

UTILIZATION OF COMBINING BIOTIC AND ABIOTIC TREATMENTS TO CONTROL BACTERIAL ANGULAR LEAF SPOT DISEASE OF CUCUMBER

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ABSTRACT

Bacterial angular leaf spot disease caused by *Pseudomonas syringae* pv. *lachrymans* is an important foliage disease of cucumber, under protected cultivation system. The present work was planned to control the disease using bio-agents and abiotic agents alone or combining, under greenhouse and commercial plastic house conditions. Data obtained indicated that application of bio- and abiotic agents decreased severity of bacterial angular leaf spot disease of cucumber, compared with the control. Fluorescent Pseudomonads (*Pseudomonas aeruginosa*, *P. fluorescens* and *P. putida* isolates) were more effectiveness than isolate of *Bacillus subtilis* to reduce the disease severity, when they were applied as soil drench treatment. Application of abiotic agents as foliar treatment was more effective than as seed treatment to decrease the disease severity. Isolates of *P. fluorescens* or *P. putida* as bio-agents and salicylic acid or ethephone as abiotic agents were the most effective against the disease. Disease severity was significantly reduced by increasing rates of abiotic agents. However, interaction between bio-agents (*P. fluorescens* or *P. putida* isolates) as soil treatment and abiotic agents (salicylic acid or ethephone) as foliar treatment greatly decreased severity of bacterial angular leaf spot disease of cucumber, under greenhouse and commercial plastic house condition, compared with the control. Combination between *P. fluorescens* isolate as soil treatment and salicylic acid as foliar treatment were the most effective against the disease.

Keywords: Bacterial angular leaf spot, Cucumber, *Pseudomonads syringae* pv. *lachrymans*, Bio-agents, Abiotic agents

INTRODUCTION

Bacterial angular leaf spot caused by *P. syringae* pv. *lachrymans* (Smith and Brain) Yang *et al* is a worldwide and destructive to cucumber. The bacterium

attacks cucumber (*Cucumis sativus*) grown under field conditions in humid and semi-humid climates (Dixon, 1981). Biological control of plant diseases by microbial agents has been extensively investigated during the past decades

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(Weller, 1988 and Schroth *et al* 1984). Palleroni (1984) observed that fluorescent Pseudomonads usually coexists with other microorganisms in diverse environments including soil, water and bio-materials and reported as potential bio-control agents of phytopathogens. Plant growth-promoting rhizobacteria (PGPR) strains were tested as seed treatments or foliage sprays for biological control against multiple cucumber pathogens under greenhouse and field conditions (Raupach and Kloepper, 1998) and were applied as seed treatment alone or as seed treatment plus as soil drench at transplanting against several diseases of cucumber (Liao, 1989). Mei *et al* (1990) applied application of some PGPR strains to foliage diseases. It was found that application of PGPR strains induces systemic resistance against cucumber diseases (Liao, 1989; Liu *et al* 1995 and Raupach and Kloepper, 2000).

Induction of systemic resistance became 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). Abiotic inducer (salicylic acid and acetyl salicylic acid) showed positive results in controlling fungal diseases (Ye *et al* 1989), Viral diseases (Van Loon and Antoniw, 1982) and bacterial diseases (Rasmussen *et al* 1991). Induced systemic resistance (ISR) can be induced in susceptible and resistant cultivars using pathogen, non-pathogen and certain chemical compounds (Madamanchi and Kuc, 1991 and Kuc and Strobel, 1992). Chemical inducers that have been reported include Oxalate, Di-basic phosphate, β -ionone, 2, 6 dichloroisonicotinic acid (INA) and its methylester derivatives (Kuc, 1995).

Chemical inducers of disease resistance hold substantial promise as agents of plant disease control (Ye *et al* 1995).

The present work aimed to study the ability of some biotic and abiotic agents alone or in combination to suppression of bacterial angular leaf spot disease of cucumber, under greenhouse and commercial plastic house conditions.

MATERIAL AND METHODS

The pathogen

Virulent isolate of *P. syringae* pv. *lachrymans* was isolated and identified from infected cucumber plants (Abd El-Ghafar, 2000). The bacterium was grown on yeast extract peptone agar (YDC) medium for 48h at 28°C. Bacterial growth was suspended in sterile distilled water (SDW) and adjusted according its optical density at A620nm = 0.1 (10^7 colony forming units (cfu)/ml) according to El-Sadek *et al* (1992) and Abd El-Ghafar (2000).

