Using bicarbonates for controlling late blight disease of potato plants under field conditions

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Abstract: Late blight disease caused by *P. infestans* of potato plants was controlled under field conditions using potassium or sodium bicarbonates alone or in combination with Citral. In laboratory experiments, results indicated that complete inhibition in linear growth of *P. infestans* was obtained with potassium or sodium bicarbonates at 2 % and Citral at 0.5 %. Moreover, in field experiments, results indicated that the most effective treatment was potassium bicarbonate at 2.0 % plus Citral at 0.5 % which reduced the disease severity by 84.4 and 82.4 % during two growing seasons.The highest reduction was obtained with potassium or sodium bicarbonate at 1.0 or 2.0 respectively plus citral at 0.5 % and Redomil which reduced the early blight incidence more than 61.8 % as compared with untreated plants. As for potato yield the highest increased was obtained with potassium bicarbonate at 2.0 % plus Citral at 0.5 % which increased tuber yield by 76.0 and 67.9 % during two growing seasons.All treatments significantly increased the chitinase and *β*-1,3 – glucanase activities. The great increased was obtained with potassium bicarbonate at 2.0 or 1.0 % plus Citral which increased the chitinase and β-1,3 – glucanase activities more than 120.0 and 220.1 % respectively. It could be suggested that combined treatments between potassium bicarbonate plus Citral might be used for controlling late blight disease of potato plants under field conditions.
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1. Introduction

Potatoes (*Solanum tuberosum* L.) are considered one of the most important vegetable crops in Egypt. Late blight caused by *Phytophthora infestans* is the most important disease attacking potato plants (Bad-El-Kareem, et *al.,* 2001; Pilet *et al.,* 2006; El-Gamal *et al.,* 2007 and Skelsey *et al.,* 2009).

There has been considerable interest in the use of baking soda (sodium bicarbonate,($NaHCO_3$) and potassium bicarbonate ($KHCO_3$) for controlling various plant fungal diseases (Karabulut *et al.*, 2003 and Smilanick *et al.*,2006). Bicarbonates are widely used in the food industry (Lind say, 1985) were fount to suppress several fungal diseases of cucumber plants (Ziv and Zitter, 1992). Baking soda sprays provided good control of several plant diseases (Horst *et al.*, 1992; Arimoto *et al.*, 1997;Palmer *et al,* 1997; Janisiewicz, and Peterson. 2005).Also potassium bicarbonate provided the best protection against plant diseases (Smilanick and Margosan, 1999; Fallilk *et al.*,1996 and Smilanick, *et al.*,2006). Sodium or potassium bicarbonates combined with oil were effective in the control plant diseases (Horst *et al.*, 1992 and Ziv and Zitter, 1992). Abd-El-Kareem (2007) reported that potassium bicarbonates combined with Nerol significantly reduce the early blight disease and increased the tuber yield of potato plant under field conditions. Citral as one fractions of citrus essential oil caused complete inhibition of the linear growth of Giotrichum *candidum*, *Penicillium digitatum* and *P. italicum* as causal agents of fruit citrus diseases (El-Mohamedy *et al.,* 2002).

The main objectives of the present research are studying the effect of sodium or potassium bicarbonates alone or in combination with Citral against late blight disease of potato plants under field conditions.

2. Materials and Methods

Source of pathogenic fungi and potato tubers

Pathogenic isolate of Phytophthora infestans the causal agent of late blight diseases was kindly obtained from Plant Pathology Dept., National Research Centre, Giza, Egypt.Meanwhile, Potato tubers cv. Sponta were obtained from Dept., of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt.

Laboratory experiments

Effect of different concentrations of potassium, sodium bicarbonates or citral on linear growth of Phytophthora infestans

Different concentrations of potassium or sodium bicarbonates and Citral as one fractions of citrus essential oil (obtained from Delta Aromatic Company, Egypt) were tested to study their inhibitory effect on linear growth of *P. infestans*. Five concentrations of potassium or sodium bicarbonate

i,e. 0.0,0.25,0.50, 1.0 and 2.0 % (w / v) and Citral at 0.10, 0.25, and 0.50 % (v/ v) were added individually to conical flasks containing sterilized rye agar medium (Cohen *et al.,* 1991) to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri dishes (9 cm – diameter). Plates were individually inoculated at the center with equal disks (6-mm- diameter) of 10-days old culture of *P. infestans*. Five plates were used as replicates for each particular treatments. Inoculated plates were incubated at 25 ± 2 °C.The average linear growth of fungus was calculated after 10 days.

