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ORIGINAL ARTICLES

Efficacy of Different Plant Resistance Inducers against Downy and Powdery mildew Diseases of Pepper under Plastic Houses Conditions

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ABSTRACT

Different plant resistance inducers treatments against Downy and Powdery mildew diseases incidence of pepper was carried out as spray treatments on growing plants under commercial plastic houses at Haram location, Giza governorates. The evaluated foliar spray treatments were applied four times with fifteen days intervals starting at thirty days after transplanting. The obtained results revealed that plant spray with treatments, [Calcium chloride (20mM) + *S. cerevisiae* $10x10^{10}$cfu/mL (10ml/L) + Chitosan (0.05mM)] and [Potassium bicarbonate (20mM) +Thyme oil (5ml/L)] resulted in the highest reduction in foliar diseases Downy and Powdery mildew diseases incidence and severity of pepper plants grown under plastic houses conditions and increased produced yield as well. The obtained results lead to suggest that the usage of combined application of the bio-agent, *Saccharomyces cerevisiae* and/or plant resistance inducers might be used as easily applied, safely and cost effective control methods against such foliar plant diseases.

Key words: Downy mildew, powdery mildew, pepper, plant resistance inducers**,** plastic house

Introduction

Pepper (*Capsicum annuum* L.) is one of widespread vegetables grown in plastic houses under protected cultivation system. Pepper plants are attacked with several root and foliar diseases. The most serious foliar diseases are Downy and Powdery mildews. It was reported that (Anonymous, 2005) downy mildew caused by the pathogen *Peronospora parasitica,* can develop during the winter vegetable season. Cool damp weather with high relative humidity and air movement stimulates disease development by promoting sporulation, spore dispersal and plant infection by the pathogen. Downy mildew severity increases as the duration of free moisture on plant leaves rises. Also, powdery mildew of pepper is caused by *Leveillula taurica*, Powdery mildew can be severe during the warmest part of summer and can cause heavy yield losses. The pathogen has a very wide host range and inoculum. Detecting powdery mildew on pepper can be difficult. The white powdery growth characteristic of powdery mildew diseases occurs only on the underside of leaves and it will turn brown rather than remaining white. Diffuse yellow spotting often develops on the upper surface. Affected leaves tend to drop off the plant, as occurs with bacterial leaf spot. Successful management of downy and powdery mildews can be achieved by planting cultivars that are tolerant or resistant to the pathogens. If susceptible cultivars are grown, it is extremely important to have chemical protection in place when environmental conditions become favorable for diseases development. Several new agrochemicals are in development that have activity on the pathogens that cause downy mildew diseases. Economical control depends on establishing an overall disease management system for the entire farm. Keeping careful records of the crops planted, the problems encountered, and the pesticides alternatives used is important. In this regards, foliar application of calcium chloride has been also reported to delay ripping and control mould disease in strawberries (Cheour *et al*., 1991). Application of sodium bicarbonate or calcium chloride significantly reduced the early blight incidence and severity (El-Mougy and Abdel-Kader, 2009). They added that Calcium chloride proved higher efficacy for reducing both disease incidence and severity than that of sodium bicarbonate when applied either alone or combined with *Saccharomyces cerevisiae.* Calcium administered to the plant through the nutrient feed has been reported to be important for resistance to bacterial wilt resistance in tomato (Yamazaki and Hoshima, 1995). Moreover, it was found that $KHCO_3$ applications were effective in reducing the severity of powdery mildew on *E. japonica* and pumpkin (Ziv and Zitter, 1992; Ziv, and Hagiladi, 1993).

Moreover, Chiosan, in recent years, the importance of chito-saccharides as plant growth promoting and disease control agents has been emphasized (Trotel-Aziz *et al*., 2006; Aziz *et al*., 2006). Chitosan has been shown to induce defense responses in different plants (Chang *et al*., 1992; Hadwiger *et al*., 1994). Also, Chitosan oligomers was found to induce defense responses in grapevine leaves and a stimulation of chitinase and β-1,3-glucanase activities (Aziz, *et al*., 2006). Furthermore, the combination of Chitosan and CuSO4

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increased phytoalexin production. This elicitor capacity of Chitosan and/or CuSO4 appeared to be associated with an induced protection of grapevine leaves against gray mold and downy mildew diseases. Atia *et al*., (2005) recorded that chitosan/copper complex retained a presence on the potato leaf surface late and early blights where infection by either *A. solani* or *P. infestans* occurs.

