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Full Length Research Paper

Hot pepper growth promotion and inhibition of fusarium wilt (*Fusarium oxysporum*) with different crop stalks

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This paper studied hot pepper growth promotion and inhibition of fusarium wilt with rice stalks, corn stalks, soybean stalks and hot pepper stalks (control). The results of this study showed that extracts of corn stalks, soybean and hot pepper stalks (CK) had a positive effect at a concentration of 0.01 g L^{-1} but had an inhibition at a concentration of 0.04 g L^{-1}. Different from 0.01 g L^{-1}, all extracts of the crop stalks had inhibitory actions on mycelia growth of *Fusarium oxysporum* at a concentration of 0.04 g L^{-1}. As the concentration of rice stalks extract increased, the inhibiting effects of *F. oxysporum* growth became obvious. At the concentration of 0.04 g L^{-1} extracts of rice stalks, corn stalks, soybean stalks and hot pepper stalks (CK) all inhibited the germination of *F. oxysporum* spore. The growth and physiological indices of hot pepper, such as plant height, stem diameter, fresh weight and root vigor, were promoted by decomposed crop stalks (DCS), besides there was a positive correlation between the effects and the amount of DCS addition. The physiological parameters of hot pepper, such as superoxide dismutase (SOD) activity, malonaldehyde (MDA) content and conductivity of relative specific, decreased obviously after the treatments with DCS were given.

Key words: Crop stalks, hot pepper, *Fusarium oxysporum*, allelopathy.

INTRODUCTION

Hot pepper (*Capsicum frutescens L.*) is an important vegetable crop. Some members of hot pepper are used as spices, vegetables and medicines. Since the 1990s, as a result of larger consumption, greater market demand, more uses of pepper, larger scale pepper processing, more breakthroughs in pepper breeding, better year- round cultivation and further industrialized operations than ever, hot pepper production has been developing relatively rapidly in the world (Zhang et al., 2008; Li et al., 2009).

China is the main grower of hot peppers in the world. The output of hot peppers in China, about 215 thousand metric tons, accounted for 9.19% of total world output (Xiong, 2008). Continuous cropping obstacle is a key topic in hot pepper cultivation. The disease severity became more and more serious with the extension of continuous cropping year. The hot pepper production would decrease 10 to 15, 20 to 30 and 30 to 50% respectively with the continuous cropping for one year, two years and three years (Zhang and Shangguan, 2005). Intercropping and rotation patterns have been used in traditional methods of overcoming continuous cropping obstacle. It may be practically infeasible to continue the tradition patterns, as the industrialization and specialization of hot pepper production have been advocated as the main production models.

Plants contain numerous active compounds, with over 0.4 million secondary metabolites. There are beneficial or deleterious effects of one plant on the other (including

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micro-organisms) through the release of chemical substances (Hisashi, 2011; Robert et al., 2011; Xu et al., 2010). That is allelopathy (Rice, 1984). Recently, certain active substances derived from natural plants are available to control fungus, bacteria, virus, nematodes, pests and weeds (Cao et al., 2006; Liu, 1995; Wang et al., 2000; Lu and Xie, 2003). The extracts of clove, cinnamomum cassia, rhubarb, aristolochia debilis and scutellaria had notable inhibitive activities that can cause fusarium wilt of *F. oxysporum* f.sp. Niveum, *F. oxysporum* Schlechtend. f.sp. Melonis, *F. oxysporum* f.sp. Lycopersici and *F. oxysporium* f.sp. Cucumerinum (Liu et al., 2011). Many plants have potentialities to promote the growth of other plants (Wang et al., 2011; Ma et al., 2011; Yang and Wu, 2011). Therefore, this paper studied the growth promotion and inhibition of fusarium wilt of hot pepper by 3 crop stalks.

