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Biodegradation of Herbicides by *Pseudomonas aeruginosa* in two Soils Types of the Bou Namoussa irrigable perimeter (Algerian Extreme Northeast): Effects on mineral nutrition (P_2O_5 and NO_3^-).

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Abstract

The high use of both herbicides (the Glyphosate and 2.4-D) from 1968 on one side and on other side, the needs in phosphorus and nitrogen (P and N) in irrigable perimeter soils of Bounamoussa, constitutes a major constraint of good economic management, agronomical and environmental. Thanks to the bio-augmentation phenomenon, this study was conducted to estimate interactions effects between herbicides Glyphosate and 2.4-D (end-use formulation) and microorganism inoculum on assimilable phosphorus production, as well as on evolution of nitrate nitrogen in both agricultural soils (S1 of Beni Ammar and S2 of Maiz Bachir). Experiment focused on both herbicides which have been added to agronomical rate after conversion. During this work, we used as inoculum Pseudomonas aeruginosa (10^9 bacteria/l), a microbial strain which we have isolated from an adapted soil to herbicides degradation. After three months of incubation, the results obtained confirm significant variable effect of herbicides Glyphosate and 2.4 D. The survey showed that there is a toxic effect in non-inoculated treatments compared to the witnesses. Also, this investigation revealed that inoculation of Pseudomonas aeruginosa in both treated soils by herbicides allows an improvement and increase of P_2O_5 and NO_3^- quantities. In effect, stimulation rates recorded allow confirming that positive effect of this bacterial intervention process.

1. Introduction

The pesticides (herbicides, insecticides, fungicides....) are essential tools to agriculture; they help to fight against harmful insects, weeds, and contribute so in great quantity to economic food production [7- 3]. By contrast, if incorrectly used these herbicides may be very dangerous for soils, water, and environment and at last to human health by their residues. This is due to the fact that most of the insecticides are persistent because of their lipophilic properties [12 - 5] and therefore toxic [10 -18]. The organo-phosphorus (Glyphosates) are chemically very stable, resistant to the degradation and remain intact in the environment during several years at the same time their presence is unavoidable in the food chain [21]. In Algeria, the most used herbicides are Glyphosate and 2.4-D, notably in the irrigable perimeter of Bou Namoussa, from 1968 [8].

The Importance of fertilizing elements such as nitrogen and assimilable phosphorus, influences considerably on crop production. For these reasons, it is important to study herbicides' impact on the biological soils functioning (biogeochemical cycle of N and P). In our survey, we tried to develop a microbiological technique of decontamination or bioremediation of the soil; it consists to introduce a bacteria able to degrade herbicide, some say about bio-augmentation. Since microbial biomass of an agricultural soil plays an essential role in degradation and in mineralisation of inputs, of the organic matter and recycling of these nutrient elements available to the plants. This work consists to study effects of this method on bioavailability of assimilable phosphorus (P_2O_5) and nitrate nitrogen (NO_3^-) in irrigable perimeter of Bou Namoussa (IPB).

2. Experimental details

2.1 General presentation of the study area

The area study located in wilaya of EL-Tarf, Northeast of Algeria (Fig 1) is a part of tellian Atlas. This region is known for its agricultural vocation notably in the irrigable boundary of Bou Namoussa (cereals, market gardening). The region is characterised by a Mediterranean climate, mild and humid. The average annual rainfalls are 682 mm, for period going from 1993 to 2015 [8- 41]. For the same period, the coldest month temperature is of 10°c and the hottest month is of 25°c.

Figure 1: Location of the study region

2.2 Sampling and soils' physico-chemical characteristics

We selected two soils, one at Beni Ammar and the other at Maiz Bachir, At level of each experimental station, we have sampled two kg of arable layer ground (0-30 cm) using a soil auger. The sample has been dried at ambient temperature, crushed and sieved at 2 mm and subdivided into two sections, one for experimentation and other for determination of soils physico-chemical parameters. The results of physico-chemical analyses of both soils showed in the table below (Tab. 1).

