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Fusarium wilt of chickpeas (*Cicer arietinum* L.) in north-west Algeria

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***Fusarium oxysporum* (Schletend: Fr) f.sp. ciceri (Padwick) (*FOC*) is a soil fungus that is a permanent threat to the chickpea (*Cicer arietinum* L.) causing wilt syndrome. During spring of 2005 to 2009 surveys at three agro-climatic zones of north-western Algeria through seven sites. The presence of the disease was found in all the 50 fields chickpea visited. The pathogen, *F. oxysporum* f.sp. ciceri was isolated from infected plants harvested. Three types of symptoms are observed on chickpea plants in fields: *Symptom-yellowing*, *Wilting* and *Root rot*- that appeared in very wet conditions on few fields from locality: *Tighenif-Maoussa-Field station* (Mascara region). The means of incidence and severity of the disease were high in all regions. From region of the Mascara; the incidence was estimated between 5 and 31% for stage 2 branching, of 10 to 60.2% for stage prebloom, and of 54 to 98.6% in maturation stage. It was higher in dry years. The severity varies from 2 to 3.56 for the three stages and the index of the disease was evaluated between 2.5 and 68.77%. The average rating was drawn between 7 sites, were incidence varies between 50 and 100%, severity from 1 to 2.88 and the disease intensity index drawn between 15 and 68.77%. *F. oxysporum* was the main species isolated from diseased parts of the plant with an average frequencies of 49 and 91% followed by *Fusarium solani* with 44 and 51%. *F. culmorum F. equiseti, Sclerotium* spp. and *Rhizoctonia solani,* are which part of the microflora isolated and could be responsible for various disease collar and root. The study of virulence on a susceptible cultivar that very susceptible to *F. oxysporum* f.sp. *ciceri*, ILC482 confirmed the presence of this special form.**

Key words: *Cicer arietinum, Fusarium oxysporum f.sp. ciceri*, incidence, North-western Algeria pathogenicity.

INTRODUCTION

The Chickpea (*Cicer arietinum* L.) has always had an important part in the farming system in the Mediterranean countries. It represents a pulse of wide consumption (Laumont and Chevassus, 1956). Its importance comes from its rich seed protein of high quality and its ability to attract atmospheric nitrogen and enrich the soil. So it is used as an excellent preceding crop for cereals. Although in Algeria in recent years have seen a rising trend of the agricultural area and production of this crop, however, yields are means (22,274 ha-178.404qx -8.0q/ha in 2009, Statistic from the Ministry of Agriculture, 2010). Chickpea in Algeria is affected by various fungal diseases and in a previous work we identified various important diseases whose primary is without doubt the blight caused by *Ascochyta rabiei* (Merzoug et al., 2009). The studies on pathogens of chickpea, have focused on anthracnose caused by *A. rabiei*, a real obstacle to culture. Now is added complex wilt and root rot, called wilt syndrome

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Table 1. Climatic characteristics of the chief towns of departments Chickpea producers in different agro-climatic zones of northwest Algeria average of the months from January to June for the years 2005- 2009).

Areas / sites	No. of field	Elevation (m)	Rainfall (mm) average annual	Temperature (°C)			Seasons of survey
				Min.	Moy.	Max.	
Coastal plains							
Mostaganem	02	104	32.02	6.9	17.33	29.51	2007
			33.57	6.2	15.75	26.52	2008
			22.24	13.08	18.32	24.03	2009
Ain- témouchent	08	250	26.50	10.78	15.76	17.00	2006
			34.20	12.1	19 .19	24 .17	2008
Internal plains							
Mascara	17	600	20.34	5.52	16.57	32.43	2005
			22.05	6.56	20.05	30.51	2006
			32.08	8.38	21.13	14.45	2007
			22.44	7.73	22.23	14.75	2008
Sidi-Bel-Abbès	08	470	42.54	8.36	14.96	21.75	2009
			22.77	5.9	19.50	32.77	2006
			17.02	7.28	16.58	22.70	2008
Relizane	04	560	56.52	9.1	21.60	28.38	2009
The highlands							
Tiaret	02	1023	52.78	5.26	18.76	21.1	2009
Saida	02	752	41.39	4.95	17.25	31.55	2009

