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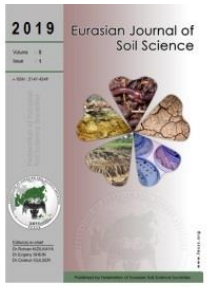

Role of soil physicochemical and microbiological properties in the occurrence and severity of chickpea's Fusarium wilt disease

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Abstract

The aim of the present study is to evaluate the relative disease severity of chickpea wilt in the most important chickpea growing areas in North Algeria and their relationship to soils properties. The physicochemical and biological parameters of 14 soils were analyzed and correlated to the disease index severity (*Dis*). Soil physicochemical factors were determined as a means of particle size distribution, pH, Electrical Conductivity (EC), $CaCO_3$ content, total Nitrogen (Total-N), Olsen-P and biological factors including Foc inoculum density (ID-Foc), *Trichoderma* spp propagule number (*TrPn*), *Pseudomonas* spp and *Bacillus* spp. The results revealed that the spread of the disease was evident in all prospected areas and recorded as low to medium with values ranging from 2.05 to3 9.8. The disease severity was positively correlated with EC (r=0.62), Total-N (r= 0.79), and ID-Foc (r=0.72), whereas negatively correlated with Olsen-P (r=-0.67), *TrPn* (r=-0.70) and *Pseudomonas* spp (r=-0.89). There was no correlation between *Dis* and soil physical (clay, loam and sand), chemical (pH, $CaCO_3$ content) and biological factors (*Bacillus* spp). As well, ID-Foc was positively correlated with Total-N and negatively correlated with Olsen-P. The results indicated that *TrPn* and *Pseudomonas* spp were positively correlated, whereas both were negatively associated with ID-Foc and D*is*. Our finding pointed out the critical role of some physicochemical and biological soil characteristics in the epidemic development of chickpea wilt under field conditions.

Keywords: Chickpea, *Fusarium oxysporum* f. sp *ciceris*, Nitrogen, Olsen-P, *Trichoderma spp*, *Pseudomonas spp*.

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Introduction

Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend: Fr. f. sp. *ciceris* (Padwick Matuo and K. Sato) is the most important constraint to production of chickpea in the worldwide, particularly in the Mediterranean area and the Indian subcontinent (Haware, 1990). Pathogen can cause yield losses, with an annual average of 10–15%, although, the disease can destroy the crop completely under specific environmental conditions (Trapero-Casas and Jiménez-Díaz, 1985).

The disease is defined by a monocyclic epidemic nature induced by chlamydospores as primary inoculum wich survive on crop residues in soil for more than 6 years in the absence of susceptible host (Jiménez-Díaz

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et al., 2015). Furthermore, the expression of epidemic *Fusarium* wilt can result from the complex interaction of population of chickpea, population of Foc, and soil properties (Mehmood et al., 2013).

Soil properties are considered as epidemiological factors that can influence the occurrence and severity of plant diseases (Ghorbani et al., 2008). Soil physicochemical properties such as pH, nitrogen and phosphorus play an important role in the growth and susceptibility of the host, multiplication and infectivity of the pathogen, or in the interaction of host plant and pathogen (Höper and Alabouvette, 1996; Elmer and Datnoff, 2014).

Research reports have demonstrated that soil intrinsic microbial communities or specific sub-populations have the potential to suppress pathogen infectivity of host plants (Shen et al., 2015). However, the degree of soil suppressiveness is associated to soil microorganism's biodiversity such as *Trichoderma* spp, *Bacillus* spp and *Pseudomonas spp*, which are commonly used as biological control agents (Lemanceau and Alabouvette, 1993), and which in turn are influenced by soil physicochemical properties (Lenc et al., 2011).

The associations between the soil properties on the behavior of microorganisms have been investigated intensively but are still imperfectly understood (Lucas, 2006). Furthermore, understanding the mechanism of the interaction between soil physicochemical properties and microorganisms is important for the successful disease control in a natural agro-system (Naseri and Hamadani, 2017).

In this context, the main objective of the present work is the study of the combined effects of soil physicochemical parameters (EC, N, P, pH) and antagonistic agents (*Pseudomonas* spp, *Bacillus* spp and *Trichoderma* spp) on the development of chickpea wilting as well as on the density of the inoculum and severity of *Fusarium* wilt in natural conditions.

Material and Methods

Farm assessment and soil sampling

This study was carried out during June 2015 indifferent cultivated fields' chickpea located in different agro-climatic zones in North Algeria, including Constantine (3 sites), Guelma (2 sites), Mascara (2 sites), Ain Témouchent (3 sites), Sidi Bel Abbes (2 sites) and Relizane (2 sites). Soil samples were collected from 14 fields' taken from the rhizosphere soil surrounding chickpea roots. Soil samples were initially sieved to remove all plant residues then air-dried and ground into 2-mm particles for physicochemical and biological analyses.