Table.1: Physico-chemical characteristics of both soils of the study region de la présentation géographique du Périmètre irrigable de Bounamoussa (PIB)

Physico-chemical characteristics	**Measure unit**	**S 1of Beni Ammar**		**S2 of Maiz Bachir**
Granulometry	%	Sand	35	78
		Silt	15	10
		Clay	40	12
Classes texturale	Textural triangle (G.E.P.P.A) [23]	Sandy clay		Sandy
pH	-	7.54		7.65
Electrical conductance	µs/cm	99.00		68
Water retention capacity	%	32.54		25.25
Total limestone	%	T		T
K	ppm	173.10		57
Na	meq/100g	0.35		0.1
Mg	meq/100g	1.84		0.32
Ca	meq/100g	17.82		5.87
CEC	meq/100g	72		16.55
P (Olsen)	Ppm	8.30		1.23
N_T	%	0.18		0.08
C	%	2.13		0.8
MO	%	3.66		1.376
C/N	-	11.83		10

2.*3 Choice and characterisation of herbicides and their rates*

Choice is focused on two weed killers, commonly used in Algeria [12]. The Glyphosate: is a weak organic acid in white powder belonging to the chemical organo-phosphorous family, of a natural amino acid analogue. The glycine, endowed of a phosphonate grouping N-(phosphonomethyl (glycine), $C_3H_8NO_5P$ is a total foliar systemic weed killer. The Glyphosate is strongly absorbed into the soils; it has been degraded by microorganisms and may be more or less persistent.

Acid 2.4-*dichlorophénoxyacetique* (also noted 2.4-D): is a weed killer of chemical organo-chlorine of basic formula $C_8H_6Cl_2O_3$**.** Colourless crystal or white powder with no odour, selective against weeds but inactive on lawn and cereals, it prevents fruit drop and acts as growth hormone (anxin) on dying plants. It is one of contaminant of water, soils, air and rains, which we find also in the internal air. We retained agronomic rate (simple) of 2.5 µg for Glyphosate and 12.1 µg for 2.4-D.

2.4 *Preparation of Pseudomonas aeruginosa's isolate*

This name comes from *monas* which means *mobile rod.* This Gram negative bacterium with rounded edges can be grouped by pair or in short chains. It is commonly present in the soil and in water [30 - 2]. We adopted suspensions-dilutions method. We carried out a dilution serial from stock solution 10 g of soil 1 (soil 1 rich in OM and in microflora) + 90 ml of sterile water. Then we have spread these different suspensions on King B agar, selective for pseudomonoas, in order to estimate the cell concentration. After incubation of 48 h at 28°c, we obtain well-isolated colonies of Pseudomonas *aéruginosa* strain, stored in tubes containing an enriching environment of King B added with glycerol (25%) at - 20°c.

2.5 Experimental set-up and inoculation

This study has been carried out under controlled conditions at temperature of 28°c on a period of 90 days (03 months) in the oven and in the darkness. The experimental soil has been divided into two parts. One of these sections has been treated and crushed with both herbicides Glyphosate (H1) and 2.4-D (H2) (0.1 mg of herbicide kg 1 of soil introduced in water quantity of 2/3 of CR), and the other without herbicides. Then, the soil has been inoculated with *Pseudomonas aéruginosa's* strain previously isolated from an irrigable perimeter soil of Bou Namoussa and able to mineralise the Glyphosate and 2.4-D. Therefore, we have introduced 10^9 of bacteria/l in both soil types already containing a microbial population (non-sterile). The experimental set-up includes 12 treatments and 3 repetitions (for 36 treatments). We compared inoculated treatments (S1P, S1H1P, S2H2P, S2P, S2H1P and S2H2P) to those non-inoculated (S1, S1H1, S1H2, S2, S2H1 and S2H2). The mineralisation kinetics has been followed during experimentation period with the following time paces: 0, 3, 7, 14, 28, 42, 60 and 90 days. Each treatment underwent two dosages (P_2O_5 and $N0_3^-$).

