



RESEARCH ARTICLE

FUNGAL SPECIES ASSOCIATED WITH COLLAPSED STRAWBERRY PLANTS CULTIVATED IN STRAWBERRIES PLANTATIONS IN MOROCCO

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ARTICLE INFO

Article History:

Received 20th January, 2016
Received in revised form
14th February, 2016
Accepted 28th March, 2016
Published online 26th April, 2016

Key words:

Decline, Fungi,
Strawberry plants,
Morocco.

ABSTRACT

Strawberry plants of Venicia variety severely affected by collapse which has leads to their total drying were brought by a farmer in the laboratory in spring 2011 from Dlalha village (Gharb-Loukkos, Northwestern Morocco). The ignorance of the causes of this decline required a mycological laboratory analysis based on the identification of fungi colonizing samples and calculating the infection percentages for different vegetative organs. The highest isolation proportions reaching 50% and 38.4% were recorded respectively by *Botrytis cinerea* and *Alternaria alternata* on strawberries, 30 and 55.5% on strawberry leaves, increasing to 56.4% and 65% on stems also hosting *Fusarium oxysporum* isolated with a frequency of 30.4% and *Fusarium* sp. (13.4%). On the aerial parts, 8 fungal species were poorly represented and whose contamination percentages ranging from 4.35% to 11.1%. Isolations made from the crown and roots allowed detection of *Macrophomina phaseolina*, *F. oxysporum*, *Rhizoctonia solani* whose proportions vary from 28.6% to 52.6%, 38% to 42.1% and 42.8% to 47.36%. However, weaker frequencies of isolation were assigned to *Cylindrocarpon descrutans*, *Pythium* sp. and *Phytophthora* sp. not exceeding 10.53% and 5.26% respectively. These telluric agents were accompanied by *Aspergillus nidulans* (19.05%), *Trichoderma* sp. (4.76%) and *Cunninghamella* sp. (9.52%).

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Citation: Najoua MOUDEN, Rachid BENKIRANE, Amina OUAZZANI TOUHAMI and Allal DOUIRA, 2016. "Fungal species associated with collapsed strawberry plants cultivated in strawberries plantations in Morocco", *International Journal of Current Research*, 8, (04), 29108-29117.

INTRODUCTION

Like many vegetable crops, strawberry cultivation that has experienced sustained growth in terms of acreage and yield in Morocco and mainly in the Gharb-Loukkos region is limited by serious diseases that can affect the root system, aerial parts causing damage to the host and significant reductions in yield. Including, root rot, crown rot caused by *Phytophthora* spp., *Verticillium* wilt of strawberry and diseases caused by species of *Colletotrichum* spp. known worldwide as specific and lethal diseases of strawberry cultures (Paulus, 1990; Freeman and Katan, 1997; Freeman et al., 1998; Duncan, 2002). Other soil borne pathogens such as *Fusarium* (Golzar et al., 2007; Juber et al., 2014), *Rhizoctonia* (Fang et al., 2012; Ceja-Torres et al., 2014), *Cylindrocarpon* (Manici et al., 2005), *Macrophomina*

phaseolina (Avilés et al., 2008; Hutton et al., 2013), *Pythium* (Abdel-Sattar et al., 2008), *Gnomonia*, *Phoma* (Moročko, 2006; Ceja-Torres et al., 2014) and *Phytophthora* (Mingzhu et al., 2011) are able to induce individually or in combination major infections in strawberry. Similarly, gray mold caused by *Botrytis cinerea*, downy mildew (*Sphaerotheca macularis*), leaf spot (*Mycosphaerella fragariae*) are devastating on this culture (Brugnara and Colli, 2014; Delhomez et al., 1995; Bulger et al. 1987; Sosa-Alvarez et al., 1995; Berrie et al., 1998). Their severity depends on cultivar susceptibility (Muller, 1965), weather conditions (Jarvis, 1964) and level of infection source. In Morocco, the strawberry planting material is usually imported from Spain (Tanji et al., 2014). During the crop year 1994/95, Morocco imported more than 60 million seedlings from Europe what generated an exit of currencies of about 50 million dirham's (Naja, 1995). Moreover, the production risks the development of various fungi lodged in the foreign seedlings and those preexisting in the strawberry fields and on the strawberry plant being the dominating previous crop grown.