Soil physicochemical characterization

Soil samples were analyzed for particle size distribution (pipette method), soil acidity (pH) with a 1:2.5 w/v and electrical conductivity (EC) (1:5 w/v). Equivalent calcium carbonate ($CaCO_3$) was determined using the Bernard calcimeter (Hulseman, 1966). Total nitrogen was determined by the Kjeldahl method as described Jones (2001). Available phosphorus (Olsen-P) was determined by the method of Olsen et al. (1954).

Soil fungal and bacterial isolation

The dilution plate method described by Bulluck et al. (2002) by means of agar media was used for the determination of the different fungal species. 10 g of each soil sample were suspended and diluted into 90 ml of sterile distilled water then mixed for 30 minutes. Successive dilutions of 10 ml from this suspension were prepared with 90 ml sterile distilled water (10^{-2} and 10^{-3}). Each suspension was transferred onto 90 ml of agar media. Various suspensions were poured into Petri dishes (90 mm) and were incubated at 25°C for 3 to 15-days. Different colony types were transferred to potato dextrose agar (PDA) media and incubated for 7-days under the same conditions for taxonomic identification. The isolated *Fusarium oxysporum* strains were purified and identified by single-spore cultures and identified based on morphological and microscopic characteristics according to specific identification keys given by Messiaen and Cassini (1981).

The pathogenicity tests were performed according to pot screening procedure as described by Nene and Haware (1980). *Trichoderma* spp colonies which developed on PDA were identified based on visual macroscopic and microscopic observations according to Gams and Bissett (1998).

Regarding bacterial isolation, 1g from each soil was suspended in 9 ml of sterile distilled water according to Bulluck et al. (2002) technique. The suspensions were shaken and then heated at 50°C for 5 min. *Pseudomonas* Selective Agar (PSA) and Nutrient Agar were used for isolation of *Pseudomonas spp* and *Bacillus spp*, respectively. Here too, successive dilutions of 10 ml from this suspension were prepared with 90 ml sterile distilled water (10^{-2} and 10^{-3}). Aliquots (1 ml) of each dilution from each soil sample were transferred into 9 ml culture medium in Petri dishes. The plates were incubated at 28-37°C for 24-48h.Each colony of *Pseudomonas* spp and *Bacillus* spp was isolated and identified based on morphology and total density per sample (colony-forming units /g soil). All experiment was carried out in four replicates.

Fusarium wilt disease assessment, Foc isolation and pathogenicity test

Disease assessment in chickpea was carried out during the flooring stage to observe symptom development and disease evolution. Disease incidence (DI) was assessed according to Traperos-Casas and Jiménez-Díaz (1985) by counting the number of plants showing symptoms in three representative 10 m lengths of row, randomly chosen from each field. Severity of *Fusarium* wilt (ISM) was assessed on a scale of 0 to 4 according to the percentage of foliage with yellowing or necrosis (0 = 0%; 1 = 1 to 33%; 2 = 34 to 66%; 3 = 67 to 100% and 4 = dead plant). DI and ISM data (rated from 0 to 4) were used to calculate disease index intensity (*Dis*) using the equation *Dis* = (DI× ISM)/4.

A total of 10 wilted plants were collected from each field, for laboratory analysis. The infected plants were placed in paper envelopes, air-dried at room and stored at 4°C until used to isolate the pathogen. *F. oxysporum* cultures isolated from wilted plant were identified microscopically based on morphological characteristics. The pathogenicity tests were performed according to pot screening procedure (Nene and Haware, 1980).

Statistical analysis

One way analysis of variance (ANOVA) with post-hoc Newman-Keuls test was used to test differences between soil samples. The association between soil physicochemical, biological properties and D*is Fusarium* wilt were also calculated using the Pearson correlations. The association between physicochemical and biological soil properties as well as Dis was made by principal component analysis (PCA). The statistical analyses were done using the software package STATISTICA 8.

Results

Physicochemical properties of soils

The results in Table 1 show the variation in physicochemical proprieties of soils from the 14 sites. In terms of physical properties, sand, loam and proportion clay showed significant differences between the studied 14 sites. Particle size distribution varied significantly among the 14 sites ($P < 0.05$). The highest values of sand (46.3%), clay (61.4%) and loam (84.4%) were observed in S14, S9 and S3, respectively. In contrary, S3 was characterized by the lowest value of clay (11%). S9 presented the lowest value of silt (11%), whereas, S3 showed the lowest value of sand (4.4%).

