Yemeni Journal of Agriculture & Veterinary Sciences **(2013) 1(1):9—20)**

Faculty of Agriculture & Veterinary Medicine - Thamar university

Yemeni Journal of Agriculture & Veterinary Sciences

Available online at; WWW.YJAVS.COM

Effect Of Overwintering On Potato Wilt Bacteria Under Sana'a Conditions

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Article history:

Received
25 September 2013

Accepted
20 October 2013

ABSTRACT

Survival of potato bacterial wilt disease was investigated in 107 infected potato samples under winter conditions in Sana'a,Yemen. Results obtained on the effect of over wintering low teperature on *Ralstonia solanacearum* showed that the highest rate of the bacterium population survival was associated more with the monoculture of potato cv. Picasso than other cultivars with or without irrigation. The number of colony forming units (CFU) dropped rapidly to low levels at low temperature but not to undetectable levels, but with temperature increase, recovery of colonies occurred. Bacterial survival occurred on various crops or in the soil of unplanted pots, with or without irrigation. Wheat plants were the most effective in reducing bacterial population, as measured by the CFU count. Such data support the assumption of using crop rotation with wheat or bean plants is more effective in reducing the bacterial pathogen population as compared to no rotation or fallow soil. There was variability among the different bacterial isolates. Isolate 5 had the highest CFU, followed by that for isolates 6 and 4 with a mean of 37.61 $x10^4$, $34.17x10^4$ and $33.00x10^4$ (CFU), respectively. In five samples collected during winter, data showed that samples differed significantly in their associated bacterial populations, except for samples collected on January 28,2010 and on March 18,2010.

Key words: winter**,** *Ralstonia solanacearum***,** potato**,** Sana'a**.**

INTRODUCTION

Potato bacterial wilt caused by *Ralstonia solanacearum* Yabuuchi (1995) has become an endemic and serious constraint disease in potato producing areas. *Ralstonia solanacearum* is recognized as a soil inhabitant and is able to persevere in the soil environment through long crop rotations. French and Gutarra (1974) found that *P. solanacearum* race 3 strains could adapt to colder temperatures *in vitro*. They showed that race 3 isolate adapted for 20 days by growth at 16 ^{o}C. It later grew faster at 16 and 20 ^{o}C and also grew at 12 ^{o}C and survived at 8 °C, whereas the original culture did not. Populations of the *Ralstonia solanacearum* decreased more rapidly at 20°C compared with 15°C in sandy soil Wolf *et al*. (1998). A sharp, still unexplained, rise in bacterial population occurred when water temperature rose above 15 °C in spring Wenneker *et al*. (1999). Bacterial wilt (*P. solanacearum*) has spread into cooler areas, this spread may have been the result of the movement man of diseased tubers with low temperature adaptation of the pathogen progressively taking place as selective pressure resulted from progressively movement to colder climate French (1983) and Hayward and Hartman (1994). A cold climate resulting in average soil temperature of 14°C or below in tropical Kenya impeded the soil survival of *P. solanacearum* race 3 after introduction with infected tubers, when wilting had occurred (French,1986). Sunaina *et. al*. (1989) showed that *P. solanacearum* survived latently in low temperature (4°C) a deleterious effect on survival of the bacterium in lenticels and surface but could not completely eliminate the pathogen from tissues even after 240 days of storage. Temperatures of 4 °C, 36 °C, or 44 °C induced accelerated declines of the culturable cell numbers. Whereas the strain was able to undergo several cell divisions at 12°C, 20°C, and 28°C Elsas *et al.* (2001).

Hsu (1991) observed that bacterial *P. solanacearum* survived much longer in moist soil than in dry or flooded soil. Soil moisture content has a direct effect on population levels of *R. solanacearum*. The sampling in February could not be detected in any plots which correspond to very low soil moisture Arthy and Akiew (1999). Dry soil to significantly reduced levels of *R. solanacearum* Elsas *et al*., (2000). Hayward and Hartman (1994) found that bacterial wilt of potato is favored by high inoculums potential and high temperature, it can infect a few other crops (such as tomato) and a few different weed . Wilt disease incidence had a direct relationship with sesame yield Hazarika and Das (1999). Accelerated decline of CFU counts to undetectable numbers, and was obtained with -beans-maize-potato treatment Elsas *et al*., (2000). Lemaga *et al*. (2001) observed that the highest wilt reduction was with potato-maize-maize-potato successives. Beans probably a symptom less carrier of *R. solanacearum* can be a good rotation crop if used before or after cereals in a potato rotation. The major crop benefit and bacterial wilt suppression effects of the summer rotation of corn and soybean, while winter cover crops had less effect on tobacco yields Leeminn (2001). Rivard (2006) distinguished crop rotation with a non-susceptible host may provide some control, but this measure is difficult because of the wide host range of the pathogen and the considerable value of local agricultural lands._The present work was planned to study the remain effect of bacterial wilt pathogen under winter condition and study the effect of rotation crops in potato rotation system on potato plants bacterial infection