Seeds and sowing

Fungicides- free seeds of cucumber cultivar Beet Alfa MR were obtained from Department of vegetable, Horticulture Research Institute, Agriculture Research center, Giza, Egypt and sown in seedling trays containing pasteurized peat moss and vermiculite (2:1 v/v). Trays were kept under greenhouse condition and irrigated regularly. Cucumber seedlings (3- week- old) were transplanted into clay pots (20 cm diameter) containing sandy- clay soil and each pot contained three seedlings.

Bioagents

Isolates of *B. subtilis* (Bs3), *P. aeruginosa* (Pa1), *P. fluorescens* (Pf5) and *P. putida* (Pp12) were obtained from bacterial disease laboratory, Department of plant pathology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. These isolates were previously evaluated as bio-control agents against phytopathogenic bacteria (Abd El-Ghafar and Abd El-Sayed 1997, Abd El-Ghafar, 2000, Abd El-Ghafar and Mosa, 2001 and Abd El-Sayed *et al* 2003). These isolates were grown on yeast extract peptone dextrose agar medium for 48hr at 28°C. The bacterial cells suspended in distilled water and centrifuged at 3000 rpm for 30 min. The precipitant was re-suspended in distilled water to reach concentration of 10⁸ cfu / ml as determined from a standard curve based on absorbance at A620 nm.

Bio-agents were used as soil drench or foliar treatments. The seedlings were treated (soil drench as soil treatment or sprayed as foliar treatment) with bio-agents (10 ml/ seedling) after 2- week from sowing trays and after 3- week from transplanting to clay pots. Untreated seedlings with bio-agents were also planted in clay pots. Treated and untreated seedlings were inoculated with bacterial suspension (10 ml/ seedlings) of the pathogen after 4-week from transplanting to clay pots. Five pots were used as replicates per treatment. All inoculated seedlings with the pathogen were placed in humidity chamber for 48-h before and after inoculation, (Abd El-Ghafar and Mosa, 2001).

Abiotic agents

Bion (benzol 1,2,3 siodiazol,7 carbo synobic acid-s-methy ester) or Ethephone (2-chloro ethyl phosphonic acid) or Gasmonic acid (alpha-linolenic acid) or Salicylic acid (2- hydroxybenzoic acid, C7H6O3) were applied as abiotic agents at 0.25, 0.50, 0.75 and 0.1 % or 2.5 ; 5; 7.5 and 10 ppm or 0.25,0.5,0.75 an 0.1% or 2.5,5,7.5 and 10 mM, respectively. These agents were used as seed soaking or foliar treatment. In case of seed treatment, seed of cucumber were soaked in abiotic agents solutions for 6 h before planting in seedling trays. In cause of foliar treatment, cucumber seedlings were sprayed with abiotic agents (10 ml/seedling) after 2-week from sowing into seedling trays and after 3-week from transplanting in clay pots. Untreated seedlings with abiotic agents were also planted in clay pots. Treated and untreated seedlings were inoculated with bacterial suspension (10ml/seedling) of the pathogen after 4-weeks from transplanting in clay pots. Five pots were used as replicates per treatment. All inoculated seedling with the pathogen were placed in humidity chamber for 48-h before and after inoculation (Abd El-Sayed *et al* 1996).

Integration treatments

Greenhouse experiments

Seedlings of cucumber were treated with bio-agents (*P. fluorescens* (Pf5) and *P. putida* (PP12) as soil drench treatment after 2-week from sowing into seedling trays, as mentioned previously. Treated

seedlings with bio-agents were retreated with abiotic agents (ethephone, at 7.5 ppm and salicylic acid, at 7.5 mM) as foliar treatment after 3-week from transplanting in clay pots, as mentioned before. Untreated seedlings were also planted in clay pots. Treated and untreated seedlings were inoculated with the pathogen as mentioned previously. All inoculated seedlings were placed in humidity chamber for 48-h before and after inoculation (Abd El-Ghafar and Mosa, 2001). Five pots were used as replicates per treatment.

