EFFECT OF SOIL NUTRIENT AND BIOTA DYNAMICS ON WILT DISEASE SEVERITY IN CHICKPEA

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A B S T R A C T

The present study was performed to investigate dynamic and combined effects of soil nutrients (nitrogen and phosphorus) and biotic factors on disease severity of *Fusarium* wilt in chickpea (AUDPC). Chickpeas were harvested, 180 days after onset of experiment, from eight natural infested soils during two years (2016 and 2017) and assessed for rhizospheric nitrogen (N-Total$_R$) and phosphorus availability (Olsen-P) in relation to *Fusarium oxysporum* f.sp *ciceris* inoculum density (ID-Foc), *Trichoderma* spp, and *Pseudomonas* spp as well as nitrogen (N-Total$_{PL}$) and phosphorus (P-Total$_{PL}$) plants uptake. The results showed that high level of ID-Foc significantly increased AUDPC values. It was found that ID-Foc was negatively correlated with Olsen-P and positively correlated with N-Total$_R$. Accordingly, AUDPC values were positively correlated with high concentrations of N-Total$_R$ ($r=0.77**$ in 2016, $r=0.94***$ in 2017) while negatively correlated with high concentrations of Olsen-P ($r=-0.73**$ in 2016, $r=-0.79**$ in 2017). In addition, AUDPC values showed negative interactions with N-Total$_{PL}$ ($r=-0.80**$ in 2016, $r=-0.78**$ in 2017), whereas positive interactions with P-Total$_{PL}$ ($r=0.80**$ in 2016, $r=0.87**$ in 2017). Moreover, AUDPC values were negatively associated with *Pseudomonas* spp and nodosity during the two years of study as with *Trichoderma* spp in 2017. The results demonstrated that wilt disease severity was strongly associated with nutrients and biological soil properties. It can be assumed that soils with highest Olsen-P levels, *Trichoderma* spp and *Pseudomonas* spp can help *Fusarium* wilt suppression.

Keywords: Chickpea; *Fusarium oxysporum* f.sp *ciceris*; *Trichoderma* spp; *Pseudomonas* spp*;* Phosphorus; Nitrogen.

INTRODUCTION

Fusarium wilt, caused by soil-borne fungus *Fusarium oxysporum* f. sp. *ciceris* (Foc), appears to be the most devastating diseases of chickpea throughout the world, particularly, in North Africa (Reddy *et al.,* 1980). The yield losses caused by this soilborne fungus have been estimated to 10 and 15% worldwide. Total losses may be increased by severe wilt epidemics under some environmental conditions (Navas-Cortes *et al.,* 2000).

Infection of susceptible host is also possible when survival chlamydospores grown in soil or embedded in plant tissue. This primary inoculum of *Fusarium* wilt in chickpea can survive on crop residues for more than 6 years in the absence of susceptible host (Haware *et al*., 1990; Jimenez-Díaz *et al*., 2015).

Soil is a fundamental medium for crop growth in all production systems. The productivity of any system depends, to a large extent, on soil characteristics such as nutrients supply and structural characteristics that affect rooting (Ghorbani *et al*., 2008). Soil also hosts a complex fauna and microbial population involved in many different biological processes, which also affects its physical and chemical properties, and ultimately the productivity of agricultural ecosystems.

Nitrogen and phosphorus are among the main nutrients for plant structure and function (Hu and Schmidhalter, 2005). Nitrogen is a key constituent of proteins and plays an important role in different enzymatic activities of plants, whereas phosphorus is involved in the energy transfer to the cells (ATP and NADPH). It is well established that nitrogen and phosphorus availability can affect the resistance of plants to pathogens (Marschner, 1995). These major constituents (N and P) afforded important roles in metabolic pathways that lead to the production of lignin, phenols, phytoalexins, and other defense-related molecules (Elmer and Datnoff, 2014). Despite the fact that N and P are among the most vital nutrients for plant, there are many reports demonstrating the effects of N and P in the development of plant disease, but still the mechanism of pathogenesis poorly understood (Hoffland *et al.*, 2000). One of the most drawbacks within these studies is that factors (N, P, oligo-elements, etc.) are often evaluated individually on culture medium, making difficult to account the interactions between these nutrients. Therefore, a proper understanding of soil physicochemical and biological characteristics and adequate interpretation of their interactions is required for a better improvement of agricultural productivity. Hence, the main objectives of this study were to evaluate the combined effects of different soil nutrients (N-Total$_R$, Olsen-P) and biotic factors (ID-Foc, *Trichoderma spp*, and *Pseudomonas* spp) on wilt disease severity in natural soils and controlled conditions during 2 years to predict the effects of the studied variables on the epidemic occurrence of *Fusarium* wilt.

MATERIALS AND METHODS

Study sites and soil samples: Eight soil samples were collected from different regions in North Algeria (Skikda, Constantine, M'ila, and Guelma). Each region is represented by two sites. A total of 100 kg of soil were collected from each site at a depth of 0 to 25 cm and were stored in closed plastic bags.

Physicochemical and biological analysis of soil: Initial soil samples were air-dried and ground into 2-mm particles for physicochemical and biological analysis. The particle size distribution was studied according to pipette method. The pH of the different soils was determined in water using a conventional pH meter (1/2.5, w/w). Calcareous content (CaCO$_3$)

was assessed using the Bernard calcimeter method. Total nitrogen (N-Total) was assessed by the Kjeldahl method (Lambert, 1977). Available phosphorus (Olsen-P) was determined by the method of Olsen *et al.,* (1954).

The microbial populations of each soil sample were examined using a serial-dilution method as described previously (Rapilly, 1968). Selective Komada's and rose Bengal culture media were used to isolate *Fusarium oxysporum* and *Trichoderma* spp, respectively. Ten grams of soil were suspended in 90 ml of sterile distilled water and shaken at 200 rpm for 30 min. Successive dilutions of 10^{-2} and 10^{-3} were made from the initial suspension. Aliquots of 10 ml from each dilution were transferred into 90ml culture medium. The prepared suspensions were poured into Petri dishes and incubated at 25°C in the dark for 5 to 14 days. Analyses were performed by counting the number of colonies per Petri dish. Results were expressed as colony-forming units per gram of dry soil (Cfu g^{-1} dry soil). Microscopic examination was used to identify *Fusarium* spp by evaluating the morphology of their spore structures and specific identification keys established by Messiaen and Cassini, (1968). *Trichoderma* spp colonies were identified based on visual macroscopic and microscopic observations according to Gams and Bissett, (2002).

Regarding *Pseudomonas* spp isolation, 10 g of each soil were suspended in 90 ml of sterile distilled water, shaken and heated at 45°C for 5 min. Aliquots of 10 ml from each dilution were transferred into 90ml of cetrimide *Pseudomonas* spp selective medium. The prepared suspensions were poured into Petri dishes and incubated at 37°C for 24 to 48 h. Each colony of *Pseudomonas* spp was isolated and identified based on morphology and total density per sample (colony-forming units g^{-1} soil). All experiments were carried out in four replicates.

Experimental set-up, disease assessment, and pathogenicity test: The pot experiment was conducted in four replications during the period of December to May 2016 and 2017 at the greenhouse of El Hammadia, Bordj Bou Arreridj, Algeria (attitude 35.979 N and longitude 4.7477780 E).

