J. Turk. Phytopath., Vol. 36 No. 1-3, 21-30, 2007 ISSN 0378 - 8024

Induction of Systemic Resistance in Eggplant Against Fusarium Wilt Disease in Polytunnel Conditions

H. Handan ALTINOK

Erciyes University, Faculty of Agriculture, Department of Plant Protection, 38039, Kayseri, Turkey

ABSTRACT

In this study, a plant activator (Actigard 50 WG; Acibenzolar-S-methyl; ASM) and an isolate of *Fusarium oxysporum* formae speciales nonpathogenic on eggplant (*F. oxysporum* f. sp. *melonis*; FOM) which were found successful against Fusarium wilt disease (*Fusarium oxysporum* f. sp. *melongenae*; *Fomg*) in previous pot experiments, were examined in polytunnel conditions. Pathogen (*Fomg*10) was applied 72 h after ASM and FOM treatments to allow resistance development. Average lengths of ASM and FOM-treated plants were 88.8 and 78.7 cm, respectively, while control plant lengths were 53.56 cm, at 67th day after inoculation (DAI). *Fomg*10 was isolated from 25th cm of ASM and FOM induced plants and from 50th cm of control plants to evaluate disease progress. The disease improved rapidly in control plants by the end of experiment (67 DAI), and disease severity was 81%. Although neither of inducers succeeded to inhibit initial penetration of the pathogen, however, induced a certain amount of resistance by reducing the disease severity in eggplants by 37.65% (ASM) and 50.61% (FOM) at 67th DAI, compared to control.

Key words: *Fusarium oxysporum*, *Solanum melongena*, acibenzolar-S-methyl, induced resistance, polytunnel

INTRODUCTION

Fusarium oxysporum Schlechtend: Fr. is one of the most important soil-inhabiting fungal species and consist of both pathogenic and nonpathogenic strains (Booth, 1971). Individual pathogenic strains are known to be phylogenetically diverse and have a high degree of host specificity within *F. oxysporum*; It is generally known as a species complex which are assigned to intraspecific groups, including formae speciales (f. sp.) and other forms (Booth, 1971; Kistler, 2001).

Fusarium wilt disease on eggplant (*Fusarium oxysporum* Schlecht. f. sp. *melongenae*) is one of the major fungal diseases causing economical yield losses in Turkey as well as several other eggplant producing countries. Under optimal infection conditions, such as temperature, high soil moisture level, soil compaction and poor soil drainage, this soil-borne pathogen can completely destroy the plant. Infected plants exhibit leaf chlorosis and slight vein clearing on outer leaflets, followed by yellowing

and dropping of leaves, then xylem browning of the stem and finally death of the aboveground parts (Altınok, 2005). The infection and the symptoms are observed when the temperature is about 25 °C. Fusarium wilt spreads widely especially on the eggplant cultivations in Eastern Mediterranean Region which causes serious yield losses. As in the other soil based disease agents, there exist no economic, accurate and practical control methods against Fusarium wilt on eggplant. In recent years many methods alternative to chemical control were improved for soil-borne pathogens by researchers. Induced resistance as a mechanism of biological control, which activating the defense responses of the plants before pathogen's attack is an effective way of disease control. As the host specificity of *Fusarium oxysporum* f. sp. is high, it causes limited number of diseases in plants. However, this agent penetrates various plant species and advances to root cortex layer (Booth, 1971; Nelson et al., 1983). There are three different wilt pathogens colonizing eggplant, *F. oxysporum* f. sp. *melongenae*, *Verticillium dahliae* (Bhat and Subbarao, 1999) and *F. oxysporum* f. sp. *radicis-lycopersici* (Rowe, 1980). These pathogens may appear together in same field or even in the same plant. Many studies worldwide have been conducted on identification of sources of resistance against Fusarium wilt disease on eggplant but currently there is no resistant varieties reported. Physiological races of *F. oxysporum* f. sp. *melongenae* are yet to be defined and there is only one record of vegetative compatibility group (VCG) on literature (Katan, 1999). Morphological, genetical, molecular and biological control studies appear to be rather limited. In a previous study, vegetative compatibility group (VCG-0320) of Turkey isolates, and compatibility with European VCGs were determined (Altınok and Can, 2010).