Commercial plastic house experiments

These experiments were carried out under commercial plastic house conditions, at Bhateem Research station, Agriculture Research center, during 2003-2005 growing seasons. The experiment design was a randomized complete block. Five rows of plants were applied per treatment and each row contained 50 plants. Generally five rows were left without treatment as control. All plants were left for naturally infection. During the growing season, conventional cultural practices were followed as recommended for irrigation and fertilizer treatments. Cucumber seedlings were treated as soil treatment with bio-agents in seedlings trays as mentioned previously through greenhouse experiment. Treated seedlings with bio-agents were planted in commercial plastic house and retreated as foliar treatment with abiotic agents as mentioned before through greenhouse experiment (Abd El-Ghafar and Mosa, 2001).

Disease assessment

In greenhouse experiments, disease incidence was recorded after 7 days from inoculation with the pathogen on 15 plants per treatment. In commercial plastic house experiments, disease incidence was recorded on 50 plants per treatment (10 plants/ row) after 4-week from planting. Disease severity was estimated as mean number of spots per leaf, where four leaves were randomly selected from each plant, and as disease index (%) according to the disease rating scale from 0 to 5, in which 0 = no lesion, 1= 1-20 % of leaf area with lesion, 2= 21-40 %, 3= 41-60 %, 4= 61- 80 % and 5= 81 -100 % (Liu *et al*1995 and Raupach and Kloepfer, 1998). Disease index (DI) was calculated by the following formula:

$$DI = \frac{\sum R \cdot T}{5XN} \times 100$$

Where, T= Total number of plants with each category.

R = Disease severity scale R (R = 0, 1, 2, 3, 4 and 5).

N = Total number of plants tested.

Also, percentage of disease reduction (PDR) was calculated as the following:

$$PDR = \frac{Ds_{ck} - Ds_{tr} \times 100}{Ds_{ck}} \times 100$$

Where, Ds_{ck} = Disease severity in check treatment

Ds_{tr} = Disease severity in treated treatment

Data were statistically analyzed using the "F" test and the value of LSD ($p=0.05$) was calculated (Snedecor and Cochran, 1967).

RESULTS

Effect of bio- and abiotic agents on disease severity

Results in Table (1) showed that application of *B. subtilis*, *P. aeruginosa*, *P. fluorescens* and *P. putida* isolates as biotic agents led to considerable decrease in severity of bacterial angular leaf spot disease of cucumber compared with the control. Fluorescent pseudomonads (*P. aeruginosa*, *P. fluorescens* and *P. putida*) isolates were more effective to reduce the disease than isolate of *B. subtilis*, when they were applied as soil treatment. Meanwhile, application of *B. subtilis* isolate as foliar treatment was more effective than fluorescent pseudomonads isolates. Meantime, isolates of *P. fluorescens* and *P. putida* were the most effective against severity of cucumber bacterial angular leaf spot disease. However, application of Bion, ethephone, gasmonic acid and salicylic acid as abiotic agents reduced severity of bacterial angular leaf spot disease compared with the control. Application of abiotic agents as foliar treatment was more effective to decrease the disease severity than application of these agents as seed treatment. Meanwhile, disease severity was significantly reduced by increasing rates of abiotic agents. Salicylic acid and ethephone were the most effective against severity of bacterial angular leaf spot disease of cucumber (Fig.1 and 2)

Effect of combination between bio- and abiotic agents on disease severity

Interaction between bioagents (*P. fluorescens* or *P. putida* isolates) as soil treatment and abiotic agent (Salicylic acid or ethephone) as foliar treatment greatly decrease severity of bacterial angular leaf spot disease of cucumber, under greenhouse and commercial plastic house conditions, compared with the control. Disease severity was more reduced under greenhouse condition than under commercial plastic house condition, where percentage of disease reduction ranged from 36.2 to 44.7 % and ranged from 23.9 to 32.5 %, respectively. Combination between *P. fluorescens* as soil treatment and abiotic agents as foliar treatment were more effective against the disease severity than combination between *P. putida* as soil treatment and abiotic agents as foliar treatment. Meanwhile, combination between *P. fluorescens* as bioagent (soil treatment) and salicylic acid as abiotic agent (foliar treatment) were the most effective against severity of bacterial angular leaf spot disease of cucumber under greenhouse and commercial plastic house conditions (Table 2 and 3).