MATERIALS AND METHODS

Extract preparation

Rice stalks, corn stalks and soybean stalks powder were smashed by pulverizer (type: FZ102, voltage: 220 V, power: 0.32 kW) after drying, and mixed with distilled water (1:20 w/w). After being shaken supersonically for 24 h (100 r/min) in number controlled oscillator, the mixture was centrifuged for 20 min (3,500 r/min). The supernatant was collected and stored at 4°C for analysis. The collected volume varied with the plant dry weight used (the collected volume is 1 ml if the plant dry weight used is 0.05 g). Rice stalks, corn stalks and soybean stalks extract concentrations (0.01 mg ml^{-1}, 0.04 mg ml^{-1}) were prepared from the mother extract with distilled water to examine the mycelium growth, and the concentration of 0.04 mg ml^{-1} for spore germination. Before the test, the extract needed sterilization by filtration with the filters of 0.22 μm. The fungus *F. oxysporum* was isolated from infected hot peppers growing in the infested seedling nursery area and verified by pathology Laboratory according to Booth's postulate.

Plant preparation

Japan mitaka hot peppers were studied as the materials through the pot cultivation experiment. The decomposition of rice stalks, corn stalks and soybean stalks were achieved with the following method: the crop stalks were smashed by pulverizer (type: FBW350, voltage: 220 V, power: 1.8 to 2.2 kW) after drying, mixed with distilled water and horses dung (10: 10: 1 w/w/w). The mixture was covered in a plastic film decayed for 50 days in solar-greenhouse. The temperature changed from 25 to 33°C during the day to 20 to 25°C at night in the greenhouse.

Mycelium growth

The mycelium growth of *F. oxysporum* was measured with growth rate method. To measure the growth of *F. oxysporum*, 5 ml of 0.01 mg ml^{-1}, 0.04 mg ml^{-1} extract was added to 5 ml thawed cultural medium (sterilized and cooled to 40°C) and made into slab. Each treatment had five replicates with pure PDA medium as control. The 6 mm-diameter pure cultured *F. oxysporum* spot was inoculated at 25°C and incubated in darkness. The colony diameter was measured with the cross method after 3-days incubation and the inhibitory rate was calculated.

Spore germination

Effects of extract on spore germination were measured with the suspending method. The preparation of the spore suspension required putting *F. oxysporum* in a small beaker, adding a small amount of distilled water and then mixing them very well. The spore suspension was filtered into a microcentrifuge tube and was then observed with a microscope. During the observation, the concentration of *F. oxysporum* spore was diluted to make sure 100 spores were observed within sight with a 600 power binocular microscope. The extract of 10 μl was taken to mix with 10 μl suspension containing *F. oxysporum* on a slide. The slide was incubated at 25°C and observed with a microscope 7, 9, and 11 h later respectively. The numbers of germinated and total spores were recorded to calculate the germination rate. Each treatment was replicated thrice with distilled water as control and each replicate was observed from 10 visions.

Pot trial

Plants were cultivated in 20 cm-diameter pots filled with mixed soils which included sieved soil from a hot pepper monocropping field for three years, horse dung and decomposing crop stalks. A series of proportion of the mixed soils (66.7: 33.3: 1, 66.7: 33.3: 5, 66.7: 33.3: 10 w/w/w) were prepared for the experiment. The total mass of the mixed soils in each pot was 2 kg. One hot pepper seedling with a true leaf was transplanted per pot. The pots were placed randomly with 3 replications with the mixed soils without decomposed crop stalks as control. Plants and roots were sampled after transplanting for 60 days for the measurement of the plant growth parameters.

Data processing

The results were expressed in RI Value as per Williamson method (Wang et al., 2005; Vokou et al., 2003):

If $T \geq C$, then $RI = 1 - C/T$

If $T < C$, then $RI = C/T - 1$

Where, C is control data; T is treatment data; RI is allelopathic index; RI >0 indicates stimulation, while RI <0 indicates inhibition. The intensity of effect was expressed in RI value and the original data were processed using DPS software.

