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Effects of Triazole and Strobilurin-Based Fungicides on *Fusarium culmorum* on Wheat

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Abstract

Fusarium culmorum is an important pathogen, that causes pre and post-emergence death in seedlings from both seed and soil-borne sources, in addition to causing root, crown root and wheat head infections on wheat. Treatment of seeds with fungicides is especially important to inhibit seed originated seedling infections when cultural methods are ineffective against the infection of the pathogen in root and crown root and the lack of completely resistant wheat cultivars against this pathogen. The efficiency of fungicides with prothioconazole + tebuconazole and triticonazole + pyraclostrobin active ingredients, licensed on *Fusarium* spp. in seeds, on seed originated seedling infections of *F. culmorum* is determined *in vitro* and *in vivo* conditions. Seeds of Flamura-85 bread wheat cultivar, naturally infected with *F. culmorum* S-14, were used in this study. The effect of treating seeds naturally infected with *F. culmorum* S-14 with fungicides on seed germination, root length, coleoptile length and disease severity was determined in the experiment *in vitro*. Germination rate of the seed was determined as 68% for triticonazole + pyraclostrobin and 43% for prothioconazole + tebuconazole, while disease severity was 42.70% and 61.30%, respectively. While both fungicides were determined to be effective on the disease severity, the effect of triticonazole + pyraclostrobin on disease severity was found to be higher than prothioconazole + tebuconazole. Because of the higher rate of effectiveness of triticonazole + pyraclostrobin to prevent seed-borne infections *in vitro* conditions, it was determined that the fungicide causes an increase in germination rate with wet and dry weight of the seed, while significantly decreasing the disease severity, in the tests to determine the effectiveness of the fungicide *in vivo* conditions.

Keywords: *Fusarium culmorum*, fungicide, wheat

1. Introduction

Fusarium culmorum (W.G. Smith) Saccardo is an important soil/seed borne pathogen and causes head blight, root and root crown rot in wheat (Scherm et al., 2013). With the germination of conidies or long living chlamydospores in the period from wheat seed germination to spike emergence, infections might occur in the root, root crown and stem. Infections in seedling period mostly occur in coleoptile and/or root crown, because of 3-4 year longevity of pathogen chlamydospores in the soil. Infection symptoms, proceeding with the development of the plant, can also be observed after the tillering period. Bleached heads start to form on the plants with water stress related to the increase in the transpiration speed of plants. In Turkey, 50% crop loss is sometimes recorded in regions with high disease severity (Akgül, 2008; Anonymous, 2008). In highly humid Thrace region, it is the cause of economical losses in wheat by causing pre and post-emergence deaths originating from soil/seed-borne seedling infections. Cultural practices are not sufficient to control *F. culmorum* and natural conditions are suitable for disease development and there is no fully resistant cultivar against the agent (Scherm et al., 2013; Arıcı, 2006). Thus, it is obligatory to use fungicides to control the agent. Fungicide treatment of seeds against root and root crown infections of the pathogen is important to prevent seed-borne infections (Cook, 1986). When the fungicides from triazole group fungicides in Sterol biosynthesis inhibitors (SBI) fungicides such as prothioconazole, tebuconazole, and triticonazole and carboxin from succinate dehidrogenase inhibitor (SDHI), with systemic impact mechanism, are applied on the seed surface, they can effectively protect the seeds from soil/seed borne infections of *F. culmorum* by moving to cotyledon, embryo, rootlet and endosperm using the humidity in the soil (Delen, 2016). While triazole group fungicides prevent the formation of mycotoxins of wheat head blight agents *F. culmorum* and *F. graminearum*, they can also be effective on yield (Scherm et al., 2013). Hence, it is known that difenoconazole, diniconazole flutriafol, metconazole, propiconazole, prothioconazole, tebuconazole, triticonazole and prothioconazole + tebuconazole increases yield (Demirci & Maden, 2006; Paul et al., 2010). First seed treatments against *Fusarium culmorum* in wheat have started with the licensing of carboxin + thiram in mixture form in 1993, in Turkey. Fungicide mixtures with the active ingredients prochloraz + triticonazole, prothioconazole + tebuconazole and triticonazole + pyraclostrobin were licensed in 2011, 2013 and 2015 respectively. Usage of these fungicides on wheat seeds is obligatory for wheat production in our region. However, it is found that the effectiveness of the seed fungicides on pathogen can change depending on soil/seed borne infections of *F. culmorum* (Köycü & Sükut 2018). Determination of the effectiveness of fungicides with the active ingredients prothioconazole + tebuconazole and triticonazole + pyraclostrobin, licensed on *Fusarium* spp., on seed-borne infections of *Fusarium culmorum*, was the purpose of this study.

