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INDUCED SYSTEMIC RESISTANCE TO CONTROL BACTERIAL SPOT OF TOMATO

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ABSTRACT

Bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is an important disease of tomato. Application of *Bacillus subtilis, Pseudomonas aeruginosa, P. fluorescens, P. putida* and *Streptomyces griseoviridis* isolates (biotic agent) and bion, ethephon and salicylic acid (abiotic agents) were applied as inducers agents alone or in combination against tomato bacterial spot disease, under artificial inoculation conditions. All aforementioned agents decreased the disease severity compared with the control. Application of bioagents as soil or seedling treatments were more effective than their application as foliar treatment. Meanwhile application of biotic agents as foliar treatment were less effective than their application as soil or seedling treatment. Fluorescent pseudomonades (*P. aeruginosa, P. fluorescens* and *P. putida*) isolates as bioagents and salicylic acid or ethephon as abiotic agents were the most effective in the disease reduction. Combination between fluorescent pseudomonades isolates as soil treatment and ethephon or salicylic acid as foliar treatment greatly reduced the disease severity and significantly increased in the disease reduction.

Key words: Tomato, Bacterial spot, *Xanthomonas campestris* pv. vesicatoria, Inducer agents

INTRODUCTION

Bacterial spot of tomato (*Lycopersicon esculentum* Mill.) and Pepper (*Capsicum annum* L.) caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is widespread. The disease causes significant losses when warm temperatures and rainy weather occur **(Jones *et al* 1991).** The disease observed worldwide in tomato and pepper production areas **(Bouzar *et al* 1994).** Different strains of the bacteria cause the disease on both pepper and tomato, pepper only, or tomato only. Induces systemic resistance (ISR) in plants was experimented by **Ross (1966).** ISR can be induced susceptible and resistant cultivars using pathogens, nonpathogens and certain chemical compounds **(Madamanchi and Kuc, 1991 and Kuc and Strobel, 1992).** The use of a chemical inducer eliminates po-

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tential problems associated with the introduction of some plant pathogens as inducer. Chemical inducers of disease resistance hold substantial promise as agents of plant disease control (**Ye *et al* 1995 and Weingart *et al* 2001**).

Zayed *et al* (2004) reported that the potentiality of these bacteria for biocontrol to produce a broad spectrum of secondary metabolites that may be toxic to other micro-organisms. In recent, there has been substantial interest issue of ethylene compound (abiotic-agents) or biocontrol agents to control of bacterial leaf spot plant pathogens was recommended (**Wilson *et al* 1998 and 2003**).

Induced resistance in plant and its significance to fundamental science and as a technology for plant disease control were developed. Induced resistance is specific, being an effective approach against a wide range of bacterial plant pathogens (**Ye *et al* 1995**). Abiotic inducer salicylic acid (SA) showed positive results in controlling bacterial plant disease (**Malamy and Klessing, 1992**). Salicylic acid (SA) is a natural constituent of plant. The involvement of salicylic acid in systemic acquired resistance (SAR) has been clearly established. The characterization of the biochemical changes associated with SAR has demonstrated a close correlation between development of resistance and induction of pathogen related proteins (PR), which play an important role in the natural defense response of plants (**Palva *et al* 1994**), salicylic acid as a putative endogenous signal for the systemic acquired resistance (SAR) response was demonstrated by (**Ye *et al* 1995**). **Desirée, (1999)** evaluated the efficacy of acibenzolar-S-methyl (Bion 50% wg BTG), as an inducer of systemic acquired resistance against bacterial and fungal disease of tobacco. Ethephon acts a signal for defense response during plant microbe interaction. Support of the involvement of ethephon in plant resistance comes its ability to induce accumulation of pathogenesis-related proteins (PR) (**Weingart *et al* 2001**). The present work aimed to study the ability of some biotic and abiotic agents alone or in combination, as inducer to investigate their potentiality in suppression of bacterial spot of tomato under artificial inoculation conditions.

