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Studies on Antagonism of *Trichoderma* Species to *Fusarium oxysporum* f. sp. *fragariae* V. Biological Control of *Fusarium* Wilt of Strawberry by a Mycoparasite, *Trichoderma harzianum*

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딸기 시들음병균에 대한 *Trichoderma*속 균의 길항작용에 관한 연구 V. 중복기생균 *Trichoderma harzianum*에 의한 딸기 시들음병의 생물적 방제

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ABSTRACT : The biological control effect of *Trichoderma harzianum* on the *Fusarium* wilt of strawberry and several factors affecting on its efficacy were examined through pot experiments. *T. harzianum* grown on wheat bran, rice straw, rice hull, sawdust or barley straw was respectively incorporated into the pathogen-infested soil, and significantly suppressed the strawberry wilt caused by *Fusarium oxysporum* f. sp. *fragariae*. The wheat bran or rice straw culture of *T. harzianum* suppressed the disease incidence more effectively than other substrates for culture, decreasing it to 68% of the untreated control. The conidial suspension of *T. harzianum* alone or the suspension mixed with crab shell also effectively reduced the disease incidence. The control effectiveness of *T. harzianum* was high in acid soil (pH 3.5~5.5). In sandy loam soil, the disease incidences and population densities of the pathogen were decreased by the treatment of *T. harzianum*, while there was no significant effect of *T. harzianum* on the pathogen in loam soil.

***Key words* : *Trichoderma harzianum*, *Fusarium oxysporum* f. sp. *fragariae*, *Fusarium* wilt of strawberry, biological control, wheat bran, rice straw, mycoparasite.**

Antagonists may be applied to destroy a pathogen inoculum, to prevent recolonization of the pathogen in soil, or to protect germinating seeds and roots from the infection by the pathogen. For the biological destruction of a pathogen inoculum, the most effective antagonists may be mycoparasites of the pathogen (5).

Since *Trichoderma viride* firstly showed significant control effects on *Rhizoctonia solani* in the infected soil as well as mycoparasitic activity in media (31), most attempts in soil treatments with mycoparasites have been concentrated on the destruction of sclerotium and hypha of *R. solani*, *Corticium rolfsii*, or *Sclerotium rolfsii* by *T. harzianum* or *T. viride* (2, 3, 4, 6, 7, 10, 17, 19, 25, 26, 28, 33). However, there are few studies on the biological control of *Fusarium* spp. by mycoparasites, especially on the *Fusarium* wilt of strawberry (13, 14, 18). Recently, the results of *in vitro* experiments on the biological control of the aforementioned pathogen were reported by Moon *et al.* (21~24). A mycoparasitic fungus having a significant inhibitory effect on the *Fusarium* wilt of strawberry was identified as *T. harzianum* (21). The antagonistic effect of *T. harzianum* and its correlation with nutritional and environmental conditions were evaluated (22). It was found that chitinase played more important roles as a mechanism of the observed antagonistic ac-

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tivity than β-1,3-glucanase (23). Several factors affecting on the antagonistic activity of *T. harzianum* to the pathogen were also examined in *in vitro* soil experiments (24).

This study was carried out in a plastic film house to examine the effect of *T. harzianum* on the control of the *Fusarium* wilt of strawberry caused by *F. oxysporum* f. sp. *fragariae*, and to evaluate several factors involved in the enhancement of disease suppression.

MATERIALS AND METHODS

Preparation of inocula and substrates for antagonists. *T. harzianum* T42, which showed a high mycoparasitic activity to the pathogen (21), was used in this study, and *T. viride* T74 and T34 producing nonvolatile antibiotics that inhibit the mycelial growth of *F. oxysporum* f. sp. *fragariae* (21) were also used in some experiments of this study. Strawberry seedlings (cv. Hokowase) with two or three leaves were transplanted in sterilized soil in 13.2-cm-d pots, and 10 ml of the conidial suspension (10^6/ml) of *F. oxysporum* f. sp. *fragariae* S1 was inoculated to the strawberry root. Then, the antagonist, which was grown for 15 days on the selected substrates, was treated into the rhizosphere (4 g/pot), and the plants were placed in the plastic film house at 25~30°C. Each pot was watered every other day.