DISCUSSION

Bacterial angular leaf spot disease of cucumber caused by *P. syringae* pv. *lachrymans* has become an important disease under commercial plastic house conditions in Egypt (El-Sadek *et al* 1992 and Abd El-Ghafar, 2000). Application of biotic and abiotic treatments alone decreased the disease severity compared

Table 1. Influence of bio-agents as soil and foliar treatments on severity of bacterial angular leaf spot disease of cucumber, under greenhouse conditions

Bio-agent	Treatment	Mean No. of Spot/Leaf	Disease index %	Disease reduction %
<i>Bacillus subtilis</i> (Bs3)	Soil	7.5	39.9	17.9
	Foliar	8.3	43	12.6
<i>Pseudomonas aeruginosa</i> (Pa1)	Soil	7	35.3	27.4
	Foliar	8.8	45.6	7.3
<i>Pseudomonas fluorescens</i> (Pf5)	Soil	6.2	30.445	37.5
	Foliar	8.6	45	8.5
<i>Pseudomonas putida</i> (Pp12)	Soil	6.5	32.6	32.9
	Foliar	8.7	45.4	7.7
Check	Soil	8.9	48.6	0
	Foliar	9.3	49.2	0

LSD at 5 %

Bio-agent	0.3	2.6
Treatment	0.7	3.2
Interaction	1.3	3.5

with the control. Biotic agents (fluorescent pseudomonads, i.e. *P. aeruginosa*, *P. fluorescens* and *P. putida* isolates) as soil drench treatment and abiotic agents (Salicylic acid and ethephone) as foliar treatment were the most effectiveness against severity of bacterial angular leaf spot of cucumber under greenhouse conditions. Results of the present study are in agreement with Liu *et al* (1995); Wei *et al* (1996); Klopper *et al* (1996); Lucase (1998); Abd El-Ghafar (2000) and Abd El-Ghafar and Mosa (2001) that application of PGPR strains to root may induce systemic resistance to foliar disease. Severity of bacterial angular leaf spot disease of cucumber was significantly reduced, when isolates of bio agents were

applied as soil treatment (Abd El-Ghafar, 2000). Induced systemic resistance (ISR) is based on plant defense mechanism that are activates by inducing agent as PGPR (Klopper *et al* 1992) or ISR once expressed activity multiple potential defense mechanisms that include increasing in activity of several defense enzymes and pathogenesis-related (PR) proteins (Lawton and Lamb, 1987 and Strobel *et al* 1996) and phytoalexins (Kuc and Rush, 1985, Ongena *et al* 2000 and Jeun *et al* 2004). Plant growth-promoting rhizobacteria (PGPR) are root colonizing beneficial bacteria and the beneficial effects include biological control and growth promotion (Weller, 1988).