Field experiments:

Effect of potassium or sodium bicarbonates alone or in combination with Citral on late blight severity of potato plants under field conditions. Experiments were carried out, in Experimental Farm of National Research Centre at El-Noubareia, Behera governorate.

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with Citral at 0.5 % in addition to Fungicides (Redomil® – plus at 2 g / l)were applied under field conditions to study the possibility of their effect against late blight disease during tow cultivation seasons. Potato yield was also determined at two cultivation seasons.

Field experiments were conducted under natural infection in plots (4x8 m) each comprised of 8 rows and 32 holes / row, was conducted in a completely randomized block design with three replicates (plots) for each particular treatment.

Treatments:

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with Citral at 0.5 % in addition to Fungicides Redomil® – plus at 2 g / l (2,6 dimethyl phenyl methoxyacetyl amino) From Syngenta Crop Protection, Company, Australia were applied as follow: -

Treatments	
Single	Combined
1- $KHCO_3$ 1 %	5- $KHCO_3$ 1 % + Citral
2- $KHCO_3$ 2 %	6- $KHCO_3$ 2 % + Citral
3- $NaHCO_3$ 1 %	7- $NaHCO_3$ 1 % + Citral
4- $NaHCO_3$ 2 %	8- $NaHCO_3$ 2 % + Citral
Control	
Redomil® plus 2 g / l Un-treated plants	

Application:

All treatments were applied as foliar application on potato plants which had 4-5 compound leaves and every 15 days up to 90 days of planting.

Disease assessment

Late blight scale from 0 to 4 according to Cohen et al., (1991) was used, as follows:

0 = No leaf lesions.
1 = 25 % or less.
2 = 26 – 50
3 = 51 –75
4 = 76-100 % infected area of plant leaf.

Late blight disease was recorded up to 90 days of planting.

Determination of tuber yield

Tuber yield of potato (kg /m^2) for each treatment was determined.

Effect of potassium or sodium bicarbonates alone or in combination with Citral on enzymes activity of potato plants.

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with Citral at. 0.5 % were tested to study their effect on chitinase and β-1,3 -glucanase activities of potato plants.

Extraction of enzymes .Chitinase and β-1,3 -glucanase activities was determined after 40 days of planting. The enzymes extracted of potato leaves and the supernatant was prepared according to method of (Tuzun *et al.,* 1989)

Chitinase assay: chitinase activity was determined by colourimetric method of Boller and Mauch (1988). Colloidal chitin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars.

Chitinase activity was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes.

β-1,3 -glucanase assay:-

The method of Abeles and Forrence (1970) was used to determine glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars.

The method was carried out as 0.5 ml of enzyme extract was added to 0.5 ml of 0.05 M of potassium acetate buffer (pH 5) containing 2% laminarin. The mixture was incubated at 50°C for 60 minutes. The reaction was stopped by adding 1 ml of dinitrosalicylic acid reagent and heating the tubes for 5 minutes at 100°C. The tubes were cooled and 3 ml of distiled water were added before assay. The optical density was read at 500 nm. 1 β-1,3-glucanase activity was expressed as mM glucose equivalent released gram fresh weight tissues / 60 minutes.

Statistical analysis :

Tukey test for multiple comparisons among means was utilized (Neler *et al.,* 1985).

3. Results

Laboratory experiments

Effect of potassium or sodium bicarbonate and citral on linear growth of *P. infestans*

Potassium or sodium bicarbonates at five concentrations *i.e* 0.0,0.25, 0.50, 1.0 and 2.0 % (w / v) and Citral at 0.1, 0.25, and 0.50 % (v/v) were

tested to study their inhibitory effect on linear growth of *P. infestans in vitro*. Results in Table (1) indicate that all treatments significantly reduced the linear growth of of *P. infestans.* Complete inhibition was obtained with potassium or sodium bicarbonates at 2 % and Citral at 0.50 %. The highest reduction was achieved with potassium bicarbonate at 1.0 % which reduced the linear growth by 93.3 % as compared with untreated plates. Meanwhile, potassium or sodium bicarbonates at 0.50 and 1.0 % respectively and Citral at 0.25 % had moderate effect. Other treatments showed less effective.

