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SALTS APPLICATION FOR SUPPRESSING POTATO EARLY BLIGHT DISEASE

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Abstract: The suppressive effect of sodium and calcium salts applied individually or combined with the yeast *Saccharomyces cerevisiae* against *Alternaria solani* the causal agent of early blight disease of potato was evaluated under laboratory, greenhouse and field conditions. *In vitro* test a complete inhibition in fungal growth was observed at concentration of 30 mg/ml of both sodium bicarbonate and calcium chloride. The commercial backing yeast *S. cerevisiae* (CBY) enhanced the inhibitory effect of tested salts reflected in increasing mycelial fungal growth reduction when combined at the rate of 1:1 at each concentration tested. In pot experiment, under artificial infestation with pathogenic fungus, application of sodium bicarbonate or calcium chloride significantly reduced the early blight incidence and severity by increasing their concentrations. Their most effective concentration were 30 mg/ml that reduced the disease incidence by 50 and 62.4%, respectively. Superior effect of sodium bicarbonate or calcium chloride in disease reduction was observed when they combined with CBY. Field trails for evaluating the most promising greenhouse treatments were preformed under natural infestations during two successive summer seasons. Calcium chloride proved higher efficacy for reducing both disease incidence and severity than that of sodium bicarbonate when applied either alone or combined with CBY. Also, it is observed that increasing concentrations of both sodium bicarbonate or calcium chloride showed parallel decrease in disease incidence and severity. Application of (CBY) enhanced the efficacy of salts spraying against early blight disease. Similar trend was also observed with the increase of potato tubers yield. On the light of the present study it could be suggested that the usage of combined application of the yeast *S. cerevisiae* with sodium bicarbonate or calcium chloride might be used as easily applied, safely and cost effective control methods against such plant diseases.

Key words: *Alternaria solani*, early blight, calcium chloride, control, potato, sodium chloride

INTRODUCTION

Early blight is a very common disease of both potato and tomato. It causes leaf spots and tuber blight on potato, and leaf spots, fruit rot and stem lesions on tomato. The disease can occur over a wide range of climatic conditions and can be very destructive if left uncontrolled, often resulting in a complete defoliation of plants. In contrast to the name, it rarely develops early, but usually appears on mature foliage. Early blight is caused by the fungus, *Alternaria solani* (Ellis & G. Martin) L.R. Jones & Grout, which survives in infected leaf or stem tissues on or in the soil. Wharton and Kirk (2007) reported that the pathogen survives between growing seasons in infested plant debris and soil, in infected potato tubers and in overwintering debris of susceptible solanaceous crops and weeds including hairy nightshade (*Solanum sarrachoides*). This fungus is universally present in fields where these crops were grown. It can also be carried on tomato seed and in potato tubers. Spores form on infested plant debris at the soil surface or on active lesions over a fairly wide temperature range, especially under alternating wet and dry conditions. They are easily carried by air currents, wind-blown soil, splashing rain, and irrigation water. Infection of susceptible leaf or stem tissues occurs in warm, humid weather with heavy dew or rain. Early blight can develop quite rapidly in mid to late season and is more severe when plants are stressed by poor nutrition, drought, or pests. Infection of potato tubers occurs through natural openings on the skin or through injuries. Tubers may come in contact with spores during harvest and lesions may continue to develop in storage.

In Egypt potato (*S. tuberosum*) is of the largest horticultural export. In most recent years the EU has accounted for about 70%–90% of Egyptian potato. Potato early blight disease occurs in most production areas to almost every year although it has a significant effect on yield only when frequent wetting of the foliage favours early and rapid symptom development. Estimating total annual crop losses due to any particular disease is difficult to do accurately. Values in the literature for crop losses due to early blight vary enormously from 5 to 78% (Waals *et al.* 2004; Pasche *et al.* 2004, 2005).

Apart from the use of crop rotation, certified disease-free seeds and resistant varieties, and control measures

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are important to minimize infection. It is usually necessary to apply fungicide sprays to fully protect plants from early blight. Fungicide alternatives that have fungicidal effect on disease incidence and development are safety of application and environmental pollution concern.

