



## التسجيل الأول للفطر *Fusarium solani* كمسبب لعفن جذور بادرات الفستق الحلبي *Pistacia vera* L. في المشاتل في سورية.

### First Report of *Fusarium solani* Causing Root Rot of Pistachio (*Pistacia vera* L.) Seedlings in Nurseries in Syria

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#### المُلخَص

لوحظ خلال عامي 2014 و2015 موت عدد كبير من غراس الفستق الحلبي *Pistacia vera* L. بعد عدة شهور من الإنبات في مشتل الكوم التابع لمديرية الزراعة والإصلاح الزراعي في محافظة السويداء (سورية). لذلك هدف هذا البحث لدراسة هذه الظاهرة وتعريف الفطر المسبب لها. أظهرت النتائج أن النسبة المئوية للغراس الميتة بلغت أكثر من 50% في عام 2014، وتجاوزت 20% في عام 2015 حتى تاريخ هذه الدراسة. إذ لوحظ وجود تقرحات على جذور الغراس الميتة، وتلونها بلون بني محمر إلى أسود، وفي بعض الحالات شمل التلون الأسود الجذر بالكامل، كما لوحظ امتداد التلون أيضاً إلى قاعدة الساق. وتبين من خلال العزل من الأنسجة المصابة أن الفطر المسبب لموت الغراس هو *Fusarium solani* Sacc. (Mart.). وتم تأكيد النتيجة من خلال تطبيق فرضية كوخ بإجراء عدوى اصطناعية على أصل الفستق الحلبي *Pistacia vera*. إذ بلغت النسبة المئوية للغراس الميتة المعدة اصطناعياً أكثر من 60%، كما أبدت أعراضاً مماثلة لتلك التي لوحظت على الغراس في المشتل. ثم عُزل الفطر *F. solani* مرة أخرى من الغراس المعدة اصطناعياً. لذلك تعد نتائج هذه الدراسة التسجيل الأول لعفن التاج والجذور لغراس الفستق الحلبي المتسبب عن الفطر *F. solani* في المشاتل في سورية.

**الكلمات المفتاحية:** موت الغراس، لعفن الجذور، المشاتل، الفستق الحلبي، *Fusarium solani*

#### Abstract

In 2014/15 growing season, It was observed a significant death rate of pistachio (*Pistacia vera* L.) seedlings after several months of germination in Al-kom nursery belonging to the Directorate of Agriculture and Agrarian Reform in the governorate of Sweida / Syria. The objectives of this research were to define the pathogen responsible for this phenomena. The inventory process showed that the percentage of seedling death in 2014 was more than 50 %, and exceeded 20 % in 2015 up to the date of this study. The dead seedlings showed lesions on roots with reddish brown to black coloration, and in some cases the entire root appear black. Discoloration was also observed at the base of stem. The isolation from infected tissues showed that the fungus causing seedling death was *Fusarium solani* (Mart.) Sacc. Koch's postulates were completed by an artificial infection on *Pistacia vera* rootstock. The seedling death percentage was more than 60%, and showed symptoms similar to those that had appeared on the nursery seedlings, then the fungus *F. solani* was reisolated from artificially infected seedlings. The results of this study are considered as the first report of root and crown rot of pistachio seedlings caused by *F. solani* in nurseries in Syria.

**Keywords:** Seedling death, Root rot, Nursery, Pistachio, *Fusarium solani* .

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## Introduction

The pistachio (*Pistacia vera* L.) is native to western Asia and Asia minor, where still found growing wild in Turkey, Syria, Iran, Iraq, India, Lebanon, Palestine, southern Europe, Asia and Africa (Eskalen *et al.*, 2001).

