EFFECT OF ABIOTIC AND BIOTIC TREATMENTS ON BACTERIAL ANGULAR LEAF SPOT AND PHENOLIC COMPOUNDS OF CUCUMBER

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ABSTRACT

Bacterial angular leaf spot disease caused by Pseudomonas syringae pv. lachrymans is one of the most important foliage disease of cucumber. The present work was planned to control the disease using six inducers i.e. salicylic acid, cobalt sulphate, di-basic potassium phosphate, lithium chloride, potassium silicate and tri-potassium phosphate, in addition two bioagents named Ps. fluorescens and Bacillus subtilis were applied as foliar treatments to induce systemic resistance in cucumber plants against bacterial angular leaf spot disease. The most effective inducers were salicylic acid, dibasic potassium phosphate and lithium chloride, respectively. On the other hand, Bacillus subtilis was least effective one followed by potassium silicate and Ps. fluorescens, respectively. While, tri-potassium phosphate, cobalt sulphate were moderate in this respect. Efficacy of used inducers prolonged about till six weeks after treatment.

The inducers were shown to increase phenolic compounds of cucumber leaves, whereas after two weeks of treatment up to six weeks, free and total phenol in the treated plants showed higher amounts than those in the untreated ones. Another trend was obtained with conjugated phenol. A positive correlation had been noticed between efficacy of inducers and accumulated amounts of free and total phenol in cucumber leaves, i.e. the higher inducer efficacy the higher free and total phenol accumulation.

INTRODUCTION

Cucumber [Cucumis sativus L] is one of the most important vegetable crops. It suffer from a bacterial disease caused by Pseudomonas syringae pv. lachrymans which effect the production [Moura & Romeiro, 1997; Staub & Crubaugh, 2001 and Shen et al 2001]. Application of bactericides hazard the environments, hazard human health and create resistant strains in the pathogen population.

firmed that concentration of 7.5 mM of salicylic acid was effective in inducing resistance in cucumber plants against angular leaf spot disease. Treatment with 50 mM of di-basic potassium phosphate reduced cucumber downy mildew incidence [Ibrahim, 1998]. El-Toony (2003) found that foliar spraying with 0.1 and 1 mM of cobalt sulphate and lithium chloride, respectively, proved good results in controlling cucumber powdery mildew disease. Application of tri-potassium phosphate as foliar spraying at the rate of 1 mM decreased severity of potato bacterial wilt disease [Zayed, 2004]. Resistance to potato black leg was achieved with tuber treatment with acibenzolar – s – methyl (ASM) [Benelli et al 2004]. Application of prohexadione – ca, as a new inducer inhibit of gibberellin biosynthesis, it leads to significant changes in the spectrum of flavonoids and their phenolic precursors in pome fruits, which causes reduced susceptibility to fire blight disease [Buban et al 2004]. Mahmoud & Gomah (2006) tested bacterial suspension [10⁶ cfu/ml] of Ps. fluorescens and B. subtilis as foliar treatment against crown gall of peach disease, the results revealed that 88.6 and 70.9% gall inhibition, respectively. Many biochemical and cellular changes occur during induction systemic resistance, where they would be encountered and act against fungal and/or bacterial pathogens at an early stage of the infection process [Kuc, 1995 and Ye et al 1995].

The aim of this investigation is to evaluate some inducers to minimize the effect of angular leaf spot disease on cucumber plants by systemic induced resistance.

MATERIALS AND METHODS

Preparation of bacterial isolates

Virulent isolate of Ps. syringae pv. lachrymans and isolates of Ps. fluorescens and B. subtilis as bioagents were obtained from Bacterial Diseases Department, Institute of Plant Pathology Research. The isolates were grown on nutrient agar medium for 48 hour at 28°C. The bacterial cells were suspended in distilled water to reach concentration of 10⁸ cfu/ml as determined from a standard curve based on absorbance at A₆₂₀ nm [Shekhawat et al 1992].

Used inducers

In untabulated data, primary experiments were carried out for tested different concentrations of each inducer [lower, equal and higher than/reviewed conc.]. Then, conc. which resulted the best disease control were used in the further experiments. Bacterial suspensions [10⁶ cfu/ml] of Ps. fluorescens and Bacillus subtilis were used as bioinducers. Salicylic acid [2-hydroxybenzoic acid, C₇H₆O₃], cobalt sulphate [CoSO₄·7H₂O], di-basic potassium phosphate [K₂HPO₄], lithium chloride [LiCl], potassium silicate [K₂SiO₃] and tri-potassium phosphate [K₃PO₄] were used as chemical inducers at the concentrations of 7.5, 0.1, 50, 1, 17 and 1 mM, respectively.

