Plant Pathology
Journal

Pathogenecity of Tunisian Isolates of Three *Verticillium* Species on Tomato and Eggplant

[1]Hayfa Jabnoun-Khiareddine, [2]Mejda Daami-Remadi, [1]Khaled Hibar,
[1]Fakher Ayed and [1]Mohamed El Mahjoub
[1]Departement of Phytopathology,
Horticultural High School and Breeding of Chott-Mariem, 4042 Sousse, Tunisia
[2]National Institute of Agronomic Research, Tunis, PRRDA-CE Chott-Mariem, 4042 Sousse, Tunisia

Abstract: In many surveys in the Tunisian Sahel, several isolates of *Verticillium* have been collected from vegetable crops showing symptoms of *Verticillium* wilt. These isolates were identified as *V. dahliae*, *V. albo-atrum* and *V. tricorpus* on the basis of their morphological and cultural characteristics. Four isolates of *V. dahliae*, two isolates of *V. albo-atrum* and two isolates of *V. tricorpus* were examined for their mycelial growth at different incubation temperatures and their virulence against tomato and eggplant cultivars. Seedlings at the three leaf stage were root-dipped in a conidial suspension (10^7 conidia mL^{-1}) and maintained in the glasshouse at 21-25°C. Disease severity was assessed through the index of leaf damage and plant height, recorded eight weeks after inoculation. Growth test showed that the majority of *Verticillium* isolates were able to grow from 10 to 30°C. All tomato and eggplant seedlings exhibited typical *Verticillium* symptoms compared to the non-inoculated control. The index of leaf damage, as well as plant height, differs significantly depending on cultivars and isolates. *V. albo-atrum* and *V. tricorpus* were moderately to highly pathogenic against eggplant and tomato plants. *V. dahliae* was the most aggressive against these hosts, showing a high level of pathogenicity to both eggplant and tomato plants.

Key words: *Verticillium dahliae*, *V. albo-atrum*, *V. tricorpus*, *Lycopersicon esculentum* MILL., *Solanum melongena* L., inoculation, alteration

INTRODUCTION

In all areas of tomato (*Lycopersicon esculentum*) cultivation, serious damage by vascular wilt pathogens is often reported (Elena, 1999). In Tunisia, the vascular wilt fungi which attack tomato are *Fusarium* and *Verticillium* (El Mahjoub, 1974; Hajlaoui *et al.*, 2003; Hibar, 2002; Hibar *et al.*, 2005; Jabnoun-Khiareddine, 2004). This last soilborne pathogen is responsible for some of the world's major diseases affecting vegetable, field, weeds, tree and ornamental crops (Barbara and Clewes, 2003; Peresse *et al.*, 1971; Robb, 2002; Rowe and Powelson, 2002; Rowe *et al.*, 1987; Schnathorst, 1981). However, *Verticillium* symptoms are generally confused with those of *Fusarium* spp., since they cause similar foliar symptoms. In fact the first symptom of the disease is yellowing of the lower leaves. The affected leaves die and the symptoms spread to younger leaves (Elena, 1999). Furthermore one of the main diagnostic features used by field pathologists is browning discoloration of the vascular tissues. Other common symptoms include stunting, leaf epinasty and abscission

(Pegg and Brady, 2002; Robb, 2002). Thus, isolation and identification of the particular wilt pathogen involved in individual instances is necessary (Elena, 1999).

Unlike *Fusarium* species, which exhibit host specificity, *Verticillium* species have not been grouped into special forms, because an isolate from one host often attacks several other unrelated plant species (Isaac, 1949). In fact, *Verticillium* has been found to colonize the roots of a wide range of plants, including species that do not become systemically invaded (Resende *et al.*, 1994). However, when isolating *Verticillium*, problems can occur with general media such as PDA, because these media support growth of many microorganisms (Platt and Bollen, 1995); furthermore, the absolute distance between the germinating structures of *Verticillium* and other microorganisms, capable to grow on the medium, could be the decisive factor whether or not *Verticillium* will form a visible colony. So that, the identification of the cause of plant diseases involves the use of various media that support good growth and sporulation of the pathogens (Gould and Termorshuizen, 2003).

