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Research Article

DIVERSITY AND DISTRIBUTION OF FUNGI FROM STRAWBERRY PLANTS GROWN IN GHARB-LOUKKOS (MOROCCO)

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ABSTRACT

Through four visits to 7 farms: Moulay Bousselham 3 farms (Gharb area) and Louamra 4 farms (Loukkos area), during 2012-2013 strawberry campaign, from February to April 2013. The mycological analysis of plants manifesting various diseases symptoms revealed the presence of 39 fungal species.The identified Fungal groups on aerial parts of cultivated varieties are mainly represented by *Botrytis cinerea,* showing the highest percentage of contamination (90.3%), followed by *Alternaria alternata* (88.1%), *Cladosporium cladosporioides* (33.3%), *C. herbarum* (53.1%), *Colletotrichum acutatum* (49.3%) on Splendor in Ghdira, *C. gleoesporioides* (25%) on Festival in Gnafda, *Epicoccum purpurascens* (35.5%) on Camarossa in Gnafda and *Mucor* sp. (33.3%) on Splendor in Ghdira. By comparison, 14 species showed a lower occurrence and lower contamination percentages ranging from 3.4 to 24.3% and including newly associated species to the aerial organs of strawberry in Morocco as *Memnoniella echinata*, *Trichothecium roseum*, *Fusarium nivale* and *Rhizoctonia solani*. The colonization of rhizosphere is marked by the presence of 12 species dominated by *Cylindrocarpon destructans* (57.8%), *Rhizoctonia solani* (46.8%) *Fusarium oxysporum* (30.8%), coexisting with three new species: *Gliomastix murorum*, *Curvularia lunata* and *Fusarium culmorum*.

The presence of the major fungal species is significantly distinct throughout the visited exploitations. *B. cinerea* contamination is higher in the surveyed plots of Moulay Bousselham both on fruits and leaves (94% in Gnafda and 91.46% in Dradra) than that of Louamra (44.4% in Ghdira and 52.8% in Ouled Hamou). As for *C. acutatum*, its percentage of contamination is around 50% on splendor fruits in Ghdira significantly higher than those recorded in Dlalha, Gnafda, Ouled Hamo and Frular (17%). However, *R. solani* has a high occurrence in Gnafda; *C. destructans* achieved higher percentages affecting 4 varieties in 5 strawberry farms while the presence of *F. oxysporum* is more restricted.

INTRODUCTION

Berries' growing is booming in Morocco. The production area is spread over 4162 hectares most of which is dedicated to strawberries. The rest is shared between Blueberry (10%) and raspberry (5%) (Anonymous, 2014). During the 2013-2014 campaign, more than 145,000 tons of strawberries were produced in 3,500 Ha and Morocco exported 74,000 tons of strawberries which represent 7% of the world production and 95% are destined for the European market (Anonymous, 2014). Over a total area of 4,900 Ha and 165,000 tons of fruit produced, the Loukkos-Gharb region has generated around 1.5 billion DH as turnover in 2012 - 2013 and ensuring 3 million working days on a 9-month period from September to May. Indeed, both irrigated area in the Gharb - Loukkos benefited from the different "Region development programs" to intensify and diversify the varieties, control production techniques, packaging, processing, exportations and use of the potential resources as suitable soils and abundant water. Large farms, medium and small ones are planted by Camarossa, Splendor, Festival, Fortuna, Sabrina and San Andreas varieties (Tanji *et al.*, 2014).

But, harmful damage can be incurred in those production systems where various microorganisms, parasites, and pests can attack and house the strawberry plants in addition to their interaction with concomitant conditions. Indeed, various aerial and soil-borne fungi are feared on strawberry crop (*Fragaria*

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ananassa) as *Botrytis cinerea* (Greathead *et al*., 1962; Sutton, 1998; Xu *et al*., 2012), *Colletotrichum* sp. (Madden and Boudreau 1997; Smith, 2008) which causes crown rot on strawberry (Denoyes - Rothan *et al*., 2003) and *Rhizoctonia* spp. (Dodge and Stevens, 1924; Martin, 1988). In New Zealand, the strawberry industry is experiencing heavy losses due to fungal diseases that can cost up to $ 4.4 million per year or 20% of the crop value (Timudo-Torrevilla *et al*., 2005).

Thus, a quantitative, qualitative study of the fungal flora inhabiting strawberry plants and the fluctuation of this component during the season and from one plot to another can be useful tool to better promote the state of contamination of strawberries plantations in the Loukkos-Gharb area, to distinguish fungal species that dominate and judge their impact on this crop.

MATERIALS AND METHODS

Locations and times of surveys

The strawberry cultivation is practiced in various localities of the rural communes of Moulay Bousselham, in the south of Loukkos and delimiting the northern Atlantic coast of the Gharb region (70 km north of Kenitra, 35 km south of Larache) and Laouamra, located at 11.46 km south of Larache (Northern Morocco).

- In February 2013, surveys were conducted in three localities of Moulay Bousselham: Dlalha, Gnafda, and Dradra.
- In mid-April 2013, two surveys have concerned a strawberry farm in Moulay Bousselham (Gnafda) and four others in Louamra (Ouled Hamo, Boucharen, Frular cooperative and Ghdira).

Sampling

A diagonal sampling was performed at each visit at the strawberry farms of Laouamra and Moulay Bousselham during 2012-2013 campaign. Fifteen samples of diseased strawberry plants were collected and put into white plastic bags.

Techniques of samples analysis of the various organs of plants of strawberry

Analysis of the mycoflora associated with fruits, leaves, stems and roots of strawberry plants was performed by using the modified Blotter method (Benkirane, 1995). Leaves and stems with different types of lesions are removed from the strawberry plants. One cm fragments of leaves and one cm length pieces of stems were prepared and washed with tap water, disinfected with sodium hypochlorite at 5% within five minutes and then rinsed with sterilized distilled water, then placed in sterile Petri dishes containing 2 slices of filter paper moistened with sterile distilled water. Plates are then incubated in continuous light. Certain fragments of leaves or stems, incubated in the same manner as above, are transferred in Petri dishes containing P.S.A. medium (Potato Sucrose Agar, 200 g potato, 20 g sucrose, 15 g Agar-agar, 1000 ml distilled water). The dishes are placed in an incubator for 7 days in dark at 28ºC.

While strawberries showing lesions, they were disinfected with sodium hypochlorite at 1%. Then the pieces of strawberries with lesions are placed in Petri dishes containing the culture medium P.S.A. and incubated for 7 days in dark at 24°C.