Table 1. Physicochemical properties of different soil samples from 14 chickpea fields in North Algeria

Field sites	Silt (%) **	Clay (%) **	Sand (%) **	pH **	EC (µScm-1) **	Olsen-P (mg kg-1) **	N (g kg-1) **	$CaCO_3$ (%) **
S1	71.1±0.20c	19.4±0.14i	09.4±0.20h	8.0±0.05de	143.2±3.14k	4.9±0.53d	1.4±0.02h	27.3±0.02c
S2	70.5±0.20c	22.2±0.35h	07.2±0.45i	8.2±0.00ab	163.5±1.73j	6.4±0.24c	1.7±0.02g	31.2±0.45b
S3	84.4±0.24a	11.0±0.13k	04.4±0.11j	8.1±0.01bcd	173.9±1.57i	8.5±0.08b	0.7±0.02j	20.2±1.04d
S4	30.9±0.59h	40.8±0.56d	28.1±0.20b	8.1±0.02cd	167.3±1.25ij	5.4±0.08d	0.8±0.06ij	27.0±0.88c
S5	54.3±0.21f	24.5±0.15g	21.1±0.27d	7.0±0.03h	181.6±3.65h	10.5±0.19a	0.9±0.00ij	7.8±0.46f
S6	63.8±0.43d	21.5±0.32h	14.5±0.42f	7.5±0.00g	226.9±2.71e	2.6±0.11gh	0.9±0.03i	7.4±0.46fg
S7	60.3±0.32e	21.6±0.41h	17.9±0.41e	8.2±0.03bc	285.7±0.89b	4.2±0.30e	2.4±0.11cd	41.6±0.52a
S8	34.3±0.22g	35.6±0.66e	30.0±0.57e	7.5±0.07g	216.2±3.08f	3.2±0.11fg	2.6±0.05bc	21.0±1.21d
S9	23.9±0.36j	61.4±0.45a	14.6±0.16f	7.5±0.07g	242.1±0.97d	5.4±0.03d	2.1±0.04ef	5.7±0.23g
S10	77.8±0.91b	13.8±0.33j	8.3±0.58hi	8.3±0.01a	297.7±0.64a	4.1±0.03e	2.9±0.06a	42.6±0.75a
S11	30.5±0.31h	43.9±0.33c	25.4±0.40c	7.2±0.04h	144.8±1.32k	3.4±0.10f	2.3±0.11de	3.2±0.45h
S12	60.5±0.58e	27.2±0.57f	12.2±0.71g	7.9±0.02e	197.3±2.20g	3.6±0.13ef	2.7±0.09b	19.6±0.44d
S13	27.0±0.87i	44.2±0.74c	28.7±0.63b	7.7±0.10f	187.1±2.31h	5.4±0.30d	2.1±0.10f	01.8±0.50h
S14	34.9±0.38g	46.3±0.26b	18.6±0.49e	8.1±0.03cd	254.3±5.11c	2.5±0.05h	2.3±0.06de	11.3±0.58e
p-Value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Values represent the mean of four replicates ± SE (standard errors). Values of probability of one-way ANOVA. Within a column different letters denote significant difference ($P < 0.01$).

Statistical analysis showed that all chemical characteristic measures were significantly affected by site locality. Results showed that pH ranged from 7 to 8.3 in the top soils. As such, fifteen percent of the sites were rated as neutral to mildly alkaline with pH ranging from 7 to 7.9. The EC ranged from 143.2 to 297.7 µS cm^{-1} and was found higher, especially, in S10 and S14. In contrast, CE was very lower in S1 and S11. Soil $CaCO_3$ content across sites was low to medium, ranging from 1.8 to 42.6 % in top soils. Total-N in the different sites was evaluated as low to medium with values ranging from 0.7 to 2.9 g kg^{-1}. Olsen-P showed a large variation between sites which increased from 2.5 to 10.5 mg kg^{-1}. Av.P (Olsen-P) was lowest in S15, while the high Olsen-P content was observed in S6.

