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COULD CABBAGE LEAF DEBRIS BE USED FOR THE CONTROL OF FUSARIUM WILT OF PEPPER?

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ABSTRACT

In intensive agricultural production with narrow crop rotations, *Fusarium oxysporum*, a plant pathogenic fungus causing the wilt disease of pepper, frequently develops an abundant population in the soil, which threatens production profitability. Reducing the inoculum level below a critical threshold is highly demanding because of the long-lasting chlamydospores that could remain in the soil for many years. Soil disinfection with methyl bromide was the most effective way to control this pathogen. However, the ban of methyl bromide uses increased interest in control alternatives. Many studies have shown that glucosinolates, secondary metabolites of plants from the Brassicaceae family, as well as their derived biologically active products, negatively affect plant pathogenic bacteria and fungi, insects, nematodes, and weeds. The aim of this research was to determine if cabbage leaf debris, as a by-product material in cabbage production, could be effectively used in the control of Fusarium wilt in pepper. Fresh cabbage leaves were blended and immediately incorporated in sterile substrate seven days before inoculation of pepper plants at the 5-7 leaf stage. Conventional fungicide captan and biofungicide based on *Trichoderma asperellum* were used as reference treatments. The assessment was performed 25 days after inoculation; the degree of wilting, plant growth parameters, and the length of necrosis were determined. Fresh cabbage leaves were highly effective in Fusarium wilt control (91.3% compared to control), indicating a great potential for their use in Fusarium wilt management.

Keywords: *Fusarium oxysporum, Brassicaceae, secondary metabolites, glucosinolates.*

INTRODUCTION

Pepper production is severely affected by soil-borne plant pathogenic fungi worldwide. *Fusarium oxysporum*, the causal agents of a wilt disease, is among the most important pathogens of pepper in terms of losses in the production (Mijatovic et al., 2005). It can infect plants at any pheno-phase. However, young plants can unknowingly be infected since wilting does not occur until several weeks after infection. The main symptoms are yellowing, wilting and shriveling of leaves, followed by plant stunting and twig or branch dieback. Disease symptoms often appear later in the growing season and are first noticed on the lower (older) leaves.

As the disease progresses, younger leaves will also be affected and the plant eventually dies. Because of endophytic growth of the pathogen, as well as long persistence of its resting survival structures (chlamydospores) in soil, wilt disease is very difficult to control (Alström, 2001).

Over the past several decades, soil fumigation with methyl bromide has been the primary method of controlling soil-borne diseases. However, in 1992 methyl bromide was listed as a Class I ozone-depleting substance (Bell, 2000) and, afterwards gradually removed from the market. Without proper control, soil-borne diseases could increase crop losses to unpredictable levels. Although crop rotation slowly reduces inoculum density, it is not always profitable in intensive cropping. In addition, resistant pepper cultivars are not commercially available, while grafting of plants on resistant rootstock would not be cost-effective under practical conditions (Gilreath et al., 2004). Phasing-out of methyl bromide brought into sharp focus the need for alternative strategies for the management of soil-borne diseases. To replace methyl bromide several direct control methods such as the use of other fumigant-like pesticides that have a long history of use (methylisothiocyanate generator metam sodium, 1,3-dichloropropene(1,3-D), chloropicrin, mixtures of these compounds) or various biologically-based options were advertised (Matthiessen & Kirkegaard, 2006). In recent years, numerous authors have highlighted the potential use of Brassicaceae plants and the related biofumigation process to control numerous plant pathogens, such as *Rhizoctonia solani*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, *Fusarium sambucinum* (Larkin & Griffin, 2007; Handiseni et al., 2017), *Pyrenochaeta lycopersici* and *Verticillium albo-atrum* (Giotis et al., 2009). Different Brassicas species are used as biofumigants against plant pathogens because of their diverse capabilities of producing biologically inactive sulfur compounds known as glucosinolates. These compounds, in the presence of water and endogenous enzyme myrosinase, are hydrolyzed into bioactive products, mainly isothiocyanates, organic cyanides, oxazolidinethiones, and thiocyanate (Manici et al., 1997; Gimsing & Kirkegaard, 2009). These volatile substances are responsible for the suppressive effects on soil-borne pathogens, a phenomenon defined as “Biofumigation”. The aim of this research was to determine if cabbage leaf debris, as a by-product material in cabbage production, could be effectively used as biofumiogants in the control of Fusarium wilt in pepper. As reference treatments for comparison, some fungicides, biofungicides and fertilizers of natural origin were included.