Corresponding Author: Mejda Daami-Remadi, National Institute of Agronomic Research, Tunis, PRRDA-CE Chott-Mariem 4042 Sousse, Tunisia

In Tunisia and especially in the Tunisian Sahel, region with market garden vocation, the monoculture of tomato using the same cultivars prevails. Furthermore, the crop rotation done by the most of the farmers of this region consists on the use of susceptible *Verticillium* crops such as potato, eggplant, pepper, cucumber. During the last few years, many *Verticillium* species have been isolated in Tunisia from several crops, including, tomato, potato and eggplant, pepper, exhibiting vascular wilt symptoms (Jabnoun-Khiareddine, 2004; Jabnoun-Khiareddine *et al.*, 2005a, b). Many *Verticillium* isolates were obtained, which are morphologically different. In fact, the *Verticillium* genus includes the virulent plant pathogenic species *V. dahliae* Kleb, *V. longisporum* (Stark) Karapapa and *V. albo-atrum* Reinke and Berth., which have low saprotrophic abilities, *V. nubilum* Pethybr. and *V. nigrescens* Pethybr. which are saprobes and weak pathogens and *V. tricorpus* Isaac which has intermediate saprotrophic ability and is pathogenic to a limited number of crops such as tomato (Isaac, 1953), snapdragon (Isaac, 1956) and potato (Thanassoulopoulos and Giapanoglou, 1994). All these species can be associated with the same crop, e.g., potato or tomato (Isaac, 1967; Pegg and Brady, 2002; Skotland, 1971). The six species can be differentiated morphologically by the types of resting structures they form in and on the surface of plant material and on many artificial agar media, such as Potato Dextrose Agar (PDA) (Gould *et al.*, 2003).

The Tunisian isolates were identified as *V. albo-atrum*, *V. dahliae* and *V. tricorpus* on the basis of cultural and morphological characteristics (Jabnoun-Khiareddine, 2004; Jabnoun-Khiareddine *et al.*, 2005b). Because of the longevity of microsclerotia and the broad host range of *Verticillium* species, *Verticillium* wilt is considered an increasing problem in the Tunisian Sahel. The objective of present research was therefore, to study the pathogenecity in green house conditions of the Tunisian isolates of *Verticillium* species, on two solanaceous crops: tomato and eggplant.

MATERIALS AND METHODS

Isolation and identification of *Verticillium* species: *Verticillium* was isolated from tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.) and pepper (*Capsicum anuum*). Crop plants showing symptoms of *Verticillium* wilt were collected for the last four years from different fields in the Sahel region. Isolations were made at several levels: roots, collars and stems, on the same day each sample was collected.

Sections (5 to 7 cm long) of plant tissue exhibiting vascular discoloration were rinsed thoroughly in tap water and cut into 0.5-1 cm pieces. After surface-disinfesting in sodium hypochlorite (10%) for 3 min, the plant pieces were rinsed three times in sterile distilled water, dried on sterile filter paper and plated on PDA (Potato Dextrose Agar) medium with streptomycin sulphate (300 mg L^{-1}). Fungal cultures were incubated for two weeks at 20°C. The fungal isolates were cleaned up by subculturing successively on antibiotic plates from the edge of actively growing colonies and single spores were then isolated. Microscopic cultures of *Verticillium* isolates were examined and species were identified by their resting-structure morphology, by means of the taxonomic key presented by Isaac (1956) and the descriptions of Hawksworth (1970) and Hawksworth and Talboys (1970a, b). Monoconidial subcultures of all *Verticillium* species were stored in 25% aqueous glycerol solution at-20°C.

Eight isolates of *Verticillium* spp were used in this study: four isolates of *V. dahliae* (two from tomato, Vdt1 and Vdt2, one from potato Vdp1 and one from eggplant, Vde1), two isolates of *V. albo-atrum* from tomato (Va1 and Va2) and two isolates of *V. tricorpus* (one isolate from potato, Vt1 and the other from tomato, Vt2).

Effect of temperature on the mycelial growth of *Verticillium* isolates: To compare optimal temperature requirements of *V. dahliae*, *V. albo-atrum* and *V. tricorpus*, mycelial growth of eight isolates of *Verticillium* (Vdt1, Vdt2, Vdp1 and Vde1, Va1, Va2, Vt1 and Vt2), was evaluated at 5, 10, 15, 20, 25, 30 and 35°C. For each isolate and temperature combination, five plates (85 mm diameter) of PDA were seeded at the center with a six mm agar plug taken from the edge of an actively growing colony of each isolate. The radial growth of all colonies was measured after 21 days. The experiment was conducted twice. Analysis of variance to evaluate the effects of isolates, temperatures, growth and the interaction between these variables on mycelial growth was made using the SPSS procedure.