Biological properties of soils

Statistical analysis showed significant differences between all biological properties of soil samples. *TrPn*, *Pseudomonas* spp, *Bacillus* spp and ID-Foc varied significantly according to site locality (P<0.05). The ID-Foc varied between 3.25 × 10^3 and 29 × 10^3 Cfu g^{-1} soil. Seven fields showed a higher level of ID-Foc which varied from 12.25 × 10^3 to 29 ×10^3 Cfu g^{-1} soil. Based on the *Trichoderma* identification criteria, 439 *Trichoderma* isolates were found. *Trichoderma* isolates were mainly divided into eight species; *T. viride*, *T. harzianum*, *T. atroviride*, *T. virens*, *T. koningii*, *T. viridеisens*, *T. citrina*, *T. placentula* and T. *polysporum*. In the soil samples, *TrPn* varied between 3.75 ×10^3 to 13.75 × 10^3 Cfu g^{-1}soil (Figure 1a). Five fields showed a higher level of *TrPn*, varying from 13.25 to 13 × 10^3 Cfu g^{-1}soil. Based on biochemical, physiological and morphological properties, selected isolates were identified as *B. subtilis*, *B. circulans, B. lentus, B. aneurinilyticus, B. firmus and B. licheniformis.* Three species of *Pseudomonas* spp were identified including *P. aeruginosa, P. luteola* and *P. fluorescens*. Data analysis showed that *Pseudomonas* spp density varied between 1.4 to 14.88 ×10^7 Cfu g^{-1} soil (Figure 1b). Highest values were recorded in S1, S2 and S3. However, *Bacillus* spp density varied between 2.35×10^7 and 40.86 ×10^7 Cfu g^{-1} soil (Figure 1b).

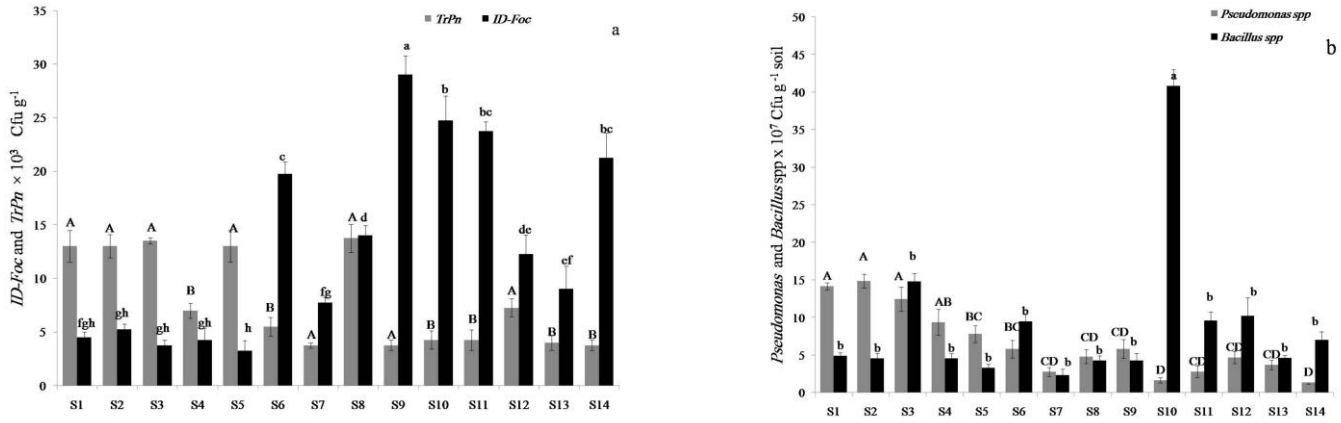

Figure 1. Biological properties of different soil from chickpea fields in Algeria a) ID- Foc and TrPn (expressed as Cfu g^{-1} soil); b) Total Pseudomonas spp and Bacillus spp communities. Data marked by different letters in a column indicate significant difference at P = 0.05 level according to Tukey test.

Disease assessment

Visual observation of symptoms in the field showed that contaminated plants exteriorize wilted or yellowed beaches (Figure 2a,b). The typical symptoms of wilting appeared mainly on the upper part of the leaves, then quickly gained the whole plant and finished with the death of the plants. In late attack, plants showed atypical symptoms of the disease where partial yellowing of the plants appeared at the lower part and then progressed to the upper part. It was found that the disease is widespread in all studied areas and his prevalence was 100%. The *Dis* values for each plot were presented in Figure 3. According to ANOVA, higher significant difference between D*is* values and soils locality was obtained (P<0.0001). The *Dis* revealed low to very high level with an average ranging between 2.05 in S1 to 39.83 in S10.

