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Management of Potato Bacterial Wilt Disease Using Abiotic Treatments

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Abstract: Bacterial wilt disease caused by *Ralstonia solanacearum* (Smith) Yabuuchi is one of the most important bacterial diseases in the world. This study aimed to test some chemical compounds such as bactericides that were applied to control bacterial wilt disease under artificial inoculation conditions and their effects on the yield of potatoes under greenhouse conditions.*In vitro*, tested chemical compounds (Cefalexin, Gentamycin, and Copper sulphate) inhibit the growth of *R. solanacerum* compared with control treatment. Copper sulphate was the most effective where the Inhibition zone diameter was 6.0 to7.4mm compared with cefalexin and gentamycin, 3.6 to 5.8mm respectively, but cefalexin was moderately effective 4.2 to 5.8mm and gentamycin was less effective 3.6 to 5.8mm against the pathogen. Inhibition zone diameter was increased with increasing concentrations of tested chemical compounds. Greenhouse experiments showed that the chemical compounds used reduced the severity of potato bacterial wilt disease and increased potato yield compared with control treatment. When using Cefalexin, the mean of wilted shoots was 20.5 and the severity of disease 16.3, while when using Gentamycin as an Abiotic, the mean of wilted shoots was 22.8 and severity of disease 17.6 which was the most effective compared with Copper sulphate treatment were wilted shoot was 31.8 and severity of disease 29.6. The application of tested chemical compounds as soil drench treatment led to a percentage of infection which ranged from16.4 to19.0 more effective than tuber treatment where the percentage of infection ranged from 18.4 to 20.1.

Keywords: Abiotic agents; Bacterial wilt; Control,Libya; Potato; *Ralstonia solanacearum.*

INTRODUCTION

Bacterial wilt is a very severe disease, in particular, due to the broad pathogenic variability of the bacterium, its capacity to survive in the soil, and the large number of its host species (Felix et al., 2010; Muthoni et al., 2013). Once introduced in an area, eradication of the bacterium is very difficult; there are reports that *R. solanacearum* persists in the soil for more than ten years in the absence of Solanaceae species by surviving in the rhizosphere of various cultivated species or weeds (Chowdappa et al., 2013; Kago et al., 2017). It is a gram-negative, rod-shaped, strictly aerobic bacterium that is primarily a soil-borne and waterborne pathogen (Adebayo & Ekpo, 2005). It infects host plants from their roots, infected from damaged wounds formed by lateral root emergence or by root damage caused by soil-borne organisms. *R. solanacearum* infect the roots or stems as they colonize the plant through the xylem in the vascular bundles. Wilting of the whole plant

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may follow rapidly if environmental conditions are favorable for the pathogen. Plant-to-plant infection can occur when bacteria shed from infected roots move to the roots of nearby healthy plants. Therefore, the management of this disease is very difficult (Nguyen & Ranamukhaarachchi, 2010; Ullas et al., 2015).

Despite decades of efforts by many national and international organizations to control bacterial wilt disease, it has continued to be a considerable problem worldwide. The variability of the pathogen and the agro-ecosystems has undoubtedly hampered progress in controlling the disease. Excessive use of pesticides to control plant diseases is a significant problem in agricultural fields. So it is a priority study for biological control, because the current production systems demand crop protection by innovative and environmentally friendly methods compatible with sustainable agriculture as an alternative to chemical applications (Almoneafy et al., 2014; Kuć, 2001). An option is to enhance the natural defensive response of plants through adequate stimulation, a phenomenon known as induced resistance (Al-Mughrabi, 2008), which provides efficient disease control and increases crop yields (Abd-El-Kareem et al., 2001). The abiotic inductors include chemical products or molecules responsible for disease-resistant signaling (Walters et al., 2005). Management of *R. solanacearum* is difficult using a single method. Hence using an integrated programmer would be a promising approach to controlling the disease (Kalpage & De Costa, 2014).

The present work aimed to test some chemical compounds as bactericides which were applied to control bacterial wilt disease under artificial inoculation conditions and their effects on the yield of potatoes.

MATERIALS AND METHODS

The pathogen: Pathogenic isolates of *R. solanacearum* were isolated and identified in the Potato Brown Rot Project (PBRP), Agriculture Search Center, Giza, Egypt.