Crowns and roots of strawberry plants are released from the soil, washed with flowing water several times and cut into segments of 1 to 2 mm. The segments were disinfected with alcohol, washed aseptically with sterile distilled water, dried between two sheets of sterile filter paper and placed in sterile Petri dishes containing the Agar water (15 g Agar-agar, 1000 ml). After incubation at 28°C in dark for 48 hours, the appeared colonies were transferred to P.S.A medium and incubated under the same conditions for 7 days (Rapilly, 1968).

Fungal species was determined after an optical microscope examination of different cultures and fragments using determination keys Gilman (1957), Tarr (1962), Ellis (1971), Chidambaram *et al.* (1974), Domsch *et al.* (1980) and Champion (1997).

The infection and / or contamination percentage 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) x 100

Where PC is the percentage of infection and / or contamination; NLI is the number of lesions infected with a fungal species and given NTL is the total number of lesions.

Statistical analysis

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

RESULTS

Fungal contamination of aerial parts of strawberry plants in Moulay Bousselham in February 2013

The fungi isolated from sampled rotten fruits, leaves and stems of strawberry varieties belonging to three separate localities in Moulay Bousselham lead to find the presence of a community gathering eight genera with incidence expressed by the average percentage contamination of the plant parts. The result shows the variation in the frequency of the fungi identified according to the organ of the plant from which the isolation was made, the variety and locality (Table 1). In the Dlalha locality, strawberries of Sabrina variety harbored *Botrytis cinerea* (69.2%), *Alternaria alternata* (7.9%), *Fusarium* sp. (22.7%), *Colletotrichum acutatum* (10.5%), *Cladosporium herbarum* (25.7%), *Penicillium* sp. (7.2%) and *Rhizopus stolonifer* (8.5%) while the fruit samples of Splendor were mostly colonized with *C. acutatum* (29.7%), *B. cinerea* (26.1%), *A. alternata* (8.6%), *R. stolonifer* (7.7%) and *Epicoccum purpurascens* (4.4%). In this locality, the detection of *A. alternata* on Splendor leaves is high up to 88.1% while *Cladosporium herbarum* (21.5%) and *E. purpurascens* (11.6%) but absent on the stems. In comparison, the four varieties grown in the area of Gnafda are less attacked. However, among the most detected fungal contaminants, *B. cinerea* is very abundant on fruits, leaves and stems with isolation percentages ranging from 71.8% to 94.6% over the Sabrina variety. On Splendor variety, its isolation frequency is low on stems (32.3%) but will be 72.9% on leaves and 90.3% on fruits.

Table 1 The occurrence of fungal contaminants in February 2013 on leaves, stems and on strawberries surface of four strawberry varieties cultivated in Moulay Bousselham (expressed by contamination/ or infection percentage %)

Fungal species	Organs	Dlalha		Gnafda				Dradra
		Sabrina	Splendor	Sabrina	Splendor	Festival	Camarossa	Camarossa
Botrytis cinerea	Strawberries	69.2c	26.1cd	71.8c	90.3a	89.4a	69.1b	89.6a
	Leaves	86.0a	70.6b	94.6a	72.9b	82.7a	81.3a	91.4a
	Stems	79.6b	74.2b	87.5b	32.3c	-	36.2c	44.3b
Alternaria aletrnata	Strawberries	7.9fg	8.6ef	36.4d	-	7.4 d	5.6h	-
	Leaves	-	88.1a	19.9ef	84.2a	-	-	-
	Stems	-	-	21.9ef	-	-	16.5de	-
Colletotrichum acutatum	Strawberries	10.5fg	29.7c	9.5gh	-	-	8.6fgh	-
	Leaves	-	5.6fg	-	-	-	-	-
	Stems	-	-	-	-	-	-	-
***Fusarium* sp.**	Strawberries	22.7de	-	13.7fg	8.2f	14.8c	11.5f	-
	Leaves	-	-	13.5fg	-	68b	-	-
	Stems	-	-	-	15.4de	-	19.2d	23.6c
***Penicillium* sp.**	Strawberries	7.2fg	-	-	12.1def	6.9d	9.7fg	8.7d
	Leaves	5.6g	-	-	-	-	-	-
	Stems	-	-	-	-	-	-	-
Cladosporium herbarum	Strawberries	25.7d	-	8.7gh	18d	23.6c	10.2fg	10.1d
	Leaves	10.1fg	21.5d	5.4h	9.6ef	6.7d	12.9ef	-
	Stems	18.3e	-	24.8e	-	-	18.5d	22.4c
Rhizopus stolonifer	Strawberries	8.5fg	7.7efg	-	-	-	-	-
	Leaves	-	-	-	-	-	-	-
	Stems	-	-	-	-	-	-	-
Epicoccum purpurascens	Strawberries	-	4.4g	-	-	-	-	-
	Leaves	-	11.6e	-	-	-	-	-
	Stems	-	-	-	-	-	-	-

(-) : genus no isolated
The results of the same colomn followed by different letters differ significantly at 5%.

Similarly, fruit and leaves of the Camarossa variety showed high contamination by this agent respectively reaching a percentage of 69.1% and 81.3% compared to 36.2% on the stems. On Festival, this fungus was isolated from the fruit and leaves with significantly identical frequencies of 89.4% and 82.7%. Also, *A. alternata* colonized significantly Splendor stems with a frequency egal to 84.2%. The frequency of isolation was lower on Sabrina, ranging from 19.9% to 36.4% on leaves against 7.4% on the fruit of Festival variety. In addition, a high percentage of *Fusarium* spp. up to 68% isolated from the leaves of the Festival variety was recorded. The isolation percentages of *Penicillium* sp., *C. herbarum* and *C. acutatum* are lower not exceeding 24.8%.

In Dradra, the contamination percentages by *B. cinerea* are higher on strawberries (89.6%) and leaves (91.4%) of Camarossa variety followed by stems (44.3%). *Fusarium* sp. and *C. herbarum* were isolated from the stems at the percentages of 23.6 and 22.4%. The strawberries were also colonized by *C. herbarum* and *Penicillium* sp. at the respective frequencies of 10.1 and 8.7%.

Fungal contamination of strawberry plants in Moulay Bousselham and Louamra in mid-April 2013

The mycological examination of strawberry plants brought from Moulay Bousselham and Louamra farms led us to conclude the existence of various fungal species with a disproportionate distribution across the five locations and various varieties in cultivation.