2. Materials and Methods

2.1 Wheat Seeds

F. culmorum S-14 isolate, determined as pathogenic in wheat (Köycü & Özer 2014), was inoculated in 1×10^5 (Haidukowski et al., 2012) conidia/ml dose on anthesis (ZGS 64) (Zadoks et al., 1974) period Flamura-85 wheat cultivar with hand atomizer (Haidukowski et al., 2012). Inoculated heads were covered with clean polyethylene bags and left for incubation for 48 hours. Infected plant material from infected heads was named as FcT (positive control) and plant material from untreated heads were named as KT (negative control) at harvest.

2.2 In Vitro Tests

Active ingredients prothioconazole 150 g/L + tebuconazole 20 g/L (Lamardor News 170 FS; Bayer Crop Science) and triticonazole 80 g/L + pyraclostrobin 40 g/L (Insure Perform; Basf Turkey), which are licensed for *Fusarium* species, were used in seed treatment. These tests were conducted to determine the most effective fungicide to use *in vivo* tests. After disinfecting the surface of the seeds of FcT and KT seed materials with 1% NaOCl solution for 3 minutes, they were cleaned in sterile distile water 2 times and dried on sterile blotting paper in sterile cabin. Dried seeds were treated with 50 ml/100 kg commercial doses of triticonazole + pyraclostrobin and prothiconazole + tebuconazole. Four layers of sterile blotting paper, wetted in sterile distile water, were placed in petri dishes with 20 seeds per petri dish. Seeds in control treatment were fungicide free. The seeds were incubated for 7 days under total darkness and 23±1°C. At the end of the incubation period, germination rate (%), root and coleoptile lengths (cm) were determined from seeds with coleoptile 2 times longer than seed length. Disease severity was determined by comparing the necrosis on the root and coleoptile parts of the seed with a modified 0-5 scala (Wildermuth & Mc Namara 1994) (Figure 1). Disease severity was calculated (Townsend & Heuberger 1943). The experiment was conducted in randomised plot design with 5 repetition containing 2 petris in each repetition. Re-isolation of lesions on roots and coleoptiles of the plants were made using PDA (Potato Dextrose Agar, Merck) after the evaluation of disease severity. The experiment has been repeated twice.

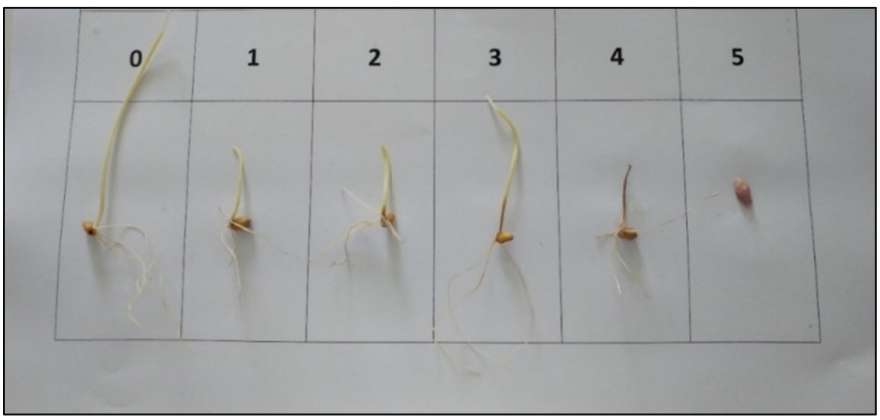

Figure 1. Descriptive scala used in the evaluation of disease severity (0: healthy plant; 1: Necrotic area is lower than 25% 2: Necrotic area is between 25-50% 3: Necrotic area is between 51-75% 4: Necrotic area is greater than 75% 5: Plant is dead)