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In 2010, a sickly strawberry plant parts of three varieties (Camarossa, Festival and Splander) collected from two strawberry farms in Moulay Bouselham (Gharb-Loukkos, Northwestern Morocco) yielded numerous fungal species as *Botrytis cinerea*, *Chaetomium globosum*, *Alternaria alternata*, *Mucor* sp., *F. avenaceum*, *F. semitectum*, *F. oxysporum*, *F. solani*, *Verticillium dahliae*, *Colletotrichum acutatum*, *Aspergillus nidulans*, *Ulocladium atrum*, *Stemphyllium botryosum*, *Gliocladium roseum*, *Thielavia terricola*, *Stachybotrys* sp. and *Rhizoctonia solani* with varied frequency (Mouden et al., 2013). In 2011, fungal isolation from senescent strawberry plants of Festival variety collected from a Dlalha beside Moulay Bouselham revealed the presence of *Pestalotia longisetula* (Mouden et al., 2014). Fungi belonging to about 40 genera were isolated from either frigo- or field-grown strawberry plants (Rigotti et al., 2003). In Spain, Avilès et al., (2008) reported drying and mortality of many strawberry cultivars following transplantation into the field in Huelva. In spring 2011, a farmer brought to the laboratory a large number of samples of strawberry plant of the Venicia variety presenting the symptoms of deterioration and wilt.

He also announced that other strawberry growers suffered from the same damages. This study was carried out with an aim of giving a mycological diagnosis on the cause of dieback and premature wilting of strawberry plants of the cultivated Venicia variety.

MATERIALS AND METHODS

Twenty samples of infected strawberry plants of the Venicia variety collected in Dlalha (Moulay Bouselham, Northwestern Morocco) and brought to the laboratory by a farmer in spring 2011 were placed in white plastic bags in a refrigerator.

Fungal isolation and identification

The analysis of the mycoflora associated with leaves, stems of strawberry plants was conducted using the modified Blotter method (Benkirane, 1995). Leaves and stems showing different types of lesions or necrosis were removed from the strawberry plants.



Figure 1. Partial or total desiccation (a), stunted root (b) and progressive browning on the crown (c) plants of strawberry variety Venicia at an advanced stage of development of a strawberry collected in Dlalha village (Moulay Bouselham, Northwestern, Morocco)

Table 1. Isolation percentages (%) of the fungal species isolated from various vegetative organs of strawberry plants of the Venicia variety collected in Dlalha village (Moulay Bouselham, Northwestern, Morocco)

Espèces fongiques	Strawberries (%)	Leaves (%)	Stem (%)	Crown (%)	Root (%)
<i>Botrytis cinerea</i>	50.0a	30b	56.4b	-	-
<i>Alternaria alternata</i>	38.4b	55.5a	65.5a	-	-
<i>Stemphyllium sarciniforme</i>	-	10c	-	-	-
<i>Cladosporium cladosporioides</i>	11.1c	-	12.5de	-	-
<i>Ulocladium botrytis</i>	-	-	4.3e	-	-
<i>Chaetomium globosum</i>	5.5d	11.1c	-	-	-
<i>Aspergillus nidulans</i>	11.1c	-	-	-	19.1c
<i>Epicoccum purpurascens</i>	-	11.1c	10.2de	-	-
<i>Bipolaris spicifera</i>	-	5.5d	4.3e	-	-
<i>Nigrospora sphaerica</i>	15.4c	-	-	-	-
<i>Coniella fragariae</i>	5.5d	-	4.3e	-	-
<i>Neofusicoccum parvum</i>	-	10c	-	-	-
<i>Torula herbarum</i>	-	-	4.3e	-	-
<i>Cunninghamella elegans</i>	-	-	-	-	9.5cd
<i>Apiosordaria hispanica</i>	-	-	-	-	4.7d
<i>Fusarium</i> sp.	-	-	13.4de	-	-
<i>Fusarium oxysporum</i>	-	25b	30.4c	42.1b	38.1a
<i>Rhizoctonia solani</i>	-	-	8.7de	47.3ab	42.8a
<i>Trichoderma harzianum</i>	-	-	-	-	4.7d
<i>Macrophomina phaseolina</i>	-	-	-	52.6a	28.6bc
<i>Cylindrocarpon destructans</i>	-	-	-	10.5c	9.5cd
<i>Pythium</i> sp.	-	10c	-	10.5c	4.4d
<i>Phytophthora</i> sp.	-	-	-	5.3c	4.7d

(-) : genus not isolated

The results of the same line followed by different letters differ significantly at 5%.