Pathogenicity tests: Pathogenicity of V*erticillium* species was determined by the root-dip method, using tomato (cv. Colibri resistant to the *V. dahliae* race 1) and eggplant (cv. Bonica, susceptible). Eight isolates of *Verticillium* (Vdt1, Vdt2, Vdp1 and Vde1, Va1, Va2, Vt1 and Vt2), were inoculated onto tomato and eggplant seedlings. Inoculum was prepared from 1-week old liquid cultures on PDB (Potato Dextrose Broth) which was filtrated and the density of each *Verticillium* isolate was adjusted to approximately 10^7 conidia mL^{-1}. Seedlings at

the three leaf stage were uprooted from the substrate; their roots washed in tap water, rinsed by sterile distilled water and dipped for 30 min in fungal suspension (Bhat *et al.*, 2003). Non-inoculated control seedlings were dipped in sterile distilled water. Seedlings were then transplanted to pots filled with the sterilized peat and perlite, mixed in equal proportions. Plants were arranged in a randomized complete factorial design and incubated in glasshouse benches at 21-25°C. They were irrigated regularly and fertilized using a nutrient solution (N, 150 ppm; P, 50 ppm; K, 150 ppm; Ca, 150 ppm; Mg, 30 ppm; Fe, 3 ppm; Mn, 1.5 ppm; Zn, 0.2 ppm; B, 0.4 ppm; Cu, 0.1 ppm; Mo, 0.05 ppm and H_2O, qsp 1l). The experiment was performed twice.

Plants were monitored for the development of the symptoms of *Verticillium* wilt and the days between the inoculation and the first observation of symptoms was recorded. Symptoms included chlorosis, necrosis and wilt (Béye and Lafay, 1985). Plant height and disease severity were recorded eight weeks after inoculation. The eight of each tomato and eggplant plants was measured from the soil line. A scale of 0 to 4 was used to assess disease severity in which: 0 = leaf of healthy aspect; 1 = epinasty or wilted leaf without chlorosis; 2 = one or several bands slightly chlorotic on one or several leaflets; 3 = Chlorotic bands on all the surface of one or several leaflets or chlorotic band with a necrotic centre on one or several leaflets and 4 = complete necrosis of several leaflets or dead leaf (Béye and Lafay, 1985). An Index of Leaf Damage (ILD) was then calculated, 60 days post inoculation, for every plant according to the following formula: ILD = notes sum/possible maximum. Possible maximum is equal to four times the number of well developed leaves carried by the plant (Béye and Lafay, 1985).

Ten plants were inoculated for each host-isolate combination. Analyses of variance were conducted for each dependant variable to determine effects of isolates and host plants and to examine the interactions among these variables. All statistical analyses were performed using SPSS.

RESULTS

Isolation and identification of *Verticillium* species: During the surveys, epinasty, chlorosis and necrosis on the leaf, defoliation, stunting and light to dark brown vascular discoloration in the roots, collar and stem of diseased plants were observed in the growing season. Sampled plants were collected from many vegetable crops (tomato, potato, eggplant and pepper) growing in the greenhouse or under field conditions. Typical cultures of *Verticillium* were isolated from representative diseased

plants in many fields in the different surveyed regions (Table 1). Sometimes, species of *Alternaria, Fusarium* and other fungi emerged from random areas on surfaces of the disinfected plant pieces. In contrast, *Verticillium* always emerged from the vascular tissue and in most isolation from the upper stem.

Verticillium isolates were identified based on their morphological and cultural characteristics. All *Verticillium* isolates produced flocculose colonies with conidiophores which were more or less erect, hyaline, with verticillate branches and with 2-4 phialides at each node. These phialides are borne in whorls on branched aerial

Table 1: Origin of isolates of *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus* collected in Tunisian regions from 2002 to 2005