The contamination of Splendor, Camarossa and Festival varieties is marked by the presence of *B. cinerea* with percentages of 58.3, 57.1 and 51.6% respectively in Frular, Boucharen and Gnafda compared to 44.4% in Ghdira where the isolation frequency of *C. acutatum* is not significantly different from that of *B. cinerea* (49.3%). *Cladosporium herbarum* was isolated from Festival strawberries in Boucharen (28.6%) and Camarossa in Gnafda (30.2%) while *C. cladosporioides* is present only in Frular (25%). A lesser occurrence marked *Mucor* sp. existing on strawberries of Splendor in Ghdira with a frequency of 33.3%. *Pestalotia longisetula* and *Coniella fragariae* were respectively isolated from Festival in

Table 2 The occurrence of fungal species contaminating the strawberries of three varieties cultivated in Louamra and Moulay Bousselham in April 2013 (expressed by contamination/ or infection percentage %)

Variety-Locality / Fungal species	Splendor				Festival		Camarossa	
	Old H	Frl	Ghd	Gnf	Bch	Gnf	Ghd	Gnf
Botrytis cinerea	-	58.3a	44.4a	-	57.1a	-	-	51.6a
Alternaria alternata	-	12.5b	-	-		-	-	
Cladosporium herbarum	-	-	-	-	28.6b	-	-	30.2b
Cladosporium cladosporioides	-	25c	-	-	-	-	-	-
Colletotrichum acutatum	-	-	49.3a	19.7	-	-	-	-
Pestalotia longisetula	-	-	-	-	14.3c	-	-	-
***Mucor* sp.**	-	-	33.3b	-	-	-	-	-
Coniella fragariae	-	5.6d	-	-	-	-	-	-

(-): species not isolated.
The results of the same column followed by different letters differ significantly at 5%.
Localities: Old H: Ouled Hamo; Frl: Frular; Bch: Boucharen; Gnf: Gnafda; Ghd: Ghdira.

Boucharen and Splendor in Frular with percentages egal to 14.3 and 5.6% (Table 2).

Table 3 The occurrence of fungal species isolated from leaves of three strawberry varieties cultivated in Louamra and Moulay Bousselham in April 2013 (expressed by contamination/ or infection percentage %)

Variety- Locality / Fungal species	Splendor				Festival		Sabrina	Camarossa	
	Old H	**Frl**	**Ghd**	**Gnf**	**Bch**	**Gnf**	**Gnf**	**Ghd**	**Gnf**
Botrytis cinerea	51.7a	81.2a	44.9a	-	59.7a	31.4b	30.1ab	46.6a	75.1a
Cladosporium Cladosporioides	4.5cd	-	11.2d	-	-	-	-	24.1b	15c
Cladosporium herbarum	-	-	-	-	-	22.4c	35.7a	-	13.8c
Alternaria alternata	13.5bc	-	30.6b	22.6a	41.6b	46.7a	30.2ab	-	-
Epicoccum purpurascens	13.8bc	-	23.2c	-	26.9c	12.6d	-	-	35.5b
Aspergillus nidulans	5.7cd	-	-	-	-	-	-	13.7c	-
Colletotrichum acutatum	10.4bc	8.3b	35.1b	21a	-	-	-	-	-
Colletotrichum gleoesporioides	-	-	-	-	-	22.2c	-	-	-
Pestalotia longisetula	13.7bc	-	-	-	-	-	-	-	-
Trichothecium roseum	6.8cd	-	11.2d	-	-	-	-	-	-
Stemphylium botryosum	6.8cd	10b	-	-	-	-	24.3c	-	-
Chaetomium globosum	-	-	-	-	-	11.5d	-	-	-
Bipolaris spicifera	3.4d	-	-	-	-	-	-	-	-
Nigrospora sphaerica	-	-	-	-	-	-	9.4d	-	-
Memnoniella echinata	-	-	5.9e	-	-	-	-	-	-
Fusarium semitectum	-	-	14.5d	-	-	-	-	-	-
Rhizoctonia solani	10.4bc	-	-	-	-	-	-	-	-

(-): species not isolated.
The results of the same column followed by different letters differ significantly at 5%.
Localities: Old H: Ouled Hamo; Frl: Frular; Bch: Boucharen; Gnf: Gnafda; Ghd: Ghdira.

from those of *Trichothecium roseum* and *R. solani* accompanied by *C. acutatum*, *P. longisetula*, *S. botryosum*, *C. cladosporioides* and *A. nidulans* (Table 3). Similarly, in the Ghdira farm, Splendor leaves sheltered *T. roseum* isolated at a frequency of 11.2% significantly equal to those of *C. cladosporioides*, *F. semitectum* but higher than that of the new species detected namely *Memnoniella echinata*. In Gnafda, the leaves of Festival variety were also contaminated with *Chaetomium globosum* at the percentage of 11.5% while on Sabrina leaves; *N. sphaerica* has appeared with a frequency of 9.4% (Table 3).

The species mostly found on the leaves are *B. cinerea* and *A. alternata* followed by *C. acutatum* and *E. purpurascens* with a clear dominance of *B. cinerea* that achieved a high percentage of contamination in the range of 81.2% in Frular, 75.1% on Camarossa in Gnafda, 59% on Festival in Boucharen and 30.1% on Sabrina in Gnafda (Table 3). In this farm, *A. alternata* and *E. purpurascens* had significantly identical frequencies to *B. cinerea* and greater than those of *Stemphylium botryosum* and *Nigrospora sphaerica*. Festival leaves colonisation by *A. alternata* reached 46.7% in Gnafda and 41.6% in Boucharen. The frequency of isolation of this fungus was 22.6% and 13.5% recorded respectively over Splendor in Gnafda and Ouled Hamo (Table 3). As for *E. purpurascens*, has contaminated the three varieties Camarossa, Festival, and Splendor on which its frequency reached 35.5% in Gnafda, 26.9% in Boucharen and 13.8% in Ouled Hamo. Other colonizers appeared for the first time as *Bipolaris spicifera* whose percentage of contamination is significantly different

In the stems, *B. cinerea* isolation frequency is lower, ranging from 23.1 to 58.3% over the three varieties from the four localities while the incidence of *Cladosporium* has increased notably that of *C. herbarum* having recorded a contamination percentage of 53.1% on Camarossa in Ghdira, 29.3% in Gnafda compared with 33.3% on Festival and 22.4% on Sabrina in the same locality (Table 4). Undetectable on these varieties, *C. cladosporioides* reached stems of Splendor variety in Frular (16.7%) and in Ouled Hamo (33.3%).