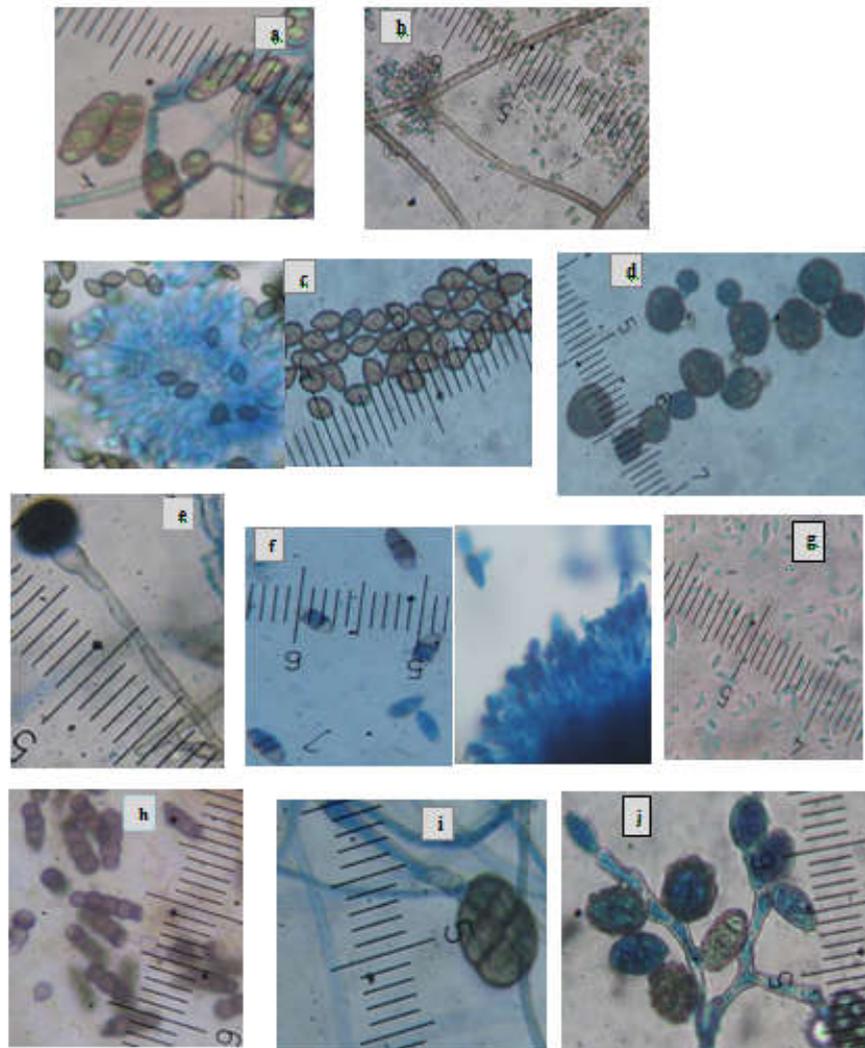


Figure 2. The microscopic appearance of fungal species isolated from organs of *Fragaria ananassa*. a: conidia of *Bipolaris spicifera*; b: conidiophore and conidia of *Cladosporium cladosporioides*; c: conidiogenous cells and conidia of *Coniella fragariae*; d: *Epicoccum purpurascens* conidia; e: *Nigrospora sphaerica*; f: *Neofusicoccum parvum*; g: *Fusarium* sp.; h: *Torula herbarum* conidia; i: *Stemphyllium sarciniforme*; j: *Ulocladium botrytis*. Optical zoom: $\times 400$. Mounting liquid: Cotton blue

