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Effect of organic amendments on *Verticillium* wilt of cotton

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Abstract

Verticillium wilt is the most devastating disease of cotton in China and a challenge for producers to find effective means of control. Here, we report the effects of different organic amendments on the incidence of this disease and on the rhizosphere microflora of cotton plants. Seven organic amendments were evaluated for their suppressive effect on cotton *Verticillium* wilt caused by *Verticillium dahliae* Kleb. The results showed that organic amendments applied to soil reduced disease severity in both inoculated pots and naturally infested cotton field plots. The most effective control was achieved with crab shell (chitin), soybean stalk and alfalfa, and in pots the efficacy was 72%, 60% and 56% for vascular tissues, respectively. Rice chaff gave moderate control, while poultry manure, peanut cake and wheat straw showed a weak suppressive effect with efficacy of 21%, 28% and 11% for vascular tissues, respectively. Organic amendments increased the population size of rhizosphere microbes (including fungi, bacteria and actinomycetes), which varied at the different ages of the cotton plants. The organic materials with the best biocontrol capacity strongly stimulated the proliferation of antagonists to *V. dahliae* in the rhizosphere. However, poultry manure, peanut cake and wheat straw caused only small changes in the total numbers of microflora and the percentage of antagonists was lower. Extracts from organic amendments were highly inhibitory to *V. dahliae*. The changes undergone by rhizosphere microbes after the addition of organic amendments may contribute to suppression of cotton wilt and help to explain the protective effect of the amendments. The results indicate that application of organic amendments is an effective control measure against cotton *Verticillium* wilt.

Keywords: Organic amendments; Cotton; *Verticillium dahliae*; Control efficacy; Rhizosphere microbes

1. Introduction

Verticillium wilt of cotton, caused by the soilborne fungal pathogen *Verticillium dahliae* Kleb, is one of the most important diseases in many cotton production areas of the world (Hampton et al., 1990; Eldon and Hillocks, 1996) with corresponding economic crop losses (Schnathorst and Mathre, 1966). Infected plants usually exhibit symptoms of marginal chlorosis or necrosis in leaves, discoloration of the stem vascular bundles, decrease in photosynthesis and increase in respiration, resulting in a significant reduction of the plant biomass and heavy loss of yield (Hampton et al., 1990; Paplomatas et al., 1992). The disease is usually managed by using tolerant cultivars and cultural practices. However, no genetic sources of resistance provide protection from infection of the vascular system, and none of the current upland cotton cultivars are immune to *V. dahliae*, although some are more tolerant than others (Colson-Hanks and Deverall, 2000). Xiao et al. (1998) found that crop rotation could be a successful practice for managing cotton *Verticillium* wilt, but was not practically applicable. Some chemical fungicides seem to be effective, but they are not environmentally friendly (Nannipieri, 1984). Furthermore, the repeated use of such chemicals encourages the development of resistance in the target pathogen (Goldman et al., 1994), and has a negative effect on beneficial organisms. Control of *V. dahliae* is especially difficult due to its ability to survive in field soil for several years as various types of mycelia, clusters of hyaline cells and microsclerotia (Schnathorst, 1981). Therefore, it is necessary to develop new methods to manage this disease.

An alternative for protecting crops from diseases is biocontrol with organic amendments. For centuries, farmers

