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Screening for *Pseudomonas* and *Bacillus* antagonistic rhizobacteria strains for the biocontrol of *Fusarium* wilt of chickpea

Hannane Abed [a], Noureddine Rouag [b,*], Dahou Mouatassem [c], Amar Rouabhi [b]

[a] Department of Microbiology, Faculty of Nature and Life Sciences, University of Ferhat Abbas Sétif 1, Sétif, Algeria
[b] Department of Agronomy, Faculty of Nature and Life Sciences, University of Ferhat Abbas Sétif 1, Sétif, Algeria
[c] Department of Agronomy, Faculty of Nature and Life Sciences, University of Mohamed El Bachir El Ibrahimi (UMBI), de Bordj Bou Arreridj, Algeria

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Abstract

The aim of this work is to study the ability of several isolates belonging to Rhizobacteria (*Pseudomonas* and *Bacillus*) collected from several chickpea growing areas in Algeria, to control the mycelium growth of *Fusarium oxysporum f. sp. ciceris.* Interesting isolates were characterized for their morphological characteristics, physiological and biochemical activities as potential bio-control agent. Fungal inhibition tests were performed using plate assay and each isolate were tested for the production of protease, cyanide hydrogen, indole acetic acid, antifungal volatile and extracellular compound. According to API 50 CH, we are able to identify six *Bacillus* species (*B. subtilis*, *B. circulans*, *B. lentus*, *B. aneurinilyticus*, *B. firmus*, *B. licheniformis*; and with API 20NE test we have identified three Pseudomonas species (*P. aeruginosa*, *P. luteola*, *P. fluorescens*). The ability of bacterial isolates was varied in production of Protease, Gelatinase, Amylase, Cellulase, Acid Indole acetic, Lipase, Catalase and Cyanid Hydrogen. This is traduced in different rate of inhibition growth due to various extracellular compounds, where B61 (*Bacillus aneurinilyticus)* and P39 (*Pseudomonas luteola*) and P70 (*Pseudomonas fluorescens)* were the most efficient with 77 and 55.5% respectively, while B39 (*Bacillus firmus*) and P41 (*Pseudomonas luteola*) were the most efficient by volatile compounds with 70.5 and 77.5% respectively. Our results indicate that these bacteria isolates can be used in the biocontrol of *Fusarium oxysporum f. sp. ciceris.*

Keywords: Antagonistic, *Bacillus*, Bio-control, Chickpea, *Fusarium oxysporum, Pseudomonas*

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries (Jukanti et al., 2012). Fungal plant pathogens are among the most important factors that cause serious losses to agricultural products annually (Ekundayo et al., 2011). Chickpea production is severely limited by Fusarium wilt which is caused by *F. oxysporum* Schlechtend. Fr. f. sp. *ciceris* (Padwick) Matuo and K. Sato. (Jalali and Chand, 1992). *Fusarium* wilt is a serious disease threat, especially in low rainfall areas, where weather conditions are favourable for disease development. From 33 countries of

* Corresponding author.
Department of Agronomy, Faculty of Nature and Life Sciences, University of Ferhat Abbas Sétif 1 (UFAS-1), Sétif, Algeria
Tel.: +213772176 508 E-mail address: rouag_rn@yahoo.fr
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the world has been reported (Nene et al., 1996) causing 10–15% yield losses annually (Singh and Dahiya, 1973) depending upon the environmental conditions. The disease is more prevalent in the Indian subcontinent, United States, Tunisia, Algeria, Turkey, Ethiopia, Spain, and Mexico (Halila and Strange, 1996; Labdi, 1990; Nene et al., 1989; Westerlund et al., 1974). In severe cases, yield losses riches 100% under favourable conditions in chickpea (Landa et al., 2004). Other species and formae specialis of *Fusarium* also cause wilt in chickpea and produce mycotoxins (Gopalakrishnan et al., 2005), which markedly reduce the potential of crop rotation as a disease management strategy. *Fusarium oxysporum* f. sp. *ciceris* (FOC) may survive in soil and on crop residues as chlamydospores for up to six years in the absence of susceptible host, and spread by means of both soil and infected seed (Haware et al., 1978).