caused by *Fusarium oxysporum* Schlechtend. f.sp. ciceri (FOC) (Rhrib, 1990). This wilting syndrome has been reported in Algeria by Bouznad (1989) and in the Mediterranean: Spain, Tunisia, Morocco and Turkey (Haware, 1990). In Algeria, its presence was observed mainly in the center and east (Labdi, 1990) with a lesser extent to the west (Bouznad et al., 1996), indicating that it could become a serious problem in this region. In Tunisia the severe wilting causes a reduction in chickpea production, about 40% of fields located in the main producing areas are infected (Sayoud et al., 1999). Based on the work of Trapero-Casas and Jiménez-Díaz (1985) and Jalali and Chand (1996), annual losses due to wilting ranged from 10 to 15%. Sometimes under specific conditions, there is a total loss of cultures (Haware and Nene, 1980; Navas-Cortés et al., 2000).

In Algeria, despite the importance of wilt syndrome, there is little quantitative assessment of the impact of wilt on chickpea production and little knowledge is acquired for the pathogen. FOC is seed-borne and soil, saprophytic fungus optional in absence of its host, and it can survive on plant debris in soil for 6 years (Haware et al., 1978). FOC causes two types of symptoms with two different pathotypes. Sudden wilting, observed in young plants, characterized by rapid death of the plant, and yellowing, which develops slowly from the bottom to the top. Variability of the pathogen in pathogenicity is reported at the levels of disease severity and type of symptoms. Several seasons of surveys (1999-2004) symptoms of yellowing and wilting are reported across areas of crops. According, having found its importance and its wide distribution, we conducted surveys on several agro-climatic zones of western Algeria to assess the extent and the importance of the disease. In this study we examined the geographic variability of disease incidence (DI), and the severity of the disease (S).

MATERIALS AND METHODS

Areas and sites surveyed

Table 1 indicates the different agro-climatic zones surveyed and representing the most important cultivated in chickpea field in north-west Algeria. Based on the occurrence of chickpea yellowing and wilt, 3 different agroclimatic zones around of 7 regions were survey for sampling. During the seasons from 2005 to 2009, 50 fields, separated by 3 to 5 km apart, were visited between March and late June. From each site, 20 plants showing symptoms of yellowing or wilting were collected for laboratory analysis. The infected plants were placed in paper envelopes, air-dried at room and stored at 18°C until used to isolate the pathogen. Isolations were made from the wilted plants of chickpea.

Calculation of disease incidence (DI)

The DI is expressed as a percentage of the number of sick units on the total number of plants considered, (Trapero-casas, 1983). In

each plot, three rows, (80 plants per row to 130) each 10 m long, were chosen arbitrarily. Plants in each row were examined and the number of plants showing symptoms of yellowing or wilting vascular noted. DI is expressed as the percentage of affected plants, counted in three rows by the total number of plants.

$$Wilt\ incidence = \frac{No\ of\ plants\ wilted}{Total\ No\ of\ plants} X100$$

For each plot values of the impact were grouped into classes of similar extent to that seen by Traperos-Casas (1983): [0% (zero), 0.1 to 0.5% (low) 1 to 20% (moderately high); 20.1 to 50% (high), > 50% (very high)].

Calculation of the severity of the disease (S)

The DI does not provide information on the severity. It is calculated based on visual assessment of the proportion of plant parts affected by wilting or yellowing: a column of three rows of plants considered an affected their severity is assigned based on the importance of symptoms: [0: no symptoms, 1 yellowing or wilting of 1/3 of the plant; 2: yellowing or wilting of 2/3; 3: yellowing and wilting of the whole plant 4: Plant dead] (Traperos-Casas, 1983; Cooke, 1998). To estimate the severity of the disease, the average index of severity (ISM) was calculated for each plot.

$$ISM = \frac{\sum nj \times ij}{\sum_j nj}$$

Where, n = number of plants characterized by the index i of severity of illness attributed to plants. ISM were grouped into classes: 0 <ISM<1 mild disease, 1 <ISM <2 moderately severe; 2 <ISM<3 serious disease;3<I SM< 4 very serious disease (El-Aoufir, 2001).