MATERIAL AND METHODS

Isolate

An isolate of *F. oxysporum* was isolated from infected pepper plant sampled in Smederevska Palanka in Serbia, using a standard laboratory method for the isolation of plant pathogens (Dhingra and Sinclair, 1995). Small fragments of diseased xylem tissue were washed under running tap water for 30 minutes, surface disinfected by 2% NaClO, placed aseptically on potato dextrose agar medium

(PDA) and incubated at 25±1°C for 5-7 days. To obtain an axenic culture, the developed mycelium was transferred to fresh PDA medium. Conidia for monoconidial purification of the isolate were produced on a synthetic nutrient-poor agar medium (SNA) (Leslie & Summerell, 2006). The isolate was maintained on PDA slants at 5°C in the culture collection of the Institute of Pesticides and Environmental Protection, Belgrade, Serbia. Preliminary identification of the isolate was based on morphological and pathogenic characteristics, according to Lević (2008). The identity of the isolate was confirmed by an amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) using the primers ITS1 and ITS4 (White et al., 1990).

Tested products

Tested products were selected based on available literature data on their modes of action, spectrum of activity, on pathogens biological features, as well as the authors' long-lasting practical experience in crop protection.

Inoculum preparation

Inoculum was prepared by growing the isolate in glass bottles containing 150 g double sterilized barley grains at 25°C for 21 days. Then, the inoculum was mixed thoroughly with 400 ml of growth substrate (Floragard®, Germany) and added into pots at the rate of 3% in all the treatments except uninoculated control (Hashem et al., 2010).

The experiment

As a test organism, pepper plants (cv. Novosadska babura) at 5-7-leaf stage, grown in 60-celled polystyrol trays were used. The experiment consisted of: cabbage leaf treatment of the growing substrate, five treatments of the substrate performed with commercial products available on the market, uninoculated and inoculated controls.

Chopped fresh cabbage leaves were added into 400 ml of inoculated growth substrate at the rate of 5%, thoroughly mixed, placed in pots, watered with 100 ml of distilled water and covered with plastic foil. The following products: Plant extracts (mixture) - Ecofluor (Ecosoil, Serbia), Complex P and K ferilizer - Phosphilux (Luxembourg, Israel), Oligosaccharide complex - FYTO11 (Fytofend, Belgium), Captan - Agrocaptan (Agromarket, Serbia), and *Trichoderma asperellum* - Trifender (Bioved, Hungary) were applied at a label rate, using 100 ml of the dilution/pot. The products were applied three times, at the same time as cabbage leaves treatment, 7 and 14 days afterwards. Uninoculated substrate and inoculated substrate watered with 100 ml of distilled water were used as controls. All pots were kept in a growth chamber (24±2°C) for 7 days. Then, the foil from cabbage leaves treatment was removed pepper plants were transplanted into each pot of all the treatments. The experimental design was a complete randomized block with five replicates per treatment and three plants per replicate.

The degree of wilting was observed daily, while the final evaluation of the disease severity and plant growth parameters was performed 25 days after the transplantation. Disease severity was estimated based on a visual 0-5 scale, where 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = slight wilting with pronounced chlorosis, 3 = slight wilting and necrosis, 4 = pronounced wilting and necrosis, and 5 = death of plant (Figure 1). The length of necrosis on longitudinal section of plants and plant growth parameters (plant height from a substrate surface to the top of plants, whole plant fresh weight and the diameter of a stem) were measured and analyzed. Disease severity index (DSI) was evaluated using Townsend-Heuberger's formula (Puntner, 1981): $DSI = \Sigma(nv)100/NV$ where: n = degree of infection rated on a scale, v = number of plants in a category, N = highest degree of infection rate, and V = total number of plants screened. Raw data were transformed using $\sqrt{x}$ or $\sqrt{(x+0.1)}$ transformation and subjected to one-way ANOVA. Means were separated by Duncan multiple range test. The analysis was conducted in Software Statistica 7 (StatSoft, USA).