MATERIAL AND METHODS

Highly virulence isolates of potato wilt bacteria (*Ralstonia solanacearum*) which selective for used in this study were isolated from naturally infected potato tubers and potato rhizosphere were collected during August 2005 and April 2006, from 15 fields and from three stores at three locations (Thyshymeran, El-Manzel and Yarim) in Ga'a Al-hagl district Ebb Governorate (Table 1).

Table (1): Source of highly virulence potato wilt bacteria collected from different potato tubers and soil from Ga'a Al-hagl samples

Identified bacteria	Isolate code	Source
Ralstonia solanacearum	4,5 and 6	Ga'a Al-hagl

Over wintring studies:

The over wintering Top Roof Experiment:

Four crops were planted in black plastic pots which were exposed to the air on the top roof of one building at Agriculture College, Sana'a University in order to study survival of bacteria (*R. solanacearum*) causing potato wilt in potted soil under Sana'a winter condition with host plants such as: potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum*) solanaceous crops. Two cultivars of potato were used in this experiment. One is Picasso cultivar which is a hypersensitive cultivar to bacterial wilt disease, the other is Diamont cultivar which is a less sensitive cultivar to potato bacterial wilt, (El-Ariqi, 1996 and Bader, 2006). Another non-host plants for *R. solanacearum* such as bean (*Vicia faba*, Fabacea) and wheat (*Tritichum aestivum*,Poacea) Table (2), some pots were to leaved out without sown with irrigated or none according to Hazarika, and Das (1999). Bacterial suspension were added to mixture of sterile peat moss and soil (1:2) as bacterial populations into planted pots. Treatments were analyzed through five intervals according to Adollfo (1981) Sunania *et al.* (1989), Hsu, (1991), Anastasia (1993), Alcalá and Besty, (1998) and Lemaga *et al*., (2001).

Seeds were planted on 20 November 2006 in plastic pots of 20 × 16 × 10 cm. filled with sterile peat moss - soil mixture.

The experiment was arranged in complete randomized design (3×3×5×13) with three replicates per treatment. On the top roof of the building under Sana'a winter conditions, 117 treatments were kept in air as follows:

Picasso, Diamont, Tomato, Bean, Wheat, intercrop such as Picasso & Tomato, Picasso & Bean, Picasso & Wheat, Diamont &Tomato, Diamont & Bean, Diamont & Wheat and no seeding pots which treated with *R. solanacearum* were irrigated or not irrigated, (Kloss and Tumapon 1987). Treatments were watered semi daily.

Table (2): Crops used for the top roof experiment for potato bacterial wilt study under Sana'a winter conditions

Common name	Scientific name	Cultivar	family
potato	*Solanum tuberosum L.*	Picasso	Solanaceae
potato	*Solanum tuberosum L.*	Diamont	Solanaceae
Tomato	*Lycopersicon esculentum*	Tomato	Solanaceae
Bean	*Vicia faba*	Bean	Fabaceae
Wheat	*Tritichum aestivum* L.	wheat	Poaceae

Preparing the Laboratory:

Preparation of *R. solanacearum* Isolates:

For preparing the inoculate used in over wintering studies, glycerol nutrient agar (GNA) medium was used for growing highly virulence of *R. solanacearum* [No. 4, 5 and 6 isolates] , at 27°C for 48 hr. Bacterial cultures were suspended in 50 ml distilled sterilized water, individually, then 2 ml from homogeneous suspension was inoculated per 50 ml sterile glycerol nutrient broth [Beaf extract, 3 grams: Peptone, 5 grams : Glycerol,

20 ml : D. W 1000 ml] sterile in bottles size 300 ml (50 bottles per isolate). All inoculated bottles were incubated at 27˚C for 48 hr.

In December 5/ 2006, mixtures of peat moss-soil in pots were infected by adding 50 ml of bacteria suspension around the base of the plants per pot. Pots were watered after inoculation and kept on the top roof under Sana'a winter conditions.

