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INTERACTION BETWEEN BIOTIC AND ABIOTIC AGENTS TO CONTROL OF POTATO BACTERIAL WILT DISEASE

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ABSTRACT

This study was planned to control the disease using interaction between biotic and abiotic agents. In vitro, tested bactericides (gentamycin, ceflaxcin and copper sulphate) inhibit the growth of R. solanacerum compared with control treatment. Copper sulphate was the most effective compared with ceflaxcin and gentamycin. Ceflaxcin was moderately effective and gentamycin was less effective against the pathogen. Inhibition zone diameter was increased with increasing concentrations of tested bactericides. Application of different bio-agent isolates (Pseudomonas fluorescence, Bacillus subti-Bacillus megaterium and Serratia mareacescne) reduced the growth of the pathogen compared with control treatment. B. subtilis was the most effective, but Ps. fluorescens and B. megaterium was moderately effective and S. marescences isolate was less effective against the pathogen. In greenhouse experiments, the disease severity decreased and potato yield increased with interaction between bio-agents (Bacillus subtilis, and Pseudomonas fluorescence) and bactericides (gentamycin and ceflaxcin), or bio-agents and resistance inducers factors (salicylic acid and jasmonic acid) or bactericides and resistance inducers factors compared with control treatment. However, interaction between bio-agents as tuber treatment and bactericides as soil drench treatment or interactions between bactericides as soil drench treatment and resistance inducers as foliar treatment were the most effective against the disease and the yield. But, interactions between bioagents as soil drench treatment and resistance inducers as foliar treatment were moderately effective to control the disease and the yield. But, interaction between cefalexin as bactericide and *Ps. fluorescens* isolate as bio-agent or salicylic acid as resistance inducer were the most effective on disease severity and the yield compared with other treatments. Interaction between *B. subtilis* isolate as bio-agent and jasminic acid as resistance inducer were less effective compared with other treatment.

Keywords: Bacterial wilt; Potato; biotic agents; abiotic agents; *Ralstonia solanacearum*; control; bactericides; bio-agents; resistance inducers.

INTRODUCTION

Potato is one of the most important crops worldwide. Potatoes are widely cultivated in Egypt. This crop considers one of the most important vegetable either for local consumption and exportation (Gado, 2013). Its commercial cultivars are highly susceptible to many fungal and bacterial diseases. Bacterial wilt caused by Ralstonia solanacearum causes significant yield loss (Park et al 2016). Bacterial wilt is a devastating plant pathogen with a global distribution and an unusually wide host range, which in the absence of host plants can also be free living as a saprophyte in water or in the soil (Genin and Denny, 2012). More recently, it has been a matter of concern for producers of protected cultivation, where crop rotation, one of the main control strategies, is not properly adopted for economic reasons (Lopes, 2015).

Despite decades of efforts by many national and international organizations to control bacterial

wilt disease, it has continued to be a considerable problem throughout the world. Excessive use of pesticides to control plant diseases is an important problem in the agricultural fields, so it is a priority study for biological control, because the current production systems demand the crop protection by innovative and environmentally methods compatible with sustainable agriculture as an alternative to chemical application (Kuc, 2001). An option is to enhance the natural defensive response of plants through adequate stimulation, phenomenon known as induced resistance (Al-Mughrabi, 2008), which provides an efficient disease control and increases crop yields (Abd-El-Kareem et al 2001). The abiotic inductors include chemical products or molecules as responsible of disease resistance signaling (Walters et al 2005).

Currently, there are two ways to induce resistance, the acquired systemic resistance (ASR) and induced systemic resistance (ISR), which can be differentiated by the nature and regulatory paths of the inductor (also called elicitor) (Kunkel and Brooks, 2002). Salicylic acid is a plant hormone that acts as a marker and regulator of plant responses against pathogens and abiotic stress is the molecule involved in ASR pathways (Mauch-Mani and Métraux, 1998). It has been found that the exogenous application of SA in plants induces stress tolerance, increases the biological response against salinity and extreme temperatures, modifies the concentrations of antioxidants, nutrients and chlorophyll (Guzmán-Téllez et al 2014) and increases the protection against pathogen attack (Wildermuth et al 2002). It has been reported that ISR (another type of resistance) is effective against viral, bacterial and fungal diseases, is dependent of Jasmonic acid(JA) ,another essential role of JA in the immunity activation against pathogens that feed on dead tissues, such as some necrotrophic fungi or bacteria (Gutjahr, and Paszkowski, 2009). In plants, the resistance against pathogenic infection can be improved by biotic and abiotic treatments, also called inductors. The biotic inductors include: such as Bacillus, Streptomyces, Pseudomonas, and Agrobacterium (Alizadeh et al 2013 and Akram et al 2013). Management of R. solanacearum is difficult using a single method hence using integrated programmer would be a promising approach control of disease (Kalpage and De Costa, 2014).

The present work aimed to evaluate some bactericides, resistance inducer and bio-agents in combination were applied to control bacterial wilt disease under artificial inoculation conditions and their effects on the yield of potato.