The calculation of the index of disease severity Disease Intensity Index (DII is very important to give the relationship between DI and ISM, is calculated as the percentage of disease incidence X severity index / index maximum scale of severity (Luo et al., 2000).

$$DII = \frac{DI\ X\ ISM}{4}$$

Isolation from the infected plant and seed chickpea

Isolations were made from stem, collar, root segments and rhizosphere areas of symptomless plants to determine the occurrence of vascular infections. The samples pieces of individual plants were cut into 5 to 10 mm-long segments, surface disinfested (0.2% NaOCl for 2 min), plated on Potato Dextrose Agar (PDA): broth 250 g potato, 20 g of agar and 20 g of dextrose per liter of distilled water), and incubated at 22°C and a 12 h photoperiod for 5 to 7 days (Erskine et al., 1990; Landa et al., 2001).

Isolation from the rhizosphere is carried of three zones (a): soil that separates easily from the roots; (b) soil adjoining the roots; (c): mycelium adhering to root. For soil isolation area(a) according to the method soil plats: 5 mg of soil are placed in Petri dishes disperse then adding a few drops of sterile water to good dispersion and then sank PDA. For area (b) roots are washed with 10 ml of sterile water. In an Erlenmeyer, suspension is stirred for 30 min and adjusted wit 90 ml of sterile water: first dilution. 10 ml of the first dilution are removed and placed in 90 ml of sterile water and so on until the 6th dilution. The roots area (c) are washed and treated as above stems. Seed samples were disinfected and incubated agar test method, under the same conditions (Rapilly, 1968; Campbell and Greaves, 1990).

Identification of isolates

The strains are identified and purified based on morphological and microscopic characteristics of *Fusarium oxyxporum*, presence of microconidia and macroconidia in typical spindles for the species. The fungus was identified according to the identification keys of *F. oxysporum* (Snyder and Hansen, 1954; Messien and Cassini, 1968; Booth, 1971; Nelson et al., 1983). Only isolates with these characteristics are considered to belong to the special form *F. oxysporum* f.sp. *ciceri.* The other fungal species were purified by *single-spore cultures* and identified (Watanabe, 2002; Leslie and Summerell, 2006).

Obtaining single-spore cultures (clones)

El-Ani (1968) showed that microconidia of are *Fo* uninucleate and haploid nuclei then are all from a single nucleus at the start. To obtain cultures from a single spore, microconidia containing a suspension of 20 spores / ml were spread on PDA. After 24 h of germination, the young fronds from single microconidia are removed aseptically and placed separately on PDA. Each frond will be a clone, which may have different morphological characteristics of the other clones.

Obtaining suspensions of conidia

Inoculate of isolates were prepared from 21-day-old cultures grown in liquid Potato Sucrose Agar (PSA): broth 250 g potato, 20 g of agar and 20 g of sucrose per liter of distilled water medium to 25°C at 100 rpm under continuous cool fluorescent light. The mycelium of the cultures was removed by passing through four layers of cheesecloth, and the concentration of spores present in the liquid medium was adjusted to 1×10^6 per ml using a hemacytometer.

Pathogenicity assays

To identify formae specialis of *F. oxysporum*, passing by a bioassay is required (Messiaen and Cassini, 1981).The *Fusarium* species contains formae specialis, group with morphologically identical forms and enfeoffed to each plant (Snyder and Hansen, 1954). Several formae specialis have been described by Messiaen and Cassini (1981), those specific to Chickpea (*Cicer arietinum* L.) is *F. oxysporum* f.sp. *ciceris* (*FOC*). To do this, susceptible hosts are faced with the fungus. The special shape *ciceri* is determined by inoculation of a variety of chickpea ILC482, highly susceptible to *Fusarium* wilt, provided by the Technical Institute of Arable Crops of Saida (I.T.G.C).