In plant protection, antibiosis, competition and hyperparasitism are primary antagonistic mechanisms on controlling the diseases with biotic factors. Latest research on this subject is indicating biotic agents are also inducing plant defence mechanisms, along with their direct antagonistic efficiency, for suppression of fungal diseases. Several studies indicate that various nonpathogenic bacteria and fungi strains are effective biocontrol agents for phytopathogenic *Fusarium*. In addition to biotic agents, many plant activators such as Messenger®, a naturally occurring bacterial protein and European-wide licensed product Actigard 50WG (acibenzolar-S-methyl; ASM), which includes 3% harpin protein, are also used to control of plant disease (Kessmann et al., 1994; Görlach et al., 1996; Cole, 1999; Tally et al., 1999; Louws et al., 2001).

In our previous studies, plant activator Actigard 50 WG and *F. oxysporum* formae speciales nonpathogenic on eggplant (*Fusarium oxysporum* f. sp. *melonis*; FOM) were successfully tested in pot experiments to control of this disease (Altınok et al., 2002). However, the evidence for successful deployment both of biotic and abiotic factors in climatized conditions is insufficient to conclude that it would control a pathogen in the field (Cavaglieri et al., 2005). The objectives of this study were to evaluate the effect of ASM and FOM of Fusarium wilt on eggplant in the polytunnel conditions.

MATERIALS AND METHODS

Plant material

Eggplant seedlings (*Solanum melongena* L. ***cv***. "***Faselis*** F_1***"***) with six full leaves were used for polytunnel experiment. A short term (67 days) experiment was arranged in a randomized complete block design and performed with four blocks as replicates with eight plants in each parcel. Seedlings were watered with drip irrigation method and fertilized according to recommendations, which is as follows; potassium nitrate (13% N, %46 K_2O; 300-700-1100 g da^{-1} for seedling-growing-harvesting stages), monoammonium phosphate (12% N, 61% P_2O_5; 200-200-200 g da^{-1}), ammonium nitrate (33% N; 200-500-600 g da^{-1}). Soil in polytunnel were solarized, prior to experiment.

Application of ASM and nonpathogenic FOM

*Fomg*10 (*Fusarium oxysporum* f. sp. *melongenae*) was detected as the most virulent isolate by means of disease severity in a former study, therefore selected for the experiments (Altınok and Can 2010).

ASM (Syngenta Crop Protection, Inc., Basel Switzerland; acibenzolar-S-methyl (benzo [1,2,3] thiadiazole-7-carbothioic acid-S-methyl ester), was dissolved in distilled water and then sprayed three times to seedlings with 15 days intervals as recommended by the producer firm. Procedure of ASM application by producer company is given in Table 1 (www.syngentacropprotection.com). Due to increasing plant canopy, incremental dosage regimen is suggested for field applications by the producers of Actigard 50WG, at minimum seven days interval, with a maximum of eight treatments.

A *Fusarium oxysporum* formae speciales nonpathogenic on eggplant (FOM; *Fusarium oxysporum* f. sp. *melonis*) was used as a biotic inducer. The fungal isolates were maintained on slant culture at 4 °C. The fungi were cultured on Potato Dextrose Agar (PDA) (Merck, Germany) and Fusarium minimal medium (FMM); for 7 days in the dark at 25 °C (Nelson et al., 1983). Each 250 ml erlenmeyer flask containing 100 ml of the synthetic liquid medium was inoculated with four mycelial discs with 5 mm diameter from the margin of the 7-day-old FOM cultures (Lecoq et al., 1991), following adjustment of pH to 7.0 with 1 M HCl, the flasks were then incubated on a rotary-shaker at 150 rpm, at 25 °C in the light for 8 days. FOM inoculum (200 ml) (10^6 spore/ml), was poured as irrigation water on seedlings at the six-leaf stage as described above. Positive control plants were inoculated with *Fomg*10. Negative control plants were dipped in sterile distilled water.

Pathogen (*Fomg*10) was inoculated with 10 g of ***wheat inoculum*** of seedling root for three days after ASM and FOM inoculations. To prepare colonized wheat inoculum, 10 mycelial PDA plugs (4 mm in diameter) of *Fomg*10 were used to inoculate 1000 ml bottles containing sterilized wheat seed. Seeds (200 g) were soaked overnight in distilled water; then water was drained and seeds autoclaved at 121 ºC for

30 min on each of two consecutive days. Inoculated wheat seeds were incubated at 24 ºC under illuminated conditions for 2 weeks, with flasks shaken daily to ensure uniform colonization of *Fomg*10. At 21th day, colonized wheat seeds was harvested, air dried in a laminar flow cabinet, and then sealed in plastic pocket and stored at 4 ºC. Pathogen progression was recorded with periodical stem isolations from excess plants with 10 days intervals during the experiment. Plant lengths were measured in centimeters, at last evaluation day of experiment. Study was conducted Kulak village in Tarsus county of Mersin province in 2004 growing season.