MATERIAL AND METHODS

1. Seeds and sowing

Tomato seeds cultivar peto 86 were obtained from Horticulture Department, Faculty of Agriculture, Ain Shams University, Egypt. Seeds were sown in seedling trays containing peat-moss and vemiculite (1:1 v/v) for 4 weeks, irrigated regularly and kept under greenhouse conditions. All seedlings were transferred to pots containing sterilized sandy-clay soil (1:1 v/v). Each pot contained 3 seedling and 5 pots were used as replicates for each treatment. Five pots per treatment were treated with water and applied as control.

2. The pathogen

Virulent isolate of *X. campestris* pv. *vesicatoria*, previously isolated from infected leaves of tomato was used through this study. This isolate was kindly obtained from Plant Pathology Department, Faculty of Agricultural, Ain Shams University. The bacterium was grown on yeast dextrose calcium carbonate agar (YDC) medium for 48 hr at 28°C. Bacterial growth was suspended in sterile buffer solution (0.85% NaCl) and adjusted according to its optical density at A_{620} nm = 0.03 to 5 X 10^6 colony forming units (cfu)/ml according to **Wang *et al* (1994)**. All tomato seedlings were inoculated (**Abd El-Ghafar and Abd El-Wahab, 2001**) by the pathogen after 30 days from planting.

3. Effect of bioagents on disease severity under artificial inoculation conditions

Isolates of *B. subtilis* (BS_3), *P. aeruginosa* (Pa1), *P. fluorescens* (Pf5), *P. putida* (PP12) and *S. griseoviridis* (Sg1) were previously isolated and evaluated as biocontrol 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**). All bioagents were grown on tryptic soy agar (TSA) medium for 48 hr at 28°C, except *S. griseoviridis* isolate grown on starch nitrates agar (SNA) medium for seven days. These bioagents were suspended in sterile distilled water (SDW) and centrifuged at 3000 g for 30 min. The precipitant was resuspended in SDW to reach the concentration of 10^8 cfu/ml as determined from a standard curve based on absorbance at 620 nm.

Bioagents were applied as soil, seedlings and foliar treatments. In case of soil treatment, each pot (25 cm diameter) was treated with 450 ml of bioagents suspension after 10 days from planting in pots (soil drench). In case of seedlings treatment, tomato root seedlings were soaked in bioagnets suspensions (450 ml treatment) for 1 hr. before directly planting. Meanwhile, in case of foliar treatment, tomato seedlings were sprayed with bioagents suspensions after 28 days from planting in pots. Inoculation of the pathogen were made after 30 days from transplanting.

4. Effect of abiotic agents on disease severity under artificial inoculation conditions

Bion (acibenzolar,s-methyl), ethephon (2-chloroethylphosphoric acid) and salicylic acid (2-hydroxybenzoic acid, $C_7H_6O_3$) were applied at 0.5, 1.0 and 1.5 ppm. These agents were applied as soil, seedlings and foliar treatments (**Zayed *et al* 2004**) as previously mentioned with bioagents treatments.

5. Effect of combination between biotic and abiotic agents

Tomato seedlings treated with fluorescent pseudomonades (*P. aeruginosa, P. fluorescens* and *P. putida* isolates) as soil drench treatment as previously mentioned. Also, these seedlings treated with ethephon or salicylic acid at 1.5 ppm as the foliar treatment as previously mentioned. (the best concentration in treatment by abiotic agents only).

6. Disease assessment

Disease incidence was recorded after 7 days from inoculation with the pathogen. The disease was determined according to **McCarter (1992)** as follows: (1) average total lesions number per leaf, where 4 leaves were randomly selected from each plant, (2) disease severity (%) according to the disease ratting scale from 0 to 5 (**McCarter, 1992**) and (3) percentage of disease reduction (PDC) was calculated from disease severity as follows :

$$PDC = \frac{(DI_{ck} - DI_{tr})}{DI_{ck}} X\,100$$

Where, DI_{ck} = Disease severity (%) in check treatment
DI_{tr} = Disease severity (%) in treated treatment

Data were statistically analyzed using the "F" test and the value of LSD ($P \leq 0.05$) was calculated according to **Cochran and Cox (1957).**

RESULTS

1. Effect of biotic agents

Application of *B. subtilis* (BS1), *P. aeruginosa* (PA1), *P. fluorescens* (Pf5), *P. putida* (PP12) and *Streptomyces griseoviridis* (Sg1) isolates as biotic agents decreased severity of bacterial spot of tomato compared with the control (Table, 1). All previous biotic agents as soil or seedling treatments were more effective than foliar treatment in disease reduction. Meantime, *P. aeruginosa, P. fluorescens* and *P. putida* isolates were the most effective in reducing the disease severity, where percentage of disease reduction was 15.3-24.0, 15.7-22 and 12.5-19.1%, respectively. Application of *S. griseoviridis* isolate was the least effective, where percentage of disease reduction was 2.0-8.9%.