Chickpea seed cultivars (Ghab 5) were surface sterilized using sodium hypochlorite (2%) for 3 min, rinsed three times with sterile distilled water and

germinated (10 seed/ disc) at 25±3°C during 8 days in Petri dishes containing two sterilized filter paper. Chickpea seedlings were carefully transferred into soil samples at the rate of five seeds per pot. Seedlings were grown under greenhouse conditions (25°C and 70% humidity) and irrigated with sterile water as needed. The experiment was achieved 180 days after sowing.

The severity of *Fusarium* wilt was assessed 8-day intervals after the appearance of symptoms using the technique described by Trapero-Casas and Jimenez-Dıaz, (1985). Disease incidence (DI) was assessed by counting the number of plants showing symptoms. Severity of *Fusarium* wilt was assessed on a scale of 0 to 4 according to the percentage of foliage with yellowing (0 = 0%, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%, and 4 = dead plant). Incidence symptoms (0 or 1) and severity data (rated from 0 to 4) were used to calculate disease index intensity (Dis) using the equation Dis = (I×S)/4. Additionally, for each site, the area under disease progress curve (AUDPC) was estimated by the following formula:

$$AUDPC = \sum_{i+1}^{n}[(x_i + x_{i+1})/2](t_{i+1} - t_i)$$

The pathogenicity tests were performed according to pot screening procedure as described by Nene and Haware, (1980). Briefly, the special forme *ciceris* was determined by inoculation of a variety of chickpea ILC482, highly susceptible to *Fusarium* wilt. Inoculum was prepared from a single-spore culture of *Fusarium oxysporum*. After 20 days of culture at 25 °C, the inoculum was thoroughly mixed in the pots and five seeds of susceptible cultivars were sown in each pot.

Plant harvest and rhizosphere soil sampling: During the harvest period, at the full productive stage, shoots of chickpea were separated from roots at the cotyledon node. Rhizosphere soil which corresponds to the fraction of soil adhering to roots was carefully separated from roots, collected and stored in a refrigerator at 4°C for more than three days prior to analysis.

Assessment of Olsen-P, N-Total$_R$, ID-Foc, *Trichoderma spp* and *Pseudomonas* spp: Nitrogen concentration in rhizosphere (N-Total$_R$) was determined by Kjeldahl technique. Soil phosphorus (Olsen-P) availability was assessed as described previously (Olsen *et al.,* 1954). To evaluate the

dynamic of rhizospheric soil in terms of fungal and bacterial communities, ID-Foc, *Trichoderma spp*, and *Pseudomonas* spp were determined using the technique of Rapilly, (1968), as described above.

Assessment of SWD, Yg, and N-Total$_{PL}$ and P-Total$_{PL}$ shoots uptake: At maturity stage of plant, grain yield (Yg) was determined in the different studied sites. Yg was determined as dry weight after drying for 48 h at 70°C. Nodules per plant were separated from roots, dried (48 h at 70°C) and weighed. N-Total$_{PL}$ was determined by the green malachite method as described by Valizadeh *et al.,* (2003). P-Total$_{PL}$ was calculated as the amount of total phosphorus taken up by the plant (Betencourt *et al.,* 2012).

STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) with post-hoc Tukey test was used to evaluate differences between soil samples. Significant difference was determined by Tukey's multiple comparison tests at P<0.05. Soil physicochemical and biological properties and AUDPC of *Fusarium* wilt were also calculated using the Pearson correlations. Statistical analyses were done using the software package STATISTICA 8.

RESULTS

Soil physicochemical and biological characteristics: Physico-chemical and biological characteristics of the eight experimental sites were given in Table 1. Physical parameters in terms of sand, loam and clay proportion showed highly significant differences between the different sites (P<0.001). Particle size distribution varied significantly among the studied sites. The highest values of clay (36.02%), loam (64.37%) and sand (13.63%) were recorded in S6, S8, and S4, respectively. In contrast, S7 was characterized by a lowest value of clay (23.49%) and loam (53.82%), while S5 showed the lowest value of sand (9.37%).

Statistical analysis showed highly significant differences in soil pH, CE, CaCO$_3$ content, Olsen-P and N-total (P<0.001). In general, CaCO$_3$ ranged from 5.37 to 25.95 % as a result most of the studied soils were calcareous. pH ranged from 7.05 to 8.3 in the different sites. Soil organic matter content ranged from 1.17% to 1.73%, with highest values in S2 and S6. N-Total was rated as low to medium with values ranging from 0.82 to 1.46 g kg^{-1}. P-Total varied between 177.15 and 251.8 mg kg^{-1} in S4 and S7, respectively. Olsen-P

showed a large variation among the studied sites, the values increased from 18.6 to 25.08 mg Kg^{-1}. Olsen-P was lowest in S4 and showed higher value in S7.

Statistical analysis showed that *Trichoderma spp.*, ID-*Foc* and *Pseudomonas* spp were significantly affected by site locality (P<0.05). ID- Foc varied between $6×10^3$ and $28.67×10^3$ Cfu g^{-1} soil. The highest levels were recorded in S1, S2 and S4. In the present study five species of *Trichoderma* spp were identified; *T. viride*, *T. harzianum*, *T. atroviride*, *T. virens*, *T. polysporum*. The studied soils showed that *Trichoderma* spp ranged

between $5×10^3$ to $26.33×10^3$ Cfu g^{-1} soil and were primarily concentrated in S7 and S8.

Morphological observations and biochemical tests revealed three species of *Pseudomonas* spp; *P. aeruginosa*, *P. luteola*, and *P. fluorescens*, ranging between $60.67×10^9$ to $228×10^9$ Cfu g^{-1} with highest levels in S3, S7, and S8.

Disease assessment and pathogenicity of Fusarium oxysporum: In the present study, we noticed atypical symptoms of the disease, where first partial yellowing begun from the basal parts and progressed to the upper part of the plants. In the case of a very severe disease, we recorded

intensive defoliation, weak flowering or broad spread of sterile flowers, fewer pods, small pods or empty pods. However, in the same case, severely affected plants showed early maturation compared to the less affected plants. Longitudinal or transversal sections of the vascular system showed a large discoloration. This phenomenon subsequently leads to the death of plants. *Fusarium oxysporum* isolates, obtained from wilted plants and soil samples inoculated on susceptible variety ILC 482, would be the special form *ciceris* responsible for the vascular wilt of chickpea, observed in the greenhouse experiment.

Table 1. Physicochemical and biological characteristics of the studied sites.