The leaves fragments of 1 cm² and stems pieces of 1 cm length were washed with tap water, rinsed with sterile distilled water, disinfected with sodium hypochlorite at 5% for five minutes. Then, the fragments were rinsed three times for 30 s in sterile distilled water. After this, they were placed in sterile Petri dishes containing three discs of blotting paper, humidified with sterile distilled water. The dishes were after incubated in continuous light. Some leaves or stems fragments incubated in the same manner as previously were put on PSA agar plates (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g Agar-agar and 1000 ml distilled water) and incubated on darkness at 28°C. The developed colonies were then observed for the species determination. Strawberries showing lesions, were disinfected with sodium hypochlorite at 1%, rinsed with sterile distilled water, air-dried on sterile blotting paper and placed on PSA agar plate. Dishes were kept at 24°C on darkness for 7 days. The roots removed from their ground gangue are washed with running water several times, cut out into small pieces of 0,5 to 1 cm, disinfected with alcohol for five minutes, put on sterile distilled water, dried with sterile filter paper, then placed in sterile Petri dishes containing water agar (15 g Agar-Agar and 1000 ml distilled water). After incubation at 28°C in the dark for 48 h, the colonies formed were transferred to PSA agar plates and incubated then in the same conditions for 7 days (Rapilly, 1968).

The observation of different cultures and fragments under the optical microscope has allowed us to identify the fungal species by using the identification keys of Gilman (1957), Tarr (1962), Ellis (1971), Chidambaram *et al.* (1974), Domsch *et al.* (1980) and Champion (1997). The percentage of infection and / or contamination by different fungal species is calculated according to the method of Ponchet (1966) which defines the frequency of isolation of different fungi from 100 lesions or 100 root rots present in the studied plants according to the formula:

$$PC = (NLI / NTL) \times 100$$

PC: Percentage of infection and / or contamination;

NLI: Number of lesions containing the fungal specie.

NTL: Total number of lesions used in the isolation.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and LSD test at 5% level. The percentages were transformed into Arcsin \sqrt{P} (where P is the proportion of percentage).

RESULT AND DISCUSSION

In its preliminary examination, sample plants show apparent senescence of leaves, stems and strawberries (Figure 1a). In addition, the root system of some plants was reduced and root tissues were damaged and black (Figure 1b). On crowns, the transverse sections allowed the observation of circular necrosis of different size, brown dark colored, developed since central vascular tissue (Figure 1c). On the other hand, they show a deep perforation at the necrotic tissue. The isolations revealed the existence of heterogeneous fungal fructifications whose identification made it possible to determine various fungi

contaminating this batch as well as the calculation of their frequency of isolation from the organs analyzed. Indeed, 15 species of the fungal complex identified are isolated for the first time in the region. This is the case of *Bipolaris spicifera* (Figure 2a), *Cladosporium cladosporioides* (Figure 2b), *Coniella fragariae* (Figure 2c), *Epicoccum purpurascens* (Figure 2d), *Nigrospora sphaerica* (Figure 2e), *Neofusicoccum parvum* (Figure 2f), *Fusarium* sp. (Figure 2g), *Stemphylium sarciniforme* (Figure 2h), *Torula herbarum* (Figure 2i), *Ulocladium botrytis* (Figure 2j), *Cylindrocarpon destructans* (Figure 3a), *Macrophomina phaseolina* (Figure 3b), *Phytophthora* sp. (Figure 3c), *Pythium* sp. (Figure 3d), *Apiosordaria hispanica* (Figure 3e), *Cunninghamella elegans* (Figure 3f) and *Trichoderma harzianum* (Figure 3g).