2. Effect of abiotic agents

Results in Table (2) show that application of Bion, ethephon and salicylic acid as abiotic agents reduced severity of bacterial spot of tomato, compared with the control. Application of Bion as soil or seedling treatment was more effective than foliar treatment in the disease reduction. Meantime, application of ethephon or salicylic acid as foliar treatment were more effective than soil or seedling treatments. Increasing doses of previously compounds led to increase their effectiveness on the disease. Meanwhile, application of salicylic acid or ethephon were the most effective at 1.5 ppm as foliar treatment, where percentage of disease reduction was 41.0 - 41.8%, respectively. Application of Bion as soil or seedling treatments was the most effective, where percentage of disease reduction was 33.7 or 35.7%, respectively.

3. Combination between biotic and abiotic agents

Interaction between *P. aeruginosa, P. fluorescens* and *P. putida* isolates (biotic agents) as soil treatment and ethephon or salicylic acid (abiotic agents) as foliar treatments greatly decreased severity of bacterial spot of tomato, compared with the control (Table, 3). Meantime, the combination between *P. aeruginosa* and salicylic acid gave the highest value of disease reduction (63.7%) followed by combination between *P. fluorescens* and salicylic acid (60.9%) and the combination between *P. putida* and salicylic acid (56.5%).

DISCUSSION

Bacterial spot of tomato may spread in several locations in Egypt due to cultivation of different hybrids and cultivars of tomato seeds from many foreign countries as USA. Where, this is known to be

Table 1. Effect of different biotic agents applied as soil or seedling or foliar treatment on severity of bacterial spot of tomato, under artificial inoculation conditions.

Biotic agent	No. of infection lesion/leaf			Disease severity (%)			Disease reduction (%)		
	A	B	C	A	B	C	A	B	C
Untreated control	6.0	6.1	6.1	24.6	24.8	24.8	0.0	0.0	0.0
Bacillus subtilis (Bs1)	5.3	5.5	6.0	21.6	21.9	23.0	12.2	11.7	7.3
Psdueomonas aeruginosa (PA1)	4.1	4.3	5.3.	18.6	18.9	21.0	24.0	23.8	15.3
P. fluorescens (Pfs)	4.3	4.5	5.6	19.2	19.6	20.9	22.0	21.0	15.7
P. putida (pp12)	4.4	4.7	5.7	19.9	20.2	21.7	19.1	18.5	12.5
Streptomyces griseoviridis (Sg1)	5.8	5.9	6.1	22.4	22.9	24.3	8.9	7.7	2.0
L.S.D. at 5%	0.9	0.6	0.4	1.2	1.4	0.9			

A = Soil treatment B = Seedling treatment C = Foliar treatment

Table 2. Effect of different abiotic agents applied as soil or seedling or foliar treatment on severity of bacterial spot of tomato, under artificial inoculation conditions

Abiotic agent	Dose (ppm)	No. of infection lesion/leaf			Disease severity (%)			Disease reduction (%)		
		A	B	C	A	B	C	A	B	C
Bion	0.5	4.2	4.3	4.9	20.8	21.0	21.4	19.4	17.6	16.4
	1.0	3.4	3.7	3.8	17.5	17.9	18.3	32.2	29.8	28.5
	1.5	3.0	3.1	3.3	16.6	16.9	17.3	35.7	33.7	32.4
Ethephon	0.5	4.4	4.2	4.0	19.2	19.2	18.9	25.6	24.7	26.2
	1.0	3.8	3.5	3.3	17.2	16.2	16.0	33.3	36.5	37.5
	1.5	3.2	3.0	2.8	16.5	15.4	15.1	36.0	39.6	41.0
Salicylic acid	0.5	4.3	3.7	3.5	18.8	18.3	18.0	27.1	28.2	29.7
	1.0	3.5	2.5	2.4	17.0	15.6	15.4	34.1	38.8	39.8
	1.5	2.9	1.9	1.7	16.4	15.0	14.9	36.9	41.2	41.8
Uncontrolled	0.0	6.1	6.2	6.1	25.8	25.5	25.6	0.0	0.0	0.0
L.S.D. at 5%		0.8	0.7	1.1	1.3	1.2	1.3			