	Clay (%) **	Loam (%) ***	Sand (%) ***	pH **	CaCO$_3$ (%) **	Organic matter (%) ***	N-Total g Kg^{-1} ***	Olsen-P mg Kg^{-1} ***	P-Total (mg kg^{-1}) **	ID-Foc ** (10^3 Cfu g^{-1})	*Trichoderma* spp *** (10^3 Cfu g^{-1})	*Pseudomonas spp* *** (10^9 Cfu g^{-1})
S1	25.43±0.08c	62.33±0.13b	12.25±0.16ab	8.3±0.00a	8.25±0.28f	1.58±0.12ab	1.24±0.03bc	21.06±0.49bc	184.49±1.58de	28.67±8.65a	13.33±0.88cd	60.67±8.69d
S2	26.71±0.02b	62.43±0.85b	10.86±0.85bc	8.02±0.02b	5.37±0.20g	1.69±0.03d	1.34±0.02b	19.09±0.24cd	226.68±3.19b	22.33±1.86ab	8±1.73cd	79±3.46cd
S3	26.32±0.28b	63.78±0.25ab	9.84±0.30c	7.63±0.01c	23.51±0.35c	1.53±0.05abc	0.82±0.01d	19.36±0.86cd	203.17±2.29c	08±0.58b	15.67±1.20bc	153.33±16.83abc
S4	23.84±0.34e	62.53±0.24ab	13.63±0.37a	7.38±0.02d	4.87±0.45g	1.17±0.02c	1.18±0.03c	18.60±0.22d	177.15±0.46e	12±1.15ab	12.67±0.33cd	102.67±18.81bcd
S5	26.185±0.06b	64.09±0.22ab	9.73±0.19c	7.24±0.03e	23.62±0.65b	1.39±0.03abc	1.46±0.03a	20.44±0.47bcd	188.69±0.47d	6.67±2.73b	8±0.58cd	72±10.15cd
S6	23.49±0.31e	63.30±0.518ab	13.13±0.82a	7.27±0.00e	19.92±0.57d	1.73±0.13a	1.23±0.02bc	21.89±0.26b	202.16±2.59c	06±1.53b	5±0.59d	150.67±10.15abc
S7	36.02±0.10a	53.82±0.32c	10.16±0.24c	7.05±0.01f	15.16±0.43e	1.31±0.14bc	0.83±0.01d	25.08±0.23a	251.80±0.71a	7.33±2.85b	26.33±0.38a	228±38.57a
S8	24.58±0.22d	64.37±0.21a	11.06+0.24bc	7.20±0.05e	25.95±0.63a	1.34±0.07abc	0.83±0.03d	20.04±0.21bcd	177.18±0.48e	8.67±1.20b	23±2.52ab	173.33±11.85ab
p	<0.001	<0.000000	<0.000008	<0.001	<0.001	<0.000001	<0.000001	<0.000001	<0.001	<0.001	<0.000001	<0.00004

Values represent the mean of 4 replicates ±SE (standard errors);**, *** indicate that the differences were significant at P < 0.01 and P < 0.001, respectively.

According to ANOVA, AUDPC values showed a very highly significant effect depending on the experimental sites (p ≤0.001). Data displayed in Figure 1 showed that AUDPC values were significantly higher in S1 (4081) and S2 (4098.5) during the first season (2016), as well as in S5 (3489.5) and S6 (4009.5) during the second season (2017). The lowest values were recorded in S7 (1372) and S8 (1229) within 2016, and in S3 (1372) and S8 (1535) within 2017.
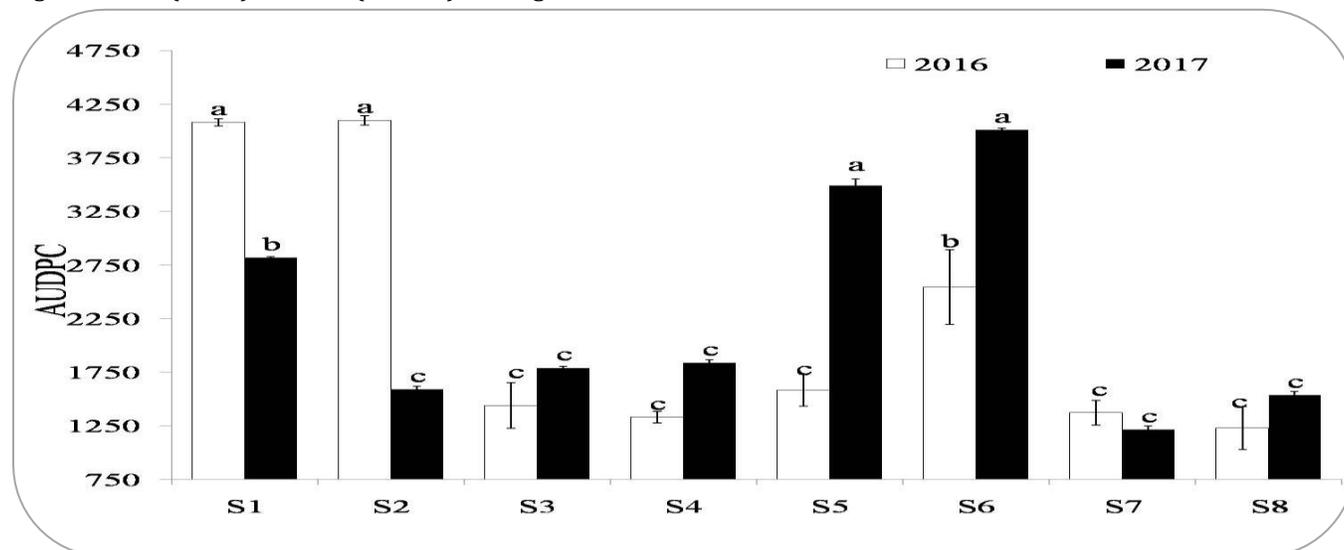
Figure 1. Evaluation of the disease severity (AUDPC) of *Fusarium* wilt. Values represent the mean of four replicates ± SE (standard errors). Values of the probability of one-way ANOVA (Site treatment) within a column with different letters denote a significant difference at P < 0.0001.

Relationship between wilt disease severity and soil properties: PCA plots represented the eight experimental sites which were distributed according to the 10 physicochemical and biological soil properties in relation to the disease severity (Figure 2). In 2016, the first and second ordination axis accounted for 81.15% and 10.19% of the total variance, while in 2017 this accounted for 73.24% and 11% of the total variance, respectively. Based on the PCA analysis, AUDPC values were negatively correlated with Olsen-P, nodosity, N-Total$_{PL}$, *Trichoderma* spp and *Pseudomonas* spp, and positively correlated with ID-Foc, N-Total$_R$, P-Total$_{PL}$, SDWt and Yg.
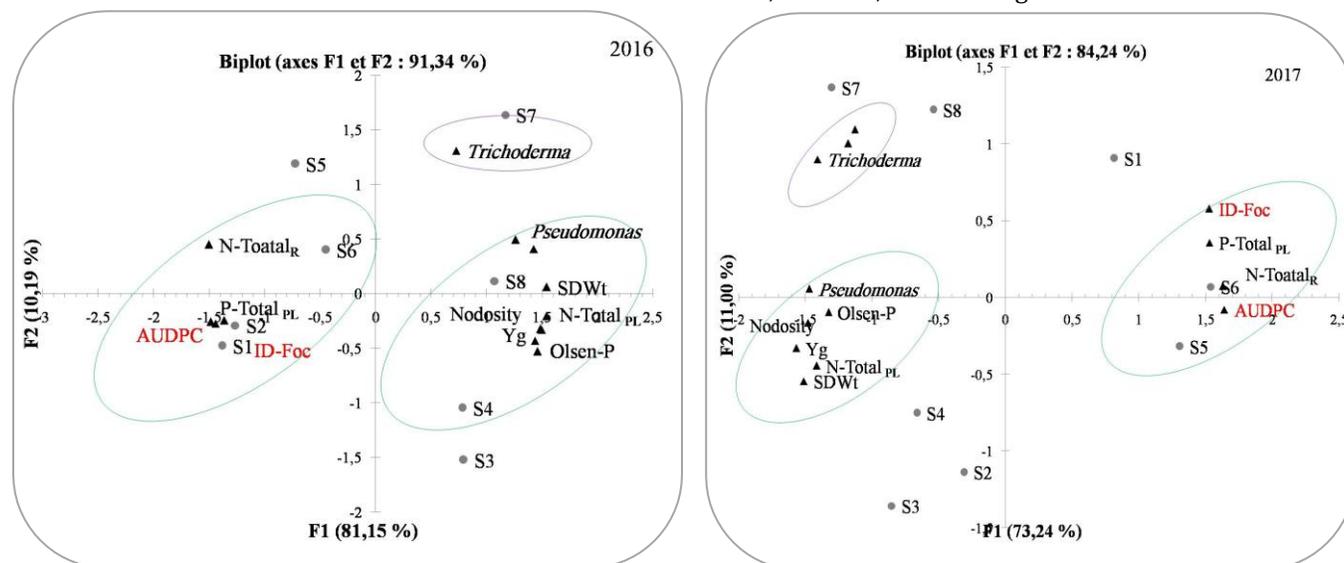
Figure 2. Bioplot of principal component analysis (PCA) of chickpea samples according to physicochemical and biological characteristics and AUDPC. 2016 (F1 accounted for 81.15% of the variance, and F2 accounted for 11%), 2017 (F1 accounted for 73.24% of the variance, and F2 accounted for 10.19%).