Statistical analysis

The experimental data were processed with the Excel software and analyzed with analysis of variance using DPS version 7.05 (Tang and Feng, 2002). Prior to an ANOVA with multiple-comparison tests, variance ratio statistics were tested for the treatments significance. The multiple-comparison tests include least significant difference (LSD) and least significant ranges (LSR) and LSR includes q test and Duncan's method. Duncan's method of LSR was used in this paper. And the significant differences of the RI of hot pepper growth, mycelium growth and spore germination of *F. oxysporum* treated by different crop stalks were tested. The small and capital letters indicate the difference in 5 and 1% level respectively by Duncan's method of LSR test.

Table 1. Effect of extract of different crop stalk on mycelium growth.

Treatment	Concentration ($g \cdot L^{-1}$)	Allelopathy index (RI)				
		3 d	4 d	5 d	6 d	7 d
T1	0.01	-0.0368^{BCbc}	-0.0741^{Bb}	-0.0580^{Bb}	-0.0512^{Bc}	-0.0645^{Bc}
T2		0.0882^{Aa}	0.1286^{Aa}	0.0542^{ABa}	0.0510^{Ab}	0.0792^{Aab}
T3		0.1177^{Aa}	0.0949^{Aa}	0.0654^{ABa}	0.0568^{Ab}	0.0507^{Ab}
CK		0.0230^{ABb}	0.1482Aa	0.0855^{Aa}	0.1331^{Aa}	0.1266^{Aa}
T1	0.04	-0.1098^{Aa}	-0.0833^{ABb}	-0.1667^{Bb}	-0.1380^{Bbc}	-0.0860^{Bb}
T2		-0.1370^{Aa}	0.0258^{Aa}	0.0122^{ABa}	-0.0265^{ABb}	-0.0860^{Bb}
T3		-0.0610^{Aa}	-0.1759^{Bb}	-0.1594^{ABa}	-0.1690^{Bc}	-0.0860^{Bb}
CK		-0.0732^{Aa}	0.0688^{Aa}	0.0066^{Aa}	0.0613^{Aa}	0.0361^{Aa}

Data are mean RI values of three repetitions. Capital letter and lower letter after the number stands for significance on the level of 1% and 5% respectively when comparing with the CK in test. T1: rice stalks; T2: corn stalks; T3: soybean stalks; CK: hot pepper stalks.

RESULTS

Fusarium wilt

Hot pepper is very susceptible to fusarium wilt due to accumulation of pathogens in the soil under continuous cropping of hot pepper. Extract of rice stalks had inhibitory actions on mycelia growth of *F. oxysporum in-vitro* culture and the growth inhibition increased with the concentration of rice stalks extract increasing. The allelopathy index was -0.0368 and -0.1098 for the concentration of 0.01 and 0.04 g L^{-1} respectively 3 days later. Extracts of corn stalks, soybean stalks and hot pepper stalks (CK) had a positive effect at low concentrations but had an inhibition at higher concentrations 3 days later. The allelopathy index were 0.0882, 0.1177 and 0.023 for the concentration of 0.01 g L^{-1} and -0.137, -0.061 and -0.0732 for the concentration of 0.04 g L^{-1} respectively. At the concentration of 0.01 g L^{-1}, the treatment of rice stalks had highly significant differences with the treatment of corn stalks, soybean stalks and hot pepper stalks (CK). However, the treatment of corn stalks and soybean stalks had no significant difference between them. For 0.04 g L^{-1} treated concentration, there were no significant differences among the four treatments. There was no positive correlation between *F. oxysporum* biomass and cultured time. The change trend of mycelia growth of different treatments for 4, 5, 6 and 7 days later respectively was the same as that of it 3 days later (Table 1).