Times of Picking up the Samples:

Viability of *R. solanacearum* in 117 top roof over wintering treatments was assessed. Samples were collected at five different times from all treatments in order to check the number of *R. solanacearum* colonies/g in bacterial mixture added into pots at different periods during winter months at mean of minimum and maximum air temperatures according to Payne and Waldran, (1983) and Sunaina, and Gupta (1998). In December 10, 2006, when the maximum temperature was 29 ˚C and the minimum temperature was 15˚C, the first sample was taken by approximately 1 gram collected from mixture (7 cm deep near rhizosphere) and added to 9 ml of sterile distilled water for every treatment (Elsas *et al.*, 2000). Samples were marked and taken to the laboratory. Then prepare a series of decimal dilutions of the suspension to 10^{-4} bacteria cell. The dilutions were done in laminar sterilized air flow chamber. About 1 ml of 10^{-4} cell suspension was transferred to marked sterile plates twice for each treatment. Then 15 ml of tetrazolium chloride (TZC) agar medium were poured into Petri dishes, then plates were moved to spread the suspension (Adolfo 1981).

Petri dishes were incubated at 27 ºC for 48 hours shaking plates after 24 h for making sure that no pollution because noticed the growth of *R. solanacearum* after 48 hr. Indirect count method was used to obtain the population densities or decreases by counting single number of colonizes. TZC to select *R. solanacearum* from the suspension according to Kelman (1954) and El-Ariqi (2001).

in January 6, 2007 when the highest temperature was 22ºC and the lowest temperature was 8ºC the second sample was taken using the same method mentioned previously.

In January 28, 2007 when the maximum temperature was 18ºC and the minimum temperature was 3ºC, the third sample was collected using the same method mentioned previously.

Similarly, in February 18, 2007 the maximum temperature was 26ºC and the minimum temperature was 13ºC, the fourth sample was collected. The lowest temperature during 29 and 30th January was 1ºC.

In March 18,2007 when the maximum temperature was 29ºC and the minimum temperature was 15ºC, the final sample was collected using the same method mentioned.

Obtained data were subjected to the one-way analysis of variance according to SAS statistical program. Furthermore, least significant difference (L.S.D) at 5 % probability was computed to compare the significant difference between means.

RESULTS AND DISCUSSION

Over wintering studies on *R. solanacearum* isolates:

An experiment was designed to determine the effect of over wintering coldness, several of planting crops (host and non-host plants) in seeded and non seeded pots, with watered pots and in pots non seeded but not watered treatments on the survival of *Ralstonia solanacearum* isolates. The study also included the microbial count analyses in soil at five different times during winter in relevance to wilt disease induction. Data representing colony forming unit presented in Plate (1).

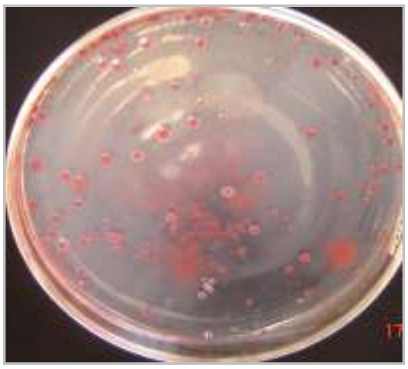

Plate (1): Numbers of *R. solanacearum* colony forming unit at 10^{-4} dilution on TZC medium.

Data in Tables (3) showed that the biggest population survival of *Ralstonia solanacearum* was associated more with the monoculture of potato Picasso cultivar followed by that associated in treatments such as: with bicultural types of Diamont & tomato, Diamont cultivar, Diamont & wheat, potato Picasso cultivar & bean, potato Picasso cultivar & tomato, potato Diamont cultivar & bean, potato Picasso cultivar & wheat, the monocultures of: tomato, no seeding without irrigation treatment, bean, no seeding but irrigating and pots seeded with wheat treatment alone. Means of colony forming unit for the previous mentioned treatments were: $39.27 x10^4$, $39.22 x10^4$, $39.22 x10^4$, $38.11 x10^4$, $36.56 x10^4$, $35.09 x10^4$, $34.73 x10^4$, $34.40 x10^4$, $33.89 x10^4$, $33.87 x10^4$, $32.22 x10^4$, $30.57 x10^4$ and $29.58 x10^4$ (CFU), respectively. There were no significant difference between treatments of potted potato Picasso cultivar alone, potato Diamont cultivar alone, potato Diamont cultivar & tomato, potato Diamont cultivar & wheat, potato Picasso cultivar & tomato and Picasso & bean treatments, but there was no significant difference between potted potato Diamont cultivar & bean, potato Picasso cultivar & wheat, tomato alone, no seeding without irrigation treatment, bean alone, no seeding and with irrigation treatment, and wheat alone treatment. However, significant difference between treatments such as: Diamont, no seeding without irrigation, bean and wheat treatments observed. In terms of isolates activities, isolate 5 had the highest colony forming unit, followed by that for isolates 6 and 4 with a mean of $37.61 x10^4$, $34.17 x10^4$ and $33.00 x10^4$ (CFU), respectively. There were significant differences between isolate 5 and the other two isolates (6 and 4). However, there was no significant difference between isolates 6 and 4. In five samples collected during winter, data showed that samples differed in their associated bacterial populations significantly except for these taken on January 28-2007 and on March 18-2007. Survived bacteria in samples taken at five different times during winter were present in tables (3) and in which they showed the following:

1- Numerous bacterial colony forming units (CFU)were observed on the first sample collected on December 10-2006 with a mean of $117.03 x10^4$ colony forming units. The effect of

December 10-2006 collection was as expected, where initial population levels showed high incidence because the samples were collected only five days after adding bacterial mixture suspension into the potted plant and the temperature was convenient. These results are identical with Jian (2007) who found that *Ralstonia solanacearum* is a soil borne plant . Data in the same tables showed that mean of (CFU) was maximum for isolate No.5 in tomato pots treatment with a mean of 143.33 $x10^4$ (CFU) and minimum in wheat treatment with a mean of 99 $x10^4$ (CFU), followed by isolate No. 4 which gave a maximum value for Diamont potato cultivar & wheat treatment with a mean of 141.67 $x10^4$ (CFU) and minimum in Diamont alone with a mean of 91 $x10^4$ (CFU) followed by isolate No. 6 performance, where its maximum activity was for no seeding without irrigation treatment as maximum mean (CFU) given was 126.00 $x10^4$ and the minimum was given by treatment of wheat alone with a mean of 92.67 $x10^4$ (CFU).

2- In January- 6-2007, samples collection showed a fast population decrease in every treatment with a mean of 21.77 $x10^4$ (CFU). The drop effect of colony forming unit viability during this time of sample collection was due to temperature decrease (22°C) at maximum and 8°C at minimum. Results obtained were in accordance with those reported by Joe (1986) who reported that populations of *Pseudomonas solanacearum* fell rapidly at lower temperatures and wilt was not clear in the assays completed. Results also were coincided with Hsu (1991) who observed that wilt was much less destructive during winter and that was supported by data observed by Elsas *et al.* (2000) and Leeminn.(2001). The results clearly indicated that (CFU) survived with larger numbers in potted host than in non host pots. More decreases were observed in non host plant potted due to root exudates of host plants which enhance and direct bacteria motility towards the plant roots. Therefore, the ability of bacteria to survive under winter conditions was enhanced. Data obtained are in agreement with Jian (2007) who reported that *Ralstonia solanacearum* is specifically attracted to diverse compounds, and especially to root exudates from a host plants. Such data are coincided with Rivard (2006) who distinguished crop rotation with a non-susceptible host may provide some control against bacterial wilt. Also data are coincided with findings reported by Leeminn (2001). The mean of colonies activity of isolates showed that the isolate No. 4 had a maximum value in case of Picasso potato cultivar treatment with mean of 42.50 $x10^4$ (CFU) and minimum activity recorded for pots with no seeding plus without irrigation treatment. Isolate No.5 had a maximum activity value for pots with Picasso potato cultivar & beans plants with mean of 35.33 $x10^4$ (CFU), and minimum in bean or
no cultivar no irrigation with mean of 9 colony forming unit. Isolate 6 got a maximum value in case of tomato with a mean of 26.33 $x10^4$ (CFU) and minimum in Diamont potato cultivar & wheat with a mean of 14.33 $x10^4$ CFU.

3- The results on Jan-28-2007, showed more decrease in (CFU) of *R. solanacearum* than on Jan-6-2007 with a mean of 13.68 $x10^4$ (CFU) this was computable with decrease of temperature 18ºC at maximum and 3ºC at minimum degree. Isolate No. 4 had a maximum value in Diamont potato cultivar & tomato treatment with a mean of 26.00 $x10^4$ (CFU) and a minimum in no seeding without irrigation pots treatment with a mean of 2.33 $x10^4$ (CFU). Also isolate No.5 showed a maximum mean of 25.00 $x10^4$ (CFU) in Picasso potato cultivar & bean and a minimum mean of 6.00 $x10^4$ (CFU)in no seeding without

