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

[40]

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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 *Pseudomonas 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.

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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.

Induction of systemic resistance become widespread in plant disease control **[Kuc, 1987].** Induced resistance is non-specific being an effective approach against a wide range of plant pathogens **[Ye *et al* 1995]. Mei *et al* (1990)** mentioned that application of some plant growth promoting rhizobacteria [PGPR] strains against foliage diseases. Application of PGPR strains induces systemic resistance against cucumber diseases **[Raupach and Kloepper, 2000].** Because of lack of a sufficient degree of resistance of cultivated plants systemic induced resistance has gained increasing interest in controlling bacterial diseases, this is true to have potential effect on bacterial wilt of tomato **[Abd El-Sayed *et al* 1996];** angular leaf spot of cucumber **[Sticher & Metraux, 2000 and Abd El-Sayed *et al* 2006];** bacterial wilt of potato **[Zayed, 2004]** and crown gall of peach **[Mahmoud and Gomah, 2006]. Dixon *et al* (1994) and Lievens *et al* (2001)** reported certain inducers to control a wide range of diseases in various plant species by inducing systemic resistance. In this respect, **Menzies *et al* (1992)** found that foliar spraying with 17 mM potassium silicate significantly reduced cucumber powdery mildew disease. **Abd El-Sayed *et al* (1996)** confirmed 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 [10^8 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 menimize 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 10^8 cfu/ml as determined from a standard curve based on absorbance at A_{620} 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/with reviewed conc.]. Then, conc. which resulted the best disease control were used in the further experiments. Bacterial suspensions [10^8 cfu/ml] of *Ps. fluorescens* and *Bacillus subtilis* were used as biotic inducers. Salicylic acid [2-hydroxybenzoic acid, $C_7h_6O_3$], cobalt sulphate [$COSO_4$-$7H_2O$], dibasic potassium phoshate [K_2HPO_4], lithium chloride [LICL], potassium silicate [$KSIO_3$] and tri-potassium phosphate [K_3PO_4] 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 [10^8 cfu/ml] five days after treatment with different inducers. All inoculated seedling 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

$$\frac{\textit{Sum of } [n \times v]}{\textit{Total number of leaves x max. gradings } [5]} \times 100$$

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:

$$PDC = \frac{[In_{ck} - In_{tr}]}{In_{ck}} \times 100$$

Where : In_{ck}= Infection in check treatment
In_{tr} = 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% $NaCO_3$ 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

Treatments	Application time								
	2 weeks			4 weeks			6 weeks		
	A	B	C	A	B	C	A	B	C
Salicylic acid	5.3	2.9	80.5	7.6	4.1	75.1	11.6	5.8	64.7
Di-basic potassium phosphate	7.5	3.3	72.4	11.8	5.7	61.3	15.4	7.2	53.2
Lithium chloride	10.4	5.6	61.8	14.3	8.1	53.1	18.7	10.9	43.2
Tri-potassium phosphate	12.7	8.2	53.3	17.0	10.3	44.3	20.3	12.6	38.3
Cobalt sulphate	15.3	9.6	43.7	19.4	13.8	36.4	22.5	15.0	31.6
Potassium silicate	21.2	17.1	22.1	25.9	18.9	15.1	29.2	19.3	11.2
Pseudomonas fluorescens	17.9	12.1	34.2	23.5	14.2	23.0	27.1	16.3	17.6
Bacillus subtilis	24.5	18.4	9.9	28.2	20.1	7.5	30.6	20.9	7.0
Control	27.2	18.8	-	30.5	20.7	-	32.9	22.3	-
L.S.D. at 0.05	1.8	N.S		2.1	N.S		1.3	0.9	

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.

Treatments	Phenolic compounds [mg catecol/100 g fresh weight] as:								
	Free			Conjugated			Total		
	A	B	C	A	B	C	A	B	C
Salicylic acid	6.7	4.9	3.5	3.2	2.9	1.1	9.9	7.8	4.6
Di-basic potassium phosphate	6.1	4.5	2.9	3.0	2.6	1.2	9.1	7.1	4.1
Lithium chloride	4.8	4.2	2.1	2.9	2.2	1.7	7.7	6.4	3.8
Tri-potassium phosphate	4.2	3.1	1.9	2.7	2.6	1.1	6.9	5.7	3.0
Cobalt sulphate	3.2	2.9	1.7	2.2	1.3	1.2	5.4	4.2	2.9
Potassium silicate	2.3	2.0	1.3	1.6	1.3	1.1	3.9	3.3	2.4
Pseudomonas fluorescens	2.8	2.6	1.6	1.3	0.9	1.0	4.1	3.5	2.6
Bacillus subtilis	2.1	1.8	1.2	1.5	1.2	1.1	3.6	3.0	2.3
Control	0.4	0.2	0.1	3.1	2.7	2.0	3.5	2.9	2.1

A = After 2 weeks from inoculation
B = After 4 weeks from inoculation
C = After 6 weeks from inoculation

total, conjugated and free phenols was obtained after four and six weeks from inoculation. Phenolic compounds in cucumber leaves were decreases with increasing the period after treatment by abiotic and biotic treatments. Generally, abiotic treatments were more effective than biotic treatments on phenolic compounds. Meanwhile, phenolic compounds as free were more concentration than as their conjugated. Salicylic acid, di-basic potassium phosphate, lithium chloride and tri-potassium phosphate were the most effective to increase concentrations of phenolic compounds.