The proportions observed in the underground parts are considerably different from those of the above ones as well by the number of detected fungi as by the values obtained. Fruit samples were colonized mainly by *Botrytis cinerea* which presented a frequency of 50 and 38.4% by *Alternaria alternata* against lower frequencies not exceeding 15.4% by *Nigrospora sphaerica*, 11.1% related to *Cladosporium cladosporioides*, *Aspergillus nidulans* in comparison with 5.5% by *Chaetomium globosum* and *Coniella fragariae* (Table 1). At the leaf level, *A. alternata* is present with a frequency reaching 55.5% exceeded *B. cinerea* (30%), *Fusarium oxysporum* (25%), *Neofusicoccum parvum* and *Stemphylium sarciniforme* which hold comparable colonization rates with those of *Pythium* sp. (10%), *C. globosum* (11.1%) and *Epicoccum purpurascens* but higher than those of *Bipolaris spicifera* (5,5%). The isolation frequencies relating to the species found on the stems remain less important in the order of 4.3% except for *B. cinerea*, *A. alternata* passing respectively to 56.4, 65.5 and 30.4% for *F. oxysporum* compared to 13.4% for *Fusarium* sp., 12.5% for *Cladosporium cladosporioides*, 10.1 and 8.7% for *Epicoccum purpurascens*, *Rhizoctonia solani* and less than 4.4% for *Ulocladium botrytis*, *Bipolaris spicifera*, *Coniella fragariae* and *Torula herbarum*. Fungi associated with crowns are represented by frequency amounting to 52.6% attributed to *Macrophomina phaseolina*, *Rhizoctonia solani* (47.3%), 42.1% to *F. oxysporum*. Added to these, *Cylindrocarpon destructans*, *Pythium* sp. and *Phytophthora* sp. have got frequencies of 10.5 and 5.3% respectively. By invading the roots, these fungal agents recorded much lower frequencies in particular *C. destructans* whose frequency is 28.6% with the detection of *Cunninghamella elegans*, *Trichoderma harzianum*, *Aspergillus nidulans* and *Apiosordaria hispanica* at the respective proportions 9.5, 4.76, 19.1 and 4.7%.

The results indicated above, reveal the coexistence of various fungi distributed unequally on both sides on the aerial and underground organs of the strawberry plant. Thus, the underground parts were colonized especially by recognized pathogenic fungi on many crops. Among which, *C. destructans* responsible for black root rot, which affects the performance of strawberry plants in California was isolated from diseased plants strawberry showing discoloration of their vascular elements (Yuen *et al.*, 1991). Studies conducted by Fang *et al.* (2012) showed the sensitivity of certain varieties of strawberry plant to this pathogen in the fields where the crown and root diseases are predominant.