4- Colony count samples collected on Feb.-18-2007 was extremely low with a mean of 7.89 $x10^4$ (CFU). The decrease in colony forming unit count in non host plant pots was more than in host plant pots, and there were no visible symptoms of wilt disease on host plants. There were higher colony counts of *R. solanacearum* population units in potato cultivar pots than in bean and wheat pots treatment. The highest decrease was recorded for irrigated but not seeded pots treatment**.** Such decrease reflects the fact that *R. solanacearum* colonies do not mutate and cold tolerant mutant colonies under over wintering conditions. Moreover, the temperature went down to 1°C before collecting the samples. Data obtained agree with these found by Elsas *et al.*, (2000), who found that *R. solanacearum* race 3 biovar 2 population was severely reduced at 4°C. Isolate NO,5 had a maximum mean survival value 18.00 colony forming unit in Diamont potato cultivar & Tomato pots treatment and a minimum value 3.33 $x10^4$ colony forming unit in case of no seeding without irrigation pots treatment, followed by isolate No.6 which gave a maximum mean of 11.33 $x10^4$ (CFU) in case of potato Picasso cultivar pots treatment, and a minimum mean of 4.00 $x10^4$ (CFU) in pots with no seeding and with irrigation treatment, followed by isolate No. 4 which yielded a maximum mean of 12.00 $x10^4$ (CFU) for Diamont potato cultivar treatment and a minimum of 2.67 $x10^4$ (CFU) in case of no seeding with irrigation pots treatment.

irrigation pots treatment, followed by isolate No.6 with a maximum mean of 21.00 $x10^4$ (CFU) in Picasso potato cultivar and a minimum mean of 6.67 $x10^4$ (CFU) for wheat treatment.

5- Samples collected on march-18-2007 showed enhancement of isolates populations in all treatments compared to these prevailed on Jan-28-2007 and Feb.-18-2007. The mean (CFU) collection mean for all treatments was 15.09 $x10^4$ value. Data in tables (3) showed that colony forming unit enhancement in potato pots treatment was in contrast with other treatments. Also, visible symptoms of wilt disease was evident on some host plant such as Picasso potato cultivar and tomato only when the temperature was 15 ^{o}C at minimum level and 29^{o}C at maximum level which is considered to be appropriate degrees for wilt disease incidence. Such results are in accordance with these reported by Arthy and Akiew **(**1999) who found that the colonies sampling in February could not be detected in any pots corresponds to very low soil moisture at the time of sampling. Following good rain and high temperatures, the level increased in March. Also data are coincided with that reported by Sunaina and Gupta (1998) who reported that the number of *R. solanacearum* colonies/g soil was much greater in samples collected during hottest and wetted summer months with mean minimum and maximum air temperatures ranging between 20.5°C and 28°C. By observing the mean of isolates function, isolate No,5 had the maximum value in Picasso potato cultivar & bean pots treatment with mean of 35.67 $x10^4$ (CFU) and a minimum in no seeding with irrigation pots treatment with mean of 4.33 $x10^4$ (CFU) followed by isolate No.6 with a maximum mean of 26.00 x10^4 (CFU) in bean treatment and a minimum mean of 2.33 x10^4 (CFU) in no seeding with irrigation pots treatment, followed by isolate No. 4 with a maximum mean of 25.00 x10^4 (CFU) in Diamont potato cultivar treatment and a minimum mean of 2.00 x10^4 (CFU) in no seeding with irrigation pots treatment.

It was concluded that, despite of bacteria survival occur in soil on February-18-2007, there were no observed symptoms of wilt disease on host plants and most of (CFU) were avirulent. However, there were visible symptoms on potato Picasso cultivar on March 18-2007. Results presented also proved that keeping bacteria at various winter temperatures demonstrate races ability to survive for a long time at low temperatures. Such results are in accordance with Hayward and Hartman (1994) who suggested that the wider distribution of strains of *Pseudomonas solanacearum* were tolerant to relatively cool temperatures. They are also in agreement with Olsson (1976) who observed at northerly latitude in which bacterial wilt has been reported during the over wintering of latent infection on *Solanum dulcamara* inoculated plants at Solna, Sweden, more than 59ºN where soil freezes for 2 months each year. Data also generally clarify that bacterial survival occurred on various crops or in unplanted pots such as no seeding with irrigation pots treatment and no seeding without irrigation pots treatment implemented. These results agree with Alcalá and Betsy (1998) who showed that survival of the bacterial wilt pathogens for long periods exist in the absence of the host. Populations of (CFU) dropped rapidly to low levels at low temperature but not to undetectable levels. By enhancing raise of temperature degrees, they can recover. Wheat plants were the most effective in reducing bacteria as mean of 39.58 x10^4 (CFU) was detected. Such data support the assumption of having rotation crops with wheat and bean plants are better than having no rotation with host plant treatment or unplanted treatment. This phenomenon should be considered when choosing rotation crops in an attempt to manage bacterial wilt disease in the field. Results obtained are coincided with Devaux *et al.,* (1987) who reported that in Rwanda a one-crop rotation with any one of the five other principles crops grown there greatly reduced wilt disease incidence. Data also proved that the reduction of colony forming unit of intercropping host & non host on Picasso potato cultivar plants is due to the effect of non host plant but this was not observed in case of Diamont potato cultivar or tomato which agrees with Kloss and Tumapon.(1987), who reported an intercropping effect in the Philippines reduced build-up of *Pseudomonas solanacearum* compared with monoculture potato system. Also, this is in agreement with data reported by Autrigque and Potts, (1987) and Lemaga *et al*. (2001). The highest and rapid progress of bacterial wilt in potato monoculture could be attributed to the increase of pathogen population. There was more progress achieved on non host pots of bean treatment than unplanted pots treatment. Data obtained were in agreement with these reported by Anastasia (1993), who found that the pathogen survive in larger numbers in planted soil rather than in non-hosted soil such as beans, cowpeas, wheat, and maize, and than in soil free of plants. Larger bacterial populations obtained in dry soil than irrigated soil indicated that dry soil may keep the bacteria survival, and such data obtained are in contrary to findings reported by Hsu (1991), who reported that *R. solanacearum* survived much longer in moist soil than in dry or flooded soil. It is also in contrary to Arthy and Akiew (1999), who suggested that population levels reflect soil moisture content in the same month. Picasso potato cultivar was associated with large numbers in soil due to being as a hypersensitive host and such finding agrees with data studed by El-Ariqi (1996) and Bader (2006).