Results and discussion

From a symptomatic pepper plant, showing yellowing, wilting and shriveling of leaves only *Fusarium*-like colonies were recovered. Since all of the derived isolates were with uniform colony appearance, one was randomly selected for further investigations. The isolate produced delicate, white to pink mycelium on PDA medium. After seven days of incubation at 25°C in the absence of light, the fungus formed a colony of 7 cm in diameter. The presence of conidia on the PDA substrate was not observed. However, in a 5-day-old culture on a SNA medium, a large number of unicellular, elliptical, oval-shaped microconidia and straight to slightly curved macroconidia with three septate were observed. Based on the studied characteristics, the test isolate was preliminary identified as *F. oxysporum.* Following successful amplification and sequencing of a 600-bp product, BLAST analysis showed that the sequence of the amplified product was identical with the

sequence of the isolate *F. oxysporum* deposited in NCBI database (Acc. No. EF495230), thus confirming species identity.

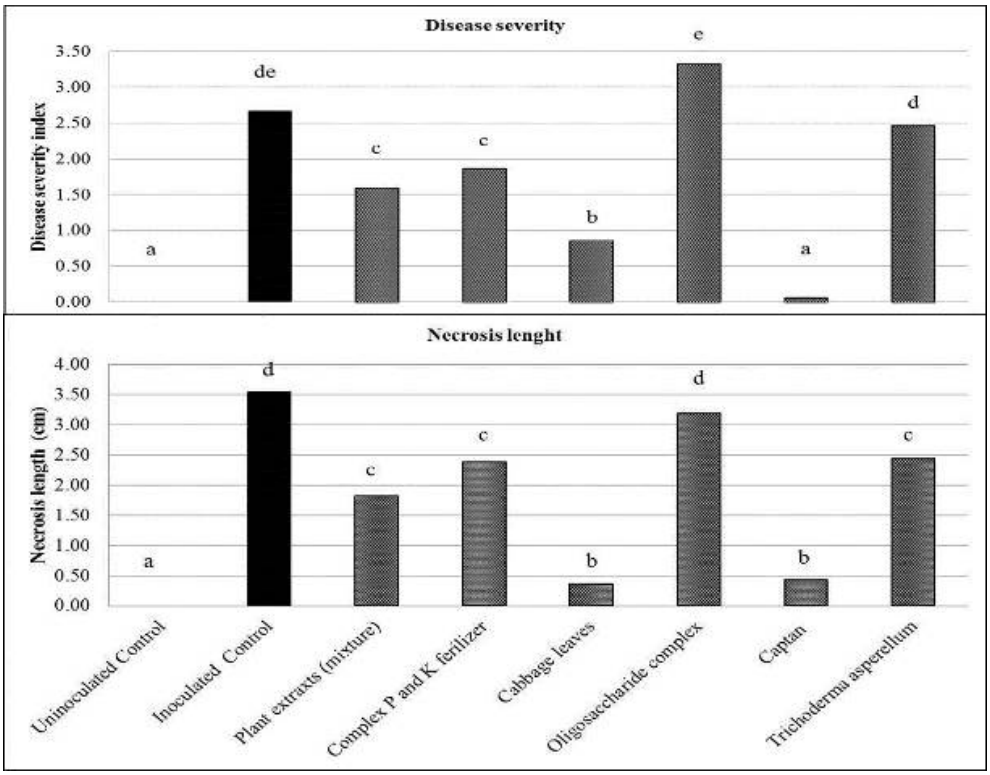

Figure 2. Fusarium wilt severity and the length of necrosis (in cm) in pepper plants inoculated with *Fusarium oxysporum* 25 days after the transplantation. Significant differences among treatments are assigned by different letters.

The effect of studied treatments on Fusarium wilt severity and the length of necrosis in pepper plants inoculated with F. oxysporum are presented in Figure 2. Based on the disease severity observation, captan and cabbage leaves were the most effective in the disease suppression; the DSIs were 0.07 and 0.87 respectively, compared to the inoculated control where DSI was 2.67. Similar results were reported by Larkin & Griffin (2007) for several Brassica species. In addition, the shortest necrosis length was recorded on the stem longitudinal section of the plants treated with captan and cabbage leaves (0.43 and 3.37 cm, respectively), compared to 3.55 cm that was noted in the inoculated control plants. Interestingly, the disease severity in the treatment with oligosaccharide complex was even higher than in the control plats, revealing stimulating effect of the studied oligosaccharide complex on the disease development.