Extracts of rice stalks, corn stalks, soybean stalks and hot pepper stalks (CK) were inhibitory to spore germination of *F. oxysporum* at the concentration of 0.04 g L^{-1}. The inhibitory effect on spore germination was in inverse proportion to the cultured time. The inhibitory of spore germinated with rice stalks and corn stalks extract was higher than that with soybean stalks and hot pepper stalks extract 7 h later. The allelopathy index was -0.7291 and -0.7223. That of latter was -0.6646 and -4313. Different cultured time of extract of crop stalks affected the inhibitory of the spore germination in the order of 7 h>9 h>11 h (Figure 1). There were significant differences in the treatments and CK. For the treatments, there were differences which increased with time.

The growth parameters (plant height, stem diameter, fresh weight (FW) of shoots and roots) and the physiology parameters (root vigor) of hot pepper increased obviously after treatment with decomposed powder of rice stalks, corn stalks, and soybean stalks (Figure 2). The increased effect on hot pepper growth was dependent on the weight of decomposed crop stalks. All the parameters (plant height, stem diameter, fresh weight (FW) of shoots and roots and root vigor) of A1 were smaller than that of A2 and A3, and all the parameters of A2 were smaller than that of A3. For the plant height, different crop stalks contributed to the extent of allelopathic effects in the order of corn stalks>rice stalks>soybean stalks. For other parameters, the change trend of the allelopathy index was the same as that of plant height. The allelopathy index of corn stalks, which was highest among all the treatments were 0.0383, 0.0999, and 0.1142 respectively for the plant height of A1, A2 and A3 (Table 2). The allelopathy index of soybean stalks, which was lowest among all the treatment were 0.0334, 0.0581 and 0.0796 respectively for the plant height of A1, A2 and A3. This could be partly explained by the increased content of soil organic matter from the powder applied into soil. The higher soil organic matter perhaps resulted in vigorous growth and dry matter accumulation.

The physiologic parameters of hot pepper (SOD activity, MDA content and conductivity of relative specific) were used to determine the tolerance to environmental stresses of the plant. The parameter values tended to increase in proportion with the growth of environmental stresses. In this paper, the physiologic parameters of hot pepper (SOD activity, MDA content and conductivity of relative specific) decreased obviously after treatment with decomposed powder of rice stalks, corn stalks and

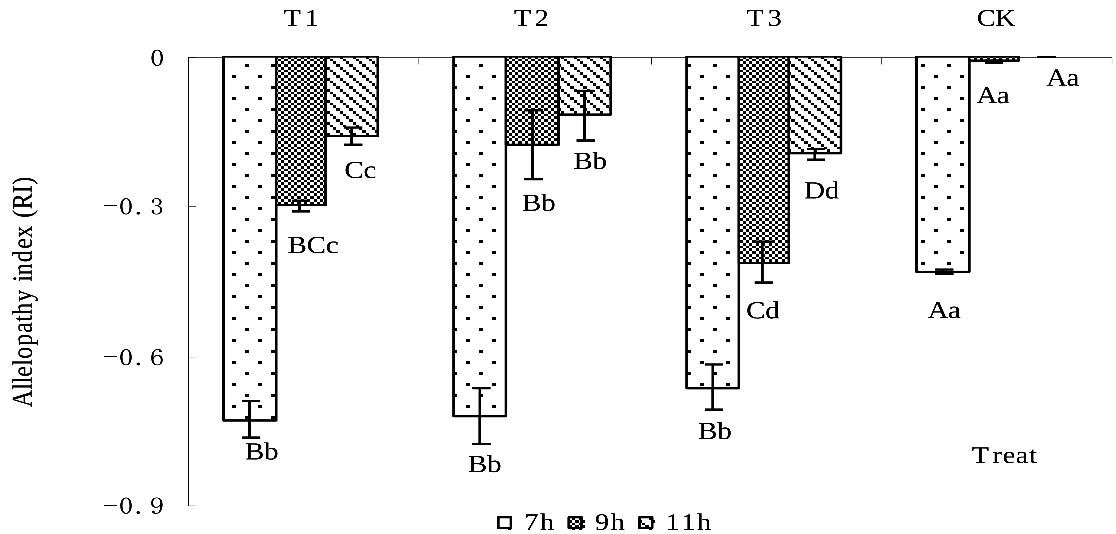

Figure 1. Effect of extract of different crop stalk on spore germination of pathogenetic fungi of seedling blight; T1: rice stalks; T2: corn stalks; T3: soybean stalks ; CK: hot pepper stalks.

