ORIGINAL ARTICLES

Application of plant Resistance Inducers for Controlling Early and Late Blights of Tomato under plastic houses conditions

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ABSTRACT

Evaluating the efficacy of different plant resistance inducers treatments against Early and Late blight foliar diseases incidence was carried out as spray treatments on growing Tomato 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, \([\text{Calcium chloride (20mM) + } S. \text{ cerevisiae } 10^9 \text{cfu/mL (10ml/L) + Chitosan (0.05mM)}] \text{ and} [\text{Potassium bicarbonate (20mM) + Thyme oil (5ml/L)}] \) resulted in the highest reduction in foliar diseases Early and Late blight diseases incidence and severity of Tomato plants grown under plastic houses conditions and increased produced yield as well. On the light of the present study it could be suggested that the usage of combined application of the bio-agent \( S. \text{ cerevisiae } \) and/or resistance plant chemicals might be used as easily applied, safely and cost effective control methods against such foliar plant diseases.

Key words: Early blight, Late blight, plant resistance inducers, Plastic houses, Tomato.

Introduction

Tomato plants (Solanum lycopersicum L.) considered one of the most important vegetable crops overall the world. Early and Late blights caused by \( \text{Alternaria solani} \) and \( \text{Phytophthora infestans} \) are the most important diseases attacking potato plants (Cook and Deahi, 1998; Khurana, 1998; El-Kareem et al., 2001& 2002). Early blight characterized as one or two spots per leaf, approximately \( \frac{1}{4} \) to \( \frac{1}{2} \) inch in diameter. Spots have tan centers with concentric rings in them and yellow halos around the edges, meanwhile symptoms of Late blight were described as spots start out pale green, usually near the edges of tips of foliage, and turn brown to purplish-black. In humid conditions, a fuzzy mold appears on the undersides of leaves.

Control of these diseases depends mainly on fungicidal treatments. In order to avoid the environmental pollution fungicide alternatives are needed (Rauf, 2000; El-Mougy et al., 2004). A successful disease-control program could involve just a single practice, but the long term reduction of disease losses generally requires the application of several control measures. The best way to ensure success of a disease- management program is to use integrated disease-control measures (Dik et al., 2002). Some chemicals were reported as resistance inducers against plant diseases. In this regard, the content of ascorbic acid in plant tissues has been associated with resistance to some diseases (Zacheo et al., 1977). 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 \( S. \text{ cerevisiae} \). 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 KHCO3 applications were effective in reducing the severity of powdery mildew on \( E. \text{ japonica} \) and pumpkin (Ziv and Zitter, 1992; Ziv, and Hagiladi, 1993). These findings encouraged us to evaluate The potential use and the efficacy of foliar sprays of single or integrates of natural compounds as biological control (\( S. \text{ cerevisiae} \), mineral salt (CaCl2, K2HPO4, CHCO3), antioxidant (ascorbic acid), resistance chemical inducers (Chitosan, Saccharin) and essential oil (Thyme) on grown vegetables were evaluated in the present study to provide acceptable control level of Tomato foliar diseases under plastic house conditions.

Therefore, the main objective of the present study was designed to evaluate some plant resistance inducers treatments as foliar spray eco-friendly treatments for controlling Late and Early blights under commercial plastic houses conditions.
Materials and Methods

Evaluation of plant resistance inducers under natural infestation with Tomato foliar diseases causal organisms against Early and Late blights infection 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 plant resistance inducers treatments against Early and Late blights infection were applied as foliar sprays treatment as follows:

- Calcium chloride (20mM) + \textit{Saccharomyces cerevisiae} 10\times10^{10}\text{cfu/mL} (10ml/L) + Chitosan (0.05mM)
- Potassium bicarbonate (20mM) + Thyme oil ( 5ml/L)
- Chitosan (0.05mM) + Thyme oil ( 5ml/L)
- Potassium monohydrogen phosphate (20mM)
- 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 vegetables were sprayed with proposed treatments 3 times with 15 days intervals after transplanting (Aleandri et al., 2010).

