International Journal of Innovation and Scientific Research
ISSN 2351-8014 Vol. 17 No. 1 Aug. 2015, pp. 99-113

http://www.ijisr.issr-journals.org/

Behavior of *Xanthomonas fragariae* in an inorganic medium enriched with N, P, or K

Tormal Djassinra, Abla Elhartiti, Zineb Mammad, and Khadija Ounine

Laboratoire de Microbiologie Appliquée, Faculté des Sciences, Université Ibn Tofaïl, BP 133, 14000, Kenitra, Morocco

ABSTRACT: In this study, the behavior of *Xanthomonas fragariae*, angular leaf spot of strawberry agent, was followed in the AB medium, enriched with nitrogen, phosphorus or with potassium, and in the soil of the Mamora forest with 14% to 28% of humidity in function of these fertilizer elements. The obtained results have shown that Na_2HPO_4 and NH_4Cl, used, 0.01 and 0.05 mol/L, respectively as a phosphorus and nitrogen source, have a significant effect on the survival of *Xanthomonas fragariae.* By contrast, KCl, used as a source of Potassium, has no significant effect on the number of culturable cells.
The three sources used NPK, 14% and 28% showed a great influence on the number of culturable cells of *Xanthomonas fragariae*, either increasing or decreasing. Potassium, at 28 to 14% of humidity, inhibited the rate growth of Xanthomonas, while the phosphorus and nitrogen stimulated its growth, greater than 28% of humidity than 14%. Similarly the bacterial growth was not affected during the incorporation of NPK at different concentrations in the soil of Mamora.

KEYWORDS: *Xanthomonas fragariae*, growth, fertilization, Nitrogen-Phosphorus-Potassium, soil, humidity.

1 INTRODUCTION

Angular leafspot, caused by *Xanthomonas fragariae* (Kennedy et King, 1962), was described in New Zealand, Australia, Asia and in Africa and in the majority of the European countries where the strawberry is cultivable (CABI/EPPO, 2013). In Morocco, angular leaf spot of strawberry, is responsible for considerable yield losses, it was reported first on strawberry in the region of Loukkous (Mdarhri, 2005). It causes the browning of sepals that dry, affecting the aesthetic quality of fruits. *Xanthomonas fragariae* attacks the leaves and migrates to the crown and roots of plants; they spread from the diseased plants to healthy plants in strawberry fields through irrigation, splashing rain, wet equipment, etc. The conditions for the disease are medium or cool temperatures during the day, night low temperatures and high humidity (Fisher, 2004).

In order to limit the damages induced by plants, several control methods are developed, mineral nutrition, for example, is much in demand as a means of prevention against many diseases (El Youssfi *et al.,* 2014; Wienhold *et al.,* 2009**)**. In this sense, despite the nitrogen is used to improve crop yields (Christiane *et al.,* 1999; Dossa *et al.,* 1991), but at the same time increases the sensitivity and susceptibility of plants to leaf diseases (Develash et Sugha, 1997; Doshi et Thakore, 1995; Vilain, 1993 ; Nacro *et al.,* 1997). Phosphorus has variable effects on performance. Repeated applications of phosphorus affect the useful microbial diversity over time and cause a drop in crop yields (Gyaneshwar *et al.,* 2002). As for potassium, it is able to reduce several plant diseases (Anderson, 2002).

The present study aims to investigate 1 / the relationship between fertilization and *in vitro* behavior of *Xanthomonas fragariae* in the AB media nitrogen-enriched, phosphorus or potassium and 2 / and its behavior depending on the humidity, 14% and 28% in soil amended nitrogen, phosphorus or potassium.

Corresponding Author: Tormal Djassinra

2 MATERIALS AND METHODS

2.1 PREPARATION OF THE BACTERIAL SUSPENSION

The used strain of *Xanthomonas fragariae* in this study is from the collection of the microbiology laboratory of the Faculty of Applied Sciences in Kenitra. It was isolated from strawberry leaves of the variety "Camarosa" grown in greenhouses of M'nasra (Gharb) (Djassinra *et al.,* 2012).

A preculture of the strain was carried out at 26 ° C on medium LPG, 24 hours later, a suspension of bacterial CFU / mL (OD = 0.1) at 620 nm was prepared (Turechek et Peres, 2009).

2.2 PREPARATION AND SOIL INOCULATION

Soil samples from Soil Mamora were taken, after eliminating the top 5 cm of the surface (15 to 25 cm of the vertical depth). The pH of the various soil samples was 7.4.

After screening, 20 g of soil are left in screw vials. They are sterilized at 121 ° C for 15 min. After cooling, the moisture content of the soil sterile tubes is adjusted to 28% or to 14% (P / V).

Each vial of soil supplemented with different concentrations of nitrogen source (NH4Cl), phosphorus (Na_2HPO_4) or potassium (KCl), was inoculated with 1 mL of the bacterial suspension of *Xanthomonas fragariae,* ajusted to 10^8UFC/mL corresponding to the exponential growth phase. Pots are then incubated in the dark at 26 ° C for 32 days.

2.3 COMPORTEMENT DE *XANTHOMONAS FRAGARIAE*

2.3.1 IN MINERAL AB MEDIUM ENRICHED WITH NITROGEN, POTASSIUM OR PHOSPHORUS

250 ml flasks containing 40 ml of AB mineral solution (1 g/L of NH_4Cl, 0.3 g/L de$MgSO_4$, 0.15 g /L of KCl, 0.01 g/L of CaCl2, 2.5 mg/L of $FeSO_4$) modified and supplemented of 1g/L of glucose (Emily Alexander *et al.,* 1999). They are complemented by different concentrations of Na2HPO4, KCl or NH4Cl, while the control is performed without these elements. They are then inoculated with a bacterial suspension of 108 CFU / mL and incubated at 26 ° C.

2.3.2 BACTERIAL COUNT

In order to achieve bacterial count of each sample, a series of successive dilution of 1/10 is carried out. After stirring, 0.1 of each dilution was cultivable on Petri dishes containing medium YPGA (Van Den Mooter *et al.,* 1990), the incubation is carried out in the dark and at 26 ° C for 24 days.

The bacterial concentration was expressed as CFU / mL using the following formula.

UFC/ml = NC / 0.1mL x Fd

CFU: colony forming units, NC: number of colonies, Fd: dilution factor. The results are expressed as log10 CFU / mL.

2.3.3 ANALYSES STATISTICS

All analyzes were performed with the software Statistics for Social Sciences (SPSS, version 21.0) and the Excel spreadsheet program (version 2013). Data were represented as mean ± standard deviation (SD) or median (interquartile range from 25 to 75).

The normality of distribution was tested by the Kolmogorov-Smirnov test. In the case of abnormal distribution, Mann-Whitney test was used to compare median between two independent samples. In the case of normal distribution and if the variances are homogeneous, the ANOVA, a factor between two or more independent samples, was used. Post hoc tests were used for multiple comparisons. P values <0.05 were considered significant.

