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# EFFECT OF THE TIMING OF FUNGICIDE APPLICATION ON YIELD AND QUALITY PARAMETERS OF WHEAT INFECTED WITH *FUSARIUM* CROWN ROT DISEASE

**Nagehan Desen KÖYCÜ[1*], Füsun SUKUT[2]**

[1]*Tekirdag Namık Kemal University, Faculty of Agriculture, Department of Plant Protection, 59030 Tekirdag, Türkiye*
[2]*Tekirdag Namık Kemal University, Institute of Natural and Applied Sciences, 59030 Tekirdag, Türkiye*

**Abstract:** Crown rot caused by *Fusarium culmorum* (FCR) is a common and important pathogen affecting the cereal industry through grain yield and quality losses. In this study, the effects of epoxiconazole plus prochloraz application and several other applications on disease severity and grain quality parameters including thousand-grain weight (TGW), grain yield (GY), protein (GP), Zeleny sedimentation (ZS), wet gluten (WG) and Grain index (GI), were assessed. The efficacy of epoxiconazole plus prochloraz, were determined in the T1 (ZGS25), T2 (ZGS34), and T3 (ZGS45) growth stages of winter wheat with seven alternative spray programs. These programs were based on (i) the application (SF) of seed fungicide to infected seeds (ii) control without fungicide (non-SF) and (iii) three different growth stages of wheat. The interaction between seed fungicide applied and fungicide application time was significant ($P \leq 0.01$) for DS, ZS, and WG. The effectiveness regarding the disease severity, TGW, and GY of epoxiconazole plus prochloraz in relation to FCR wheat showed significant ($P \leq 0.01$) changes depending on the application time. The disease severity resulted in lower T1-T2 (9.66%) and T1-T2-T3 (9.91%) stages than the other stages. The highest yields were obtained when the fungicide was applied twice at the T1-T2 stages. DS/TGW and DS/GY were negatively correlated and TGW/GY was positively correlated in SF.

**Keywords:** *Fusarium culmorum*, Fungicides, Grain quality, Wheat

***Corresponding author:** Tekirdag Namık Kemal University, Faculty of Agriculture, Department of Plant Protection, 59030 Tekirdag, Türkiye
**E mail:** dkoycu@nku.edu.tr (N.D. KÖYCÜ)
Nagehan Desen KÖYCÜ https: //orcid.org/0000-0003-2511-6096
Füsun SUKUT https: //orcid.org/0009-0004-1488-0085

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## 1. Introduction

*Fusarium* species such as *Fusarium culmorum* (WG Smith) Sacc. and *F. graminearum* Schwabe the major causal agents of Fusarium crown rot (FCR) causing necrotic lesions in the root, sub-crown, and stem tissues of wheat are also the causal agent of Fusarium head blight (FHB), a globally important wheat disease (Cook, 1992; Burgess et al., 2001; Backhouse et al., 2004; Tunalı et al., 2006). There is resistance to FCR among commercial cultivars, but the disease severity can be high in suitable effects of climatic conditions, even in resistant cultivars (Scherm et al., 2013, Özdemir, 2022). *Fusarium culmorum* has been reported as the most prevalent and virulent pathogen causing crown rot in Türkiye (Demirci and Dane, 2003; Tunalı et al., 2008; Akgül and Erkılıç, 2016; Tok and Arslan, 2016; Gebremariam et al., 2018; Köycü and Özer, 2019). *F. culmorum*, has the lowest requirements for the high relative humidity to infect wheat, (Klix et al., 2008) and shows high disease severity in dry soils and areas with high temperatures (Balmas et al., 2015). Therefore, *F. culmorum* is also the most predominant pathogen and most frequently isolated crown rot disease in the Trakya region (Hekimhan and Boyraz, 2011; Köycü and Özer, 2019).

The grain quality characteristics such as wet gluten (%), particle size index (PSI), Zeleny sedimentation (mL), and gluten index (%) were significantly decreased in infected grains compared to control after Fusarium head blight (Köycü, 2021). Cultural practices such as good tillage for the decomposition of infected plant residues against these seed/soil-borne and residue-borne pathogens, crop rotation, adjusting the planting date, and limiting nitrogen for proper fertilization are recommended for FCR disease control (Montanari et al., 2006; Evans et al., 2010), but these practices are insufficient (Cook, 2010). Seed treatments containing the active ingredients tebuconazole, metalaxyl-M, thiram, difeconazole, fludioxonil, triadimenol, and carboxin alone or in combinations can increase seedling growing and grain yield by decreasing the FHB infections in highly susceptible varieties of wheat (Schaafsma and Tamburic-Ilincic, 2005; Akgül and Erkılıç, 2016; Köycü and Sukut, 2018). However, the application of fungicide to the seed is also inadequate in the prevention of FCR infections in the seedling growth and stem elongation stages (Smiley et al., 2005; Hudec, 2007; Beccari et al., 2011; Akgül and

Erkılıç, 2016). The effectiveness of fungicides may vary depending on whether seedlings are infected with seed/soil-borne disease (Sukut and Köycü, 2019). Few studies have reported the effects of fungicide application against foliar diseases on wheat grain quality (Gooding et al., 2000; Dimmock and Gooding, 2002; Wang et al., 2005; Blandino et al., 2009; Rodrigo et al., 2015). Studies have not been undertaken on changes in the quality parameters of the grain of fungicide application number at different growth stages with *Fusarium* crown rot-infected wheat seeds. Thus, the goals of this study were to determine a) the effectiveness of seed fungicide application/non-fungicide on fungicide applications, b) the effect of fungicide applications and their number on FCR disease, grain yield, and quality parameter characters of grain at the different stage of bread wheat.