2.6. Assimilable phosphorus determination

The assimilable phosphorus is made according to Olsen's method [26 - 27 - 23]. Extraction of phosphorus soluble forms is made thanks to the formation of carbonic acid by dissolution of sodium bicarbonate: orthophosphates anions react with ammonium molybdate in acid medium to give phosphomolybdic acid that is reduced by ascorbic acid in molybdenum-blue where absorbance is proportional to the concentration in phosphorus at 660 nm, by Spectrophotometr Genesys UVVis 10.

2.7. Nitrogen determination in the soil

Mineral nitrogen determination is made according to Drouneau and Gooney's method. The extraction solution of nitric nitrogen is calcium chloride ($CaCl_2$, 1N) or potassium chloride (KCl, 2N). On protocol plan, we collect an aliquot of 20 ml of filtrated solution to which we add 20 ml of sodium (NaOH, 6N). Afterwards we distil ammonium which is trapped by boric acid (2%) in presence of mixed colour indicator, the reactive of TACHIRO (purple in medium acid and in green in basic medium). Distilled nitrogen represents in fact nitrate nitrogen NO_3^- of the soil. Results of the mineralizing activity of germs are expressed in mg/kg [23].

2.8. Inhibition rate of herbicide or stimulation

The following formula for evaluation of inhibition percentage or of stimulation of given treatment compared to the witness is:

% of inhibition or of stimulation = % of treatment degradation / % of witness's degradation

When:

The rate is inferior at 1 ⟶ an inhibition (negative effect)
The rate is superior at 1 ⟶ a stimulation (positive effect)
The rate is equal ⟶ no effect

2.9. Statistical treatments
Static analysis has one factor and two controlled factors:
factor 1: soil
factors 2: herbicides and bacteria
The graphic representation of results has been made by Excel 2010 software.

3. Results and Discussion

3.1. Effect of Glyphosate and 2.4-D on bioavailability of assimilable phosphorus (P_2O_5).
During experimental incubation period of 90 days in laboratory, influence of both herbicides Glyphosate H1) and 2.4-D (H2) in both soils (SI Beni Ammar and S2 Maiz Bachir) on assimilable phosphorus production are recorded (Fig 2 and 3). After 90 days of incubation, cumulated contents produced in P_2O_5 are respectively 14.83, 13.87, 4.83 and 4.34 ppm in S1H1, S1H2, S2H1 and S2H2 compared to witnesses, for 18.86 and 6.33 ppm in soils S1 and S2 (Fig. 2 and 3). These herbicides seem to exert toxic effects against mineralising microflora of organic phosphorus in soils S1 and S2. This effect is more significant in treated soils by 2.4-D on phosphorus availability. Analysis of our results reveals that mineralisation of organic phosphorus depends on type and on physico-chemical quality of used herbicides types. Impact of Glyphosate and 2.4-D on bioavailability of assimilable phosphorus in the soil, justifies the decrease of acid-alkaline phosphatase enzyme activities [39]. Elimination of microbial strains is probably due to the herbicides toxic effect, on telluric micro floras [39 – 37 - 8]. In effect, [25 - 28] observed that effect of herbicides can reduce microbial biomass activity on a transitional basis in the first days after application. We also identified the presence of degrading microflora adaptation period (fig. 2 and 3). This difference is more noted for treatment with sandy soil S2 (Maiz Bachir), this is explained by a low content in humified organic matter in soils converts into humus thanks to living microorganisms called humificaters and by absence of loamy colloids.
After three months of incubation under controlled conditions, we distinguish two phases: The first one is called: phase of intense mineralisation after a month (from 1st day to 28 day), for treatments (S1, S2, S1P and S2P). From 14 days until 60 days for treatments S1H1, S1H2, S2H1 and S2H2 and between 7 - 42 days for treatments S1H1P, S1H2P, S2H1P and S2H2P. Flow of mineralisation during about 2 months of incubation release significant amounts of phosphorus (fig 3 and 4). During the period that spread between 60 - 90 days, we observe phenomenon of retrogradation which that means reductions and decreases of quantities of P_2O_5 of 0.9, 1.29, 1.73, 0.92, 1.17, 1.59, 1.84, 0.98, 4.48, 2.31, 1.86 and 2.24 ppm for treatments S1, S1H1, S1H1P, S1H2, S1H2P, S1P, S2, S2H1, S2H1P, S2H2, S2H2P and S2P. This slight decrease is due to the fact that in soil, phosphates enter in chemical reactions with sesquioxydes, calcium carbonate, and magnesium and turn into phosphate less assimilable or non assimilable but also to phenomena of chemical absorption and of retrogradation. Also it is due to phosphates ions fixation by microorganisms [4- 26 - 24].