DISCUSSION

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 defence 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

Abd El-Sayed, Wafaa M.; N.Y. Abd El-Ghafar and S.A.M. Shehata (1996). Application of salicylic acid and aspirin for induction of resistance to tomato plants against bacterial wilt and its effect on endogenous hormones. **Annals of Agriculture Science, Ain Shams University, Cairo, 41: 1007-1020.**

Abd El-Sayed, Wafaa M.; E.A.M. Gado and N.Y. Abd El-Ghafar (2006). Utilization of combining biotic and abiotic treatments to control bacterial angular leaf spot disease of cucumber. **Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 14(1): 381-194.**

Avdiushko, S.A.; X.S Ye and J. Kuc (1993). Detection of several enzymatic activities in leaf prints of cucumber plants. **Physiological and Molecular Plant Pathology, 42: 441-454.**

Benelli, A.I.; N.D. Denardin and C.A. Forcelini (2004). Action of acibenzolar-s-methyl applied on potato tubers and plants to prevent black leg. **Fitopathologia-Brasileira, 29(3): 263-267.**

Buban, T.; L. Foldes; Z. Fekete and W. Redemacher (2004). Effectiveness of the resistance inducer prohexadione-ca against fire blight in shoots of apple trees inoculated with *Erwinia amylovora*. **Bulletin-OEPP, 34(3): 369-376.**

Chester, K.S. (1933). The problem of aquired physiological immunity in plants. **Q. Biol. Rev., 8: 129-154.**

Dixon, R.A.; M.J. Harrison and C.J. Lamb (1994). Early events in the activation of plant defense responses. **Annual Review of Phytopathology, 32: 479-501.**

El-Toony, A.M.E. (2003). Novel control methods by using systemic resistance inducers. **Minufiya J. Agric. Res., 28(6): 1821-1836.**

Ibrahim, F.A.E. (1998). Induction of Resistance to Some Diseases of Cucumber Plants, p. 37. Ph.D. Thesis, Fac. Agric., Ain Shams Univ., Cairo.

Irving, H.R. and J. Kuc (1990). Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by K_2HPO_4. **Physiological and Molecular Plant Pathology, 37: 355-366.**

Kalaichelvan, P.T. and N. Elangovan (1995). Effect of phenolics on *Drechslera oryzae*. **Indian Phytopathol., 48(3): 271-274.**

Kuc, J. (1987). Plant Immunization and Its Applicability for Disease Control, pp. 255-274. Chet I. ed. John Wiley and Sons, New York.

Kuc, J. (1991). Phytoalexins: perspective and prospects. **Mycotoxins and Phytoalexins, pp. 595-603.**

Kuc, J. (1993). Non pesticide control of plant disease. **Proc. 10th International Symp. on Systemic Fungicides and Antifungal Compounds, Eugen Ulmer, Stuttgart, pp. 225-237.**

Kuc, J. (1995). Induction Systemic Resistance. An overview, pp. 169-175. Kluwer Academic Publishers, Netherlands.

Lievens, B.; K. Vaes and J. Ryckeboer (2001). Systemic resistance induced in cucumber against pythium root rot. **Compost Science & Utilization, 9(3): 221-229.**

Mahmoud, S.M. and A.A. Gomah (2006). Induced systemic resistance against *Agrobacterium tumefaciens* by certain biotic and abiotic agents. **Egypt. J. Agric. Res., 84(3): 655-663.**

Mei, R.; B. Chen; S. Lu and Y. Chen (1990). Field application of yield increasing bacteria (YIB). **Chin. J. Microbiol. Ecol. 2: 45-49.**

Menzies, J.; P. Bowen and D. Ehret (1992). Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber. **J. Amer. Soc. Hort. Sci., 117(6): 902-905.**

Moura, A.B. and R. Romeiro (1997). Inoculation of cucumber seeds and detection of *Pseudomonas syringae* pv. *lachrymans* by biological baits. **Revista Brasileira de Sementes, 19(2): 380-383.**

Raupach, G.S. and J.W. Kloepper (1998). Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. **Phytopathology 88: 1158-1164.**

Raupach, G.S. and J.W. Kloepper (2000). Biocontrol of cucumber disease in the field by plant growth-promoting rhizobacteria with and without methyl bromid fumigation. **Plant Dis. 84: 1973-1075.**

Shekhawat, G.S.; K. Chakrabarti; V. Kishore; V. Sunaine and V. Gadewar (1992). Possibilities of biological management of potato bacterial wilt with strain of *Bacillus* sp., *B. subtilis* and actinomycetes. **Proceeding of an International Conference, Taiwan, pp. 327-330.**

Shen, D.; Y. Yin; C. Qi and J. Song (2001). A new cucumber F_1 hybrid. **Acta Horticulturae Sinica, 28(5): 481.**

Snell, F.D. and C.T. Snell (1953). Colorimeteric Methods of Analysis Including Some Turbidimeteric and Nephelometeric Methods, 111:606. D. Van Nostrad Company, Inc., New York.

Staub, J.E. and L.K. Crubaugh (2001). Cucumber inbred line USDA 6632E. **Cucurbit Genetics Cooperative, 24: 6-7.**

Sticher, L. and J.P. Metraux (2000). Inhibitors of N-linked glycosylation induce systemic acquired resistance in cucumber. **Physiological and Molecular Plant Pathology, 56(6): 245-252.**

Subba Rao, P.V.; J.P. Geigen; J. Einhorn; B. Rio and A. Ravise (1988). Host defence mechanisms against groundnut rust. **Internat. Arahis Newslett., 4: 16-18.**

Ye, X.C.; N.E. Strobel and J. Kuc (1995). Induced systemic resistance [ISR]. **Novel Approaches to Integrated Pest Management, pp. 95-113.** Plenum Press, New York.

Zayed, K.A.M. (2004). Pathological Studies on Potato Bacterial Wilt Disease, p. 44. M.Sc. Thesis, Fac. Agric., Ain Shams Univ., Cairo.