Table 4 The occurrence of fungal species isolated from stems of three strawberry variéties cultivated in Louamra and Moulay Bousselham in April 2013 (expressed by contamination/ or infection percentage %)

Variety-Locality / Fungal species	Splendor				Festival		Sabrina	Camarossa	
	Old H	**Frl**	**Ghd**	**Gnf**	**Bch**	**Gnf**	**Gn**	**Ghd**	**Gnf**
Botrytis cinerea	46.6a	50a	29.4a		40.6a	37.2a	23.1a	30.6b	58.3a
Cladosporium cladosporioides	13.3b	33.3b	-	-	-	-	-	-	-
Cladosporium herbarum	-	-	-	-	16.7c	33.6a	22.4a	53.1a	29.3b
Alternaria alternata	-	12.5c	-	20.1	23.4b	19.6b	-	-	-
Epicoccum purpurascens	-	-	-	-	22.7b	-	-	-	-
Colletotrichum gloeosporioides	-	-	-	-	14.3cd	-	-	15.4c	-
Gliocladium roseum	-	-	-	-	-	-	11.2b	-	-
Ulocladium atrum	-	-	-	-	-	5.2c	-	-	-
Pestalotia longisetula	13.3b	-	-	-	-	5.4c	-	-	-
Memnoniella echinata	-	-	-	-	7.7d	-	8.3b	-	-
Fusarium nivale	-	-	-	-	-	9.1c	-	-	-
Thielavia terricola	-	-	14.3b	-	-	-	-	6.6d	10.5c
Fusarium semitectum	-	-	5.56c	-	-	-	-	-	-

(-): species not isolated.
The results of the same column followed by different letters differ significantly at 5%.
Localities: Old H: Ouled Hamo; Frl: Frular; Bch: Boucharen; Gnf: Gnafda; Ghd: Ghdira.

Similarly, *A. alternata* is less frequent at the stems, observed only on Festival in Boucharen and Gnafda (23.4 and 19.6%). The stems were weakly colonized by *Thielavia terricola* detected on Camarossa in Gnafda and Ghdira (6.6 and 10.5%) and on Splendor in Ghdira (14.3%). *M. echinata* reappeared in Boucharen on Festival at a frequency of 7.7% accompanied with *C. gloeosporioides* (14.3%) and *E. purpurascens* (22.2%) and on Sabrina in Gnafda at 8.3%. In addition, a very small occurrence is noticed in *Gliocladium roseum*, *U. atrum,* and *F. semitectum* against which *F. nivale* newly detected, presented a frequency of around 9.1% on Festival in Gnafda significantly identical to that of *P. longisetula* also found on Splendor in Ouled Hamo (Table 4).

The analysis of the underground organs allowed the identification of 10 fungal species on the crowns strawberry varieties (Table 5). *C. destructans* was detected in the five strawberry production farms with a higher degree recorded on Festival in Gnafda of about 56.4% but noted absently on Sabrina witch was also colonized by *R. solani* reaching a percentage of contamination in Gnafda of 45.1% on Festival and 36.4% on Camarossa, inexistent in Ghdira and less frequently on Splendor in Ouled Hamo and Frular (Table 5). Hamo and Frular not exceeding 12.3% and increased to 18.2% on Sabrina in Gnafda and 22.4% on Camarossa in Ghdira face to 26.6% by *F. solani*. In front of these species, *C. lunata*, *G. murorum*, *P. cactorum* and *C. globosum* only detected in one locality are scarcely present compared to *M. phaseolina* isolated with a frequency of 29.1% on Festival in Boucharen and 16.4% for *T. harzianum* found in Ghdira on Splendor and Camarossa (13.4%) (Table 5).

Similarly, the presence of *C. destructans* and *R. solani* was very important on the roots of four varieties expressed by percentages of up to 57.3% followed by *F. oxysporum* more frequent on Festival and Sabrina roots in Gnafda (Table 6). *F. culmorum* was encountered only on Splendor in Gnafda with a proportion of 13.5%. In Frular, the Splendor roots have hosted *F. solani* and *Phytophthora cactorum* which also appeared in Ouled Hamo at an equal percentage of 11.1% to that of *M. phaseolina*, *Pythium* sp. and *Stachybotrys atra*. Comparatively, *Mucor* sp., *G. roseum* and *C. globosum* showed low frequencies incomparable to that of *T. harzianum* encountered in three locations on two varieties (Table 6).

Table 5 The occurrence of fungal species isolated from crowns of strawberry plants of four varieties cultivated in Louamra and Moulay Bousselham in April 2013 (expressed by contamination/ or infection percentage %).

Variety- Locality / Fungal species	Splendor				Festival		Sabrina	Camarossa	
	Old H	Frl	Ghd	Gnf	Bch	Gnf	Gnf	Ghd	Gnf
Fusarium oxysporum	12.3b	10.1b	-	-	-	-	18.2a	22.4a	22.1b
Fusarium graminearum	7.7c	-	-	-	-	-	-	-	-
Fusarium solani	-	-	-	-	-	-	-	26.6a	-
Cylindrocarpon destructans	39.4a	28.6a	37.5a	24.1	34.9a	56.4a	-	20.2a	18.5b
Rhizoctonia solani	10.1bc	17b	-	-	24.8b	45.1b	22.2a	-	36.4a
Phytophthora cactorum	7.7c	-	-	-	-	-	-	-	-
Macrophomina phaseolina	-	-	-	-	29.1ab	-	-	-	-
Trichoderma harzianum	-	-	16.4b	-	-	-	-	13.8b	-
Curvularia lunata	-	-	-	-	14.5c	-	-	-	-
Gliomastix murorum	-	-	-	-	16.6c	-	-	-	-

(-): species not isolated.
The results of the same column followed by different letters differ significantly at 5%.
Localities: Old H: Ouled Hamo; Frl: Frular; Bch: Boucharen; Gnf: Gnafda; Ghd: Ghdira.

Three representatives species of the Fusarium genus encountered have determined low levels of contamination recorded by *F. graminearum* (7.7%) on Splendor crown in Ouled Hamo, by *F. oxysporum* on Splendor crown in Ouled

In Louamra, the identified fungal communities have several species in common with those of Moulay Bousselham. The 16 species detected on the aerial organs can be divided into three

Table 6 The occurrence of fungal species isolated from roots of strawberry plants of four varieties cultivated in Louamra and Moulay Bousselham in April 2013 (expressed by contamination/ or infection percentage %)