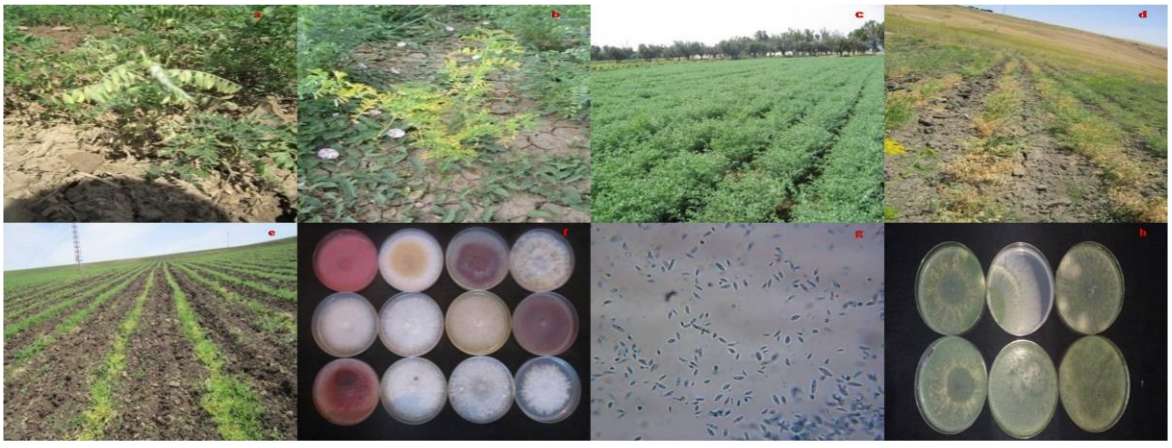

Figure 2. Symptoms of Fusarium wilt regarded in different fields (a- Typical symptoms b- atypical symptoms. Disease wilt regarded in different fields with different intensity (c, d and e). Morphotypical variation in Foc isolates obtained from wilted chickpea (f). Microscopic observation of Foc (g). Variation in Trichoderma isolates (h).

F. oxysporum and *F. solani* were most consistently isolated from stems showing symptoms of yellowing and wilting. Quantitative analysis of the fungi isolated from the stems effectively showed dominance of the *F. oxysporum* species with a rate of 95.14%. Nevertheless, a low occurrence was recorded for *F. solani* (4.86%). The selected Foc isolates (Figure 2f,g) obtained from the different wilted plants and soils completely expressed the symptoms of vascular wilt after inoculation of the latter on the susceptible variety ILC 482. Thus, isolates inoculated with this variety are certainly special forms *ciceri*, and constitute the isolates responsible for the vascular wilt of chickpea, noticed *in vivo*.

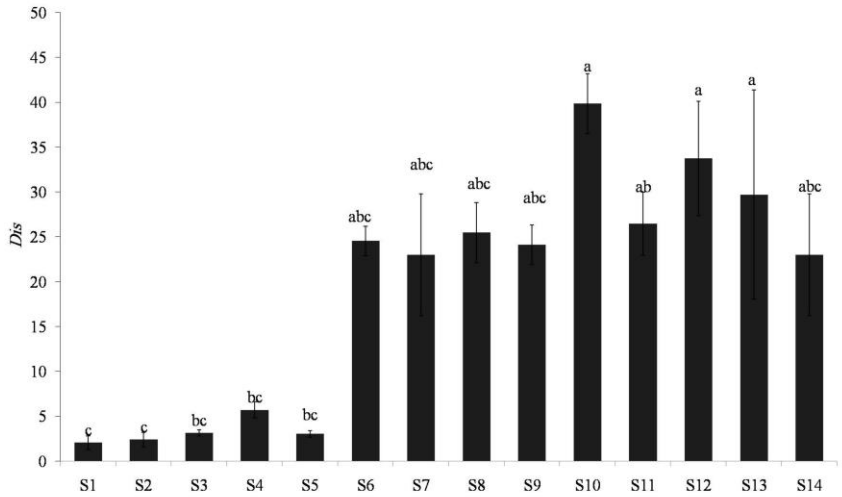

Figure 3. Evaluation of disease Index severity (Dis) of Fusarium wilts of chickpea in 14 investigated field during 2015 in North Algeria. Data were obtained in maturation point. Values represent the mean of four replicates ± SE (standard errors). Values of probability of one-way ANOVA (Site treatment).Data marked by different letters in a column indicate significant difference at $P < 0.0001$.

Relationship between wilt disease and soil properties

Relationship between soil physicochemical and biological properties and *Dis*

PCA plots represented the 14 experimental sites which were distributed normally according to 13 physicochemical and biological soil properties in relation to the disease index severity of wilt disease (Figure 4). The first and second ordination axis accounted for 41.19 and 28.96% of the total variance, respectively. Based on PCA analysis, Olsen-P, Total-N, ID-Foc, *Dis*, *TrPn* and *Pseudomonas* spp clustered together toward of the right side of the biplot, whereas loam, clay, sand, pH, EC, $CaCO_3$, and *Bacillus* spp were clustered at the opposite side. The D*is* was negatively correlated to Olsen-P, *TrPn, Pseudomonas* spp, and positively correlated to soil EC, ID-Foc and Total-N.