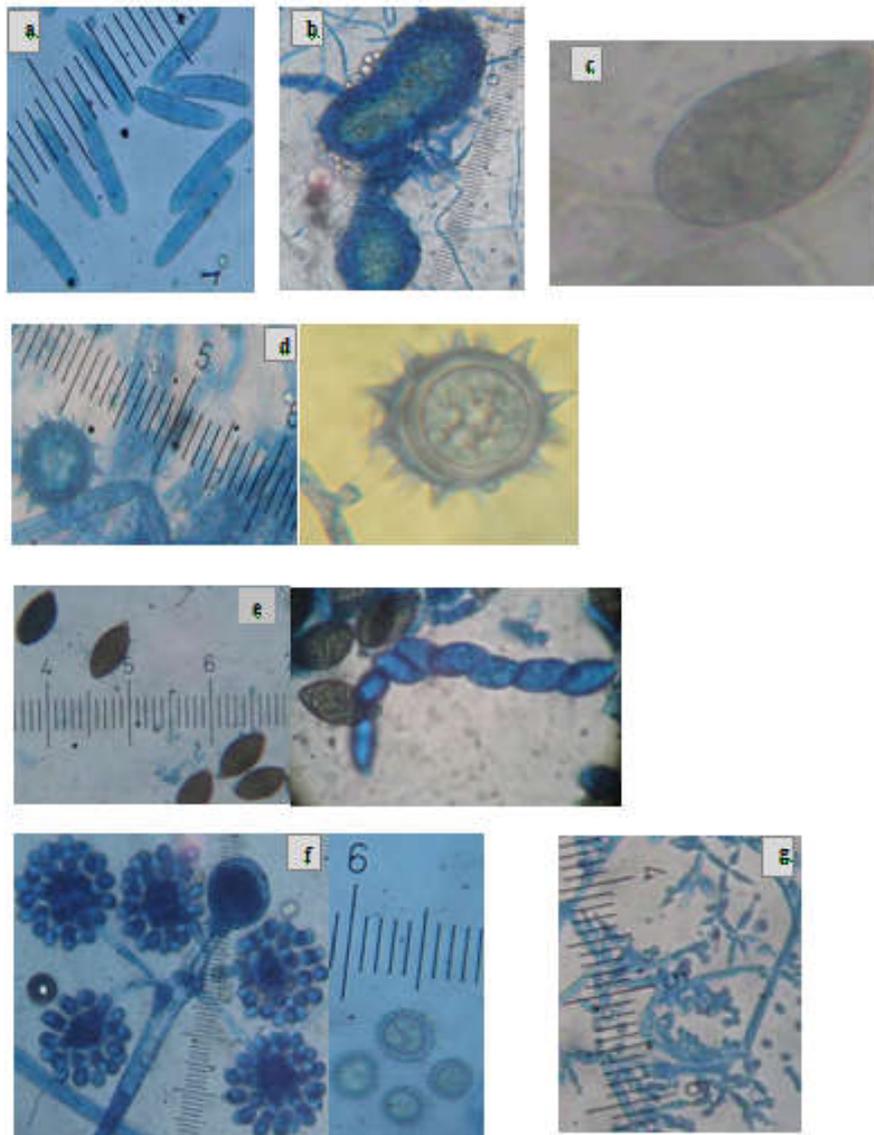


Figure 3 The microscopic appearance of fungal species isolated from underground organs of strawberry plants; a : Conidia of *Cy lindrocarpon destructans* ; b: Sclerotia of *Macrophomina phaseolina*; c : Sporangia of *Phytophthora* sp. ; d : Sporangia of *Pythium* sp.; e : Asci with 8 ascospores and ascospores of *Apiosordaria hispanica* ; f : Sporangiophores and Sporangioles of *Cuninghamella elegans* ; g : *Trichoderma harzianum* conidia and conidiophore. Optical zoom: $\times 400$. Scale: en μm . Mounting liquid: Cotton blue

According to Halleen *et al.* (2006), *C. destructans* is the main causal agent of black foot disease of grapevines and root rot of *Panax ginseng* (Jang *et al.*, 2011). In Argentina, it was reported for the first time on *Rumohra adiantiformis* causing leaf blight; root and rhizome rot (Palmucci and Grijalba, 2009). Indeed, this fungus and *Macrophomina phaseolina* are combined to crown and root disease of strawberry (Fang *et al.*, 2012). It was isolated from an economically important plant known as *Euphorbia lathyris* (Young and Alcorn, 1982). So far in Pakistan it has been reported to cause disease on 67 economic hosts including field crops, pulses, flowers and vegetable (Khan, 2007). According to this author, *M. phaseolina* generally affects the fibrovascular system of the roots and basal internodes and the severity of the disease is directly related to the population of viable sclerotia in the soil. In Spain, at the end of the 2006 season (May–June) collapsed and dying strawberry plants were observed on several cultivars in four fields (Avilés *et al.* 2008).

Cut crowns of affected plants revealed dark brown necrotic areas on the margins and along the woody vascular ring. Roots of these plants were also shown to be necrotic. For Zveibil *et al.* (2012), crown and root rot caused by this fungus has become predominant in Israel. In present study, *R. solani* witch remains the most virulent of its genus is well represented among communities of soil-borne fungi, it was highlighted on stems, crowns and roots. The tests carried out by Botha *et al.*, (2003) indicate that *R. solani* was pathogenic to strawberry roots causing severe stunting, wilting and collapse on young strawberry plants. Abad *et al.* (1999) consider the black root rot a major disease of strawberry plant in States and in other countries; it is induced according to Christlyn *et al.*, (2005) by a complex including *Rhizoctonia fragariae* and *Pythium* sp. Although they are slightly represented, the presence of both genera *Phytophthora* and *Pythium* joined previous studies. Indeed, the genus *Phytophthora* is one of the strong parasites dreaded on strawberry that many species have been designated.

P. cactorum responsible of bitter rot of strawberries (Chang, 1987; Sharma et al., 2005; Iribarren et al., 2012). According to Latorre and Viertel (2004), *P. cactorum* is the cause of root and crown rot of strawberry recently found in Chile and there is a potentially higher risk of dissemination and development of root and crown rot in plants kept under cool conditions before planted. Bhat and Brown (2010) have detected it on the roots, crowns and petiole tissues of strawberry plants. Also, considerable damage was recorded in the field, in tonnage transported following the invasion of strawberries by *P. cactorum* and *P. citrophthora* (Kao and Leu, 1979). In Japan, *P. cactorum* and *P. nicotiana* were encountered in three strawberry greenhouses (Li and al., 2013). According to De los Santos (2002), affected plants by *P. cactorum* exhibit an internal red-brown discoloration of the upper crown, a bluish discoloration of leaves, and the plants were wilted. Eventually, plants collapsed and died. Another species named *P. fragariae* is the causal agent of typical symptoms of red stele on strawberry (Milholland and Daykin, 1993). In addition, isolations from plants have yielded *Fusarium oxysporum* and *Pythium* sp. that were previously reported from strawberry and known to produce a root rot (Wilhelm, 1952).