3 RESULTS

3.1 BEHAVIOR OF *XANTHOMONAS FRAGARIAE* IN THE AB MINERAL MEDIUM ENRICHED N, P OR K

The evolution of the growth of *Xanthomonas fragariae* in the AB mineral medium, supplemented with different concentrations of Na2HPO4, KCl or NH4Cl, (0.01, 0.05 and 0.15 mol / l) is followed by counting the number of culturable cells in CFU / mL (Table I and Figure 1).**Table I: Evolution of the number of cultirable cells of *Xanthomonas fragariae* in the mineral AB medium supplemented with different concentrations of N, P, or K over time without moisture .The results are expressed as log10 CFU / mL.**

mol/L / Days	Control	KCl			Na_2HPO_4			NH_4Cl		
		0.01	0.05	0.15	0.01	0.05	0.15	0.01	0.05	0.15
T1	6,32	6.34	6.88	7.2	6.39	6.4	6.72	6.82	6.51	6.89
T2	6,9	6.42	6.51	6.5	6.4	6.45	7.91	7.26	7.5	7.72
T3	7	6.45	6.98	6.55	6.45	6.52	8.52	7.98	8.52	8.63
T4	7,8	7.81	7.1	7.56	6.51	6.71	8.77	8.77	9.2	9.44
T16	8,25	8.2	9	7.82	6.58	6.86	8.98	9.53	9.73	9.84
T24	9,11	8.88	9.6	9.98	6.66	6.98	8.92	9.61	9.88	9.98

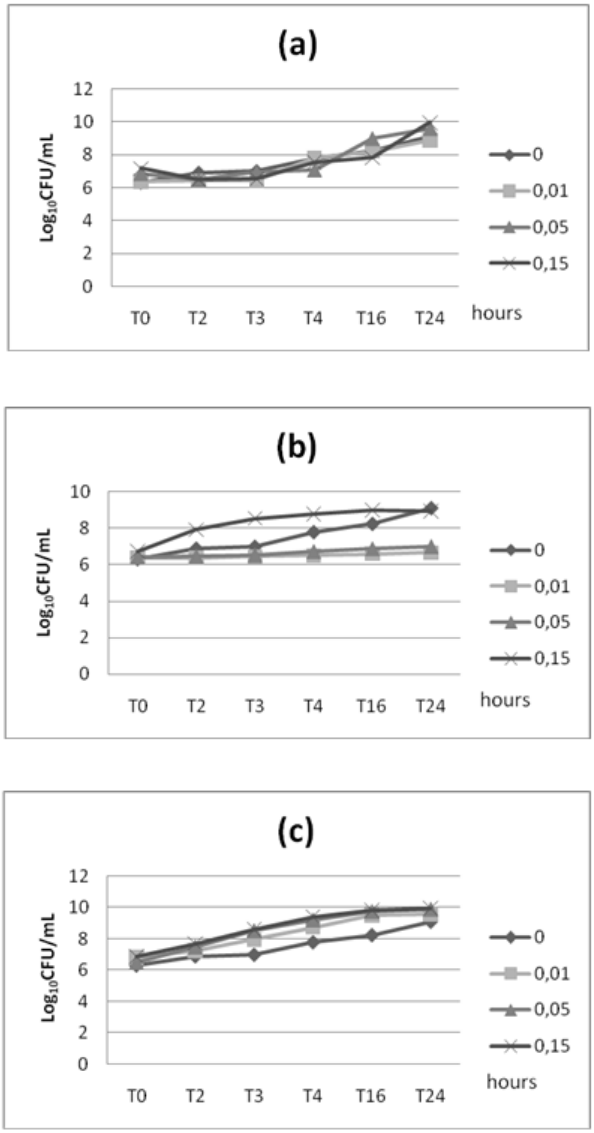


Figure 1: Growth of Xanthomonas fragariae in the AB mineral medium enriched N, P, or K; (a): KCl; (b): Na2HPO4; (c): NH4Cl.

The results in Table N° I show that the number of culturable cells of *Xanthomonas fragariae,* in the AB mineral medium modified and enriched with KCl, increases identically, both the control in media containing different concentrations of KCl (0.01 ; 0.05 ; and 0,15mol/l). As for Na2HPO4, there is a maximum increase in the number of culturable cells for concentration 0.15 mol / L and reaches a high level compared to the control, whereas the level of the increase is less than the control at concentrations 0.01 and 0.05 mol / L.

However, for the NH4Cl, there is an increase in the number of cultivable cells with a maximum of 9.61, 9.88 and 9.98 Log10UFC / ml after 24th days respectively for concentrations 0.01; 0.05 0,15mol / L (Figure 1 (c)).

3.1.1 EFFECT OF AB MINERAL MEDIUM ENRICHED WITH N, P AND K ON THE BEHAVIOR OF *XANTHOMONAS FRGARIAE.*

According to a multiple comparison by the Games-Howell test, the variance of the analysis to a single classification factor, shows that the survival of *Xanthomonas fragariae* is maximal at the concentrations of 0.01 to 0.05 mol / L of Na2HPO4 and NH4Cl. This analysis shows that these concentrations have a highly significant effect of 5% error on the survival of *X. fragariae* while the medium supplemented with different concentrations of KCl has no significant effect on the survival of this bacteria.

Table 1: Effect of different concentrations of potassium, nitrogen or phosphorus on the behavior of Xanthomonas fragariae

concentrations	solutions	signification	Std. Deviation	95% of the average confidence interval		Probability (P)
				minimum	Maximum	
0 (n=6)	**Na2HPO4**	7.56	1.02	6.49	8.64	1.000
	KCL	7.56	1.02	6.49	8.64	
	NH4CL	7.56	1.02	6.49	8.64	
0.01 (n=6)	**Na2HPO4**	6.50	0.11	6.39	6.61	0.015
	KCL	7.35	1.09	6.20	8.50	
	NH4CL	8.33	1.17	7.10	9.55	
0.05 (n=6)	**Na2HPO4**	6.65	0.23	6.41	6.90	0.021
	KCL	7.68	1.29	6.33	9.03	
	NH4CL	8.56	1.33	7.16	9.95	
0.15 (n=6)	**Na2HPO4**	8.30	0.87	7.39	9.21	0.248
	KCL	7.60	1.28	6.26	8.94	
	NH4CL	8.75	1.24	7.45	10.05	

ANOVA with one factor correction Welch.

Table 2: Comparison of multiple concentrations 0.01 and 0.05 mol /L potassium, nitrogen or phosphorus on the behavior of Xanthomonas fragariae.

Variable	I	J	significant difference (I-J)	Std. Error	Probability (p)	95% confidence interval	
						minimum	maximum
0.01	Na_2HPO_4	KCL	-0.85	0.45	0.231	-2.30	0.60
		NH_4CL	-1.83000*	0.48	0.027	-3.38	-0.28
	KCL	Na_2HPO_4	0.85	0.45	0.231	-0.60	2.30
		NH_4CL	-0.98	0.65	0.333	-2.77	0.81
	NH_4CL	Na_2HPO_4	1.83000*	0.48	0.027	0.28	3.38
		KCL	0.98	0.65	0.333	-0.81	2.77
0.05	Na_2HPO_4	KCL	-1.03	0.53	0.222	-2.72	0.67
		NH_4CL	-1.90333*	0.55	0.037	-3.66	-0.15
	KCL	Na_2HPO_4	1.03	0.53	0.222	-0.67	2.72
		NH_4CL	-0.88	0.76	0.500	-2.95	1.19
	NH_4CL	Na_2HPO_4	1.90333*	0.55	0.037	0.15	3.66
		KCL	0.88	0.76	0.500	-1.19	2.95

Both sources have a significant effect on *Xanthomonas fragariae* survival are Na2HPO4 and NH4Cl concentrations 0.01 and 0.05 mol / L.