The objective of the present study was designed to evaluate efficacy of some plant resistance inducers as foliar spray against downy and powdery mildew diseases of pepper plants growing under plastic house conditions.

Materials and Methods

Efficacy of *Saccharomyces cerevisiae* and/or plant resistance inducers under natural infestation with pepper foliar diseases causal organisms were evaluated against downy and powdery mildews infection. This experiment was performed under protected cultivation system conditions in commercial plastic houses of Ministry of Agriculture and Soil Reclamation, A.R.E. at Haram location, Giza governorate.

Different treatments against were applied as foliar sprays as follows:

1. Calcium chloride (20mM) + *Saccharomyces cerevisiae* $10x10^{10}$cfu/mL (10ml/L) + Chitosan (0.05mM)
2. Potassium bicarbonate (20mM) + Thyme oil (5ml/L)
3. Chitosan (0.05mM) + Thyme oil (5ml/L)
4. Potassium monohydrogen phosphate (20mM)
5. Control (received only the recommended fungicide TAZOLEN 72% [mancozeb 64% + metalaxyl 8%])

The experimental plastic house consists of 5 rows, each (0.9 x 60m, width x long) divided into 3 parts 20m long each, and every part considered as one replicate. Three replicates were used for each particular treatment in complete randomized block design.

The growing Pepper plants were sprayed with proposed treatments 3 times with 15 days intervals after transplanting (Aleandri *et al*., 2010).

The growing pepper plants in the experimental plastic houses received the recommended pesticides against harmful insects, *i.e*. aphids, trips, white fly, etc. as needed. Meanwhile, the check control received traditional programs for controlling diseases and pests which recommended by following up committee of Protected Cultivation Administration Office, Ministry of Agriculture and Soil Reclamation. The growing pepper received traditional agricultural practices, *i.e*. irrigation, fertilization, etc.

Monitoring and scouting of foliar diseases incidence, *i.e*. downy and powdery mildews of Pepper were recorded. Percentages of diseases incidence and severity were recorded at 60, 90 and 120 days of transplanted date. At the end of growing season the obtained accumulated yield was calculated.

Disease assessment:

- *Disease incidence:*

Percentage of each foliar disease incidence was recorded as the number of diseased plants relative to the number of growing plants for each row, then the average of disease incidence was calculated.

- *Disease severity:*

Percentage of each foliar disease severity was recorded as following equation:

$$D.S.\% = \frac{\Sigma (n \times c)}{N} X100$$

Whereas: D.S. = Disease severity %
n = Number of infected leaves per category
c = Category number
N= Total examined leaves

Disease severity scale from 0 to 4 according to Cohen *et al*.,(1991) was followed, whereas: 0 = No leaf lesions; 1 = 25% or less; 2 = 26-50 %; 3 = 51-75 %; and 4 = 76-100% infected area of plant leaf.

At the end of growing season the accumulated yield was calculated for each particular treatment.

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Results and Discussion

Pepper plants grown in plastic house at Haram location showed reduction in Downy and Powdery mildews disease when treated with different formula of chemical plant resistance inducers (Tables 1 and Fig 1). Presented data revealed that all applied treatments could successfully delay both downy and powdery mildews more than 60 days of plant growth. Data also, showed that the percentage of downy mildew was higher than that of powdery mildew throughout the growing season. The percentage of Downy and Powdery mildews incidence increased as the plant growth is increased up to 120 day of growth. At this age Downy mildew incidence was recorded as 18.4, 14.2, 20.6 and 26.4% at the applied treatments, [Calcium chloride (20mM) + *S. cerevisiae* $10x10^{10}$cfu/mL (10ml/L) + Chitosan (0.05mM)], [Potassium bicarbonate (20mM) +Thyme oil (5ml/L)], [Chitosan (0.05mM) + Thyme oil (5ml/L)] and [Potassium monohydrogen phosphate (20mM)], in respective order, comparing with 39.6% in control check plants. As for Powdery mildew, the recorded disease incidence was 12.4, 9.6, 16.4 and 18.6%, in respective order with the above mentioned treatments comparing with 38.6% in check control. Also, it was observed that at 120 day of growth, the highest reduction in diseases incidence of downy and powdery mildews was recorded as 64.1, 75.1% at [Potassium bicarbonate (20mM) +Thyme oil (5ml/L)] treatment. Meanwhile, treatment, [Potassium monohydrogen phosphate] revealed the lowest reduction records, 33.3 and 51.8% for downy and powdery mildews respectively over the control treatment.