In Egypt, Abdel-Sattar et al. (2008) attributed the black root rot and crown to *P. cactorum*, *C. fragariae*, *R. solani* and *F. oxysporum*. The latter grows and survives for long periods on organic matter in soil and in the rhizosphere of many plant species (Fravel et al., 2003). Wilt-inducing isolates of *F. oxysporum* have been divided into more than 120 different formae speciales (f. spp.) according to their host range across a wide range of plant families (Fravel et al., 2003; Michielse and Rep, 2009). *F. oxysporum* f. sp. *fragariae* penetrates strawberry plants through roots, severely affecting roots and crowns, and resulting in rapid wilting and eventually death of strawberry plants (Fang et al., 2011; Koike et al., 2009). The crown and root deterioration can also result from an interaction between non-parasitic factors (Milholland et al., 1989) and root infections by fungi such as *Pythium* (Watanabe et al., 1977) and / or plant pathogenic nematodes (Mervosh and Lamondia, 2004). As soil-borne fungi, *Trichoderma harzianum* and *Cunninghamella elegans* encountered on the roots with a low frequency were accompanied by *Apiosordaria hispanica*. Indeed, *T. harzianum* is widespread in soil (Gaddeyya et al., 2012; Sharma et al., 2011; Rakesh Sharma, 2013), seeds (Hannin, 2003) and waters (Zehhar, 2011). *Cunninghamella elegans* is one of keratinophilic fungi isolated from soils of two tanneries in Jos metropolis in Nigeria (Nwadiaro et al., 2015).

One species of this genus was found on the skin and pulp of banana fruit (Meddah et al., 2010). Concerning *Apiosordaria hispanica*, it could be proposed as a new fungal resident of vegetative organs. According to Stchigel et al. (2000), the genus *Apiosordaria* belonging to *Ascomycetes* class comprises 21 species, including mainly soil-borne and coprophilous. In Spain, two species *Apiosordaria hispanica* sp. nov and *A. Globulosa* sp. nov have been isolated from soil of *Quercus ilex* L. and *Pinus halepensis* vegetations (García et al., 2003). Another species called *A. antarctica* was found in Antarctica besides *A. nigriensis* isolated from soil of Nigeria (Stchigel et al., 2003). Isolations made from senescent leaves without

apparent typical symptoms revealed the dominant presence of some fungi that were previously reported as virulent colonizing strawberry leaves (Saber et al., 2003), it is mainly *B. cinerea* and *A. alternata* which infection leads respectively to blight (Hausbeck and Moorman, 1996) and black spots (Wada et al., 1996). Although the detection of other species is less frequent, their presence would indicate a weakness of the plant and an obvious source of nuisance for their impact on many plant species. In the present study, *Stemphylium sarciniforme* is reported for the first time on the strawberry. However, it was revealed pathogenic on *Cicer arietinum* (Nene et al., 1996). Its occurrence on *Trifolium pretense* L. and *T. repens* L. generates considerable damage in wet periods (Cho and Yu, 2000). Concerning *Epicoccum purpurascens*, its detection on strawberry plant dates for a long time, it was cited by Maas (1984) and Rigotti (2003).