It is difficult to manage the disease either through crop rotation or application of chemicals because of soil nature persistence and its capacity to survive for long time even in the absence of host (Haware et al., 1996). Efficacy of wilt management was improved when bio-control agents were combined with cultural practices such as sowing date (Landa et al., 2004). Biological control provides an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and reduced environmental impact (Reino et al., 2008). However, management of fungal diseases using antagonistic microorganisms, known as biological control, has been the focus of intense research worldwide (Killani et al., 2011). The use of bacteria as biocontrol agents of soil borne plant pathogens, as an alternative or complementary strategy to physical and chemical disease management, has been investigated for over 70 years (Weller, 1988). The lack of consistency in performance of beneficial bacteria such as *Pseudomonas* spp. or *Bacillus* spp. under field conditions has limited their use in commercial agriculture (Raaijmakers et al., 2002). Much of that inconsistency has been attributed to variability in physical and chemical properties within niches occupied by biocontrol agents that affect both colonization and expression of biocontrol mechanisms such as antibiosis, parasitism, competition and induced resistance.

Various mechanisms are involved in the biological control of fungal pathogens by Plant Growth Promoting *Rhizobacteria* (PGPR). These mechanisms include the production of secondary metabolites such as antibiotics, siderophores, hydrolytic enzymes, volatile extracellular metabolites, hydrogen cyanide and competition for nutrients, promotion of plant growth and, finally, induced resistance within the plants (Moeinzadeh et al., 2010; Kloepper et al., 1992)

Therefore, biological control offers potential for suppression of *Fusarium* wilt under field conditions, particularly when used in combination with cultivars with partial resistance to the disease and choice of sowing date. Species of *Bacillus* have also been known to produce compounds which promote plant growth directly or indirectly, hydrogen cyanide (HCN), siderophores, indole acetic acid (IAA), solubilize phosphorous and antifungal activity (Saharan and Nehra, 2011; Wahyudi et al., 2011; Godinho et al., 2010). The objectives of this research were: (1) to characterize and select *Pseudomonas* and *Bacillus* isolates from rhizospheric and rhizoplanic soils infested with chickpea wilt, and (2) to determine their antagonistic activity *in vitro* in dual cultures against *Fusarium oxysporum ciceris*.

Material and Methods

Preliminary screening

One hundred and forty for bacterial isolates were tested for their ability to produce antifungal substances against *Fusarium oxysporum* f. sp. *ciceris* using a dual-culture *in vitro* assay on PDA plates. Twenty µl of each bacterial suspension (10^8 cfu/ml) was placed on the plate. After 48h incubation at 28°C, a single 6 mm diameter mycelial disc was placed at the extremity of plates. Then, plates were incubated at 27-29 °C in darkness and after 5 days the growth diameter of the pathogen (distance between the point of placement of fungal disk and actively growing edges of the fungus) was measured. The percentage of growth inhibition was calculated using the method described by Erdogan and Benlioglu (2010). This experiment was conducted twice. Bacteria with inhibitory potential were selected for further experiments.

Identification of bacterial antagonist

Initially, the selected isolates were identified based on gram positive, spore forming, and fluorescent pigment production, aerobic or anaerobic growth. To identify *Pseudomonas* species, oxidase, catalase, amylase, protease, cellulase, indole acetic acid, lipase and gelatinase, growth at 41°C, growth at 4°C tests were further performed. To identify *Bacillus,* motility, growth at 45˚C, anaerobic growth in glucose broth,

were assessed (Shaad, 1988). The Analytical Profile Index (API), particularly API 20E and API 5OCHB were used as supplementary tests, for the identification and differentiation of *Pseudomonas, Bacillus* and related species, respectively (Logan and Berkeley, 1984).

Protease production

Bacterial isolates were tested for protease production by growing them on skim milk agar (SKM) (Chantawannakul et al., 2002). The ability to clear the skim milk suspension in the agar was taken as evidence for the secretion of protease. Non- bacteria inoculated plates were used as control.

Hydrogen cyanide production

Production of hydrogen cyanide was determined on nutrient agar medium+ 4.4g of glycine. 100 µl of bacterial culture (48 h) were streaked on the surface of medium, and then sterilized filter papers were soaked in 2.0% Na_2CO_3 in 5.0% (w/v) picric acid and placed in the upper lid of the Petri dish. The Petri dishes were sealed with parafilm and incubated at 30 °C for 4 days. A change in the colour of the filter paper from yellow to reddish brown was accepted as an index for cyanogenic activity. Non - inoculated plates with bacteria was used as control (Alstrom, 1987).

Indole acetic acid production (IAA)

The production of IAA was determined as described by Bric et al. (1991). Bacterial strains were inoculated into nutrient broth (peptone, 5 g; yeast extract, 1.5 g; beef extract, 1.5 g; and NaCl, 5 g; each per liter) and incubated at 30 °C for 5 days. A 5 ml culture was removed from each tube and centrifuged at 10,000 rpm for 15 min. An aliquot of 2 ml supernatant was transferred to a fresh tube to which 100 µl of 10 mM orthophosphoric acid and 4 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. The mixture was incubated at room temperature for 25 min, and the absorbance of pink colour developed was read at 530 nm using a spectrophotometer.