Table 1: Percentage of Downy and powdery mildew diseases incidence in response to application of different formula against foliar diseases of Pepper grown in plastic houses under protected cultivation system (Haram location)

Treatment	Foliar diseases incidence (%)					
	Downy mildew			Powdery mildew		
	Days of transplanting			Days of transplanting		
	60	90	120	60	90	120
Calcium chloride + *S. cerevisiae* +Chitosan	0.0 b	8.2 cd	18.4 d	0.0 b	6.4 c	12.4 d
Potassium bicarbonate +Thyme oil	0.0 b	8.4 cd	14.2 e	0.0 b	5.2 cd	9.6 e
Chitosan + Thyme oil	0.0 b	9.1 c	20.6 c	0.0 b	6.3 c	16.4 c
Potassium monohydrogen phosphate	0.0 b	12.8 b	26.4 b	0.0 b	11.2 b	18.6 b
Control	18.6 a	29.4 a	39.6 a	14.6 a	32.2 a	38.6 a

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$).

Disease severity of Downy and Powdery mildews of Pepper plants dramatically reduced in response to chemical plant resistance inducers as foliar application. Data presented in Table (2) and Fig (2) showed high suppress in Downy and Powdery mildews diseases severity recorded at 120 day of plant growth as 0.6, 1.2, 1.3, 1.4% and 0.5, 0.8, 1.0, 1.2% for both diseases at the applied treatments, [Potassium bicarbonate (20mM) +Thyme oil (5ml/L)], [Calcium chloride (20mM) + *S. cerevisiae* $10x10^{10}$cfu/mL (10ml/L) + Chitosan (0.05mM)], [Chitosan (0.05mM) + Thyme oil (5ml/L)] and [Potassium monohydrogen phosphate (20mM)], respectively comparing with 2.8 and 2.1% in untreated control check plants.

Illustrated data by Fig (2) showed that at 120 days of plant growth foliar treatment, [Potassium bicarbonate (20mM) +Thyme oil (5ml/L)] could cause the highest reduction in disease development calculated as 71.4, 76.1% followed by 57.1, 61.9% at treatment [Calcium chloride (20mM) + *S. cerevisiae* $10x10^{10}$cfu/mL (10ml/L) + Chitosan (0.05mM)], for downy and powdery mildews, in respective order. Meanwhile, the lowest reduction in diseases development was recorded as 50.0, 42.8% for downy and powdery mildews at the treatment, [Potassium monohydrogen phosphate (20mM)], respectively.

Table 2: Percentage of Downy and powdery mildew diseases severity in response to application of different formula against foliar diseases of Pepper grown in plastic houses under protected cultivation system (Haram location)

Treatment	Foliar diseases incidence (%)					
	Downy mildew			Powdery mildew		
	Days of transplanting			Days of transplanting		
	60	90	120	60	90	120
Calcium chloride + *S. cerevisiae* + Chitosan	0.0 b	0.6 b	1.2 b	0.0 b	0.4 c	0.8 c
Potassium bicarbonate +Thyme oil	0.0 b	0.4 bc	0.6 c	0.0 b	0.3 c	0.5 d
Chitosan + Thyme oil	0.0 b	0.4 bc	1.3 b	0.0 b	0.4 c	1.0 b
Potassium monohydrogen phosphate	0.0 b	0.5 b	1.4 b	0.0 b	0.8 b	1.2 b
Control	0.6 a	2.0 a	2.8 a	0.8 a	1.4 a	2.1 a

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$).