MATERIALS and METHODS

Test organisms

Pathogenic isolates of *Ralstonia solanacearum* were obtained from Potato Brown Rot Project (PBRP), Agriculture Search Center, Giza, Egypt.

One strain from each the bacterial **antagonists**, *Pseudomonas fluorescence, Bacillus subtitlis*, *Bacillus megaterium and Serratia mareacescne* were obtained from Bacterial Diseases Laboratory, Plant Diseases Department, Faculty of Agriculture, Ain Shams University.

In vitro experiments

Bactericides efficiency

The antimicrobial activity of the antibiotics, gentamycin and ceflaxcin in addition to the copper sulphate were evaluated against R. solanacearum in vitro. Gentamycin and ceflaxcin were applied at concentrations of 25, 50, 75 and 100 ppm while copper sulphate was applied at concentrations of 50, 100, 150 and 200 ppm. Cell suspension of R. solanacearum (48- hours-old) was swabbed on the surfaces of Petri dishes containing nutrient agar (NA) medium using sterile cotton swab. A sterile uniformly size filter paper discs (5 mm in diameter) were soaked separately in the specific bactericides solutions for 5 minutes and then placed on the surface of inoculated plates. A discs soaked in sterile distilled water were served as control. After 48 hours of incubation at 28° C, the plates were observed for the presence of inhibition zones around the disks (Thornberry, 1950 and Ali, 2010). The test was repeated twice and the mean of inhibition zone for each tested compound were calculated from four replications per once (Thornberry, 1950).

Biocontrol agents efficiency

Cell suspension of *R. solanacearum* was swabbed on the surfaces of NA medium in Petri dishes using sterile cotton swab. Subsequently, uniform size filter paper disks (6 mm in diameter) were impregnated by 10 μ l of cell suspension (10 CFU/ml) of the specific antagonistic isolate and left to dry in laminar flow cabinet. After drying, the discs were placed on the surface of each inoculated plate. A disc impregnated by 10 μ l of sterile distilled water were served as control. The plates were incubated in the upright position at 28 $^{\circ}$ C for 3

days. Three replicates were carried out for each isolate. After incubation period, the diameters of the growth inhibition zones formed around the disk were measured.

In planta experiments

Biocontrol activity in planta Experimental conditions and inocula preparation

The experiments were carried out under artificial inoculation conditions in greenhouses of plant pathology department, Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

Potato (*Solanum tuberosum* cv., Nicola) that was confirmed to be free from *R. solanacearum* bacterium using PCR analysis were used to assessment the effect of biotic and abiotic agents on severity of bacterial wilt disease. Prior to cultivation, the potato tubers were placed in trays at room temperature in dark until the eyes (buds) protrude. The germinated tubers were planted (one tuber each) in 40 cm, diameter plastic pots containing sterilized sandy-clay soil (7kg/pot) where it was nurtured during the duration of experiment as usual with this crop.

To prepare the inoculum of the pathogen (*R. solanacearum*), the bacterium was grown on sucrose peptone agar medium at 28°C for 48 hr. Bacterial cells were suspended in buffer solution (PH, 7.2) and adjusted to 3.2 x 10⁸ Colony Forming Units (CFU)/ml, using a spectrophotometer (optical density of 0.12 at 590 nm).

To prepare the inoculum of the antagonistic bacteria, each isolate was grown on nutrient agar (NA) medium for 48 hr at 28°C. The resulted bacterial growth was suspended in buffer solution (PH, 7.2) and adjusted to 10⁹ Colony Forming Units (CFU)/ml, using a spectrophotometer (optical density of 0.3 at 600 nm) according to **Ciampi and Sequiera (1980)**.

Inoculation procedures and control agent treatments

To infection of potato plants by the pathogen, soil infestation was carried out by adding 500 ml of the bacterial suspension 5 day before planting (Michel and Mew, 1998).

Bactericides (gentamycin and ceflaxcin) were applied at the rate of 75 ppm to control the disease as soil drench or tuber treatments. Inducers factors (salicylic acid and jasmonic acid) were applied at the rate of 1.0 Mm to control the disease as foliar or tuber treatments. Bio-agents (*Bacillus subtilis*,

and Pseudomonas fluorescence isolates) were examined to control the disease as soil drench or tuber treatments. In case of drench soil, suspension of bactericides and /or bio-agent's solutions were added to infested soil at the rate of 500 ml / pot at the same time of planting potato tubers. While, in case of tubers treatments, the tubers were soaked in suspension of bactericides and /or inducer factors solutions for 3-hrs before planting, but with bio-agents treatments the tubers were soaked in the suspension of antagonistic bacteria plus 1.0% carboxy methyl cellulose (1:1, v:v) and 0.1 M maganisum sulphate for 15 minute and dried at room temperature for 6-hrs before sowing in the soil infestation. Meanwhile, in case of foliar treatments, suspension of inducer factors solutions was spread on plant foliage's after 20 days from planting. Five pots were applied as replicates for each treatment (Zayed et al 2004b).