Disease Assessment

ASM, FOM and (+) control plants were assessed according to leaf symptoms for 10 days intervals of 7 days after inoculation (DAI) of pathogen, with a *Fusarium* yellow rating of 0 to 4, in which 0 = no lesions, 1 = slight leaf chlorosis and necrosis, 2 = vein clearing on outer leaflets, 3 = yellowing and dropping of leaves, 4 = dead plant. Disease severity percentage was calculated based on the scale values, according to Townsend-Heuberger formula below and compared with controls (Townsend and Heuberger, 1943).

$$P = \Sigma \frac{n \times v}{Z \times N} \times 100$$

In formula, P; percentage of disease severity, n; Number of plants in the disease scale, v; Numerical value of disease score, Z; Highest score number, N; Total number of plants. The data were subjected to an analysis with Levene's homogeneity of variance test then grouped by Duncan's multiple range test ($P \geq 0.05$) contained in the SPSS software (SPSS Inc., Chicago, IL, USA). Percent efficiency of treatments were calculated with Abbott's formula.

Table 1. Producer's suggestions for Actigard 50WG applications on weekly basis, after transplanting of seedlings.

ASM dose (g ha^{-1})	Treatment (ha L^{-1})	Weeks after transplanting
23	290-480	0-2
35	570-670	3-4
53	670-950	5-8

RESULTS AND DISCUSSION

Evaluation of disease progression during experiment was determined by isolation of pathogen from leaf stalks. Pathogen was isolated first from 15th cm at 17 DAI from treated (ASM and FOM) and (+) control plants. Latest pathogen isolations were from 27th cm at 27 DAI from ASM and FOM-treated plants, and from 55 cm at 57th DAI, from control plants. Disease progression was also obviously slower in ASM and FOM-treated plants than (+) control (Table 2). Both abiotic and biotic inducers were failed to

inhibit penetration of pathogen, on the other hand, significantly decreased the infection rate.

Table 2. The effect of ASM and FOM treatments to disease progress.

Treatments	Days					
	17	27	37	47	57	67
	Disease Progress (cm)					
	20	*25*	*35*	*45*	*50*	*55*
ASM	+	+	–	–	–	–
FOM	+	+	–	–	–	–
(+) CONTROL	+	+	+	+	+	–

Results of ASM, FOM and (+) control plants were assessed as leaf symptoms for 10 days intervals by 7 DAI of pathogen. ASM and FOM-treated plants were not shown wilting symptoms at 17th DAI but, disease severity was found 10% in positive control plants at the same DAI (Figure 1). First symptoms of ASM and FOM-treated plants were slightly observed at 27th DAI and disease severity were determined as 18.0 and 12.0%, respectively. Disease progress was significantly decreased in ASM and FOM-treated plants while the disease rapidly progressing in (+) control plants, during experiment (Figure 1). ASM was found not as effective as FOM according to 67th DAI results (Table 2). Reduction in disease severity ranged from 40 to 50% relative to control. Both treatments were found effective and reduced disease severity by 37.65 and 50.61%, respectively (Table 2). Plants inoculated with pathogen (+ control) showed a disease severity of 81%, while healthy plants (- control) displayed no symptoms, as expected.

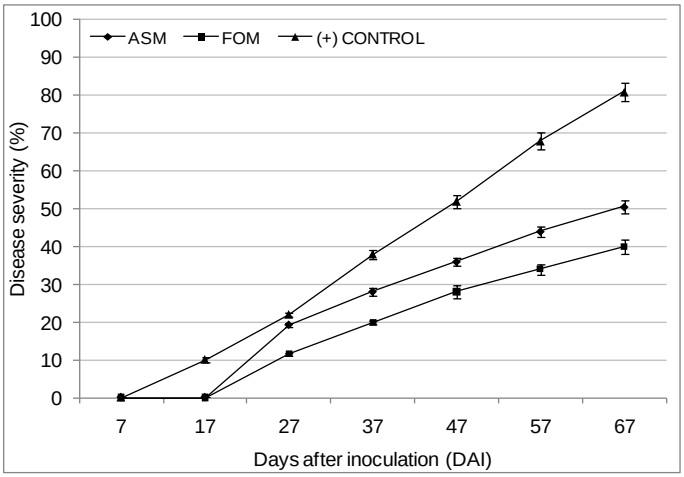

Figure 1. Effect of 3 DAI treatments of ASM and FOM on Fusarium wilt disease progression on eggplant. Vertical bars indicate standard error of four replicates.