3.2. Interactions effects between herbicides and inoculation of Pseudomonas aeruginosa on assimilable phosphorus production in the soils
A review of results shows in The histogram (Fig 3), use of an inoculation rate of *Pseudomonas aeruginosa* (10^9 bacteria/l) entails a speed increase of assimilable phosphorus production (fig 1 and 2) which allows gains of around 10.08, 2.07, 6.06 and 11.43 ppm in the treatments S1H1P, S1P, S2H1P and S2P against witnesses S1 and S2. It is linked to the chemical nature of Glyphosate herbicide that contains phosphorus. Content's increase of assimilable phosphorus is in accordance with works of [34- 35- 22 - 14]. In this case, interaction effect between 2.4-D and the inoculated bacteria is positive in both experimented soils (fig.2 and 3). Generally, injection of inoculum contributes to a significant, positive modification of mineralisation kinetics of organic phosphorus in the soils [1]. At the picture of results, contents in P_2O_5 in loamy soils**,** inoculated, treated with herbicides H1 are very high compared to sandy soils non-inoculate linked to (H2).
Concerning the relative importance of the interactive effect of Glyphosate and 2.4-D, and intervention of *Pseudomonas aeruginosa* on assimilable phosphorus bioavailability (P_2O_5) in soils (S1 and S2), the statistical

study (Table 2 and 3) based on variance analysis with two factors (*Pseudomonas aeruginosa* and herbicide) shows existence of significant difference (Fobs 1 = 2.87* and Fobs 2 = 2.97*) at mineralisation plan of organic phosphorus between systems (S1 and S2) and soils – herbicides - inoculum systems).

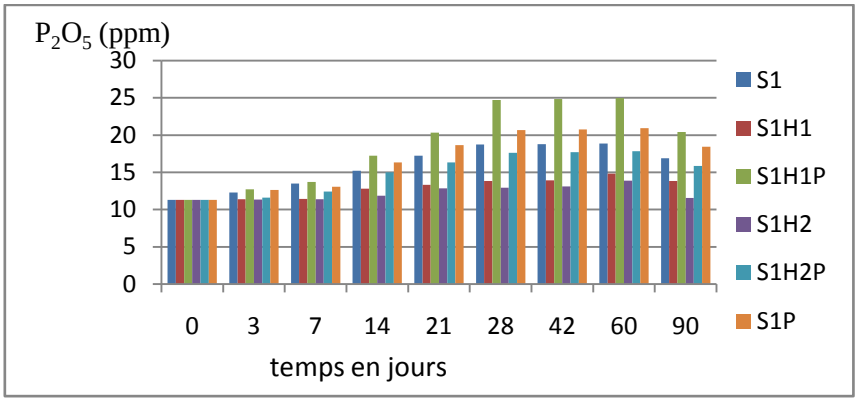

Figure 2: Interaction between inoculum of *Pseudomonas aéruginosa* and 2.4-D (H2) and the Glyphosate (H1) on bioavailability of assimilable phosphorus (P_2O_5) in the soil S1 (Beni-Ammar) of irrigable perimeter of Bou Namoussa (IPB) during an incubation period of 90 days.