Disease assessment

The disease severity was assessed after 90 days from planting. Severity of potato bacterial wilt was evaluated as percentage of sprouts which showing wilting symptoms per plant and as disease index (%) according to 0 - 5 rating scale, 0= No visible symptoms; 1= 1-25% of the plant is wilting; 2= 26-50% wilt; 3= 51-75% wilt; 4= more than 75% wilt and 5= plant died (Kemp and Sequeira, 1983). Percentage of Disease severity (DS) was calculated as follow:

DS= $(\Sigma R.T / 4 \times N) \times 100$

R= Disease severity scale (1, 2, 3, 4 and 5)

T= Number of plants per degree rating R (0-4)

N= Total number of tested plants

However, percentage of disease reduction (PDR) was calculated from disease index using the following formula:

 $PDR = DI_{ck} - DI_{tr} / DI_{ck} \times 10$

Where: DI_{ck} = Disease index in check treatment.

DI_{ts}= Disease index in treated treatment.

Meanwhile, number of tubers and their weights (g) per plant were assessed after 100 days from planting for all treatments.

Potato yield

Number of tubers and their weights (g) / plant were assessed after 100 days from planting for all previously treatments.

Statistical analysis

Analysis of variance (ANOVA) was carried out according to **Snedecor and Cochran (1982).** ANOVA was carried out using compatible computer basic language. LSD test was used to compare treatment means at 5% level of significances

RESULTS

In vitro experiments

Bactericides efficiency

Application of bactericides i.e. ceflaxcin, gentamycin and copper sulphate, at different concentrations were examined against growth of bacterial wilt pathogen (Ralstonia solanacerum), In vitro (Table 1). All tested bactericides decreased the growth of R. solanacerum compared with the control treatment, where the mean inhibition zone ranged from 3.6 to 7.4mm. Copper sulphate was the most effective against the growth of R. solanacerum compared with ceflaxcin and gentamycin, where the mean inhibition zone was 6.8, 5.2 and 4.7 mm, respectively. While, ceflaxcin was moderately effective and gentamycin was less effective against the pathogen, where mean inhibition zone was 5.2 and 4.7 mm, respectively. Efficacy of tested bactericides increased with increasing their concentrations against the growth of R. solanacerum, where the mean inhibition zone increased from 6.0 to 7.4mm with copper sulphate, from 4.2 to 5.8mm with ceflexin and from 3.6 to 5.5 mm gentamycin.

Biocontrol efficiency

Bacillus megaterium, Bacillus subtilis, Pseudomonas fluorescence and Serratia marescences isolates as biocontrol agents were examined against the growth of Ralstonia solanacerum under in vitro conditions (Table 2). Application of different bio-agent isolates reduced the growth of the pathogen compared with the control treatment, where the mean inhibition zone ranged from 2.2 to 4.3 mm. While B. subtilis was the most effective against the growth of R. solanacerum, where the mean inhibition zone was 4.3mm, but isolates of Ps. fluorescence and B. megaterium were moderately effective against the pathogen growth, where mean inhibition zone were 3.8 and 3.3 respectively. Also S. marescences was less effective against the pathogen, where mean inhibition zone was 2.2mm.

Table 1. Efficacy of some bactericides at different concentrations on the growth of bacterial wilt pathogen (*Ralstonia solanacearum*) under *in vitro* conditions

Bactericide	Concentration (PPM)	Mean of inhibition zone (mm)		
Ceflaxcin	25	4.2		
	50	5.0		
	75	5.6		
	100	5.8		
Gentamycin	25	3.6		
	50	4.3		
	75	5.3		
	100	5.5		
Copper sulphate	50	6.0		
	100	6.5		
	150	7.1		
	200	7.4		
Control	0.0	0.0		

LSD at 5% 0.8

Table 2. Efficacy of some bio-agents on the growth of bacterial wilt pathogen (*Ralstonia solanacea-rum*) under *in vitro* conditions

Bio-agent	Mean of inhibition zone (mm)
Bacillus megaterium	3.3
Bacillus subtilis	4.3
Pseudomonas fluorescence	3.8
Serratia marescences	2.2

LSD at 5% 1.0

In planta experiments

Biocontrol activity in planta

Interaction between bio-agents (*B. subtilis and Ps. fluorescence* isolates) as tuber treatment and bactericides (ceflaxcin and gentamycin) as soil drench treatment was examined against potato bacterial wilt disease under artificial inoculation conditions (**Table 3**). Disease severity significantly decreased with interaction between bio-agents and bactericides compared with the control treatment, where percentage of wilted shoots ranged from 13.4 to 14.8% with interaction treatments and was 31.8% with control treatment, but percentage of disease index ranged from 13.2 to 14.5% with interaction treatments and was 29.6% with control treatment. While, interaction between *Ps. fluorescence* isolate

as bio-agent and bactericides was more effective than interaction between B. subtilis isolate and bactericides against the disease, where percentage of disease control was 53.7 and 52.0%, respectively. Also, interaction between ceflaxcin as bactericide and bio-agents were more effective than interaction between gentamycin and bioagents on the disease, where percentage of disease control were 53.0-55.4 and 51.0-25.0%, respectively. Also, interaction between Ps. fluorescence isolate was the most effective against potato bacterial wilt disease compared with other treatment, where percentage of disease control was 55.4% but interaction between B. subtilis isolate as bio-agent and gentamycin as bactericide was less effective compared with other treatment, where percentage of disease control was 51.0%.