The seeds were disinfected and germinated in Petri dishes between two sheets soaked in sterile water and then incubated under the same conditions. Seedlings are transplanted into pots containing sand autoclaved at 120°C (1 h for 3 consecutive days). The pots are placed in a chamber illuminated between 20 and 22°C under daylight illumination. Inoculation is carried out on plants aged 8 to 10 days (stage two first leaves unfolded) by immersing the roots in the conidial suspension containing 10^6 conidia / ml, during 30 min. The control seedlings were immersed in a solution of sterile distilled water. Then, the seedlings are transplanted into pots containing a mixture of 500 g sterilized sand+ peat + soil (1: 1: 1). Isolates and three replicates per 5 plants per pot were performed. Incubation is carried out as before. Reading

Table 2. Evaluation of Fusarium wilt in three physiological stages in the sites of the region of Mascara: Average Impact (ID), severity (GD) and the intensity of the disease (DII).

Seasons	Stade végétatif	DI%	ISM	DII%
	Ramification	54	3.5	47.25
2004-2005	Prebloom	62	3.49	54.09
	Maturation	75	3.34	62.62
	Ramification	8.75	3.56	7.78
2005-2006	Prébloom	54.25	3.30	44.75
	Maturation	62.00	3.25	51.15
	Ramification	5	2	2.5
2006-2007	Prebloom	10	2.5	6.25
	Maturation	54	3	40.6
	Ramification	31.2	3	23.4
2007-2008	Prebloom	60.2	2	30.1
	Maturation	98.6	2.79	68.77
	Ramification	46	2.69	30.93
2008-2009	Prebloom	69	2.25	38.81
	Maturation	98.33	2	49.16

the results is done by raising the daily symptoms on cultivar ILC 482 for 40 days. To confirm the involvement of *F. oxysporum* f.sp. *ciceri* in infection, a reisolation of the fungus is carried on stems and PDA cultures obtained are compared to their parent strain.

RESULTS

Three types of symptoms are observed on chickpea plants in fields:

1. *Symptom*-yellowing observed in all fields. Discoloration of leaves that are a light green changing to a progressive yellowing of leaves. The appearance is seen as early as seedling stage or later stage or flowering branch. This symptom is observed in the apical leaves and progresses to the basal leaves. In recent dry up and fall the plant can dry out completely or partially. The release of full or partial petioles of the leaves which had reached the lower leaves and progressing up (most common). This symptom appeared early results in stunting of the plant and its growth is stopped. Appeared before the plant growth is slowed.
2. *Wilting* - symptom appears very early it causes early death of the seedling which remains at ground level. It is present in all sites visited with a frequency between 1 to 10%. In 2 fields this symptom is dominant: Locality from *Oued-abtal* and from *Tighenif* (Mascara); called early wilting by different authors, and causes more losses than the yellowing late. This symptom is observed up to the stage or pod plants dry out completely. The pods are smaller and empty. In general the roots showed a healthy appearance but with a reduced root system. A cross section shows a color system. Pod filling and grain quality, is reduced (small size and wrinkled).
3. *Root rot*-in very wet conditions on some fields from locality: *Tighenif-Maoussa-field station* (Mascara); and during springs of 2007 and 2008, aboveground show yellowing and wilting sided leaflets and leaves at the base of plants. Symptoms that can be generalized to whole plants. Plants are stunted and the root brown and decomposes, it is a root rot. The crown area has some browning with the presence of sclerotia after a heavy rain and continued high humidity. The plant breaks easily revealed to the crown and root rot remain in the soil. Finally, the death of the plant can be observed. This symptom can be confused with wilting and may occur simultaneously.

Disease evaluation

The incidence increase with age of plant but the score of severity was steady (Table 2). For maturation stage of vegetation varies in all plots of chickpea during the seasons of 2005 to 2009 about 5 to 100% (*Tiaret Mostaganem, Ain Temouchent, Saida*, *Mascara*) (Table 3). We note DI moderately high to very high with average ranked between 50 and 100%. The range severity score was between 1 and 4 with a mean value of 1 to 2.88 considered according to the scale of medium severity for *Mostaganem, Relizane, Tiaret* and *Saida*, to severe for

Table 3. Evaluation of Fusarium wilt in maturation point in the north-western Algeria and agroclimatic zones between 2005 and 2009: Average Impact (DI), severity (ISM) and the intensity of the disease (DII).