In vitro experiments

Bactericides efficiency: The antimicrobial activity of the bactericides (cefalexin and gentamycin) in addition to the copper sulphate were evaluated against *R. solanacearum in vitro* in the department of botany, the faculty of science El-Gubba, Derna University, Libya. Cefalexin and gentamycin 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 (using a microliter pipette). Cell suspension of *R. solanacearum* (48 hours old) was swabbed on the surfaces of Petri dishes containing nutrient agar (NA) medium using a 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 disc soaked in sterile distilled water served as control. After 48 hours of incubation at 28^{o}C, the plates were observed for the presence of inhibition zones around the disks (Balouiri et al., 2016; Shaheen, 2010). The test was repeated twice, and the mean inhibition zone for each tested compound was calculated from four replications per once.

In plant experiments: These experiments were carried out under artificial inoculation conditions in greenhouses of the Plant Pathology Department, Faculty of Agriculture, Ain shams university, Cairo, Egypt.

Experimental conditions, inoculum preparation: Potato (Solanum tuberosum cv., Nicola) that was confirmed to be free from *R. solanacea-rum* bacterium using PCR analysis was used to assess the effect of abiotic agents on the severity of bacterial wilt disease. Prior to cultivation, the potato tubers were placed in trays at room temperature in the dark until the eyes (buds) protruded. The germinated tubers were planted (one tuber each) in 40 cm diameter plastic pots containing sterilized sandy-clay soil (7kg/pot) in a specialized oven, where they were nurtured during the duration of this experiment as usual with this crop (Maier *et al*., 2009). To prepare the inoculum of the pathogen *R. solanacearum*, the isolate (HL2) was grown on sucrose peptone agar medium at 28°C for 48 hr. Bacterial cells were suspended in a buffer solution (PH, 7.2) and adjusted to 108 (cfu)/ml) using a spectrophotometer (optical density of 0.12 at 590 nm). The infestation procedure (500 ml/Pot) was made 5 days before planting according to (Michel & Mew, 1998).

Effect of abiotic agents on severity of bacterial wilt disease: Bactericides (cefalexin, gentamycin) were applied at the rate of 50 and 100 ppm, and at the rate of 100 and 200 ppm for copper sulphate using a microliter pipette, to control the disease as soil drench or tuber treatments. In the case of drench soil, suspension of bactericides solutions was added to infested soil at the rate of 500 ml/pot at the same time of planting potato tubers. While in the case of tubers treatments, the tubers were soaked in a suspension of bactericides (Shaheen, 2010).

Disease assessment: The disease severity was assessed 90 days after planting. Severity of potato bacterial wilt was evaluated as the percentage of sprouts that showed wilt symptoms per plant. The (%) according to 0 - 5 rating scale, was used as 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 (Kempe & Sequeira, 1983). The percentage of Disease index (DI) was calculated as follows:

DI (%) = (Σ R.T / 5×N) × 100

T= total number of plants with each category (R) disease severity scale (1, 2, 3, 4, and 5)

N= Total number of tested plants

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

PDR (%) = DIck-DItr/DIck×10

Where: Dick = Disease index in check treatment.

DIts = Disease index in treated treatment.

Also, the number and weight of tubers were recorded after 100 days from planting for each treatment.(Kehil, 2002).

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

RESULTS

In vitro experiments

Bactericides efficiency: Cefalexin, gentamycin, and copper sulphate were applied as bactericides against the growth of bacterial wilt pathogen (*Ralstonia solanacerum)*, at different concentrations, *in vitro* (Table, 1). All tested bactericides decreased the growth of *R.solanacerum* in comparison 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 cefalexin and gentamycin, where the mean inhibition zone was 6.8, 5.2, and 4.7 mm, respectively. While, cefalexin was moderately effective and gentamycin was less effective against the pathogen, where the mean inhibition zone was 5.2 and 4.7 mm, respectively. Inhibition zone diameter was increased with increasing concentrations of tested antibiotic and copper sulphate against the growth of *R. solanacerum*, where the mean inhibition zone was increased from 6.0 to 7.4mm with copper sulphate, from 4.2 to 5.8mm with cefalexin and from 3.6 to 5.5 mm gentamycin.

