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THE DETERMINATION OF THE EFFICACY OF SOME MICROBIAL PREPARATIONS AGAINST APPLE SCAB DISEASE (*VENTURIA INAEQUALIS* (CKE) WINT.) IN ISPARTA

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Abstract

The effect of *Trichoderma harzianum* (T-22), *Bacillus subtilis* (BS), mycorrhizal preparations (Endo Roots Soluble) and combinations was investigated against apple scap disease [*Venturia inaequalis* (Cke.) Wint.]. Biological preparations were applied as foliar and soil application in different combinations. The experiment was established in gardens with a Scarlet-Spur apple variety of 3 years old in Eg�irdir. Biological preparations were applied at 3 times in April-May 2017, taking into account the warnings of the early warning system at the recommended doses. Dictotom blue (Mancozeb) + Nimbus (Micobuthanil) was used as commercial fungicide. After 21 days of application, disease severity was assessed according to scale 0-4. The results obtained according to the scale values were calculated to the % disease severity by applying the Towsend-Heuberger formula and the% effects of the biological preparations according to the Abbott formula. Test results were evaluated by Tukey multiple comparison test using variance analysis with SPSS 21 statistical program. As a result, the difference between all the applications was found significant ($p<0.05$). Based on the results of the experiment, the lowest disease severity was obtained from T-22 (foliar application) (12.2%) against *Venturia inaequalis.* Disease severity was determined in the application of Dicotom blue + Nimbus (13.2%) and Mycorriza (14.8%) respectively. The percentage of disease severity in control plants was observed as 40.2%. In the experiment, it was determined that the highest % efficacy values of the biological preparations were for T-22 (foliar application) (69.65%), T-22 (foliar application) + *B. subtilis* (foliar application), (67.66%) Dikotom blue+Nimbus (67.19%) and mycorrhiza (soil application) + *B. subtilis* (foliar application), (64.18%) respectively. The lowest % efficacy values of the biological preparations were determined in the application of *B. subtilis* (foliar application) (48.76%), *T. harzianum* (soil application) (53.23%) and T-22 (soil application) + *B. subtilis* (foliar application) (58.71%). As a result, biological preparations could be used as an alternative to chemical control, because of its positive aspects in terms of environment and food safety.

Keywords: Mycorrhizza, *Trichoderma hazrianum, Bacillus substilis*, Biological control, Apple scab, Scarlet-spur

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1. Introduction

Apple is a temperate climate fruit and it can grow in every province of our country. Isparta province has an important place in our country due to its ecological conditions in terms of apple cultivation. Especially Egirdir, Gelendost, Senirkent, Yalvaç and Gönen districts, especially apple production are cultivated in

Isparta region. According to the Turkish Statistical Institute in 2015, apple production in our country is 2.569.759 tons, of which 435.938 tons is produced in Isparta (TUIK, 2015).

There are many pests, diseases and weeds which cause economic losses in apple orchards in our country (Birişik et al., 2009). A serious disease of apples and ornamental crabapples, apple scab (*Venturia inaequalis*: [(Cooke) G. Winter 1875] (Pleosporales: Venturiaceae)) attacks both leaves and fruit. Apple scab occurs everywhere in the world where apples are grown and causes more losses than any other apple disease. It is most serious in areas that have cool, wet weather during the spring and may not be economically important in warm or dry climates. This disease causes a 60% depreciation of the eternal apple orchards (Agrios, 1997) in terms of yield and quality (Turkoglu, 1978, Kaymak, 2012).

Control of the disease currently depends on frequent application of fungicides. Its significance is indicated by the fact that up to 20 fungicide treatments are performed per season to control apple scab disease. Resistance of the Venturia fungus to an increasing number of chemical fungicides in major apple growing areas has arisen over recent years.

The development of novel antagonists for biological control of apple scab may offer alternative options for disease control. Research on biological control of V. inaequalis mainly focussed on the overwintering stage of the pathogen in fallen leaves (Carisse et al., 2000). Only few reports described preliminary work on the possible use of biological control against apple scab (Fiss et al., 2000; Burr et al., 1996, Köhl et al., 2006).

Apple scab disease (*Venturia inaequalis*), one of the most important fungal diseases of apple trees, can cause serious loss of fruit production. In a study conducted by Boyraz in 2005, it was reported that during the rainy season in Eğirdir, Isparta, trees were sprayed 20-25 times against apple scab disease (Boyraz et al., 2005). Similarly, in Switzerland, it has been reported that 15-20 times of spraying against apple scab disease (Gygax et al., 2004).

It is becoming compulsory to control fungicides for the struggle of plant diseases which causes considerable loss of crops in culture plants. For this reason, the risk of resistance against pathogens, but also their negative effects on the environment and people who used pesticide is another negative factor arising from the implementation of this method (Demirci, 1996).