2.3 In Vivo Tests

Method for triticonazole + pyraclostrobin treatment of seeds infected with *F. culmorum* S-14 (FcT) *in vitro* was repeated *in vivo*. Seeds were sowed into sterile plastic pots (12 × 10 cm) with 1/3 mixture of sand and peat (Klasman-Deilmann), sterilized in otoclav, with 20 seeds in each pot. Untreated seeds were sowed into control pots. Pots were placed into a climate chamber with 23±1 °C 14:10 light: dark, %80-90 r.h. and 10 000 lux light conditions and periodically irrigated with tap water. The experiments were conducted in randomised plot design with 5 repetition containing 2 pots in each repetition.

Plant emergence rates (%) and (ZGS 12) (Zadoks et al., 1974), 15 and 30 days after sowing date, evaluation of disease severity (%) on the roots and coleoptile parts of the seedlings according to the scala used in seed tests, plant height (cm) (after root formation), wet and dry weights of plants were determined. Plants were dried in paper bags in a 50 °C stove for 72 hours and weighted to determine the dry weight. Re-isolation of lesions on roots and coleoptiles of the plants were made using PDA nutrient medium after the evaluation of disease severity.

2.4 Statistic

Effect of fungicides *in vitro* and *in vivo* was tested with Tukey test in SPSS (Version 18; IBM Corp., Armonk, NY).

3. Results

F-85 wheat seeds infected with *F. culmorum* FcT (pozitive control) and uninfected seeds KT (negative control) were used in field conditions for *in vivo* and *in vitro* tests.

3.1 In Vitro Tests

In vitro tests were made with the purpose of determining the most effective fungicide between prothioconazole + tebuconazole and triticonazole + pyraclostrobin active ingredient fungicides to use *in vivo* tests. After the germination of the naturally infected (*F. culmorum* S-14) some abnormal germinations in seeds (Figure 2) and necrosis in roots and coleoptile of the seeds were observed. In these observed abnormal germinations, only root or only coleoptile has formed in the some germinated seeds. In seeds with root formation, very short root development, only main root, main root + 1 side root was observed and all roots have developed weakly.

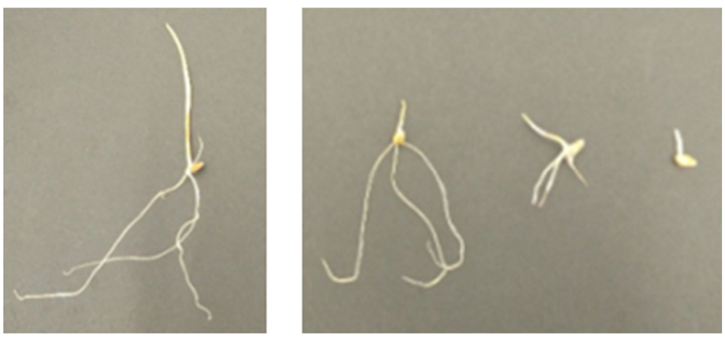

Figure 2. Germination of seeds a) control (left) b) infected with *F. culmorum* S-14 (right)