Table (1): Effect of the three bactericides at different concentrations on the growth of bacterial wilt pathogen (*Ralstonia solanacearum*) under in vitro conditions

Treatment	Concentration (PPM)	Mean of inhibition zone (mm)
Cefalexin	25	4.2
	50	5.0
	75	5.6
	100	5.8
Mean		5.2
Gentamycin	25	3.6
	50	4.3
	75	5.3
	100	5.5
Mean		4.7
Copper sulphate	50	6.0
	100	6.5
	150	7.1
	200	7.4
Mean		6.8
Control	0.0	0.0
LSD at 5% 0.8		
Cefalexin	50	5.0
	100	5.8
Mean		5.2
Gentamycin	50	4.3
	100	5.5
Mean		4.7
Copper sulphate	50	6.0
	100	6.5
Mean		6.8
Control	0.0	0.0

In plant experiments: Effect of abiotic agents on severity of bacterial wilt disease: Evolution of bactericides on severity of potato bacterial wilt disease: Cefalexin, gentamycin, and copper sulphate agents were applied at different concentrations using two methods of application (soil drench or tubers treatments) to evaluate the disease severity of bacterial wilt on potato tubers, under artificial inoculation conditions. Results in (Tables 2 and 3) showed that the tested bactericides reduced the severity of potato bacterial wilt disease compared with the control, where disease severity ranged from 19.4 to 28.4% with the percentage of wilt shoots, from 15.3 to 26.7% with the percentage of disease index, and from 9.8 to 48.3% with the percentage of disease control. But the control treatment was 31.8, 29.6 and 0.0%, respectively. Cefalexin and gentamycin were the most effective in controlling potato bacterial wilt disease, followed by copper sulphate, compared with control treatments. It could be noticed that disease severity was decreased by increasing doses of abiotic agents, and there were differences between antibiotics and copper sulphate.

Results in (Table 2) revealed that the application of cefalexin and gentamycin at (50 and 100 ppm) as soil treatment were the most effective to control potato bacterial wilt disease compared with copper sulphate. Where disease severity ranged from 21.6 to 19.4 % with the percentage of wilted shoots, from 17.2 to 15.3 % with the percentage of disease index, and from 41.9 to 48.3% with the percentage of disease control for cefalexin, while disease severity ranged from 23.8 to 21.7 % with the percentage of wilted shoots ranging from 18.0 to 17.2% with the percentage of disease index, and from 35.8 to 39.2%, with the percentage of disease control for gentamycin. While copper sulphate was moderately effective to control the disease, where disease severity ranged from 26.0 to 24.9 % with the percentage of wilted shoots, from 20.1 to 19.0 % with percentage of disease index, and from 32.1 to 35.8% with the percentage of disease control, when were applied at (100 and 200 ppm).

Table (2): Effect of the three bactericides at different concentrations as soil drench treatment on severity of potato bacterial wilt disease, under artificial inoculation conditions.

Treatment	Concentration (PPM)	Disease severity		Efficacy (%)
		Wilted Shoot (%)	Disease index (%)	
Cefalexin	50	21.6	17.2	41.9
	100	19.4	15.3	48.3
Mean		20.5	16.3	45.1
Gentamycin	50	23.8	18.0	39.2
	100	21.7	17.2	35.8
Mean		22.8	17.6	40.6
Copper sulphate	100	26.0	20.1	32.1
	200	24.9	19.0	35.8
Mean		25.5	19.6	34.0
Check	0	31.8	29.6	0.0
LSD at 5%			1.6	1.7

Application of cefalexin, gentamycin, and copper sulphate as tubers treatment, the lowest effectiveness in disease reduction was in decreasing severity of potato bacterial wilt as in (Table 3). When applied at (50 and 100 ppm) respectively as tubers treatment for each of the (cefalexin, gentamycin) disease severity ranged from 25.2 to 23.1 percent of wilted shoots, from 21.9 to 19.4% with the percentage of disease index, and from 26.0 to 34.5 % with the percentage of disease control for cefalexin, while disease severity ranged from 27.3 to 26.0 % with the percentage of wilted shoots ranging from 23.1 to 21.6% with percentage of disease index, and from 22.0 to 26.0%, with percentage of disease control for gentamycin. When applied at (100 and 200 ppm) by copper sulphate, disease severity ranged from 28.4 to 27.5 % with the percentage of wilted shoots, from 26.7 to 24.8 % with the percentage of disease index, and from 9.8 to 16.2 % with the percentage of disease control.