Influence of different inducers against bacterial angular leaf spot of cucumber

Seeds of cucumber [cv. Bet-Alpha] used in these experiments were obtained form Department of Vegetable Research, Agricultural Research Centre. Five seeds were planted in 30 cm in diameter pot containing sandy clay soil [1:1], 10 ml/seedling foliar spraying with different inducers were carried out on cucumber seedlings [30 old days] with each of the different inducers. Five pots were replicated for each inducer. Five pots were sprayed with distilled water served as a control. The leaves of all plants were sprayed with Ps. syringae pv. lachrymans suspension [10⁶ cfu/ml] five days after treatment with different inducers. All inoculated seedlings were placed in humid chamber for 48 h.

Disease severity was recorded after two, four and six weeks from inoculation as percentage of disease index according to the disease rating scale from 0 to 5, in which 0 = no lesions, 1 = 1-20% of leaf area with lesions, 2 = 21-40%, 3 = 41-60%, 4 = 61-80% and 5= 81-100% [Raupach and Kloepper, 1998]. The disease index [DI] was calculated using the following formula:

\[ \text{Sum of } [n \times v] \times 100 \]

\[ \text{Total number of leaves } \times \text{max. gradings [5]} \]

Where: \( n \) = number of infected leaves in each category

\( v \) = numerical value of each category

Also, percentage of disease control [PDC] was calculated as the following:

\[ PDC = \frac{[\text{In}_{tk} - \text{In}_t]}{\text{In}_{tk}} \times 100 \]

Where:

\( \text{In}_{tk} \) = Infection in check treatment

\( \text{In}_t \) = Infection in treated treatment
Obtained data was statistically analyzed and the least significant differences [L.S.D.] at 5% level was calculated.

**Determination of phenolic compounds**

Phenolic compounds were colorimetrically determined using the phosphotungistic phosphomolybdic acid (Folin Ciocalteau) phenol reagent according to Snell and Snell (1953).

**Preparation of samples**

100 g of cucumber leaves treated or non-treated control, were separately bottled in 100 ml of 95% ethanol. The sample ethanol mixture was boiled in a water bath for five minutes, then extracted for 16 hrs by Soxhle’s apparatus. The extracted samples were then dissolved in 5 ml 70% ethanol, centrifuged at 3000 rpm for 10 mins to separate the ethanol fraction and let to dry at room temperature. The dried samples was dissolved in 5 ml of isopropanol and used in the following analyses.

**Total phenols**

Ten drops of concentrated HCL were added to 0.1 ml of the sample, heated quickly to boiling point and placed in a boiling water bath for 10 min. After cooling, 1.0 ml of the folin reagent and 5 ml of a 20% NaCO₃ were added. The mixture was diluted to 10 ml with distilled water and determination was carried out using spectrophotometer (UV 2600) at + 520 nm after 30 min.

**Free phenols**

Free phenols were determined by adding 1.0 ml of the folin reagent and 3 ml of a 20% solution of sodium carbonate to 0.1 ml of the sample, then diluted to 10 ml with distilled water. Data was measured using spectrophotometer (UV 2600) at 520 nm after 30 min.

**Conjugated phenols**

Conjugated phenols were determined by subtracting values of free phenols from the values for total phenols. All these determinations were expressed as mg/g fresh weight of plant sample.

**RESULTS**

**Effect of abiotic and biotic treatments on disease incidence**

Results in Table (1) indicated that all treatments significantly reduced the disease incidence when compared with the control treatment. There were significant differences when salicylic acid, di-basic potassium phosphate, lithium chloride, tri-potassium phosphate, cobalt sulphate, Ps. fluorescens, potassium silicate and B. subtilis were applied as foliar spraying after two weeks from inoculation, where percentage of disease infection were 5.3, 7.5, 10.4, 12.7, 15.3, 17.9, 21.2 and 24.5, respectively. The same trend was realized after four and six weeks from inoculation. There was a positive correlation between disease infection and severity. In all treatments efficacy of used inducers remained till six weeks. Efficiency of abiotic and biotic treatments were reduced with increasing the period after treatment. Abiotic treatments were more effective than biotic ones to decrease the disease. Salicylic acid and di-basic potassium phosphate were the most effective against the disease, where percentage of disease control was 64.7 – 80.5 and 53.2 – 72.4%, respectively. Meanwhile, lithium chloride, tri-potassium phosphate and cobalt sulfate were moderately effective, where percentage of disease control 43.2– 61.8, 38.3 – 53.3 and 31.6 – 43.7%, respectively. Pseudomonas fluorescens, potassium silicate and Bacillus subtilis were less effective, where percentage of disease control was 17.6 – 34.2, 11.2 – 22.1 and 7.0 – 9.9%, respectively.

