A. tenuissima*	2	+
8	*A. alternata*	2	+	**91**	*A. tenuissima*	3	+
11	*A. tenuissima*	2	+	**97**	*A. alternata*	2	-
20	*A. tenuissima*	2	+	**98**	*A. tenuissima*	3	+
24	*A. tenuissima*	3	+	**99**	*A. tenuissima*	1	+
32	*A. tenuissima*	3	+	**100**	*A. alternata*	3	-
30	*A. tenuissima*	2	-	**102**	*A. tenuissima*	3	-
32	*A. alternata*	5	+	**104**	*A. tenuissima*	4	+

36	*A. tenuissima*	5	+	**105**	*A. alternata*	4	-
38	*A. tenuissima*	3	+	**107**	*A. alternata*	3	+
41	*A. tenuissima*	2	+	**109**	*A. tenuissima*	4	+
42	*A. alternata*	8	+	**120**	*A. tenuissima*	1	+
45	*A. tenuissima*	1	-	**121**	*A. tenuissima*	5	-
46	*A. tenuissima*	1	+	**126**	*A. tenuissima*	5	+
47	*A. tenuissima*	3	+	**129**	*A. alternata*	5	+
49	*A. tenuissima*	4	+	**130**	*A. tenuissima*	4	+
51	*A. tenuissima*	6	-	**131**	*A. alternata*	7	+
52	*A. tenuissima*	6	-	**132**	*A. tenuissima*	8	+
54	*A. tenuissima*	4	+	**135**	*A. alternata*	2	-
55	*A. tenuissima*	3	-	**137**	*A. tenuissima*	5	-
56	*A. tenuissima*	8	-	**138**	*A. tenuissima*	2	+
57	*A. alternata*	2	+	**139**	*A. tenuissima*	4	+
58	*A. tenuissima*	5	+	**140**	*A. tenuissima*	2	-
59	*A. tenuissima*	1	+	**141**	*A. alternata*	2	+
60	*A. tenuissima*	5	+	**142**	*A. tenuissima*	1	-
65	*A. arborescens*	5	+	**143**	*A. alternata*	4	+
69	*A. alternata*	2	+	**150**	*A. tenuissima*	2	+
70	*A. tenuissima*	6	-	**151**	*A. tenuissima*	2	+
71	*A. alternata*	3	+	**153**	*A. tenuissima*	2	+
72	*A. tenuissima*	2	+	**155**	*A. tenuissima*	1	+
73	*A. tenuissima*	4	-	**156**	*A. alternata*	7	+
75	*A. tenuissima*	5	+	**157**	*A. tenuissima*	4	+
77	*A. alternata*	5	+	**161**	*A. alternata*	4	+
79	*A. alternata*	1	+	**164**	*A. tenuissima*	4	+
81	*A. tenuissima*	2	+	**165**	*A. alternata*	4	+
82	*A. tenuissima*	8	+	**167**	*A. alternata*	7	+
83	*A. tenuissima*	2	-	**168**	*A. alternata*	8	+
84	*A. alternata*	4	+	**174**	*A. tenuissima*	5	+
85	*A. alternata*	7	+	**194**	*A. alternata*	4	-
86	*A. tenuissima*	3	+	**229**	*A. alternata*	8	+
87	*A. tenuissima*	2	+				

[a]Symptoms: Brown necrosis with yellow halo=1; Brown necrosis in bordures of leaves =2; Brown necrosis with or without yellow halo; yellowing of basal leaves =3; Brown necrosis; yellowing of basal leaves= 4; Brown or dark necrosis with or without yellow halo = 5;Dark spots= 6; yellow basal, Brown diffuse necrosis with yellow halo or dark spotsleaves =7; Brown necrosis in bordures of the leaf with or without yellow halo; yellowing of leaves= 8.[b]Lesion diameter increased (+) or remained constant (-) between 7 and 21 days after-inoculation.

The pathogenicity assays of the study raveled a high degree of variation in virulence of the different isolates. It was reported that many small spore *Alternaria* species from the *alternata* section cause blight disease in the members of *Solanceae* family and causing leaf spot disease in vegetables belonging to other families (Grogna et al., 1975; Verma & Verma, 2010; Mamgain et al., 2013). The *A. arborescens* isolate induced more disease, on host cultivars used and this concludes that AAL-toxin might be synthesized by isolate 65. However, among 27isolated *A. alternata* isolates, 40,74% of the isolated were rated as virulent with severe blight symptoms and 62,96% of the strains did not lead to higher infection levels, while from the 53 *A. tenuissima* isolates only 16,98% isolates were able to produce severe blight symptoms and 83,01% were low virulent under the different conditions used. It may be associated with variability of the strains and their ability to produce toxins. *Alternaria* produced lytic enzymes such as polyglacturonase, pectin lyase, pectin methylestrase, cellulose and two categories of toxin, namely, host, specific toxins (HST) and non-host specific toxin (NHST). Among the first, toxins such as the AM, AC, AK, AF and AL have been identified and their role in pathogenesis verified (Ruehle, 1964). It was also reported that pathotype *A. alternata* f. sp. *Lycopersicum* Syn. *A. arborescens* (Simmons, 2000), produces AAL-toxin (Grogan et al., 1975, Weir et al., 1998; Mesbah et al., 2000). In addition, many species of *A. alternata* and *A. tenuissima* are known for the production of host specific-toxins (HST), which are essential virulence factors and determine their host range (Nutsugah et al., 1994; Kohmoto et al., 1995). Plants respond by deposition of lignin to the cell wall of infected cells (Von Ramma, 1962). In present study production of HST was not investigated however, further studies are needed to investigate the small spore *Alternaria* isolates based on their secondary metabolites patterns to check their potential to produce (HST) and NHST. Less virulent strains tested are considered as weak pathogens that might be able to colonize only old and injured tissue which resulted from sunburns, or cracking of leaves by the wind and lesions on ripe fruit (black mold) or green fruit but only one or two epidermal cells are affected and the lesions do not develop further even after fruit ripens. All this facilitate the entry to tissues and cause opportunistic infections (Cassol & St.