Although these treatments reduced pathogen's severity over 80% in previous pot experiments on eggplant (Altınok et al., 2002), under farmers conditions, current results point out that, investigation of interactions between FOM and eggplant may provide important clues on the mechanisms of resistance induction, against Fusarium wilt disease.

In addition to assessment of disease severity of Fusarium wilt, plant length was also measured during the vegetation period. Compared to negative control plant lengths, positive controls exhibited a growth retardation rate of about 57%, while ASM and FOM applications are displaying a reduction about 6 and 16%, respectively. This indicates both applications positively effected plant growth in the existence of pathogen. FOM was more effective than ASM in suppression of disease, but as a plant activator, ASM treatments resulted significantly better plant growth, compared to FOM (Table 3).

Table 3. Effect of 3 DAI applications of ASM and FOM on disease severity and plant growth at 67th DAI.

Treatments	Disease Severity (%)	Effect (%)	Plant Length (cm)
ASM	50.50 b*	37.65	88.80 b
FOM	40.00 c	50.61	78.70 c
(+) Control	81.00 a	-	53.56 d
(–) Control	-	-	94.06 a

* Means within columns followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P \geq 0.05$).

Induced resistance has been reported for many crops with fungal and bacterial pathogens, along with inoculation methods (Gessler and Kuc, 1982; Erzurum and Maden, 1995; Fuchs et al., 1997). Both of the ASM and FOM obviously induced disease resistance in eggplants. For systemic acquired resistance (SAR), plants require a time period to develop resistance, before the pathogen challenge. In many studies, this interval was reported as about 3 days before inoculation of pathogen (Biles and Martyn, 1989; Soylu et al., 2003). In this research, 72-hr interim was selected for development of resistance in eggplants to disease. Biles and Martyn (1989) have reported enhanced response from avirulent races of *Fusarium oxysporum* f. sp. *niveum* and observed localized and systemic resistance for 72-hr interim. In the same study, pathogen challenge after 24 and 72 hr induction periods provided significant local protection on plants in greenhouse. Nonpathogenic *F. oxysporum* strains have different modes of action. These strains affecting the rate of chlamydospore germination of the pathogen, enhances induced systemic resistance and compete for nutrients by colonizing cortex layer of roots (Benhamou et al., 2002). Mechanisms of wilt biocontrol activity for *F. oxysporum* (Fo47) include induced resistance, microbial competition for nutrients and inhibition of spore germination (Lemanceau et al., 1993).

Benhamou and Belanger (1998) and Louws et al. (2001) have also reported enhanced response by ASM, in many other vegetables especially against fungal and

bacterial disease agents. In a study involving pre-treatment of plants with ASM, activities of peroxidase (POX) and glutathione peroxidase (GPX) were determined as markers of resistance. It has been also reported that active oxygen species (AOS) may induce expression of defence-related genes (Soylu et al., 2003). Accumulation of β-1,3-glucanases and chitinases are important plant defence responses. These antifungal hydrolases are in enhanced cooperation with pathogenesis-related proteins in systemic acquired resistance responses (Boller, 1987). ASM is currently registered as a commercial product for use on many vegetables for control of fungal and bacterial leaf diseases (Cole, 1999; Louws et al. 2001; Soylu et al., 2003). But, some researches showed that the ASM is an effective inducer against soil-borne diseases also on several other crops (Benhamou and Belanger 1998; Elmer, 2006). Fine-tuned applications of ASM to local conditions would greatly support development of environment-friendly, efficient and economical plant protection strategies, as demanded by integrated pest management programs for a sustainable growing of eggplant.

Various formae speciales of *Fusarium oxysporum* have ability to colonize on root cortex layer but can only reveal symptoms on their own hosts. Plant's resistance mechanism constituted by nonpathogenic *Fusarium oxysporum* inoculations may exhibit a good level of protection against pathogens. In this manner, following studies on FOM should focus on mechanisms of disease suppression. Investigations on FOM-activated plant resistance genes and enzymes involved in induced resistance may provide valuable information on fungal resistance induction mechanisms of nonpathogenic *Fusarium oxysporum* formae speciales on eggplant.