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have consciously and unconsciously manipulated the ecology of the soil by using organic matter. Organic amendments are known to affect soil aeration, structure, drainage, moisture holding capacity, nutrient availability and microbial ecology (Davey, 1996). These practices influence pathogen viability and distribution, nutrient availability and the release of biologically active substances from both crop residues and soil microorganisms. Developing disease suppressive soils by introducing organic amendments and crop residue management takes time, but the benefits accumulate across successive years by increasing beneficial soil microorganism population and structure. Organic materials can be used to control many soilborne plant pathogens. *Fusarium* crown and root rot in tomato plants has been controlled with the addition of chitin and chitosan from crab shell (Benhamou and Theriault, 1992, 1994) and *Pythium ultimum* by poultry manure and wheat straw (Bettiol et al., 1997) or pine bark compost (Zhang et al., 1996). *Verticillum* wilt of crops can be suppressed by applying alfalfa to soil (Marshunova and Muromtsev, 1975) and *Fusarium oxysporum* has been controlled by using municipal solid waste compost containing antagonistic microorganisms such as *Bacillus subtilis*, *Trichoderma* and *Pseudomonas* (Serra-Wittling et al., 1996). Tenuta and Lazarovits (2002) demonstrated that ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *V. dahliae*. Jordana et al. (1994) and Iglesias et al. (1999) suggested that organic amendments could stimulate natural defence and improve development of seedlings growing in poor soils and providing an ecological and efficient means for the control of pepper-wilt caused by *V. dahliae* (García-Mina et al., 1996). There are sufficient data to indicate that organic materials reduce disease incidence caused by a wide range of soilborne plant pathogens. However, there have been fewer reports of controlling cotton *Verticillium* wilt by applying organic amendments (Li et al., 2002). Confirmation of the potential of organic amendments for the control of plant diseases requires a detailed study of soil ecology and antagonistic potential.

The purpose of our study was to evaluate, under controlled conditions, the effects of seven organic materials on cotton *Verticillium* wilt and to test microorganisms in the rhizosphere of cotton plants for their contribution to disease control.

2. Materials and methods

2.1. Preparation of organic amendments

Organic amendments were prepared as follows. Crab collected from the sea was heated in an oven at 60 °C for 3 h for crab shell. Alfalfa grown in plots for 8 weeks was cut at root level, chopped and cut into small pieces. Poultry manure collected from feedstock was composted in a pile, passively aerated over a period of approximately 32 weeks until the average core compost temperature declined to 45 °C. Water was added during the process. The manure was then harvested and air-dried in sunlight. Peanut cake obtained as residue after extraction of peanut oil was air-dried, as was soybean stalk, rice chaff and wheat straw. All organic amendments described above were macerated in an integrator to 3–5 mm pieces and applied to soil 7 d before cottonseeds were planted.

2.2. Cottonseeds treatment

A cotton cultivar susceptible to *V. dahliae*, Zhongmian 17, was kindly provided by the Institute of Economic Crops, Henan Academy of Agricultural Science. In order to remove surface pathogens, cottonseeds were first delinted by sulphuric acid (12 g sulphuric acid /100 g cottonseeds, shaken together for 2–3 min) and then washed. The delinted seeds were kept in an autoclaved mixture of perlite and peat (1:1, v/v) and allowed to germinate at 20–30 °C, and germinating seeds with approximately equal radical lengths were selected for sowing.

2.3. Fungal inoculation

The strain of *V. dahliae* used in this experiment was isolated in 1998 from the naturally infested cotton growing areas at Fengqiu Ecology Station, the Chinese Academy of Science, where cotton *Verticillium* wilt was serious for several years. The fungus was grown in 9-cm Petri dishes on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) medium at 25 °C in the dark for 3 d (primary plates). Subsequently, four colonized plugs of agar from a single isolate were cut out and placed in a 500 ml glass flask (loosely closed by cotton wool) containing autoclaved 95 g sand, 5 g porridge oats and 20 ml distilled water. The flasks were incubated at 25 °C for 7 d and shaken by hand for 5 min twice a day. The oats inoculum was dried by air and the contents were homogenized in a blender before inoculation of soil.

2.4. Experiment design

The experiment was conducted in both pots and field plots. In the pot experiment, the application rate to soil (from the garden) for crab shell (chitin), poultry manure and peanut cake was 2% (w/w), while the rate for rice chaff, soybean stalk, wheat straw and alfalfa was 1% (w/w). The soil was shown to be free of *V. dahliae* Kleb (by the procedure described by Yang and Shang (2002). After the soil and organic amendments were thoroughly mixed, the soil was inoculated with the pathogen by mixing with the oat inoculum at a concentration of 4% (w/w). Nine 10-L pots for each treatment were filled with the mix. Soil inoculated with the pathogen without any organic amendments was used as the control. Each pot was sown with four geminated healthy cottonseeds and then placed in a growth chamber (20 °C/28 °C, night/day and 12 h photoperiod) and then moved to a greenhouse (20 °C/28 °C,