The growing tomato plants in the experimental plastic houses received the recommended pesticides against harmful insects, \textit{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 tomato in all plastic houses received traditional agricultural practices, \textit{i.e.} irrigation, fertilization, etc.

Monitoring and scouting of foliar diseases incidence of Early and Late blights of tomato were recorded. Percentages of disease incidence and severity were recorded at 60, 90 and 120 days of transplanted date.

\textit{Disease assessment:}

- \textbf{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 treatment, then the average of disease incidence was calculated.

- \textbf{Disease severity:}

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

  \[
  \text{D.S. \%} = \frac{\sum \left( n \times c \right)}{N} \times 100
  \]

  Whereas: \text{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 \textit{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.

\textbf{Statistical Analysis}

All experiments were set up in a complete randomized block design. One-way ANOVA was used to analyze differences between applied treatments. A general linear model option of the analysis system SAS (SAS Institute Inc. 1996) was used to perform the ANOVA. Duncan’s multiple range test at \(P < 0.05\) level was used for means separation (Winer 1971).
Results and Discussion

The obtained results in Table (1) and Fig (1) showed the Early and Late blights incidence of Tomato plants grown in plastic house at Haram location. Presented data revealed that all applied treatments caused significant effect on foliar diseases resulted in high reduction in both Early and Late blights diseases incidence comparing with check control. Data also showed that Early blight disease started to occur after 90 days of plant growth with records of 3.3, 5.4 and 7.2% at the applied treatments, [Potassium bicarbonate (20mM) + Thyme oil (5ml/L)], [Calcium chloride (20mM) + *S. cerevisiae* 10x10^10cfu/mL (10ml/L) + Chitosan (0.05mM)] and [Chitosan (0.05mM) + Thyme oil (5ml/L)], respectively. Meanwhile, the disease incidence was recorded as 12.3, 16.4 and 23.2% in control at 60, 90, 120 days of plant growth. Similar effect was also observed with treatment, [Potassium monohydrogen phosphate (20mM)] that it recorded disease incidence as 5.2, 11.4% at 90 day of plant growth, respectively.

Concerning Late blight incidence treatments, [Calcium chloride (20mM) + *S. cerevisiae* 10x10^10cfu/mL (10ml/L) + Chitosan (0.05mM)], [Potassium bicarbonate (20mM) + Thyme oil (5ml/L)] and [Chitosan (0.05mM) + Thyme oil (5ml/L)] could delay the occurrence of disease incidence up to more than 60 day of Tomato plant growth. The recorded disease incidence was 3.3-7.4%; 3.4-7.8% and 4.2-8.2% at 90 and 120 day of plant growth. Meanwhile, at the applied treatment, [Potassium monohydrogen phosphate (20mM)] the recorded disease incidence as 2.2, 7.4, 14.2% at 60, 90 and 120 day of plant growth comparing with 12.4, 14.3, 28.2% in untreated control, respectively.

### Table 1: Percentage of Early and Late blight diseases incidence in response to application of different formula against foliar diseases of Tomato grown in plastic houses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Early blight</th>
<th>Late Blight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days of transplanting</td>
<td>Days of transplanting</td>
</tr>
<tr>
<td>Calcium chloride + <em>S. cerevisiae</em> + Chitosan</td>
<td>0.0 b</td>
<td>0.0 c</td>
</tr>
<tr>
<td>Potassium bicarbonate + Thyme oil</td>
<td>0.0 b</td>
<td>0.0 c</td>
</tr>
<tr>
<td>Chitosan + Thyme oil</td>
<td>0.0 b</td>
<td>0.0 c</td>
</tr>
<tr>
<td>Potassium monohydrogen phosphate</td>
<td>0.0 b</td>
<td>5.2 b</td>
</tr>
<tr>
<td>Control</td>
<td>12.3 a</td>
<td>16.4 a</td>
</tr>
</tbody>
</table>