There was a considerable interest in the use of sodium bicarbonate ($NaHCO_3$) and potassium bicarbonate ($KHCO_3$) for controlling various fungal diseases in plants (Karabulut *et al.* 2003; Smilanick *et al.* 2006). Bicarbonates are widely used in the food industry (Lindsay 1985) and were found to suppress several fungal diseases of cucumber plants (Ziv and Zitter 1992). Spraying plants with $NaHCO_3$ solution provided good control of several plant diseases (Horst *et al.* 1992; Arimoto *et al.* 1997; Palmer *et al.* 1997; Janisiewicz and Peterson 2005). Also, spraying with $KHCO_3$ solution provided the most effective protection against plant diseases (Fallilk *et al.* 1996; Smilanick *et al.* 1999, 2006). Sodium or potassium bicarbonate combined with oil were effective in controlling plant diseases (Horst *et al.* 1992; Ziv and Zitter 1992).

Calcium chloride as $CaCl_2$ was reported to suppress growth of the citrus mould pathogen *Penicillium digitatum* (Droby *et al.* 1997). Calcium chloride effectively reduced silver scurf lesions on potato tubers, but not sporulation of *Helminthosporium solani.* It is known that addition of calcium chloride can also improve the activity of biocontrol agents (Droby *et al.* 1997; McLaughlin *et al.* 1990).

Certain strategies, such as pre- or postharvest application of calcium salts, hydrogen peroxide and chitosan against fruit decay are proposed (Conway *et al.* 1992, 1994). Pre- and postharvest calcium applications have been used to delay ageing or ripening to reduce postharvest decay and control of many diseases in fruits and vegetable (Poovaiah 1986). Saftner *et al.* (1997) reported that postharvest calcium treatment of apples provided broad-spectrum protection against the postharvest pathogens of *Penicillium expansum* and *Botrytis cinerea.*

Biological control using either natural products or antagonistic microorganisms proved to be successful for controlling various plant pathogens in many countries (Papavizas and Lumsden 1980). It is still not expensive and is easy in application, however it can serve as the best control measure under restricted conditions. In addition, its application is safe, un-hazardous for human and avoids environmental pollution (Sivan and Chet 1989). *Saccharomyces cerevisiae* was used as biocontrol agent and systemic resistance mechanisms (El-Sayed 2000). Attyia and Youssry (2001) reported that a local isolate of *S. cerevisiae* had a reduction potential against radial growth of pathogenic fungi *Macrophomina phaseolina* and *Fusarium solani*, the cause of root rot diseases in tomatoes and eggplants.

The objective of this research was to evaluate the effects of some salts in addition to the commercial backing yeast as natural products and bio-pesticides on *in vitro* inhibition of *A. solani* in laboratory assays and determine their effect on development and suppression of early blight disease under field conditions.

MATERIALS AND METHODS

Fungal and yeast cultures

A virulent pathogenic isolate of *A. solani* was obtained from the Plant Pathology Department, National Research Centre, Egypt. The fungal cultures were maintained on PDA medium at 5±1°C as stock cultures until use. In addition, one of local mixture isolates of the backing yeast *S. cerevisiae* was also used in the present work. This yeast mixture is produced commercially for the purpose of backing and food industries.

Potato

Potato tubers cv. Diamond were used in the present study. Potato tubers were stored in the dark at 4°C until use.

Chemicals

Sodium bicarbonate ($NaHCO_3$) and calcium chloride ($CaCl_2$) were purchased from Sigma-Aldrich.

***In vitro* tests**

The inhibitory effect of sodium bicarbonate and calcium chloride, individually or combined, with commercial backing yeast (CBY) was evaluated while measuring the linear growth of *A. solani in vitro*. For each of the tested salts four concentrations were prepared by dissolving in sterilized distilled water. They were added individually to conical flasks containing sterilized PDA medium before solidification to obtain the proposed concentrations of 5, 10, 20 and 30 mg/ml. The supplemented media were poured into Petridishes (9 cm Ø), nearly 20 ml per each. A separate PDA plates free of salts were used as control treatment. Mycelial discs (5 mm Ø) were taken from the periphery of an actively growing PDA culture of *A. solani* and placed at the centre of the prepared Petri dishes.