For preparing the substrates and incubating the antagonist, dried wheat bran, rice straw, barley straw and rice hull were respectively added with tap water in the ratio of 1 : 1.5 (v/v), and mixed. A wheat bran-sawdust-tap water mixture (3 : 1 : 4, v/v/v) was also prepared. All of the substrates were autoclaved for 1 hr at 121°C two times on two successive days. The prepared substrates were inoculated with *T. harzianum*, and incubated for 15 days with the light for 12 hours a day. For the treatment of crab shell, 0.23 g of crab shell containing 0.3% chitin was added to each pot prior to the inoculation of the antagonist and the pathogen. To prepare the conidial suspension of the antagonist, 4 g of the rice straw culture of the antagonist was washed by tap water, eliminating rice straw, and used for inoculum.

Preparation of soils. Loam soil (pH 5.21) containing 2.14% organic matter was collected from strawberry fields in Kimhae, Kyungnam, and was mixed with sand at the ratio of 1 : 1. The sandy loam soil (pH 6.16, 0.76% organic matter) was used for the whole experiments of this study. In the soil texture experiment related to the biological control, both the original loam soil and the sandy loam soil were used.

Adjustment of soil pH. For the experiment of the effect of soil pH on the suppression of the *Fusarium* wilt of strawberry by *T. harzianum* T42 and *T. viride* T74, different soil pH values were obtained by adding 0.1 N NaOH or 0.1 N HCl into soil preparations. Soil pH was estimated by the electrometric method using a pH meter (Mattler Delta 340) (24). The acidity of watering water for the strawberry seedlings was also adjusted according to the aforementioned different soil pH values.

Examination of disease incidence. Disease incidence was examined at one-week intervals from the 3rd through the 12th week after inoculation. The disease indices are as follows; 0 : healthy plant, 1 : stunted plant without any symptoms on leaf, 2 : plant with 1~2 deformed or yellowish leaves, 3 : plant with more than 3 deformed or yellowish leaves, 4 : withered plant, and 5 : dead plant.

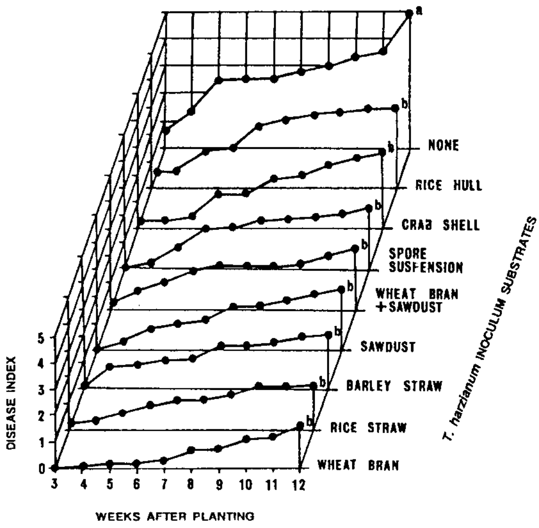

Fig. 1. Effects of substrates for *T. harzianum* inoculum on the biological control of the strawberry wilt caused by *F. oxysporum* f. sp. *fragariae* in a pot experiment. Disease index: 0-healthy plant, 1-stunted plant, 2-plant with 1~2 deformed or yellowish leaves, 3-plant with more than 3 deformed or yellowish leaves, 4-withered plant, and 5-dead plant. Treatments with different letters are significantly different (p=0.05) according to Duncan's multiple range test.