Isolates	Species	Host of Origin	Geographical origin	Year of isolation
Vdt1	*V. dahliae*	Tomato	Chott-Mariem	2002
Vdt2	*V. dahliae*	Tomato	Chott-Mariem	2002
Vdt3	*V. dahliae*	Tomato	Chott-Mariem	2002
Vdt4	*V. dahliae*	Tomato	Chott-Mariem	2002
Vdt5	*V. dahliae*	Tomato	Chott-Mariem	2002
Vdt6	*V. dahliae*	Tomato	Chott-Mariem	2002
Vdt7	*V. dahliae*	Tomato	Chott-Mariem	2002
Vdt8	*V. dahliae*	Tomato	Chott-Mariem	2003
Vdt9	*V. dahliae*	Tomato	Chott-Mariem	2003
Vdt10	*V. dahliae*	Tomato	Chott-Mariem	2003
Vdt11	*V. dahliae*	Tomato	Chott-Mariem	2004
Vdt12	*V. dahliae*	Tomato	Chott-Mariem	2004
Vdt13	*V. dahliae*	Tomato	Téboulba	2004
Vdt14	*V. dahliae*	Tomato	Téboulba	2004
Vdt15	*V. dahliae*	Tomato	Kalaa Kébira	2004
Vdt16	*V. dahliae*	Tomato	Kalaa Kébira	2005
Vdt17	*V. dahliae*	Tomato	Kalaa Kébira	2005
Vdt18	*V. dahliae*	Tomato	Kalaa Kébira	2005
Vdp1	*V. dahliae*	Potato	Kalaa Kébira	2002
Vdp2	*V. dahliae*	Potato	Sidi Bou Ali	2002
Vdp3	*V. dahliae*	Potato	Sidi Bou Ali	2003
Vdp4	*V. dahliae*	Potato	Sidi Bou Ali	2004
Vdp5	*V. dahliae*	Potato	Sidi Bou Ali	2004
Vdp6	*V. dahliae*	Potato	Chott-Mariem	2005
Vdp7	*V. dahliae*	Potato	Chott-Mariem	2005
Vde1	*V. dahliae*	Eggplant	Chott-Mariem	2002
Vde2	*V. dahliae*	Eggplant	Kalaa Kébira	2004
Vde3	*V. dahliae*	Eggplant	Kalaa Kébira	2004
Vdpe1	*V. dahliae*	Pepper	Téboulba	2003
Vdpe2	*V. dahliae*	Pepper	Chott-Mariem	2004
Vdpe3	*V. dahliae*	Pepper	Chott-Mariem	2004
Va1	*V. albo-atrum*	Tomato	Chott-Mariem	2002
Va2	*V. albo-atrum*	Tomato	Chott-Mariem	2002
Va3	*V. albo-atrum*	Tomato	Chott-Mariem	2002
Va4	*V. albo-atrum*	Tomato	Chott-Mariem	2002
Va5	*V. albo-atrum*	Tomato	Chott-Mariem	2004
Va6	*V. albo-atrum*	Tomato	Chott-Mariem	2004
Va7	*V. albo-atrum*	Tomato	Chott-Mariem	2004
Va8	*V. albo-atrum*	Tomato	Chott-Mariem	2004
Va9	*V. albo-atrum*	Tomato	Chott-Mariem	2004
Vt1	*V. tricorpus*	Tomato	Chott-Mariem	2002
Vt2	*V. tricorpus*	Tomato	Chott-Mariem	2002
Vt3	*V. tricorpus*	Tomato	Chott-Mariem	2002
Vt4	*V. tricorpus*	Tomato	Sidi Bou Ali	2004
Vt5	*V. tricorpus*	Potato	Sidi Bou Ali	2004
Vt6	*V. tricorpus*	Potato	Sidi Bou Ali	2004
Vt7	*V. tricorpus*	Potato	Chott-Mariem	2005
Vt8	*V. tricorpus*	Potato	Chott-Mariem	2005

These phialides are borne in whorls on branched aerial hyphae. Conidia arise singly at the apices of the phialides and are formed in clusters in a mucilaginous slime. These conidia are for the most part single-celled and haploid. Since conidial size differed in various culture media, at different temperatures and after different periods of growth and that conidial size alone was not a reliable character to use in species determination in *Verticillium*, the Tunisian *Verticillium* species were differentiated morphologically by the types of resting structures they form on PDA media. Three species of *Verticillium* were identified: *V. dahliae*, *V. albo-atrum* and *V. tricorpus*. In fact, *V. dahliae* forms microsclerotia which are clusters of thick-walled heavily melanized cells which separate as discrete bodies from the parent mycelium (Fig. 1); In *V. albo-atrum*, hyphal sections differentiate into thick-walled melanized cells, the dark resting mycelia (Fig. 2) while *V. tricorpus* forms microsclerotia, resting mycelium and chlamydospores (Fig. 3). On PDA, the microsclerotia of *V. tricorpus* are large and irregularly shaped, usually with melanised hyphae growing from them, whereas *V. dahliae* forms smaller and oval to elongate microsclerotia which are sharply differentiated from the hyaline mycelium and hyaline conidiophores. Moreover, *V. tricorpus* often causes a yellow discoloration of the PDA medium up to 1-2 weeks.

In *V. albo-atrum*, the bases of conidiophores were darkened whereas in *V. dahliae* and *V. tricorpus*, conidiophores were completely hyaline. This difference is especially noticeable on the host and in strains which recently have been brought into artificial culture; the character may be lost in *V. albo-atrum* on prolonged culture, however. The conidia of *V. albo-atrum* are longer and slightly wider than those of *V. dahliae*.