soybean stalks (Figure 2). The decreased effect on hot pepper growth was dependent on the weight of decomposed crop stalks. All the physiologic parameters (SOD activity, MDA content and conductivity of relative specific) of A1 were higher than that of A2 and A3 and that of A2 were higher than that of A3. For SOD activity, different crop stalks contributed to the extent of allelopathic effects in the order of corn stalks>soybean stalks> rice stalks. For other parameters, the change trend of the allelopathy index was the same as that of SOD activity. The allelopathy index of rice stalks, which was highest among all the treatment, were -0.0390, -0.0580 and -0.1190 respectively for SOD activity of A1, A2 and A3. The allelopathy index of corn stalks, which was lowest among all the treatment, were -0.0520, -0.0970 and -0.1360 respectively for SOD activity of A1, A2 and A3. All aforementioned treatments showed no significant difference except MDA index.

DISCUSSION

Continuous cropping obstacle has been a key topic in crop cultivation (Mazzola, 2000; Hou et al., 2006). A lot of research results showed that the main causes of continuous cropping obstacle are accumulation of harmful microbes, changes of micro-organisms in soil, salinization of secondary soil and auto-toxicity of vegetable crops, etc (Singh et al., 1999; Sun et al., 2005). Some measures to prevent continuous cropping obstacles include choosing fine variety, crop rotation, rational fertilization, grafting, biological control were studied (Wang et al., 2005). In recent years, allelopathic inhibition on the continuous cropping obstacle was attracting increasing interest (Aryaa et al., 1995; Yu, 1999). Zhang et al. (2006) reported that the extracts of *Cnidium monnieri* and *Sophora flavescens* had dose-dependent inhibitory effects on the mycelium growth of *Verticillium dahliae*. The research of Wang et al. (2005) showed the antifungal activity of root exudates against the verticillium wilt. Similarly, Lu et al. (2003) found that the incidence of fusarium wilt of cotton and the spore germination were decreased by mint volatiles. Studies showed that the plants contain biochemical substance (called Allelochemicals) against phytopathy. This paper studied the allelopathic effects of crop stalks and the results showed that *F. oxysporum* was suppressed by some allelochemicals in crop stalks. Not only the mycelia but also the spore germination was inhibited. Crop stalks are more available than herbal medicine of *C. monnieri* and *S. flavescens* and helpful for overcoming continuous cropping obstacle.

The decomposition of crop stalks biomass in the soil made the soil fertile by supplying organic matter, plant nutrients and improving the soil texture (Yu and Song, 2003). The sequence of nutrients release rates of rice straw, wheat straw and rapeseed straw were that C release rate of three crop straws were up to 57.53, 66.58 and 52.54%, and N were up to 42.05, 49.26 and 57.83%, and P were up to 68.28, 59.93 and 67.32% after 124 days incubation, respectively. For all three crop straws, K release rate was 98% within the first 12 days of incubation (Dai et al., 2010) and above all, the decomposed crop stalks in the soil released a large amount of chemical that could influence soil microbial structure and population. The decomposed crop stalks and the microbial collectively influence the plant growth (Glinwood et al., 2011). The results of this test showed that hot pepper growth was promoted by rice stalks, corn stalks or soybean stalks.