Variety-Locality / Fungal species	Splendor				Festival		Sabrina	Camarossa	
	OldH	Frl	Ghd	Gnf	Bch	Gnf	Gnf	Ghd	Gnf
***Mucor* sp.**	7.1d	-	-	-	-	-	-	-	-
Gliocladium roseum	-	-	-	-	-	13.5c	-	-	-
Chaetomium globosum	-	-	-	-	-	9.1c	-	-	-
Fusarium oxysporum	-	-	-	-	20.1c	30.2b	30.8a	22.6a	-
Fusarium culmorum	-	-	-	13.7b	-	-	-	-	-
Fusarium solani	-	16.6b	-	-	-	-	-	-	-
Rhizoctonia solani	42b	22.4ab	-	-	33.7b	53.3a	38.4a	-	46.8a
Cylindrocarpon destructans	50.5a	16.3b	-	32.2a	57.3a	57.8a	21.4b	27.7a	45.3a
Phytophthora cactorum	11.1d	8.3c	-	-	-	-	-	-	-
***Pythium* sp.**	22.2c	-	-	-	-	-	-	-	-
Stachybotrys atra	22.2c	-	13,3	-	-	-	-	-	-
Macrophomina phaseolina	11.1d	-	-	-	-	-	-	-	-
Trichoderma harzianum	-	16.6b	-	-	-	-	-	33.3a	6.4b

(-): species not isolated.
The results of the same colum followed by different letters differ significantly at 5%.
Localities: Old H: Ouled Hamo; Frl: Frular; Bch: Boucharen; Gnf: Gnafda; Ghd: Ghdira.

groups, the first includes *B. cinerea*, *A. alternata* and *C.herbarum* abundantly appearing on the different varieties in five locations compared to the second including *C. acutatum*, *C. gloeosporioides*, *Mucor* sp., *E. purpurascens*, *S. botryosum* which are relatively less frequent besides *C. globosum*, *U. atrum*, *B. spicifera*, *A. nidulans*, *P. longisetula* and *C. fragariae* while the third gathers *T. roseum*, *M. echinata* and *F. nivale* that are reported for the first time on strawberry plants in Morocco.

Distribution of *B. cinerea* and *C. acutatum on* aerial parts of strawberry plants of varieties cultivated in different localities of Laoumra and Moulay Bousselham during 2012-2013 compaign

The examination of mycoflora associated to aerial organs of strawberry plants in the surveyed farms allowed us to appreciate a number of fungi often incriminated to be harmful to the strawberry culture.

The contamination percentages achieved by *B. cinerea* and *C. acutatum* varied significantly on the basis of the varieties and locations. Except for Splendor in Ouled Hamo and Camarossa in Ghdira, the attack of strawberries is very important in Gnafda amounted to 94% or 90.3% on Festival and Splendor respectively, rising up to 71.4% on Sabrina and 54.5% on Camarossa which has expressed a higher percentage of contamination (90.1%) in Dradra. In Dlalha, *B. cinerea* is profuse on Sabrina (70.2%) greatly exceeding Splendor (23.3%), whereas, in Ghdira, its proportion rises to 44.4%, to 58.3% in Frular which is comparable to Boucharen where the percentage is around 57.1% on Festival (Figure 1A).

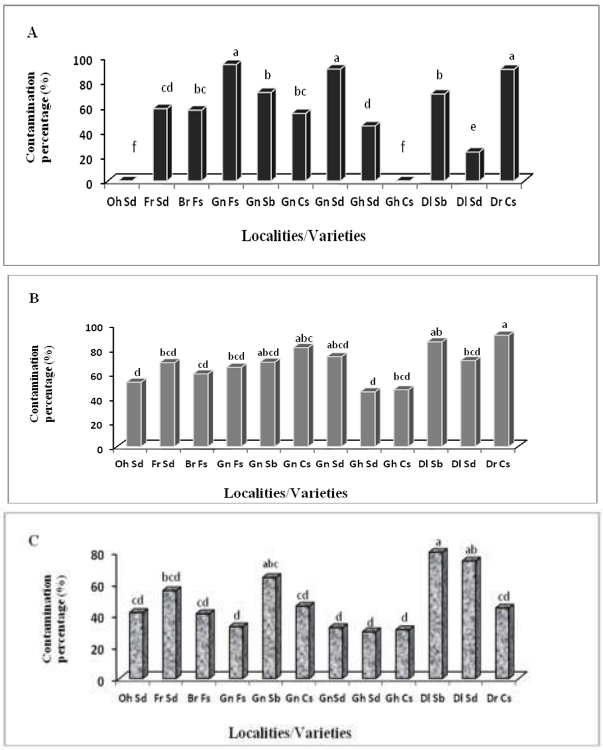

Figure 1 Distribution of *B. cinerea* on strawberries (A), leaves (B) and stems of varieties cultivated in different localities of Laoumra and Moulay Bousselham during 2012-2013 compaign.

Varieties: Sd: Splendor, Fs: Festival; Sb : Sabrina ; Cs : Camarosa
Localities, Oh: Ouled Hamo; Fr: Frular; Br: Boucharen; Gn: Gnafda; Gh: Ghdira; Dl: Dlalha; Dr: Dradra.

The results followed by different letters differ significantly at 5%.

The presence of *B. cinerea* was high in all locations and especially on the leaves of the Camarossa variety in Dradra up to 91.46%, 86% on Sabrina in Dlalha and 81% on Camarosa in Gnafda where the three varieties Splendor, Sabrina and Festival presented contamination percentage from 73.8% to 65.1%, lowering to 59.6% on Festival in Boucharen and 52.8% in Ouled Hamo on Splendor showing a contamination rate of 68, 9% in Frular (Figure 1B).

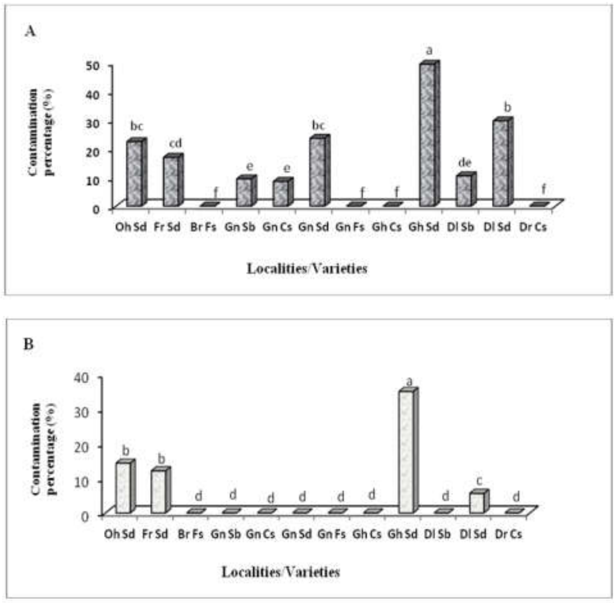

Figure 2 Distribution of *C. acutatum* on strawberries (A) and leaves (B) of varieties cultivated in different localities of Louamra and Moulay Bousselham during 2012-2013 compaign.