Table(1) Linear growth (mm) of P. infestans as affected with different concentrations of potassium or sodium bicarbonates and citral

Treatments	Concent. %	Linear growth (mm)	Reduction %
$KHCO_3$	0.25	42.0 [c]	53.3
	0.50	31.0 [d]	65.6
	1.0	6.0 [f]	93.3
	2.0	0.0 [f]	100.0
$NaHCO_3$	0.25	53.0 [b]	41.1
	0.50	37.0 [c]	58.9
	1.0	12.0 [e]	86.7
	2.0	0.0 [f]	00.0
Citral	0.1	32.0 [d]	64.4
	0.25	10.0 [e]	88.9
	0.50	0.0 [f]	0.0
Control		90.0 [a]	-----

Figures with the same letter are not significantly different (*P*= 0.05)

Field experiments

Effect of potassium or sodium bicarbonate alone or in combination with citral on late blight incidence of potato plants under natural infection

The promising treatments in laboratory experiments were applied under field conditions during tow successive growing seasons.

Potassium or sodium bicarbonate at 1.0 or 2.0 % alone or in combination with Citral at 050 %. In addition to Fungicides (Redomyl® – plus at 2 g / l) were applied to study their effect on late blight severity of potato plants. Results in table (2) indicate that all treatments significantly reduced the disease incidence. The most effective treatment was potassium bicarbonate at 2.0 % plus Citral at 0.5 % which reduced the disease severity by 84.4 and 82.4 % during two growing seasons.The highest reduction was obtained with potassium or sodium bicarbonate at 1.0 or 2.0 respectively plus Citral at 0.5 % and Redomyl® which reduced the early blight incidence more than 61.8 % as compared with untreated plants. Single treatments with potassium bicarbonate at 2.0 % reducer the disease incidence by 47.1 and 50.0 % during two growing seasons. Other treatments showed moderate effect.

Table (2) Late blight incidence [(2)] on potato plants as affected with potassium or sodium bicarbonates alone or in combination with citral under field conditions

Application (%)	First season		Second season	
	Disease incidence	Reduction %	Disease incidence	Reduction %
Single treatment				
$KHCO_3$ 1.0	2.0 [b]	37.5	2.3 [b]	32.4
$KHCO_3$ 2.0	1.6 [c]	50.0	1.8 [c]	47.1
$NaHCO_3$ 1.0	2.3 [b]	28.1	2.3 [b]	32.4
$NaHCO_3$ 2.0	1.9 [b]	40.6	1.9 [c]	44.1
Citral 0.5	2.1 [b]	34.4	2.0 [c]	41.2
Combined treatment				
$KHCO_3$ 1.0 + Citral	1.1 [e]	65.6	1.3 [ed]	61.8
$KHCO_3$ 2.0 + Citral	0.5 [f]	84.4	0.6 [f]	82.4
$NaHCO_3$ 1.0 + Citral	1.5 [d]	53.1	1.6 [d]	53.0
$NaHCO_3$ 2.0 + Citral	1.0 [e]	68.8	1.2 [e]	64.7
Redomyl – plus 2 g / l	1.0 [e]	68.8	1.1 [e]	67.6
Control	3.2 [a]	—	3.4 [a]	—

(1) Figures with the same letter are not significantly different (*P*= 0.05)

(2) Late blight scale from 0 to 4 according to Cohen *et al*. (1991).

Effect of potassium or sodium bicarbonates alone or in combination with Citral on tuber yield of potato plants under field conditions

Potassium or sodium bicarbonate at 1.0 or 2.0 % alone or in combination with Citral at 0.50 %. In addition to Fungicides (Redomyl® – plus at 2 g / l) were applied to study their effect on tuber yield of potato plants. Results in table (3) indicate that all treatments significantly increased the tuber yield. The high increased was obtained with potassium bicarbonate at 2.0 % plus citral at 0.5 % which increased tuber yield by 76.0 and 67.9 % during two growing seasons. Moderate increased was obtained with potassium bicarbonate at 2.0 as single treatment, potassium bicarbonate at 1.0 % plus citral at 0.5 %, sodium bicarbonate at 2.0 plus Citral at 0.5 % and Redomyl® which increased the potato yield more than 40.0 %. Other treatments showed moderate effect.

Table (3) Tuber yield of potato plants as affected with different concentrations of potassium or sodium bicarbonates alone or in combination with citral under field conditions.