Production of volatile antibiotics

Firstly, 100 µl of bacterial suspension (1×10^7 cfu/ml) from each isolate were sprayed on the surface of a Petri plate containing nutrient agar medium and incubated at 27-30 °C for two days. In another Petri plate containing PDA medium, a 5 mm disk of a 7 days-old pure culture of *Fusarium oxysporum* f. sp. *ciceris* was placed at the centre. Then both half plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension. Plates were sealed with parafilm. In the control plates, bacterial suspension was replaced with sterile water. Plates were incubated at 27-29 °C for 5 days and the percentage of inhibition zone was calculated for each isolates (Fiddaman and Rossall, 1993). For each treatment, there were four replicates and the experiment was repeated twice.

Data analysis

The results obtained were statistically processed through the analysis of variance ANOVA and B Tukey test at *P<0.05 and **P<0.01 to evaluate the significance between treatments. Correlation matrices have also been developed to define the interactions between the various parameters studied using SPSS version 18.0 and Microsoft Excel software 2010.

Results

Bacterial isolates Screening and characterization

Sixteen isolates for each genus (*Pseudomonas* and *Bacillus*) out of one hundred and forty for bacteria strains isolated from chickpea rhizosphere have shown substantial inhibition zones against and revealed a high antifungal activity against *Fusarium oxysporum* f. sp *ciceris* in *in vitro* tests (Figure 1). Based on biochemical, physiological and morphological properties, selected isolates were identified as *Bacillus subtilis (*B40, B45, B48, B62 and B65), *Bacillus circulans* (B72 and B73), *Bacillus lentus* (B79, B69, B53 and B41), *Bacillus aneurinilyticus* (B64, B61 and B47), *Bacillus firmus* (B39) and *Bacillus licheniformis* (B59) (Table 1). Three species of *Pseudomonas* were identified: *Pseudomonas aeruginosa* (P29, P37, P44 and P50), *Pseudomonas luteola* (P31, P36, P39, P41, P53, P59, P61, P64 and P65) and *Pseudomonas fluorescens* (P66, P70 and P45) (Table 2).

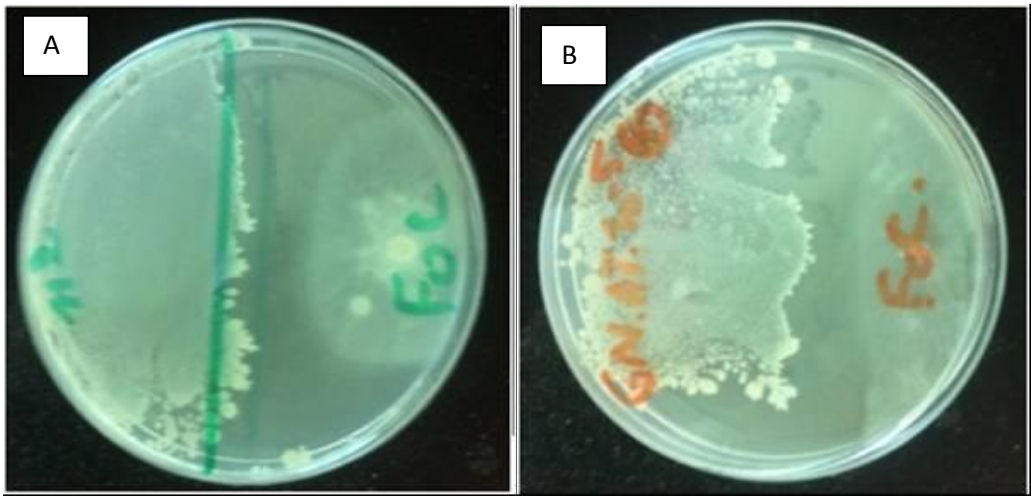

Figure 1. Inhibition of mycelium growth of *Fusarium oxysporum* f. sp. *ciceris* by bacterial isolates on PDA media by dual-culture assay (A *Pseudomonas*; B *Bacillus*).

