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Determination of Mycorrhizae Interactions and Pathogenicity of *Rhizoctonia solani* Kühn Isolated from Strawberry and *Xanthium strumarium*

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Abstract: The effects of mycorrhizal fungi on the disease severity of *Rhizoctonia solani* Kühn., and the role of mycorrhizal fungi on strawberry (*Fragaria vesca* L.) growth were investigated. Strawberry seedlings were inoculated with BioOrganics as a mycorrhizal preparation against 2 *R. solani* isolates, which were isolated from strawberry (RsFv) and *Xanthium strumarium* (RsXs). The highest disease severity in strawberry roots was observed after inoculation with RsFv at a rate of 67%. On the other hand, the lowest disease severity in strawberry roots was at 31% for 15BRsFv (15 days before inoculation with BioOrganics and RsFv). Similarly, the highest disease severity in plants was seen with RsXs inoculated plants at a rate of 77%. The disease severity in plants was at 35% for 15BRsXs (15 days before inoculation with BioOrganics and *R. solani* isolated from *Xanthium strumarium* L.).

When strawberry leaf and shoots were evaluated, the disease severity varied as follows: 45% for RsFv, 40% for RsXs, 15% for BRsFv, 10% for BRsXs, 15% for 15BRsFv, and 5% for 15BRsXs. According to these results, there was no significant difference between the pre-inoculation of mycorrhizae (15 days) and simultaneous inoculation with a pathogen.

Based on our findings, we concluded that when all plant growth parameters were treated with mycorrhizae (either alone or with *R. solani*) strawberry plant growth was greatly improved, and our observations showed the beneficial effects of mycorrhizae on yield.

Key Words: Strawberry, mycorrhizae, *Rhizoctonia solani*

Çilek ve Domuz Pıtrağından (*Xanthium strumarium*) İzole Edilen *Rhizoctonia solani* Kühn.'nin Patojenisitesi ve Mikoriza Etkileşimi

Özet: Bu çalışma çilekte (*Fragaria vesca*) *Rhizoctonia solani* Kühn.'nin neden olduğu hastalık şiddetine ve çilek bitkisinin gelişimine mikorizal fungusların etkisini değerlendirmek amacıyla ele alınmıştır. Çilekte patojen oldukları bilinen biri çilekten (RsFv), diğeri domuz pıtrağından (*Xanthium strumarium* L., RsXs) izole edilmiş 2 *Rhizoctonia solani* izolatına karşı mikorizal bir preparat olan BioOrganics ile inokule edilen çilek fideleri kullanılmıştır. En yüksek hastalık şiddeti RsFv ile inokule edilen bitkilerin köklerinde % 67 olarak saptanırken, en düşük hastalık şiddeti 15BRsFv (RsFv inokulasyonundan 15 gün önce BioOrganics uygulaması) uygulamasında % 31 olarak saptanmıştır. RsXs ile inokule edilen çilek bitkilerin köklerinde de hastalık şiddeti yine yüksek olmuş ve % 77 olarak saptanmıştır. 15BRsXs (RsXs uygulamasından 15 gün önce BioOrganics uygulaması) uygulamasında ise hastalık şiddeti % 35 olmuştur. Çilek bitkilerinin yaprak ve sürgünlerinde görülen hastalık şiddeti değerlendirildiğinde ise RsFv uygulamasında % 45, RsXs uygulamasında % 40 olurken, BRsFv % 15, BRsXs % 10, 15BRsFv % 15 ve 15BRsXs % 5 olmuştur. Bu sonuçlara göre patojen uygulamasından 15 gün önce mikoriza uygulamasıyla, mikoriza ile birlikte patojen uygulaması arasında istatistiki olarak bir farklılık bulunmamıştır. Elde ettiğimiz bulgulara göre mikoriza ile inokule edilen bitkilerin (tek başına veya *R. solani* ile birlikte) gelişimlerinin artığı ve verim üzerine de olumlu etkilerinin olduğu gözlemlenmiştir.