In our study was to assess the effects of mycorrhizae (ERS i.e., *Glomus intraradices, Glomus aggregatum, Glomus mosseae, Glomus clarum, Glomus monosporus, Glomus deserticola, Glomus brasillianum, Glomus etunicatum* and *Glomus margarita* mycorrhizal fungi as live content 23.5%), *Trichoderma harzianum* (T-22 Planter Box i.e. 1.15% *Trichoderma harzianum* Rifai strain KRL-AG2) and *Bacillus subtilis* [Serenade SC i.e. 1.34% *Bacillus subtilis* strain QST 713 (min. 1x109 cfu/ml)] and their combinations against *Venturia inaequalis* disease on orchard conditions.

2. Materials and Methods

The study was carried out on apple orchard in Eğirdir, Turkey during 2016-2017. In this study scarlet spur apple (figure 1) variety known to be susceptible to apple scab disease was used (Planting year 2013). All trials were conducted in a fully randomized block design. *Trichoderma harzianum* Rifai strain KRL-AG2 (T-22 Planter Box), *Bacillus subtilis* strain QST 713 (Serenade) and Mycorrhiza spp. (ERS, i.e, 23.5% in terms of live content) of *Glomus intraradices, Glomus aggregatum, Glomus mosseae, Glomus clarus, Glomus monosporus, Glomus deserticola, Glomus brasillianum, Glomus etnicatum* and *Glomus margarita* mycorrhiza fungi were tested in orchards.

Figure 1. Orchards of Scarlet spure

2.1. Treatment and assessments

T. harzianum, B. subtilis, Mycorrhiza and combination were applied as biological preparations (Table 1). *T. harzianum, B. subtilis* applications were sprayed on the apple trees and applied from soil. Mycorrhiza was applied only from soil. Preperats were applied at doses recommended by the company at regular intervals usually 7 days for 5 times. The basic treatments in the trials were; 1: untreated control 2: *T. harzianum, B. subtilis,* Mycorrhiza and combination 3: A reference fungicide schedule (figure 2). Fungicides used were Myclobutanil (Nimbus 24 EC -Hektaş) Mancozeb (Dikotan Blue-Koruma Tarım).

21 days after last application, plants were evaluated according to scale 0-4 (Table 2) (Anonymus, 1996). The disease severity was evaluated using Townsend-Heuberger's formula (Towsend, Heuberger, 1943). The

percentage effect of the applications was calculated using the Abbott formula (Abbott, 1925).

Table 1. Combination table of applications against *V. inaequalis*

Application of trial materials	Number of plant
Mycorrhiza (ERS) (soil)	5
T. harzianum (T-22) (Foliar)	5
T. harzianum (T-22) (Soil)	5
B. subtilis Qst 713 strain (Foliar)	5
Mycorrhizae (ERS) (Soil)- *B. subtilis* Qst 713 strain (Foliar)	5
T. harzianum (T-22) (Foliar) - *B. subtilis* Qst 713 strain (Foliar)	5
T. harzianum (T-22) (Soil) - *B. subtilis* Qst 713 strain (Foliar)	5
Fungicide (Foliar)	5
Control (water)	5

Table 2. Apple scab scale value.

Scale Value	Definition of disease
0	No spot
1	Up to 5 spots with less than 5 mm
2	Up to 5 spots greater than 5 mm or more than 5 spots with less than 5 mm
3	More than 5 spots greater than 5 mm
4	More than half of the leaf covered with spots

2.2. Statistics

Data from each trial in Eğirdir, Isparta were analyzed by analysis of varience (ANNOVA) to detect differences between treatments. Mean comparisons were made using Duncan's tests; all statistical tests were conducted at a probability level of P ≤ 0.05. All analyses were performed using the SPSS 21 software.

3. Results

T. harzianum, *B. subtilis,* Mycorrhiza and combination were applied to leaves and soil the production of conidia by *V. inaequalis* was quantified on treated leaves. All applications reduced significantly diseases. The lowest disease severity was obtained from T-22 (foliar application) (12.2%) against *V. inaequalis*. Disease severity was determined in the application of Dicotom blue+Nimbus (13.2%) and Mycorriza (14.8%) respectively. The percentage of disease severity in control plants was observed as 40.2%. In the experiment, it was determined that the highest % efficacy values of the biological preparations were for T-22 (foliar application) (69.65%), T-22 (foliar application) + B. subtilis (67.66%), Dikotom blue+Nimbus *B. subtilis* (67.66%), Dikotom blue+Nimbus (67.19%) and mycorrhiza+*B. subtilis* (64.18%) respectively. The antagonist *T. harzianum* (foliar application) was found to significantly reduce sof *V. inaequalis*, the fungus causing apple scab. The lowest % efficacy values of the biological preparations were determined in the application of *B. subtilis* (48.76%), *T. harzianum* (soil application) (53.23%) and T-22 (soil application) + *B. subtilis* (foliar application) (58.71%) (Table 3). Similar results were obtained in both years.