3.2 BEHAVIOR OF *XANTHOMONAS FRAGARIAE* IN THE SOIL WITH 14% AND 28% OF HUMIDITY IN FUNCTION OF THE POTASSIUM SOURCE

In order to study the survival of *Xanthomonas fragariae* 14% and 28% humidity and the different concentrations of KCl, a comparison of the number of cultivable cells (log_{10}UFC / ml) is performed. The results are reported in Table II and Figure 2.

Table II: Evolution of the number of cultirable cells of Xanthomonas fragariae, in the soil of Mamora at 14% and at 28% of humidity and in function of different concentrations of potassium (KCl). Results are expressed in log_{10} CFU/ml.

	Numbre of cultirable cells (log_{10} CFU mL-1)							
Humidity	**14%**				**28%**			
mol/L / **days**	**0**	**0.01**	**0.05**	**0.15**	**0**	**0.01**	**0.05**	**0.15**
T0	1.18	1.3	1.44	1.64	1.26	1.5	1.62	1.73
T4	6.81	6.3	6.57	6.26	6.26	7.11	7.3	7.49
T8	6.94	8.32	8.5	8.76	7.06	8.46	8.61	8.95
T12	7.33	6.79	6.92	7.09	7.73	6.64	6.84	6.97
T21	7.64	6.24	6.46	6.52	7.90	6.57	6.53	6.6
T32	9.18	6.04	6.11	6.34	9.66	6.24	6.32	6.51

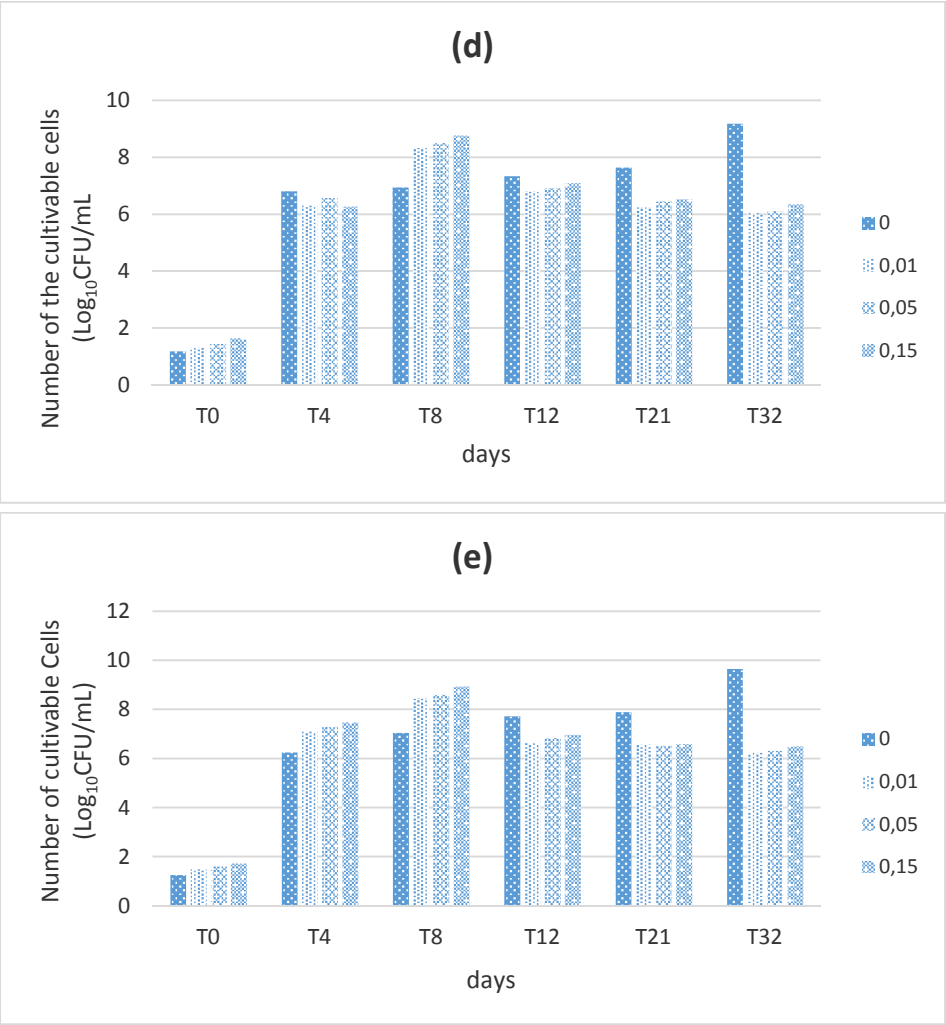


Figure 2 : Bahavior of Xanthomonas fragariae in the soil of the forest of Mamora enriched with potassium ; (d) : 14% Humidity, (e) : 28% Humidity

The evaluation of the survival of cultirable cells of *Xanthomonas fragariae*, in the presence of different concentrations of KCl, at 14% of humidity, passes through two phases during 32 days of incubation. For 0.01; 0.05 and 0.15 mol / L of KCl, there is a phase of growth where the number of cultirable cells increases for all three concentrations, with significant growth from 6.3 to 8,32$\log_{10}$UFC / mL and 6.57 to 8.5 $\log_{10}$UFC / mL and 6.26 to 8,76$\log_{10}$UFC / mL respectively for the above concentrations, followed by a regression phase where culturability *Xanthomonas fragariae* decreases from 8.32 to 6,04$\log_{10}$UFC / mL, from 8.5 to 6.11 $\log_{10}$UFC / ml and from 8.76 to 6,52$\log_{10}$UFC / ml for all three concentrations.

The same remark is observed at 28% of humidity, we note an increasement of the *Xanthomonas fragariae* growth during 8 days, this growth reached a level of the order of 8.46; 8.61 and 8,95log10UFC / mL, followed by a decrease in number of cultivable cells.

• Average Analysis

In order to study the behavior of *Xanthomonas fragariae* in the mineral AB medium in function of humidity, a comparison of the average is carried out, the results are shown in Figure 3.