Anahtar Sözcükler: Çilek, mikoriza, *Rhizoctonia solani*

Introduction

Strawberries are largely grown in the Aegean, Mediterranean, and Marmara regions of Turkey (1). Aydın province is one of the most important centers in the Aegean region, which is responsible for 60% of strawberry production; this province provides 8.4% of the total strawberries grown in Turkey. Sultanhisar, a town in Aydın province, supplies 92% of total strawberry

production from the province, which amounts to 12,320 t (2,3).

There are various fungal diseases that are responsible for significant strawberry yield losses in Turkey. The most important soil-borne fungal diseases found in Aydın are *Phytophthora* spp., *Rhizoctonia solani* Kühn., and *Verticillium dahliae* (4,5). Existing treatment approaches to such problems can be costly, difficult, and even discouraged due to synthetic pesticides. As a result, alternative treatments have been investigated for overcoming soil-borne fungal problems. The focus of alternative treatments of soil-borne fungi is on the replacement of widely used synthetic pesticides. One of the most important alternative treatments revolves around mycorrhizae (6,7). Vesicular arbuscular mycorrhizae (VAM) have been shown to be an important tool in the biological control of soil-borne pathogens including *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Verticillium*, and *Thielaviopsis* as well as various nematodes not only in sustainable but also in organic agriculture (8).

The positive effects of mycorrhizal fungi on plant development are well known. In a study on the favorable effects of mycorrhizae varieties on *R. solani* in 2 different potato varieties it was reported that disease due to *R. solani* was reduced in shoots and crowns by 60%-71.2% in plants inoculated with *Glomus etunicatum* (9). In-vitro studies showed that strawberry plant roots inoculated with *G. etunicatum* and *G. monosporum* reduced *Phytophthora fragariae* sporulation by 67% and 64% respectively in 48 h (10). Strawberry plants inoculated with a preparation that included *Glomus intraradices* and *G. etunicatum* showed increased crown width, leaf area, and dry root weight (11). It has also been reported that strawberry and clover plants inoculated with 4 different *Glomus* spp. showed better growth in the presence of mycorrhizae (12) and mycorrhizal fungi, resulting in increased fruit count per strawberry plant (13).

We have been focusing on the benefits of mycorrhizae in various agriculturally important products. This study is important to highlight the possibility and success of using mycorrhizae for disease prevention in strawberrys grown in Aydın against a common pathogen like *R. solani,* especially isolated *Xanthium strumarium* L. Specific findings pertain to the effects of mycorrhizal fungi on the disease severity of *R. solani*, and the role of mycorrhizal fungi on plant development.

Materials and Methods

Strawberry plants (*Fragaria vesca* cv. Camarosa) constituted the plant component of our study. We used a mycorrhizal preparation as symbiont named BioOrganics Root Dip Inoculant (BioOrganics Inc.) (*Glomus aggregatum*, *G. clarum*, *G. deserticola*, *G. intraradices*, *G. monosporus*, *G. mosseae*, *Gigaspora margarita*, and *Paraglomus brasilianum*) and as a pathogen we used 2 *R. solani* (previously described as AG4) isolates: RsFv isolated from strawberry and RsXs isolated from *X. strumarium*. Both were identified as pathogenic to strawberry.

Strawberry seedlings were grown in a plastic pot (18 × 18 cm) containing a sterilized mixture of soil and sand (1/1, v/v). This study was designed to study BioOrganics alone (B), *R. solani* (RsFv isolated from strawberry; RsXs isolated from *X. strumarium*), BioOrganics Root Dip Inoculant and *R. solani* (BRsFv, BRsXv), and control (C) applications. In a parallel study, plants inoculated with mycorrhizae 15 days before pathogen inoculation were planted in pots filled with *R. solani* (15BRsFv, 15BRsXs) contaminated soils. Plants were periodically watered with deionized water during the study. In addition, each week, the pots were supplied with 100 ml of a nutrient solution containing (mg l^{-1}): 720 mg of $MgSO_4.7H_2O$, 12.2 mg of KH_2PO_4, 295 mg of $Ca(NO_3)_2.4H_2O$, 240 mg of KNO_3, 0.75 mg of $MnCl_2.4H_20$, 0.75 mg of KI, 0.75 mg of $ZnSO_4.7H_2O$, 1.5 mg of H_3BO_3, 0.001 mg of $CuSO_4.5H_20$, 4.3 mg of FeNaEDTA, and 0.00017 mg of $Na_2MoO_4.2H_2O$ (14).