Application (%)	First season		Second season	
	Yield (kg /m2) Mean	Increase %	Yield (kg /m2) Mean	Increase%
Single treatment				
KHCO3 1.0	3.0 df	20.0	3.6 cd	28.6
KHCO3 2.0	3.7 bc	48.0.	4.2 b	50.0
NaHCO3 1.0	3.3 cd	32.0	3.5 cd	25.0
NaHCO3 2.0	3.3 cd	32.0	3.8 c	35.7
Citral 0.5	3.0 df	20.0	3.2 f	14.2
Combined treatment				
KHCO3 1.0 + Citral	4.0 b	40.0	4.2 b	50.0
KHCO3 2.0 + Citral	4.4 a	76.0	4.7 a	67.9
NaHCO3 1.0 + Citral	3.5 cd	40.0	3.8 cd	35.7
NaHCO3 2.0 + Citral	3.7 bc	48.0.	4.2 b	50.0
Redomyl – plus 2 g / l	3.7 bc	48.0	4.2 b	50.0
Control	2.5 g		2.8 g	

Figures with the same letter are not significantly different (*P*= 0.05)

Effect of potassium or sodium bicarbonates alone or in combination with Citral on chitinase activity of potato plants under field conditions

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with Citral at 0.50 %. were applied to study their effect on chitinase activity of potato plant. Results in table (4) indicate that all treatments significantly increased chitinase activity. The most effective treatment was potassium bicarbonate at 2.0 or 1.0 % plus Citral at 0.5 % which increased the activity by 126.7 and 120.0 % respectively as compared with untreated plants. Potassium bicarbonate at 2.0 as single treatments and sodium bicarbonate at 2.0 % in combination with Citral increased the enzyme activity by 100.0 %. Other treatments show moderate effect.

Effect of potassium or sodium bicarbonates alone or in combination with Citral on β-1,3 – glucanase activity of potato plants under field conditions

Potassium or sodium bicarbonates at 1.0 or 2.0 % alone or in combination with citral at 0.50 % were applied to study their effect on **β**-1,3 – glucanase activity of potato plant. Results in table (5) indicate that all treatments significantly increased β-1,3 – glucanase activity.The most effective treatment was potassium bicarbonate at 2.0 or 1.0 % plus Citral at 0.5 % which increased the activity by 333.3 and 220.1 % respectively as compared with untreated plants. Potassium bicarbonate at 2.0 as single treatments and sodium bicarbonate at 2.0 % in combination with Citral increased the enzyme activity by 195.8 and 200.0 %. Other treatments show moderate effect.

Table (4) Chitinase activity of potato plants as affected by potassium or sodium bicarbonate alone or in combination with citral

Application (%)	Chitinase activity (2) Mean	Increase %
Single treatment		
$KHCO_3$ 1.0	2.7 [c]	80.0
$KHCO_3$ 2.0	3.0 [b]	100.0
$NaHCO_3$ 1.0	2.6 [c]	73.0
$NaHCO_3$ 2.0	2.6 [c]	73.0
Citral 0.5	2.5 [c]	66.7
Combined treatment		
$KHCO_3$ 1.0 + Citral	3.3 [a]	120
$KHCO_3$ 2.0 + Citral	3.4 [a]	126.7
$NaHCO_3$ 1.0 + Citral	2.6 [c]	73.0
$NaHCO_3$ 2.0 + Citral	3.0 [b]	100.0
Control	1.5 [d]	—

(1) Figures with the same letter are not significantly different (*P*= 0.05)

(2) Chitinase activity expressed as mM N-acetyl glucose amine equivalent released / gram fresh weight/ 60 min.

Table (5) β-1,3 – glucanase activity of potato plants as affected by potassium or sodium bicarbonate alone or in combination with Citral

Application (%)	*β*,1-3 – glucanase [(2)] Mean	Increase %
Single treatment		
$KHCO_3$ 1.0	6.5 [c (1)]	170.8
$KHCO_3$ 2.0	7.1 [b]	195.8
$NaHCO_3$ 1.0	6.4 [c]	166.7
$NaHCO_3$ 2.0	6.6 [c]	175.0
Citral 0.5	6.0 [d]	150.0
Combined treatment		
$KHCO_3$ 1.0 + Citral	7.7 [a]	220.1
$KHCO_3$ 2.0 + Citral	8.0 [a]	233.3
$NaHCO_3$ 1.0 + Citral	6.4 [c]	166.7
$NaHCO_3$ 2.0 + Citral	7.2 [b]	200.0
Control	2.4 [e]	—

(1) Figures with the same letter are not significantly different (*P*= 0.05)

(2) *β-1*,3-glucanase activity was expressed as mM glucose equivalent released gram fresh weight tissues / 60 minutes.