Variation of SDWt, Yg, and nodosity in relation to AUDPC: In this study, Yg, SDWt and nodosity varied significantly according to the studied sites (p<0.0001) (Table 2).

Table 2: Effect of wilt disease on SDWt, Yg, and nodosity in chickpea.

	2016			2017		
	SDWt (g plant[-1]) ***	Yg (g plant[-1]) ***	Nodosity (mg plant[-1]) ***	SDWt (g plant[-1]) ***	Yg (g plant[-1]) ***	Nodosity (mg plant[-1]) ***
S1	0.79±0.08c	0.34+0.20c	1.73±1.01b	1.35+0.08bcd	1.88+0.80cd	24.33±0.52c
S2	0.96±0.10c	0.03+0.02c	1.50±0.87b	2.88+0.47a	2.33+0.20bc	30.20±3.43bc
S3	2.11±0.10ab	3.38+0.14a	46.25±4.93a	2.91+0.39a	4.95+0.71a	159.61±2.99a
S4	1.94±0.10ab	3.28+0.17ab	48.40±2.87a	3.26+0.26a	4.35+0.21ab	130.87±17.71a
S5	1.29±0.47bc	0.35+0.21c	3.83±2.29b	0.33+0.10d	1.90+0.20cd	0.00+0.00c
S6	1.62±0.20abc	0.77+0.22c	12.93±3.24b	0.75+0.14cd	0.13+0.13c	0.00+0.00c
S7	2.21+0.01a	3.27+0.12ab	51.08±3.08a	1.96+0.19abc	5.05+0.29a	152.67±15.77a
S8	2.28+0.02a	2.61+0.10b	49.80±3.07a	2.56+0.33ab	3.43+0.41abc	56.98±15.77b
p	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001

Values represent the mean of 4 replicates ± SE (standard errors);**, *** indicate that the differences were significant at P < 0.01 and P < 0.001, respectively.

The results showed that SDWt varied between 0.79 g and 2.28 g during 2016. Chickpea cultivars were higher in S8 (2.28 g plant[-1]) and S7 (2.21 g plant[-1]), when compared to S1 (0.79 g plant[-1]) and S2 (0.96 g plant[-1]). During 2017, a significant increase in SDWt was observed which ranged from 12.28% to 70.8%. Yg showed a significant decrease from 2016 to 2017 (from 23.9 to 98.71%). The highest Yg was found in S3 (3.38 mg.plant[-1]) and S4 (3.28 mg plant[-1]) during 2016, whereas, in S8 (5.05 mg plant[-1]) and S6 (4.95 mg plant[-1]) during 2017. The lowest Yg was found in S1 (0.79 mg plant[-1]) and S2 (0.96 mg plant[-1]) within 2016, and in S1 (1.88 mg plant[-1]) and S6 (0.13g plant[-1]) within 2017. Nodosity varied between 1.5 to 51.08 mg plant[-1] during 2016. S1 (1.73 mg plant[-1]) and S2 (1.50 mg plant[-1]) showed the lowest values compared to S7 (51.08 mg plant[-1]) and S8 (49.80 mg plant[-1]). In 2017, a significant increase in nodosity was recorded which evolved from 12.6 to 95.03%. When investigating the relationship between Yg, SDWt, and AUDPC values, significant negative correlations were established (Figure 3a, b, c, d). In terms of Yg, the strong correlation of determination (COD) was recorded in 2017 ($r^2 = 0.73$) compared to 2016 ($r^2 = 0.59$), while for SDWt, the COD was higher in 2017 ($r^2 = 0.69$).

Significant negative correlations were also found between nodosity and AUDPC values which are represented by strong COD in 2016 ($r^2 = 0.62$) and 2017 ($r^2 = 0.52$) (Figure. 3e, f). Our results suggest that an increase in nodosity is associated with a decrease in AUDPC levels.
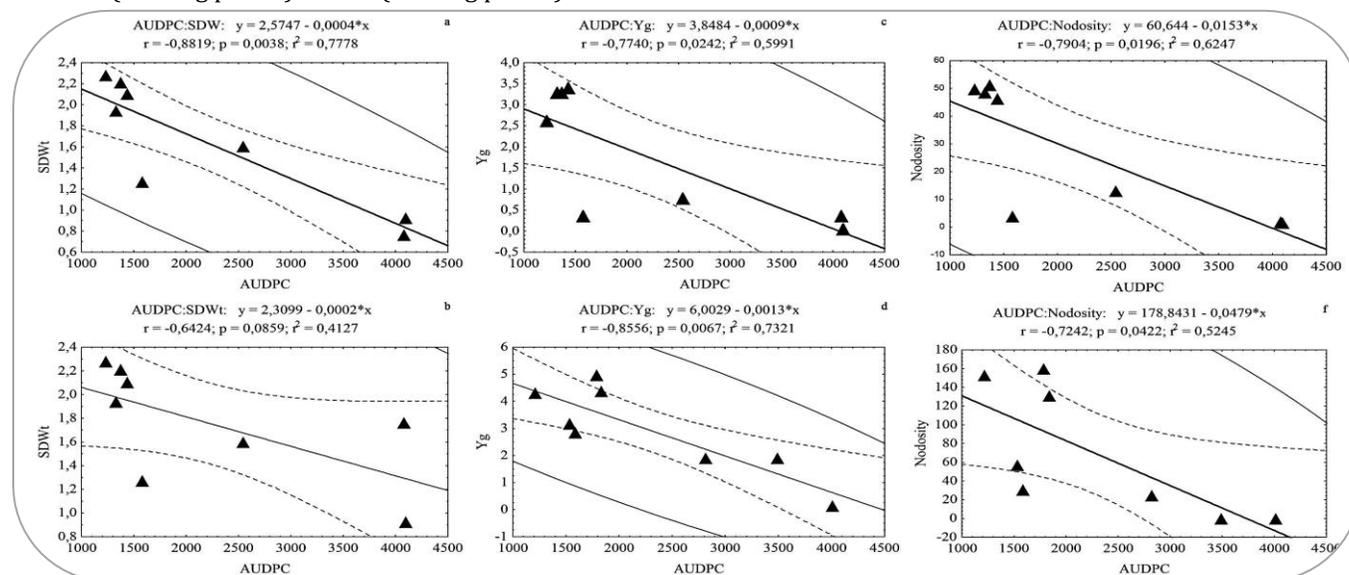
Figure 3. Correlation between SDWt, Yg, nodosity, and AUDPC values. Pearson's linear correlation coefficients (*r*), coefficients of determination (*r*²) and *P* values are given in each plot. Values are the means of four replicates of plant cultivars collected at the full productive stage.