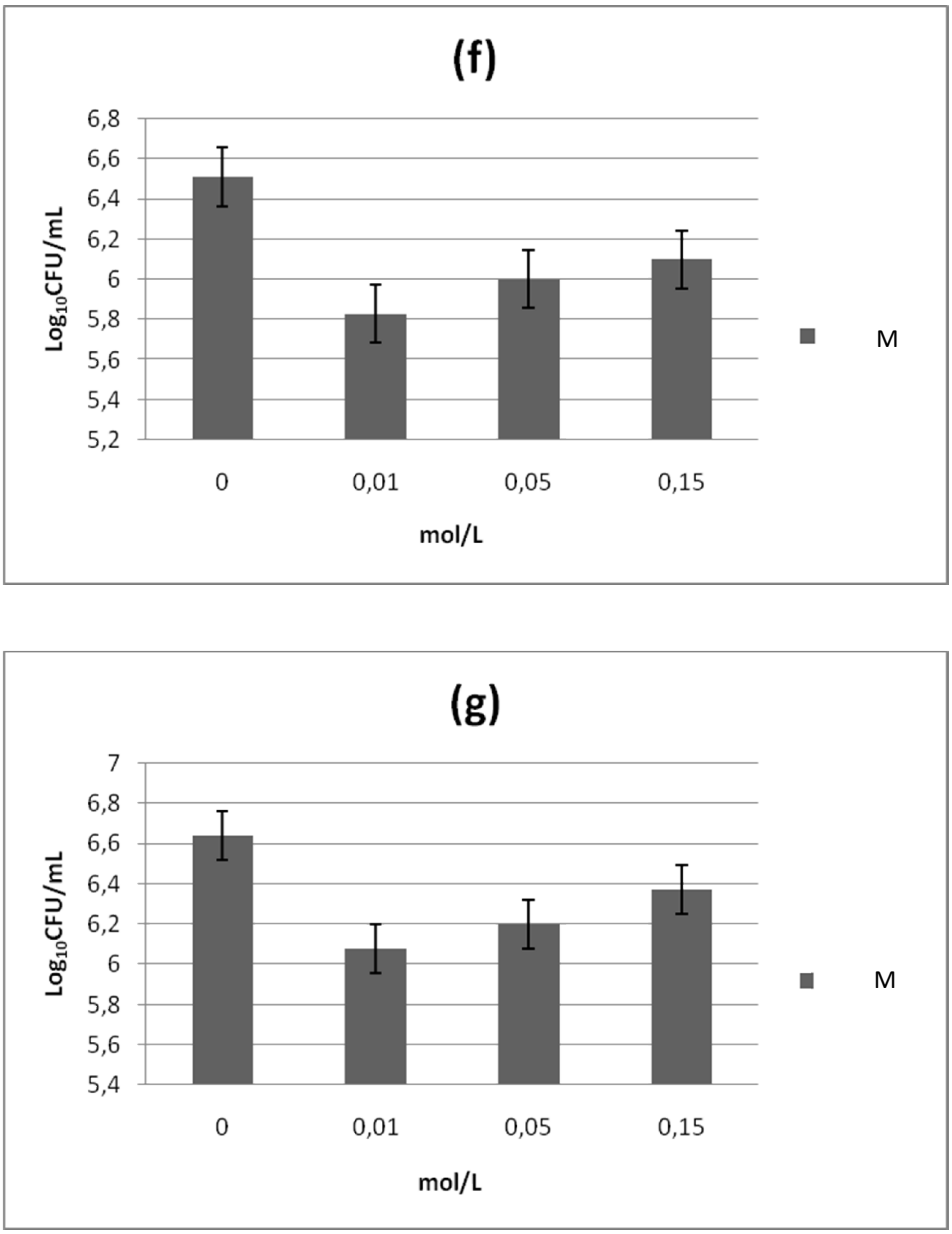


Figure 3 : Average of cultivable cells of Xanthomonas fragariae to the concentartions 0.01 ; 0.05 ; 0.15mol/L of KCl ; at 14% (f) and 28% of humidity (g).

The analysis of the average surviving cells of *Xanthomonas fragariae* in function of the concentrations of KCl, shows that the growth of the bacteria depends on the effect of the KCl concentration in combination with humidity. Indeed, at 14% of humidity, we observe a slight decrease in the average number of culturable cells compared to control that number is less important to 0,01mol / L, while it is important to 0.05 and 0.15 mol / L, but remains lower for the control.

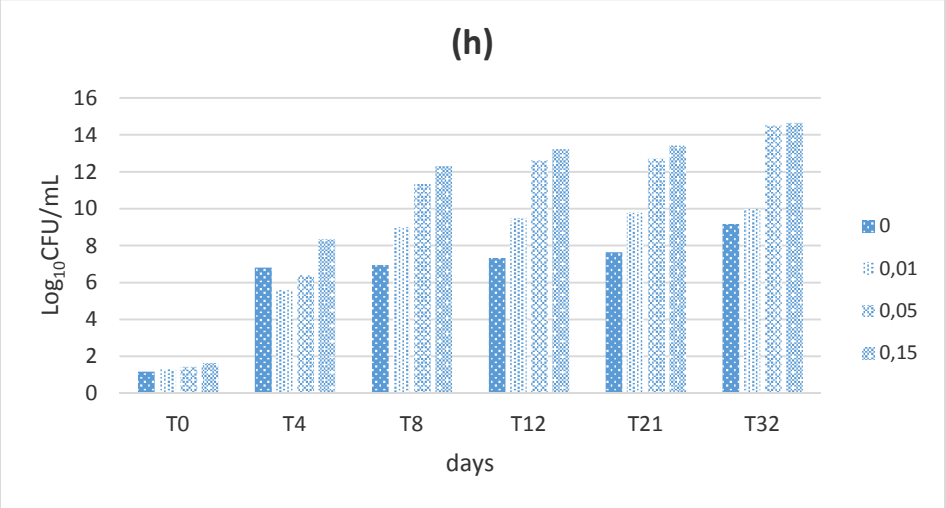
The same remark was observed in 28% of humidity, there is a decrease in the cells number of the bacteria to the concentrations 0.01 compared to control, while the maximum number of cultirable cells was noted for concentration of 0.05 and 0.15mol / L.

3.3 BEHAVIOR OF *XANTHOMONAS FRAGARIAE* IN THE SOIL AT 14% AND 28% OF HUMIDITY IN FUNCTION OF THE NITROGEN SOURCE

The results of the *Xanthomonas fragariae* survival, in the soil of Mamora at 28% and at 14% of humidity in function of different concentrations of NH4Cl are reported in Table III and Figure 4.

Table III: Evolution of the cultirable cells number of Xanthomonas fragariae, in the soil of Mamora at 14% and at 28% of humidity in function of different concentrations of nitrogen. The results are expressed in log_{10} CFU / mL.

	Number of cultivable cells (log_{10} CFU ml-1)							
Humidity	**14%**				**28%**			
mol/L / days	0	0.01	0.05	0.15	0	0.01	0.05	0.15
T0	1.18	1.3	1.44	1.64	1.26	1.5	1.76	1.9
T4	6.81	5.6	6.4	8.34	6.26	8	10.5	12
T8	6.94	9	11.34	12.3	7.06	12.01	11.78	13.2
T12	7.33	9.5	12.62	13.22	7.73	12.46	12	12.9
T21	7.64	9.79	12.71	13.41	7.9	13.8	13.8	14.7
T32	9.18	9.98	14.5	14.65	9.66	13.9	14.45	15