The efficacy of combined inhibitory effect of tested salts and CBY against the growth of *A. solani* was also evaluated. This test was carried out using Petri dishes containing PDA supplemented with the above mentioned salts concentrations. Growth inhibition of pathogenic fungus affected by CBY in the presence of sodium bicarbonate or calcium chloride in the growth medium was evaluated following a dual culture technique after Ferreira *et al.* (1991). Different amounts of CBY as 5, 10, 20 of 30 mg/ml were suspended in sterilized distilled water, shacked using a vortex for 5 min, then the cell concentrations were determined with a haemocytometer slide. The CBY cell concentrations were 6, 8, 10 and $12x10^8$ cell/ml in respective order to the weight used. A loopful of different CBY concentrations were streaked individually on one side of 9 cm Petri dishes containing PDA supplemented with different salt concentrations, and 5 mm disks of fungal pathogen were placed on the opposite side of the yeast inoculated plates. Both tested microorganisms were placed 2 cm from the plate edges. A set of inoculated with the fungus plates was used for control treatment.

All plates were incubated at 25±2°C until full fungal growth in control plates. Five replicates were used for each treatment. The diameter of the fungal colonies was

measured and percentage reduction in fungal growth was calculated in relative to its growth in control treatment.

***In vivo* tests**

The efficacy of different concentrations of sodium bicarbonate and calcium chloride, individually or combined with CBY against early blight incidence on potato plants was tested under greenhouse and field conditions.

Preparation of spraying solutions and fungal suspension

Four concentrations of each sodium bicarbonate and calcium chloride were prepared by dissolving in sterilized distilled water to obtain the proposed concentrations of 5, 10, 20 and 30 mg/ml. Also, different weights of CBY 5, 10, 20 and 30 mg/ml were suspended in sterilized distilled water, shacked using a vortex for 5 min, and then they were ready to use.

The fungus *A. solani* was grown on PDA medium at 25±2°C until an abundant heavy growth of conidia was evident. Conidia were harvested by scraping the surface of the colonies with a spatula and transferred to sterilized distilled water and filtered through nylon mesh, then spore suspension was adjusted with sterile water to give a spore concentration of 10^6–10^7 per millilitre.

Greenhouse experiment

The same treatments preformed in laboratory test were evaluated in the greenhouse. The present experiment was carried out in the greenhouse of Plant Pathology department, National Research Centre, Egypt. Potato tubers cv. Diamond were grown in plastic pots (30 cm diameter) containing sandy loam soil at 22–25°C and RH 75–80%. The usual agricultural practices of irrigation and fertilization were followed. When plants had 4–5 compound leaves, three plants/pot and ten pots for each treatment were used. Solutions of sodium bicarbonate and calcium chloride were applied individually or combined with CBY with respective concentrations as foliar spray to potato plants. *S. cerevisiae* (CBY) was applied three days after salts spray. Plants sprayed with sterilized distilled water served as a control treatment. Plant inoculation was carried out 5 days after of (CBY) treatments by spraying with spore suspensions (10^6–10^7 spore/ml) of *A. solani*.