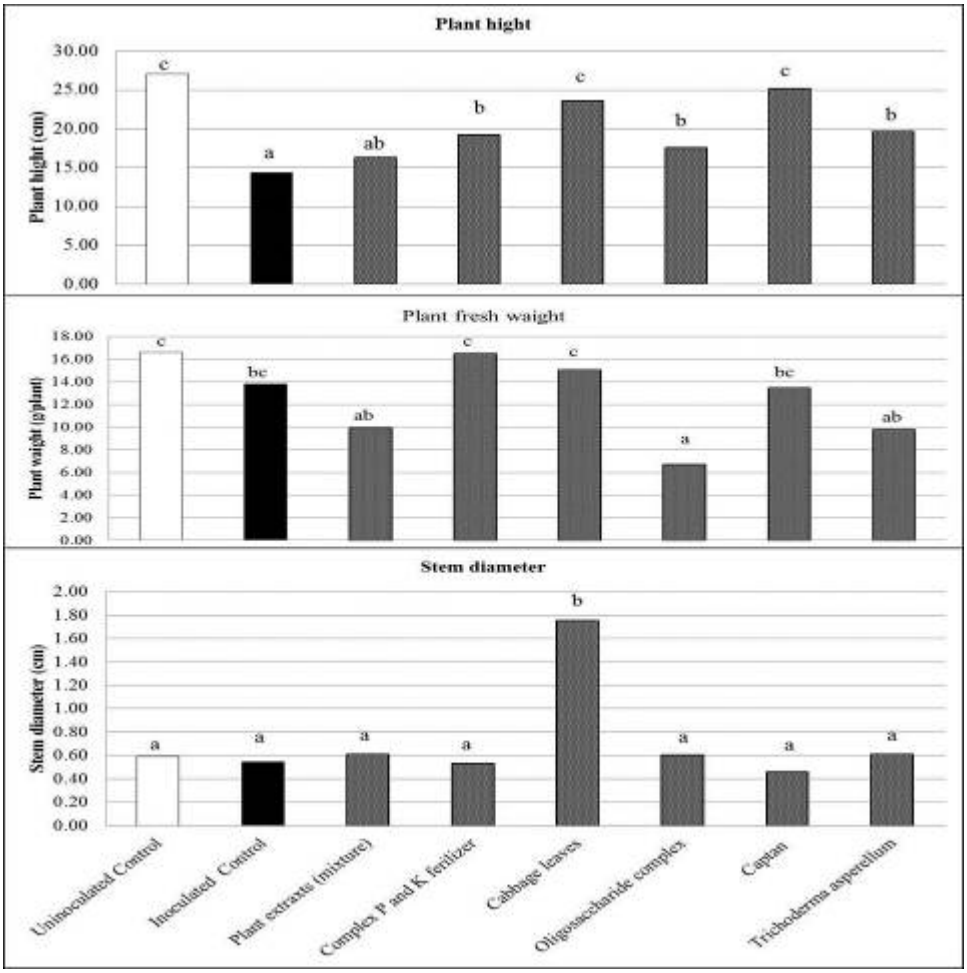

Figure 3. Height (in cm), fresh weight (in g) and stem diameter (in cm) of pepper plants inoculated with *Fusarium oxysporum* 25 days after the transplantation. Significant differences among treatments are assigned by different letters.**Figure 1.** A visual 0-5 scale for Fusarium wilt severity estimation in pepper: 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = slight wilting with pronounced chlorosis, 3 = slight wilting and necrosis, 4 = pronounced wilting and necrosis, and 5 = death of plant.

Not only fresh cabbage leaves were effective in Fusarium wilt control (91.3% compared to uninoculated control, or 67.4% compared to inoculated untreated control), but also they increased the diameter of the stem that was significantly wider than in uninoculated control plants (Figure 3), revealing simulative effect of cabbage leaves on plant growth.

CONCLUSION

A great potential of biofumigation by cabbage fresh material in the management of Fusarium wilt disease of pepper was determined.

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