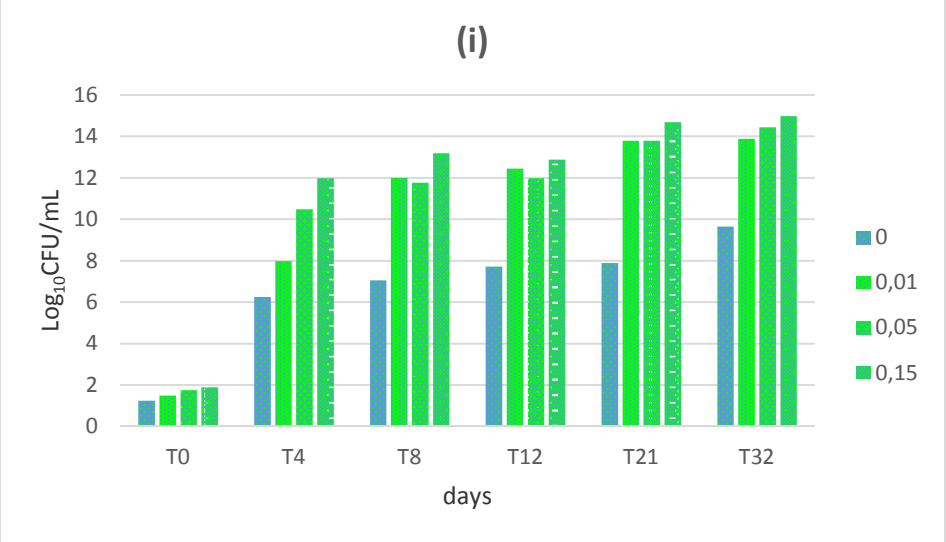


Figure 4 : Behavior of Xanthomonas fragariae in the the soil of Mamora floor enriched with nitrogen; (h) : 14% Humidity, (i) : 28% Humidity.

In general, we observe that the used three concentrations of NH_4CL in function of 14 and 28% of humidity constitute a favorable media to the cellular survival of *Xanthomonas fragariae*. In fact, the cultirable cellular number after 32 days of incubation vary from 14.5 to 14.65 log10 CFU/mL at 14% of humidity for the concentrations 0.05 and 0.15 mol/L and from 14.45 to 15 mol/L at 28% of humidity for the same concentrations. By cons, at the concentration 0.01 mol/L, the bacterial survival is totally different in function of humidity, at 28% of humidity, the number of the cultirable cellular number has known a maximal increase and attains a level in the order of 13.9; 14.45 and 15log10 CFU/mL respectively 0.01; 0.05; 0.15mol/L of NH_4CL that is not comparable with those of 14% that stays less important.

- **Means analyzes**

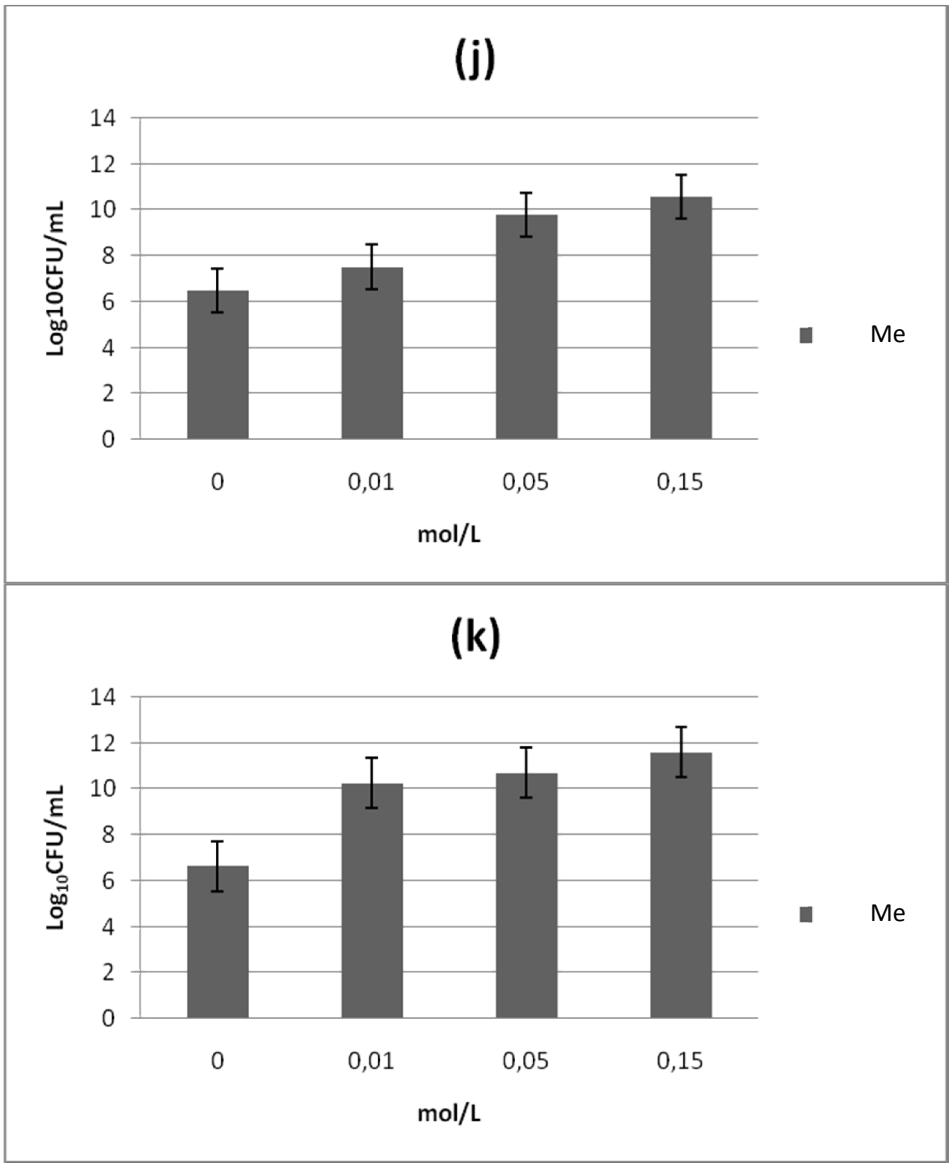


Figure 5 : Means number of the cultirable cells Xanthomonas fragariae at the concentrations of 0.01 ; 0.05 ; 0.15mol/L of NH_4Cl ; at 14% of humidity(j) et at 28% of Humidity (k) .

The analyzes of the cultirable cells in function of the NH_4Cl, shows that the growth of the *Xanthomonas fragariae* bacteria, in function of the humidity, depends of the NH_4Cl concentrations. In fact, about the figure number 5 ((j) and (k)) we notice an increase of the mean cultivable cells relative to the control, thus more the concentration of NH_4Cl increases more the mean of the cultirable cells increases.

3.4 BEHAVIOR OF *XANTHOMONAS FRAGARIAE* IN THE SOIL AT 14% AND AT 28% OF HUMIDITY IN FUNCTION OF THE PHOSPHATE SOURCE.

The survival of *Xanthomonas fragariae* is monitored during time, in different concentrations of phosphorus at 14% and 28% of humidity. The results of these experiences are consigned in the table IV and in the figure 6.

Table IV: Evolution of the cultirable cells of Xanthomonas fragariae, in the soil of Mamora at 14% and at 28% of humidity and in function of different concentration of phosphorus concentration. The results are expressed in log_{10} CFU/mL.