**Effect of abiotic and biotic treatments on contents of phenolic compounds in cucumber leaves**

Data in Table (2) indicated that all used inducers increased total phenols in leaves compared to this in the control. After two weeks of inoculation, the highest level of total phenols [9.9 mg catecol/100 g fresh weight] was obtained with salicylic acid which also reflected the lowest percentage of disease infection and severity. The lowest one [3.6 mg catecol/100 g fresh weight] was obtained with B. subtilis which reflected the highest percentage of disease incidence and severity. As regards to conjugated phenols, all treatments decreased its levels in treated leaves compared with the control. Vice versa, they sharply increased free phenols levels over the control. The same trend of
Table 1. Influence of abiotic and biotic treatments on incidence of bacterial angular leaf spot disease of cucumber, at different periods under artificial inoculation conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Application time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>5.3</td>
</tr>
<tr>
<td>Di-basic potassium phosphate</td>
<td>7.5</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>10.4</td>
</tr>
<tr>
<td>Tri-potassium phosphate</td>
<td>12.7</td>
</tr>
<tr>
<td>Cobalt sulphate</td>
<td>15.3</td>
</tr>
<tr>
<td>Potassium silicate</td>
<td>21.2</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>17.9</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>24.5</td>
</tr>
<tr>
<td>Control</td>
<td>27.2</td>
</tr>
<tr>
<td>L.S.D. at 0.05</td>
<td>1.8</td>
</tr>
</tbody>
</table>

A = Percentage of infection.  
B = Disease index [%] according to disease rating [0-5]  
C = Percentage of disease control [PDC]

Table 2. Influence of abiotic and biotic treatments on phenolic compound contents [free, conjugated and total] of cucumber leaves, at different periods.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Phenolic compounds [mg catecol/100 g fresh weight] as:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>6.7</td>
</tr>
<tr>
<td>Di-basic potassium phosphate</td>
<td>6.1</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>4.8</td>
</tr>
<tr>
<td>Tri-potassium phosphate</td>
<td>4.2</td>
</tr>
<tr>
<td>Cobalt sulphate</td>
<td>3.2</td>
</tr>
<tr>
<td>Potassium silicate</td>
<td>2.3</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>2.8</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>2.1</td>
</tr>
<tr>
<td>Control</td>
<td>0.4</td>
</tr>
</tbody>
</table>

A = After 2 weeks from inoculation  
B = After 4 weeks from inoculation  
C = After 6 weeks from inoculation
Diseases are of the most important factors affecting cucumber production. Survey of cucumber diseases indicated that angular leaf spot was a serious disease. The importance of it as a limiting factor in cucumber production increased during the two last decades. Many reports have been published in this concern by Moura and Romeiro (1997), Sticher and Metraux (2000), Staub and Crubaugh (2001) and Shen et al (2001). Sticher and Metraux (2000) used ISR as a true attempt to control cucumber angular leaf spot disease far away polluting the environment conditions. As well as, Lievens et al (2001) used it in cucumber against root rot disease. In this research, some biotic and chemical agents were tested as inducers against aforementioned disease.

Among biotic agents, Ps. fluorescens and B. subtilis were tested in this investigation. The first bacterium was effective than the second one when applied as foliar treatment. Similar result was recorded by Mahmoud and Gomah (2006). Chester (1933) reported that biotic inducers were the first agents used to induce resistance against diseases. In this respect, induction of resistance using biotic agents triggers defense mechanisms and plants could be immunized against pathogens by selected nonpathogens [Kuc, 1991].

Results indicated that application of salicylic acid, cobalt sulphate, di-basic potassium phosphate, lithium chloride, potassium silicate and tri-potassium phosphate as chemical inducers, decreased disease infection and severity, whereas, salicylic acid was the most effective in conferring ISR. Utility of these chemicals as inducers with its used concentrations was reported by Abd El-Sayed et al (2006), El-Toony (2003), Ibrahim (1998), El-Toony (2003), Menzies et al (1992) and Zayed (2004), respectively. In present work, efficacy of used inducers prolonged till six weeks after treatment. In this connection, Kuc (1993) mentioned that efficiency of disease resistance mechanisms could be expressed systemically for extended periods of times by using biotic agents and chemicals which themselves were not antimicrobial.

Results revealed that exogenous application of used inducers caused considerable increase in free and total phenols after two weeks of treatment and continued till six weeks. These results are in harmony with those of Irving and Kuc (1990) and Avdiushko et al (1993). The toxic phenolic compounds in plant cells were found to act through enhancing host resistant by stimulating host defense mechanisms [Subba Rao et al 1988] and penetrating pathogens and causing considerable damage to the cell metabolism [Kalaichelvan and Elangovan, 1995].

REFERENCES