Pistachio nut has been grown in Syria for centuries. Traditionally, Aleppo which is in the northern part of Syria, is the main growing area of pistachios, which later spread to all countries and known worldwide by "Aleppo pistachio". According to FAO statistics (1993), Syria is classified as the fourth Pistachio nut producing country in the world after Iran, U.S.A. and Turkey. This tree occupies about 6% of the total area of fruit trees in Syria, with an area of about 56 thousand hectares, and is concentrated in six governorates: Aleppo 44%, Idlib 13%, Damascus countryside 6%, Alraka 1% and Sweida 1%. There are some trees aging more than 500 years in Ain-El-Thainah near Damascus (Hadj-Hassan and Kardouch, 1995).

In Syria several *Pistacia* species such as *P. atlantica* (in the East and South), *P. palaestina* and *P. lentiscus* (in the West) can be found. Seedlings of *P. vera* and *P. atlantica* are used as rootstocks. The nurseries of Ministry of Agriculture and Agrarian Reforms produce mostly one year old pistachio seedlings and distribute them to the orchardists. Some private nurseries produce nursery plants of pistachio nut in commercial scale.

There are many fungal diseases known to afflict pistachio trees in east-Mediterranean regions. Several diseases have been identified on this crop, some of them causing considerable damage. This was due mainly to new knowledge on the causal agent of pistachio diseases, new farming practices and also to the cultivation of pistachio in new areas with different environmental conditions resulting in changes in the epidemiology of the different diseases. Pistachio growing mostly in dry areas are rarely infected by root rotting fungi (Eskalen *et al.*, 2001).

*Fusarium solani* (Mart.) Sacc. (teleomorph = *Nectria haematococca* (Berk. & Br.) is a phytopathogenic fungus and is an important causal agent of several crop diseases, such as root and fruit rot of *Cucurbita* spp., root and stem rot of pea, sudden death syndrome of soybean, foot rot of bean and dry rot of potato (Cho *et al.*, 2001; Desjardins, 2006), and crown disease of oil palm (Hafizi *et al.*, 2013). There are at least 111 plant species from 87 genera that are commonly infected by *F. solani* (Kolattukudy and Gamble, 1995).

During 2014/15, it was observed significant death of pistachio seedlings after several months of germination in Al-kom nursery belonging to directorate of agriculture and agricultural reform in governorate Sweida. The present study was conducted to identify the pathogen responsible for this phenomena.

## Material and methods

**Sampling:** The percentage of dead seedlings was estimated. Ten dead seedlings were taken randomly from the nursery on 18/05/2014, put in a plastic bag, and transferred to the laboratory of phytopathology belonging to the Directorate of Agriculture and Agrarian Reforms in Al-Kom region. The roots were washed with running water, then pathological symptoms were described.

**Fungal isolation:** Infected roots showing typical symptoms of brown colored tissues had been removed from dead seedlings, superficially sterilized for 5 minutes in 6% sodium hypochlorite, washed 3 times with sterile water, cut into small pieces, then put on potato dextrose agar (PDA) medium supplemented with antibiotic Amoxicillin (100 ppm). Dishes were incubated at  $22 \pm 2^{\circ}\text{C}$ . Pure cultures were made by additional transfers. Fungal isolates were classified basing on their morphology according to Commonwealth Mycological Institute (C.M.I., 1990).

**Preparation of spore suspension:** 15 ml of sterilized water were added to a Petri dish containing a fungal culture of 10 days old. The conidia were liberated by scraping the surface of growing cultures using sterilized needle, and filtered through cheesecloth to remove most of the mycelial fragments. The concentration of the spore suspension was adjusted to  $10^6$  spore/ml by the aid of a haemocytometer.