	Number of the cultirable cells (log_{10} CFU mL-1)							
Humidity	**14%**				**28%**			
mol/L / Days	**0**	**0.01**	**0.05**	**0.15**	**0**	**0.01**	**0.05**	**0.15**
T0	1.18	1.59	1.64	1,8	1.26	1.24	1.37	1.30
T4	6.81	4.45	5.11	6.45	6.26	5.45	6.81	8.14
T8	6.94	6.22	6.24	8.8	7.06	6.6	7.22	9.42
T12	7.33	7.35	7.86	9.3	7.73	7.36	8.65	9.8
T21	7.64	8.53	9.11	10.22	7.9	8.85	9.95	11.2
T32	9.18	9.32	10	12	9.66	9.11	11	13.13

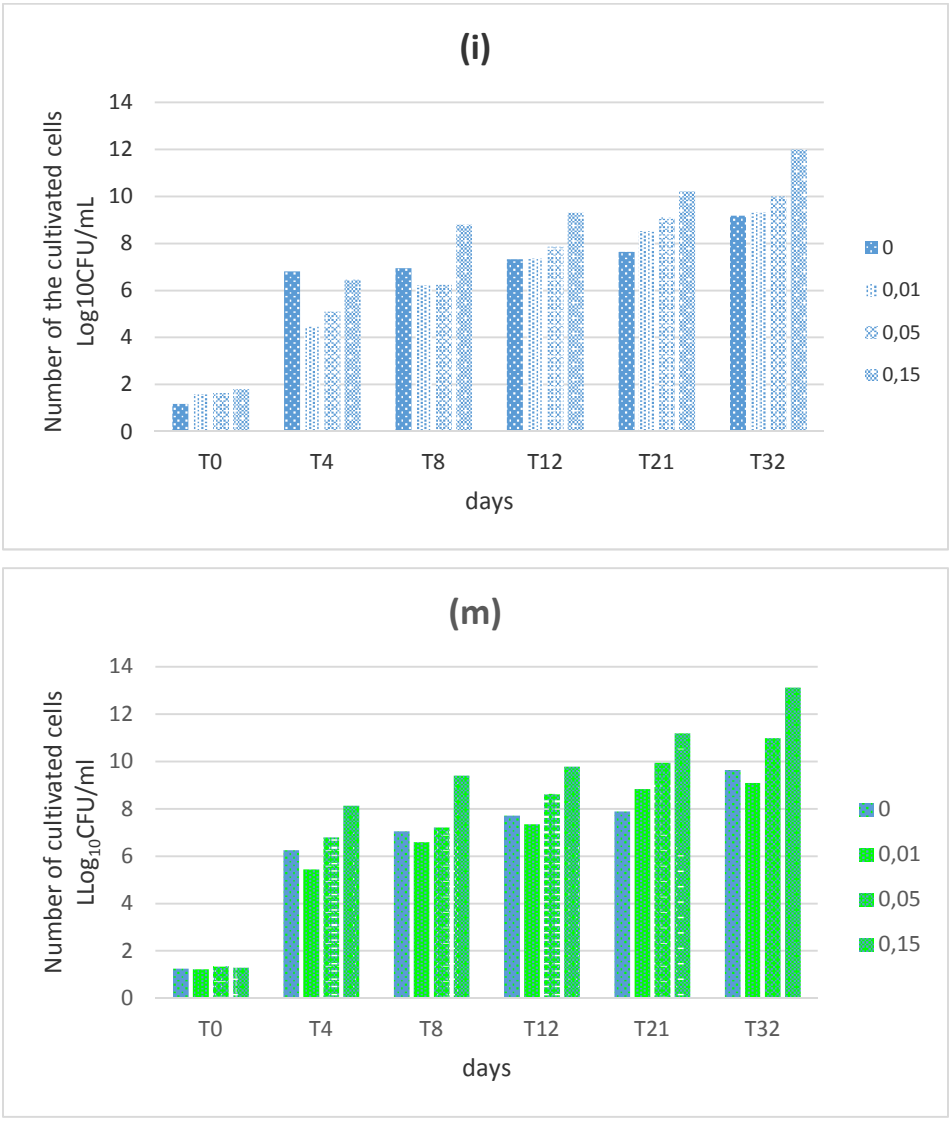


Figure 6: Behavior of Xanthomonas fragariae in the soil of the Mamora forest enriched with phosphorus; at 14% (l) and at 28% of humidity (m).

The analyzes of the table IV shows that the mean number of the cultivable cells has varied according to the tested concentrations of Na_2HPO_4 in function of humidity.

At 14% of humidity, during the first days of incubation, the number of *Xanthomonas fragariae* cells knows an increase of the cultirable cells number that attains a maximum in the 32th days. By cons, for the concentrations 0.01 and 0.05mol/L, the growth of *Xanthomonas fragariae* stays less important relative to the control (figure 6 (i)).

At 28% of humidity, we note that the cultirable cells number increases in function of the Na_2HPO_4 concentration incorporated in the medium, this increase seems that it depends on the concentration de Na_2HPO_4 in the modified AB mineral medium,: more the concentration increases more the rate of the growth increases.