Germination rate, root and coleoptile length, disease severity of the seeds after treatment with triticonazole + pyraclostrobin and prothioconazole + tebuconazole were given in Table 1. While germination rates for these two fungicides were found to be 68% for triticonazole + pyraclostrobin and 43% for prothioconazole + tebuconazole, the difference between the germination rates of triticonazole + pyraclostrobin and positive control was found to be significant ($P < 0.05$). The increase in germination of the seeds after treatment with triticonazole + pyraclostrobin was 38%, while it was 13% after treatment with prothioconazole + tebuconazole. When root lenghts were evaluated, it was determine that the root length in positive control was 3.60 cm, while it was respectively 5.07 and 4.74 cm in triticonazole + pyraclostrobin and prothioconazole + tebuconazole, with a significant difference between positive control and fungicide treatments ($P < 0.05$). The effect of fungicides on coleoptile length has shown a significant difference between triticonazole + pyraclostrobin and positive control ($P < 0.05$). Also, coleoptile length was found to be higher in fungicide treated seeds than negative control and fungicide treatment in infected seeds caused an increase in coeloptile length. The highest increase was in triticonazole + pyraclostrobin with 2.87 cm.

When the effect of fungicides on disease severity was evaluated, it was found that disease severity on fungicide treated seeds were respectively 61.30% and 42.70% in prothiconazole + tebuconazole and triticonazole + pyraclostrobin with a significant difference between fungicide treatments and positive control (P < 0.05). Disease severity for triticonazole + pyraclostrobin and prothiconazole + tebuconazole was 49.22% and 27.11%, respectively.

Table 1. The effect of fungicides on germination (%), root and coleoptile length (cm) and disease severity (%) in infected seed with *F. culmorum* S-14

Treatment	Germination rate (%)	Root length (cm)	Coleoptile length (cm)	Disease severity (%)
Triticonazole + pyraclostrobin	68b*	5.07b	2.87a	42.70b
Prothioconazole + tebuconazole	43c	4.74b	2.63ab	61.30b
FcT (positive control)	90a	3.60c	1.86b	84.10a
KT (negative control)	91a	7.26a	2.58ab	11.70c

Note. *Differences between means with different letters in the same column are significant according to Tukey multiple comparison test (P < 0.05).

3.2 In VivoTests

Deformations were observed in the root crown of the seedlings caused by the pathogen in the pots. Root crown was found to be weaker in positive control seedlings than negative control seedlings and side roots were formed in addition to main root in some plants with brown necrosis on these roots (Figure 3). In some seedlings developed from fungicide treated seeds, even though the root crown was weakly developed, there wasn't any necrosis on these root crowns.

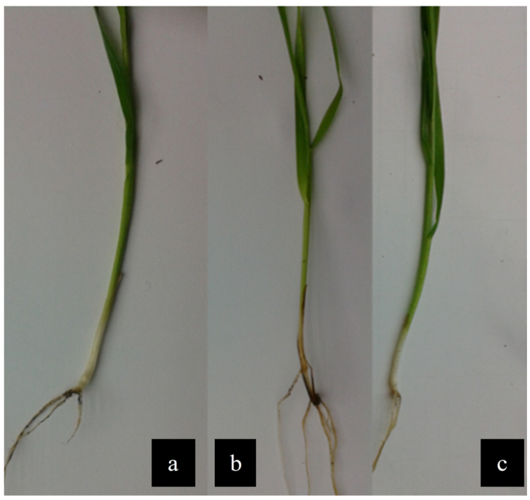

Figure 3. (a) negative control; (b) positive control; (c) triticonazole + pyraclostrobin

Effect of triticonazole + pyraclostrobin on seedling emergence after 15 days from sowing, seedling height after 30 days from sowing (cm), disease severity (%), wet and dry weight (g) was determined (Table 2). Seedling emergence rate was 96% in negative control, 46% in positive control, 79% in triticonazole + pyraclostrobin. There was a 50% decrease in seedling emergence in seeds infected with *F. culmorum* when compared to negative control. Fungicide treatments on infected seeds were 44.17% effective and caused a 33% increase in seedling emergence. When the effect of fungicide on seedling height was examined, the difference between negative and positive control was not significant, while it was significant between triticonazole + pyraclostrobin and positive control (P < 0.05). Plant height was approximately 34 cm in positive and negative control, while it was 36.04 cm in triticonazole + pyraclostrobin, which showed that the fungicide helps in increasing the plant height.