Table (3): Effects mean of winter weather on colony forming unit CFU (10^4) of *Ralstonia solanacearum* monthly during winter season

Host	Isolates	Tim					Isolates effect	Plants effect
		10\12\06	**6\1\07**	**28\1\07**	**18\2\07**	**18\3\07**		
Potato	4	108.00	42.50	15.33	10.33	22.33	33	
(Picasso cv.)	5	130.00	31.67	17.33	14.00	27.33	37.61	39.27
	6	115.00	28.33	21.00	11.33	16.33	34.17	
Potato	4	91.00	16.67	16.00	12.00	25.33	33	
(Diamond)	5	123.67	22.00	20.33	15.00	28.33	37.61	39.22
	6	119.00	20,33	13.33	9.67	16.00	34.17	
	4	96.33	16.33	6.33	433	6.67	33	
Tomato	5	143.33	24.33	16.00	8.33	17.00	37.61	33.89
	6	109.67	26.33	13.00	5.33	10.00	34.17	
	4	109.67	15.67	10.00	6.33	4.33	33	
Bean	5	126.67	9.67	13.67	7.67	9.67	37.61	32.22
	6	109.33	19.00	10.00	5.67	26.00	34.17	
	4	108.00	10.33	6.67	7.00	10.00	33	
Wheat	5	99.00	27.67	13.00	7.33	16.00	37.61	29.58
	6	92.67	24.00	6.67	5.67	9.67	34.17	
Potato	4	109.00	29.00	10.00	7.33	11.67	33	
(Picasso)&	5	121.00	32.00	13.33	8.00	12.33	37.61	35.09
Tomato	6	112.00	23.67	18.33	6.00	11.67	34.17	
Potato	4	107.33	16.67	11.33	4.00	16.00	33	36.56
(Picasso)&	5	121.00	35.33	25.00	8.67	35.67	37.61	
Bean	6	114.33	25.00	8.67	5.33	14.00	34.17	
Potato	4	134.33	17.33	5.67	4.33	10.00	33	34.40
(Picasso)&	5	130.67	20.33	14.67	10.00	16.00	37.61	
Wheat	6	109.33	16.67	12.33	4.67	9.67	34.17	
Potato	4	117.00	28.67	26.00	10.33	20.33	33	39.22
(Dimond)& Tomato	5	114.67	22.33	15.00	18.00	13.67	37.61	
	6	125.00	21.67	19.67	12.33	23.67	34.17	
Diamond	4	101.33	16.33	17.67	9.00	11.00	33	
&	5	126.33	17.67	13.67	11.67	20.67	37.61	34.73
Bean	6	117.33	17.67	16.00	6.33	18.33	34.17	
Potato	4	141.67	35.00	24.67	8.33	25.33	33	
(Diamond)&	5	118.33	24.00	15.67	7.00	9.33	37.61	38.11
Wheat	6	105.00	14.33	11.67	9.67	21.67	34.17	
without sowing with	4	110.00	15.33	8.00	2.67	2.00	33	
irrigation	5	129.33	21.33	5.00	4.00	4.33	37.61	30.57
	6	118.00	19.33	12.67	4.00	2.33	34.17	
without (sowing& irrigation)	4	141.00	4.33	2.33	4.00	8.00	33	
	5	135.00	9.00	6.00	3.33	5.33	37.61	33.87
	6	126.00	24.33	21.33	8.67	15.33	34.17	
Tim effect		**117.03**	**21.77**	**13.68**	**7.89**	**15.09**		-