- **Means analyzes**

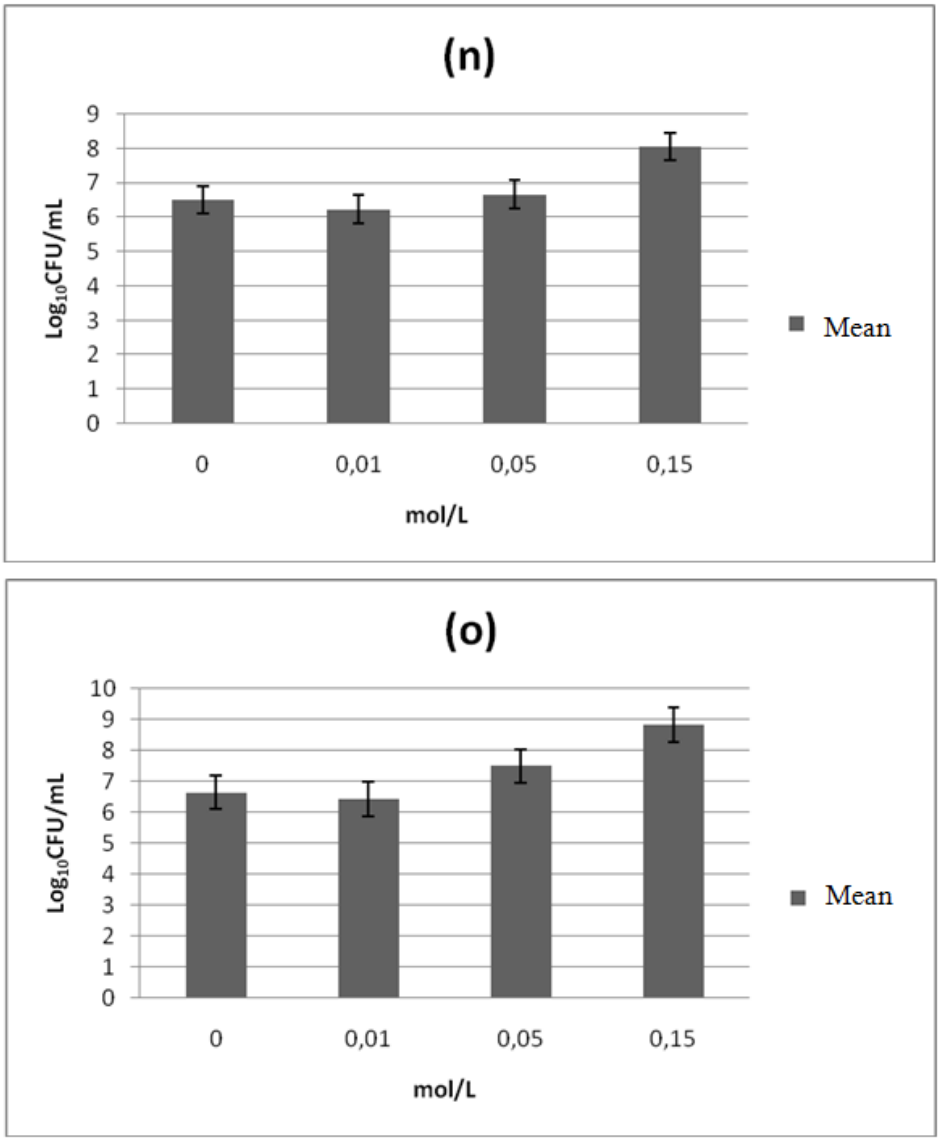


Figure 7 : Means of the cultirable cells of Xanthomonas fragariae in the concentrations 0.01; 0.05; 0.15mol/L of Na_2HPO_4; at 14% (n) and at 28% (o) of humidity.

The analysis of the cultirable cells at 14% of humidity, in function of different concentrations of Na_2HPO_4, revealed a decrease of the cells number of *Xanthomonas fragariae* in the concentration 0.01 relative to the control, but this number increases for the concentrations of 0.05 and 0.15mol/L.

At 28% of humidity, and at a concentration 0.01 of Na_2HPO_4, we note an accentuated decrease of the mean number of the cultirable cells of *Xanthomonas fragariae* relative to the control. By cons, in the case of the concentration 0.05 and 0.15 mol/L, the mean number of the cultivable cells is higher than the control.

3.5 EFFECT OF AZOTE, PHOSPHORUS, OR OF POTASSIUM COMBINED AT HUMIDITY OF 14% AND/OR 28% ON THE BEHAVIOR *XANTHOMONAS FRAGARIAE*

Table3 : Effect of different concentrations of azote, potassium or phosphorus combined with humidity.

solutions	Concentrations	14%	28%	Probability (p)
Na2HPO4	0	7.14(5.40, 8.03)	7.40(5.01, 8.34)	0.631
	0.01	6.24±2.86	6.44±2.89	0.910
	0.05	6.66±3.05	7.50±3.40	0.662
	0.15	8.10±3.58	8.86±4.10	0.738
KCL	0	7.14(5.40, 8.03)	7.40(5.01, 8.34)	0.631
	0.01	5.83±2.37	6.09±2.38	0.856
	0.05	6.00±2.38	6.20±2.39	0.886
	0.15	6.10±2.38	6.38±2.44	0.848
NH4CL	0	7.14(5.40, 8.03)	7.40(5.01, 8.34)	0.631
	0.01	7.53±3.46	10.28±4.81	0.282
	0.05	9.84±4.95	10.72±4.62	0.757
	0.15	10.59±4.89	11.62±4.89	0.725

The results of the analysis of variance in a single classification factor between the various concentrations of Na2HPO4, KCl or NH4Cl according to humidity.

4 DISCUSSION

The mineral or organic fertilization, pesticide application and useful microbial inoculations, aim to increase the productivity and economic performance (Shaharoona *et al.,* 2006). However, the side effects of these factors on bacterial organisms are often overlooked.

In our work we tested in one hand, the effect of different concentrations of nitrogen, potassium or phosphorus on the behavior of *Xanthomonas fragariae* and secondly the same combined effect humidity 14% and 28%.

Xanthomonas fragariae is able to survive in the soil sterile of the Mamora forest at 0.15 mol/L of Na_2HPO_4, and of NH_4Cl so this growth is inhibited in the concentrations 0.01, 0.05mol/l. We can suggest that 0.15mol / L concentration that gives cells a greater protective effect. The analysis of variance for a single classification factor shows that the concentrations 0.01 and 0.05mol / L causes an inhibition of *Xanthomonas fragariae* growth so that the growth is stimulated at 0,15mol/L. These results are similar to those reported par Shaharoona *et al*. (2006) that mentioned which specifically referred to the efficacy of *Pseudomonas* sp. with a significant increase in maize yields when plants receive adequate amounts of nitrogen. Martyniuk *et al.* (2009) have shown that the fertilized soil with mineral fertilizers improve microbiological activity of bacteria (number and respiratory activity of bacteria) and the biochemical properties of the soil. Furthermore, Meysam Beigzade *et al*. (2013) showed that there was a significant interaction between the application of phosphate fertilizer and bacterial growth. By cons, potassium has no significant effect on the growth of many bacteria of *Xanthomonas fragariae*.

Numerous studies have shown that potassium application is able to reduce the incidence of several pathogen genera of plants, case *Verticillium, Rhizoctonia, Fusarium, Plasmopa* (Anderson, 2002; Develash and Sugha, 1997; Angadi and Vijayakumar, 2000). Kelman (1953) noted that its application reduces high levels of glutamine and glutamic acid in tobacco plants sensitive to *Alternaria*, *Cercospora*, and *Sclerotinia*, which makes them less susceptible to these pathogens plants. The survival of several pathogenic bacteria in plant debris and soil has been studied by various authors. In many studies, survival varies with the pathogen in question and it is influenced by environmental factors such as pH, soil humidity, temperature, aeration and the interactions between them and between the microorganisms. Our study examined the survival of *Xanthomonas fragariae* in soil humidified to 14% or 28%.

The results showed that *Xanthomonas fragariae* is able to survive in different tested experimental conditions. Indeed, factors such as temperature, time, the sterility of the ground, do not affect the growth of these bacteria in the forest floor of Mamora (Kenza *et al*., 2014). Generally, the cells of *Xanthomonas fragariae* persist more time in the conditions of high humidity the in the conditions of low humidity of soil. The effect of humidity on the survival of *Xanthomonas fragariae* was not significant*;* this can be explained by the type of the used soil by a partial loss of soil organic matter for sterilization. However, the characteristic of a ground which seems to have more impact on the survival of the bacteria and water

retention, which is related to particle size distribution, organic matter content, which is confirmed by a study by Sinisa *et al*. (2007) that compared mortality *E. coli* in two soil types. In this case, bacterial mortality was mainly influenced by soil type (sterile and non sterile). They also noted that the improvement of moisture retention depends on the size of soil particles which may therefore increase bacterial survival due to increased water capacity. Tate, in 1978, also reported that the survival of *E. coli* in an organic soil over a period of 8 days after application of manure was larger than in sandy soil (soil Mamora). This was attributed in part to the richness in organic matter in the soil containing manure and greater ability to retain humidity.

However, other authors have reported a reduction in the severity and bacterial decay of *Ralstonia solanacearum* after application of a high dose of nitrogen in sandy soil fertilized with ammonia compounds was more effective (Kelman, 1953; Michel and Mew, 1998)**.** Beringer and Kay (1993) have made long-term studies on the survival of *Bradyrhizium japonicum* in Italian clay soil type and they found that the humidity in the soil is the most obvious factor affecting the level of population. The flooding leads to a dramatic decrease in population in the soil. In addition, fertilized soils with sufficient usable nitrogen maintain survival of Rhizobium in soil (Lochead and Thexton, 1953).