The appearance of *Verticillium* cultures on PDA or on other artificial medium differs among the species (Fig. 4-6). and among the isolates of the same species. In *V. dahliae*, the abundance or not of the microsclerotia and their distribution in the media make many differences on the morphology of *V. dahliae* isolates. The use of different media can also show the morphological difference between *Verticillium* species such as oats medium which permit a very faster development of *V. albo-atrum* isolates compared to those of *V. dahliae* (Jabnoun-Khiareddine, 2004).

Effect of temperature on the mycelial growth of *V. dahliae*, *V. albo-atrum* and *V. tricorpus*: Temperature had a significant influence on the growth of *Verticillium* isolates. Analyses of variance indicated that the interaction between the temperatures of incubation and the *Verticillium* isolates significantly affected the mycelial colony diameter. The three *Verticillium* species showed

similar growth at temperatures between 10 to 35°C (Table 2). Temperature of 20°C was optimum for maximum growth of all *Verticillium* species (Table 2). Important growth rates were observed also at 15 and 25°C.

Both *V. albo-atrum* isolates and *V. tricorpus* isolate Vt1 were able to grow at 5°C except for *V. dahliae* isolates and *V. tricorpus* isolate, Vt2. All *Verticillium* species were tolerant to 30°C, except the *V. dahliae* isolate, Vdp1. Although these isolates were tolerant to 30°C, their mycelium was thin and irregular. Neither *Verticillium* species grew at 35°C. All *V. dahliae* isolates were relatively slow-growing, whereas, *V. albo-atrum* and *V. tricorpus* isolates were fast-growing.

Pathogenicity of *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus*: Pathogenicity of all *Verticillium* isolates (four *V. dahliae*, two *V. albo-atrum* and two *V. tricorpus*)

Table 2: Effect of temperature on radial growth of *Verticillium* spp after 21 days on PDA

Isolate	Colony diameter (cm)						
	5°	10°	15°	20°	25°	30°	35°
Vdt1	0	2.1	3.9	4.6	4.4	2.6	0
Vdt2	0	2	4.2	4.8	3.4	2.4	0
Vdp1	0	2.1	4.4	5.1	3.9	0	0
Vde1	0	1.75	3.8	4.4	2.8	1.2	0
Va1	1.2	3.2	4.6	5.7	5.2	2.1	0
Va2	0.7	2.8	4.8	5.5	4.8	2	0
Vt1	0.9	2.8	4.6	5.5	4.5	1.9	0
Vt2	0	2.7	3.9	5.1	4.1	0.5	0

LSD (p≤0.05) = 0.18 cm, Isolates of *V. dahliae* are labelled with: Vdt1, Vdt2, Vdp1 and Vde1. Isolates of *V. albo-atrum* are labelled with Va1 and Va2; those of *V. tricorpus* are Vt1 and Vt2

Table 3: Pathogenicity of *Verticillium* isolates inoculated onto tomato (cv. Colibri, resistant to *V. dahliae* race 1) and eggplant (cv. Bonica, susceptible) plants in the glasshouse eight weeks after inoculation (21°<T<25°C)

Isolates	Tomato		Eggplant	
	Plant height (mm)	Disease severity (ILD)[a]	Plant height (mm)	Disease severity (ILD)[a]
Vdt1	126h	2.75b	40h	2.95b
Vdt2	130g	4.05b	47g	3.95a
Vdp1	151f	1.75c	53f	3.05c
Vde1	168f	1.25b	36f	5.00b
Va1	316e	2.50d	95c	0.85d
Va2	350b	3.10c	119b	1.33c
Vt1	228e	1.10d	122e	1.87d
Vt2	284d	1.50d	105d	1.47d
Noninoculated control	495a	0e	205a	0e