Table 1. Antimicrobial metabolites activities of *Bacillus* isolate against *Fusarium oxysporum* f. sp. *ciceris*

Isolates	DC (%)	IZ (mm)	VC (%)	HCN	Prot	Gel	Amy	Cel	IAA	Lip	Cat
B39	70,5	9	70.5	++	+++	-	+	-	+	+	+
B40	69,5	5	60.5	++	+++	+	+++	+	+	-	+
B41	59,5	7	30	++	+++	-	+	++	+	+	+
B45	64	12	60.5	++	+++	+	+++	+	+	-	+
B47	55	10	35	+	+++	+	+++	++	-	-	+
B48	48	7	57	++	+++	+	++	++	+	-	+
B53	40	10	50	++	++	-	++	-	++	+	+
B59	54,5	5	14.5	++	+	-	+++	+	+	+++	+
B61	77[c]	3	9	-	++	+	+++	++	+	-	+
B62	45	1	20	+	+++	+	+++	+	+++	++	+
B64	68,5	7	46.5	+	+	-	+++	-	+++	+	+
B65	61	10	55	+	++	-	+++	-	+	+	+
B69	52,5	5	15.5	++	+++	+	+++	+	+	++	+
B72	60	5	50	+	+	+	++	++	+	++	+
B73	64,5	7	40.5	+	+	+	+++	+	+	+	+
B79	64,5	4	34	-	+++	+	++	++	++	-	+
Average	59,63	6,69	37,78								
Gaptype	9,99	2,96	16,55								

DC = Percent growth inhibition in dual culture method, IZ : diameter of Inhibition Zone in dual culture, VC= Percent growth inhibition in volatile compound, HCN : Cyanid Hydrogen, Prot : Protease, Gel : Gelatinase, Amy : Amylase, Cel : Cellulase, IAA : Indole acetic Acid, Lip : Lipase, Cat : Catalase.

In vitro antifungal activity

Extracellular and volatiles compounds

In dual culture test, *Bacillus* strains showed to have more inhibition of the pathogen growth in the PDA medium than *Pseudomonas* isolates. Indeed, with extracellular compounds diffused in the PDA medium, *Bacillus* strains (Table 1) gave an average of inhibition equal to 59.63 ± 9.99% vastly superior to 47.16 ± 9.32% recorded with Pseudomonas strains (Table 2). While, with volatiles compounds, *Pseudomonas* appears to be more effective in inhibition of the pathogen mycelium development. Thus, we obtained 42.15 ± 22.77% of mycelial growth inhibition by *Pseudomonas* isolates (Table 2) compared to 37.78 ± 16.55% recorded by *Bacillus* isolates (Table 1). If we look to gap-type calculated (22,77% and 16,55%), we must note

here the wide intraspecific variability in both genera studied, particularly with volatile compounds it is more pronounced.

Table 2. Antimicrobial metabolites activities of *Pseudomonas* isolate against *Fusarium oxysporum* f. sp. *ciceris*

Isolates	DC (%)	IZ (mm)	VC (%)	HCN	Prot	Gel	Amy	Cel	IAA	Lip	Cat
P29	52	2	45	+	+++	+	-	-	++++	+	+
P31	55	18	21	+	+++	-	-	-	+	-	+
P36	41	10	37	+	-	-	+	+	++	+	+
P37	49	7	39.5	+	+	-	-	-	++++	-	+
P39	55,5	18	68	+	-	+	+	++	+	-	+
P41	45	7	77.5	++		-	+++	-	++++	+	+
P44	40,5	7	66	++	-	-	-	+	+	-	+
P45	46,5	0	66	++	+++	+	+++	+	+	+	+
P50	49	7	17	+	-	-	-	++	-	-	+
P53	71	8	52	+	+	+	+++	-	+	+	+
P59	34,5	1	4	+	++	-	-	++	-	-	+
P61	40,5	4	13	+	+	-	++	+	++++	++	+
P64	45,5	8	72	+	+	-	++	+	++	-	+
P65	38	15	9.5	++	+	+	-	+	+++	++	+
P66	36	10	42	++	++	+	+++	-	+++	+	+
P70	55,5	6	45	+	++	+	+++	-	+++	+	+
Average	47,16	8,00	42,15								
Ecartype	9,32	5,35	22,77								

DC = Percent growth inhibition in dual culture method, IZ : diameter of Inhibition Zone in dual culture, VC= Percent growth inhibition in volatile compound, HCN : Cyanid Hydrogen, Prot : Protease, Gel : Gelatinase, Amy : Amylase, Cel : Cellulase, IAA : Indole acetic Acid, Lip : Lipase, Cat : Catalase.

As regards the connection between the percentage inhibition of mycelial growth and the inhibition zone, the results obtained do not show a direct bond because the largest zone of inhibition was not observed with the strains giving the higher level of inhibition either with *Bacillus* or *Pseudomonas* strains. Indeed, it was the strain B45, P31 and P39 that have gave the high read inhibition zones with 12 and 18mm respectively (Table 1 and 2). We can conclude, the general inhibition is due to the combined effects of several extracellular and volatile compounds.

