

Chemical and Organic Amendments for Controlling Potato Bacterial Wilt

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Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the most important and widespread bacterial diseases of solanaceous crops in tropical, sub-tropical areas and some warm regions. The present research was aimed to evaluate some chemical and organic amendments, individually or in combinations, for controlling potato bacterial wilt disease under greenhouse and field conditions. Application of chemical amendments, *i.e.* urea, ammonium nitrate and ammoniated superphosphate, as well as organic amendments, *i.e.* garlic, cabbage and camphor dry leaves, either individually or in mixture, reduced severity of potato wilt under greenhouse and field conditions compared to the check treatment. Efficiency of tested amendments increased with increasing their application rates. In greenhouse experiments, chemical amendment was more effective than organic ones in disease control. Moreover, mixed treatments was more effective than their individual applications. Meantime, urea (as a chemical amendment) and dry garlic leaves (as an organic amendment), either individual or in mixture, were the most effective. Under field conditions, the population of *R. solanacearum* in rhizosphere, crown and potato tubers, along with severity of wilt were decreased, meanwhile potato yield was increased with mixing chemical and organic amendments compared to the check. Mixing urea with dry garlic leaves was the most effective to reduce population of *R. solanacearum* and to increase the yield, but other mixtures were moderately effective.

Keywords: Bacterial wilt, chemical amendment, organic amendment, potato and *Ralstonia solanacearum*.

Bacterial wilt *Ralstonia solanacearum*, phylotype II sequevar (Tomlinson *et al.*, 2011), is one of the most important quarantine plant diseases as listed in A2 European Plant Protection Organization during exportation (Anonymous, 2004). Potato is one of the four worldwide major food crops after wheat, maize and rice. Integrated pest management (IPM) is the important approach in sustainable agricultural development which has a role in reducing the crop losses, increasing productivity and minimizing contamination and health hazards (Nwilene *et al.*, 2008). Kelaniyangoda *et al.* (1997) reported that soil amendments with urea (200kg/ha), CaO (2t/ha), cow dung (10t/ha) and poultry manure (10t/ha) affected the bacterial wilt incidence. Urea (200 kg of N/ha) and CaO (5000 kg/ha) suppressed the survival of *Ralstonia solanacearum* (Michel and Mew, 1998). Mixing urea with calcium oxide at the rate of 80: 800 kg/rai reduced the artificial infested soil with

2.83×10⁷ CFU/ml of *R. solanacearum* bacteria (Vudhivanich, 2002). Moreover, three different levels (1, 5 and 10%) of cocopeat, farmyard manure (FYM) compost and green compost were used to control bacterial wilt (Yadess *et al.*, 2010). Different doses of Gypsum were tested on disease severity of *R. solanacearum* (Ajjappalavara *et al.*, 2008). Use of NPK fertilization (Messiha *et al.*, 2007), compost-amended (Ogai *et al.*, 2009 and De Brito, 1995), mushroom compost (Chellemi *et al.*, 1992) and leaf juices of hemp-agrimony (*Eupatorium cannabinum*) have shown a good results against soilborne diseases (Kumar and Tripathi, 1991). Cruciferous plants as soil biofumigants (Gamliel and Stapleton, 1993), ammonium sulfate, potassium sulfate, urea as well as dried leaves of cabbage, camphor and garlic were also used to control bacterial wilt (Abd El-Ghafar and Abd El-Sayed, 2002). Camphor (*Cinnamomum camphora*) was used as soil fumigant (Chen *et al.*, 1988). Essential oils namely thyme, camphor, caraway and lemon oils were tested against *R. solanacearum* (Frag, 2013).