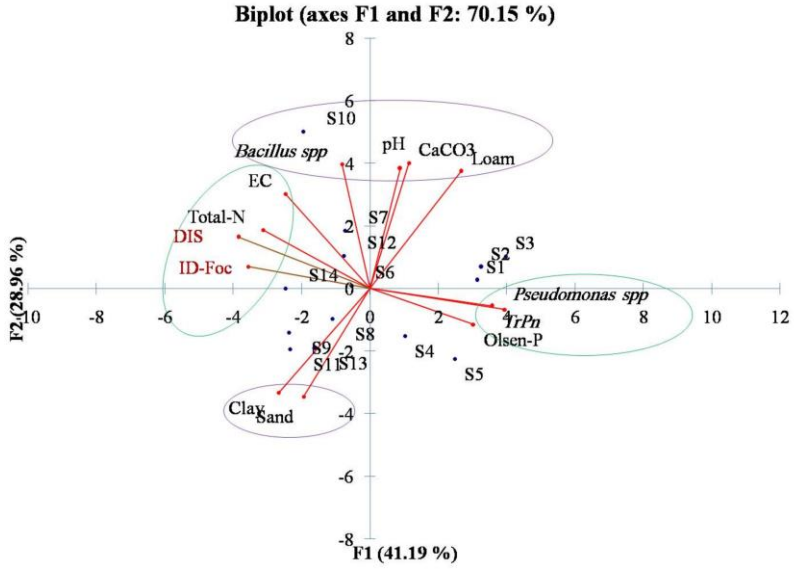

Figure 4. Principal component analysis of 14 chickpea fields: correlations among physicochemical (Laom, sand, Clay, olsen-P, Total-N, pH, CE) and biological characteristics (*Bacillus* spp, *Pseudomonas* spp, ID-Foc, *TrPn*) and D*is*. F1 accounted for 41.19% of the variance, and F2 accounted for 28.96%.

The Pearson correlation analysis (Table 2) of the total data from the 14 soil samples confirmed the results of PCA analysis and showed that D*is* was positively associated with EC, Total-N rate and ID-Foc, whereas negatively correlated with Olsen-P, *TrPn* and *Pseudomonas* spp. There was no correlation between D*is* and soil physical parameters (Loam, clay, and sand), chemical parameters (pH, $CaCO_3$ content) and biological factors (*Bacillus spp*). The analysis carried on EC showed a positive correlation with D*is* (r=0.62**). The increase in EC significantly increased D*is*, this was particularly evident in S7 and S8, where EC values were 285.72 and 216.28 µS cm^{-1}, respectively. While, low EC and D*is* values were recorded in S1 and S2. The studied soils showed a highly significant positive correlation between Total-N rate and D*is* (r= 0.79***). Accordingly, highest D*is* was observed in plots with a high concentration of Total-N particularly in S10 and S12. The results showed that Olsen-P deficiency increased significantly D*is* values, there was a negative relationship between Olsen-P and D*is*values (r = -0.67**). Analysis of S6 and S14 plots also showed lowest values of Olsen-P with values of 2.64 and 2.58 mg kg^{-1}, respectively. However, D*is* values were higher in the same sites. Pearson correlation indicated that D*is* was positively correlated with ID-Foc. Increased rate of ID-Foc increased significantly D*is*. The correlation analysis showed that *TrPn* were negatively correlated with D*is* (r=-0.70***). D*is* values decreased with the increase of *TrPn*. This was observed in S1and S2, while, D*is* values increased in S10 and S12 when the *TrPn* decreased. Analysis of bacterial outcomes, especially, *Pseudomonas* spp with Pearson correlation showed a negative correlation with D*is* (r=-0.89***). The results revealed highest concentrations of *Pseudomonas* spp in S2 and S3 while D*is* were lowest in same sites. In contrast, highest values of D*is* were observed both in S10 and S13 with lowest levels of *Pseudomonas* spp.

Relationship between soil physicochemical and biological parameters with ID-Foc

The correlation analysis showed that soil ID-Foc was significantly affected by Olsen-P, Total-N and *TrPn* (Table 2). Data showed that ID-Foc was positively correlated with the level of Total-N and negatively correlated with contents of Olsen-P. It was found that the rate of Total-N affected significantly ID-Foc in the soil, where a positive correlation was observed (r=0.56*). In fact, high levels of Total-N significantly increased ID-Foc. Inversely, the rate of Olsen-P was negatively correlated with ID-Foc (r=-0.58*). High level of Olsen-P significantly decreased ID-Foc. The latter significantly decreased in S6 and S14 when compared to S5 and S3. The results showed that *TrPn* were also negatively correlated with ID-Foc (r=- 0.65**). The concentration of ID-Foc decreased when *TrPn* increased. This was observed in S3 and S5 with high *TrPn* values. Otherwise, a negative correlation between ID-Foc and *Pseudomonas* spp (r =-0.65*) was recorded. These findings can be noticed in S1 and S2 with high rates of *Pseudomonas* spp.