Varieties: Sd: Splendor, Fs: Festival; Sb: Sabrina; Cs: Camarosa
Localities, Oh: Ouled Hamo; Fr: Frular; Br: Boucharen; Gn: Gnafda; Gh: Ghdira; Dl: Dlalha; Dr: Dradra.
The results followed by different letters differ significantly at 5%.

On stems, *B. cinerea* invasions are increasing on Sabrina and Splendor varieties in Dlalha than on Camarossa in Ghdira and Gnafda where Sabrina was significantly affected (63.6%). They were moderately detected on Splendor in Frular (55%) or in Ouled Hamo (41.5%) and in Boucharen on Festival (40.7%) and much less in Ghdira, and Gnafda (Figure 1C).

As for *C. acutatum*, frequency isolation from strawberries is greater on Splendor with a percentage of about 49.4% in Ghdira, 29.7% in Dlalha, 23.6% in Gnafda, 22 4% in Ouled Hamo and 17% in Frular against low frequencies of less than 11% in Dlalha, Gnafda or 0% on Festival (Boucharen and Gnafda) and Camarossa in Drader (Figure 2A). Similarly, this fungus specifically affects the leaves of Splendor variety (35.1%) in Ghidra, 14.4% in Ouled Hamo, 12.1% in Frular and 5.6% in Dlalha while it was abscent in other localities (Figure 2B).

Distribution of *Rhizoctonia solani*, *Cylindrocarpon destruction* and *Fusarium oxysporum* on underground organs of strawberry varieties cultivated in different localities of Laoumra and Moulay Bousselham in mid-April 2013

Rhizosphere were mainly attacked by four various soil-borne fungi that expressed contamination percentages on 5 varieties of strawberry production farms.

The colonisation of the crowns by *R. solani* is greater in Gnafda, 45.2% on Festival, 36.4% on Camarossa and 22.1% on Sabrina, decreases to 24.6% in Boucharen, 16.9% in Frular and 10.6% in Ouled Hamo but disappears on Splendor in Gnafda and Ghdira (Figure 3A).

R. solani appeared more likely in the roots than in the crowns with percentages amounting to 53.3% in Gnafda, 46.8%, and 38.4% respectively on Festival, Camarossa and Sabrina, none on Splendor in both strawberry plots of Ghdira. It was detected in Ouled Hamo with a percentage of 42%, exceeding that of Boucharen (33.6%) and Frular (22.4%) (Figure 3B).

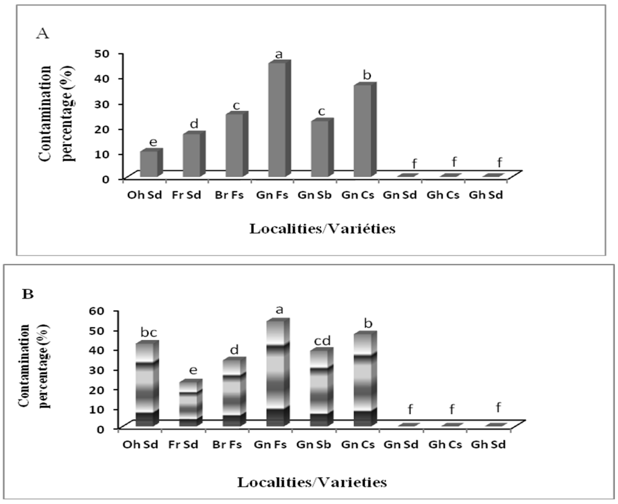

Figure 3 Distribution of *R. solani* on crowns (A) and roots (B) of strawberry plants cultivated in 5 localities in Louamra and Moulay Bousselham in mid-April 2013.
Varieties: Sd: Splendor, Fs: Festival; Sb: Sabrina; Cs: Camarosa
Localities, Oh: Ouled Hamo; Fr: Frular; Br: Boucharen; Gn: Gnafda; Gh: Ghdira.
The results followed by different letters differ significantly at 5%.

On another hand, *C. destructans* has been found abundantly in different localities.

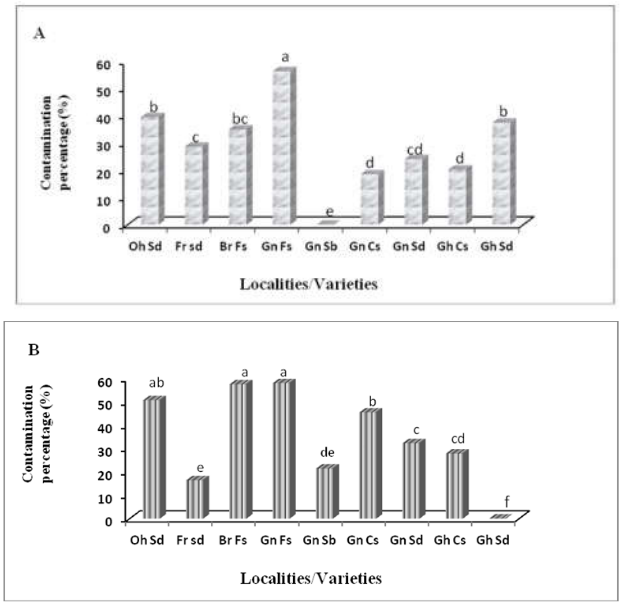

Figure 4. Distribution of *C. destructans* on crowns (A) and roots (B) of strawberry plants cultivated in Louamra and Moulay Bousselham in mid-April 2013.

Varieties: Sd: Splendor, Fs: Festival; Sb: Sabrina; Cs : Camarosa
Localities, Oh: Ouled Hamo; Fr: Frular; Br: Boucharen; Gn: Gnafda; Gh: Ghdira.
The results followed by different letters differ significantly at 5%.

Its isolation frequency in Gnafda reached 56.37% on the crowns of Festival, significantly different from those recorded on Splendor in Ouled Hamo and in Ghdira reaching 39.47% and 37.5% respectively, but at the same time comparable to the percentage recorded in Boucharen on Festival (Figure 4A).

The contamination degree of Splendor variety is lower in the Frular farm (28.63%) and Gnafda (24.07%) but exceeding the variety Camarossa in Gnafda and Ghdira with contamination percentages reaching 18.5% and 20.23 % respectively. However, the contamination was not observed on the crowns of the plants of Sabrina variety in Gnafda (Figure 4A) or plant roots of Splendor variety in Ghdira (Figure 4B).

On the roots level, fungus presence was important in Boucharen, Gnafda and Ouled Hamo where isolation frequencies were significantly identical exceeding 50%. Contamination percentages of Camarossa variety were 32.2% recorded in Gnafda, 27.76% in Ghdira and with significant differences from those recorded in Frular on Splendor and on Sabrina in Gnafda (Figure 4B).