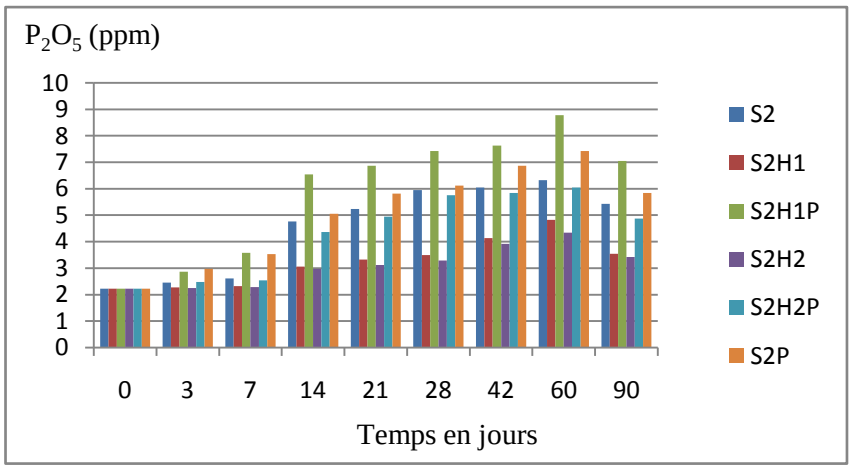

Figure 3: Interaction between inoculum of *Pseudomonas aéruginosa* et le 2.4-D (H2) et le Glyphosate (H1) on bioavailability of assimilable phosphorus (P_2O_5) in the soil S2 (Maiz el Bachir) of irrigable perimeter of BouNamoussa (IPB) during an incubation period of 90 days.

Table 2: Variance analysis (experience 1, organic phosphorus mineralisation in soil of Maiz Bachir)

Variation source	ddl	SCE	Middle square (MS)	F Calculated
factor	5 (K 1)	32150.01	4552.85	
Residual	42 (K2)	34932.3	1586.36	2.87*
totals	47	67082.31		

Table 3: Variance analysis (experience 1 organic phosphorus mineralisation in soil of Beni Ammar

Variation source (VS)	ddl	SCE	Middle square (MS)	F Calculated observed
factor	5 (K 1)	25075.99	20144.20	
Residual	42 (K2)	63167.1	2105.57	2.97*
totals	47	88243.09		

Tables 4 and 5 confirm inhibition and stimulation of *Pseudomonas aeruginosa* bacteria in treatments (soil – herbicides H1and H2) and P) towards the production of assimilable phosphorus (P_2O_5):

Table 4: Stimulation rate of *Pseudomonas aéruginosa* on assimilable phosphorus bioavailability (P_2O_5 in soil S1 (Beni Ammar) and soil S2 (Maiz Bachir) treated by Glyphosate (H1) and 2.4-D *Pseudomonas aéruginosa* (H2) of irrigable perimeter of Bou Namoussa during incubation period of 90 days

	SIHIP	SIP	S2H2P	S2P
% of simulation of pseudomonas	+ 32.29 %	+ 10.97 %	+ 38.70 %	+ 17.37 %

Table 5: Inhibition rate of *Pseudomonas aéruginosa* on assimilable phosphorus bioavailability P_2O_5) in soil S1 (Beni Ammar) and soil S2 (Maiz Bachir) treated by Glyphosate (H1) and 2.4-D (H2) of irrigable perimeter of BouNamoussa during an incubation period of 90 days.