Inoculum production and inoculation of *R. solani*

Corn meal and sand culture (135 g of soil, 15 g of corn meal, and 20 ml of potato juice) was used in the production of *R. solani* inoculum. The corn meal and sand cultures were sterilized in an autoclave at 121 ºC on 2 separate occasions lasting 60 min each (15). Mycelia containing disks removed from colonies grown on potato dextrose agar (PDA) for 1 week were mixed with soil culture in 500 ml sterile erlenmeyer flasks and were allowed to develop for 4 weeks in an incubator at 20 ºC. Pots filled with sterile media were then inoculated with *R. solani* inoculum at a ratio of 1/19 (v/v) (16).

Simbiont inoculation was carried out by dipping strawberry seedlings into a solution prepared with 42 cc BioOrganics Root Dip Inoculant in 1 l of deionized water. Strawberry transplants were dipped into the mycorrhizal inoculums and planted in sterile and *R. solani* inoculated soil.

Experimental design and evaluations

This study was carried out in an unheated glasshouse under natural daylight from April to June 2006 in Aydın. The experiment was arranged 5 replicates with 5 plants in each replicate. Four months after inoculation, the plants were evaluated for morphological attributes including number of leaves and daughter plants and shoots, and root fresh and dry weights (dried 70 ºC for 24 h) and the results recorded. Disease severity of shoots and leaves was rated on a scale of 0-4 (0 = no wilt symptoms; 1 = fewer than 25% of the leaves wilted; 2 = 26%-50% of the leaves wilted; 3 = 51%-75% of the leaves wilted; 4 = 76%-100% of the leaves wilted) (17), while a universal 0-5 scale (0 = no wilt symptoms; 1 = lesions fewer than 3 mm of the crown; 2 = 3-10 mm lesions; 3 = 10-20 mm lesions; 4 = <20 mm lesions on crown; 5 = dead plant with necrotic crown) was used in assessing the disease severity of roots (18). The data were evaluated by Fisher's least significant difference (LSD) test, using the JUMP IN program (SAS Institute, Cary, NC, USA) at P = 0.05.

Results and Discussion

The effect of mycorrhizal fungi on severity of disease in *Rhizoctonia solani*-strawberry pathosystem

Our observations during the growth period of plants revealed that mycorrhizae applications significantly reduced disease severity (caused by *R. solani*) in strawberry plants. RsFv and RsXs resulted in varied virulence (Table 1). Based on these results, disease severity in strawberry plant roots inoculated with RsFv was 67%, while this level was 77% in RsXs inoculated plants. In addition, green parts of plants had disease severity of 45% for RsFv and 40% for RsXs inoculated plants. All mycorrhizae treatments reduced disease severity in the strawberry plants (Table 1). Similar findings were reported in previous studies involving various plants grown in the presence of other pathogenic fungi (8,9,19,20).

It should be noted that *R. solani* isolated from *X. strumarium* showed high disease severity compared to *R. solani* isolated from strawberry. Inoculation of strawberry seedlings with mycorrhizae (BioOrganics) either 15 days pre-inoculation or at the same time with a pathogen inhibited disease severity. These results indicate that the type of beneficial effects offered by mycorrhizae is very rapid and independent of the pathogen. Therefore, allowing mycorrhizae to grow in the absence of pathogen does not enhance the impact of mycorrhizae on the pathogen.

Effects of mycorrhizal fungi on strawberry plant growth

Analysis of variance of the complete data set indicated significant differences among mycorrhizae and *R. solani* treatments. Mycorrhizae and *R. solani* treatments were compared with respect to strawberry plant leaf and daughter plant count, fresh and dry leaf weight, root fresh, and dry weight. Absolute values of plant fresh and dry weights as well as root fresh and dry weights were considerable lower in the presence of the pathogen. The decreases in fresh root weight, dry plant weight, and dry root weight were statistically significant in the presence of RsFv (Table 2).