This contaminant was found on senescent stems and debris from post harvest *Cicer arietinum* (Dugan et al., 2005). In addition, he is involved in biological control against one of rice pathogens (Motlagh, 2011), on strawberry leaves towards multiple parasites of the plant (Card, 2005), against *Pythium irregular* affecting legumes (Koutb and Ali, 2010). *C. cladosporioides* contaminated as well strawberries as the stems. Indeed, it can affect strawberry leaves (Gubler et al., 1999), it was associated with fungi encountered on the fruit of the date palm (*Phoenix dactylifera* L.) in conservation (Atia, 2011). According to Fatima et al. (2009), it is responsible for the deterioration of certain fresh fruit and vegetables after harvest. *Ulocladium botrytis* present on the stems was able to colonize bean seeds in Spain, desert plants distributed in the south of Iraq (Muhsin et Zwain, 1989). Similarly, it was isolated from infected leaves of *Scutia buxifolia* (Saparrat et al., 2007), plant debris (Ismail, 2006), soil in Iraq (Al Duboon and Mashhad, 2012). According to Müller-Stöver and Kroschel (2005), its use as mycoherbicide is of limited effectiveness against *Orobanche* spp. In addition, the strawberry leaves were hosted for the first time by *Bipolaris spicifera* which can cause fungal diseases.

In Morocco, it has been reported by Kadri et al. (2011) on *Punica granatum*, on *Ficus nitida retusa* (Drider et al., 2011), on *Citrullus lanatus* (El Mhadri et al., 2009). Its host range also includes *Eucalyptus tereticornis* (Mohanan and Sharma, 1986) and sorghum (Ünal et al., 2011) on which it induces leaf spot. A new resident has also been detected. It's *Coniella fragariae* who was found in strawberries and stems. Similarly, Deremiens et al. (1996) reported a high incidence estimated 50% of this fungus on strawberries in one of the communities surveyed in Manitoba. This species can also reach the petioles and the crown of strawberry plants (Rigotti, 2003). According to Mohan and Manokaran (2013), *Coniella* genus is recognized as pathogenic, causing visible leaf lesions on different clones of *Eucalyptus* spp. from separate locations in India. Along with all isolated species, it's also *Torula herbarum* that was previously enumerated in mycoflora associated with strawberry plants on farms in Brazil (Medeiros and al., 2007). In Michigan, the *Torula* genus was announced on strawberries (Beneke et al., 1954). The species *Torula herbarum* was also reported on *Eucalyptus microtheca* (Abbas et al., 2010). It showed aggressiveness towards *Aloe barbadensis* (Ayodele

and Ilondu, 2008). *Nigrospora sphaerica* is recognized pathogenic on other plant species as *Glycyrrhiza glabra* where it can cause the drying of the leaves and defoliation (Verma and Gupta, 2008), on *Cucurbita wenyujin* causing severe leaf scorch (Zhang *et al.*, 2011). It's represented with a frequency of 6% among the isolated fungi and bacteria from the decay of the ginger rhizome after harvest (Moreira *et al.*, 2013), on sorghum seeds (Panchal and Dhal, 2011). In India, *N. sphaerica* was recorded in a fungal population of air spora inside and outside some plantations (Panda *et al.*, 2009). The fungal community identified on strawberry plants contains saprophytic, necrotrophic and pathogenic microorganisms but whose existence and the fluctuating frequency of isolation during the strawberry crop season and from one year to another could determine a diagnosis of plant health areas planting for a long time, the survival and productivity of vegetable cultivation.

Indeed, fungi are able to use different mechanisms to survive, multiply and spread as hydrolytic enzymes and toxins that allow their penetration and invasion of young plants. The study conducted by Lugauskas *et al.*, (2003) confirmed the abundance of plant pathogenic fungi in the soil of the same plot where strawberry cultivation was practiced for longer time as *Ascochyta fragaricola*, *Cercospora fragariae*, *Fusarium equiseti*, *F. oxysporum*, *F. solani*, *Perenospora fragariae*, *Phytophthora cactorum*, *Pythium intermedium*, *P. ultimum*, *Plasmodiophora brassicae*, *Sclerotium rolfsii* and *Verticillium albo-atrum* that have gradually accumulated from one year to another in the same plot of strawberry. Possibly, the dominance of some fungal species and their coexistence with pathogenic fungi would promote tissue damage and leads probably to this collapse and death of plant examined.

Acknowledgments

This study was conducted under the national program to support sector research, project RS-23: "Phytosanitary status of strawberry farming in Morocco and looking for alternative control means: Production and formulation of a *Trichoderma* spp. Biofungicide", funded by National Centre for Scientific and Technical Research (CNRST), Rabat, Morocco.

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