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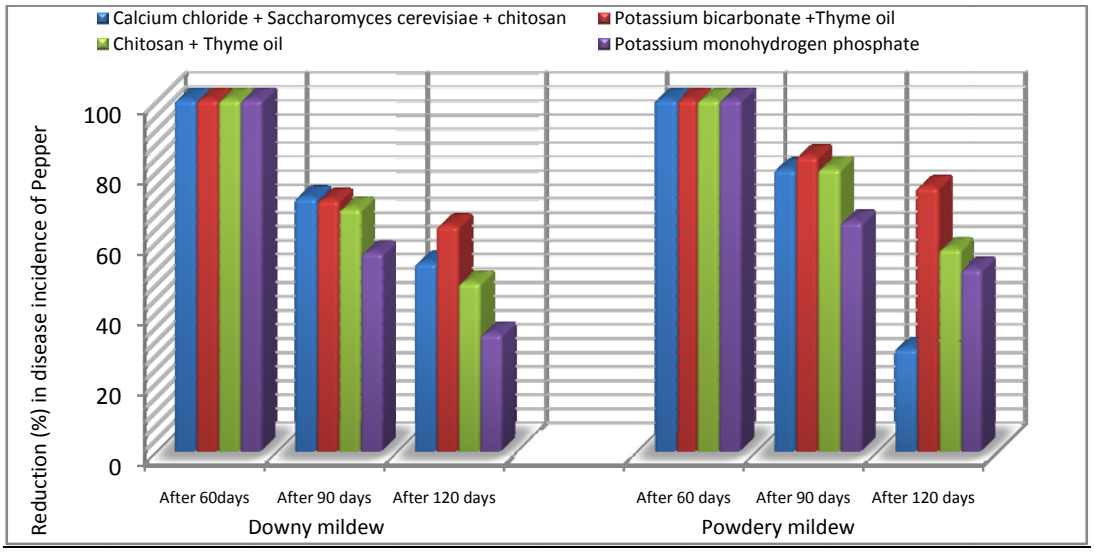

Fig. 1: Reduction (%) in Downy and powdery diseases incidence in response to application of different formula against foliar diseases of Pepper grown in plastic houses under protected cultivation system (Haram location)

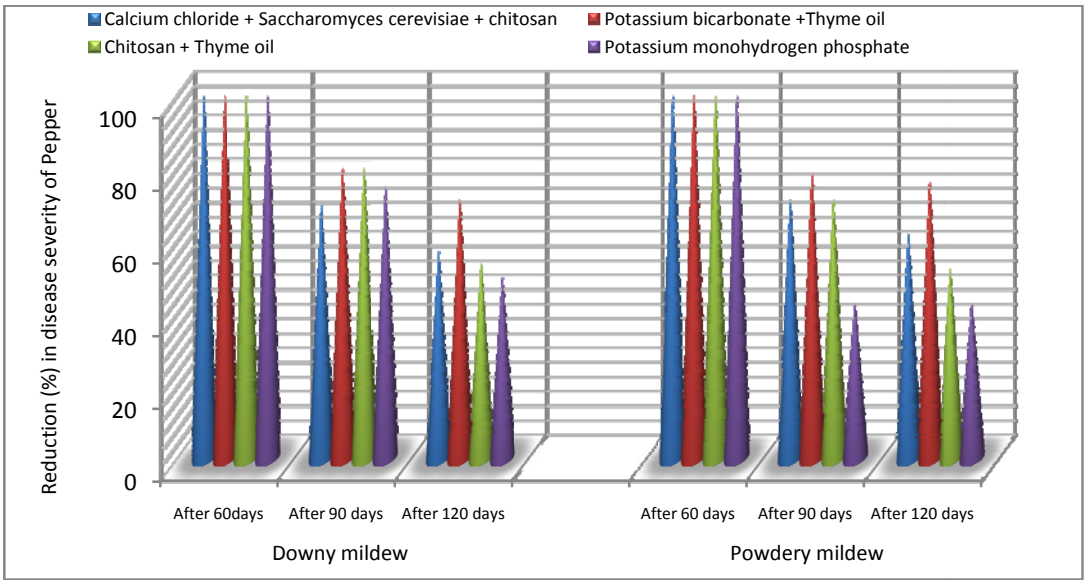

Fig. 2: Reduction (%) in Downy and powdery diseases severity in response to application of different formula against foliar diseases of Pepper grown in plastic houses under protected cultivation system (Haram location)