Areas/Sites	DI (%)		ISM		DII(%)	MF. Foc (a) (%)
	Mean	Range	Mean	Range		
Costal plains						
Mostaganem	100	(-)	1.5	(1-3)	37.5	46.24
Ain-témouchent	100	(-)	2.5	(2 -4)	62.5	70.02
Mean	100		2		50	56.13
Internal plains						
Mascara	77.58	(54-98.6)	2.88	(2- 3.56)	55.85	60.50
Sidi- Belabbes	65	(20-90)	2.79	(2-3)	45.33	55 .90
Relizane	80	(10-100)	1.21	(1-4)	24.2	69.90
Mean	74.19		2.29		41.79	62.1
Highlands						
Tiaret	50	(30-60)	1.21	(1-3)	15.12	50.12
Saida	100	(-)	1.06	(1-3)	26.5	62.54
Mean	75		1.18		20.81	56.33

(a)-MF, Mean frequencies of *F. oxysporum* f .sp.*ciceri.*

*Mascara, Ain-Temouchen*t, and *Sidi-Bel-Abbes*.

The index of disease severity is high and varied between 15.12 and 62.5%. From sites of regions *Relizane, Tiaret* and *Saida* was relatively low between 15 and 26%.The dominant pathotype in prospecting was the gradual yellowing observed in all fields.

Pathogenicity of *F. oxysporum*

From stems showing symptoms of yellowing and wilting we could isolate the species *F. oxysporum*. Its inoculation on chickpea cultivar ILC 482 has identified the special form *ciceri*. The first symptoms appeared after the 3rd day after inoculation for some very aggressive isolates. The symptoms are identical to those described in the field. Cultures derived from reisolation from inoculated seedlings were morphologically identical to the parent strain. The frequencies of isolation of FOC was constant for the different survey sites and varies from 50 to 70%.

Pathogenic fungal species isolated from infected plants

The analysis of the isolated microflora quantitatively and qualitatively varies according to the treated parts of infected plants .The fungal species isolated was associated with the complex of Fusarium wilt. The presence of *F. oxysporum* was always dominant and was isolated from all parts of the plant (Table 4) with an average of 43.26% and followed by *Fusarium solani* with 31.61%.The remaining species colonized different parts of the plant: *F. culmorum* (stem, collar, root); *F. equiseti* (collar), *Sclerotinia* spp. (root and rhizosphere) *Rhizoctonia solani* (collar and rhizosphere). It *would* cause collar rot and root rot of chickpea.

DISCUSSION

Identification of morphological and pathogenic *F. oxysporum* showed that *Fusarium* wilt is present in all plots surveyed in the north-western Algeria. Symptoms are present at all physiological stages with varying degrees from one field to another and from one season to another. These symptoms are similar to those reported in Tunisia, Morocco, Spain, California and India (Westerlund et al., 1974; Nene and Reddy, 1987; Jimenaz-diaz and Trapero-Casas, 1988; Halila and Strange, 1996; El Aoufir, 2001).

Wilt disease was favored by periods of drought and less intense rainy seasons. DI is high at all sites; in 5 of 7 sites is between 80 and 100% to classes it at very high incidence. In 40% of sites ISM was valued of 2.5 to 2.88 so ranked as “serious disease” and in some localities it reaches 4 maximum value, so reveled “very serious disease”. DII which gives the relationship between DI and ISM and is between 15 and 60% an important indicator of the intensity explains the low yield. Although these values is variable from one site to another and from one field to another. The development of this disease seems

Table 4. Average frequencies (%) of pathogenic species isolated from different plant parts of chickpea affected between 2005 to 2009.