Clair, 1994; Manjunath et al., 2010), Many *S. lycopersicum* cultivars are resistant to this saprophytic form.

3.4 Symptomatology and Koch's postulates

Differences in symptom expression were observed when using different aggressiveness strains (Figure 3). Leaf spots were circular, and dark to light brown spots occur singly or in large numbers on the leaf, mainly ≥ to 5mm in diameter in the first week post inoculation. The leaf may turn yellow, then brown and fall off. Older leaves are usually affected before the disease works up the plant as observed in Kumar & Srivastava (2013). Apparently the leaves looked healthy but the lesions were visible only if the leaves were kept against the source of light. Periodic changes in size, shape and color of the lesions were also observed and the results are summarized in Table 5.

The lesion progression was initially slow in first week post inoculation, which it showed the maximum lesion size of 10.4 mm was recorded in the third week post inoculation dark to brown spots or lesions were produced as a result of irregular growth patterns by the organism in the leaf tissue giving the lesion the same appearance as in natural infection field. There were often narrow spots with yellow halo around that sometimes led to an extended necrosis surrounded by yellowing observed on diseased leaves and were presumed to be due to the pathogen's toxin(s) Nutsugah et al.(1994). Observations on periodical disease development are more or less identical to those described by Stammler et al. (2014).

After inoculation of tomato cultivars with the small spore *Alternaria* species, symptoms were observed on all tested plant (Figure 2 and Table 5). Brown necrosis with a yellow halo developed on all cultivated tomato with *A. tenuissima* strains (45, 46, 59, 99, 120, 142 and 155). Leaf blight symptoms with brown diffuse necrosis and yellow halo or dark spots and yellow basal leaves were very distinctive on the *A. alternata* isolates (85, 131, 156 and 167). On tomato inoculated with (42, 168 and 229) A. *tenuissima* strains and (56, 82 and 132) *A. alternata* strains, brown necrosis in bordures of the leaf with or without yellow halo; yellowing of leaves were observed, while brown lesions in leaf bordures were present on plants inoculated with 14 *A. tenuissima* (11, 20, 30, 41, 42, 81, 83, 87, 89, 138, 140, 150, 151 and 153)and 7 *A. alternata* strains (8, 57, 69, 79, 97, 135 and 141) as typical symptom observed in natural infected leaves. However, dark spots were observed only on less virulent *A. tenuissima* strains (1, 51 and 70) without an increase in lesion size. *A. arborescens* strain 65 induced dark necrosis with yellow halo on leaves and lethal dark brown cankers on stems, leaf necrosis and wilting in the three tomato cultivars (figure 3), probably by the action of the AAL toxin. The three tomato cultivars, cherry tomato, Saint Pierre' and Rio Grande showed almost same symptoms for each isolate.