4. Discussion

Potatoes (*Solanum tuberosum* L.) are considered one of the most important vegetable crops in Egypt. Late blight caused by *Phytophthora infestan*s is the most important disease attacking potato plants (Pilet *et al.,* 2006; El-Gamal *et al.,* 2007 and Skelsey *et al.,* 2009)

Controlling this disease depends mainly on fungicidal treatments (Pasche *et al.,* 2005). Bicarbonates are widely used in the food industry **(Lind say 1985)** have antifungal activity (Ziv and Zitter, 1992). In present study, results indicate that potassium or sodium bicarbonates and Citral showed great inhibitory effect in linear growth of *P. infestans.* Complete inhibition was obtained with potassium or sodium bicarbonates at 2 % and Citral at 0.50 % *in vitro*. In this respect Ziv and Zitter (1992) reported that Potassium and sodium bicarbonates have inhibitory effects against several pathogenic fungi. In addition to bicarbonates salts has been shown to have a profound inhibitory effect on several fungi and causes the collapse of hyphal walls and shrinkage of conidia (Punja and Grogan, 1982 and Ziv and Zitter, 1992).

Potassium and sodium bicarbonates were fount to control several plant diseases (Smilanick and Margosan, 1999; Janisiewicz and Peterson. 2005;, and Smilanick *et al.*, 2006). In present study, in field experiments, results indicate that all treatments significantly reduced the disease incidence. The most effective treatment was potassium bicarbonate at 2.0 % plus Citral at 0.5 % which reduced the disease severity by 84.4 and 82.4 % during two growing seasons. As for potato yield the highest increased was obtained with potassium bicarbonate at 2.0 % plus Citral at 0.5 % which increased tuber yield by 76.0 and 67.9 % during two growing seasons. In the present study bicarbonate and Citral oil are effective for controlling early blight disease.Their effectiveness is quite good when applied as single treatments but improved when used in combination. This result may be due to: the antifungal activity of bicarbonate and citral, synergistic effect between bicarbonate and citral and increased the activity of chitinase enzyme of potato against fungal pathogen. In this respect Punja and Grogan (1982) reported that the improved effectiveness of control with combinations of bicarbonates plus oil was attributed to fungicidal characteristics of bicarbonates ions.Moreover, the fungicidal and spreader sticker characteristics of oil that keep the bicarbonate ions on foliar surfaces (Homma *et al.,* 1981 and Ziv and Zitter, 1992).

Hypothesis have been proposed for the inhibitory mechanisms of bicarbonates and oil. Hydrogen ion concentration of bicarbonates salts has been shown to have a profound inhibitory effect on sclerotia and conidia germination of *S. rolfesii* and *S. fuliginea* respectively (Punja and Grogan, 1982 and Homa *et al.,* 1981).Furthermore, film-forming polymers may form a physical barrier on leaf surfaces against germ tube penetrations (Elad *et al.,* 1989 and Ziv, and Zitter 1992). The bicarbonate causes the collapse of hyphal walls and shrinkage of conidia, (Punja and Grogan, 1982 and Ziv and Zitter, 1992).On the other hand the role of potassium (Potassium bicarbonate) in increasing crop resistance to diseases caused by bacteria and fungi was widely reviewed by Perrenoud (1990). In general, potassium application improves plant health and vigour, making infection less likely or enabling a quick recover (Perrenoud, 1993).Potassium probably exerts its greatest effects on disease through specific metabolic functions that alter compatibility relationships of the host-parasite environment and increases the production of disease inhibitory compounds, such as phenols, phytoalexins and auxins around infection sites of resistant plants. (Kiraly, 1976). In the present study results indicate that all treatments increased the chitinase and B -1,3-glucanase activities.In this respect, B-1,3-glucanases and chitinases are able to hydrolyze B -1,3-glucan and chitin, respectively, the major components of fungal cell walls (Kauffmann *et al.*, 1987, Legrand *et al.,* 1987 and Abd-El-Kareem *et al.,* 2009). Sodium or potassium bicarbonates combined with oil were effective in the control of several plant fungal diseases (Horst *et al.,* 1992 and Ziv and Zitter, 1992).

It could be suggested that combined treatments between potassium bicarbonate plus citral might be used for controlling late blight disease of potato plants under field conditions.

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