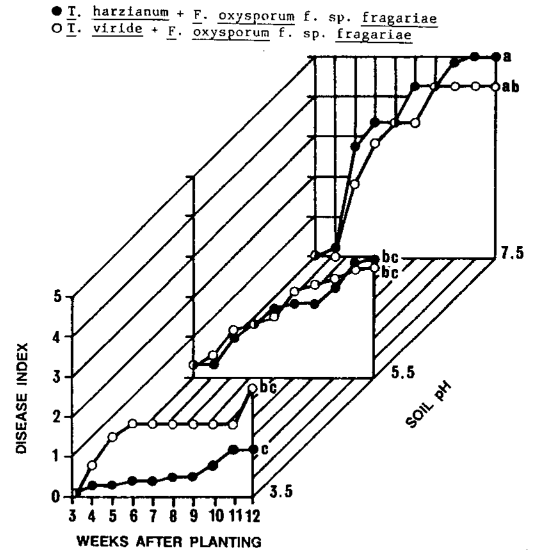

Fig. 2. Effects of soil pH on the biological control of the strawberry wilt disease by *T. harzianum* (isolate T42, ●—●) and *T. viride* (isolate T74, ○—○) (rice straw preparations) in a pot experiment. Disease index: 0-healthy plant, 1-stunted plant, 2-plant with 1~2 deformed or yellowish leaves, 3-plant with more than 3 deformed or yellowish leaves, 4-withered plant, and 5-dead plant. Treatments with different letters are significantly different (p=0.05) according to Duncan's multiple range test.

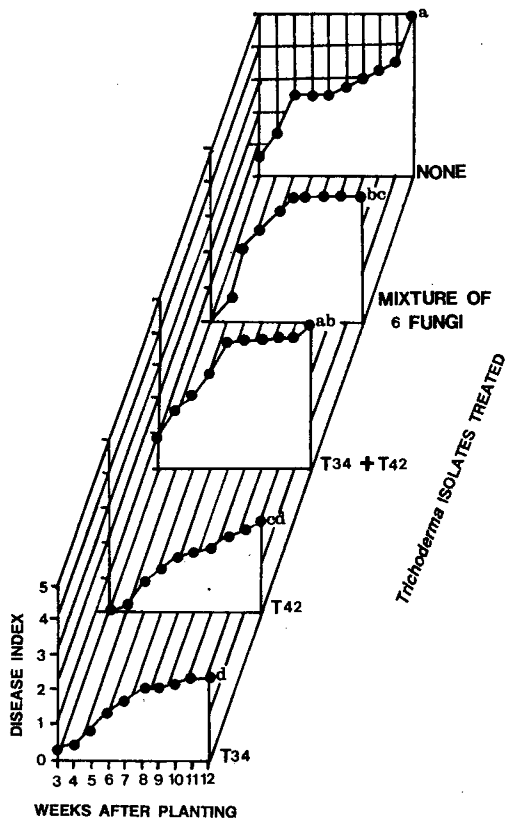

Fig. 3. Effects of different isolates *Trichoderma* spp., which were grown on rice straw, on the biological control of the strawberry wilt caused by *F. oxysporum* f. sp. *fragariae* in a pot experiment. Mixture of 6 fungi: *T. harzianum* (isolates T42 and T17), *T. viride* (isolates T74 and T34) and other two isolates of *Trichoderma* species. Disease index: 0-healthy plant, 1-stunted plant, 2-plant with 1~2 deformed or yellowish leaves, 3-plant with more than 3 deformed or yellowish leaves, 4-withered plant, and 5-dead plant. Treatments with different letters are significantly different (p=0.05) according to Duncan's multiple range test.

RESULTS

Effect of substrates on the biological control. Fig. 1 illustrates the effect of substrates for *T. harzianum* on the biological control of the strawberry wilt caused by *F. oxysporum* f. sp. *fragariae* in the pot experiment. Disease indices in the treatments with *T. harzianum*, regardless of the substrates used for the antagonist, were lower than the untreated control which had been treated only with the pathogen. There were no significant differences among the substrates used in this study, even though wheat bran and rice straw inocula of *T. harzianum* showed the highest effect against the *Fusarium* wilt disease among the substrates, reducing 68% of the disease severity (1.6 as for disease index) compared to the control. Inoculation of the conidial suspension of *T. harzianum* alone was also effective, showing 54% of disease reduction compared to the control.

Effect of soil pH. Fig. 2 shows the effect of soil pH on the suppression of the *Fusarium* wilt of strawberry by *T. harzianum* T42 and *T. viride* T74 which were grown on rice straw, an effective substrate for *T. harzianum*. In case of the treatment of *T. harzianum*, disease indices at pH 3.5 and pH 5.5 were lower than at pH 7.5 at 12 days after inoculation, although there were no significant differences in disease index among soil pH's for *T. viride* T74.