A = Soil treatment B = Seedling treatment C = Foliar treatment

Table 3. Effect of combination between soil treatment with biotic agents and foliar treatments with abiotic agents on severity of bacterial spot of tomato, under artifical inoculation conditions

Soil treatment	Foliar treatment (ppm)	No. of infection lesion/leaf	Disease severity (%)	Disease reduction (%)
P. aeruginosa (Pa1)		1.5	11.2	54.8
P. fluorescens (Pf5)	Ethephon	1.8	11.9	52.0
P. putida (PP12)	(1.5)	2.0	12.2	50.8
Non		3.1	15.7	36.7
P. aeruginosa (Pa1)		1.0	9.0	63.7
P. fluorescens (Pf5)	Salicylic acid	1.2	9.7	60.9
P. putida (PP12)	(1.5)	1.6	10.8	56.5
Non		2.3	15.1	39.1
P. aeruginosa (Pa1)		3.8	18.5	25.4
P. fluorescens (Pf5)	Untreated	4.1	20.2	18.5
P. putida (PP12)		4.2	21.0	15.3
Non		5.9	24.8	0.0
LSD at 5%		0.7	1.1	

seed borne and cause epidemics on tomato. Meanwhile, high relative humidity and warm temperature which favoured the disease were found, when tomato plants grown under plastic sheet **(Abd El-Ghafar and Abd El-Wahab, 2001).** The results reported here indicated that application of bioagents decreased severity of bacterial spot of tomato compared with the control. Application of all bioagents as soil or seedling treatments were more effective than foliar treatment in the disease reduction. Fluorescent pseudomonades (*P. aeruginosa, P. fluorescens* and *P. putida*) isolates were the most effectiveness and *S. griseoviridis* isolate was less effective. Plant growth promoting rhizobacteria (PGPR) isolates could induce systemic resistance in tomato plants against the challenge-inoculated pathogen (*X. campestris* pv. *vesicatoria*), when bioagents isolates were applied as soil drench treatment. These PGPR could root-colonizing beneficial bacteria and the beneficial effects include biological control and growth promotion **(Weller, 1988 and Fravel and Engelkes, 1994 and Wilson *et al* 2003).** Induced systemic resistance (ISR) is based on plant defense mechanism that are activated by inducing agent as PGPR **(Kloepper *et al* 1992),** or ISR, once expressed, activates multiple potential defense mechanisms that include increases in activity of several defense, and pathogensis related protein **(Lawton and**

Lamb, 1987) enzyme phytoalexins **(Kuc and Rush, 1985)**.

Application of Bion, ethephon and salicylic acid as abiotic agents reduced severity of bacterial spot of tomato compared with the control. Application of Bion as soil or seedling treatments was more effectiveness than foliar treatment and at the same time application of ethephon or salicylic acid foliar treatment was more effectiveness than soil or seedling treatments in the disease reduction. **Palva *et al* (1994)** suggested that three possible ways for salicylic acid capability to induced resistance to some pathogenic bacteria. These are

(1) Salicylic acid could directly affect to bacteria as a chelating agent.
(2) Salicylic acid could act as inducer of plant defence compound such as pathogenesis-related proteins.
(3) The inhibition could be a combination of both effects. However, salicylic acid has been established as a putative signal molecule that induces plant defense and systemic acquired resistance (SAR).