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

Moreover, the highest reduction in disease incidence (Fig 1) calculated as 100, 100, 85.7% and 100, 76.2, 73.7% for Early and Late blights at the treatment, [Calcium chloride (20mM) + *S. cerevisiae* 10x10^10cfu/mL (10ml/L)] followed by treatment, [Potassium bicarbonate (20mM) + Thyme oil (5ml/L)] which recorded 100, 100, 76.7% and 100, 76.2, 72.3% for both diseases at tomato growth periods of 60, 90 and 120 day, respectively. The other applied treatments, [Chitosan (0.05mM) + Thyme oil (5ml/L)] followed by [Potassium monohydrogen phosphate (20mM)] showed similar effect on the diseases incidence throughout the growth period in a lesser effect that at 120 days of plant growth they recorded the lowest reduction in diseases incidence calculated as (68.9, 50.8%); (70.9, 49.6%) for Early and Late blights, in respective order.
Regarding Early and Late blights disease severity, data in Table (2) and Fig (2) showed high suppressive effect of all applied treatments causing reduction in disease express on infected Tomato plants comparing with control. In this concern, the highest reduction in disease development was recorded at treatments, [Calcium chloride (20mM) + S. cerevisiae 10x10^6cfu/mL (10ml/L)] and [Potassium bicarbonate (20mM) +Thyme oil (5ml/L)] followed by [Chitosan (0.05mM) + Thyme oil (5ml/L)] and [Potassium monohydrogen phosphate (20mM)], respectively.

**Table 2: Percentage of Early and Late blight diseases severity in response to application of different formula against foliar diseases of Tomatogrown in plastic houses**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar diseases severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early blight</td>
</tr>
<tr>
<td>Days of transplanting</td>
<td>Days of transplanting</td>
</tr>
<tr>
<td>Calcium chloride + S. cerevisiae + Chitosan</td>
<td>0.0 b 0.0 c 0.2 d</td>
</tr>
<tr>
<td>Potassium bicarbonate + Thyme oil</td>
<td>0.0 b 0.0 c 0.4 c</td>
</tr>
<tr>
<td>Chitosan + Thyme oil</td>
<td>0.0 b 0.0 c 0.6 b</td>
</tr>
<tr>
<td>Potassium monohydrogen phosphate</td>
<td>0.0 b 0.2 b 0.6 b</td>
</tr>
<tr>
<td>Control</td>
<td>0.3 a 1.2 a 1.6 a</td>
</tr>
</tbody>
</table>

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

Furthermore, the applied treatments showed significant suppressive effect on the Early and Late blights comparing with check control (Table 2 and Fig. 2). Presented data revealed at 60 days of plant growth an drastic suppressive effect against disease development of Early blight and Late blight recorded as 100% reduction in disease severity (DS) when the treatment [Calcium chloride (20mM) + S. cerevisiae 10x10^6cfu/mL (10ml/L) + Chitosan (0.05mM), [Potassium bicarbonate (20mM) + Thyme oil (5ml/L)] and [Chitosan (0.05mM) + Thyme oil (5ml/L)]] was applied. Meanwhile at 120 days of plant growth, the highest reduction in diseases severity were recorded at [Calcium chloride (20mM) + S. cerevisiae 10x10^6cfu/mL (10ml/L)] and [Potassium bicarbonate (20mM) + Thyme oil (5ml/L)] foliar treatments as 87.5, 75.0% and 82.6, 82.6% for Early blight and Late blights, respectively.

**Fig. 2: Reduction (%) in Early and Late blights diseases severity in response to application of different formula against foliar diseases of Tomato grown in plastic houses**

As for Tomato plants grown in plastic house, the accumulated obtained yield (Table 3) was recorded as 1,713; 1,622; 1,441 and 1,312 Ton/ plastic house for the applied treatments, [Calcium chloride (20mM) + S. cerevisiae 10x10^6cfu/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. Meanwhile, the produced yield of untreated control plants was recorded as 1,234 Ton/ plastic house.
One the other hand, the applied treatments at the same previous order caused an increase in accumulated yield over that obtained of untreated Tomato plants (Fig 3) calculated as 44.6, 36.9, 21.7 and 10.8%, respectively.