*** Least Significant Difference = 9.4 (significant at p=0.05)**

EFERENCES

Adolfo, G.G. 1981. Selective medium for *Pseudomonas solanacearum* and its application in the study of survival of the bacterium in soil, rhizosphere, and plant roots Ph.D. The University of Wisconsin - Madison, 1981, 117 pp. AAT 8123202 agar medium. Phytopathology 44: 693-695

Alcalá, M.D. and Betsy, L. 1998. Estudio de la marchitez bacteriana de la papa en tres localidades del estado lara. *Agronomia Trop*.48: 275-289.

Anastasia, S.C. 1993. Some aspects of the ecology and survival of *Pseudomonas solanacearum* E. F. Smith (race 1): Causal agent bacterial wilt of tomato Ph.D., Cornell University , 163 pp.

Arthy J. and Akiew K.1999.. Effect of Short term rotation on *Ralstonia solanacearum* population in soil . *Bacterial wilt Newsletter*, 16: 13-14

Badr, A. A.Y. 2006. Studies on some bacterial diseases of potato. M.Sc Thesis, Faculty of Agriculture Sana'a university. Yemen. 136 pp.

El-Ariqi, S.N.S. 1996. Studies on some bacterial diseases of certain Solanacious vegetable crops. M.Sc. Thesis, Faculty of Agriculture, Alexandria University. Egypt. 84 pp.

El-Ariqi, S.N.S. 2001.. Studies on biological control of brown rot diseases of potato. Ph. D. Thesis, Faculty of Agriculture, Alexandria University. Egypt 124 pp.

Elsas, J. D. De-Vries,P. M. and Van-Overbeek, L. S. 2001. Effects of ecological factors on the survival and physiology of *Ralstonia solanacearum* bv. 2 in irrigation water. *Canadian Journal of Microbiology*. 47 (9) : 842-854.

Elsas, J.D. van, Kastelein, P., Bekkum, P.van, Wolf, J. M. van-der and Vries, P. M. de 2000. Survival of *Ralstonia solanacearum* biovar 2, the causative agent of potato brown rot, in field and microcosm soils in temperate climates .*Phytopathology*. 90 (12): 1358-1366.

French, E. H. 1983 Ecological behavior of *Pseudomonas solanacearum*. Fourth international congress of plant pathology. Melbourne, Australia, 17-24 August.

French, E. H., and Gutarra, L. 1974**.** Adaptabilidad de *Pseudomonas solanacearum* razas 1 y 3 al frio. Resumenes, II Congreso Nacional de Investigadores Agrarios del Perú, Lima 1 (2-16): 44.

French, E.R.1986. Interaction between strain of *Psudomonas solanacearum*, its hostes and the environment. In :Persley,G. j.(ed.) Bacterial wilt disease in Asia and the south Pacific ACIAR . Proceedings 13: 99-104, ACIAR, Canberra.

Hayward, A. C. and Hartman, G. L 1994. Bacterial wilt the disease and its causative agent, *Pseudomonas solanacearum*. CAB. International in association with the Asian vegetable Research and Development center. 259pp.

Hazarika, D.K. and Das, K.K. 1999. Incidence of bacterial wilt of sesamum in relation of different sowing dates and varieties. *Plant Disease* 14 (2): 130-133

.

Hsu, S.T. 1991. Ecology and control of *Pseudomonas solanacearum* in Taiwan. *Plant Protection Bulletin* (Taiwan) 33: 72-79

Jian, Y. 2007. The role of bacterial taxis in *Ralstonia solanacearum*-host interactions. Ph.D., The University of Wisconsin - Madison, , 191 pp.

Joe, M. R. 1986. Evalution of an a virulent, non- bacteriocinogentic strain of *Pseudomonas solanacearum* for biological control of bacterial wilt of potato (brown rot) Ph.D., The University of Wisconsin-Madison,1986,166 pp.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a teterazolium medium. *Phytopathology* 44: 693-695.

Kloss, J. P. and Tumapon, A. S. 1987). Effects of intercropping potato on bacterial wilt. *Philippin Agriculturist* 70: 1-2.

Leeminn, O. K. 2001. Bacterial wilt in South Carolina tobacco production system: Winter cover summer crop rotation, microbial communities, and the diversity of the causal agent *Ralstoni*a *solanacearum*, in the southeastern United States Ph.D., Clemson University 171 pp.