Interaction between resistance inducers (Jasmonic acid and salicylic acid) as foliar treatment and bactericides (ceflaxcin and gentamycin) as soil drench treatment was evaluated against potato bacterial wilt disease, under artificial inoculation conditions. Results in **Table (4)** showed that,

interaction between resistance inducers and bactericides was the most effective against the disease compared with control treatment, where percentage of wilted shoots ranged from 15.3 to 16.8% with interaction treatments and was 31.8% with control treatment, but percentage of disease index ranged from 12.8 to 15.0% with interaction treatments and was 29.6% with control treatment. While, interaction between salicylic acid as resistance inducer and bactericides were more effective than interaction between Jasmonic acid and bactericides against the disease, where parentage of disease control were 53.3 and 49.7%, respectively. Also, interaction between salicylic acid as resistance inducer and celfaxcin as bactericide were the most effective against the disease compared with other treatments, where percentage of disease control was 56.8%, but interaction between Jasmonic acid as resistance inducer and gentamycin as bactericide were less effective against the disease compared with other treatments, where percentage of disease control was 39.3%.

Table 3. Effect of interaction between bio-agents as tuber treatment and bactericides as soil drench treatment on severity of potato bacterial wilt disease under artificial inoculation conditions

Bio-agent	Bactericide	Wilted shoot (%)	Disease index (%)	Efficacy (%)	Mean	
Bacillus subitilis	Ceflaxcin	13.9	13.9	53.0	52.0	
	Gentamycin	14.8	14.5	51.0	52.0	
Pseudomonas fluorescence	Ceflaxcin	13.4	13.2	55.4	53.7	
	Gentamycin	14.3	14.2	52.0	55.7	
Check	Check	31.8	29.6	0.0	0.0	

LSD at 5% 1.0 1.2

Table 4. Effect of interaction between resistance inducers as foliar treatment and bactericides as soil drench treatment on severity of potato bacterial wilt disease under artificial inoculation conditions.

Bio-agent	Bactericide	Wilted shoot (%)	Disease index (%)	Efficacy (%)	Mean
Jasmonic acid	Ceflaxcin	15.3	14.8	50.0	40.7
	Gentamycin	16.6	15.0	49.3	49.7
Salicylic acid	Ceflaxcin	15.9	12.8	56.8	50.0
	Gentamycin	16.8	14.9	49.7	53.3
Check	Check	31.8	29.6	0.0	0.0

LSD at 5% 1.4 1.7

Interaction between bio-agents (B. subtilis_and Ps. fluorescence isolates) as soil drench treatment and resistance inducers (Jasmonic acid and salicylic acid) as foliar treatment were examined to control potato bacterial wilt disease, under artificial inoculation conditions. Data in Table (5) showed that interaction between bio-agents and resistance inducers were the most effective against potato bacterial wilt disease compared with control treatment, where percentage of wilted shoots ranged 16.4 to 17.9% with interaction treatments and was 31.9% with control treatment, but parentage of disease index ranged from 16.6 to 17.9% with interaction treatments and was 29.6% with control treatment. While, interaction between Ps. fluorescence isolate and resistance inducers was more effective than interaction between B. subtilis isolate and resistance inducers to control the disease, where percentage of disease control were 42.2 and 41.1%, respectively. Also, interaction between Ps. fluorescence isolate as bio-agent and salicylic acid as resistance inducer were the most effective against the disease compared with other treatments, where percentage of disease control was 43.9%, but interaction between B. subtilis isolate and Jasmonic acid were less effective to control the disease compared with other treatments, where percentage of disease control was 39.5%.

However, interaction between bio-agents as tuber treatment and bactericides as soil drench treatment and interaction between bactericides as soil drench treatment and resistance inducers as foliar treatment were the most effective against the disease, where percentage of disease control ranged from 52.0 to 53.7% and from 49.7 to 53.3%, respectively. But, interaction between bioagents as soil drench treatment and resistance inducers as foliar treatment were moderately effective to control the disease, where percentage of disease control ranged from 41.1 to 42.2%. Also, interaction treatments were more effective to reduce severity of potato bacterial wilt disease than alone treatments, where percentage of disease control ranged from 41.1 to 53.7% with interaction treatments and ranged from 3.4 to 45.1% with alone treatments.