Increasing doses of previously abiotic agents (Bion, ethephon) led to increase their effectiveness on the disease. Induced systemic resistance (ISR) would give results that susceptible cultivars become resistant to pathogens and resistant cultivars become more resistant. Many biochemical and soil changes occur during (ISR) i.e. pathogenesis-related protein (PR proteins). Acidic PR proteins including acidic B 1,3-glucanase and chitinase are secreted into intercellular space, where they would be encounted and act against fungal and/or bacterial pathogens at an early stage of the infection process. Bacic β 1,3-gluconase and chitinase accumulate in the vacuole, may interact with pathogens at a later stage of infection during host cell deterioration **(Kuc, 1995 and Weingart *et al* 2001)**.

Combination between fluorescent pseudomonads (*P. aeruginosa, P. fluorescne* and *P. putida*) isolates as soil treatment and abiotic agents (ethephon and salicylic acid) as foliar treatment greatly decreased severity of bacterial spot of tomato, compared with the control. Interaction between *P. aeruginosa* and salicylic acid gave the highest value of disease reduction. Biotic and abiotic agents caused phytoalexin synthesis and accumulation. Specificity with phytoalexin probably resides in the regulation of the rapidity and magnitude of their synthesis and accumulation and this is under genetic control of host and pathogen. As with phytoalexins suggested defense compounds produced by a given plant (lignin, phenolic, cross-linked cell wall poilymerase, hydroxy prolin rich glycoproteins, callose, thionins, chilinase, β 1,3-glucanase and peroxidases-related proteins) can be produced equally well by susceptible and resistance cultivars giving the proper conditions for elicitatiopn **(Ye *et al* 1995, Wilson and Bachman, 1999 and Zayed *et al* 2004)**.

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مكافحة التبقع البكتيرى فى الطماطم مستخدماً عوامل الحث

[٥٠]

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يسبب مرض التبقع البكتيرى فى الطماطم العديد من المشاكل للمزارعين فى مصر فى الآونة الأخيرة وذلك مع زيادة المساحات المنزرعة لهذا المحصول تحت نظم الزراعة المختلفة وتباين الأصناف ومصادرها ونقل هذا المرض عن طريق البذور . أجريت هذه الدراسة بغرض مكافحة هذا المرض باستخدام عوامل حث مختلفة منفردة أو متداخلة معا حيث استخدمت عزلات بكتريا الـ *Bacillus subtilis, P. putida, P. fluorescens, Pseudomonas aeruginosa* ، بالإضافة إلى عزلة *Streptomyces griseoviridis* كعامل حث حيوى واستخدم كل من البيون والأيثفون وحمض السلسليك كعامل حث كيماوى ضد مرض التبقع البكتيرى فى الطماطم تحت ظروف العدوى الصناعية ، ومن أهم النتائج المتحصل عليها أن جميع عوامل الحث المختلفة خفضت شدة المرض بالمقارنة مع معاملة المقارنة وأن استخدام عوامل الحث الحيوى كمعاملة تربة أو شتلات كانت أكثر كفاءة من استخدامها كمعاملة رش على المجموع الخضرى ، فى حين أن استخدام عوامل الحث الكيماوى كانت أكثر كفاءة فى خفض المرض عند استخدامها كمعاملة رش على المجموع الخضرى بالمقارنة معاملة التربة أو الشتلات . أظهرت بكتريا السيدوموناس الفلورسنتية وكل من الاثيفون وحمض السلسليك كفاءة عالية فى خفض شدة المرض بالمقارنة مع العوامل الأخرى. وأوضحت النتائج أيضا أن التداخل بين عوامل الحث الحيوى وعلى الأخص بكتريا السيدوموناس الفلورسنتية كمعاملة تربة وكلا من الاثيفون أو حمض السلسليك (عامل حث كيماوى) كمعاملة مجموع خضرى أدت الى خفض شدة المرض بدرجة كبيرة وزيادة معنوية فى مكافحة المرض تحت ظروف العدوى الصناعية ، ولذلك يمكن الاستفادة من تلك النتائج بأختبارها تحت ظروف الاصابة الطبيعية للإقلال بقدر الإمكان من استخدام المبيدات فى مكافحة هذا المرض والعديد من الأمراض الأخرى التى قد تصيب محصول الطماطم تحت ظروفنا المصرية .

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