Variation of N-Total$_R$, N-Total$_{PL}$ in relation to AUDPC: The concentration of N-Total$_R$ and N-Total$_{PL}$ were significantly affected by site locality (p <0.05) (Figure 4a, b). In addition, a very important variability was observed among the studied sites according to the season of study.
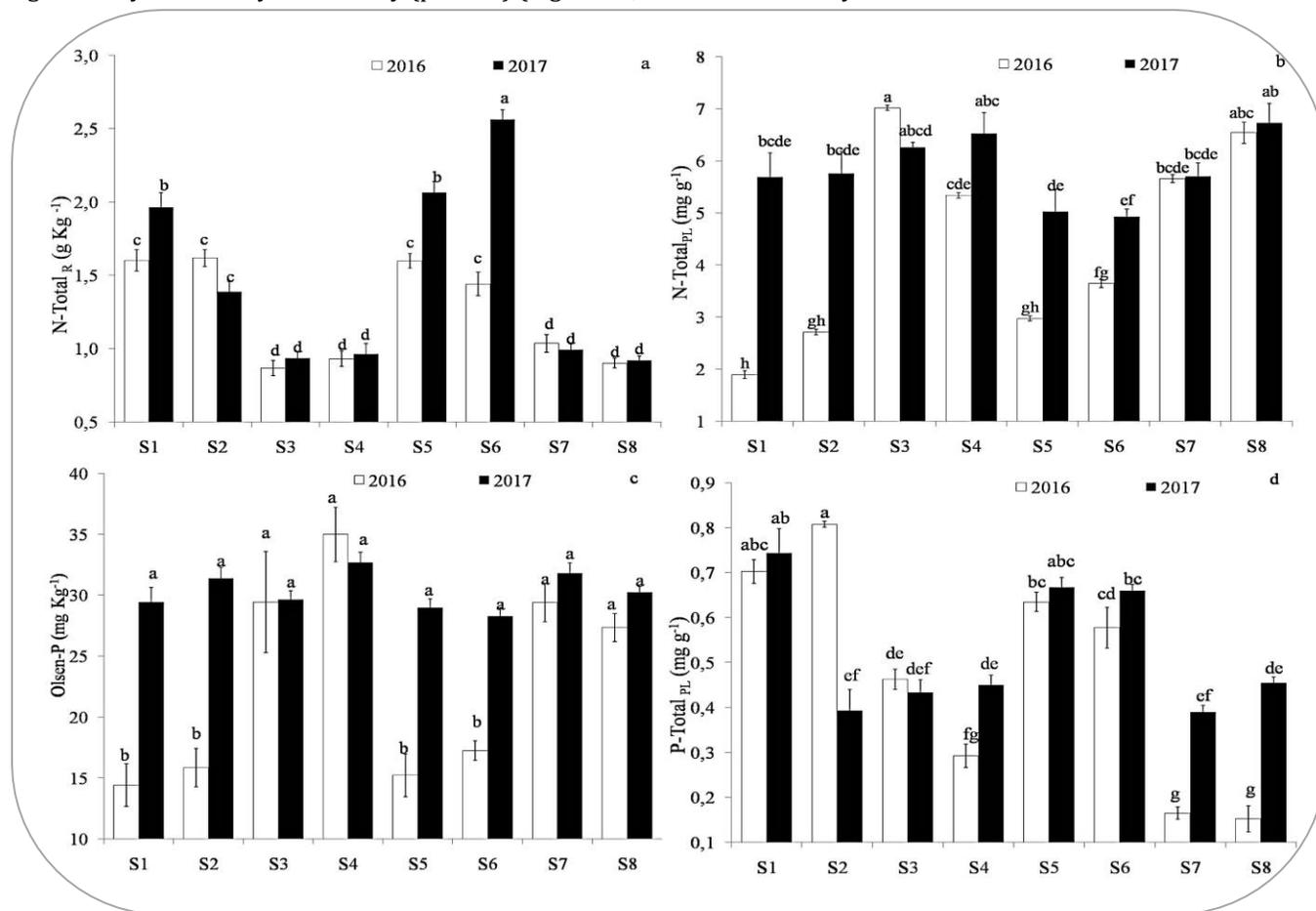
Figure 4. Variation of N-Total$_R$ (a), N-Total$_{PL}$ (b), Olsen-P (c), and P-Total$_{PL}$ (d) in rhizosphere and shoots of chickpea. Values correspond to the mean calculated of five replicates. Letters show significant differences between cropping systems (P<0.05).

A clear increase of N-Total$_R$ was observed in the majority of the studied sites with levels ranging from 5.57 to 28.82% during 2016. An exception was noticed for S4 with 20.88% of N-Total$_R$ decrease. As well, a significant increase of N-Total$_R$ was recorded in S1, S2, S5 and S6 during 2017.

Figure 4b showed a significant variability of N-Total$_{PL}$ plants uptake between the different sites. N-Total$_{PL}$ was found to be highest in S3 (7.02 mg plant^{-1}) and S8 (6.54 mg.plant^{-1}), and very low in S1 and S2 during 2016. The levels of N-Total$_{PL}$ uptake significantly increased in plants from S1, S2, S6, and S7 during 2017 to reach 52.9%.

A positive relationship was found between N-Total$_R$ and AUDPC with higher COD in 2017 (r^2 = 0.89) (Figure 5a, b). However, AUDPC was negatively correlated with N-Total$_{PL}$ (Figure 5c, d).

Variation in Olsen-P and P-Total$_{PL}$ in relation to AUDPC: According to the ANOVA, the studied sites showed a significantly variation of Olsen-P concentration within the soils during the two years of experimentation (p ≤0.001) (Figure 4c, d). A significant increase in Olsen-P was found in S3, S4, S7 and S8 with values ranging from 14.69 to 46.82% during 2016, unlike S1, S2, S5 and S6 which showed a significant decrease in Olsen-P (from 16.98 to 31.5%). During 2017, a clear increase of Olsen-P was noticed in the majority of the studied sites with values ranging from 4 to 35.97%.

Regarding P-Total$_{PL}$, the results showed also an increase in terms of P-Total$_{PL}$ plants uptake in the different sites during 2017. The highest concentrations of P-Total$_{PL}$ uptake were recorded in

chickpea cultivars from S1 (0.7 mg plant^{-1}) and S2 (0.81 mg plant^{-1}) during 2016, and in shoot chickpeas from S1 (0.74 mg plant^{-1}) and S6 (0.67 mg plant^{-1}) during 2017. The lowest levels of P-Total$_{PL}$ were found in shoot chickpeas from S7 and S8 during 2016 as well as S3 and S7 during 2017.