When all evaluation criteria were considered, improved plant growth was observed with mycorrhizae

Table 1. Disease severity (%) of shoot and roots of strawberry inoculated with *R. solani*.

Disease severity (%)	Treatments							
	B	RsXs	RsFv	BRsXs	BRsFv	15BRsXs	15BRsFv	C
Shoot	0	40	45	10	15	5	15	0
Root	0	77	67	37	35	35	31	0

B: Strawberry plants inoculated with BioOrganics; RsXs: Strawberry plants inoculated with *R. solani* isolated from *X. strumarium*; RsFv: Strawberry plants inoculated with *R. solani* isolated from strawberry; BRsXs: Strawberry plants inoculated with BioOrganics and RsXs; BRsFv: Strawberry plants inoculated with BioOrganics and RsFv; 15BRsXs: Strawberry plants inoculated with BioOrganics 15 days before pathogen inoculation were planted in pots filled with RsXs; 15BRsFv: Strawberry plants inoculated with BioOrganics 15 days before pathogen inoculation were planted in pots filled with RsFv; C: Control

Table 2. The average values of investigated characteristics in *R. solani* strawberry pathosystem.

Treatment	Number of leaves	Fresh plant weight (g)	Fresh root weight (g)	Number of daughter plants	Dry plant weight (g)	Dry root weight (g)
B	12.3 ab*	11.9 a*	16.9 a*	2.2 ab*	4.7 ab*	5.3 a*
15BRsXs	13.7 a	8.9 abc	9.8 bc	1.0 c	4.7 ab	4.3 ab
15BRsFv	8.2 bc	7.6 bc	13.1 abc	2.7 a	2.2 c	3.7 ab
BRsXs	13.5 a	9.8 abc	14.3 abc	1.0 c	5.2 a	4.2 ab
BRsFv	9.3 abc	10.9 ab	16.9 a	2.7 a	3.1 b	4.8 ab
RsXs	13.0 ab	6.5 c	10.5 bc	1.7 b	4.3 ab	4.3 ab
RsFv	4.8 c	6.6 c	8.8 c	1.8 b	1.8 c	2.7 b
C	11.2 ab	10.22 abc	15.4 ab	1.8 b	4.6 ab	6.0 a

(*) There is no statistical difference among entries with the same letter in each column, LSD test ($P < 0.05$).

treatment. It has also been reported that strawberry plants inoculated with a preparation that included *Glomus intraradices* and *G. etunicatum* showed increased crown width, leaf area, and dry root weight (11), and strawberry and clover plants inoculated with 4 different *Glomus* spp. showed better growth in the presence of mycorrhizae (12). When mycorrhizae inoculation was performed 15 days prior to *R. solani* (RsXs, RsFv), it was compared to simultaneous inoculation of mycorrhizae and *R. solani* and no significant differences were observed.

When RsXs treated plants were compared to RsFv treated plants statistically significant differences among plant leaf count and plant dry weights were confirmed. We also observed that plants treated with mycorrhizae showed better growth compared to control plants. *R. solani* reduced strawberry plant and root weight. *R. solani* isolated from weeds also caused high disease severity in strawberry. This finding is very important in our strawberry production system, because there is no cultivation in strawberry fields in the summer except for irrigation. For this reason the weed population increased in the fields, as a host of pathogens, from July to February. Therefore, mycorrhizae inoculation is important after considering the plant, *R. solani* incidence, and mycorrhizae benefits. Our results demonstrated that inoculation with BioOrganics reduced development of *R. solani* disease in strawberry plants and improved strawberry plant growth.

The exact mechanisms by which AM fungal colonization confers protective effects are not completely understood. A more in-depth understanding of these beneficial interactions is necessary for the exploitation of mycorrhizal fungi within organic and/or sustainable farming systems (8). In this study, we discuss the potential effect of mycorrhizal fungi with respect to its contribution to bioprotection against plant soil-borne pathogens. Bioprotection within AM fungal-colonized plants is the outcome of complex interactions between plants, pathogens, and AM fungi. Future studies will focus on determination of indigenous mycorrhizae species and investigation of the possibility of using these species against pathogenic fungi.

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