The use of composts to suppress soilborne plant pathogens has been extensively reviewed by many researchers (De Ceuster and Hoitink, 1999; Mikhail *et al.*, 2005 and Youssef, 2007). Processed compost is used to develop marginal soils for crop production, and revegetate wasted and disrupted lands, prepare potting mixes, improve existing crop land, stimulate growth and suppress diseases caused by soilborne plant pathogens (Hoitink and Fahy, 1986 and Mays and Giordano, 1989). Application of organic amendments is an effective measure for controlling soilborne disease (Frag, 1976; Baker, 1981; Huang and Huang, 1993 and Abd El-Ghafar *et al.*, 2004). Bacterial wilt in tomato was suppressed in the poultry and farmyard manure added soil and higher microbial activity was likely responsible (Frag, 1976 and Islam and Toyota, 2004). The suppression or enhancing effect of composts depend on their chemical and biological composition (Litterick *et al.*, 2004) as well as on the pathogen involved (Termorshuizen *et al.*, 2006).

The aim of this study is to evaluate some chemical and organic amendments, individually or in combinations, for controlling potato bacterial wilt disease under greenhouse and field conditions, and to emphasize the effect of organic amendments and balanced nutrition in disease control.

Materials and Methods

1. Greenhouse experiments:

These experiments were carried out under greenhouse conditions at Plant Pathol. Dept., Fac. of Agric., Ain Shams Univ. Certified potato tubers (cv. Lady Rosetta) were kindly provided by Potato Brown Rot Project (PBRP), Egypt. Sterilized sand-clay soil (1:1 v/v) was used for planting in pots (5 kg/pot). Virulent isolate of *Ralstonia solanacearum* bacterium was selected and grown on King's B medium at 28°C for 48h. Bacterial growth was suspended in sterilized water and adjusted, according to its optical density (at A_{600nm} = 0.3), to 5.3 × 10⁸ CFU/ml, according to Michel and Mew (1998). Soil infestation was done by adding 250ml of the bacterial suspension to each pot containing sterilized sandy-clay soil. One of germinated potato tubers was sown for each pot containing infested soil. Chemical amendments, *i.e.* ammonium nitrate (33.3%-nitrogen), urea (46%-nitrogen) and ammoniated

superphosphate (46% P₂O₅ + 16% nitrogen), as well as organic amendments (dry leaves of garlic, camphor and cabbage) were applied, either individually (at rate of 0.5, 1 and 1.5 g/kg soil) or in mixture (at rate 1g/kg soil), to the infested soil 7 days before planting (Abd El-Ghafar *et al.*, 2004). A set of pots untreated with organic and/or chemical amendments was kept as check treatment. Five pots were used as replicates for each treatment.

Disease severity was assessed 40 and 60 days after plating. Severity of bacterial wilt was calculated as percentage of wilted potato shoots [(No. of wilted shoots/total No. of tested shoots) X 100] and as percentage of disease index from disease rating for individual plant using scale of six grades (based on the visual observation of the percentage of foliage wilt), *i.e.* 0= no symptoms, 1= up to 25%, 2= 26-50%, 3= 51-75%, 4 = 76-100% and 5= dead plants, according to Kemp and Sequeira (1983). Disease index percentage (DI%) was calculated using the following formula:

$$DI (\%) = [(R \times T) / (p \times N)] \times 100$$

Whereas; T= Number of plants counted within each category.

R= Disease rating scale (R = 0, 1, 2, 3, 4 and 5).

N= Total number of tested plants.

Also, disease reduction percentage (DC%) was calculated from DI% as the following formula:

$$DC (\%) = [(A - B) / A] \times 100$$

Whereas; A= Percentage of disease index in the check.

B= Percentage of disease index in the treatment.

2. Field experiments:

These experiments were carried out in Talia region, EL-Minufiya Governorate, Egypt. This region was chosen because of its history of infection with *R. solanacearum* bacterium. The experimental layout was split plot design with five replicates for each treatment. The plots (9 m²/replicate) consisted of five rows. Certified potato tubers (cv. Lady Rosetta) bought by Environmental Friendly Program for Controlling Potato Brown Rot project in Egypt (STDF 2905), Dokki, Egypt, were used for field planting and were. Mixtures of chemicals, *i.e.* urea and ammonium nitrate, as well as organic amendments, *i.e.* dry leaves of garlic and cabbage, were applied at two different rates, *i.e.* 360kg N/ha for chemical fertilizer and 12 ton/ha for organic amendments, as recommended by Ministry of Agriculture (Zaki, 2009) and 170kg of Nitrogen (1:1, weight)/Ha for mixed chemicals and organic amendments, as recommended by European Union (EU) (Messiha *et al.*, 2007).