Correlation of *TrPn* and *Pseudomonas* spp with soil characteristics

There was no correlation between *TrPn* and physical factors of soil (soil bulk), chemical factors (pH) and biological factors (*Bacillus spp*). Moreover, significant positive correlations were found between EC (r=-0.54*), Olsen-P (r=0.55*) and *TrPn*. The detailed summary of physical, chemical, and biological factors of the soils affecting *Pseudomonas* spp population was given in Table 2. The bacterial activity was negatively correlated with CE and Total-N. However, there was a positive correlation between *Pseudomonas* spp and *TrPn*.

Discussion

This study aimed to determine the impact of physicochemical and biological properties of soils on *Fusarium* wilt disease in chickpea growing in North-Algeria areas under commercial production conditions. A geographical variation in the occurrence of wilt chickpea was observed during the survey, with an important predominance of the disease in the investigated areas. The obtained result showed variation in disease levels within large geographical area which indicates that the soil physicochemical and biological proprieties affected significantly the ID-Foc and consequently the D*is*. The presence of Foc in the field can be irregular because of the nature of its dissemination and the variability of soil properties. Moreover, the variation of population size of Foc and D*is* in the studied fields might be attributed to variations in physicochemical and biological soils factors. The correlation analysis of 14 field's data showed that D*is* was positively correlated with EC, Total-N and ID-Foc, and negatively correlated with Olsen-P, *TrPn* and *Pseudomonas* sp. There was no correlation between D*is* and clay, loam, sand, pH, $CaCO_3$ and *Bacillus*.

The information regarding the effect of soil EC on Foc population and disease severity has been neglected. In our study, EC was positively correlated to D*is* and ID-Foc. This was probably due to the favorable environment for conidia germination and mycelium growth of Foc. Shim et al. (2002) reported a positive correlation between EC and germination rate of macroconidia and a consequent increase disease incidence of cucumber *Fusarium* wilt. In experimental conditions, results obtained by Naseri and Hamadani (2017) provided the importance of soil EC, as a population indicator for *F. oxysporum* in the soil under bean production conditions.

Table 2. Pearson correlations among Dis, soil physicochemical (Laom, Sand, Clay, Olsen-P, Total-N, pH, EC) and biological characteristics (*Bacillus* spp, *Pseudomonas* spp, ID-Foc, *TrPn*). The analysis is based on the total data set of 14 field plots analyzed during 2015.

	Loam	Clay	Sand	pH	EC	Olsen-P	N	$CaCO_3$	*Trichoderma spp*	*Bacillus spp*	*Psudomonas spp*	ID-Foc	*Dis*
Loam	1.00												
Clay	**-0.94*****	1.00											
Sand	**-0.83*****	**0.60*****	1.00										
pH	0.49^{ns}	-0.40^{ns}	-0.52^{ns}	1.00									
CE	0.05^{ns}	-0.01^{ns}	-0.10^{ns}	0.30^{ns}	1.00								
Olsen-P	0.26^{ns}	-0.26^{ns}	-0.19^{ns}	-0.14^{ns}	-0.37^{ns}	1.00							
Total-N	-0.22^{ns}	0.25^{ns}	0.11^{ns}	0.14^{ns}	0.52^{ns}	**-0.59***	1.00						
$CaCO_3$	**0.58***	**-0.59***	-0.40^{ns}	**0.78*****	0.35^{ns}	-0.05^{ns}	0.20^{ns}	1.00					
Trichoderma spp	0.43^{ns}	-0.48^{ns}	-0.23^{ns}	-0.03^{ns}	**-0.54***	**0.55***	-0.44^{ns}	0.15^{ns}	1.00				
Bacillus spp	0.47^{ns}	-0.42^{ns}	-0.43^{ns}	0.37^{ns}	0.43^{ns}	-0.13^{ns}	0.31^{ns}	**0.39***	-0.20^{ns}	1.00			
Pseudomonas spp	0.43^{ns}	-0.36^{ns}	-0.44^{ns}	0.19^{ns}	**-0.66****	**0.54***	**-0.65***	0.16^{ns}	**0.76*****	-0.30^{ns}	1.00		
ID-Foc	-0.35^{ns}	0.48^{ns}	0.03^{ns}	-0.20^{ns}	0.51^{ns}	**-0.59***	**0.56***	-0.27^{ns}	**-0.65***	0.40^{ns}	**-0.65***	1.00	
Dis	-0.25^{ns}	0.25^{ns}	0.20^{ns}	-0.04^{ns}	**0.62***	**-0.67****	**0.79*****	-0.07^{ns}	**-0.70****	0.46^{ns}	**-0.87*****	**0.72****	1.00

The table showed Pearson correlation coefficients and their level of significance. Pearson's correlation coefficients (r) are calculated by monowise comparison. Asterisks "*. ** and ***"denote significant difference at P< 0.05 and P < 0.01, respectively.