In each column, values followed by the same letter are not significantly different as determined by ANOVA (p≤0.05), Isolates of *V. dahliae* are labelled with: Vdt1, Vdt2, Vdp1 and Vde1. Isolates of *V. albo-atrum* are labelled with Va1 and Va2; those of *V. tricorpus* are Vt1 and Vt2, [a]Index of Leaf Damage (ILD) is the index of leaf damage caused by *Verticillium* spp. and evaluated for each plant on a scale of 0 to 4, where 0 = leaf of healthy aspect; 1 = epinasty or wilted leaf without chlorosis; 2 = one or several bands slightly chlorotic on one or several leaflets; 3 = Chlorotic bands on all the surface of one or several leaflets or chlorotic band with a necrotic centre on one or several leaflets and 4 = complete necrosis of several leaflets or dead leaf
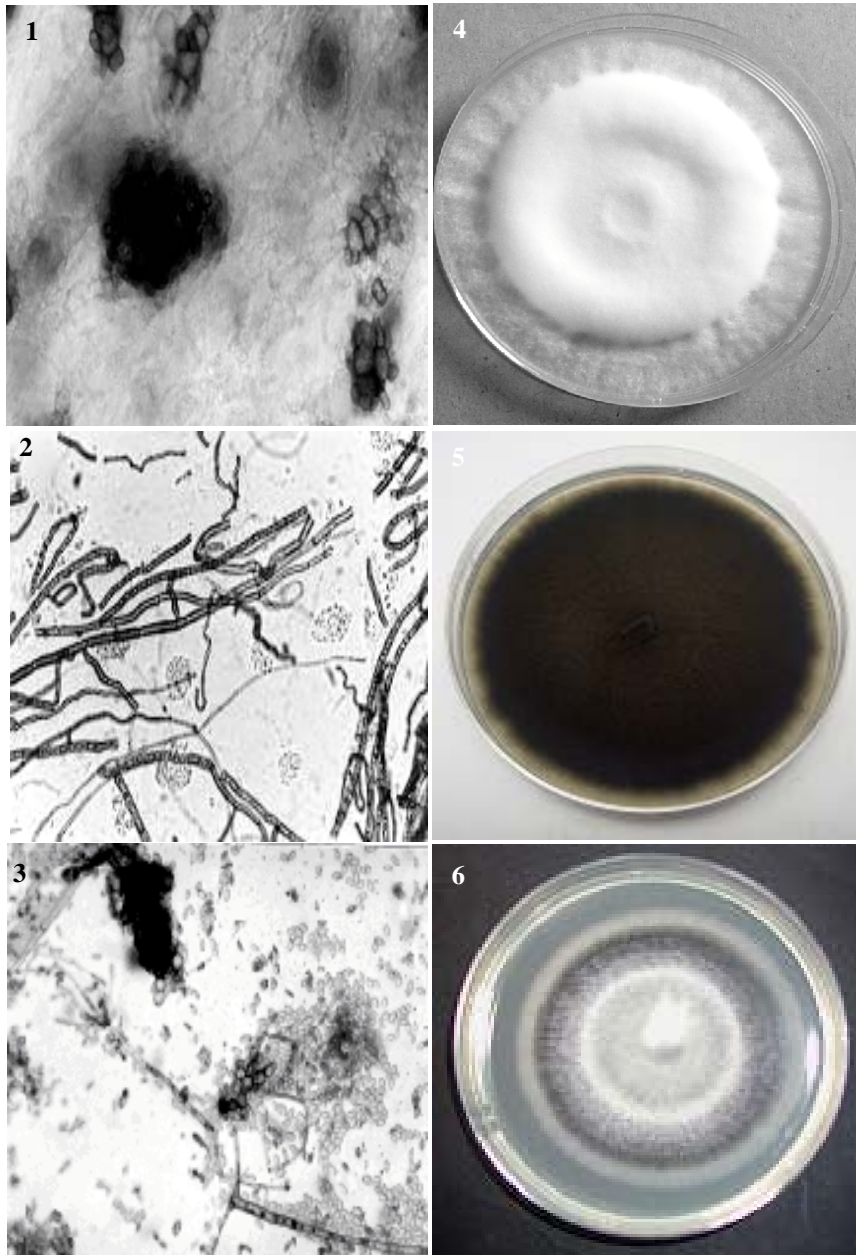
Fig 1-6: Morphology of *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus* on PDA medium.
      Fig. 1: Globose to elongate *V. dahliae* microsclerotia on PDA
      Fig. 2: Dark mycelium of *V. albo-atrum* on PDA
      Fig. 3: Oval to irregularly shaped *V. tricorpus* microsclerotia with dark hyphae on PDA
      Fig. 4: *V. dahliae* colony on PDA
      Fig. 5: *V. albo-atrum* colony on PDA
      Fig. 6: *V. tricorpus* colony on PDA

on tomato (cv. colibri, resistant to *V. dahliae* race 1) and eggplant (cv. bonica, susceptible to *V. dahliae*) was determined by the root dip method. The same inoculum concentration and root dipping period was used for the three *Verticillium* species. The criteria used to assess pathogenicity were leaf damage and plant height (Table 3). Each species was tested in two experiments with similar results.