Effect of the mixture of antagonists. An experiment was carried out to evaluate the effect of the treatment with the mixture of different *Trichoderma* isolates on the disease suppression. The results showed that the disease index in a single treatment of *T. har-*

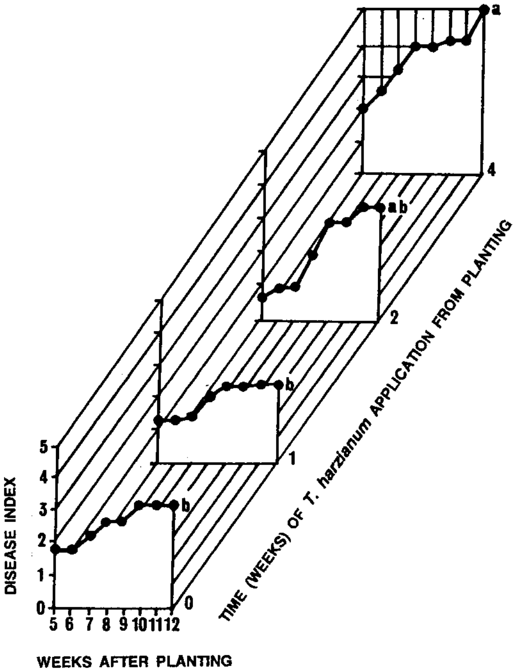

Fig. 4. Effects of application time of *T. harzianum* (isolate T42), which was grown on rice straw, on the biological control of the strawberry wilt caused by *F. oxysporum* f. sp. *fragariae* in a pot experiment. Disease index: 0-healthy plant, 1-stunted plant, 2-plant with 1~2 deformed or yellowish leaves, 3-plant with more than 3 deformed or yellowish leaves, 4-withered plant, and 5-dead plant. Treatments with different letters are significantly different (p=0.05) according to Duncan's multiple range test.

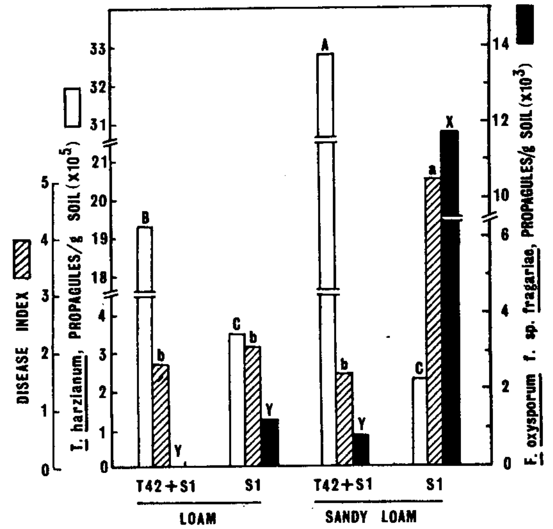

Fig. 5. Disease severities induced by *F. oxysporum* f. sp. fragariae, and the population densities of *T. harzianum* and *F. oxysporum* f. sp. *fragariae* in two different soil types 12 weeks after treatment with or without the rice straw inoculum of *T. harzianum*. T42: *T. harzianum* (isolate T42). S1: *F. oxysporum* f. sp. *fragariae* (isolate S1). Disease index: 0-healthy plant, 1-stunted plant, 2-plant with 1~2 deformed or yellowish leaves, 3-plant with more than 3 deformed or yellowish leaves, 4-withered plant, and 5-dead plant. Treatments with different letters are significantly different (p=0.05) according to Duncan's multiple range test.

zianum T42 isolate or *T. viride* T34 isolate was significantly lower than that in the treatment with the mixtures of isolates (Fig. 3).

Effect of application time of the antagonist. *T. harzianum* T42, grown on rice straw, was applied in soil at different periods of time before planting and inoculation of the pathogen. There were significant differences in disease severity (disease index) among the application times, showing that the disease was controlled more effectively in the simultaneous and 1-week-previous treatments than in the 4-week-previous treatment (Fig. 4). No suppressive effect on the disease was noted in the 4-week-previous treatment.

Effects of soil type. Fig. 5 illustrates the disease indices and the population densities of the pathogen and the antagonist in two soil types (loam soil and sandy loam soil). In the loam soil, there were no significant differences in the disease severity and the reduction of the pathogen population between the treatment of *T. harzianum* T42 and the untreated control. However, in the sandy loam soil, the antagonist treatment showed an antagonistic effect, reducing 68% of the disease severity and decreasing over 90% of the pathogen population. In the loam soil with the pathogen treatment alone, the disease severity and the population density of the pathogen were decreased as much as in loam soil with the antagonist treatment.