	S1H1	S1H2	S1H2P	S2H1	S2H2	S2H2P
% Inhibition	- 21.37 %	- 26.46 %	- 5.41 %	- 33.70 %	- 31.44 %	- 4.58 %

Results recorded in table 4 show that the simulator inoculation effect of *Pseudomonas aeruginosa* assimilable phosphorus production **(**P_2O_5**)** in both soil took place. The simulation rate increases in the direction S1P < S2P < S1H1P < S2H2P. Overall, inhibition rates are always superior in the sandy soils than in loamy ones. This result is similar with works of [15 - 40].By contrast, [16] proves that degradation of Glyphosate is stimulated by fungi. Previous work of [11 - 20 - 13] prove that Pseudomonas sp ‘s bacteria PG 2982 degrade Glyphosate in sarcosine then in glycine by a cleavage of carbon-phosphorus bond.
Compared to the witness, this bacterium has stimulated degradation of Glyphosate and probably has used it as energy source. The stimulation by pseudomonas and other microflora contributes to the bioavailability of assimilable phosphorus; this phenomenon is called Bio-augmentation. Results of table 5, shows an inhibitor effect which is linked to the soils type. In non inoculated treatments the 2.4-D and Glyphosate may have no stimulator effect on evolution of P_2O_5 or imply an increase of significant inhibition, By contrast, we note that interaction effect between *Pseudomonas aéruginosa* and 2.4-D is very weak.

3.3. Effect of Glyphosate and 2.4-D on nitrate nitrogen production (NO_3^-).
After an incubation period of 90 days, analysis of cumulative histograms represented in figures 4 and 5 reveals that nitrate nitrogen quantity noted after 90 days of incubation is relatively higher. It is of 670 mg/kg in the soil 1 (loamy) and of 370 mg/kg in soil 2 (sandy). This difference is certainly due to effect of some physico-chemical and biological factors such as nature of nitrogen compounds, soil type, and structure and clay rates [35]. So, curves analysis pointed out those quantities of 470 and 410 mg/kg of nitrogen nitrate are respectively produced in non inoculated systems S1H1 and S1H2. However, we record in systems S2H1 and S2H2, quantities of 230 and 270 mg/kg (fib 4 and 5) This means that the effect of herbicide 2.4-D is relatively more depressive that effect presented by the Glyphosate in both soil S1, Ben Ammar and S2 Maiz Bachir. This seems to be due to the nature of intrinsic chemical constituents of these products. Works of [41- 39] prove that sulfonylureas induce a toxic effect against nitrifying germs, where growth and activity are much affected by this type of pesticide. This negative effect for tellien microflora has been observed in different works [38- 17].

3.4. Interactions effects between herbicides (H1 and H2) and inoculation of Pseumonoas aeruginosa on organic nitrogen mineralisation in nitrate (NO_3^-)
In the histogram (fig 5), we represented the kinetic mineralisation of organic nitrogen in nitrate (NO_3^-) obtained in both soils treated by Gluyphosate and 2.4-D, of Beni Ammar and S2, of Maiz Bachir). The average quantities of nitrate are significantly different and are superior in the inoculated treatments. It would be a high tolerance between this bacterium and the Glyphosate, used as a nutritive substrate by *Pseudomonas aeruginosa* (fig 4 and 5). Whatever soils type and physico-chemical characteristics of used herbicides (H1 and H2), input of this bacterium provokes increases of microbial activities, that is the Bio-augmentation [29 - 31- 32]. Decrease during the third month in linked to the phenomena of chemical absorption and immobilisation (the denitrification)