Application of different formula of chemical plant resistance inducers as foliar spray resulted in reduction of foliar diseases incidence and severity which reflected positively in plant stand and its healthy growth as well as its produced yield. Pepper plants grown in plastic house at Haram location sprayed with [Calcium chloride (20mM) + *S. cerevisiae* $10x10^{10}$cfu/mL (10ml/L) + Chitosan (0.05mM)] produced the highest significant accumulated yield recorded as 1,018 Ton/ plastic house (Table 3). Data also showed that significant accumulated yield was obtained from plants sprayed with either [Potassium bicarbonate (20mM) +Thyme oil (5ml/L)] or [Chitosan (0.05mM) + Thyme oil (5ml/L)] which recorded as 0.968 and 0.952 Ton/ plastic house. On the other hand, plants sprayed with [Potassium monohydrogen phosphate (20mM)] produced 0.891 Ton/ plastic house which it also significantly differed than 0.786 Ton/ plastic house in untreated control plants

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Table 3: The obtained yield of Pepper in response to foliar application of different formula in plastic houses under protected cultivation system (Haram location)

Treatment	Average Yield Kg/row	Average Yield Ton/plastic house
Calcium chloride + *S. cerevisiae* + Chitosan	203.6 d	1,018
Potassium bicarbonate +Thyme oil	193.5 c	0.968
Chitosan + Thyme oil	190.4 c	0.952
Potassium monohydrogen phosphate	178.2 b	0.891
Control	157.2 a	0.786

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$).

Illustrated data by Figs (3) present increase (%) in the obtained yield of Pepper in response to foliar application with different formula of chemical plant resistance inducers in plastic houses under protected cultivation system. The calculated accumulated yield of Pepper plants was as 29.5, 23.0, 21.1, 13.3% over that obtained of untreated control plants sprayed in respective order with applied treatments, [Calcium chloride (20mM) + *S. cerevisiae* $10x10^{10}$cfu/mL (10ml/L) + Chitosan (0.05mM)], [Potassium bicarbonate (20mM) + Thyme oil (5ml/L)], [Chitosan (0.05mM) + Thyme oil (5ml/L)] and [Potassium monohydrogen phosphate (20mM)], respectively.

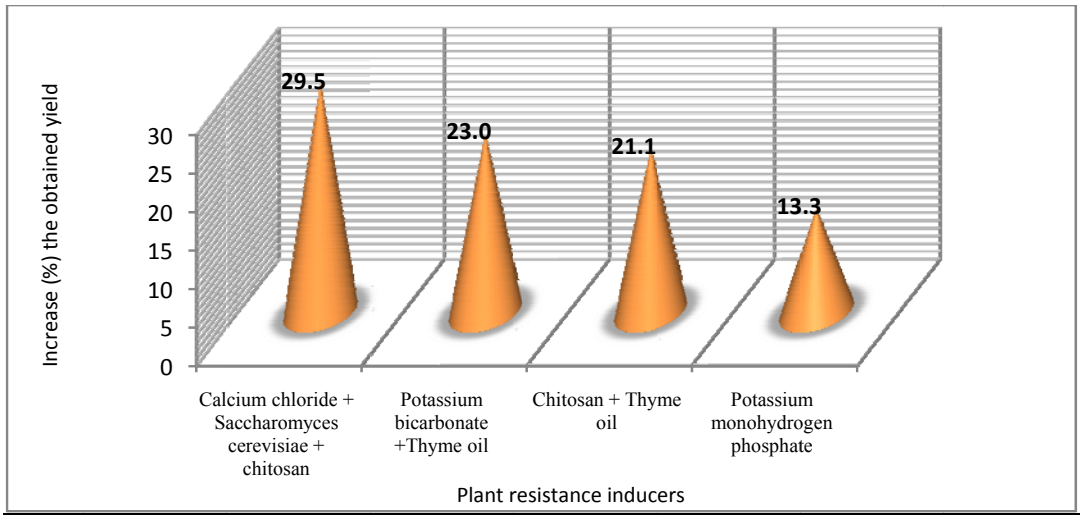

Fig. 3: Increase (%) in the obtained yield of Pepper in response to foliar application with different formula in plastic houses under protected cultivation system at Haram locations