Field experiment

The most promising treatments were applied under field conditions. Field experiment was carried out at at the Experimental Farm of National Research Centre at Al-Kanater country, Kaliobia Governorate, Egypt during 2007 and 2008 summer (January–April) growing seasons to evaluate the efficacy of sodium bicarbonate and calcium chloride individually or combined with CBY on the incidence and severity of early blight disease of potato plants. A field experiment consisted of plots 21 m^2 (3 x 7 m) each and comprised of 7 rows and 15 holes/row and was conducted in completely randomized block design with five plots as replicates for each particular treatment as well as untreated control. Potato tubers cv. Diamond were planted in all treatments. All plots received traditional agricultural practices as irrigation, fertilization and soil plunging. The used mineral fertilization of NPK was 180, 75 and 95 unit/4 200 m^2. In addition to 20 m^3 organic manure and 150 kg/4 200 m^2 agricultural sulphur were added to the soil before planting. Solutions of sodium bicarbonate and calcium chloride individually or combined with CBY with respective concentrations of 10 and 20 mg/ml for all tested factors were applied as foliar spray of potato plants. *S. cerevisiae* (CBY) was applied three days after salts spray. Plants sprayed with sterilized distilled water served as a control treatment. All treatments were applied when plants had 4–5 compound leaves and repeated again after 15 days. For comparison fungicide Ridomil *MZ* 72 WP was applied as foliar spray at the recommended dose (2.5 g/l). Early blight disease incidence and severity were recorded after 30 days of the second plant spray application. The average harvested yield was calculated for all applied treatments as kg/m^2 at the end of each growing season.

Disease assessment

Early blight incidence was estimated as the number of infected plants showing disease symptoms in relation to the whole number of potato plants. The average of records of the surveyed replicates for each particular treatment was calculated. Disease severity was estimated following the scale from 0 to 4 suggested by Cohen *et al.* (1991) as follows:

0 = no leaf lesion; 1 = lesions occupied < 25% of leaf area; 2 = lesions occupy between 26–50% of leaf area; 3 = lesions occupy between 51–75% of leaf area and 4 = lesions occupy 76 up to 100% of leaf area. Then the following formula was applied:

$$D.S. = \frac{\sum(n \times c)}{N}$$

where:
D.S. = disease severity, n = number of infected plants per category, c = category number and N = total number of examined plants.

Statistical analysis

One way analysis of variance (ANOVA) was used to analyze differences between toxic concentrations of fungicide and the linear growth of *A. solani* as well as differences between toxicity of fungicide and early blight incidence at different applied concentrations under laboratory and field conditions. MSTAT-C program (V2.1) was used to perform the analysis of variance between toxicity of fungicide and early blight incidence at different applied concentrations under field conditions. Duncan's Multiple Range Test was used for means separation (Winer 1971).

RESULTS

***In vitro* tests**

Inhibitory activity against the mycelial growth of *A. solani* was observed at all concentrations of sodium bicarbonate and calcium chloride used either individually or combined with CBY (Table 1). Fungal mycelial growth decreased significantly with the increase in concentrations

of used salts to reach minimum mycelial growth with the highest concentration used. Complete inhibition in fungal growth was observed at concentration of 30 mg/ml of both sodium bicarbonate and calcium chloride. The yeast *S. cerevisiae* (CBY) enhanced the inhibitory effect of tested salts reflected in increasing mycelial fungal growth reduction when combined at the rate of 1 : 1 at each concentration tested. Superior complete inhibitory effect was observed at both salts concentration combined with CBY at 20+20 mg/ml and 30+30 mg/ml.

Table 1. *In vitro* linear growth of *A. solani* in response to different concentrations of sodium bicarbonate and calcium chloride used individually or combined with *S. cerevisiae*

Treatment	Concentration [mg/ml]	Linear growth [mm]	Reduction [B] [%]
Sodium bicarbonate	5	48.8 b	45.77 [A]
	10	24.4 f	72.88
	20	12.8 j	85.77
	30	0 k	100
Sodium bicarbonate + CBY	5+5	44.4 d	50.66
	10+10	17.7 h	80.33
	20+20	0 k	81.78
	30+30	0 k	100
Calcium chloride	5	46.8 c	48.0
	10	22.2 g	75.33
	20	16.4 hi	81.78
	30	0 k	100
Calcium chloride + CBY	5+5	42.2 e	53.11
	10+10	18.7 h	79.22
	20+20	0 k	100
	30+30	0 k	100
Control		90 a	0

[A] mean values within columns followed by the same letter are not significantly different at $p < 0.05$
[B] reduction in fungal growth at different treatments, calculated relatively to its growth in control

***In vivo* tests**

Greenhouse experiment

The different concentrations of sodium bicarbonate or calcium chloride significantly reduced the early blight incidence. This observed reduction was increased by increasing salts concentrations (Table 2). Their most effective concentration were 30 mg/ml that reduced the disease incidence by 50 and 62.4%, respectively.