The wet weight of the seedlings was 0.51 g in negative control, 0.31 g in positive control and 0.52 g after triticonazole + pyraclostrobin treatment, with a significant difference between triticonazole + pyraclostrobin and positive control (P < 0.05). Even though the difference between fungicide treatment and negative control was not significant, wet weight of the seedling after fungicide treatment was found to be higher than negative control. When the effect of fungicide on dry weight of the seedlings was investigated, it was determined that the dry weight of the seedling was 0.06 g in negative control, 0.04 g in positive control and 0.06 g after triticonazole + pyraclostrobin treatment, with a significant difference between positive control and triticonazole + pyraclostrobin (P < 0.05). Disease severity was 6.18% in negative control, 32.03% in positive control and 8.75% in triticonazole + pyraclostrobin treatment. The effect of the fungicide on the disease was found to be 72.68% and there was a significant difference between the fungicide and positive control (P < 0.05). Fungicide treatment was found to be very effective at decreasing the develpment of the disease and there was no significant difference between the fungicide and negative control. Pathogen was obtained from the re-isolation of necrosis on the root and root crown of the seedlings.

Table 2. The effect of triticonazole + pyraclostrobin on seedling emergence rate (%), plant height (cm), wet and dry weight (g), disease severity (%)

Treatment	Seedling emergence rate (%)	Plant height (cm)	Wet weight (g)	Dry wet (g)	Disease severity (%)
Triticonazole + pyraclostrobin	79b*	36.04a	0.52a	0.06a	8.75b
FcT (positive control)	46c	34.32b	0.31b	0.04b	32.03a
KT (negative control)	96a	34.49ab	0.51a	0.06a	6.18b

Note. * Differences between means with different letters in the same column are significant according to Tukey multiple comparison test (P < 0.05).

4. Discussion

F. culmorum causes regression in development of root and stem, decrease in tillering, head formation and kernel formation by limiting water and nutrition movement in the plant with relation to infection severity (Wojciechowski et al., 1997; Bağcı et al., 2001; Hekimhan et al., 2005; Mert-Türk et al., 2013; Scherm et al., 2013). Crop losses are economically important because of the severe infections especially in seedling period, in our country (Finci, 1979; Demirci, 2003; Arıcı, 2006; Araz et al., 2009; Köycü & Özer, 2014). Thus, knowing the effectiveness of fungicides used against *F. culmorum* on disease severity is important to prevent plant deaths from seed-borne infections especially in pre and post-emergence in infected seeds. Hence, treatment of the seeds infected with *F. culmorum* by fungicides with carboxin, difenoconazole, diniconazole, tebuconazole, thiabendazole, fludioxonil + metalaxyl-M and tebuconazole + metalaxyl-M active ingredients is known to be effective on disease severity and increases product quality (Arslan & Baykal 2002; Balmas et al., 2006; Hekimhan et al., 2007; Pariyar et al., 2014; Toçan, 2014; Köycü & Sukut, 2018), while the effectiveness of the fungicides can change in relation to the sensitivity of the cultivar and different isolates of the pathogen (Akgül 2008; Spolti et al., 2013; Serfling & Ordon, 2014). Likewise, Akgül (2008) has found that the effect of prothioconazole + tebuconazole (Lamardor FS 400, Bayer Crop Science) on seedlings developed from treated seeds against *F. culmorum* has changed between 11-12%. In our study, we found that the same fungicide's effectiveness was around 27% on the pathogen.

Water content of the wheat seedlings can be determined with wet weight measurement, while the organic and inorganic compounds (nutritinal elements like phosphorus, potassium, calcium, copper, magnesium) without water can be determined with dry weight measurement (Karman, 2012). According to the results of our study, we concluded that the increase in wet and dry weight of the seedlings caused by the positive effect of triticonazole + pyraclostrobin would also increase the yield in field conditions.

5. Conclusion

In conclusion, fungicide with triticonazole + pyraclostrobin active ingredient is effective at the seed-borne infections of the pathogen on seedling development. Also, it was determined that the effect of the fungicides on the pathogen may change with the virulence of the pathogen, active ingredient of the fungicide used on the seed, weath cultivar and the seed infection method of the pathogen.

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