2.1. Population of *R. solanacearum*:

Random samples of potato rhizosphere, shoot's crown and tubers, collected from Talia region, were used to study the population of *R. solanacearum* as latent infection on semi selective medium of South Africa (SMSA medium) according to Elphinstone *et al.* (1996). Rhizosphere soil samples (100g/replicate) were homogenized in the laboratory, then suspended by adding 10g of soil to 90ml of

sterilized phosphate buffer (0.1M, PH 7.2). Meanwhile, samples of shoot's crown (50 crown/replicate) and potato tuber (200 tuber/replicate) were crushed with 0.1M sterilized phosphate buffer (pH7.2). Approximately 0.1 ml of each suspension was spread onto SMSA agar plates (Michel and Mew, 1998), then incubated at 28°C for 3-5 days. Typical colonies of *R. solanacearum* were recorded to calculate the population (Sunaina *et al.*, 1989).

2.2. Disease severity and yield assessment:

Disease severity was assessed 80 days after planting. Severity of bacterial wilt was calculated as percentage of disease index (DI) as previously mentioned. The yield was assessed as number of tubers per plant and as weight of tubers (kg) per plant with harvesting the tubers. Five plants were randomly selected per treatment to assess the yield (Yadess *et al.*, 2010).

3. Statistical analysis:

Analysis of variance (ANOVA) was carried out according to Snedecor and Cochran (1967). ANOVA was carried out using compatible computer basic language. LSD test was used to compare treatment means at 5% level of significance.

Results

1. Effect of soil amendment on potato bacterial wilt disease:

1.1. Under greenhouse conditions:

Application of chemical amendments led to the reduction severity of the bacterial wilt disease after artificial inoculation (Table 1). Disease severity was decreased with increasing the rate from 0.5 to 1.5 g/kg soil for chemical amendment, where percentage of disease reduction was progressed from 52.9 to 63.6% with ammonium nitrate, from 43.5 to 54.8% with ammoniated superphosphate and from 59.8 to 70.1 with urea, 60 days after planting. Urea was the most effective treatment in disease reduction (being 70.1%). Super phosphate was less effective one compared with other chemical amendments, where the percentage of disease reduction reached 54.8%, at the rate 1.5 g/kg soil 60 days after planting.

In addition, organic amendments led to reduce the disease severity compared to the check treatment under artificial inoculation condition (Table 2). Disease severity was decreased with increasing the rate from 0.5 to 1.5g organic amendment/kg soil where disease reduction increased from 44.3 to 57.7% with cabbage and from 39.2 to 49.3% with camphor, as well as from 56.0 to 66.7% with garlic 60 days after planting. Dried leaves of garlic was the most effective to reduce the disease (being 66.7%). Meanwhile, dried leaves of cabbage were moderately effective followed by the dried leaves of camphor (being 57.7% and 49.3%, respectively).

Mixture of chemical fertilizers with any organic amendment (at rate 1g/kg soil) was more effective in reducing potato bacterial wilt severity under artificial inoculation conditions in greenhouse compared to the check treatment. Data in Table (3) indicate that applying urea mixture with any organic amendment was more effective in reducing the disease compared to other chemical amendment treatments, when the disease reduction reached 63.2, 65.5 and 74.8% with camphor, cabbage

Table 1. Effect of different rates of Chemical fertilizers, on severity of bacterial wilt 40 and 60 days after planting under greenhouse conditions

Treatment	Rate (g/kg soil)	Disease severity (%) after (days)					
		40 days			60 days		
		A*	B	C	A	B	C
Urea	0.5	10.5	6.0	39.4	18.9	16.8	59.8
	1.0	9.3	6.2	37.4	14.5	13.7	67.2
	1.5	9.0	5.9	40.4	11.7	12.5	70.1
Ammonium nitrate	0.5	12.7	7.1	28.3	21.7	19.7	52.9
	1.0	11.2	6.8	31.3	18.6	16.7	60.0
	1.5	10.4	6.4	35.4	16.6	15.2	63.6
Ammoniated super phosphate	0.5	13.2	8.4	15.2	26.1	23.6	43.5
	1.0	10.1	7.2	27.3	21.6	20.0	52.2
	1.5	9.2	7.5	24.2	18.4	18.9	54.8
Check	0.0	14.3	9.9	0.0	45.3	41.8	0.0
LSD at 5%		1.2	1.1	----	1.4	1.6	---

*A= Wilted shoots (%), B= Disease index and C= Disease reduction (%).