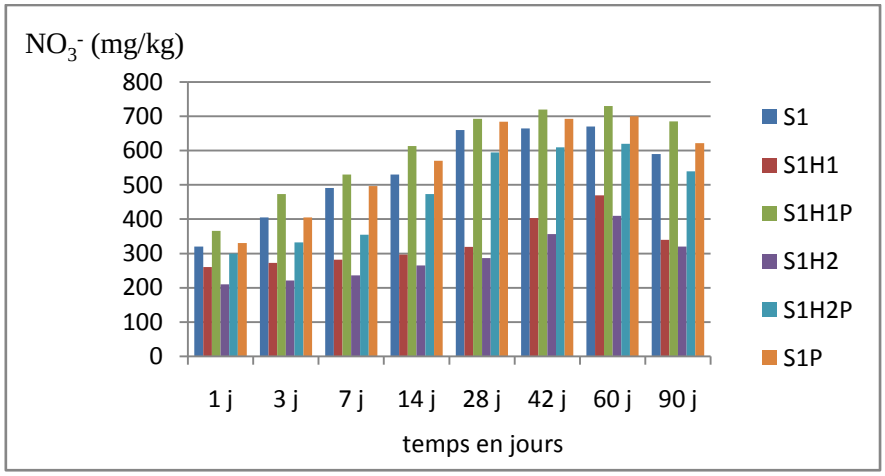

Figure 4: Interaction between inoculum of *Pseudomonas aéruginosa* and the 2.4-D (H2) and Glyphosate (H1) on the nitrification (NO_3) in soil S1 (Beni Ammar) of the irrigable perimeter of Bou Namoussa during incubation period of 90 days.

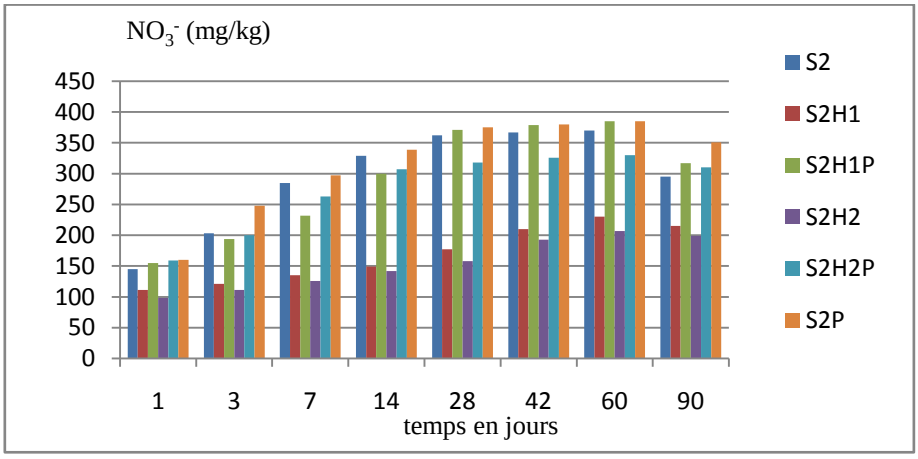

Figure 5: Interaction between inoculum of *Pseudomonas aéruginosa* and the 2.4-D (H2) and Glyphosate (H1) on the nitrification (NO_3) in soil S2 (Maiz Bachir) of the irrigable perimeter of Bou Namoussa during incubation period of 90 days.

Concerning the relative importance of the interaction effect of Glyphosate and 2.4-D and the intervention of *Pseudomonas aeruginosa* on nitrification (NO_3) in soils (S1 and S2), a statistical study based on variance analysis at two factors (*Pseudomonas aeruginosa* and herbicides) reveal existence of significant difference ($Fobs_1$ =4.74* and Fobs 2= 3.59*) on level of organic nitrogen mineralisation in nitrate (NO_3^-) between systems (S1 and S2) and systems (soils-herbicides-inoculum) in both following tables (tab.6 and 7).

Table 6: Variance analysis (experience 2 mineralisation of organic nitrogen in NO_3 in soil 2 of Maiz Bachir)

Variation source (VS)	ddl	SCE	Middle square (MS)	F calculated (observed)
factor	5 (K1)	32150.01	7116.8266	
residual	42 (K2)	34932.3	1498.25	4.74*
totals	47	67082.31		

Table. 7: variance analysis (esperience 2 mineralisation of organic nitrogen NO_3 in soil 1 of Beni Ammar)

Variation source (VS)	ddl	SCE	Middle square (MS)	F calculated (observed)
factor	5 (K1)	25076.99	8927.18	
residual	42 (K2)	63167.1	2486.68	3.59*
totals	47	88243.09		