F. oxysporum is less common on crowns of strawberry plants, detected only in Ghdira and Gnafda with respective proportions of 22.4% and 17.9% on Camarossa, on Splendor in Frular (10.1%) and Ouled Hamo (12.3 %) (Figure 5A). Additionally, the occurrence of this species is smaller on the roots being limited in Gnafda on Festival and Sabrina with a percentage of about 30%, 22.7% in Ghdira on Camarossa and on Festival in Boucharen (20.1%) (Figure 5B).

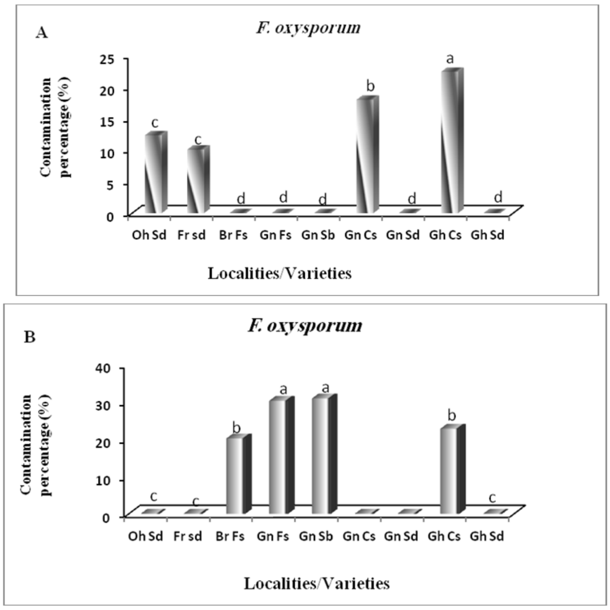

Figure 5 Distribution of *F. oxysporum* on crowns (A) and roots (B) of strawberry plants cultivated in Louamra and Moulay Bousselham in mid-April 2013.

Varieties: Sd: Splendor, Fs: Festival; Sb: Sabrina; Cs: Camarosa
Localities, Oh: Ouled Hamo; Fr: Frular; Br: Boucharen; Gn: Gnafda; Gh: Ghdira.
The results followed by different letters differ significantly at 5%.

DISCUSSION AND CONCLUSION

The mycological examination of strawberry plants sampled from the rural communes of Moulay Bousselham and Louamra revealed the existence of various fungal assemblages combining the three classes of fungi: Zygomycetes, Deuteromycetes, and Oomycetes whith a disproportionate distribution across the locations and concerned varieties in cultivation.

Earlier in the strawberry crop season, the quantitative evaluation of fungi altering fruits, foliage and stems in three localities of Moulay Bousselham will reveal the presence of *Penicillium* sp., *Rhizopus stolonifer*, *Cladosporium herbarum*, *Epicoccum purpurascens* and *Fusarium* sp. commonly found on the vegetative organs of strawberry plants (Jenkinson *et* Parry, 1994; Kwon *et al*., 2001; Rigotti *et al*., 2003; Timudo-Torrevilla *et al*., 2005; Walter *et al*., 2007) with *B. cinerea* (Moročko, 2003; Laugale *et al*., 2004), *Alternaria alternata* (Maas, 1984; Nao, 1995; Wada *et al*., 1996; Lee *et al*., 2001; Ito *et al*., 2004; Ko *et al*., 2008) and *Colletotrichum acutatum* (Timudo-Torrovilla *et al*., 2005). In addition, in mid April, the microflora spectrum identified on aerial organs of strawberry plants from Louamra and Moulay Bousselham also included *C. cladosporioides* witch was encountered at the anthers (Koike *et al*., 2003) and indicated as causal agent of strawberry flower blight by Gubler *et al*. (1999), *Mucor* sp., *Stemphylium botryosum*, *Aspergillus nidulans*, *Chaetomium globosum*, *Ulocladium atrum* and *Bipolaris spicifera* with various capacities of competition, antagonism or pathogenicity. The saprophytic and antagonistic fungi to *Botrytis cinerea, Alternaria* sp., *Ulocladium* sp. and *Epicoccum* sp. are provided with a great ability to survive for more than 16 weeks on the kiwifruit leaves in the field (Boyd-Wilson *et al*., 1998). The occurrence of *C. gloeosporioides* and *C. acutatum* is obviously related to the ability of these species to survive in the soil under various types of inoculum which can come from soil debris, plants or contaminated clothing and other host plants (Freeman *et al*., 2002). Indeed, other species have settled which likely reflect a rising sensitivity of the grown varieties in this perimeter to endophytic fungi or less competitive and emerging fungi with the most devastating fungal decay of this culture or in the presence of favorable weather conditions. This is the case of *Pestalotia longisetula*, previously reported on strawberry in the United States (Howard and Albregts, 1973), Israel (Howard, 1973), India (Shitole *et al*., 2000) and recently in Morocco (Mouden *et al*., 2014), *Trichothecium roseum*, *Fusarium nivale* and *Memnoniella echinata* who were cited for the first time in Morocco. According to Barnett and Hunter (1972), *T. roseum* is primarily a saprophyte or parasite of weakness, only he acquired the interest of several authors by its ability to induce necrotic areas on the surface of fruits and tomato leaves (Yun *et al*., 2013), to generate a pale pink strawberry rot in Korea (Kwon *et al*., 2010), as it was isolated from the infected dried pod surface of chickpea in Bangladesh (Shamsi et Soltana, 2008). In Morocco, its isolation was reported on pears during storage in the cold room of Oulmès (Bouigoumane *et al*., 2008), in Cantaloupe melons (Zemmouri *et al*., 2012) and stems of olive tree (Chliyeh *et al*., 2014). It has been reported on strawberries, matured fruit of *Cucumis melo* (Kwon *et al*., 1998; 2010) and of tomato (Yun *et al*., 2013). *Fusarium nivale* was encountered on perennial grasses (Smith, 1983), sunflower (*Helianthus annus*) (Roberts *et al*., 1986), barley (Richardson, 1990), it was reported for the first time on mango fruits in Pakistan (Khaskheli *et al*., 2008), and on leaves and stems of banana (Meddah *et al*., 2010). As to *M. echinata*, it was isolated from decaying wood (Hande and Kadu, 2015) from different grades of paper (Das *et al*., 1997). This species was found to be responsible for the deterioration of jute fibres during storage (Mishra and Misra, 2009).