Interspecific inhibition efficiency

In the case of *Bacillus* genera, the most efficient strains in the inhibition of mycelial growth were B61 with 77% of efficiency in extracellular compounds and B39 with 70.5% of efficiency in volatiles compounds (Figure 2). In the case of Pseudomonas, P53 was the most efficient in extracellular compounds (71%) and P41 (77.5%) in volatiles compounds tests (Figure 3). Regarding the interspecific efficiency, strain identified as *Bacillus firmus* appears as the more efficient (70,5%) in the inhibition of mycelia growth of *Fusarium oxysporum* f. sp. *ciceris*, both in extracellular and volatiles compounds tests. But the failure to identify a single strain, these results should be confirmed by interspecific comparison tests. With regard to Pseudomonas, there is very little difference between the three species identified by studying the effect of extracellular compounds, while in the case of volatile compounds, *Pseudomonas fluorescens* isolates seem to be more efficient with an average of 51 % of efficiency.

Both *Bacillus* and *Pseudomonas* isolates has gave a significant effect at 1% level according Tukey's test in inhibiting mycelia growth by extracellular compounds. These results were traduced by the appearance of three homogeneous groups and the distinction of B53, P31, P39, P53 and P70 isolates.

Production of antifungal metabolites

In vitro test using 32 bacterial strains against *Fusarium oxusporum fs ciceris* showed that different strains exhibited different combinations of antimicrobial metabolites. If all *Bacillus* strains have produced protease (Figure 4), amylase, catalase and Indole acetic acid excepting B47 isolate, however, they showed a great

variability in the production of gelatinase, cellulase and lipase as an enzyme products and HCN as a volatile compound (Table 1). With regard to Pseudomonas strains, except catalase and HCN which all strains gave a positive reaction, we note a high qualitative and quantitative variability between species and strains of Pseudomonas, in protease production (Figure 4), gelatinase amylase, lipase and IAA products (Table 2).

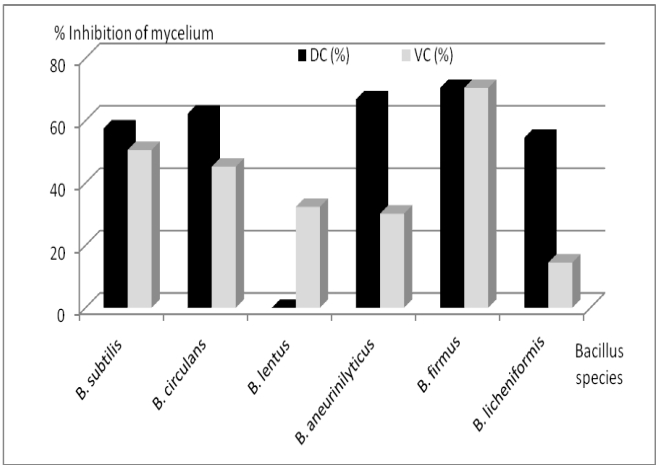

Figure 2. Inhibition of mycelium growth of *Fusarium oxysporum* f. sp. *ciceris*, by extracellular and volatiles compounds of *Bacillus* species.

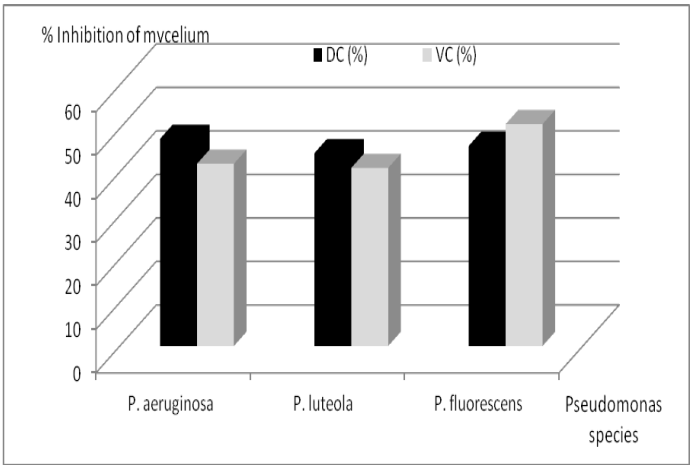

Figure 3. Inhibition of mycelium growth of *Fusarium oxysporum* f. sp. *ciceris*, by extracellular and volatiles compounds of *Pseudomonas* species.