The obtained results in the present study showed high efficacy of application plant resistance inducers Calcium chloride, Potassium bicarbonate, Potassium monohydrogen phosphate, Thyme oil, Chitosan, *S. cerevisiae* as foliar spray treatment during the growing growth season against downy and powdery mildews of Pepper plants. Sprayed treatments, [Calcium chloride + *S. cerevisiae* + Chitosan], [Potassium bicarbonate + Thyme oil] and [Chitosan + Thyme oil] could suppress both incidence and severity of downy and powdery mildews and increase the obtained yield over control of pepper plants (Table 1 & 2 and 3). These results are confirmed with those obtained by several investigators. The obtained reduction in invaded vegetable plants with downy and powdery mildews pathogens may be attributed to the role of sprayed chemical induce resistance which act as elicitors of plant defense reactions against pathogenic fungi. Similar explanation was reported by various workers. In this concern, some chemicals were reported as resistance inducers against plant diseases. Yoon *et al*., (1996) stated that the induced resistance or the acquired resistance is a kind of resistance produced by plant infected by pathogens or treated by chemistrical materials, which can protect plant from the subsequent infection of plant pathogens. Plants can acquire partial or systematic resistance when they were induced by different biological or abiotic elicitors, and these elicitors can activate a special dot of signal web by different signal route, then induce different plants to produce special protein for resisting the attack of pathogens and pests. Systematic acquired resistance (SAR) is a kind of special induced resistance, which has three charactoritics: systematic, persisting and broad spectrum of resist disease. It has significant theoretical and practical value to research and find materials that can induce plant resistance. The effects of foliar salt applications have not been previously studied using the *E. orontii* tomato patho-system, but foliar salt applications have been studied in other patho-systems. Reuveni *et al.* (1994, 1995, 1996) found that foliar single applications of K_2HPO_4, KH_2PO_4 at pH 4.5 or 9.3, KNO_3, KCl, K_2SO_4, and $NH_4H_2PO_4$ at concentrations ranging from 20 to 100 mM were effective in significantly reducing *Sphaerotheca fuliginea* on cucumber. With the exception of K_2HPO_4, we did not find these salts to be effective in reducing tomato powdery mildew as

single applications. In further experiments, Reuveni *et al*. (1996) found that foliar applications of 25 mM of K_2HPO_4 or KH_2PO_4 on a 7- or 14-day schedule were highly effective in controlling natural infection of cucumber powdery mildew. Our multiple application experiments with 40 and 100 mM K_2HPO_4 on tomato gave similar results (we did not use KH_2PO_4 in the multiple-application experiments). The mode of action of these foliar applications was not studied in our experiments. Treatments may have been effective because of osmotic, pH, or specific-ion effects. Clearly some applications were more effective than others, even though osmotic potential and ionic concentrations were similar. For example, $CaCl_2$ was generally more effective than $MgSO_4$ in the first multiple-application experiment (M1), despite the fact that osmotic potentials were very similar, and the anions (Cl–1 and SO_4 –1) were of the same concentration. Similarly, it is difficult to explain treatment effects on the basis of pH. Reuveni *et al*. (1995) reported a direct effect of phosphates on the mycelia and conidia of *Sphaerotheca fuliginea* on cucumber, which were collapsed and of irregular shape. The suppressive effect of these applications did not appear to be caused by a pH effect. Reuveni *et al*. (1996) found that using KOH to alter the pH of K_2HPO_4 foliar treatments from 4.5 to 9.4 did not influence the effectiveness of the applications. The effectiveness of foliar salt applications in controlling powdery mildew fungi gives commercial producers environmentally friendly options for disease control, and if used in combination with fungicides, these could be useful in reducing fungicide use or preventing fungicide-insensitive isolates of pathogens from increasing. Reuveni and Reuveni (1998) used 1% (mass/volume) KH_2PO_4 alternatively with fungicides on nectarine trees to control *Sphaerotheca pannosa* (Wallr.:Fr.) and were able to reduce the application of the fungicide by 50%. We found no evidence of phytotoxicity at the concentrations used. The possibility exists that the growers may be able to use foliar-applied nutrients such as Ca for disease control. Deficiencies in specific plant nutrients are often corrected through foliar application of minerals such as B, Fe, and Ca. El-Mougy and Abdel-Kader (2009) studied 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. They found that, 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. Superior effect of sodium bicarbonate or calcium chloride in disease reduction was observed when they combined with the yeast *S. cerevisiae*. They added that, under field conditions, Calcium chloride proved higher efficacy for reducing both disease incidence and severity than that of sodium bicarbonate when applied either alone or combined with *S. cerevisiae*. Also, they observed that increasing concentrations of both sodium bicarbonate or calcium chloride showed parallel decrease in disease incidence and severity. They conclude that application of the yeast *S. cerevisiae* enhanced the efficacy of salts spraying against early blight disease and increase of potato tubers yield. 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). 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). Furthermore, bicarbonate salts have broad-spectrum antimicrobial properties and are recognized by the food industry as compounds innocuous to human health. Therefore, bicarbonates are promising compounds for use with fresh vegetables that are eaten uncooked. This would eventually allow to replace or reduce the use of synthetic fungicides, whose use is increasingly questioned because of their potential danger to human health (Spotts and Cervantes, 1986). Also, Corral *et al*., (1988) stated that bicarbonates are effective against food bacterial and yeast infections and are important in controlling buccal pathogens (Miyasaki *et al*., 1986). Likewise, some of the effects of bicarbonates on microorganisms are associated with CO_2 activity (De Pasquale and Montville, 1990; Montville and Goldstein, 1989; Daniels *et al*., 1985). For example, sodium, potassium, and ammonium bicarbonate made it possible to control some fungal infections of the cucumber during preharvest (Homma *et al*., 1981; Ziv and Zitter, 1992). Similarly, they control among others, powdery mildew caused by *Leveillula taurica* (Lév.) Arn. (Fallik *et al*., 1997 b), *Oidium euonymi japonici* (Arcang.) Sacc. (Ziv and Hagiladi, 1993), and *Sphaerotheca pannosa* (Wallr.:Fr.) Lév. var. *rosae* Woronichin (Horst *et al*., 1992). Moreover, Fallik *et al*., 1997a) reported that the inhibitory effect of $KHCO_3$ on *B. cinerea* in tomato fruit has also been observed in *Capsicum annuum* (pepper plant). On the other hand, the antioxidant Chitosans is reported to influence the production of substances related to stress response, such as phytoalexins (Walker-Simmons *et al.* 1983) and chitinases (Dornenburg and Knoor 1994, O'Herlihy *et al*. 2003). It is suggested that chitosan can be used commercially for controlling tomato root rot diseases under field conditions (El-Mougy *et al*. 2006). Trials conducted in tomatoes (Walker *et al*. 2004) showed that foliar applications of chitosan resulted in yield increase of nearly 20% and a significant improvement in powdery mildew disease control. Chitosan treatments have plant growth promoting effects,