ÖZET

YÜKSEK TÜNEL KOŞULLARINDA PATLICANDA FUSARİUM SOLGUNLUK HASTALIĞINA KARŞI SİSTEMİK DAYANIKLILIĞIN TEŞVİKİ

Bu çalışmada, daha önceki saksı denemelerinde Fusarium solgunluk hastalığına karşı her ikisi de başarılı sonuçlar veren bir bitki aktivatörü (Actigard 50 WG; Acibenzolar-S-methyl; ASM) ile patlıcanda patojen olmayan bir *Fusarium oxysporum* izolatı (*F. oxysporum* f. sp. *melonis*; FOM), yüksek tünel koşullarında denenmiştir. Patojen (*Fomg*10), dayanıklılığın gelişebilmesi amacıyla ASM ve FOM uygulamalarından 72 saat sonra inokule edilmiştir. inokulasyondan sonraki 67. günde ASM ve FOM uygulanan bitkilerin boyları sırasıyla Ortalama 88.8 ve 78.8 cm iken kontrol bitkileri 53.56 cm olarak kaydedilmiştir. Hastalık ilerlemesini değerlendirmek için yapılan izolasyonlarda *Fomg*10, ASM ve FOM uygulanan bitkilerde 25 cm'den, kontrol bitkilerinde ise 50 cm den izole edilmiştir. Kontrol bitkilerinde hastalık hızla gelişmiş ve denemenin son gününde (inokulasyondan sonraki 67. gün) hastalık şiddeti %81 olarak belirlenmiştir. Uygulamaların her ikisi de başlangıç penetrasyonunu engelleye-

memiş, bununla birlikte hastalık şiddetini inokulasyondan sonraki 67. günde kontrole göre %37.65 (ASM) ve %50.61 (FOM) oranlarında azaltarak patlıcan bitkilerinde dayanıklılığı belirli düzeyde teşvik etmiştir.

Anahtar Kelimeler: *Fusarium oxysporum*, *Solanum melongena*, acibenzolar-S-methyl, uyarılmış dayanıklılık, yüksek tünel

ACKNOWLEDGEMENTS

The author is grateful to the late Dr. Yeter Canıhoş and to Dr. M. Kamberoğlu from Çukurova University. This study was supported by the Academic Research Projects Unit of Çukurova University and Süleyman Demirel University.

LITERATURE CITED

ALTINOK, H. H., 2005. First report of Fusarium wilt of eggplant caused by *Fusarium oxysporum* f. sp. *melongenae* in Turkey. **Plant Pathology**, 54: 577.

ALTINOK, H. H., A. ERKILIÇ, Y., CANIHOŞ, S. AKGÜL, 2002. Patlıcanda Fusarium solgunluğuna karşı patojen olmayan *Fusarium* türü ve Actigard'ın etkinliklerinin belirlenmesi. Türkiye 5. Biyolojik Mücadele Kongresi. 4-7 Eylül, Erzurum, 429-436s.

ALTINOK, H. H., and C. CAN, 2010. Characterization of *Fusarium oxysporum* f. sp. *melongenae* isolates from eggplant in Turkey by pathogenicity, VCG and RAPD analysis. **Phytoparasitica**, 38:149-157.

BENHAMOU, N. and R. BELANGER, 1998. Benzothiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato, *Plant Physiology***,** 118: 1203-1212.

BENHAMOU, N., C. GARAND and A. GOULET, 2002. Ability of nonpathogenic *Fusarium oxysporum* strain Fo47 to induced resistance against *Phytium ultimum* infection in cucumber. **Applied and Environmental Microbiology**, 68: 4044-4060.

BHAT, R. G. and K. V. SUBBARAO, 1999. Host range specifity in *Verticillium dahliae*. **Phytopathology**, 89: 1218-1225.

BILES, C.L. and R.D. MARTYN, 1989. Local and systemic resistance induced in watermelons by formae speciales of *Fusarium oxysporum.* **Phytopathology**, 79: 856-860.

BOLLER T, 1987. Hydrolytic enzymes in plant disease resistance. (Kosuge T, Nester EW, eds.), Plant-Microbe Interactions. Molecular and Genetic Perspectives, Vol. 2. New York, NY: Macmillan, 385-413.