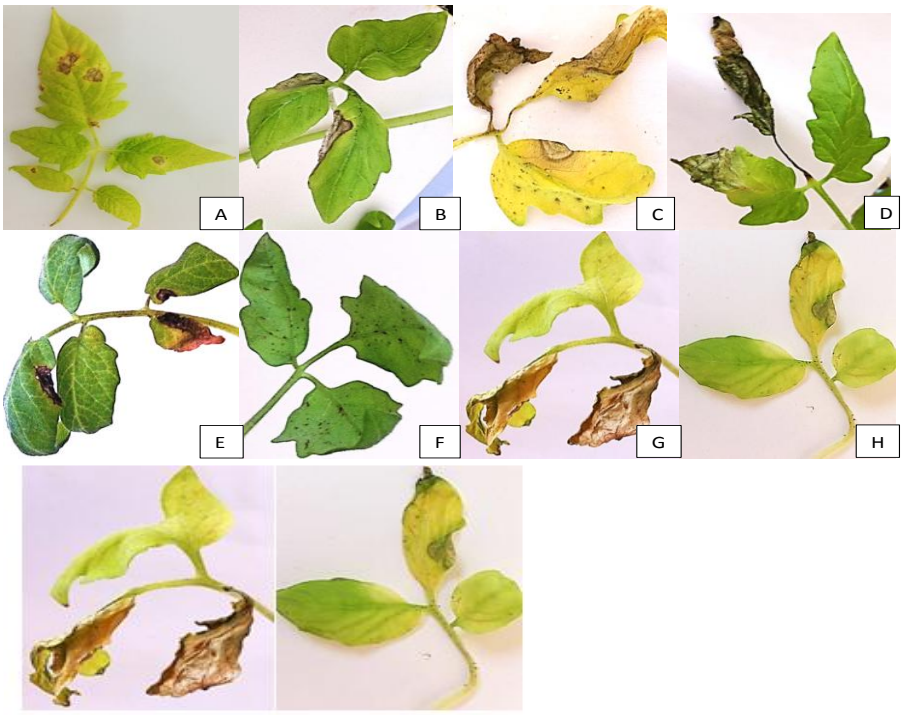

Figure 2 Symptoms observed after 21 days post-inoculation with small spore *Alternaria*isolates. A. Brown necrosis with yellow halo; B. Brown necrosis in bordures of leaves; C. Brown necrosis with or without yellow halo; yellowing of basal leaves; D. Brown necrosis; yellowing of basal leaves; E. Brown or dark necrosis with or without yellow halo; F. Dark spots; yellow basal, Brown diffuse necrosis with yellow halo or dark spots leaves; G. Brown necrosis in bordures of the leaf with or without yellow halo; yellowing of leaves.

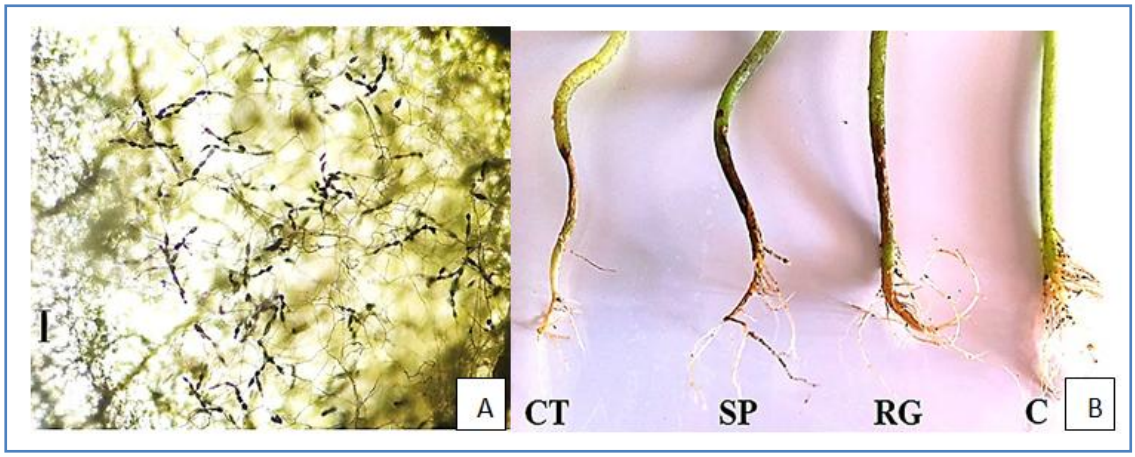

Figure 3 A. conidia chain and mycelium produced on the upper side of tomato infected leaf with *A. alternata* strain156. Bar = 100 µm; B. typical symptoms of collar rot on three tomato cultivars induced by *A. arborescens* strain 65. CT: cherry tomato, SP: Saint Pierre, RG: Rio Grande, C: control.

Observations regarding the pathogenicity test the fungus revealed the initiation of typical symptoms of the disease appeared after 5 days of inoculation on the aerial parts of tomato cv. Saint Pierre and Rio Grande. However, in cherry tomato cv. symptoms appeared 7 days after inoculation. Re-isolutions from infected leaves yielded typical cultures of the fungus thus satisfied the Koch's postulates. Similar observations were also recorded by Grogan et al. (1975) and Stammler et al. (2014) on tomato plants.

4 Conclusion

Identification of *Solanaceae* blight disease pathogens was conducted and isolates were differentiated as pathogenic *Alternaria* species from morphologically similar non-pathogenic *Alternaria* species.

Results and disease symptoms of glasshouse infection assays revealed that both types of small spore *Alternaria* occurred and had significantly different degrees of aggressiveness and symptoms. This suggest that the dominance of virulent small-spore *Alternaria* species producing host-specific toxins has probably been due to the strong selection pressure resulting from modern monocrop agriculture and newly developed susceptible genotypes (Chou & Wu, 2002), leading to rapid increases in their population from less virulent small-spore *Alternaria* species to a causal agent of blight disease which were widely underestimated and have the potential to become a serious threat for crop production. This is believed to be the first report of small spore *Alternaria* isolates causing leaf blight in tomato plants in Algeria. This information has direct applications in conducting periodic surveys of *Solanaceae*to determine pathogen populations. However, it should be achieved by additional analyses such asgenetic diversity assessment within small-spore *Alternaria*species that occur in *Solanaceae*, and their potential to produce toxins.

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