As such, in 2017 a negative relationship was found between AUDPC and Olsen-P values (Figure 5 e, f). Here, COD was higher (r^2 = 0.62). In contrast, a significant positive correlation was observed between P-Total$_{PL}$ and AUDPC values with a higher COD (r^2 = 0.76) (Figure 5 g, h).
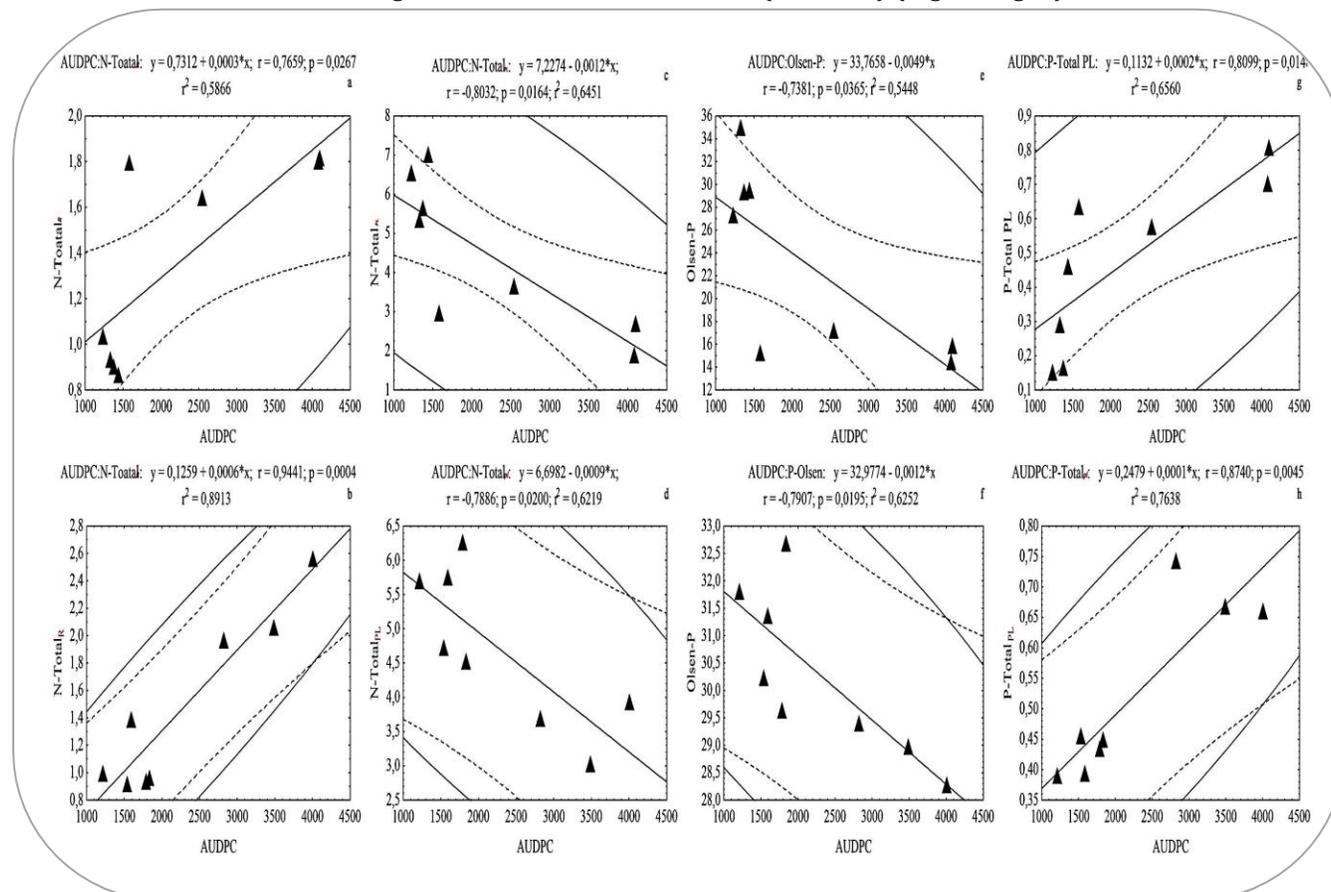
Figure 5. Correlation between N-Total$_R$, Olsen-P, N-Total$_{PL}$, P-Total$_{PL}$, and AUDPC. Values are the means of four replicates at the full productive stage. Pearson's linear correlation coefficients (r), coefficients of determination (r^2) and P values are given in each plot.

Analysis of correlations between Olsen-P, N-Total$_R$, and nodosity: Obtained results showed a negative correlation between N-Total$_R$ and Olsen-P with a higher COD in 2016 compared to 2017. Nodosity was also negatively correlated with N-Total$_R$ during the two years of study and positively correlated with Olsen-P.

Variation of microbial communities and its relation to AUDPC: Figure 6 showed a significant increase in ID-Foc, *Trichoderma* spp, and *Pseudomonas* spp in all sites during all period of study.

The results showed an increase in ID-Foc with values ranging between 32.29 to 82.69 % during 2016 and between 25 to 81.44% during 2017. In contrast, a

decrease in ID-Foc was recorded in S1 and S2 with an average of 33.72 and 38.8% in 2017, respectively.

A significant higher increase in *Trichoderma* spp levels in the different sites was observed in 2017 (73.93 to 89.82%) compared to 2016 (31.42 to 48.27%).

In the rhizosphere, *Pseudomonas* spp significantly increased in the different sites during all period of study. In 2016, the highest levels of *Pseudomonas* spp were found in S7 and S8 which correspond to 26.09 and 21.69% of total increase, respectively, and up to 40.52 and 53.62%, in S1 and S3 in 2017, respectively. Whereas S5 and S6 showed a decrease of 31.94 and 57.96% of *Pseudomonas* spp in 2017, respectively.
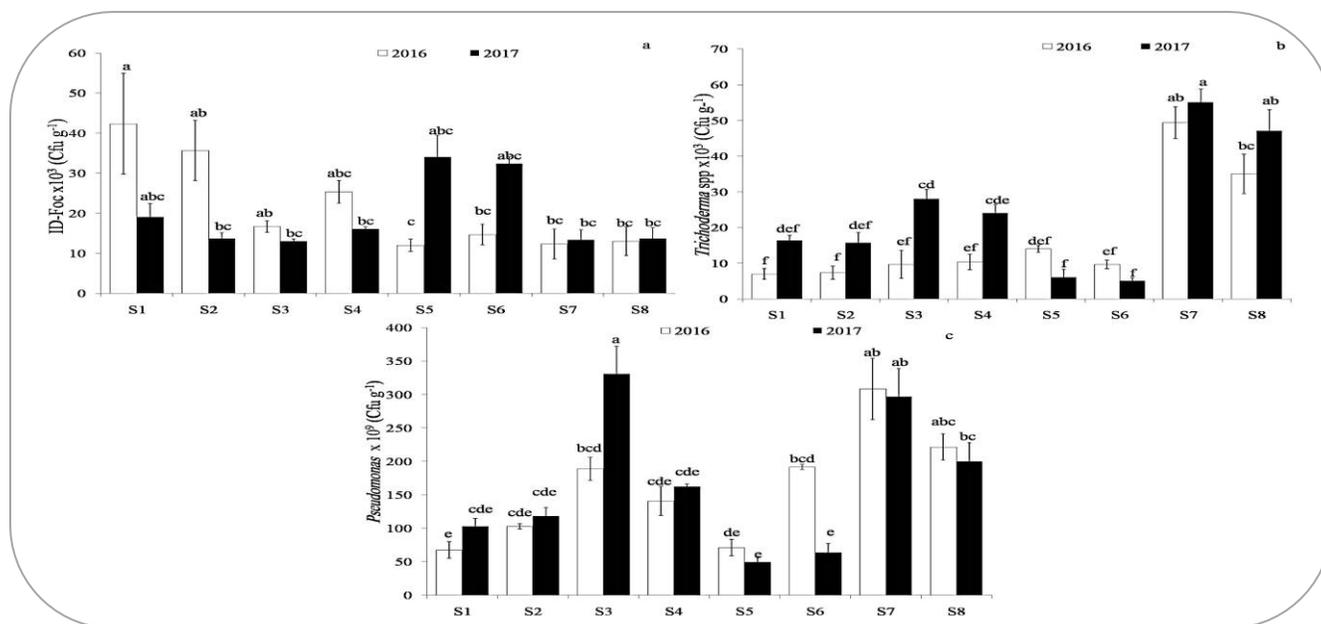
Figure 6. Variation of ID-*Foc* (a), *Trichoderma* spp (b) and *Pseudomonas* spp (c) in the rhizosphere of chickpea. Values correspond to the mean calculated with five replicates. Letters show significant differences between cropping systems (P<0.05)

A positive correlation was found between ID-Foc and AUDPC (Figure 7a, b). The strong COD is observed in 2016 (r^2=0.81). In contrary, a negative correlation was found between *Trichoderma* spp, *Pseudomonas* spp and AUDPC in 2017 (Figure 7c, d). This could be explained by the significant decrease of disease with the increase in *Trichoderma* spp and *Pseudomonas* spp.