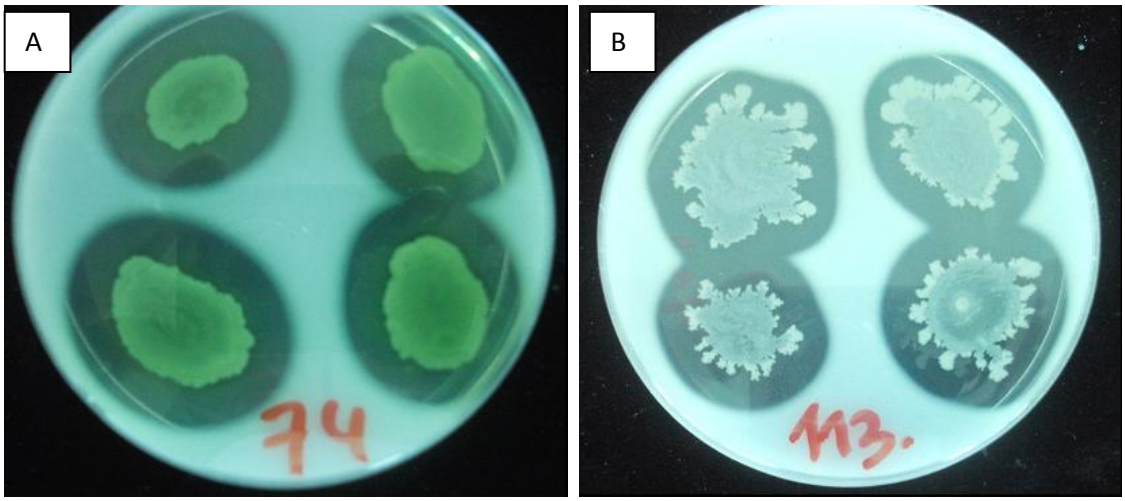

Figure 4. Enzyme activities: Protease production by bacterial isolates on PDA media (A *Pseudomonas* isolate; B: *Bacillus* isolate).

In the case of Bacillus strains, ANOVA analysis gave is a positive correlation between mycelia growth inhibition and inhibition zone, as well as for enzymatic activity between amylase and gelatinase. While, in the case of *Pseudomonas* strains, it has registered several positive correlations among different biochemical activities, example: the amylase is positively correlated with gelatinase and lipase, whereas the production of IAA is correlated with the cellulase and lipase production. From the principal components analysis (PCA), it emerges an entirely consistent correlation between biochemical tests and identification of bacterial species by API 50 CH and API 20NE.

Bacterial antagonism towards Fusarium species and strains

The results obtained show a great variability in inhibiting mycelial growth of the three FOC strains tested. Indeed, the results of direct confrontation in dual cultivation give the isolate FOC1 of the pathogen as the most sensitive fungal strain to antagonism of Bacillus isolates with an average reduction of mycelial growth equal to 58.16%. In addition, the rest of the Bacillus isolates do not exhibit a great difference in the effectiveness of the antagonism because the standard deviation is equal to 10.26 between the four deviations calculated. In contrast, the FOC2 behaves very differently when it is confronted with the Bacillus isolates,

giving an average of reducing in mycelial growth equal to 40.87%. We have obtained zero inhibition with three strains of *Bacillus* ie B48, B65 and B69. This has resulted in a standard deviation of about 23.26. The FOC3 and *Fusarium solani* isolate gave intermediate sensitivity resulted in average reduction of mycelial growth of about 43.81% and 49.47%, respectively (Figure 5, 6).

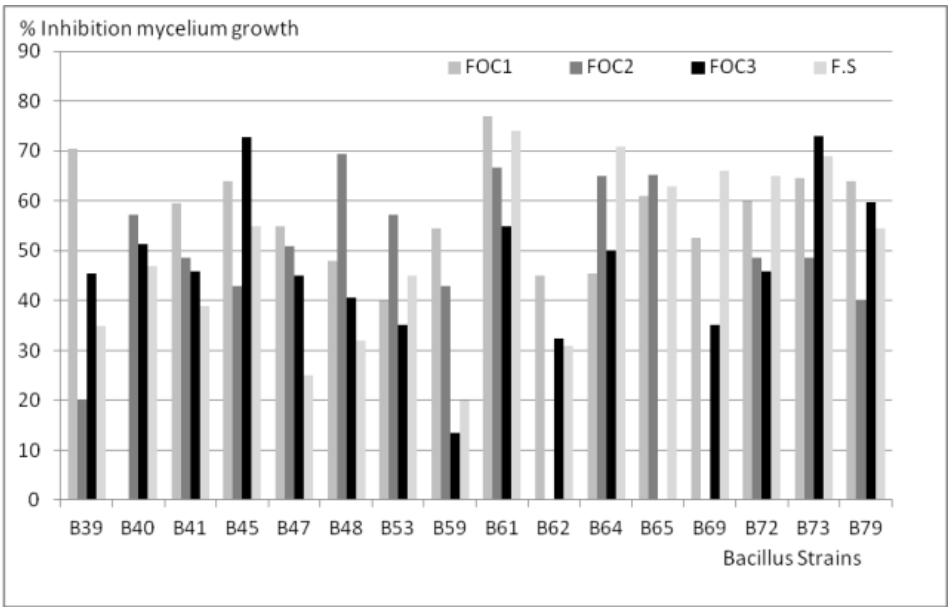

Figure 5. *Bacillus* strains antagonistic effect on the hyphal growth in dual culture of *Fusarium oxysporum* f. sp. *ciceris* strains (FOC1, FOC2 and FOC3) and *Fusarium solani*.