Table 2. Effect of different organic amendments rates, on the severity of potato bacterial wilt, after 40 and 60 days from planting, under greenhouse conditions

Treatment	Rate (g/kg soil)	Disease severity (%) after (days)					
		40 days			60 days		
		A*	B	C	A	B	C
Garlic	0.5	11.8	7.0	29.3	22.8	18.4	56.0
	1.0	8.7	6.2	37.4	18.7	15.8	62.2
	1.5	6.2	5.0	49.5	15.6	1.9	66.7
Cabbage	0.5	13.1	8.3	16.2	25.6	23.3	44.3
	1.0	10.3	7.5	24.2	22.7	19.0	54.5
	1.5	8.4	6.3	36.4	21.6	17.7	57.7
Camphor	0.5	14.0	9.2	7.1	28.8	25.4	39.2
	1.0	12.7	8.8	11.1	25.4	22.5	46.2
	1.5	11.3	7.9	20.2	23.3	21.2	49.3
Check	0.0	14.3	9.9	0.0	45.3	41.8	0.0
LSD at 5%		1.3	1.0	----	2.3	2.0	----

* As desccribed in footnote of Table (1).

and garlic, respectively. Meanwhile, mixture of ammonium nitrate or ammoniated super phosphate with any organic amendment gave moderate disease reduction effect. In case of ammonium nitrate mixture with camphor, cabbage and garlic, disease reduction reached 61.7, 64.2 and 70.6%, respectively. Meanwhile, in case of ammoniated super phosphate mixture camphor, cabbage and garlic, disease reduction reached 56.8, 59.2 and 65.3%, respectively.

Table 3. Influence of interaction between Chemical and organic amendments (at the rate of 1g/kg soil) on severity of potato bacterial wilt 40 and 60 days after planting, under greenhouse conditions

Chemical fertilizer	Organic amendment	Disease Severity (%) after days					
		40			60		
		A*	B	C	A	B	C
Urea	Garlic	8.6	6.9	56.1	18.0	13.9	74.8
	Camphor	10.6	9.2	41.4	22.4	20.3	63.2
	Cabbage	9.9	8.0	49.0	21.0	19.0	65.5
Ammonium nitrate	Garlic	9.2	7.2	54.1	19.0	16.2	70.6
	Camphor	11.4	9.8	37.6	23.3	21.1	61.7
	Cabbage	10.2	8.4	46.5	21.5	19.7	64.2
Ammoniated super phosphate	Garlic	10.1	7.9	49.7	21.3	19.1	65.3
	Camphor	13.6	10.4	33.8	25.4	23.8	56.8
	Cabbage	11.8	8.9	43.3	24.2	22.5	59.2
Check	None	18.4	15.7	0.0	58.7	55.1	0.0
LSD at 5%		1.0	1.2	----	1.9	1.7	----

* As described in footnote of Table (1).

1.2. Under field conditions:

On the basis of obtained results from greenhouse experiments, the most effective mixture treatments of urea or ammonium nitrate with dried leaves of cabbage or garlic were selected to evaluate their effect on reducing potato bacterial wilt severity under naturally infested field conditions.