Fungi organs / Organs	*Fo*	*Fs*	*Fe*	*Fc*	*Sc*	*Rh*
Leaf	55.23	22.22	0	0	0	0
Stem	70.00	23 .02	0	05.00	0	0
Collar	21.00	22.3	06.66	03.66	0	43.22
Rhyzosphere						
Area(a)	28.74	34.05	0	0	1.02	7.33
Area(b)	38.39	33.04	0	0	0.33	5.61
Area(c)	46.24	43.18	0	0	0	0
Means	43.26	31.61	1.11	09.44	0.22	12.19

(a)- Fo, Fusarium oxysporum; Fs, Fusarium solani; Fe, Fusarium equiseti; Fc, Fusarium culmorum; Sc, Sclerotinia sp.; Rh, Rhizoctonia solani

important and no part is spared, it seems to be favored mainly by seed health relative to other factors (Singh et al., 2007). Indeed the seeds tested, and used by farmers, reveal the presence of the pathogen. These seeds from local cultivar are often used without treatment by farmers to season to another which contributes to increasing the inoculums level plots. Ghosh et al. (2013) noted same problem in India and were found than local cultivar had a higher incidence of wilt disease .

On the other hand, the movement of seeds in the market allows movement of the pathogen via seed. It contributes to spread of disease. However, the health status of the plots is another constraint that contributes to falling yields. Indeed the rate of inoculums in the soil affects the annual variation of disease severity according to Navas-Cortés et al. (2000). According to this author production is controlled by planting date, cultivar and year of experimentation, production decreases with the spring planting compared to the winter sowing and to a lesser degree by virulence. The importance of the disease in our region seems strongly influenced by sowing date which in most cases and in our investigation is performed in mid-March which puts the plant to conditions of low water at ground level and high temperatures.

Labdi (1990) conducted at Sidi-Bel-Abbes, are records of 70% increase in production and climate dependence of 20 to 100% for spring planting. Dry years favor the disease or the impact, because water stress aggravates symptoms of yellowing so dominant in all plots. Indeed temperatures around 28°C accentuate water stress and lead to higher DI (Landa et al., 2006) according to that study the temperature has a significant influence on the development of the disease, the metabolic process and plant development and pathogen and its virulence. The study of Andrabi et al. (2011) reported environmental factors that influence the development of pathogens as moisture and temperature. In other work according to Mehmood et al. (2013) rain fall and soil variables (temperature and moisture) also had a significant positve effect on fusarium wilt disease.

The genotype of the cultivar, inoculum in the soil, the virulence of the pathogen in addition to humidity and temperature influences the severity and development of the disease and increasing the intensity of the disease significantly reduces the production (Gupta et al., 1986; Landa et al., 2006; Navas-Cortes et al., 1998, 2000). According to Navas-Cortes et al. (2000) winter sowing or planting early spring pushes the epidemic significantly, slowed the development level and reduces the final amount of disease. This practice can be applied in all sites surveyed and in Mediterranean areas.

The symptoms produced during test of pathogenicity were exactly identical to those described earlier by Westerlund et al. (1974) and Cabrera et al. (1985). Reisolation studies revealed the presence of the same fungus identical to the original one obtained from naturally wilted plants. The morphological and cultural characteristics of the *F. oxysporum* f. sp. *ciceris* obtained were similar to those reported earlier by several (Gupta et al., 1986). Characteristic wilt symptoms such as drooping of leaflets and yellowing of the leaves starting from apical part, progressing downward and final wilting of the whole plant were observed. Affected plant roots when split opened showed discoloration of internal tissues. The symptoms of chickpea wilt observed were similar to those recorded from all study on wilt (Westerlund et al., 1974; Haware et al., 1986). The presence of *F. oxysporum* was associated to other species *Fusarium solani (FS).* Like other pathogenic soil as *F. equiseti (FE), F. culmorum (FC), Sclerotium* spp *(SC)* and *Rhizoctonia solani (RS). (FS), (SC),* and *(RS)* was causal agents of root rot and collar rot and wet root rot respectively. It have been reported by different authors in the study on wilting of legume worldwide and are most active when high

humidity conditions and are high in humid climates (Nene and Reddy, 1987; Jimenaz-diaz and Trapero-Casas, 1988; Belabid et al., 2000). In our present study, the presence of root rot and collar is less important than the wilt in all sites because areas are located in a semi-arid to arid climate.

Conclusion

This study highlights the importance of the expansion of wilting and its impact on low yields and the role of factors related to crop management. These results can help generate interest in the management of this crop. Other studies may be conducted to identify other key factors who interfering with the production and seek ways to improve performance.

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