Pseudomonas spp was found to be negatively correlated with ID-Foc during the two years of study (Figure 7e, f); however, it is not correlated with *Trichoderma* spp. The obtained results also showed positive correlations between *Pseudomonas* spp and nodosity.
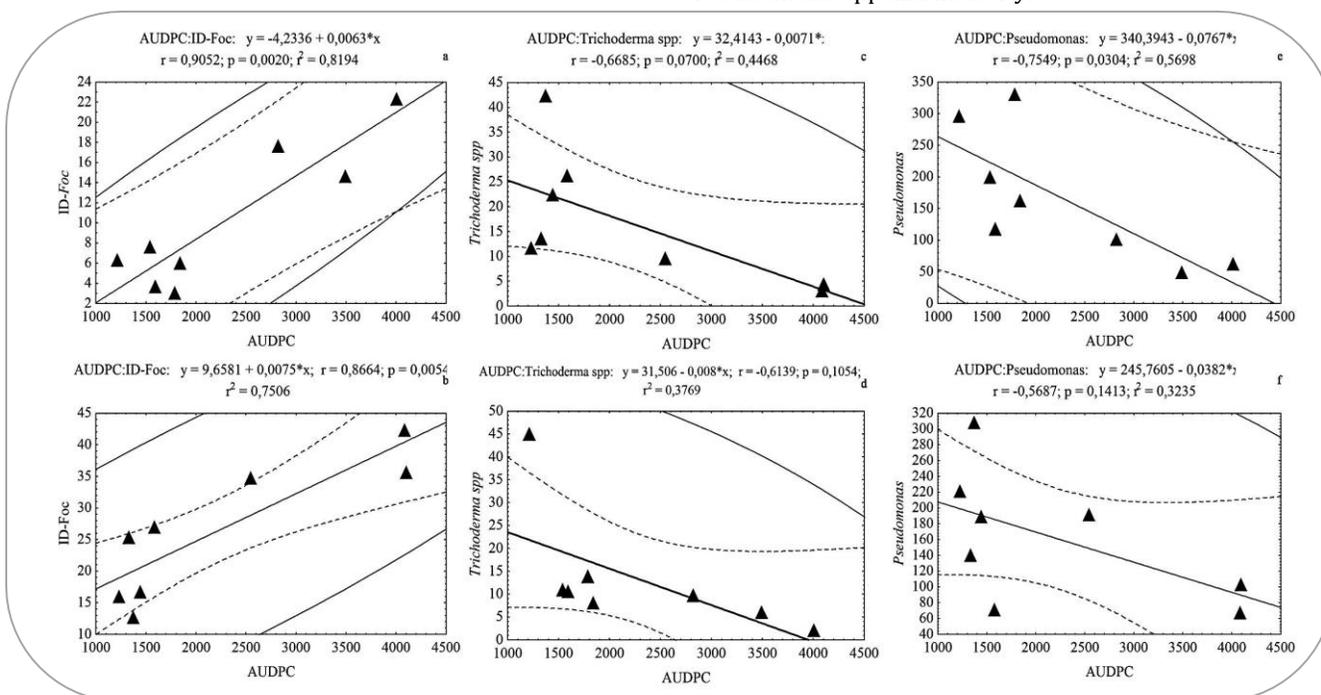
Figure 7. Correlation between ID-Foc, *Trichoderma* spp, *Pseudomonas* spp and AUDPC. Pearson's linear correlation coefficients (*r*), coefficients of determination (r^2) and *P* values are given in each plot. Values are the means of four replicates collected at the full productive stage.

Effect of Olsen-P and N-Total$_R$ on variations of microbial communities: Pearson correlation analysis showed significant positive correlations between N-Total$_R$ and ID-Foc with a higher COD in 2017 (r^2=0.84). Whereas, significant negative correlations were recorded between ID-Foc and Olsen-P. The data also revealed a negative correlation between ID-Foc and nodosity with a high COD in 2016 (r^2=0.75) than in 2017 (r^2=0.51). In addition, a negative correlation between N-Total$_R$ and *Trichoderma* spp (r^2=0.61) was recorded during 2017. These two biological factors were found to be uncorrelated in 2016.

DISCUSSION

Most of the studies regarding the epidemiology of wilt disease of chickpea have been restricted to controlled conditions with short research periods rather than under natural conditions. However, it is well known that controlled media do not represent the natural conditions that prevail in the rhizosphere of crops (Musyoki *et al.,* 2016). Therefore, this study was conducted in greenhouse experiments during two cropping seasons in eight infested natural's soil for a better understanding of some combined interactions between *Fusarium oxysporum* population, soil diversity of microorganism (*Rhizobia*, *Pseudomonas* spp, and *Trichoderma* spp) in relation to nutrient elements, especially N and P and their combined effect on the severity of vascular wilt disease in chickpea.

A great variability in terms of physicochemical and biological properties was observed in the experimental sites. The results showed a real diverse spatial organization of microbial communities within chickpea rhizosphere in relation to nutrients. Accordingly, chickpea rhizosphere showed specific temporal changes, and dynamic of mineral elements and microorganisms affected either positively or negatively the severity of the disease.

During the experiment, atypical symptoms were observed. It consists of late yellowing, severe defoliation, low flowering and low seed yield. AUDPC values varied significantly according to the geographical origin of soil during the two seasons. AUDPC values were very high in 2016 especially in S1 (4081) and S2 (4098.5) compared to 2017, where the highest values were recorded in S5 (3489.5) and S6 (4009.5). The results showed significant differences in terms of DWSt and Yg among different sites. These results could be attributed to the variations in soil properties and the differences recorded in the disease severity. This study showed a negative effect of AUDPC values on DWSt and Yg. This is in accordance with previous

research reporting a significant decrease of chickpea yields under advanced severity of vascular wilt (Jimenez-Diaz *et al.,* 2015).

A large variation of the population size of ID-Foc and disease levels was found within the studied large geographical area which means that physicochemical and biological proprieties of soil affected significantly AUDPC values. We can assume that there is a very complex interaction between physicochemical and biological properties of soil and disease severity. This finding is mainly confirmed by correlation analysis which demonstrates that N-Total$_R$ and P-Total$_{PL}$, ID-Foc were positively correlated with AUDPC, whereas Olsen-P, N-Total$_{PL}$, *Pseudomonas spp* and *Trichoderma* spp were negatively associated with AUDPC.