resulting in improved yields and plant health in numerous crops and fruits. The activation of protective mechanisms in plant tissues with chitosan inhibited the growth of taxonomically different pathogens (Vasyukova *et al*. 2001). It has been considered as an alternative to chemical fungicides (Benhamou *et al*. 1994, El-Ghaouth 1994, O'Herlihy *et al*. 2003). Similar inhibitor effect of essential oils against foliar diseases were recorded. El-Kot (2010) evaluate some essential oils as biocontrol agents against powdery mildew on *Zinnia elegans*. He found that the highest significant decrease in disease incidence and severity and the best growth and flowering parameters were recorded with ginger, cinnamon and clove oils, respectively. In addition, the activities of peroxidase (POX) and polyphenol oxidase (PPO) enzymes were increased as a result of oils sprayed on plants. Sodium or potassium bicarbonate combined with oils was effective in controlling plant diseases (Horst *et al.* 1992; Ziv and Zitter 1992). Also, Thyme and Egyptian geranium oils are considered antimycotic natural compounds may be useful for inhibition of mold fungi on wood in service or during storage of building materials (Yang and Clausen, 2007). Moreover, Momol *et al*., 2005 had the first report on the use of Thymol for controlling a plant disease under field conditions, which indicated that this compound provided effective control of bacterial wilt on susceptible tomato cultivars. Also, Thymol has been reported to have fungicidal activities and fumigation with thymol has been used for control of postharvest fungal diseases (Paster *et al*., 1995; Liu *et al.,* 2002). Modes of action of the antibacterial property of thymol appeared to include disruption of bacterial cell membrane integrity by altering protein reactions (Juven *et al*., 1994; Walsh *et al*., 2003).

With results such as those reported here, foliar application of plant resistance inducers could become effective components of an integrated disease management system for pepper. It is also suggested that such application trials with mildew diseases on other vegetable crops be conducted.

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