Typical *Verticillium* wilt symptoms were observed on both tomato and eggplant plants. These symptoms consist of a general decline of plant growth, chlorosis of the leaves, stunting followed by wilting (Fig. 7 and 8). These symptoms were first observed from 15 to 30 days post inoculation depending on cultivars and *Verticillium* species.

In fact, plants infected by all *V. dahliae* isolates were the first to develop disease symptoms, about 16 to 23 days postinoculation respectively for eggplant and tomato; while *V. albo-atrum* and *V. tricorpus* isolates developed symptoms approximately 25 to 30 days post inoculation, respectively for eggplant and tomato cultivars.

A significant interaction (p≤0.05) between *Verticillium* isolates and host cultivars was noted, showing a differential response of inoculated cultivars toward *Verticillium* isolates. *V. dahliae* isolates were more aggressive (judging by the magnitude of reduction in height and disease severity) than *V. albo-atrum* and *V. tricorpus* isolates when inoculated to eggplant and tomato plants (Table 3). The tomato isolate of *V. dahliae*, Vdt2, was the most severe on both tomato and eggplant cultivars, causing important leaf damage while the tomato isolate Vdt1 caused the most severe stunting. The potato and eggplant isolates of *V. dahliae* caused also an important leaf damage and reduction in the height of the inoculated eggplant and tomato plants, showing the aptitudes of *V. dahliae* isolates to make a cross pathogenicity. In fact, it has been proven recently that isolates of *V. dahliae* from olive were able to severely attack tomato plants (Jabnoun-Khiareddine *et al.*, unpublished data).

The tomato *V. albo-atrum* isolate, Va2 and the potato isolate of *V. dahliae*, Vdp1, caused moderate leaf damage to both eggplant and tomato plants. All *V. albo-atrum* and *V. tricorpus* isolates caused reduction in the plant height in both plant hosts. The minimal reduction was noted with *V. albo-atrum* isolates.

Inoculated eggplant and tomato plants had significantly (p≤0.05) more dead and yellow leaves than the non-inoculated control plants which remain symptomless.

Fig. 7: Comparison between non-inoculated control plant and eggplant plant inoculated with *Verticillium* sp. 30 days post-inoculation (cv. Bonica, 21<T<25°C). Stunting, wilting and chlorosis are noted on the inoculated plant

Fig. 8: Comparison between non-inoculated control plant and tomato plant inoculated with *Verticillium* sp. 25 days post-inoculation (cv. Colibri, 21<T<25°C). Wilting and chlorosis are noted on the inoculated plant

Inoculated plants developed severe vascular discoloration in the roots and stems by eight weeks after inoculation. The mean height of inoculated plants was significantly (p≤0.05) lower than the mean height of non-inoculated control plants.

All *Verticillium* isolates were readily re-isolated from inoculated eggplant and tomato plants.

DISCUSSION

Verticillium isolates obtained from vascular discoloured tissue of tomato, potato, eggplant and pepper were identified as *V. dahliae*, *V. albo-atrum* and *V. tricorpus* on the basis of cultural and morphological characteristics. *V. tricorpus* formed young yellow colonies on PDA up to 1 to 2 weeks and produce three types of resting structures: chlamydospores, dark resting mycelia and microsclerotia. *V. albo-atrum* produce only dark resting mycelia while *V. dahliae* forms only microsclerotia (Barbara and Clewes, 2003; Gould *et al.*, 2003; Isaac, 1967; Pegg and Brady, 2002; Robb, 2002). *V. dahliae* is distinguished morphologically from *V. albo-atrum* and *V. tricorpus* by a totally hyaline conidiophores, small conidia and subspherical to elongate microsclerotia. These results, similar to those obtained by several other authors (Barbara and Clewes, 2003; Ebihara *et al.*, 2003; Hawksworth, 1970; Korolev and Katan, 1999; Pegg and Brady, 2002; Robb, 2002; Termorshuizen *et al.*, 1998; Veronese *et al.*, 2003) confirm well presence in Tunisia of three *Verticillium* species (Hajlaoui *et al.*, 2003; Jabnoun-Khiareddine, 2004; Jabnoun-Khiareddine *et al.*, 2005b, 2006).

The optimal mycelial growth of all *Verticillium* isolates was 20°C. The majority of *Verticillium* isolates were tolerant to 30°C as it has been pointed out previously (Jabnoun-Khiareddine *et al.*, 2005b, 2006).

