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Integration of *Pseudomonas fluorescens* and acibenzolar-*S*-methyl to control bacterial spot disease of tomato

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Abstract

Xanthomonas axonopodis pv. *vesicatoria* was isolated from infected tomato seedlings grown in an open field in Egypt. All the tested isolates infected tomato plants but with different degrees of disease severity. In an attempt to manage this disease, tomato seeds and/or seedlings were treated with an antagonistic local isolate of *Pseudomonas fluorescens* as a suspension or its formulation or acibenzolar-*S*-methyl (BTH). When the above three treatments were applied to tomato seeds under laboratory conditions, they improved seed germination and seedlings vigour relative to control seeds treated with sterile distilled water and pathogen but *P. fluorescens* culture was the most effective. Under greenhouse and field conditions, combinations of the above treatments were used. All treatments significantly reduced disease severity of bacterial spot in tomato relative to the infected control. The biggest disease reduction compared to seedlings inoculated with the pathogen alone resulted from a foliar application of *P. fluorescens*. Combined application of *P. fluorescens* or its formulation with BTH reduced the pathogen population and increased seedling biomass and tomato yield relative to control seedlings.

Keywords: BTH; *Pseudomonas fluorescens*; *Xanthomonas axonopodis* pv. *vesicatoria*; Biological control; Tomato

1. Introduction

In Egypt, tomato (*Lycopersicon esculentum* Mill.) is considered one of the most important vegetable crops. The cultivated area is 195.00 ha producing 70,600 tonne with a productivity of 38,974.40 kg ha^{-1} (FAO, 2005). Unfortunately, it is attacked by several diseases including bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (*Xanthomonas axonopodis* pv. *vesicatoria*, Vauterin et al., 1995; Jones et al., 1998; Abd El-Ghafar and Mosa, 2001; Abd El-Sayed and Abd El-Ghafar, 2004; El-Hendawy et al., 2005; El-Meneisy et al., 2005). The disease results in leaf lesions, defoliation, fruit lesions and finally yield loss of marketable fruit (Louws et al., 2001). This bacterium readily acquires resistance to streptomycin and fixed copper bactericides (Stall and Thayer, 1962; Marco and Stall, 1983; Cuppels and Elmhirst, 1999). In addition, there is no commercial cultivar of tomato resistant to this pathogen. The lack of effective pesticides and host resistance led to the development of alternative strategies for the management of this disease. Strategies include the use of biocontrol agents such as antagonistic bacteria (El-Hendawy et al., 2005; Kavitha and Umesha, 2007) and the use of bioactive products referred to as plant activators (Louws et al., 2001) or integration of these two strategies (Abd El-Ghafar and Mosa, 2001; Anith et al., 2004).

Fluorescent *Pseudomonas* spp. are considered one of the most important biocontrol agents (Daniel and Fergal, 1992); they inhibit microorganisms by several mechanisms such as antibiotic production (Howell and Stipanovic, 1979, 1980; Dahiya et al., 1988; Howie and Suslow, 1991), production of secondary metabolites (Dunne et al., 1996) or siderophore production (Loper and Buyer, 1991; Meyer, 2007). They have been reported to play a role in controlling many plant diseases caused by bacteria, fungi and nematodes (El-Hendawy et al., 1998; Obradovic et al., 2004; Siddiqui et al., 2005; Kavitha and Umesha, 2007).

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Acibenzolar-*S*-methyl has been reported to induce plant resistance against many fungal and bacterial pathogens (Kessmann et al., 1994; Ishii et al., 1999; Abo-Elyousr, 2006).

This investigation was carried out to determine the efficiency of *Pseudomonas fluorescens* (as a pure culture or formulation) and acibenzolar-*S*-methyl (BTH) in reducing the bacterial spot incidence in tomato under greenhouse and field conditions. The combined effect of BTH with *P. fluorescens* was also evaluated against bacterial spot in tomato.

2. Materials and methods

2.1. Bacterial isolates

X. campestris pv. *vesicatoria* (Doidge) Dye (*X. a.* pv. *vesicatoria*, Vauterin et al., 1995; Jones et al., 1998) isolates were isolated from diseased tomato seedlings growing in an open field in Giza, Egypt, and identified by consulting Bradbury (1984), Lelliott and Stead (1987) and Vauterin et al. (1995).