Superior effect of sodium bicarbonate or calcium chloride in disease reduction was observed when they were combined with *S. cerevisiae*. The highest record of disease reduction in respective order of 75.0 and 68.8% was obtained for the applied concentration of 30+30 mg/ml.

Similar trend was recorded concerning the severity of early blight disease. Data in Table 2 indicate that all potato plants receiving both salts treatments have significant reduction in disease severity which increased when combined with CBY application. High reduction as 42.3 and 61.4% was obtained at 30 mg/ml of sodium bicarbonate and calcium chloride and reached up to 65% when both treatments combined with CBY. Individual application of sodium bicarbonate and calcium chloride or combination with CBY either at concentration of 20 mg/ml or 30 mg/ml showed no significant reduction in disease incidence as well as severity.

Field experiment

The promising treatments for reducing both disease incidence and severity in pot experiment was applied under natural field conditions during two successive growing seasons (Table 3). Although the fungicide treatment reduced significantly early blight incidence and severity (14.3 and 34.7%) compared with control (17.6 and 39.3%), all applied treatments had a superior effect in this respect. Presented data revealed that calcium chloride had higher efficacy for reducing both disease incidence and severity than that of sodium bicarbonate when applied either alone or combined with *S. cerevisiae*. Also, it was observed that increasing concentrations of both sodium bicarbonate or calcium chloride showed parallel decrease in disease incidence and severity. This reduction was also observed when the applied salts were combined with CBY. Obtained data also showed that the application of (CBY) enhanced the efficacy of salts spraying against early blight disease. Application of sodium bicarbonate at 10 and 20 mg/ml could reduce the disease incidence and severity by 30.6, 17.3% and 47.7, 31.8%, while these records raised to reach 41.4, 20.1 and 52.2, 45.8%, respectively when combined with CBY treatment. As for calcium chloride treatments, at the same concentration, the reduction in disease incidence and severity was recorded in respective order:

Table 2. The influence of sodium bicarbonate and calcium chloride individually or combined with CBY on early blight disease incidence and severity of potato plants under greenhouse conditions

Treatment	Concentration [mg/ml]	Disease incidence	Reduction [%]	Disease severity	Reduction [%]
Sodium bicarbonate	5	43.3 b [A]	18.7	51.4 b [A]	12.2
	10	36.6 c	31.3	45.7 c	22.0
	20	30.0 d	43.7	40.6 cd	30.7
	30	26.6 d	50.0	33.8 de	42.3
Sodium bicarbonate + CBY	5+5	20.0 de	62.4	36.4 d	37.8
	10+10	16.6 e	68.8	31.5 de	46.2
	20+20	13.3 ef	75.0	23.2 ef	60.4
	30+30	13.3 ef	75.0	20.5 f	65.0
Calcium chloride	5	40.0 b	24.9	42.8 cd	26.9
	10	33.3 cd	37.5	38.8 d	33.7
	20	23.3 d	56.2	28.4 e	51.5
	30	20.0 de	62.4	22.6 ef	61.4
Calcium chloride + CBY	5+5	36.6 c	31.3	37.4 d	36.1
	10+10	26.6 d	50.0	31.4 de	46.4
	20+20	20.0 de	62.4	24.3 ef	58.5
	30+30	16.6 e	68.8	20.5 f	65.0
Control		53.3 a	0	58.6 a	0

[A] mean values within columns followed by the same letter are not significantly different at $p < 0.05$

Table 3. The influence of sodium bicarbonate and calcium chloride applied individually or combined with CBY on early blight disease incidence and severity of potato plants under field conditions during two successive summer seasons 2007/2008 [A]