The results have shown the prevalence and the high occurrence of *B. cinerea*. The prevalence of this species compared with *C. acutatum* joined the work of Laugale *et al*. (2009) who reported high levels of infection with *B. cinerea* in 26 farms in Latvia. Many studies have described its importance as a pathogen in the fields of strawberry (Bulger *et al*., 1987; Sosa-Alvarez *et al*., 1995; Stromeng *et al*., 2009) and it is also a common problem in the tomato fields (Chastagner *et al*., 1978), raspberry (Xu *et al*., 2012), grape (Gubler *et al*., 1987; Mundy *et al*., 2012) and pear orchards (Spotts and Cervantes, 2001; Spotts and Serdani, 2006). This prevalence is due to the existence of optimal weather conditions favoring disease progression.

Indeed, gray mold is one of the most devastating diseases reducing productivity, quality pre- and post harvest strawberries in all production areas (Berrie *et al*., 2000; Legard *et al*., 2002; Rigotti and Viret, 2004). In New Zealand, the preliminary grower investigations on the relative importance of the three fruit rot pathogens (grey mould, anthracnose, and leak) in commercial strawberry production suggests that fruit infection and postharvest storage rot can be caused by these diseases (Timudo-Torrovilla *et al*., 2005). The average fruit rot incidence (expressed per day) was 3.6, 2.2 and 1.6% for grey mould, leak and anthracnose respectively (Timudo-Torrovilla *et al*., 2005).

Moreover, contamination levels vary depending on the varieties and plots. These could be linked among other things, the dose of inoculum present on farms. The relationship between the incidence of pear fruit decay and inoculum dose has been determined for conidia of *B. cinerea* in air (Spotts and Cervantes, 2001). According to Strømeng *et al*. (2009), overwintering strawberry plant debris constituted by far the most important source of conidial inoculum of *B. cinerea* in the spring in perennial strawberry fields. Boff *et al*. (2003) cited the role of petals adhering to the fruit surface as an effective and reliable source of inoculum for fruit infection by *B. cinerea* before they had dropped naturally by the end of flowering. Variation of *B. cinerea* survival in different plant parts may be related to the drip irrigation system that may change local microclimatic conditions (Araújo *et al*., 2005). Moreover, spore spread and production will be dependent on weather conditions. The infection of flower and fruit by *B. cinerea* and subsequent sporulation are favored by extended periods of leaf wetness (Bulger *et al*., 1987; Sutton, 1990; Wilcox and Seem, 1994). Similarly, Xiao *et al*. (2001) attributed the significant differences in the incidence of Botrytis fruit rot in tunnels and open field plots to daily humidity, temperature as well as rainfall.

On the other hand, many studies have shown a significant impact of agricultural practices throughout the installation of the strawberry crop to reduce the incidence of gray mold (Dalman, 1993; Legard *et al*., 2000; Mertely *et al*., 2000; Plekhanova and Petrova, 2002; Lille *et al*., 2003).

The lower frequency isolation of *C. acutatum* which differs according to locality and variety in production system seems to suggest the likely involvement of the inoculum dose, humidity of foliage and temperature to promote contamination and infection of plant tissues as has been indicated by Verma (2007). Indeed, wet conditions prevent conidia and sclerotia survival of *C. acutatum* on strawberry plant debris or in soil (Norman and Strandberg, 1997). Similarly, Smith and Black (1987) have shown that at elevated temperature (35°C) and in the presence of maximum relative humidity, the severity of infection by *Colletotrichum fragariae* exceeds that obtained at 25°C or 30°C. On the other hand, Madden and Boudreau (1997) found the reduction in the incidence of anthracnose strawberries with increasing the density of strawberry plants.

Regarding fungal mycoflora of underground parts, it is represented specifically by *Cylindrocarpon destructans* and *Rhizoctonia solani* followed by Fusarium species especially *F. oxysporum* and modestly *Macrophomina phaseolina*, *Pythium* sp., *Phytophthora cactorum* with smaller frequencies, previously undetectable even in Moulay Bousselham (Mouden *et al*., 2013). These observations would imply the increase of the degree of infestation of strawberry plants in this region by these fungi probably due to the variability in susceptibility of cultivars in culture, climatic conditions, and cultural practices. Against *R. solani*, *F. oxysporum*, *M. phaseolina* and Oomycetes, strawberry cultivars showed differences in susceptibility. According to Fang *et al*. (2011), *F. oxysporum*, binucleate *Rhizoctonia* and *M. phaseolina* are the most virulent pathogens causing crown and root diseases of strawberry production and that the virulence of these pathogens is influenced by the prevailing seasonal temperature regime and soil pH. Moreover, disease severity will vary considerably on fumigated field beds (Subbarao *et al*., 2007) or regard to soil type (Wong *et al*., 1985). It is equally important to report the presence of *Curvularia lunata* and *Gliomastix murorum* on crowns. *Curvularia* spp. are ubiquitous and are typically considered to be weak pathogens or saprophytes, however, they have been reported as minor pathogens of several plants (Hodges and Campbell, 1995). In India, *Curvularia lunata* causes root rot of strawberry (Verma and Gupta, 2010). *G. murorum* was found on the stems of seedlings of eggplant (Cwalina-Ambroziak and Nowak, 2011) and was isolated from the leaves of herbaceous plants as endophyte (Gange *et al*., 2007). Challenged by *R. solani* and *F. oxysporum*, *C. destructans* is quantitatively the most majority. This could be due to the high susceptibility of the varieties cultivated in different localities to fungi recently emerged in this region where probably control methods opposing its development are lacking or it only target commonly fungi reported in the example of *F. oxysporum* being identified in its special form *Fusarium oxysporum* f. sp. *fragariae* in the face of witch strawberry cv. Camarosa was the most susceptible cultivar, while strawberry cv. Festival was the most resistant cultivar (Fang *et al*., 2012). Furthermore, Kirschbaum and Hancock (2000) reported red stele root rot as major disease problem in the summer production system where South American producers use raised beds, plastic mulch, Calfornia cultivars and crop management practices.

This study confirms the existence of multiple fungi which house various organs of strawberry and their occurrence is apparently similar across the communities visited especially when it comes to the prevalence of the causal agent of gray mold *B. cinerea* which could colonize fruits, leaves and stems of different varieties compared to *C. acutatum* that have low frequency of occurrence. In addition, the difference of isolation frequencies of all fungi detected may give us an overview of the state of plots health where protection measure are probably not efficiently used in all of them or that our sampling surveys meet highly favorable conditions for several fungus expansion. Moreover, the soil-borne fungus was also very common. This, testify firstly insufficient preplanting control of crops and during the strawberry production season. Also, growers must reconsider the susceptibility of the varieties cultivated in this region and origin of seedlings transplanted. The variously identified fungus might show high potential infection in relation to the prevailing weather conditions and possibly even a worldwide dissemination of isolates through international plant exchanges.

Acknowledgments

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