### Table 3: The obtained yield of Tomato in response to foliar application of different formula in plastic houses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average Yield Kg/row</th>
<th>Yield Ton/plastic house</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium chloride + S. cerevisiae + chitosan</td>
<td>342.6 de</td>
<td>1,713</td>
</tr>
<tr>
<td>Potassium bicarbonate + Thyme oil</td>
<td>324.4 d</td>
<td>1,622</td>
</tr>
<tr>
<td>Chitosan + Thyme oil</td>
<td>288.2 bc</td>
<td>1,441</td>
</tr>
<tr>
<td>Potassium monohydrogen phosphate</td>
<td>262.4 b</td>
<td>1,312</td>
</tr>
<tr>
<td>Control</td>
<td>236.8 a</td>
<td>1,234</td>
</tr>
</tbody>
</table>

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

Present research focuses on finding compounds that are safe to human and environment. An alternative to pesticide application is that, it may be possible to utilize a scheme of inducible plant defense which may provide protection against a broad spectrum of disease-causing pathogenic microorganisms. Biological control using microbial antagonists has shown potential as an alternative for natural control of plant pathogens instead of synthetic chemical fungicides (Pradhanang et al., 2003; Droby et al., 1989). The mode of action of antagonistic yeasts may be competition for space and nutrients (Droby et al., 1989, Janisiewicz et al., 2000), production (El-Ghaouth et al., 1998), and induction of host resistance (Arras, 1996; Droby et al., 2002). In large-scale tests, the use of biological control often needed to be combined with low doses of synthetic fungicides to obtain a level of disease control equivalent to synthetic fungicides (Droby et al., 2003). In order to completely eliminate the use of synthetic fungicides, more environmentally friendly and harmless compound(s) should be explored to improve the activity of the antagonist. Selected chemicals such as calcium chloride (Wisniewski et al., 1995; Tian et al., 2002) in combination with biological control agents have been demonstrated to give beneficial effects on control of fruit decay. The control is not total, but in combination with bioactive additives it is possible to obtain the efficacy of the chemical standard (Lima et al., 2005; Conway et al., 2005).

![Fig. 3: Increase (%) in the obtained yield of Tomato in response to foliar application with different formula in plastic houses](image)

### Fig. 3: Increase (%) in the obtained yield of Tomato in response to foliar application with different formula in plastic houses

Another alternative control method is given by enhancing natural resistance of plants towards the pathogen. Compounds which are triggering plant’s own defense mechanisms are termed elicitors. In the present study, application of different formula of chemical plant resistance inducers as foliar spray resulted in reduction of tomato Early blight and Late blight diseases incidence and severity which reflected positively in plant stand and its healthy growth as well as its produced yield. The obtained results 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 foliar diseases of tomato plants. In this regards, many investigators studied the influence of various salts on microorganisms. There was a considerable interest in the use of sodium bicarbonate (NaHCO₃) and potassium bicarbonate (KHCO₃) for controlling various fungal diseases in plants (Karabulut et al., 2003; Smilansick 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 NaHCO₃ solution provided good control of several plant diseases (Horst et al. 1992; Janisiewicz et al., 2005). Also, spraying with KHCO₃ solution provided the most effective protection against plant diseases (Smilansick et al., 1999, 2006). Sodium or potassium bicarbonate combined with oils was effective in controlling plant diseases (Horst et al. 1992; Ziv and Zitter 1992). Furthermore, many researchers have shown that calcium plays an important role in the inhibition of postharvest decay of fruits (Conway and Sams, 1985; Conway et al., 1992) and in enhancing the efficacy of postharvest biocontrol agents (Conway et al., 1991; Wisniewski et al., 1995, Tian et al., 2002). Postharvest calcium treatment of apples provided broad-spectrum protection against the postharvest pathogens of Penicillium expansum and Botrytis cinerea (Saffner et al., 1997). The addition of CaCl₂ (2% w/v) to the formulation of the yeast biocontrol agent, Candida oleophila, enhanced the ability of this yeast to protect apples against postharvest decay (Wisniewski et al., 1995). The efficacy of controlling grey mould and blue mould rots in apples was enhanced when Trichosporon sp., even at a low concentration of 105 CFU mL⁻¹, was applied in the presence of CaCl₂ (2% w/v) in an aqueous suspension (Tian et al., 2001).