Treatment	Disease incidence	Reduction [%]	Disease severity	Reduction [%]
Sodium bicarbonate (10 mg/ml)	12.2 c [B]	30.6	32.5 c	17.3
Sodium bicarbonate (10 mg/ml) + CBY (10 mg/ml)	10.3 d	41.4	31.4 c	20.1
Sodium bicarbonate (20 mg/ml)	9.2 de	47.7	26.8 d	31.8
Sodium bicarbonate (20 mg/ml) + CBY (20 mg/ml)	8.4 e	52.2	21.3 e	45.8
Calcium chloride (10 mg/ml)	10.2 d	42.0	25.4 d	35.3
Calcium chloride (10 mg/ml) + CBY (10 mg/ml)	7.8 e	55.6	20.6 e	47.5
Calcium chloride (20 mg/ml)	6.3 f	64.2	19.1 e	66.6
Calcium chloride (20 mg/ml) + CBY (20 mg/ml)	5.2 g	70.4	16.2 f	58.7
Ridomil *MZ* 72 WP (2.5 g/l)	14.3 b	24.4	34.7 b	11.7
Control	17.6 a	0	39.3 a	0

[A] the recorded data of the two successive seasons were presented as average per cent
[B] mean values within columns followed by the same letter are not significantly different at $p < 0.05$

42, 35.3% and 64.2, 66.6% that increased up to 55.6, 47.5% and 70.4, 58.7% by combination with CBY treatment.

The average of harvested potato tubers, data in table 4 showed a considerable increase in potato yield at all applied treatments during the two cultivation seasons. A significant increase in harvested yield was observed for calcium chloride treatments of 10, 20 mg/ml either as individual application or combined with CBY which was recorded as 38.4, 50.0% and 47.6, 50%, respectively. These results were higher than those obtained by sodium bicarbonate at similar treatments recorded as 7.6, 26.9% and 19.2, 30.7%, respectively. The lowest increase in potato yield of 3.8% was observed for fungicide treatment.

Table 4. The influence of sodium bicarbonate and calcium chloride individually or combined with CBY on potato yield during two successive summer seasons 2007/2008 [A]

Treatment	Yield ([kg/m^2] [A]	Increase [%]
Sodium bicarbonate (10 mg/ml)	2.8 a [B]	7.6
Sodium bicarbonate (10 mg/ml) + CBY (10 mg/ml)	3.1 b	19.2
Sodium bicarbonate (20 mg/ml)	3.3 bc	26.9
Sodium bicarbonate (20 mg/ml) + CBY (20 mg/ml)	3.4 cd	30.7
Calcium chloride (10 mg/ml)	3.6 cd	38.4
Calcium chloride (10 mg/ml) + CBY (10 mg/ml)	3.8 d	47.6
Calcium chloride (20 mg/ml)	3.9 d	50.0
Calcium chloride (20 mg/ml) + CBY (20 mg/ml)	3.9 d	50.0
Ridomil *MZ* 72 WP (2.5 g/l)	2.7 a	3.8
Control	2.6 a	0

[A] the recorded data of potato yield for the two successive seasons were presented as average kg/m^2
[B] mean values within columns followed by the same letter are not significantly different at $p < 0.05$

DISCUSSION

Although cultural practices such as crop rotation and appropriate application of chemical management of potato can help reduce diseases incidence, alternative control strategies are needed. An interesting alternative to fungicide application for plant disease control involves the use of some organic and inorganic salts with antimicrobial properties generally used in food processing and preservation.

Selected organic and inorganic salts are active antimicrobial agents and have been widely used in the food industry. Many of these salts are effective against a range of microorganisms; most of them have low mammalian toxicity and therefore have potential for postharvest disease control. Salt treatments can inhibit plant pathogens or suppress mycotoxin production (Roinestad *et al.* 1993; Singh and Chand 1993). Sodium and ammonium bicarbonate were shown to inhibit fungal pathogens of fruits, field crops, vegetables, and ornamentals (Ziv and Zitter 1992; Palmer *et al.* 1997).