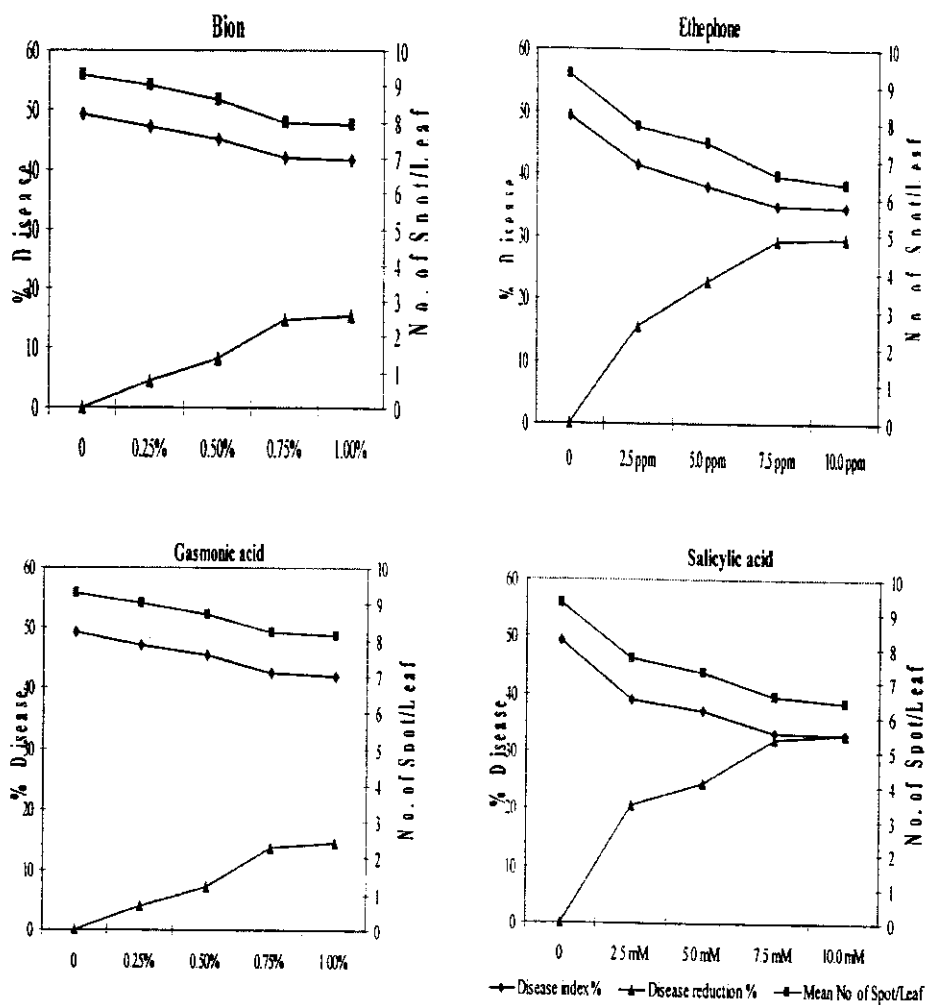


Fig. 1. Effect of abiotic agents, at different rates as foliar treatment on severity of bacterial angular leaf spot disease of cucumber, under greenhouse conditions