Application of cefalexin, gentamycin, and copper sulphate bactericides as soil drench treatment was more effective than tuber treatment against potato bacterial wilt disease under artificial inoculation conditions. Where the percentage of disease control ranged from 34.0 to 45.1%, with soil drench treatment and from 13.0 to 30.3% with tuber.

Table (3): Effect of three bactericides at different concentrations as tubers treatment on severity of potato bacterial wilt disease, under artificial inoculation conditions.

Treatment	Concentration (PPM)	Disease severity: Wilted Shoot (%)	Disease severity: Disease index	Efficacy (%)
Cefalexin	50	25.2	21.9	26.0
	100	23.1	19.4	34.5
Mean		24.2	20.7	30.3
Gen-	50	27.3	23.1	22.0
tamycin	100	26.0	21.6	26.0
Mean		26.7	23.6	24.0
Copper	100	28.4	26.7	9.8
sulphate	200	27.5	24.8	16.2
Mean		28.0	25.8	13.0
Check	0	31.8	29.6	0.0
LSD at 5%			1.3	1.5

Effect of biotic and abiotic agents on potato yield: Data in table (4 and 5) showed that potato yield was increased with the application of bactericides compared with control treatment, applied at different concentrations and using two methods (soil drench, tubers treatment).

Cefalexin and gentamycin were more effective in increasing the yield than copper sulphate, where the percentage of actually infected tubers was decreased compared with the control treatment, where the percentage mean were (16.5-18.6%) with cefalexin, (16.8-18.5%) with gentamycin, (18.6-20.0 %) with copper sulphate, and (25.7%) with control treatment. While, mean tubers weight was (234.9- 250.9), (228.4-245.6), and (194.0- 218.4) g/plant, respectively for each cefalexin, gentamycin, and copper sulphate. But mean number of tuber were (8.3-8.8) tuber/plant, for cefalexin, and (8.2-8.7) tuber/plant, for gentamycin and (7.6-7.8) tuber/plant, for copper sulphate applied at different concentrations and using methods (soil drench, tubers treatment). Control treatment was 121.9 g/plant and 4.6 tuber/plant.

Also, the application of bactericides as soil drench treatment was more effective to increase the potato yield compared with tuber treatment, where mean number of tubers were (7.8- 8.8) tuber/plant and mean tubers weight were (218.4-250.9) g/plant for soil drench treatment, but mean number of tubers (7.6- 8.3) tuber/plant and mean tuber weight were (194.0-234.9) g/plant for tubers treatment. Also, the percentage of actually infected tubers was more severe with tuber treatment than with soil drench treatment, where the severity was 18.6-20.0 and 16.5-18.6% respectively.

Table (4): Effect of three bactericides at different concentrations as soil treatment on percentage of actually infected tubers bacterial wilt and potato yield, under artificial inoculation conditions.

Treatment	Concentration (PPM)	Actually, infected tubers (%)	Mean Tubers yield	
			weight (g)/plant	Numbers/ plant
Cefalexin	50	16.7	248.2	8.7
	100	16.4	253.6	8.9
Mean		16.5	250.9	8.8
Gentamycin	50	16.9	240.9	8.6
	100	16.6	250.3	8.8
Mean		16.8	245.6	8.7
Copper	100	19.0	215.9	7.7
Sulphate	200	18.2	220.6	7.9
Mean		18.6	218.4	7.8
Check	0	25.7	121.9	4.6
LSD at		1.8	6.7	0.9

Table (5): Effect of three bactericides at different concentrations as tuber treatment on percentage of actually infected tubers bacterial wilt and potato yield, under artificial inoculation conditions.

Treatment	Concentration (PPM)	Actually, infected tubers (%)	Mean Tubers yield	
			weight (g)/plant	numbers/ plant
Cefalexin	50	18.3	230.0	8.2
	100	18.9	239.7	8.4
Mean		18.6	234.9	8.3
Gentamycin	50	18.6	226.2	8.1
	100	18.4	230.6	8.3
Mean		18.5	228.4	8.2
Copper	100	20.1	189.4	7.5
Sulphate	200	19.8	198.5	7.7
Mean		20.0	194.0	7.6
Check	0	25.7	121.9	4.6
LSD at 5%		2.1	8.3	0.8