In natural pathosystem, *Fusarium oxysporum* inoculum survives in soil as an active form in tissues or in the form of chlamydospores. This increased in the presence of its host plant (Haware *et al.,* 1990). A same outcomes were observed in the present study, showing a clear increase in ID-Foc in all rhizosphere soils after sowing. Therefore, the increased disease severity was, to some extent, associated with the increase of ID-Foc. This is in accordance with previous findings reporting a significant increase of disease severity with increasing ID-Foc (Shuga *et al.,* 1994).

N-Total$_R$ was positively correlated with ID-Foc indicating a positive effect of N on the *Fusarium oxysporum* population. This may be due to the favorable conditions of *Fusarium oxysporum* germination and sporulation, therefore, a significant increase in ID-Foc resulted in an advanced disease severity. This is in agreement with previous study demonstrating that higher N availability decreased mycelial growth and increased the number of produced conidia (Hoffland *et al.,* 2000). Snoeijers *et al.,* (2000) suggested that high levels of N could have an impact on plant-pathogen interactions by activating virulence factors and enhancing the metabolic adaptation of the pathogen. The positive effect of N-Total$_R$ on disease severity could be attributed to changes in soil pH. It has been demonstrated that N fixation can increase P availability in the rhizosphere through rhizosphere acidification mechanisms resulting from an increase in H$^+$ protons by nodulated roots, organic acids or acid phosphatases (Latati *et al.,* 2014).

Our results corroborate previous findings reporting a significant increase of disease severity in association to N availability in the rhizosphere (Shuga *et al.,* 1994; Rao and Krishnappa 1996). These authors reported that increasing N played a determining role in vascular wilt in chickpea. As

well, high N availability promoted disease and contributed to the increase in the incidence and severity of the disease.

In the present study, a significant decrease of AUDPC was associated with a high N-Total$_{PL}$ plant uptake. Also, increased N-Total$_{PL}$ was positively associated to nodosity. It has been reported that an increase in N source induced an accumulation of amino acids, which in turn can affect the activation of plant defense mechanisms (Rojas *et al.,* 2014). However, Dutta *et al.,* (2008) showed that the intensity of the disease was significantly increased with increasing N content in plant tissues grown in *Fusarium udum* infested soils.

The results showed positive effects of N-Total$_R$ on wilt disease severity, whereas it is negatively correlated with nodosity. The decreased in N-Total$_R$, to some extent, is related to the stimulation of efficiency in the rhizobial symbiosis. Previous research reports have mentioned the effect of N-Total in decreasing nodule growth (Alkama *et al.,* 2009). In the other hand, the significant relationship among nodosity and Olsen-P in the rhizosphere of chickpea induced a positive effect of nodule growth. This is in accordance with the previous findings of Betencourt *et al.,* (2012). Similarly, a study by Korir *et al.,* (2017) showed that PGPR possesses the ability of phosphate solubilization improvement by increasing P content in the soil and enhancing nodulation and N fixation in the common bean. In addition, to their useful N$_2$-fixing activity with legumes, rhizobia can improve plant P nutrition by mobilizing inorganic and organic P (Alikhani *et al.,* 2006). Also, in chickpea rhizosphere, increased Olsen-P was shown to be linked to the decrease of disease severity, probably, through the inhibition of chlamydospores germination and *Fusarium oxysporum* sporulation. This is in line with the results of the present study showing a clear decreasing of ID-Foc under strong P availability. Rao and Krishnappa, (1996) suggested the association of P with Foc-chickpea interaction and noted a negative correlation between outbreaks caused by *Fusarium oxysporum* and P content of the soil. Prabhu *et al.,* (2007) reported that improved plant growth through P nutrition allows the plant to "escape" from attack by fungal pathogens in the soil.

Disease severity was associated with an increase of P-Total$_{PL}$ plant uptake. This could be due to the energy balance after infection. In this regard, Bolton, (2009) reported that the complexity of plant defense responses required an abundant supply of energy, mainly derived from primary metabolic processes. At the same time, these metabolic changes caused by P, may be a general feature of plant-pathogen interactions that therefore affect both the immune system of plants, through the formation and accumulation of secondary metabolites.

In soil-plant-pathogen interactions, microbial communities are responsible for the biological processes that influence the occurrence of plant diseases (Wallenstein, 2017). Microbiological analysis of the rhizospheric soils of the different studied sites showed a positive effect between Rhizobia and *Pseudomonas* spp population in decreasing disease levels. It is worth noting that the increase in production and yield of chickpea, associated with the improvement of the nutritional quality of the plant, is due to the biological amendment of N and the bioavailability of Olsen-P.

A significant decrease in disease severity associated with nodosity and elevated *Pseudomonas spp* was noticed. ID-Foc was negatively correlated with high levels of nodosity and *Pseudomonas spp*. This is in accordance with previous findings reporting that *Pseudomonas* spp caused a significant decrease in fungal mycelium density and number of macroconidia of *Fusarium culmorum* population (Strunnikova *et al.,* 2015). *Pseudomonas* spp has frequently been reported to be responsible for the natural suppression of *Fusarium* wilt disease (Mazzola, 2002). This process is associated to several mechanisms which include volatile compounds by siderophore production, antibiotics, chitinases, β1,3-glucanases and cyanides, asymbiotic nitrogen fixation and solubilization of mineral and other nutrients (Narendra *et al.,* 2015).

In this study negative relationships between ID-Foc, AUDPC values and *Trichoderma* spp were found, which confirms that *Trichoderma* species contribute in the process of natural wilt disease management. These species were commonly isolated from the rhizosphere of chickpea and the level of population in the soil is necessary for the control of the disease (Sumeet and Mukerji, 2000). In this respect, *Trichoderma* spp species probably limit sporulation and *Fusarium oxysporum* mycelial growth via competition for nutrients and antifungal compounds production. In addition, Dubey *et al.,* (2007) reported that *Trichoderma* spp contributes to *Fusarium oxysporum* suppression through a variety of mechanisms such as hyperparasitism, antibiosis, and induction of host resistance. Griffin, (1994) pointed out that mineral nutrition is essential for the growth and the stimulation of fungal secondary metabolites production. This may also explain the positive correlation recorded between N-Total$_R$ and *Trichoderma* spp.

Results of particular interest from this present study are those concerning the positive correlation between *Trichoderma* spp and *Pseudomonas spp,* which were negatively correlated with ID-Foc and AUDPC. Our results demonstrated positive significant correlations between nodosity and *Pseudomonas spp*. This would undoubtedly be due to the synergistic effect between the population of *Pseudomonas spp* and *Rhizobia*. Interestingly, Zaidi *et al.,* (2003) reported that chickpea yield and nutrient uptakes were significantly increased following inoculation of chickpea plants with *Rhizobium* sp. and *Pseudomonas striata* solubilizing phosphorus in combination.

CONCLUSION

This study demonstrates the relationship between soil nutrients and the process of infection of chickpeas by the pathogen. The results in our study are relevant for wilt disease control. Our work demonstrates that chickpea cultivars undergo significant nutrient status changes in relation to AUDPC values. Olsen-P, *Trichoderma spp*, nodosity, and *Pseudomonas* were negatively correlated with ID-*Foc* and consequently reduced severity of disease. N-Total$_{PL}$ was negatively correlated with AUDPC whereas P-Total$_{PL}$ was positively correlated with AUDPC. It can be assumed that effective management of available nitrogen and phosphorus and its balance with other nutrients should be considered in an integrated strategy for *Fusarium* wilt disease management.

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