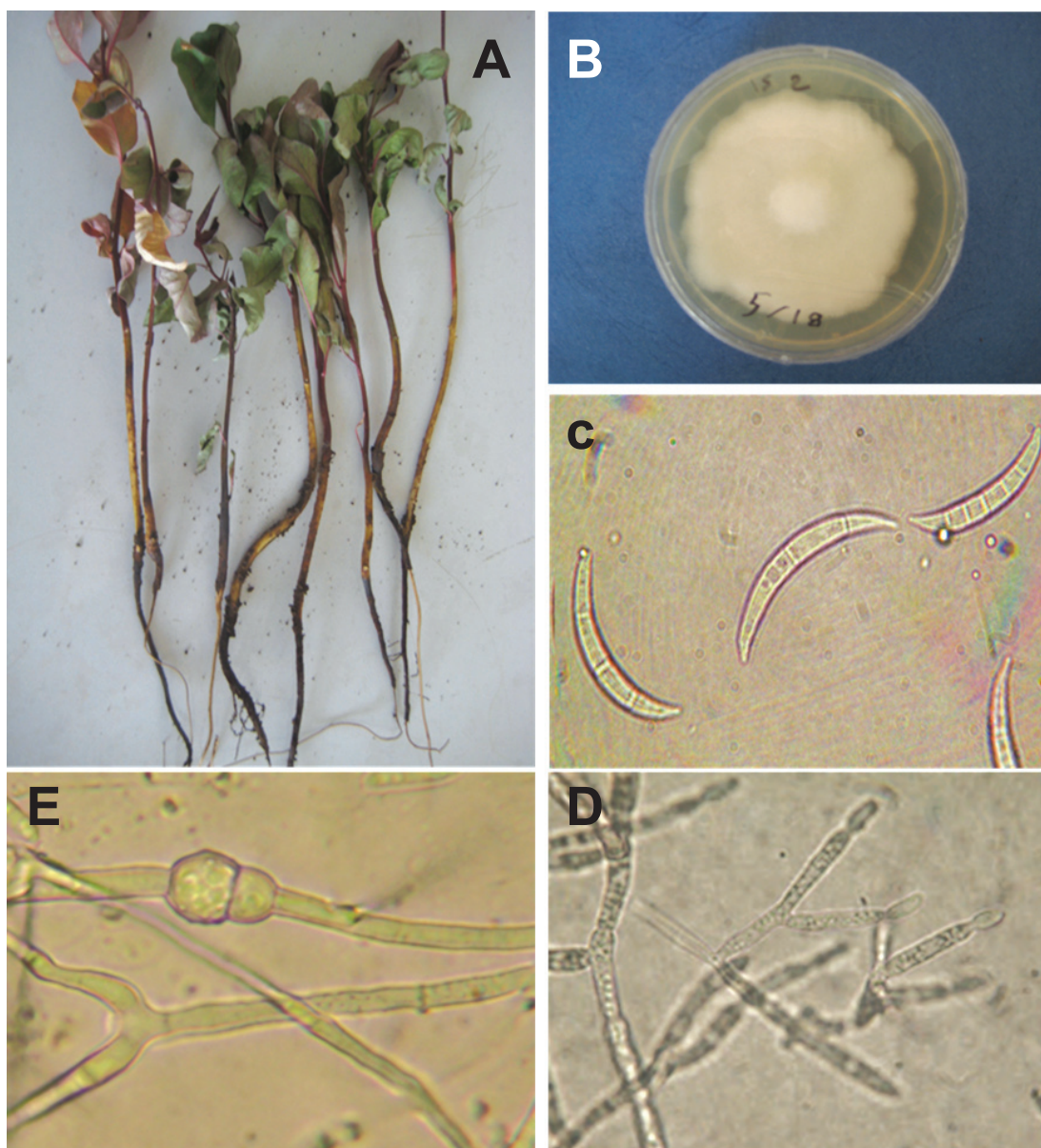
**Artificial infection:** The experiment was carried out in plastic sterilized black bags (30 x 15 cm), each contains 3 kg of soil, which is composed of a mixture of clay, black sand and organic fertilizer (2 : 1: 1 v), sterilized in the autoclave for 20 minutes at a temperature of  $121^{\circ}\text{C}$ . Pistachio seeds were surface sterilized by immersing in 6% sodium hypochlorite

for 5 minutes, and rinsed three times into sterile distilled water, then left to dry on sterilized filter paper. Twenty seeds were planted (one seed in each bag), and 10 ml of spore suspension were added around each seed, while the same volume of sterilized water was added to each of 10 seeds used as a control.