Potato yield

Interaction between bio-agents (B. subtilis and Ps. fluorescence isolates) as tuber treatment and bactericides (ceflaxcin and gentamycin) as soil drench treatment was applied to study their effect

on potato yield under artificial inoculation conditions with bacterial with pathogen (R. solanacearum). Results in Table (6) showed that application of interaction between bio-agents and bactericides led to increase potato yield compared with control treatment, where mean number of tubers were 12.5-13.6 tuber/plant with interaction treatments and was 4.6 tuber/plant with control treatment, but mean tubes weight were 312.3-388.7 g/plant with interaction treatments and was 121.9 g/plant with control treatment. While, interaction between Ps. fluorescence isolate and bactericides were more effective to increase the yield than interaction between B. subtilis isolates and bactericides, where mean number of tubers were 13.3 and 12.9 tuber/plant and mean tubers weight were 329.3 and 319.3 g/plant, respectively. But interaction between Ps. fluorescence isolate as bio-agent and ceflaxcin as bactericides were the most effective on the yield compared with other treatments, mean number of tubers was 13.6 tuber/plant and mean tubes weight was 338.7 g/plant, but interaction between B. subtilis isolate and gentamycin were less effective on potato yield compared with other treatments, where mean number of tubers was 12.8 tuber/plant and mean tubs weight was 312.3 g/plant. Meanwhile, actually infected tubers decreased with interaction between bio-agents and bactericides compared with control treatment, where percentage of actually infected tubers was 15.7-15.9% with interaction treatments and was 25.7% with control treatment.

Interaction between resistance inducers (Jasmonic acid and salicylic acid) as foliar treatment and bactericides (ceflaxcin and gentamycin) as soil drench treatment was examined on potato yield, under artificial inoculation conditions with *R. solanacearum* (Table, 7). Interaction between resistance inducers and bactericides increased the potato yield compared with control treatment, where mean number of tuber were 12.0-13.2 tuber/plant with interaction treatments and was 4.6 tuber/plant with control treatment, but mean tubers weight were 301.2-322.8 g/plant with interaction treatments and was 121.9 g/plant with control treatment.

While, interaction between salicylic acid as resistance inducers and bactericides were more effective than interaction between Jasmonic acid and bactericides on the yield, where mean number of tubers were 12.5 and 12.1 tuber/plant and mean tubers weight were 312.0 and 309.9 g/plant, respectively, while, interaction between salicylic acid

Table 5. Effect of interaction between bio-agents as soil drench treatment and resistance inducers as foliar treatment on severity of potato bacterial wilt disease, under artificial inoculation conditions

Bio-agent	Resistance induce	Resistance induce Wilted shoot (%) Diseas		Efficacy (%)	Mean	
Bacillus subitilis	Jasmonic acid	17.9	17.9	39.5	41.1	
	Salicylic acid	16.9	17.0	42.6		
Pseudomonas	Jasmonic acid	17.4	17.6	40.5	40.0	
fluorescence	Salicylic acid	16.4	16.6	43.9	42.2	
Check	Check	31.8	29.6	0.0	0.0	

LSD at 5% 1.3 1.6

Table 6. Effect of interaction between bio-agents as tuber treatment and bactericides as soil drench treatment on potato yield and percentage of actually infected tubers, under artificial inoculation conditions

Bio-agent	Bactericide	Mean tubes number/plant	Mean	Mean tubers weight g/plant	Mean	Actually infected tubers (%)	Mean
Bacillus subitilis	s Ceflaxcin 13.0 12.9 326.3		326.3	319.3	15.8	15.7	
	Gentmycin	12.8	12.9	312.3	319.3	15.4	15.7
Pseudomonas	Ceflaxcin	13.6	13.3	338.7	329.3	16.1	15.9
fluorescence	Gentmycin	12.5	13.3	319.9	329.3	15.6	
Check	Check	4.6	4.6	121.9	121.9	25.7	0.0

LSD at 5% 2.4 9.4 0.6

Table 7. Effect of interaction between resistance inducers as foliar treatment and bactericides as soil drench treatment on potato yield and percentage of actually infected tubers, under artificial inoculation conditions

Bio-agent	Bactericide	Mean tubes number/plant	Mean	Mean tubers weight g/plant	Mean	Actually infected tubers (%)	Mean
Jasmonic	Ceflaxcin	12.2	12.1	311.3	309.9	15.5	15.5
acid	Gentamycin	12.0	12.1	308.5	309.9	15.0	15.5
Salicylic	Ceflaxcin	13.2	12.5	322.8	312.0	15.9	15.7
acid	Gentamycin	2.0	12.5	301.2	312.0	15.3	13.7
Check	Check	4.6	4.6	121.9	121.9	25.7	0.0

LSD at 5% 1.8 9.9

as resistance induces and ceflaxcin as bactericides were the most effective to increase the yield compared other treatment, where mean number of tuber was 13.2 tuber/plant and mean tubers weight was 322.9 g/plant, but interaction between Jasmonic acid and gentamycin were less effective on potato yield, where mean number of tubers was 12.0 tuber/plant and mean tubes weight was 308.5 g/plant. Also, actually infected tubers were decreased with interaction between resistance inducers and bactericides compared with control treatment where percentage of actually infected tubers was 15.3-15.7% with interaction treatments and was 25.7% with control treatment.