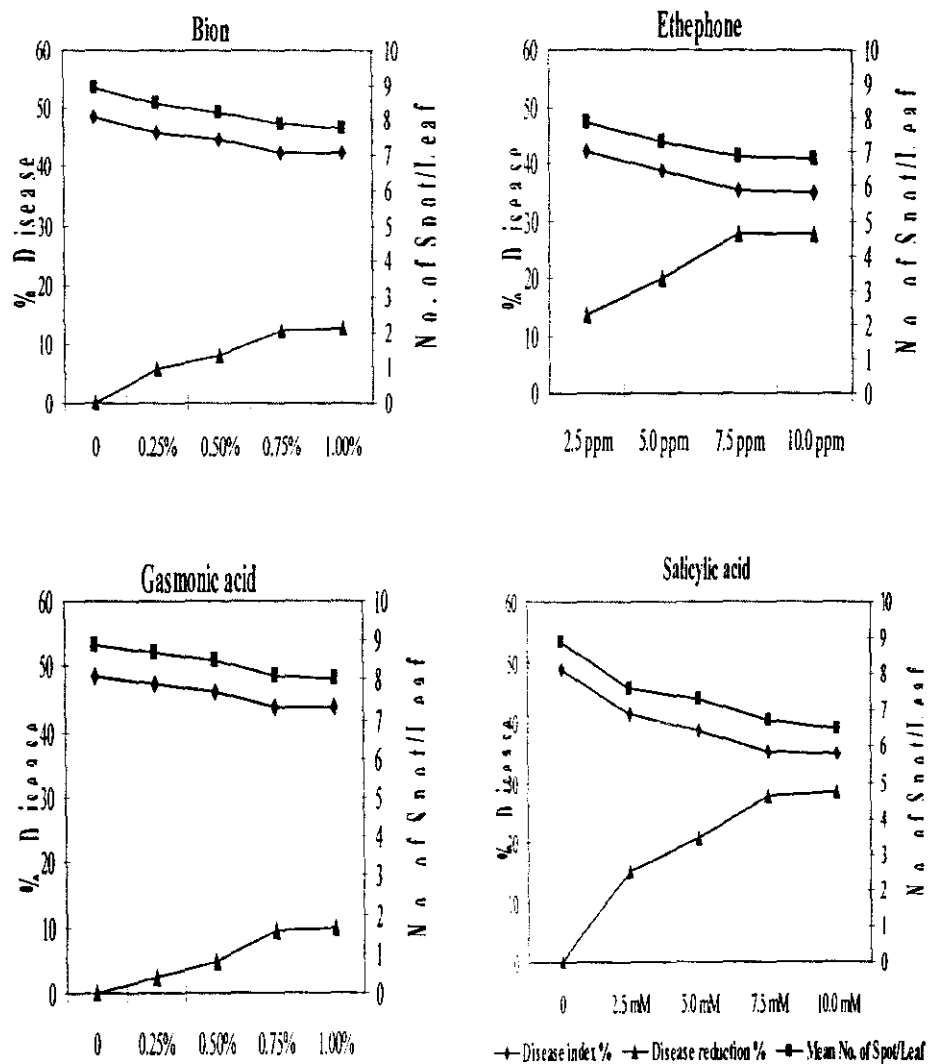


Fig 2. Effect of abiotic agents, at different rates as seed treatment on severity of bacterial angular leaf spot disease of cucumber, under greenhouse conditions

Table 2. Influence of combination between bio-agents as soil treatment and abiotic agents as foliar treatment on severity of bacterial angular leaf spot disease of cucumber, under greenhouse conditions

Soil treatment	Foliar treatment	Mean No. of Spot/Leaf	Disease index %	Disease reduction %
<i>Pseudomonas fluorescens</i> (Pf5)	Ethephone (7.5 ppm)	5.9	29.7	39.6
	Salicylic acid (7.5 mM)	5.6	27.2	44.7
	None	6.2	30.4	38.2
<i>Pseudomonas putida</i> (Pp12)	Ethephone (7.5 ppm)	6.1	31.4	36.2
	Salicylic acid (7.5 mM)	5.9	30.6	37.8
	None	6.5	32.6	33.7
None	Ethephone (7.5 ppm)	6.6	34.8	29.3
	Salicylic acid (7.5 mM)	6.6	33.3	32.3
	None	9.3	49.2	0.0

LSD at 5%

Soil	0.3	1.0
Foliar	0.3	1.3
Interaction	0.6	1.8

Table 3. Influence of combination between bio-agents as soil treatment and abiotic agents as foliar treatment on severity of bacterial angular leaf spot disease of cucumber, under commercial plastic house conditions

Soil treatment	Foliar treatment	Mean No. of Spot/Leaf	Disease index %	Disease reduction %
<i>Pseudomonas fluorescens</i> (Pf5)	Ethephone (7.5 ppm)	4.3	15.0	28.2
	Salicylic acid (7.5mM)	4.0	14.1	32.5
	None	4.8	16.7	20.1
<i>Pseudomonas putida</i> (Pp12)	Ethephone (7.5 ppm)	4.6	15.9	23.9
	Salicylic acid (7.5 mM)	4.2	14.8	29.2
	None	5.0	17.6	15.8
None	Ethephone (7.5 ppm)	4.8	16.9	19.1
	Salicylic acid (7.5 mM)	4.5	16.0	23.4
	None	5.8	20.9	0.0

LSD at 5%

Soil	0.2	0.5
Foliar	0.1	0.3
Interaction	0.5	0.9

The effect of salicylic acid (SA) or its derivatives on inducing resistance in plants against pathogens was reported by **Malamy and Klessing (1992)** who stated that the effect of the SA was not caused by direct action on the growth of pathogens, but the effect of SA application was rather a consequence of induction of plant defense response. Many biochemical and soil changes occur during ISR i.e. pathogenesis-related (PR) proteins. Acidic PR-proteins including acidic β 1, 3-glucanase and chitinase are secreted intercellular space, where they would be encountered and act against fungal and/or bacterial pathogens, at an early stage of infection process. Basic β 1,3-glucanase and chitinase accumulate in the vacuole, may interact with pathogens, at a later stage of infection, during host cell deterioration (**Ye et al 1995** and **Kuc, 1995**). **Meuwly et al (1995)** showed that SA is an essential component in the signal transduction pathway leading to systemic acquired resistance (SAR) is synthesized from phenylalanine (phe) and benzoic acid in cucumber plants inoculated with *P. syringae* pv. *lachrymans*. **Abd El-Sayed et al (1996)** and **Zayed et al (2004)** mentioned that application of abiotic agents i.e. salicylic acid or aspirin or Bion have been used successfully as resistance inducer for controlling bacterial wilt of solanaceous.