Table. 8: Stimulation rate of *Pseudomonas aéruginosa* on nitrification of nitrogen (NO_3^-) in soil S1 (Beni Ammar) and soil S2 (Maiz Bachir) treated by the Glyphosate (H1) and the 2.4-D (H2) of irrigable perimeter of Bou Namoussa during an incubation period of 90 days

	S1H1	S1H2	S1H2P	S2H1	S2H2	S2H2P
% of Pseudonoas inhibition	- 29.85	- 38.81	- 7.46	- 37.84	- 44.15	- 10.82

Table. 9: Inhibition rate of *Pseudomonas aéruginosa* on nitrification of nitrogen (NO_3) in soil S1 (Beni Ammar) and soil S2 (Maiz Bachir) treated by the Glyphosate (H1) and the 2.4-D (H2) of irrigable perimeter of Bou Namoussa during an incubation period of 90 days.

	S1HP	S1P	S2H2P	S2P
% of pseudomonas simulation	+ 8.95	+ 4.48	+ 4.05	+ 4.05

It is noted that the effect of the Glyphosate and 2.4-D in loamy soils (S1) and sandy ones (S2) is traduced by an inhibition rate of the nitrification germs activity (table 8). We also noted that the mineralisation inhibition rates increase in the treatments by the following order: S1H2P < S2H2P < S1H1 < S1H2 < S2H1 < S2H2 (tab. 8). According to [17 - 21] a significant inhibitory effect provoked by Glyphosate has been observed. This decrease of microbial activity under effect of 2.4-D reminds trifluraline effect *(*sulfonylureas family*)* towards nitrification and nitrogen fixation [15 -19]. Concerning influence of inoculum in both soils spread by the Glyphosate and 2.4-D, we observe low inhibition rates going from 7.46% for S1H2 until 10.82 % for S2H2 where effect of the bio-augmentation.

The stimulation rate varies significantly (Tab 9). This microfauna's bio-stimulation by *Pseudomonas aeruginosa* demonstrates that this product can be used as nitrogen source for microorganism's nutrition firstly and secondly for vegetal growth [6] showed that Glyphosate stimulates nitrification. The similar observations have also been made by [33- 9 -2] concerning effect of this herbicide on nitrogen mineralisation in the soils. [6 - 13] observed that effect of Glyphosate input. Let's increase carbon biomass and soils contents in $N\text{-}NO_3$ are of 18% much higher than the non irrigated plots.

Conclusion

The Glyphosate and 2.4-D have a negative effect on production of P_2O_5 and NO_3^- in both soils, while herbicide 2.4-D exerts a more depressive action than that of Glyphosate towards microflora in both related soils from the point of view of texture and structure, loamy ground and other sandy in the irrigable perimeter of Bou Namoussa. Inhibition by these two herbicides decreases the two microbial activities concerning mineralisation of assimilable phosphorus and organic nitrogen in nitrate. By contrast, inoculation of *Pseudomonas aeruginosa* in both soils treated by herbicides allows the improvement and increase of P_2O_5 and NO_3^-quantities. In fact, stimulation rates recorded allow in agronomical and bioecological terms, influence of these two herbicides appears different on carbon and organic nitrogen mineralisation in the soils. The Glyphosate stimulates both microbial activities in loamy soils at bacterium's intervention.

Consequently, it would be interesting to use in perimeter of Bou Namoussa this kind of pseudomonas particularly in soils with loamy texture spread by the Glyphosate since the clay-humic complex of the soil contributes to a good mechanism management of organic matters mineralisation. Also, it should be avoided using of weed-killers particularly in sandy grounds, where its effect is very toxic, or then we should look into enriching soil in organic matter to reduce the negative impact of herbicides.

The strain of *Pseudomonas*, used for soils inoculation studied has significantly increased production of assimilable phosphorus (P_2O_5) and nitrates (NO_3^-). This important result encourages use of bacteria (*Pseudomonas aeruginosa*) on agricultural lands during desired periods where plants are in needs of fertilisers NPK (P_2O_5 and NO_3^-) to avoid pollution of surface and ground water by phosphorus and by nitrates.

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