Interaction between bio-agents (*B. subtilis* and *Ps. fluorescence* isolates) as soil drench treatment and resistance inducers (Jasmonic acid and salicylic acid) as foliar treatment were evaluated on potato yield under artificial inoculation conditions with *R. solanacearum*. Results in **Table (8)** raveled that interaction between bio-agents and resistance inducers led to increase the yield compared with control treatment, where mean number of tubers were 10.0-10.4 tuber/plant with interaction treatments and was 4.6 tuber/plant with control treatment, but mean tubers weight were 269.6-290.6 g/plant with interaction treatment and was 121.9 g/plant with control treatment. While, interaction

between *Ps. fluorescence* isolate as bio-agent and resistance inducers were more effective than interaction between *B. subtilis* isolate and resistance inducers on the yield, where mean number of tubers were 10.2 and 10.1 tuber/plant and mean tubes weight were 280.5 and 275.5 g/plant, respectively. Meanwhile, interaction between *Ps. fluorescence* isolate as bio-agent and salicylic acid as resistance inducers were the most effective to increase the yield compared with other treatment, where mean number of tubers was 10.4 tuber/plant

and mean tubes weight was 290.6 g/plant, but interaction between *B. subtilis* isolate and Jasmonic acid were less effective on the yield, where mean number of tubes was 10.0 tuber/plant and mean tubers weight was 269.6 g/plant. While, actually infected tubers were decreased with interaction treatments compared with control treatment where percentage of actually infected tubers were 12.5% with interaction treatments and was 25.7 with control treatment.

Table 8. Effect of interaction between bio-agents a soil drench treatment and resistance inducers as foliar treatment on potato yield and percentage of actually infected tubers, under artificial inoculation conditions

Bio-agent	Resistance induce	Mean tubes number/plant	Mean	Mean tubers weight g/plant	Mean	Actually Infected tubers (%)	Mean
Bacillus subitilis	Jasmonic acid	10.0	10.1	269.6	275.5	12.3	12.5
	Salicylic acid	10.2	10.1	281.4		12.6	
Pseudomonas	Jasmonic acid	10.0	10.2	270.3	280.5	12.0	12.5
fluorescence	Salicylic acid	10.4	10.2	290.6	200.5	12.9	12.5
Check	Check	4.6	4.6	121.9	121.9	25.7	0.0

LSD at 5% 2.1 10.3 0.6

DISCUSSION

All tested bactericides decreased growth of R. solanacerum compared with the control treatment. Copper sulphate was the most effective compared with ceflaxcin and gentamycin. While, ceflaxcin was moderately effective and gentamycin was less effective against the pathogen. Inhibition zone diameter increased with increasing concentrations of tested bactericides. Bio-agent isolates reduced the growth of pathogen compared with the control treatment. B. subtilis isolate was the most effective, while isolates of Ps. fluorescens and B. megaterium were moderately effective and S. marescences isolate was less effective against the pathogen. Also, these results are in agreement with those reported by Kempe and Sequeira, 1983; Anuratha and Gnanamanickam (1990); Sunaina, et al (1997); Moura and Romeiro (2000); Ahmed (2006) and Seafelyazel (2008). It is important to note that the results of this work were in line with those obtained by **Zayed**, (2004a) who applied B. subtilis; P. aeruginosa; P. fluorescence, P. putida and Streptomyces spp. against R. solanacerum, In vitro. He found that isolates of B. subtilis, P. fluorescence and P. aeruginosa inhibited the growth of R. solanacearum while Streptomyces spp. was unable to inhibit the growth of the pathogen. Also,

he showed that *P. fluorescence* gave more inhibitory effect on KB medium while *Bacillus subtilis* was more effective on NA medium.

Kloepper et al (1980) mentioned that fluorescent pseudomonads produce fluorescent siderophore (pseudobactin) and antibiotics and suggested three lines evidence associated with these effects previously (1) Antibacterial activity effect against most strains of phytopathogenic bacteria can be detected in media limiting the siderophore production; (2) The Tn5-generated mutant produces a normal and (3) This mutant strains have full capacity to inhibit growth of R. solanacearum and Ps. marginalis. Amar (2010) observed that antagonistic microorganisms were isolated from the rhizosphere of healthy potato plants. Out of 40 bacterial isolates and nineteen actinomycetes isolates, only two bacterial isolates (Bacillus subtilis (B20) and Pseudomonas fluorescence) and four actinomycetes isolates were inhibited the growth of R. solananacearum, In vitro. Vidaver (1983) and Fravel (1988) reported that antagonistic effect (B. subtilis) may be due to production of antimicrobial compounds (peptide antibiotics or bacteriocins), that it is able to generate either by simple breakdown of external molecules or by direct synthesis of specific of specific antibiotics, dependence on a specific growth medium. Also, effect of cuprous

oxide and copper oxychloride were work mainly as surface protectants and as enzyme inhibitors on several of phytopathogens (Lye, 1977). Treatment of bacterial cells with Cu-compounds lead to marked changes in elemental composition with a toxic effect at the cell surface, leading to large scale efflux of K+ and influx of Ca2+ and Cu2+. However, the bactericides did not act directly on the bacterium, but appears to have an indirect effect on disease development, possibly mediated by the plant metabolism. Meantime, inorganic bactericides have a strong activity on phytopathogenic bacteria In vitro and In vivo, where Cu2+ ions were toxic agent in inorganic bactericides, which showed direct inhibition of bacterial growth leading to cell death (Sigee, 1993).