Lemaga, B., Kanzikwera, R., Kakuhenzire, R., Hakiza, J. J. and Manzi, G. 2001. The effect of crop rotation on bacterial wilt incidence and potato tuber. yield. *African Crop Science Journal*. 9 (1): 257-266.

Olsson, K. 1976. Over wintering of *Pseudomonas solanacearum* in Sweden. In: Sequeira, L., and Kelman, A., (ed). Proceedings of the first international planning conference and workshop on the ecology and control of bacterial wilt caused by *Pseudomonas solanacearum* Raleigh, N. C. 18-24 July 1976, 105-109.

Payne, G. A., and Waldron, J.K. 1983. Over wintering and spore release of *Cercospora zea-maydis zea maydis* in corn debris in North Carolina. *Plant Disease* 67: 87-89.

Rivard, C. l. 2006. Grafting Tomato to manage soilborne diseases and improve yield in organic production systems M. Sc. thesis, Faculty of North Carolina State University U.S.A
.

Sunaina, V., and Gupta, P. K. 1998. Relationship between seasons and severity of bacterial wilt of potato under field condition. *Indian phytopathology* 51:92- 94.

Sunaina, V., Gupta, P. K., Shekhawat, G. S. and Kishore, V. 1989. Latent survival of *Pseudomonas solanacearum* in potato tuber and weeds. *Zeitschrift fur p flanzenkrankheiten und p flanzenschutz Journal of Plant Diseases and Protectuon* 96 (4): 361-364.

Wenneker, M., Verdel, M. S. W., Kermpenaar, C. and Janse, J. D. 1999). *Ralstonia* (*Pseudomonas*) *solanacearum* race 3 (biovar 2), causal agent of potato browen rot, in surface water and natural weed hosts. Mededelingen Faculteit Landbouwkundinge en Toegepaste Biologische Wetenschappen Universiteit Gent, 64 (3b): 573-582
.

Yabuuchi, E., Yashimasa, Ikuya, K., Hisako, H. and Yukiko, N. 1995). Transfer of two *Bukholderia* and an Alcaligenes species to *Ralstonia* Gen. Nov: proposal of *Ralstonia pichettii* (*Ralstonia Palleroni* and *Doudoroff*, 1937) comb. Nov., and *Ralstonia solanacearum* (Smith 1989) comb. Nov. and *Ralstonia eutropha* (Davis 1969) comb. Nov *Microbiology Immunol*., 39 (11): 897-903.

Wolf, J. M. van-der, Bekkum, P.J-van, Elsas-J. D-van, Nijhuis, E. H., Vriend, S. G. C., and Ruissen, M. A. 1998). Immunofluorescence colony staining and selective enrichment in liquid medium for studying the population dynamics of *Ralstonia solanacearum* (race 3) in soil- .*Bulletin OEPP*. 28 (1-2): 71-79.

تأثير البيات الشتوي على بكتيريا ذبول البطاطس في منطقة صنعاء

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الملخص :

أظهرت النتائج إلى تأثر بكتيريا الذبول على البطاطس *Ralstonia solanacearum* بالبيات الشتوي تحت ظروف منطقة صنعاء، فقد ازداد أعداد البكتيريا مع تواجد عائلها (البطاطس) وبالذات الصنف بيكاسو مقارنة بالمعاملات الأخرى المزروعة والمعاملات غير المزروعة المروية منها وغير المروية. أنخفض تعداد المستعمرات البكتيرية سريعاً مع انخفاض درجات الحرارة ولكن ليس لدرجة زوال البكتيريا، ثم بعد تحسن درجات الحرارة أرتفع تعداد المستعمرات البكتيرية. لوحظ بقاء البكتيريا في كل المعاملات المزروعة وغير المزروعة، بينما كان لنباتات القمح تأثير واضح على خفض تعداد البكتيريا، لذا من الأفضل إتباع دورة زراعية يزرع فيها القمح والفاصوليا. وجد أن أقوى العزلات البكتيرية بقاءً وإظهاراً للذبول هي العزلة رقم ٥ يليها العزلتين رقم ٦ و ٤ بمتوسطات ٣٧,٦١، ٣٤,١٧ و ٣٠ وحدة مستعمره، على التوالي. لوحظ أن هناك اختلافات معنوية في أعداد المستعمرات البكتيرية لفترات أخذ قرأت العينات وعددها خمس قرأت ماعدا القراءات المأخوذة في يناير ٢٠٠٧/٢٨ ومارس ١٨/ ٢٠٠٧ حيث لا توجد فروق معنوية بين القراءتين.

كلمات مفتاحية: الشتاء، *Ralstonia solanacearum*، البطاطس، صنعاء.