DISCUSSION

Bacterial wilt (brown rot) disease of potato caused by *Ralstonia solanacearum* has been reported since many years ago (Briton-Jones, 1925). The disease causes economic problems that decrease the productivity of potato yield and hinders potato exportation to European Union (Abd El-Ghafar et al., 1995; Farag et al., 1999; Felix et al., 2010; Kehil, 2002; Muthoni et al., 2013). The disease is known to be favored by warm climates, therefore serious outbreaks have been reported (Grousset et al., 1998; Kago et al., 2017; Stead et al., 1996; Walker, 1992). Therefore, the origin of the disease in Egypt is thought to be the potato seeds imported from Europe (Balabel, 2006). In view of the fact that this disease is tubers borne, infected tubes may cause a disease outbreak in many localities if they are cultivated without careful inspection for eliminating infected tubers (Nion & Toyota, 2015).

All the tested chemical factors (cefalexin, gentamycin, and copper sulphate) were active in decreasing the growth of *R. solanacerum* compared with the control treatment. Copper sulphate was the most effective, but cefalexin was moderately effective, and gentamycin was less effective against the growth of R. solanacerum. Inhibition zone diameter was increased with increasing concentrations of tested chemical factors.

Treatment of bacterial cells with Cu-compounds leads 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 do not act directly on the bacterium, but appear 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 a toxic agent in inorganic bactericides, which showed direct inhibition of bacterial growth leading to cell death (Almoneafy et al., 2014; Sigee, 1993).

Application of chemical factors (cefalexin, gentamycin, and copper sulphate) as soil drench and/or tuber treatments, led to a decrease in the severity of potato bacterial wilt disease and percentage of actually infected tubers and it increased potato yield compared with control treatment under artificial inoculation conditions. Cefalexin and gentamycin were the most effective in controlling the disease. Application of chemical factors (cefalexin, gentamycin, and copper sulphate) as soil drench treatment was more effective than tuber treatment against potato bacterial wilt disease.

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مكافحة مرض الذبول البكتيري على البطاطس باستخدام معاملات غير حيوية

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المستخلص: مرض الذبول الجرثومي الناجم عن *Ralstonia solanacearum* (Smith) Yabuuchi. من أهم الأمراض البكتيرية على مستوى العالم، هدفت هذه الدراسة إلى اختبار بعض المركبات الكيميائية مثل المضادات الحيوية، وتم تطبيقها لمكافحة مرض الذبول البكتيري في ظل ظروف التلقيح الاصطناعي معملياً، وتأثيرها على إنتاج محصول البطاطس في ظل ظروف البيوت المحمية. حيث تم اختبار المركبات الكيميائية (سيفاليكسين، جنتامايسين، وكبريتات النحاس) وبينت النتائج خفض نمو بكتيريا *R. solanacerum* مقارنة بمعاملة التحكم. وقد كانت كبريتات النحاس هي الأكثر فاعلية حيث كان قطر منطقة التثبيط 6.0 إلى 7.4 ملم مقارنة مع جنتامايسين، 3.6 إلى 5.8 ملم على التوالي. ولكن سيفالكسين كان فعالاً بشكل معتدل 4.2 إلى 5.8 ملم، والجنتامايسين كان أقل فعالية من 3.6 إلى 5.8 ملم ضد العامل الممرض. كما بينت النتائج علاقة طردية بين قطر منطقة التثبيط مع تركيزات المركبات الكيميائية المختبرة. أظهرت تجارب البيوت المحمية أن المركبات الكيميائية المستخدمة أدت إلى تقليل شدة مرض الذبول الجرثومي للبطاطس، وزيادة محصول البطاطس مقارنة بمعاملة التحكم.عند استخدام Cefalexin كان متوسط الذبول 20.5، وشدة المرض 16.3، بينما كان متوسط الذبول 22.8، وشدة المرض 17.6 عند استخدام الجنتاميسين، وهو الأكثر فاعلية مقارنة بمعالجة كبريتات النحاس حيث كان الذبول 31.8، وشدة المرض 29.6. استخدام المركبات الكيميائية المختبرة كمعالجة غمر التربة أدت لنسبة تتراوح من 16.4 إلى 19.0 أكثر فاعلية من معالجات الدرنات، والتي كانت من 18.4 إلى 20.1.

الكلمات المفتاحية: العوامل الحيوية؛ الذبول البكتيري؛ التحكم؛ ليبيا؛ البطاطا؛ *Ralstonia solanacearum* .

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