Interaction between bactericides as soil drench treatment and bio-agents as tuber treatment or resistance inducers as foliar treatment significantly reduced severity of potato bacterial will disease and actually infected tubers and significantly increased potato yield compared other treatment, but interaction between bio-agents as soil drench treatment and resistance inducers as foliar treatment were moderately effective to decrease disease severity and actually infected tubers and to increase potato yield. But, interaction between ceflaxcin as bactericide and Ps. fluorescence isolate as bio-agent or salicylic acid as resistance inducer were the most effective on disease severity, percentage of actually infected tubers compared with other treatments, but interaction between B. subtilis isolate as bio-agent and jasmonic acid as resistance inducer were less effective compared with other treatment. Zayed, (2004) found that B. subtilis isolates decreased disease severity and increased tubers yield while Streptomyces spp. isolate was very less effective. Also, he found that application of the fluorescent Pseudomonads and B. subtilis isolates as soil treatments were more effective than tuber treatments to reduce the disease severity and increase tubers yield. Interest in biological control has increased by public concerns over the use of chemicals in the environment in general and the need to find alternatives to the use of chemicals for disease control (Whipps, 2001).

Plant growth-promoting bacteria (PGPB) may affect plant growth either directly or indirectly. Direct promotion of plant growth occurs when either (1) The PGBR facilitates the acquisition of resources from the environment including nitrogen, phosphorous and iron or (2) modulates plant growth by providing or regulating various plant

hormones including auxin, cytokinin or ethylene. Indirect promotion of plant growth by PGBR occurs when a bacterium limits or prevents the damage to plants that might otherwise be caused by various pathogenic agents including bacteria, fungi and nematodes. There are a large number of common mechanisms that PGBR to use indirect promote plant growth including the production of antibiotics, cell wall-degrading enzymes, lowering plant ethylene levels, induced systemic resistance, decreasing the amount of iron available to pathogens and the synthesis of pathogen-inhibiting volatile compounds (Glick, 2015). Induced systemic resistance (ISR) is based on plant defense mechanism that are activates by inducing agent as PGPR (Kloepper et al 1992) or ISR once expressed activity multiple potential defense mechanisms that include increasing in activity of defense enzymes and pathogenesis-related (PR) proteins (Lawton and Lamb, 1987 and Strobel et al 1996) and phytoalexins (Kuc and Rush, 1985; Ongena et al 2000 and Jeun et al 2004). Plant growthpromoting rhizobacteria (PGPR) are root colonizing beneficial bacteria and the beneficial effects include biological control and growth promotion (Weller, 1988). Control of a wide spectrum of pathogens were studied by application of antagonists largely remain an unfulfilled goal for biological control. There are three main approaches to achieve this goal: (1) Modify the genetics of the bio-control agent to add mechanisms of disease suppression that are operable against more than the pathogen; (2) Alter the environment microflora and (3) Develop strain mixtures with superior biocontrol activity (Janisiewicz, 1988). One of the methods of reducing bacterial diseases is the induction of plant resistance. Induced resistance (IR) as a general phenomenon in plants has been studied in many host plant-pathogen-interactions. Plants can be induced to enhance their defense against pathogen infection by treatment with various biotic and abiotic inducers (Walters et al 2005).

The effect of salicylic acid (SA) or its derivatives on inducing resistance in plants against pathogens was reported by **Malamy and Klessing** (1992) who stated that the effect of SA was not caused by direct action on the growth of pathogens, but the effect of SA application was rather a consequence of induction of plant defense response. **Palva et al (1994)** suggested that three possible ways for salicylic acid capability to induced resistance to some pathogenic bacteria. There are (1) Salicylic acid could directly effect to

bacteria as a chelating agent, (2) Salicylic acid could act as inducer of plant defense compound such as pathogenesis-related (PR) proteins and (3) The inhibition could be a combination of both effects. However, salicylic acid has been established as a putative signal molecule that induces plant defense and systemic acquired resistance (SAR). Biotic and abiotic agents caused phytoalexin synthesis and accumulation. Specificity with phytoalexin probably resides in the regulation of the rapidity and magnitude of their synthesis and accumulation and this is under genetic control of host and pathogen. As with phytoalexins suggested defense compounds produced by a given plant (lignin, phenolic, cross-linked cell wall polymerase, hydroxyl prolin rich glycoproteins, callose, chilinase, thionins, B 1,3-glucanase and peroxidasesrelated proteins) can be produced equally well be susceptible and resistance cultivars giving the proper conditions for elicitation (Wilson and Bachman, 1999). Abiotic compounds such as DL-3-aminobutyric acid (BABA) have been reported to induce resistance in a variety of plants against a wide range of microbial pathogens without possessing any direct antimicrobial activity. It is a simple, non-protein amino acid which, when sprayed onto the leaf surface or drenched into the soil, inducer resistance against various foliar and root pathogen (Jakab et al 2001 and Cohen, 2002).