The results from greenhouse and commercial plastic house experiments conducted over 2- year period clearly revealed that combining bioagents (*P. fluorescens* or *P. putida* isolates) as soil treatment and abiotic agents (salicylic acid or ethephone) as foliar treatment was highly effective for controlling bacterial angular leaf spot disease of cucumber than either treatments alone. Meanwhile,

combination between *P. fluorescens* as soil treatment and salicylic acid as foliar treatment was most effectiveness against the disease severity. Thus, the results indicated that both *fluorescens* pseudomonads isolates as bioagents and salicylic acid or ethephone as abiotic agents hold promise for practical disease control. These may be possible to apply a combination of both to enhance their activity and to reduce number of foliar sprays with abiotic agents or bactericides. These results will be of economic importance and applicable in an integrated disease management strategies for cucumber crop.

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الاستفادة من تداخل المعاملات الحيوية وغير الحيوية لمكافحة مرض التبقع البكتيري الزاوي في الخيار

[٢٥]

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Pseudomonas fluorescens and *P. putida* فاعلية أكثر في مكافحة المرض. ولقد وجد أن المعاملات غير الحيوية كانت أكثر فاعلية في مكافحة المرض عند استخدامها كمعاملة رش علي المجموع الخضري من استخدامها كمعاملة بذرة. تزداد فاعلية المعاملات غير الحيوية في مكافحة المرض كلما زاد معدل استخدامها. وأظهر كلا من حمض الساليسليك والاثيونون فاعلية أكبر في مكافحة المرض من العوامل الأخرى. وكذلك وجد أن معاملات التداخل بين العوامل الحيوية (*Pseudomonas fluorescens* or *P. putida*) كمعاملة تربة والعوامل غير الحيوية (حمض الساليسليك أو الأثيونون) كمعاملة رش علي المجموع الخضري أظهرت نتائج مشجعة بدرجة كبيرة في مكافحة المرض مقارنة بمعاملة المقارنة. ووجد أن التداخل بين بكتريا *Pseudomonas fluorescens* كعامل حيوي ومعاملة تربة وحمض الساليسليك كعامل غير حيوي ومعاملة رش علي المجموع الخضري كانت الأكثر فاعلية في الحد من خطورة هذا المرض. وتحت

زاد انتشار مرض التبقع البكتيري الزاوي في الخيار تحت نظم الزراعة المحمية مما سبب مشاكل كثيرة للمزارعين في مناطق زراعة الخيار تحت الظروف المصرية. وقد أجريت هذه الدراسة بغرض تقييم فاعلية التداخل بين المعاملات الحيوية وغير الحيوية في مكافحة هذا المرض تحت ظروف العدوي الصناعية والصوب البلاستيكية التجارية. وأظهرت النتائج المتحصل عليها أنه تحت ظروف العدوي الصناعية المعاملات الحيوية وغير الحيوية أعطت نتائج مشجعة في مكافحة هذا المرض بالمقارنة مع معاملة المقارنة. ووجد أن البكتريا الفلورسنتية (*Pseudomonas aeruginosa*, *P. fluorescens* and *P. putida*) كانت أكثر فاعلية من بكتريا *Bacillus subtilis* في مكافحة المرض عندما استخدمت كمعاملة ري للتربة في حين أن جميع العوامل الحيوية المختبرة كانت أكثر فاعلية في مكافحة المرض عندما استخدمت كمعاملة تربة من استخدامها كمعاملة رش علي المجموع الخضري. وأعطت عزلات

ظروف الصوب البلاستيكية التجارية أمكن الحصول علي نتائج مشابهة لنتائج التداخل بين المعاملات تحت ظروف العدوي الصناعية ولذلك من الممكن الاستفادة من تلك النتائج في الحد من خطورة هذا المرض تحت ظروف الاصابات الطبيعية في الصوب البلاستيكية الانتاجية .

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