Fifteen antagonistic bacterial isolates were isolated from the rhizosphere of tomato plants grown in an open field at Assiut, Egypt. Under UV light, these isolates showed a green-yellow fluorescence on King's medium B (King et al., 1954), which is a characteristic of *P. fluorescens*; the identification of these bacteria was confirmed by conducting various tests specific to *P. fluorescens* (Stainer et al., 1966). *P. fluorescens* and *X. a.* pv. *vesicatoria* were maintained on slopes of King's medium B and LB medium, respectively, stored at 4 °C and subcultured at monthly intervals.

2.2. Antagonistic activity

The antagonistic activity of *P. fluorescens* towards *X. a.* pv. *vesicatoria* was tested by the well diffusion method according to Zeller and Brulez (1987). A suspension of *X. a.* pv. *vesicatoria*, prepared from an overnight shaken culture, was spread over the surface of plates containing KB medium. After drying, 0.05 ml of an overnight culture of *P. fluorescens* was pipetted into a 9 mm well. Plates were then incubated at 27 °C for 2 d and examined for inhibition zones. There were four replicates for each treatment and the experiment was repeated twice.

2.3. Seeds and growth of seedlings

Seeds of tomato (*L. esculentum* Mill) cultivar Super Strain B were obtained from the Ministry of Agriculture, Egypt, and used in this study. Seeds were sown in plastic pots, each of 20 cm diameter and containing a mixture of sand (1.5 kg/pot) and slow-release fertilizer 1% NPK (12:4:6). All pots were placed on a bench in a climate-controlled greenhouse at 30 ± 5 °C with 68–80% relative humidity (RH), and watered as required.

2.4. Inoculum preparation and inoculation

Inoculum was prepared from a 48 h shaken culture of the most virulent isolate of *X. a.* pv. *vesicatoria* incubated at 28 ± 2 °C. Cultures were centrifuged at 6000*g* for 20 min at room temperature and then suspended in sterile distilled water. The number of cells in the suspension was determined from the optical density measurements at OD_{610}. The required number of cells ml^{-1} was obtained by diluting the suspension.

Four-week-old tomato plants were inoculated by spraying each plant with 30 ml of bacterial suspension containing 5×10^6 cfu ml^{-1}. After inoculation, plants were kept in a climate chamber with 30 °C day temperature and 85% RH. Disease development was recorded 7 and 14 d after inoculation. Disease index was based on a scale from 1 (no disease) to 7 (100% disease) according to Abbasi et al. (2002). Greenhouse experiments were repeated twice.

2.5. Preparation of talc-based formulation of *P. fluorescens*

The formulation of *P. fluorescens* (Fr) was prepared as described by Vidhyasekaran and Muthamilan (1995). The bacterium was grown in liquid King's medium B at 28 ± 2 °C in a rotary shaker at 150 rpm for 48 h. A 400 ml bacterial suspension containing 1×10^8 cfu ml^{-1} was mixed with 1 kg of talc powder (sterilized at 105 °C for 12 h), 15 g of calcium carbonate (to obtain the pH value of 7) and 10 g of carboxymethyl cellulose. After drying the formulation overnight under sterile conditions, it was packed in polypropylene bags.

2.6. Effect of *P. fluorescens*, its formulation and BTH on seed germination and seedling vigour in vitro

Seeds were immersed in a bacterial suspension containing $1 \times l0^8$ cfu ml^{-1} of *P. fluorescens* (Pf), or an aqueous solution of BTH (0.5 g l^{-1}), (BTH) or in sterile distilled water (DW, control) and were gently shaken for 24 h then, blot dried, plated on wet blotters and germination was tested by the filter paper method. The treated seeds were incubated at 28 °C for 1 week. Periodically, the water contents were readjusted and samples were taken for assaying germination. The viability of seedlings was determined by calculating the vigour index (VI) of the seeds. VI was calculated using the formula of Abdul Baki and Anderson (1973): (mean shoot length + mean root length) × percentage of germination. Ten grams of freshly prepared Fr was added to 1 kg of tomato seeds and germination was tested as described previously. A set of each treatment (containing 50 seeds) was replicated four times and the VI calculated as mentioned before.

2.7. Effect of *P. fluorescens*, its formulation and BTH on disease severity and pathogen population

2.7.1. Greenhouse experiments

In the following experiments, Pf was used at a concentration of 1×10^8 cfu ml^{-1} and Fr was used at a