

THE EFFECT OF SALICYLIC ACID AND *Bacillus subtilis* ON CUCUMBER ROOT AND STEM ROT, CAUSED BY *Fusarium oxysporum* F.SP. *radicis cucumerinum**

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Abstract

In this study, the effects of exogenous application of salicylic acid (SA) and *Bacillus subtilis* on cucumber plants against, *Fusarium oxysporum* f.sp. *radicis cucumerinum* (Forc) were investigated. *In vitro*, the effect of different levels of SA (2-8 mM) on growth of *B. subtilis* was negative, while concentrations more than 5 mM SA completely inhibited the mycelial development of Forc. Applications of SA (3,5 and 7 mM) and *B. subtilis* post or / and prior inoculation with the pathogen with regard to evaluation of disease severity, root and shoot fresh weight and length of shoot were conducted under greenhouse conditions. The results indicated that cucumber plants treated by combination of 7 mM SA (foliar spray) and *B. subtilis* (soil drench), prior to fungal infection, exhibited reduction of fungal infection and increased plant growth as compared to control and other treatments. Evaluation of total phenol content and polyphenol oxidase activities showed that the combined application of SA and *B. subtilis* significantly increased the above plant defense compounds compared to SA and *B. subtilis* alone and control. The peak levels of them were observed in 7 and 5 days after elicitors application, respectively. According to these results, SA as a chemical elicitor and *B. subtilis* as biocontrol agent and plant growth promoter can be integrated for effective protection of cucumber plants against Forc infection.

Keywords: Salicylic acid, *Bacillus subtilis*, Polyphenol oxidase, *Fusarium oxysporum* F.SP. *radicis cucumerinum*.

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Reference

- BRADFORD, M. M. 1976. A rapid and sensitive method for quantification of protein utilizing the principle of protein-dye binding. **Anal. Biochem.** 72:248-254.
- CONSTABLEL, C. P., YIP, L., PATTON, J. J. and CHRISTOPHER, M. E. 2000. Polyphenol oxidase from hybrid poplar, cloning and expression in response to wounding and herbivory. **Plant Physiol.** 124: 285–95.
- DURRANT, W. E. and DONG, X. 2004. Systemic acquired resistance. **Ann. Rev. Phytopathol.** 42:185-209.
- ETEBARIAN, H. R., SHOLBERG, P. L., EASTWELL, K. C. and SAYLER, R. 2005. Biological control of apple blue mold with *Pseudomonas fluorescens*. **Can. J. Microbiol.** 51: 591-598.
- FAYZA, A. F. and SABREY, Y. M. 2006. Induction of resistance in *Phaseolus vulgaris* against TNV by salicylic acid and kinetin. **Intl. J. Agric. & Biol.** 8:47-51.
- GIRISH, N. and UMESHA, S. 2005. Effect of plant growth promoting rhizobacteria on bacterial canker of tomato. **Arch. Phytopathol. Plant Protect.** 38:235 – 243.
- JALALI, B. L., BHARGAVA, S. and KAMBLE, A. 2005. Signal transcriptional regulation of plant defense responses. **J. Food Quality** 28(1): 3.
- KAMAL, A., ABO-ELYOUSR, M., HUSSEIN, M. A. M., ALLAM, A. D. A. and HASSAN, M. H. 2009. Salicylic acid induced systemic resistance on onion plants against *Stemphylium vesicarium*. **Plant Pathol.** 42: 1042 – 1050.
- KLOPPER, J. W., LEONG, J., TEINZE, M. and SCHROTH, M. N. 1980. Enhanced plant by siderophores produced by plant growth-promoting rhizobacteria. **Nature** 286:885-886.
- KURATA, K. 1994. Cultivation of grafted vegetables and developing of grafting robots in Japan. **Hort. Sci.** 29:240-244.
- LI, S. M., HUA, G. G., LIU, H. X. and GUO, J. H. 2008. Analysis of defence enzymes induced by antagonistic bacterium *Bacillus subtilis* strain AR12 towards *Ralstonia solanacearum* in tomato. **Ann. Microbiol.** 58 (4): 573-578.
- MANDAVIA, M. K., PATEL, C. M., MARAVIA, G. V. and PARAMESWARAN, M. 1997. Role of phenolic compounds in resistance to *Fusarium* wilt in chickpea. **Ind. J. Agric. Biochem.** 10:11-13.
- MAYER, A. M., HAREL, E. and SHAUL, R. B. 1965. Assay of catechol oxidase a critical comparison of methods. **Phytochemistry** 5:783-789.
- MOHAMMADI M. and KAZEMI, H. 2002. Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. **Phytopathology** 10: 1016 – 1032.
- MORENO, A., ALFEREZ, A., AVILES, M., DIANEZ, F., BLANCO, R., SANTOS, M. and TELLO, J. C. 2001. First report of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* on cucumber in Spain. **Plant Dis.** 85:1206.
- MURPHY, A. M., HOLCOMBE, L. J. and CARR, J. P. 2000. Characteristics of salicylic acid induced delay in disease caused by a necrotrophic fungal pathogen in tobacco. **Physiol. Mol. Plant** 31:139-145.
- OKA, Y., COHEN, Y. and SPEIGEL, Y. 1999. Local and systemic induced resistance to the root – knot tomato by DL – β -amino-n-butyric acid. **Phytopathology** 89: 1138 – 1143.
- OZGONEN, H., BICICI, M. and ERKILIC, A. 2001. The effect of salicylic acid and endomycorrhizal fungus *Glomus etunicatum* on plant development of tomatoes and *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *lycopersici*. **Turk. J. Agric. For.** 25:25-29.
- PODILE, A. R. and PRAKASH, A. P. 1996. Lysis and biological control of *Aspergillus niger* by *Bacillus subtilis* AF1. **Can. J. Microbiol.** 42:533-538.
- PRITHIVIRAJ, B., BIS, H. P., JHA, A. K. and VIVANCO, J. M. 2005. *Staphylococcus aureus* pathogenicity on *Arabidopsis thaliana* is mediated either by a direct effect of salicylic acid on the pathogen or by SA-dependent, NPR1 independent host responses. **Plant J.** 42:417-432.
- PUNJA, Z. K. and PARKER, M. 2000. Developing of *Fusarium* root and stem rot, a new disease on greenhouse cucumber in British Columbia, caused by *Fusarium oxysporum* f.sp. *radicis cucumerinum*. **Can. J. Plant Pathol.** 22:349-363.
- RAJU, S., JAYALAKSHMI, S. K. and SREERAMULU, K. 2008. Comparative study on the induction of defence related enzymes in two different cultivars of chickpea (*Cicer arietinum*) genotypes by salicylic