DISCUSSION

It was found from the previous *in vitro* experiments that the major factors affecting on the mycoparasitism of *Trichoderma* species were the nutritional components in media, and that the plant organic materials containing a high carbon content and a low nitrogen content improved the mycoparasitism (22). In the present study, all the tested substrates for culturing *T.*

harzianum, especially wheat bran and rice straw, were effective in increasing the biological control activity of *T. harzianum*. The results are similar to those of other studies (6, 7, 8, 10) and *in vitro* experiments showing that corn powder improved the mycoparasitic activity of *T. harzianum* in medium (22) as well as the comparative saprophytic ability in soil (24), and that maltose was a major factor in the mycoparasitism and the enzyme activity of *T. harzianum* (22, 23). Thus, if corn powder or malt with a high maltose content is used as a substrate for *T. harzianum*, the biological control activities of *T. harzianum* may be increased.

Chitin and crab were used for the biological control of soilborne diseases and showed significant control effects (1, 9, 16). Also in our study, crab shell reduced the incidence of the *Fusarium* wilt disease. However, the reduction of the disease incidence by the crab shell treatment was not significantly different from other substrates used, and even slightly less than that by wheat bran or rice straw. The reason was not examined, but it may be due to the low concentration of chitin in the crab shell treatment (i.e. 0.23 g of crab shell containing only 0.3% chitin was treated in a pot), or the improper time of the application. In the present study, crab shell was applied at the transplanting time; however, Khalifa (16) reported that *Fusarium* wilt of peas was significantly reduced when the crab shell was treated at 3~8 weeks prior to transplanting, compared with that treated at the time of transplanting.

The results in our study that the antagonistic effect of *T. harzianum* was increased in acid soil are generally consistent with those of other studies (4, 11, 12, 17, 19, 20, 27, 30, 32), in which the soil acidification promoted the biological control of diseases by *T. harzianum* and *T. viride*. The results also agreed with those of our previous studies on the influence of soil pH on the mycoparasitism, enzyme activity and antagonistic activity of *T. harzianum* in soil (22, 23, 24), suggesting that soil acidification may be an important factor for improving the biological control effect of the antagonists. On the contrary, there are some reports (15, 34) that soil alkalization by using lime resulted in controlling *Fusarium* diseases, and this might be due to the enhanced survival and activity of antagonistic bacteria in less acidic conditions (29).

In this study, the biological control of the *Fusarium* wilt of strawberry by the antagonist, *T. harzianum* T42, was more effective in sandy loam soil than in loam soil, significantly decreasing the disease severity and the pathogen population, and increasing the antagonist population. The disease severity and the population density of the pathogen were lower in the loam soil than in the sandy loam soil, regardless of the antagonist treatment, suggesting that the activities of the pathogen were suppressed in the loam soil. The decrease of the disease in the loam soil may be related to other factors such as physical and chemical properties of soil.

요 약

*Trichoderma harzianum*의 딸기 시들음병균(*Fusarium* oxysporum f. sp. *fragariae*)에 대한 생물적 방제 효과를 제고하기 위한 여러 가지 요인을 폿트실험에서 검정하였다. 밀기울, 볏짚, 톱밥, 왕겨 또는 보리짚 등 유기물에 *T. harzianum*을 배양하여 병원균 접종 토양에 처리하였을 때 유기물에 관계없이 딸기 시들음병이 유의적으로 감소하였으며, 유기물 중에서 밀기울 또는 볏짚 처리에서 특히 방제효과가 우수하여 무처리에 비하여 발병정도가 68% 억제되었다. 또한 게껍질 또는 분생포자 부유액만을 처리하여도 병방제에 효과적이었다. *T. harzianum*의 생물적 방제는 산성토양 (pH 3.5~ 5.5)에서만 그 효과가 나타났으며, 사양토에서도 발병율과 병원균의 밀도가 감소되어 그 효과가 높았던 반면, 양토에서는 *T. harzianum*의 처리에 의한 방제효과가 나타나지 않았다.

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