In fact, the temperature difference for growth and survival of the resting structures of *V. albo-atrum* (dark resting mycelia) and *V. dahliae* (microsclerotia) constitutes the most important character for the separation of *V. albo-atrum* and *V. dahliae* as biologically distinct species (Pegg and Brady, 2002). However, our Tunisian isolates of *V. albo-atrum* were able to grow at 30°C as well as *V. dahliae* and *V. tricorpus*, so that, our *Verticillium* isolates were identified mainly on the basis of the types of resting structures they form.

In fact, *V. albo-atrum* is commonly found in northern latitudes were cool wet weather prevails. *V. dahliae* prefers the warmer latitudes, while *V. tricorpus* occasionally produce mild symptoms in very specialized niches (Robb, 2002). However, Jabnoun-Khiareddine *et al.* (2006) signalled that these Tunisian isolates of *V. albo-atrum*, first reported in Chott Mariem region in spring 2002 (Hajlaoui *et al.*, 2003) were able to be adapted to the local climate of this region and to express their pathogenic capabilities. They showed also, that Tunisian *V. albo-atrum* isolates were virulent to many tomato cultivars under 17-21°C and 21-30°C temperatures ranges.

The current study demonstrates that isolates of *Verticillium* vary in pathogenicity on tomato and eggplant. In fact, the three *Verticillium* species and especially *V. dahliae* isolates were relatively highly pathogenic to tomato plants, in spite of the use of a tomato cultivar carrying the *Ve* gene. In fact, the tested isolates were able to reduce significantly the height of the inoculated tomato plants and to induce severe foliar damage. Vigouroux (1971) pointed out that isolates from a region of permanent monoculture are similar and all display high virulence against this particular crop (preferential host) but generally a weak virulence against other species which they can, nevertheless, infect (occasional host). In addition, *V. dahliae* isolates were very destructive to eggplant, causing severe wilt and stunting on the inoculated plants compared to the noninoculated control plants. These results agree with those of Vigouroux (1971) and other researchers (Korolev and Katan, 1999; Resende *et al.*, 1994) who postulated that eggplant is a preferential host for *V. dahliae*. These results confirmed well those previously obtained by Jabnoun-Khiareddine *et al.* (2005a).

In this current study, *V. albo-atrum* was more aggressive to tomato plants than eggplant. However, *V. tricorpus* showed relatively high virulence to both eggplant and tomato plants. In fact, Jabnoun-Khiareddine *et al.* (2005b) have shown that Tunisian *V. tricorpus* isolates were able to attack tomato, eggplant and potato causing wilt and stunting on the inoculated plants.

Furthermore, isolates of *V. dahliae* from potato were able to highly attack eggplant and tomato plants as well the potato isolate of *V. tricorpus*.

In the current study a high level of aggressiveness of *Verticillium* isolates was noted towards tomato plants, in spite of the use of cultivar carrying the *Ve* gene. This high virulence may be due to the permanent monoculture and the short rotation with susceptible hosts that may have increased selection pressure on strains of *Verticillium* that colonize and reproduce on tomato plants more effectively, resulting in an increase of inoculum levels causing significant yield losses (Bhat *et al.*, 2003). Tjamos (1981) suggested that the broadening of host range could occur in the field after introduction of potential hosts into the same area. In addition, the introduction of a preferential host in a crop rotation would be expected to increase greatly the amount of inoculum of a wide range of strains. Such inoculum would be available for successive crops, some of which may be occasional hosts (Vigouroux, 1971).

Bender and Shoemaker (1984) signalled that the slow decline of microsclerotia numbers in soil and the infection of weed hosts and other host crops may increase the incidence of *V. dahliae* race 1 on tomato race 1 resistant cultivars. They added that if the use of tomato cultivars with the *Ve* gene for resistance to race 1 continues, the prevalence of race 2 will probably increase and serious yield losses may occur. In fact, race 2 pathotypes may be a natural component of the *V. dahliae* population in Chott Mariem region and could have arisen as a mutation from tomato race 1 isolates or evolved from *V. dahliae* isolates pathogenic on other hosts. Furthermore, apart from the intrinsic resistance of the plant attacked, there are a number of factors known to influence the incidence and severity of *Verticillium* wilt outbreaks. In fact, *V. dahliae* are favoured by moderate to high temperature, although temperatures above 30°C are inhibitory for most strains of the pathogen (Schnathorst, 1981). In addition, *Verticillium* wilt may be exacerbated by plant pathogenic nematodes, particularly of the genus *Pratylenchus* (Harris, 1998). These factors are, in fact, present in the Tunisian Sahel.

ACKNOWLEDGMENTS

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