Table 3. Efficiency of the biological preparations used in the experiment against *V. inaequalis*

Applications	Disease Severity %	Effect %
Mycorrhiza (ERS) (soil)	14.8	63.18[ab]
T. harzianum (T-22) (Foliar)	12.2	69.65[a]
T. harzianum (T-22) (Soil)	18.8	53.23[ab]
B. subtilis Qst 713 strain (Foliar)	20.6	48.76[b]
Mycorrhizae (ERS) (Soil)- *B. subtilis* Qst 713 strain (Foliar)	14.4	64.18[ab]
T. harzianum (T-22) (Foliar) - *B. subtilis* Qst 713 strain (Foliar)	13	67.66[a]
T. harzianum (T-22) (Soil) - *B. subtilis* Qst 713 strain (Foliar)	16.6	58.71[ab]
Fungicide (Foliar)	13.2	67.16[a]
Control (water)	40.2	

Figure 2. a) T-22 (leaf) application b) ERS (soil)-BS application (foliar) c) T-22 (soil) - BS application (foliar) d) Controllable seedlings with *V. inaequalis.*

As a result of the applications, high efficacy results have been obtained in T-22 (foliar application) (69.65%), T-22 (foliar application) + *B. subtilis* (Foliar application) (67.66%) and mycorrhiza + *B. subtilis* (Foliar) (64.18%) applications (P<0.05). In the application mycorrhiza (ERS), *T. harzianum, B. subtilis*, it was found that they suppressed the *V.inaequalis* disease. As a result, biological preparations could be used as an alternative to chemical control, because of its positive aspects in terms of environment and food safety.

4. Discussion

Biological control of foliar diseases has not attracted as much attention as biological control of soilborne. Furthermore, tolerance of apple scab to certain key fungicides, accelerating cost of chemical controls and increasing restrictions make development of biocontrol options attractive. Because of that, we decided to investigate the biological control to apple scab disease. The result of our work showed that mycorrhiza (ERS), *T. harzianum, B. subtilis* reduced scab on Scarlet Spur. Many studies of the effects of AMFs against fungal diseases on different plants and pathogens have found that in some cases there was effect, as well as suppressive, inhibitory, or enhancing effects of disease (Schenck, 1987). Schaefer et al., (2008) cultivated plants of the apple cv. 'Pinova' and plants of two transgenic 'Pinova' lines (T386 and T389) in a sand-vermiculite substrate and inoculated them with the AMF *Glomus intraradices* and *G. mosseae.* After four months of growth both transgenic lines showed significantly reduced root colonization rates. The influences of *Microsphaeropsis* sp. *M. arundinis, Ophiostoma* sp. *Diplodia* sp. and *Trichoderma* sp. all antagonists of *Venturia inaequalis,* on ascospore production were evaluated under natural conditions by Carisse et al., (2000). All treatments, except those with *Ophiostoma* sp. resulted in a significant reduction in *V. inaequalis* ascospore production on the leaf disks incubated under controlled conditions or in the orchard. Leaves treated with *Microsphaeropsis* sp. *Trichoderma* sp. *A. bombacina*, and *M. arundinis* reduced ascospore production by 84.3, 96.6, 75.2, 96.6, and 52.2%, respectively.

Bolar et al., (2000) found that the chitinases of *Trichoderma harzianum* cause an increase in resistance to apple scab. Bolar et al., (2001), obtained antifungal proteins from *Trichoderma atroviride* and transferred to the endocytinase and exocitinase (N-acetyl-β-D-hexosaminidase) Marshall McIntosh applespeed, singly and in combination.

Köhl et al., (2015) tested Cladosporium *Cladosporium cladosporioides* H39, originating from a sporulating colony of *V. inaequalis,* to control apple scab development was in eight trials during 2 years in orchards in Eperjeske (Hungary), Dabrowice (Poland), and Bavendorf (Germany) planted with different cultivars. Treatments were conducted as calendar sprays or after infection periods. The overall results of the field trials consistently showed for the first time that stand-alone applications of the antagonist *C. cladosporioides* H39 reduced apple scab in leaves and fruit. The antagonist was also effective if applied one or even several days (equivalent to approximately 300 to 2,000 degree h) after infection events in several field trials and a trial conducted in Randwijk with single-spray applications at different intervals before or after infection events.

As a result; the development of novel antagonists for biological control of apple scab may offer alternative options for disease control.

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