- acid, spermin and *Fusarium oxysporum* f.sp. *ciceri*. **Aust. J. Crop Sci.** 2(3):121-140.
- RAUPACH, G. S. and KLOEPPER, J.W. 2000. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. **Plant Dis.** 84:1073-1075.
- REUVENI, R.1995. Biochemical marker for disease resistance, Pp. 99-144. *In*: Singh, R. P., Singh, U. S (Eds.), **Molecular Methods in Plant Pathology**.CRC Press , Boca Raton , FL.
- RICKER, A. S.1963. **Introduction to Research. Plant Disease. CRC Press.** London,England.
- RYALS, J. A., NEUENSCHWANDER, U. H., WILLITS, M. G., MOLINA, A., STEINER, H. Y. and HUNT, M. D. 1996. Systemic acquired resistance. **Plant Cell.** 8: 1809–1819.
- RYALS, J., UKNES, S. and WARD, E.1994. Systemic acquired resistance. **Plant Physiol.**104:1109-1112.
- SAILAJA, P. R., PODILE, A. R. and REDDANNA, P. 1997. Biocontrol strain *Bacillus subtilis* AF1 rapidly induces lipoxygenase in groundnut (*Arachis hypogaea*) copared to crown rot pathogen *Aspergillus niger*. **Eur. J. Plant Pathol.**104:125-132.
- SHAHRIYARI, D. and ZARE, R. 2007. *Fusarium* root and stem rot of cucumber. **Proc. 17th Iran. Plant Protec. Cong., Karaj, Iran.** (Abst.).
- SHOUWEI, L., FENGZHI, W. and YANLING, M. 2009. Effects of *Fusarium* wilt pathogen on the enzyme activity of cucumber cultivars of different resistance. **Plant Protec.** 35:82-85.
- STEWART, R. J., SAWYEER, B. J. B., BUCHELI, C. S. and ROBINSON, S. P. 2001. Polyphenol oxidase is induced by chillingand wounding in pineapple. **Aust. J. Plant Physiol.** 28:181–191.
- THOMPSON, D. C. 1996. Evaluation of bacterial antagonists for reduction of summer patch symptomism Kentucky blue grass. **Plant Dis.** 80: 850 – 862.
- VAKALOUNAKIS, D. J. 1996. Root and stem rot of cucumber caused by *Fusarium oxysporum* f.sp. *radicis cucumerinum*. **Plant. Dis.** 80:313-316.
- VAN LOON , L. C., BAKKER, P. A. H. M. and PIERTERSE, C. M. J. 1998. Systemic resistance induced by rhizosphere bacteria.**Ann. Rev. Phytopathol.** 36:453-483.
- VAN LOON, L. C. 2007. Plant responses to plant growth-promoting rhizobacteria. **Eur. J. Plant Pathol.** 119:243–254.
- VAN PEER, R. and SCHIPPERS, B. 1992. Lipopolysaccharides of plant-growth promoting *Pseudomonas* sp.strainWCS417r induce resistance in carnation to *Fusarium* wilt. **Nether. J. Plant Pathol.** 98:129-139.
- WANG, Y., ZHANG, Y. and ZHANG, J. 2010. Effects of salicylic acid on the behavior of Yali pear infected by *Alternaria kikuchiana* Tanaka.**Front. Agric. China** 4(1): 79–83.
- YU, T. and ZHENG, X. 2006. Salicylic acid enhances biocontrol efficacy of the antagonist *Cryptococcus laurentii* in apple fruit. **J. Plant Growth Regul.** 25: 166-174.