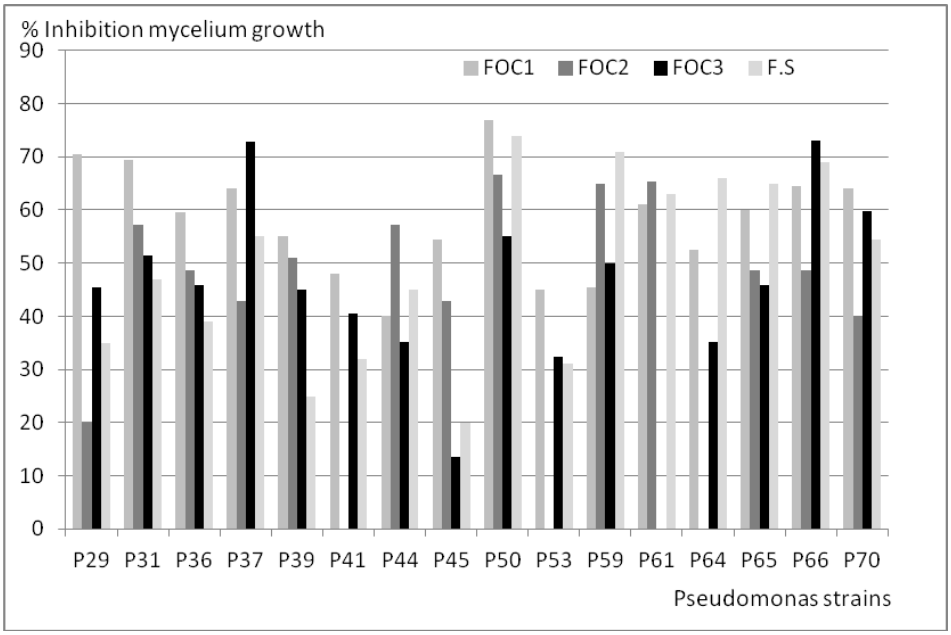

Figure 6. *Pseudomonas* strains antagonistic effect on the hyphal growth in dual culture of *Fusarium oxysporum* f. sp. *ciceris* strains (FOC1, FOC2 and FOC3) and *Fusarium solani*.

Tukey's test provides a significant effect at 1% and 5% level in the inhibition of mycelia growth with *Pseudomonas* and *Bacillus* isolates, respectively; thus resulting in the emergence of three distinct groups for each bacterial genus. While the effect of volatiles compounds was not significant at 5% level, that's mean, there is little quit diversity between strains tested.

Discussion

Fusarium oxysporum f. sp. *ciceris* is economically significant disease agent on chickpea. Due to the soil-borne nature of the disease, chemical applications for controlling the disease are rarely successful. Inconsistencies in biocontrol under varying environmental conditions have been a common limitation of soil borne

pathogens. The present research was conducted to evaluate the efficacy of *Pseudomonas* and *Bacillus* species and strains against *Fusarium oxysporum* f. sp. *ciceris*. The bacterial strains are in most cases correlated and more efficient in the inhibition of the mycelail growth of Fusarium oxysporum f. sp.ciceris isolates, if they have the same agro-ecological origin; this makes them very effective in the biocontrol. Our results corroborate with those of Weller (1988) which states that the use of rhizobacteria in the control of plant diseases is most effective when rhizobacteria were isolated from the same rhizosphere of host plant. In this study, all bacterial isolates were selected from rhizosphere of chickpeas fields. Some bacterial isolates showed high inhibition activity on pathogen, whereas others showed only mild or no activity at all. About 39% (57 of 144) of isolates showed antagonistic activity against *Fusarium oxysporum* f. sp. *ciceris* in *in vitro* tests and giving more than 30% of inhibition of mycelia growth. The rest of isolates tested exhibited no or weak antagonistic activity against the pathogen on PDA plates assay. Similar results have previously been reported (Erdogan and Benlioglu, 2010; Tjamos et al., 2004; Khot et al., 1996).