1.2.1. Population of *R. solanacearum* bacterium:

Data presented in Table (4) show that mixture either urea or ammonium nitrate with cabbage or garlic amendments, decreased the densities of *R. solanacearum*. Urea with the low rate of garlic decreased the densities from log 5.4 in the check to log 3.1 in sampled rhizosphere soil. The corresponding figures in potato rhizosphere were log 5.4 to log 3.6 in case of urea cabbage treatment. Similar trend was recorded with ammonium nitrate with either dried cabbage or garlic amendments. The densities of *R. solanacearum* in the crown area of potato showed a pronounced decrease from log 2.3 to log 1.6 and log 1.4 in urea treatment mixed with either dried cabbage or garlic at the high level, and similar trend could be recognized in case of the ammonium nitrate treatments. Moreover, the population in tubers followed the same sequence being more pronounced with cabbage and urea treatments.

1.2.2. The yield:

Data in Table (5) show that mixing chemical and organic amendment led to decrease severity of potato bacterial wilt disease and to increase potato yield compared to the check treatment, where the percentage of disease reduction ranged between 16.5 to 24.2% and the yield was increased from 1.39 to 2.14 kg/plant.

Table 4. Effect of mixed chemical fertilizers and organic amendments at different rates on count of *R. solanacearum* in rhizosphere, crown and tuber samples under natural infection conditions

Chemical fertilizer	Organic amendment	Rate*	Count of <i>R. solanacearum</i> (log 10 ⁵ /g/sample)		
			A**	B	C
Urea	Cabbage	EG	3.7	1.6	1.3
		EU	3.6	1.6	1.2
	Garlic	EG	3.4	1.5	1.0
		EU	3.1	1.4	1.0
Ammonium nitrate	Cabbage	EG	4.6	1.9	1.6
		EU	4.4	1.8	1.5
	Garlic	EG	3.9	1.7	1.4
		EU	3.8	1.5	1.3
Check	Non	untreated	5.4	2.3	1.8

* EG= Ministry of Agriculture recommendations: 360 kg N/ha for chemical fertilizer and 12 ton /ha for organic amendments. EU= EU recommendations: 170 kg of Nitrogen/ha (1:1, weight) for mixed chemical and organic amendments.

** A= Population in rhizosphere; B= Population in crown and C= Population in tuber.

Table 5. Effect of mixed chemical fertilizers and organic amendments at different rates on severity of potato bacterial wilt disease and potato yield, under natural inoculation conditions

Chemical fertilizer	Organic amendment	Rate*	Disease severity (%)	Disease reduction (%)	Tuber yield/plant	
					No. of tubers	Weight of tubers (kg)
Urea	Cabbage	EG	18.3	2.8	8.60	1.90
		EU	18.0	22.1	8.72	1.99
	Garlic	EG	17.8	22.9	8.92	2.08
		EU	17.5	24.2	9.23	2.14
Ammonium nitrate	Cabbage	EG	19.3	16.5	8.24	1.57
		EU	19.0	17.7	8.30	1.62
	Garlic	EG	18.7	19.0	8.39	1.78
		EU	18.3	20.8	8.49	1.83
Check	None	Untreated	23.1	0.0	8.21	1.39
LSD at 5%			2.4	-----	0.42	0.94

* As described in footnote of Table (4).

Mixture of urea and dried leaves of garlic was the most effective in reducing the disease and increasing the yield, the percentage of disease reduction was 22.9-24.2% and the yield was 2.08-2.14 kg/ plant. Mixture of ammonium nitrate and dried leaves of garlic were moderately effective, where percentage of disease control was 18.0-18.7% and the yield was 1.78-1.99 kg/plant. Meanwhile, mixture of ammonium nitrate and dried leaves of cabbage were less effective, where percentage of disease control was 19.0-19.3% and the yield was 1.57-1.62 kg/plant.

Discussion

Bacterial wilt disease of potato was reported in Egypt many years ago. The disease causes economic problems that decrease the productivity of potato yield and hinders potato exportation to European Union (Farag *et al.*, 1999 and Kehil, 2002). Application of chemical and organic amendments individually or mixed led to decrease in severity of potato bacterial wilt and population densities of *Ralstonia solanacearum* in rhizosphere, crown and potato tubers along with increase in potato yield compared to the check. Urea as chemical amendment and dried leaves of garlic either individually or in mixture with organic amendements were the most effective to reduce the disease severity and population *R. solanacearum* and to increase potato yield compared to other treatments. These results are in agreement with (Abd El-Ghafar and Abd El-Sayed 2002; Abd El-Ghafar *et al.*, 2004; Islam and Toyota, 2004 and Michel and Mew, 1998).