5 CONCLUSION

At the end of this work, we deduce that nitrogen and phosphorus stimulates growth rate of *Xanthomonas fragariae*. These are necessary for growing strawberries, but we must reason with the nature of nitrogen and phosphorus to be used to control the growth of this bacterium in greenhouses. This reasoning also embracing the potassium which has an inhibitory effect of the growth of *Xanthomonas fragariae,* and this with a view for improving performance. It is also interesting to know the effect of fertilizing elements on the growth of the bacteria and the development of the disease in order to consider ways to control much more adequate.

REFERENCES

[1] Anderson S.(2002). The Relationship between nutrients and other elements to Plant.diseases. Diseases Management pp. 26-32.

[2] Angadi, S.G., Vijayakumar, N.(2000). Influence of different sources and rates of potassium fertilizers on the disease incidence in Anab-e-Shahi grapes. Karnataka J. Agric. Sci. 13, 783–786.

[3] Beigzade. M., Maleki. A., Siaddat. S.A., Masoume. M.M.(2013). Effect of combined application of phosphate fertilizers and phosphate solubilizing bacteria onyield and yield components of maize single cross704. International Journal of Agriculture and Crop Sciences. 6 :17.1179-1185.

[4] Beringer JE. And Key HE.(1993). Monitoring the survival of Bradyrhizobium released into Italian soils First Meeting on Microbial ecology, Granada October PP: 24-27.

[5] Cabi and Eppo.(1998).Distribution maps of quarantine pests for Europe. CABI publishing, Oxon, UK. 355 pp.

[6] CABI/EPPO, 2013. Xanthomonas fragariae. [Distribution map]. Distribution Maps of Plant Diseases, No. October. Wallingford, UK: CABI, Map 520 (Edition 4).

[7] Christiane Raynal-Lacroix, Alain Bardet, Elisabeth Freixinos. (1999). La fertilsation azotée. Ctifl n°149.34-39.

[8] Develash, R.k., Sugha, S.K.(1997). Factors affecting development of downy mildew (Peronospora destructor) of onion (Allium cepa). Indian J. Agric. Sci. 67, 71–74.

[9] diseases. Diseases Management pp. 26-32.

[10] Djassinra T. Khouidi S., OulkheirS et Ounine K. (2012). Detection de Xanthomonas fragariae au niveau des serres de la région du Gharb, proceeding du 8éme congrès de l'association marocaine de protection des plantes-AMPP. Nov. rabat. 139-153.

[11] Doshi, A., Thakore, B.B.L. (1995). Influence of host nutrition on the development of downy mildew disease of opium poppy. Indian Phytopathol. 48, 335–338.

[12] Dossa, D.K. (1991). Contribution à l'étude de l'incidence de l'engrais azotée, de la rotation culturale et de la gestion des résidus de récolte sur le rendement du maïs (Zea mays), Mémoire d'ingénieur agronomie, UB-ESA, Lomé, 133 p.

[13] EL Youssfi B., Damir A. B oukchabine K. (2014). Vers une gestion raisonnée de la fertilisation pour le control des pourritures racinaires du blé causées par le Fusarium culmorum et le Bipolaris sorokiniana. proceeding du 9 éme congrès de l'association marocaine de protection des plantes-AMPP.449-459.

[14] Ezzirary. K, Ounine. K. (2014).Effet in vitro de *Xanthomonas fragariae* dans le sol de la forêt de Maamora. Science Lib Editions Mersenne, 6:2111-4706.

[15] Fisher. P.(2004). Les maladies des petits fruits et les stratégies de lutte, journées AGRI. Ministère de l'Agriculture et de l'Alimentation de l'Ontario (MAAO).

[16] Gyaneshwar, P., L. J. Parekh, G. Archana, P. S. Podle, M. D. Collins, R. A. Hutson and K. G. Naresh.(1999). Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphate solubilization by Enterobacter asburiae. FEMS Microbiol. Lett. 171:223-229.

[17] Kelman A. (1953). The bacterial wilt caused by Pseudomonas solanacearum. North Carolina Agricultural Experimental Station Technical Bulletin 99.

[18] Kennedy B.W., King T.H.(1962).Angular leaf spot of strawberry caused by *Xanthomonas fragariae* sp. nov. Phytopathology, 52: 873-875.

[19] Lochead A.C and Thexton R.H. (1953). Quantitative differences in three species of Rhizobium in soil of different fertilizer treatments. Journal of Bacteriology, 29:77p.

[20] Martyniuk S, Oron J, Martyniuk M. (2005).Diversity and numbers of root-nodule bacteria (rhizobia) in Polish soils, ActaSocietatis Botanicorum Poloniae. 74: 83–86.

[21] Mdarhri M. (2005). Isolement et identification de l´agent responsable de la maladie bactérienne des tâches angulaires du fraisier (Xanthomonas fragariae). Thèse 3ème Cycle Agronomie, Option Phytiatrie, Complexe Horticole, Institut Agronomique et Vétérinaire Hassan II, Agadir (Maroc), 38 pp.

[22] Michel V.V., Mew T.W.(1998). Effect of soil amendment on the survival of Ralstonia solanacearum in different soils.Phytopathology 88: 300-305.

[23] Nacro H.B.(1997). Hétérogénéité fonctionnelle de la matière organique dans un sol de savane humide (LAMTü, Côte-d'Ivoire): Caractérisation chimique et étude, in vitro, des Activités microbiennes de minéralisation du carbone et de l'azote. Thèse de Doctorat, Spécialité Ecologie Générale. Université Pierre et Marie Curie, Paris, 302p.

[24] Shaharoona AAZ, Muhammad Arshazachir B, Azeem Kalid A. (2006). Performance of *Pseudomonas spp*. containing ACC deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil Biology and Biochemistry*., 38(9): 2971-2975.

[25] Sinissa vidonic, Hushton C. Block., Drreu R. Korber. 2007. Effect of soil composition, Temperature, indigenous micoflore and Environmental conditions on the survival of Escherichia coli O157:H7.Journal of Microbial. 53:822-829.

[26] Tate, R.L.(1978).Cultural and environmental factors affecting the longevity of Escherichia coli in histosols. Appl Environ Microbiol 35: 925-929.

[27] Turechek, W. W., and Peres, N. A. (2009). Heat treatment effects on strawberry plant survival and angular leaf spot, caused by Xanthomonas fragariae, in nursery production. Plant Dis. 93:299-308.

[28] Van den Mooter M & Swings J. (1999). Numerical analysis of 295 phenotypic features of 266.

[29] Vilain, M. (1993). La production végétale. Vol 1 : Composantes de la production végétale, 205 p.

[30] Wienhold, B.J., G.E. Varvel, and W.W. Wilhelm. (2009). Container and installation time eff ects on soil moisture, temperature, and inorganic nitrogen retention for an in situ nitrogen mineralization method. Commun. Soil Sci. Plant Anal. 40:2044–2057. doi:10.1080/00103620902.