Several studies dealt with the use of different salts to control various postharvest diseases of potato and other crops. Treatment of citrus fruit with sodium carbonate or sodium bicarbonate was shown to reduce the incidence of postharvest disease caused by *Penicillium digitatum* (Smilanick *et al.* 1999, 2006). Postharvest application of ammonium bicarbonate, sodium bicarbonate, potassium carbonate, calcium propionate, and potassium sorbate reduced black root rot on carrots caused by *Chalara elegans* (Punja and Gaye 1993). Recent studies suggest that salt application reduces postharvest potato diseases. Olivier *et al.* (1998) and Hervieux *et al.* (2002) showed that application of potassium sorbate, calcium propionate, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, and ammonium bicarbonate at 0.2 M reduced silver scurf lesion development and *Helminthosporium solani* sporulation on inoculated and naturally infected potato tubers. These reports confirm the obtained results in the present study showing that sodium bicarbonate was found to be strongly inhibiting to mycelial growth of *A. solani* at concentrations of 20 and 30 mg/ml either under *in vitro* or *in vivo* trails. Moreover, the efficacy of the inhibitory effect showed parallel correlation with increasing the applied concentrations. Similar trend was also observed for the increase of potato tubers yield throughout the two successive seasons by increasing the applied salts concentrations. A significant increase in harvested yield was observed for calcium chloride treatments either as individual application or combined with CBY. These results were higher than that obtained for sodium bicarbonate at similar treatments, while the lowest increase in potato yield was observed for fungicide treatment.

Previous studies demonstrated that increasing concentrations of sodium bicarbonate resulted in a corresponding increase in efficacy (Mlikota and Smilanick 1998, 2001). The inhibitory effect of sodium bicarbonate on microorganisms may be due to reduction of cell turgor that causes a collapse and shrinkage of hyphae and spores, resulting in fungistasis (Fallik *et al.* 1997).

The use of sodium bicarbonate alone to control postharvest decays of fruit has its limitations (Palou *et al.* 2001), but it can be combined with other alternative treatments to synthetic fungicides, resulting in the control that is superior to individual treatments alone. For example, sodium bicarbonate was successfully used in combination with bacterial and yeasts biocontrol agents to enhance control of postharvest decays on citrus, pome, and stone fruits (Smilanick *et al.* 1999; Wisniewski *et al.* 2001; Janisiewicz and Peterson 2005). These reports are clearly demonstrated in the present study and show that the application of *S. cerevisiae* (CY) enhanced the control of early blight incidence and severity of potato plants when combined with either sodium bicarbonate or calcium chloride spray. Petersson and Schnurer (1995) reported that *S. cerevisiae* required an inoculum of 105 cfu/g to inhibit the growth of *Penicillium roqueforti* in non-sterile high-moisture wheat grains. In this concern

inoculum used in the present work was much more effective if reached 6, 8 and 10x10^8 cell/ml in respective order to the weights of 10, 20 and 30 mg/ml. Moreover, the present study clearly indicated the antagonistic activity of yeast *S. cerevisiae* on early blight pathogen under *in vitro* and *in vivo* conditions. The biocontrol activity of *S. cerevisiae* against *A. solani* might have possibly resulted from mycoparasitism (Hajlaoui and Belanger 1993), secretion of lytic enzymes such as β –1, 3 glucanase (Punja 1997) and production of antibiotics (Beyagoub *et al*. 1996). The present study clearly indicated the antagonistic activity of yeast *S. cerevisiae* on early blight pathogen under *in vitro* and *in vivo* conditions. Also**,** Attyia and Youssry (2001) reported that a local isolate of *S. cerevisiae* had a reduction potential against radial growth of pathogenic fungi *Macrophomina phaseolina* and *Fusarium solani*, the cause of root rot diseases in tomatoes and eggplants. They added that scanning electron microscopy revealed interaction between *S. cerevisiae* and both fungi. Also, calcium has been considered to increase biocontrol efficacy of antagonists. It may also replace the current requirement for addition of low concentrations of fungicides to ensure consistent performance of yeast control agents under large-scale and commercial conditions (Droby *et al.* 1993).