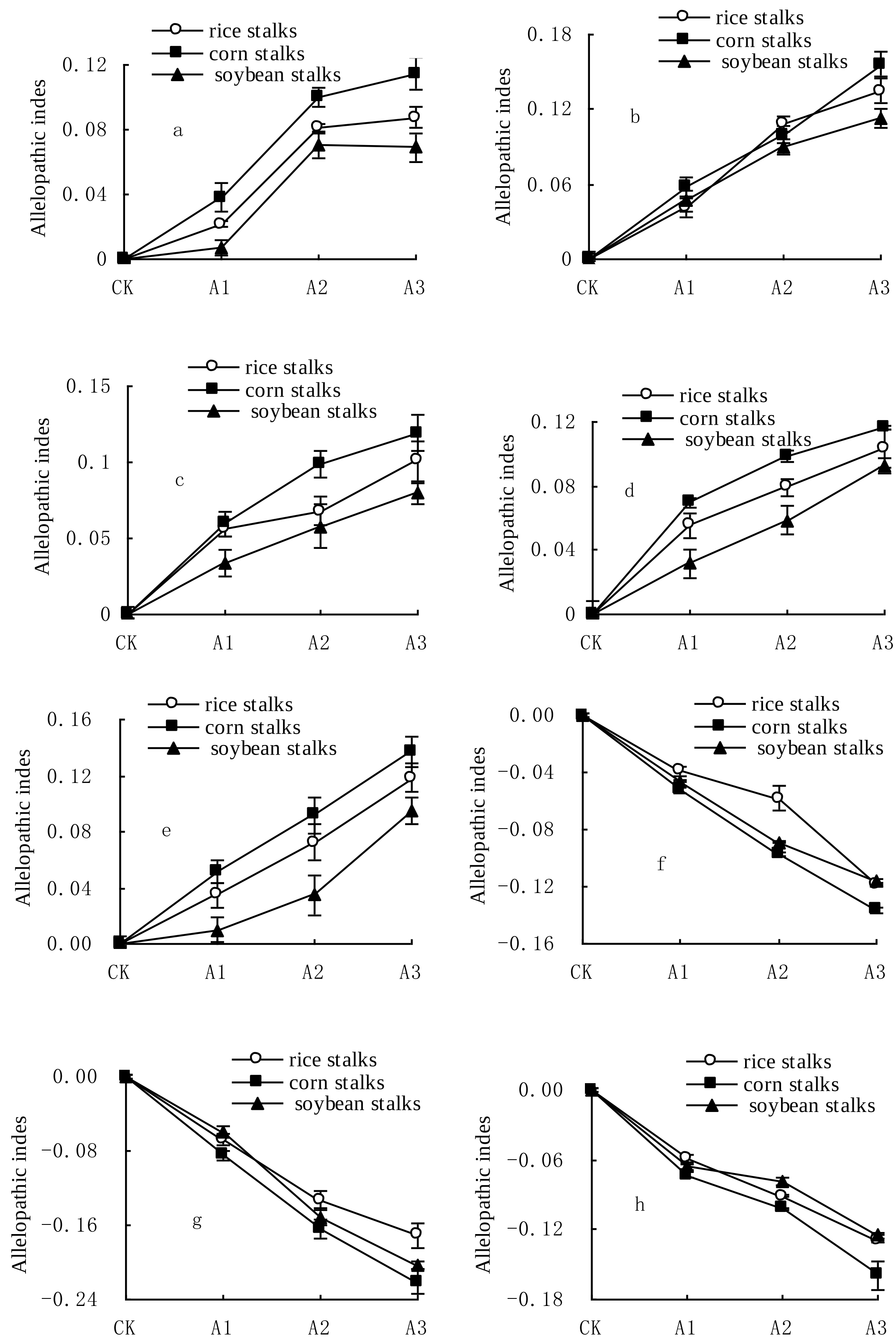

Figure 2. Effect of decomposing substance of different crop stalk on (a) plant height, (b) stem diameter, (c) FW of hot pepper shoots, and (d) FW of root, (e) root vigor, (f) SOD activity, (g) MDA content, (h) and conductivity of relative specific of hot pepper plants Note: A1: replant soil: manure: decomposing crop stalks (66.7:33.3:1, w/w/w); A2: replant soil: manure:decomposing crop stalks (66.7: 33.3: 5, w/w/w); A3: replant soil: manure: decomposing crop stalks (66.7: 33.3: 10, w/w/w).