Nitrogen has been intensively studied in relation to host nutrition and disease severity because of its essential requirement for plant growth and its limited availability in soil (Ghorbani et al., 2008). The availability of N probably increased greatly the sporulation and mycelial growth of Foc isolates. Most studies on the effect of soil N on fungus sporulation have reported that high N content in the soil enhances sporulation of telluric pathogenic plant (Hoffland et al., 2000). Otherwise, abundant N enhanced succulent growth, prolonged vegetative period, and delayed maturity of the plant, which increased the period of susceptibility to pathogens (Ghorbani et al., 2008). In the present study, a positive correlation was obtained between Total-N, ID-Foc and *Dis*. A similar result was obtained by Sugha et al. (1994) who reported that the increase of N rate favored the frequency of wilt disease.

There are many researchers associating the level of soil P to the crop disease development. Ghorbani et al. (2008) reveled that subsequent careful monitoring and management of available P and its equilibrium with other nutrients could be considered as an important strategy for crop disease control. In the present study, a negative correlation was obtained between Olsen-P, ID-Foc and *Dis* of wilt disease in chickpea. These results could probably be explained by the indirectly role of Olsen-P in the inhibition of conidial germination and mycelial growth of Foc by enhancing biological control agents. Our results showed that Olsen-P was positively correlated with *Pseudomonas* spp. This is in agreement with the observations reached by Postma et al. (2013) who found that high P availability improved by *Pseudomonas chlororaphi* were able to control *P. aphanidermatum* and *F. oxysporum* f. sp. *radicis-lycopersici* in tomato plants. Prabhu et al. (2007) reported that improved root development by P nutrition may induce the plant to 'escape' attack by soil-borne fungal pathogens.

In our result a negative correlation between ID-Foc, D*is* and *TrPn* was observed, suggesting that *Trichoderma* species participate in the process of natural disease management. Indeed, Species of *Trichoderma* spp are probably limited sporulation and growth of Foc via various mechanisms such as hyperparasitism, antibiosis and induction of host resistance or through a combination of such mechanisms (Dubey et al., 2007). Otherwise, mineral nutrition is indispensable for growth and, within a narrower range, stimulatory of fungal secondary metabolism (Griffin, 1994). In this study, a positive relationship was recorded between Olsen-P and *TrPn*. The highest rate of Olsen-P in soil represents a positive factor for the growth and the antagonistic activity against Foc. This is in accordance with previous findings reporting that *Trichoderma* species increasing significantly the concentration of soluble phosphate (Saravanakumar et al., 2013).

Pseudomonas spp has habitually been showed to be responsible for the natural suppression of *Fusarium* wilt disease (Mazzola, 2002). In the present study, *Pseudomonas* spp strains were negatively correlated with D*is*, and contribute to the disease suppression of chickpea *Fusarium* wilt disease. The mechanism of action would be through direct antagonism such as production of bioactive metabolites, rapid exploitation of root exudates, colonization and multiplication in the environment and aggressive antagonism with other microbes (Thomashow and Weller, 1988). Abed et al. (2016) tested *Pseudomonas* spp for their antagonism ability against Foc *in vitro* conditions. The results showed a great variability in inhibiting mycelial growth of Foc isolates. The ability of bacterial *Pseudomonas* spp strains varied in terms of production of protease, gelatinase, amylase, cellulase, AIA, lipase, catalase and cyanid Hydrogen. The results obtained by Saikia et al. (2009) demonstrated that the strain of *Pseudomonas* controlled the severity of wilt disease of chickpea by systemically inducing resistance against *Fusarium* wilt of chickpea and decreased the disease severity up to 26–50% as compared to control.

The results obtained in this study showed a positive correlation between *TrPn* and *Pseudomonas* spp strains and both were negatively correlated with ID-Foc and D*is*. In a controlled experiment Liu et al. (2008) demonstrated that the incidence of Southern blight of tomato decreased while the *TrPn* and *Pseudomonas* spp increased.

Conclusion

In conclusion, the results from this study confirmed a great influence of soil physicochemical and biological characteristics in the occurrence and severity of the epidemic *Fusarium* wilt of chickpea. These results contribute in order to develop more efficient management strategies and exploitation of soil nutrients that might be indispensable in the context of an integrated pest management strategy and may even contribute significantly to reduce the abusive fungicides and synthetic fertilizers utilization.

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