## Results and Discussion

During 2014/ 2015, it was observed that pistachio seedlings died after several months of germination in the Al-Kom nursery in Sweida in southern of Syria . The inventory process showed that the percentage of died seedlings in 2014 was more than 50%, 10000 out of 20000 seedlings were died. In 2015, the percentage of seedlings death exceeded 20 % up to the date of this study, where 2000 out of 10000 seedlings died, and it has also been noted that this phenomenon was growing continuously with high temperatures.

Infected seedling roots showed reddish brown or dark to light brown discoloration and decay. In some cases the entire root appear black. Discoloration was also observed at the base of stem, and the lesion area was cankered and thin (Fig. 1).



**Figure 1. (A) Pistachio seedling roots showing reddish brown to dark discoloration and decay. (B) Nine days old colony of *F. solani*. (C) Macroconidia of *F. solani* with 35- septa. (D) branched conidiophore of *F. solani*. (E) intercalary chlamydospores of *F. solani*.**

These symptoms are common for many fungal root rot and crown rot diseases. Similar symptoms were described for *Phytophthora* spp. and

*Fusarium equiseti* on roots of pistachio trees (Eskalen *et al.*, 2001), for *Fusarium* spp. on wheat (Bently *et al.*, 2006; Saremi *et al.*, 2007; Bockus *et al.*, 2007) and on pea (Hall, 1991; Schwartz and Yuen, 2005), for *Thielaviopsis basicola* on tobacco (Shew and Shoemaker, 1993).

The isolation from infected tissues showed that the fungus causing seedlings death was *Fusarium solani* (Mart.) Sacc. according to its morphology: colonies are white to cream, growing rapidly. Macroconidia are fusiform, often moderately curved, with an indistinctly pedicellate foot cell and a short blunt apical cell. They are 3 to 5 septate (usually 3 septate). Microconidia are usually abundant, cylindrical to oval, one to two celled and formed from long lateral, sometimes branched, conidiophore. Chlamydospores are hyaline, globose, borne singly or in pairs on short lateral hyphal branches or intercalary (Fig.1). These characteristics of *F. solani* are in accordance with those described by Booth (1977), Burgess and Liddell (1983) and Aoki *et al.*, 2003.

The results were confirmed through an artificial infection on *Pistacia vera* rootstock. The seedlings death percentage was more than 60%, 12 out of 20 seedlings artificially inoculated died, and the seedlings of tested rootstock showed symptoms similar to those that appeared on the nursery seedlings, and the fungus *F. solani* was reisolated from artificially infected seedlings. Many studies showed that several *Fusarium* pathogens cause essentially similar symptoms on different crops such as cortical decay of roots, root rot, wilting, yellowing and premature death on infected plants (Summerell *et al.*, 2001; Saremi 2005). In fact, *F. solani* is a facultative parasite that causes root rot, stem cankers, crown rot and storage rots of a variety of host plants in Iran and other countries (Hooker, 1990; Saremi, 2000; Saremi *et al.*, 2010). Saremi *et al.* (2011) showed that dry rot disease on tuber potato and wilting of bean were caused by *F. solani*. Generally, the fungus can be root rot pathogen on many economical plants all over the world. Our results are in accordance with those of Triki *et al.* (2013), who reported for the first time that root rot of Pistachio trees *Pistacia vera* in Tunisia was caused by *F. solani*. In contrast, Eskalen *et al.* (2001) showed that root and crown rot of pistachio growing in East-Mediterranean and Southeast Anatolian regions were caused by *Phytophthora* spp. and *Fusarium equiseti*.

The results of this study are the first report of root and crown rot of pistachio seedlings caused by *F. solani* in nurseries in Syria.

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