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التداخل بين عوامل حيوية وغير حيوية لمكافحة مرض الذبول البكتيرى في البطاطس [130]

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الموجــــز

مجيتريم فعالية متوسطة ولكن عزلة السريتي مرسنيس كانت الاقل فعالية ضد الممرض.

اوضحت النتائج المتحصل عليها ان معاملة التداخل بين عوامل المكافحة الحيوية (عزلات بكتيريا باسلس ستلس والبسيدموناس فللورنسس) والمبيدات البكتيرية (سفلكسين والجنتاميسين) أو التداخل بين عوامل المكافحة الحيوية وعوامل الحث (حمض السلسيلك وحمض الجاسمونيك) أو التداخل بين المبيدات البكتيرية وعوامل الحث تحت ظروف العدوى الصناعية خفضت بدرجة واضحة من شدة المرض والنسبة المئوية للدرنات المصابة وزادت من محصول البطاطس بالمقارنة مع معاملة المقارنة. في حين أن معاملات التداخل بين عوامل المكافحة الحيوية كمعاملة درنات والمبيدات البكتيرية كمعاملة رى للتربة أو التداخل بين المبيدات البكتيرية كمعاملة ري للتربة وعوامل الحث كمعاملة رش على المجموع الخضري كانت الاكثر فعالية في خفض المرض وزيادة المحصول. بينما التداخل بين عوامل المكافحة الحيوية كمعاملة درنات وعوامل الحث كمعاملة رش على المجموع الخضري كانت معتدلة الفعالية في مكافحة المرص وزيادة المحصول. واظهرت معاملة التداخل بين مبيد السفلكسين وكلا من حمض السلسيلك كعامل حث و/ او عزلة بكتيريا البسيدموناس فللورنسس كعامل حيوى اكثر كفاءة في خفض المرض والدرنات المصابة وزيادة محصول البطاطس بالمقارنة مع معاملات

يتسبب مرض الذبول البكتيري (العفن البني) عن بكتيريا الراستونيا سولاناسيرم ومن أكثر الامراض البكتيرية خطورة وإنتشاراً حول العالم. استهدفت هذه الدراسة امكانية مكافحة المرض بالتداخل بين معاملات حيوية وغير حيوية تحت ظروف العدوى الصناعية. تحت ظروف المعمل. اوضحت النتائج ان المبيدات البكتيرية المختبرة (سفلكنس وجنتامسين وكبريتات النحاس) خفضت من نمو بكتيريا الراستونيا سولاناسيرم بالمقارنة مع معاملة المقارنة ، في حين زادت كفاءة المبيدات مع زيادة تركيزها. واظهرت كبريتات النحاس كفاءة اكثر في تثبيط نمو البكتيريا الممرضة بالمقارنة مع المبيدات البكتيرية الاخرى المختبرة، بينما اظهر مبيد السفلكسين كفاءة متوسطة ومبيد الجنتامسين كفاءة اقل بالمقارنة مع المبيدات الاخرى. زادت فعالية المبيدات المختبرة مع زيادة تركيزها. كما اظهرت النتائج ان عزلات بكتيريا الباسلس ستلس والباسلس ميجتريم والسيدموناس فلورنستين والسريتي مرستيس كانت فعالة في تثبيط نمو البكتيريا الممرضة بالمقارنة مع معاملة المقارنة في حين ان عزلة بكتيريا الباسلس ستلس كانت الاكثر فعالية في تثبيط البكتيريا الممرضة معمليا بالمقارنة مع العزلات الاخرى من العوامل الحيوية، في حين اظهرت عزلات البسيدموناس فلورونسس والباسلس

تحكيم: ا.د إبراهيم حسن طلبه

ا.د مدحت کامل

التداخل الاخرى ولكن التداخل بين عزلة بكتيريا المرض وزيادة المحصول بالمقارنة مع المعاملات الباسلس ستلس (عامل حيوي) كمعاملة ري للتربة الأخرى. وحمض الجاسمونيك (عامل حث) كمعاملة رش على المجموع الخضرى اقل معاملات التداخل فعالية ضد المرض والدرنات المصابة ومحصول البطاطس بالمقارنة مع المعاملات الاخرى. ولكن معاملة التداخل بين عزلة بكتيريا الباسلس ستلس كعامل حيوى وحمض حث مقاومة الجاسمونيك كعامل حث كانت الأقل فعالية في مكافحة

الكلمات الدالة: مرض الذبول البكتيري، مرض العفن البني، عوامل مكافحة حيوية، عوامل مكافحة غير حيوية، بكتيريا الراستونيا سولاناسيرم، البطاطس، عوامل