Application of calcium chloride in the present study either individually or combined with *S. cerevisiae* (CY) proved to have a suppressive effect against the linear growth of the pathogen *A. solani in vitro* and its disease incidence and development under greenhouse and field trails. Similarly, calcium chloride at 2% (20 mg/ml) obviously inhibited spore germination and germ tube growth of *R. stolonifer* PDA medium (Tian *et al.* 2002). This result further supports the results of Wisniewski *et al.* (1995), who found that calcium chloride might reduce fungal infection through direct inhibition of spore germination and growth. Maouni *et al.* (2007) reported that *in vitro,* calcium chloride significantly reduced pear fruit decay caused by *A. alternata* and *Penicillium expansum* when used at 4 and 6%.

Under field conditions multiple spray applications of calcium chloride in the orchard was reported to reduce postharvest *Alternaria* infection as well as postharvest development of bitter rot following orchard infection by *Colletotrichum* spp. in apple (Biggs *et al.* 1993; Biggs 1999). In pear orchard, calcium chloride sprays were shown previously to enhance pear fruit resistance to blue mould decay and side rot (Sugar *et al.* 1991, 2003). The precise mechanism by which calcium reduces fungal infection is not yet understood, but the role of calcium in resistance may be one of interference with the activity of pectolytic enzymes (Conway *et al.* 1992) and may be partially attributable to a decrease in maceration of cell walls by polygalacturonase (PG) due to the improved structural integrity caused by the increase in calcium content (Conway *et al.* 1998). Furthermore, previous studies indicated that the increase in cell walls of apple reduces the activity of polygalacturonase extracted from the pathogenic invader fungus *P. expansum* (Conway *et al.* 1988; Wisniewski *et al.* 1995; Saftner *et al.* 1997).

The present findings demonstrate that sodium and calcium salts may have important implications for the future use of antagonistic microorganisms on a commercial scale for controlling such diseases especially under organic cultivation regime.

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POLISH SUMMARY

ZASTOSOWANIE SOLI W CELU OGRANICZENIA ALTERNARIOZY ZIEMNIAKA

W warunkach laboratoryjnych, szklarniowych i polowych, badano ograniczające działanie soli sodu i wapnia, użytych indywidualnie lub łącznie z grzybem drożdżoidalnym *Sacharomyces cerevisiae*, przeciwko *Alternaria*

solani, który wywołuje wczesną alternariozę ziemniaka. W teście *in vitro* obserwowano całkowitą inhibicję wzrostu grzyba przy użyciu stężenia węglanu sodu lub chlorku wapnia wynoszącego 30 mg/ml. Handlowe drożdże piekarnicze (*S. cerevisiae*) stymulowały działanie inhibicyjne badanych soli, co wyrażało się redukcją ilości wytwarzanej przez patogena grzybni, wykorzystywanej w badaniach w stosunku: 1 : 1 dla każdego testowanego stężenia. W doświadczeniu wazonowym, w warunkach sztucznej inokulacji patogenem, zastosowanie jednej z badanych soli powodowało istotne ograniczenie występowania alternariozy, a jej nasilenie wzrastało wraz ze wzrostem wykorzystanych stężeń soli. Najbardziej efektywne było stężenie 30 mg/ml, ograniczające występowanie choroby, odpowiednio o 50 i 62,4%. Lepszy efekt zastosowania węglanu sodu lub chlorku wapnia w ograniczaniu choroby obserwowano wtedy, gdy zabiegi były wykonywane w warunkach naturalnego zakażenia, podczas dwóch kolejnych sezonów letnich. Chlorek wapnia był skuteczniejszy zarówno w ograniczaniu występowania choroby i jej nasilenia, niż węglanu sodu, zarówno gdy stosowano go oddzielnie lub w połączeniu z *S. cerevisiae*. Zwiększając stężenia obydwóch soli uzyskano równoległe ograniczenie choroby i jej nasilenia. Zastosowanie drożdży stymulowało skuteczność opryskiwania roślin ziemniaka solami przeciwko alternariozie. Podobną tendencję obserwowano również dla wzrostu plonu bulw. W świetle wykonanych badań można sugerować, że wykorzystanie łącznej aplikacji *S. cerevisiae* i węglanu sodu lub chlorku wapnia mogłoby być wykorzystane jako łatwa w użyciu, bezpieczna i tania metoda zwalczania tej choroby.