Table 2. Effect of decomposing substance of different crop stalk on hot pepper plants.

Treatment		Allelopathy index (RI)		
Tested index	Soil additives	A1	A2	A3
Plant height	Corn stalks	0.0383^{Aa}	0.0999^{Aa}	0.1142 ^{Aa}
	Rice stalks	0.0222^{Aa}	0.0837^{Aa}	0.0904 ^{Aa}
	Soybean stalks	0.0334^{Aa}	0.0581^{Aa}	0.0796 ^{Aa}
Stem Diameter	Corn stalks	0.0586^{Aa}	0.1025^{Aa}	0.1574 ^{Aa}
	Rice stalks	0.0413^{Aa}	0.1091^{Aa}	0.1393 ^{Aa}
	Soybean stalks	0.0483^{Aa}	0.0909^{Aa}	0.1142 ^{Aa}
FW of hot pepper shoots	Corn stalks	0.0639^{Aa}	0.0975^{Aa}	0.1211 ^{Aa}
	Rice stalks	0.0585^{Aa}	0.0688^{Aa}	0.1066 ^{Aa}
	Soybean stalks	0.0303^{Aa}	0.0605^{Aa}	0.0807 ^{Aa}
FW of root	Corn stalks	0.0706^{Aa}	0.0998^{Aa}	0.1177 ^{Aa}
	Rice stalks	0.0585^{Aa}	0.1009^{Aa}	0.1101 ^{Aa}
	Soybean stalks	0.0303^{Aa}	0.0605^{Aa}	0.0942 ^{Aa}
Root vigor	Corn stalks	0.0527^{Aa}	0.0933^{Aa}	0.1378 ^{Aa}
	Rice stalks	0.0357^{Ab}	0.0744^{ABa}	0.1216 ^{Aa}
	Soybean stalks	0.0097^{Bc}	0.0348^{Bb}	0.0957^{Aa}
SOD	Corn stalks	-0.0520^{Aa}	-0.0970^{Aa}	-0.1360^{Aa}
	Rice stalks	-0.039^{Aa}	-0.0580^{Aa}	-0.1190^{Aa}
	Soybean stalks	-0.0475^{Aa}	-0.0918^{Aa}	-0.1174^{Aa}
MDA content	Corn stalks	-0.0851^{Aa}	-0.1654^{Aa}	-0.2226^{Aa}
	Rice stalks	-0.0698^{Aa}	-0.1342^{Aa}	-0.1718^{Aa}
	Soybean stalks	-0.0595^{Aa}	-0.1562^{Aa}	-0.2109^{Aa}
Conductivity of relative specific	Corn stalks	-0.0736^{Aa}	-0.1029 ^{Aa}	-0.1607^{Aa}
	Rice stalks	-0.0595^{Aa}	-0.0946^{Aa}	-0.1338^{Aa}
	Soybean stalks	-0.0681^{Aa}	-0.0794^{Aa}	-0.1251^{Aa}

A1: replant soil: manure: decomposing crop stalks (66.7:33.3:1, w/w/w); A2: replant soil: manure: decomposing crop stalks (66.7:33.3:5, w/w/w); A3: replant soil: manure: decomposing crop stalks (66.7:33.3:10, w/w/w). The little and capital English letters indicate the difference in 5% and 1% level, respectively , by LSR test.

Conclusion

By the methods of bioassay and pot experiment, the effects of overcoming ccontinuous cropping obstacle with rice stalks, corn stalks, soybean stalks were studied. All crop stalks inhibited mycelium growth and spore germination of *F. oxysporum* at 0.04 g L^{-1}, though mycelium growth was promoted at 0.01 g L^{-1}. Compared with the control of decomposed hot pepper stalks, the decomposed crop stalks promoted hot pepper growth.

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