Reduction of fungal growth by certain PGPR and formation of inhibition zones were presumably due to the antifungal substances and/or cell wall degrading enzymes; released by the bacteria into the culture medium (Fatima et al., 2009). Also, Sarhan et al. (2001) and Montealegre et al. (2005) pointed that the cell free culture filtrate of *B. subtilis* inhibited the mycelial growth, radial growth, and spore germination and germ-tubes length of *F. oxysporum* f.sp*. ciceris*. Many strains of *Bacillus* strains have been found to be potential biocontrol agents against fungal pathogens. This antifungal action involves the production of antibiotics, especially within soil microsites (Fravel, 2005).

Cyanide is a toxic and dreaded chemical produced by many rhizobacteria. Some bacteria synthesis it, others excrete it and yet others metabolize it in other to avoid predation and competition (Zeller et al., 2007). All *Bacillus* species and strains efficiency of inhibition of mycelium growth is due to the extracellular products than volatiles compounds. It has been reported that *Bacillus* strains have been able to inhibit the growth of a variety of fungal pathogens because of their ability to produce a vast array of antibiotics such as Zwittermicin, Bacillomycin, Fengycin, Bacilysin and Difficidin (Athukorala et al., 2009; Chen et al., 2009). While with *Pseudomonas* species and strains, *Pseudomonas aeruginosa* and *Pseudomonas luteola* join *Bacillus* in their action mode, whereas *Pseudomonas fluorescens* inhibits the development of the mycelium much more with volatile compounds. These results are in agreement with the research previously carried out by Romanenko and Alimov (2000). Production of Hydrogen cyanide in *Bacillus* is about 50% in both rhizospheric soils and nodules compared to *Pseudomonas* that is over 80% (Ahmad et al., 2005). This result demonstrated that cyanide hydrogen was one of the most important volatile compounds of *Pseudomonas* spp. Plant growth was enhanced in vitro by most of the rhizospheric isolated that produced HCN (Wani et al., 2007).

The majority of selected *Pseudomonas* and *Bacillus* strains showed amylase and protease activity pronounced comparatively to other metabolites productions. The protease is known that has interference in wall degrading of fungal pathogen (Ahmadzadeh and Sharifi-Tehrani, 2009). The *Pseudomonas* isolates had shown a high level of production of indole acetic acid comparatively to *Bacillus* isolates. Xie et al. (1996) indicated that compared to other strains, *Pseudomonas* strains had higher levels of IAA production. According to Joseph et al. (2007), while working with chickpea, all *Bacillus* isolates produced IAA. Even though some microorganisms produce high concentration of auxin, that is, IAA and this helps to increase plant growth and yield in wheat crop, others producing low concentration of IAA also improve plant growth (Tsavkelova et al., 2007).

Regarding bacterial antagonism towards *Fusarium* species and strains, our results agree with Adebayo and Ekpo (2005), because *B. subtilis* inhibited fungal growth and also promoted the growth of tomato plant in screen house trial. *B. subtilis* has been shown to have a broad spectrum of antimicrobial activities over diverse fungal and bacteria pathogen (Grover et al., 2009). This may be as a result of production of antibiotic, competition with pathogen for nutrients and direct antagonism (Akhtar et al., 2010). *Bacillus* spp. are known to reduce wilting index in *F. udum*, increase plant growth and cause rapid colonization of tomato tissue in order to induce systemic resistance against *F. oxysporum* (Kloepper et al., 2004).

As regards the behavior of FOC isolates and *Fusarium solani* in direct confrontation with *Pseudomonas* isolates in dual culture, it appears that the *F. solani* was the most sensitive with 53.06% of mycelia growth reduction followed by FOC1 isolate. The FOC2 is confirmed as the isolate which has the most variable behavior when confronted with different *Pseudomonas* isolates. Indeed, we noted that three isolates (P37, P53 and P65) did not any impact on mycelia growth. Besides that, we found that *Bacillus* and *Pseudomonas*

strains are more effective in inhibiting mycelia growth if they came from the same geographical area with *Fusarium* strains. In general, *F.oxysporum* f.sp *ciceris* was more sensitive to bacterial metabolites and antagonisms than *F. solani* evidenced by the inhibition percentages recorded (Mudawi and Idris, 2014).

In conclusion, this study shows that some species and strains of *Bacillus* and *Pseudomonas* are very beneficial and effective as agents of biocontrol *in vitro* tests. The most interesting bacterial strains are being evaluated *in vivo* tests in the presence of the host plant for the study of their antagonistic faculty. Research must continue to be able to produce industrially as microbial biocontrol agents enjoying the characteristic of being respectful of human health of the environment in general.

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