Huber and Watson (1970) concluded the influence of organic amendments on soilborne disease by increasing the biological buffering capacity of the soil, reducing pathogen numbers during the hydrolysis of organic material, affecting nitrification which influences the form of nitrogen predominating. Application of organic amendments are effective treatment for controlling soilborne disease (Baker, 1981; Huang and Huang, 1993 and Abd El-Ghafar *et al.*, 2004). Chen *et al.* (1988) found that the suppressive compost possess a higher microbial activity causes a depletion in essential nutrient for the survival and multiplication of the pathoen, the compost have potential to provide a conducive environment for the proliferation of Rhizobacteria antagonistic to certain root pathogens. Population and survival of *R. solanacearum* was declined at the early stage of composting. The compost pH during the early period of composting process was decreased due to production of organic acids causing further acidification as a result of microbial activity (Ginting *et al.*, 2003 and Venglovsky *et al.*, 2005). The effect of compost on *R. solanacearum* is biotic and related to shift in soil microbial community structure towards a community with enhanced antagonism (Farag, 1976 and Schonfeld *et al.*, 2003). Recheigl (1995) recorded that organic soil amendment led to physical effects on soil properties including (a) reducing the bulk density of soil (b) increasing water holding capacity (c) increasing water infiltration and drainage in fine-textures and (d) improved soil aggregation. Resistance by mineral amendment can be increased by three mechanisms: (i) changes in anatomy (ex. a higher degree of lignification), (ii) physiological and biochemical changes leading to higher production of inhibitory or repelling substances, and (iii) restriction of nutrient transfer to the pathogen which it requires for growth or development (Marschner, 2012). Garlic has lethal mechanism against soil borne diseases where contains on volatile organic chemical components alyel disulphyde, Dimethyl disulfide and Allyl propyl disulfide. Also, contain on Allicin which works as antibacterial, antifungal, antiviral, and antiprotozoal activity (Salama *et al.*, 2014).

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إستخدام الاضافات الكيماوية والعضوية لمكافحة مرض الذبول البكتيري في البطاطس

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ي بول البكتيري المتسبب عن بكتيريا *Ralstonia solanacearum* من اكثر الامراض البكتيرية اهمية وانتشارا في محاصيل العائلة الباذنجانية في المناطق الاستوائية وتحت الاستوائية والدافئة. الهدف من تلك الدراسة تقييم بعض الاضافات الكيماوية (نترات الامونيوم البوريا) والعضوية (الاوراق الجافة من الكرنب والكافور والثوم) منفردة خليط لمقاومة مرض الذبول البكتيري في البطاطس تحت ظروف الصوبة والحقل. من أهم النتائج المتحصل عليها تحت ظروف الصوبة أن إستخدام الاضافات الكيماوية والعضوية منفردة أو في خليط معاملة المقارنة. وتزداد كفاءة الاضافات الكيماوية والعضوية مع زيادة معدلات استخدامها. كما أن الاضافات الكيماوية كانت أكثر كفاءة من الاضافات العضوية الخليط

. حيث وجد ان اليوريا كإضافة كيماوية والاوراق الجافة للثوم كإضافة عضوية فردية أو في خليط كانت الاكثر فاعلية في

أن زيادة أعداد الميكروب الممرض *R. solanacearum* في منطقة الريزوسفير ومنطقة التاج وكذلك لدرنات لنباتات البطاطس كان منخفضا بدرجة معنوية كما أن استخدام الاضافات الكيماوية والعضوية في خليط ادت الي زيادة محص البطاطس وهذا قياسا علي معاملة المقارنة. باليوريا والاوراق الجافة للثوم كانت من أفضل المعاملات في خفض أعداد البكتيريا الممرضة وشدة المرض وزيادة كمية المحصول بالمقارنة مع باقي المعاملات الاخرى.