Thyme essential oil as natural alternatives that demonstrate low toxicity to humans is desirable to be used in the present work. Thyme oil applied in combination with potassium bicarbonate or chitosan showed effective reduction in foliar diseases incidence more than 50% reduction in Tomato Early and Late blights. In this regards, several investigators reported the antifungal effect of essential oils. Also, Thyme and Egyptian geranium oils are considered antinfective 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 pro-vided 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).

Chitosan had shown effective influence, in the present work, for reducing foliar diseases when sprayed in combined treatment with Thyme oil. These records were confirmed with previous reports. Chitosan, in recent years, the importance of chitosaccharides as plant growth promoting and disease control agents has been emphasized (Trotel-Aziz et al., 2006; Aziz et al., 2006). CHN (β-1-4 linked N- gluco-samine) has been shown to induce defense responses in different plants (Chang et al., 1992; Hadwiger et al., 1994). Chitosan oligomers was found to induce defense responses in grapevine leaves, as evidenced by an accumulation of stilbene phytoalexins, trans- and cis-resveratrol, ε-viniferins, and piceids, and a stimulation of chitinase and β-1,3-glucanase activities (Aziz, et al., 2006). Furthermore, the combination of Chitosan and CuSO₄ 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. Furthermore, 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. Moreover, Chitosan could enhance the accumulation of pathogenesis related-proteins such as ss-1,3-glucanase, chitinase and PR14 in treated and upper untreated tomato leaves (Atia et al., 2005). The studies with chitosan against tomato late blight suggested that chitosan displays dual effects: (a) direct interference in developmental stages of P. infestans and (b) by lesion formation, leading to disease resistance mechanisms. Moreover, several workers suggested two different mechanisms of chitosan molecule and target microorganism interaction: the first is the adsorption of chitosan to cell walls leading to the cell wall covering, membrane disruption and cell leakage; the second is the penetration of chitosan into living cells leading to the inhibition of various enzymes and interference with the synthesis of mRNA and proteins (Chircov, 2002; Zheng and Zhu, 2003).

As for the produced yield, in the present study, sprayed tomato plants with all plant resistance inducers treatments reflected on announced increase of the yield obtained ranged between 10.8-44.6% for grown tomato plants at Haram plastic houses location. The highest increase over control recorded was 44.6 and 36.9% was obtained by treatments, [Calcium chloride + S. cerevisiae + chitosan] followed by [Potassium bicarbonate + Thyme oil], respectively. Meanwhile, treatments of [Chitosan + Thyme oil] and [Potassium monohydrogen phosphate] caused an increase of the obtained yield calculated as 21.7 and 10.8% over control treatment, respectively. In this concern, several investigators reported that application of plant resistance inducers had positive effect on both plant stand and yield production. Abd-El-Kareem, et al., (2001) reported that, treated potato plants with chitosan induced resistance against late and early blight diseases and increased tuber yield.
under field conditions. Also, El-Gamal et al., (2007) stated that Calcium chloride showed an increase of potato yield which was between 36.4 to 50.0 % over control at two cultivation seasons, respectively. Moreover, Potato tuber harvested yield, in certain essential oils applied as foliar spray treatments, was significantly higher than control. Highly effective treatment induced the obvious increase of tuber yield being for 1% of thyme oil (22.1 and 40.0%) in 2006 and 2007 cultivation seasons, respectively (El-Mougy, 2009).

The present study lead to conclude that integration between salt, essential oil and bioagents is considered an applicable, safe and cost-effective method for controlling such foliar diseases.

Acknowledgement

This work was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No. 1059.

References