BOOTH, C., 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, 237p.

CAVAGLIERI, L., J. ORLANDO, M. I. RODRÍQUEZ, S. CHULZE and M. ETCHEVERY, 2005. Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides in vitro* and at the maize root level. **Research in Microbiology**, 156: 748-754.

COLE, D.L., 1999. The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance against bacterial and fungal diseases of tobacco, **Crop Protection**, 18: 267-273.

ELMER, W.H., 2006. Efficacy of preplant treatment of gladiolus corm with combination ect of acibenzolar-S-methyl and biological and chemical fungicides for control of Fusarium corm rot. **Canadian Journal of Plant Pathology**, 28: 609-614.

ERZURUM, K. and S. MADEN, 1995. Evaluation of various treatments of inducing resistance to Fusarium wilt on melon. **Journal of Turkish Phytopathology**, 24 (3): 121-134.

FUCHS, J.G., Y. M. LOCCOZ and G. DÉFAGO, 1997. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt in tomato. **Plant Disease**, 81: 492-496.

GESSLER, C. and J. KUC, 1982. Induction of resistance to Fusarium wilt in cucumber by root and foliar pathogens. **Phytopathology**, 72: 1439-1441.

GÖRLACH, J., S. VOLRATH, G. KNAUF-BEITER, G. HENGY, U. BECKHOVE, K.H. KOGEL, M. OOSTENDORP, T. STAUB, E. WARD, H. KESSMAN and J. RYALS, 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. **The Plant Cell**, 8: 629-643.

KATAN, T., 1999. Current status of vegetative compatibility groups in *Fusarium oxysporum.* **Phytoparasitica**, 27: 51-64.

KESSMANN, H., T. STAUB, C. HOFMANN, T. MAETZKE, J. HERZOG, E. WARD, S. UKNES and J. RYALS, 1994. Induction of systemic acquired disease resistance in plants by chemicals. **Annual Review of Phytopathology**, 32: 439-459.

KISTLER, H. C., 2001. Evolution of host specificity in *Fusarium oxysporum* (B.A. Summerel, J.F. Leslie, D. Backhouse, W.L. Bryden and L.W. Burgess, eds.). *Fusarium*. Paul E. Nelson Memorial Symposium. APS Pres, St. Paul, Minn., p. 70-82.

LECOQ, H., F. BLANCARD, P. BERTRAND, A. GLANDARD and P., MOLOT et MAS, 1991. Techniques d'Inoculation artificielle du melon avec differents agents pathogénes pour la sélection de variétés resistances. I. N. R. A. Domaine Saint Maurice BP 94 84143 Montfavet Cedex.

LEMANCEAU, P., P. A. H. M. BAKKER, W. J. de KOGEL, C. ALABOUVETTE, and B. SCHIPPERS, 1993. Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. *dianthi*. **Applied and Environmental Microbiology**, 59: 74-82.

LOUWS, F.J., M. WILSON, H.L. CAMPBELL, D.A. CUPPELS, J.B. JONES, P.B. SHOEMAKER, F. ŞAHIN and S. MILLER, 2001. Field control of bacterial spot and bacterial speck of tomato using a plant activator. **Plant Disease**, 85: 481-488.

NELSON, A. J., T. A. TOUSSOUN and W. F. O. MARASAS, 1983. *Fusarium* species. The Pennsylvania State University Press University Park and London, 190p.

ROWE, R. C., 1980. Comparative pathogenicity and host ranges of *Fusarium oxysporum* isolates causing crown and root rot of greenhouse and field-grown tomatoes in North America nad Japan. **Phytopathology**, 70: 1143-1148.

SOYLU, S., B. ÖMÜR and E. M. SOYLU, 2003. Induction of disease resistance by the plant activator, acibenzolar-S-methyl (ASM), against bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) in tomato seedlings **Plant Science**, 165: 1069-1075.

TALLY, A., M. OOSTENDORP, K. LAWTON, T. STAUB and B. BASSI, 1999. Commercial development of elicitors of induced resistance to pathogens. (A.A. Agrawal, S. Tüzün and E. Bent, eds.) Induced Plant Defences Against Pathogens and Herbivores. Biochemistry, Ecology and Agriculture. APS Press St. Paul, Minnesota., p.357-369.

TOWNSEND, G. K. and J. W. HEUBERGER, 1943. Methods for estimating losses caused by diseases in fungicide experiments. **Plant Disease Report**, 27: 340-343.