## 2. Materials and Methods

### 2.1. Fungicides

In the field, we used technical-grade 40 g/L pyraclostrobin plus 80 g/L triticonazole a. i. (Insure® Perform FS BASF, Türkiye; 50 mL 100kg/seed) for the seed coating and 42 g/L epoxiconazole plus 150 g/L prochloraz a.i. (Tocata® TR BASF, Türkiye, 200 g/da), applied with an atomizer, in the control of FCR. The soil was fertilized with 184 kg/ha N and 40 kg/ha $P_2O_5$. Standard practices for weed control with herbicides were followed.

### 2.2. Field Experiment

The field experiment was established in the 2017/2018 wheat growing season at the Tekirdağ Namık Kemal University research area (40° 59′ 36.5028" and 27° 34′ 53.0724"). In the experiment, a *Fusarium culmorum* (S-14)-infected Flamura 85 (F-85) cultivar was used. A detailed description of that field experiment using the infected Flamura-85 seed cultivar was provided by Köycü (2018). *F. culmorum*-infected seeds were either coated with seed fungicide (SF) or not (non-SF). Wheat plants were sprayed at the T1 (ZGS25; main shoot and 5 tillers, 14 February), T2 (ZGS34; fourth node detectable, 22 March), and T3 (ZGS 45; boots swollen, 15 April) growth stages (Table1).

**Table 1.** A winter wheat crown root experiment with three spray timing stages (T1, T2, and T3) at which the plots were untreated or treated with 200 g/da epoxiconazole plus prochloraz with seed fungicide application or non-seed-fungicide application

| Treatment | Seed fungicide[a]* | T1 (ZGS 25) | T2 (ZGS 34) | T3 (ZGS 45) |
|---|---|---|---|---|
| Check | No Fungicide | Untreated | Untreated | Untreated |
| 1 | No Fungicide | Fungicide[b]* | Untreated | Untreated |
| 2 | No Fungicide | Untreated | Fungicide | Untreated |
| 3 | No Fungicide | Untreated | Untreated | Fungicide |
| 4 | No Fungicide | Fungicide | Fungicide | Untreated |
| 5 | No Fungicide | Untreated | Fungicide | Fungicide |
| 6 | No Fungicide | Fungicide | Untreated | Fungicide |
| 7 | No Fungicide | Fungicide | Fungicide | Fungicide |

a= 40 g/L pyraclorostrobin plus triticonazole 80 g/L; Insure® Perform; b= 42 g/L epoxiconazole plus prochloraz 150 g/L, Tocata®
*****BASF; commercial fungicide.

Seeds were sown on November 10, 2017, by hand at the rate of 500 seeds per $m^2$. The experimental design involved a split plot in randomized complete blocks with three replications. The plots were surrounded by 2.5 m of bare-soil borders, which were 5 x 1 m wide. Seed fungicide was applied in the main plot; each other fungicide was applied in the subplot. For disease severity (%), the methodology derived from Townsend and Heuberger (1943) involved the selection of plants with a well-developed sub-crown internode (>2 cm) from each wheat plot during the late milk to early dough stage of development (Zadoks Growth Scale 77–84) (1974). A modified 0-5 scale (0: Healty plant, no color change in the mentioned areas; 1: Necrotic area less than 25%; 2: Necrotic area between 25-50%; 3: Necrotic area between 51-75%; 4: Necrotic area more than 75%; 5: Plant dead) proposed by Wildermuth and McNamara (1994) was utilized for assessment, encompassing a total of 60 plants in each fungicide application program. The winter damage rate was calculated by comparing the count of wheat seedlings before and after winter, utilizing the formula: winter damage rate (%) = (number of seedlings before winter - number of seedlings after winter/number of seedlings before winter) × 100. The grains were harvested at 13% grain relative humidity (RH) (ZGS 89) using a small-plot harvester (Hege mod. 125B, Maschinenbau, Germany). The thousand-grain weight (TGW) and grain yield (GY) (ton/ha) were determined following the official methods of the Approved Methods of the American Association of Cereal Chemists (AACC 2000).

### 2.3. Grain Quality Parameters

Grain protein rate (GP) (%) by ICC 1995, Zeleny sedimentation value (mL) (ZS) by ICC method No: 166/1 (ICC 1972)), wet gluten rate (%) (WG) and gluten index (GI) (%) values by ICC method No. 155 (ICC, 1994) were determined in 1 kg seed samples taken from each plot.

### 2.4. Statistical Analysis

Significant differences between the genotypes were determined based on Fisher's least significant difference (LSD). Data were analyzed with the JMP software version 5.0.1 model.

## 3. Results and Discussion

### 3.1. Climatic Condition and Disease Relationship

The climatic conditions (temperature, rainfall, and high RH) were favorable for germination at the end of November and spike emergence at the beginning of May (Figure 1). During this period, the total rainfall was approximately 281 mm, and the average daytime temperature and relative humidity were approximately 14°C, and 79%, respectively. February (T1) was rainy. The natural conditions regarding average rainfall and RH determine the critical period for FCR.

The yield losses can be severe, even for resistant cultivars, when climatic conditions are favorable to disease development, due to the partial resistance in commercial cultivars against FCR (Scherm et al., 2013). In our study, the weather conditions were also suitable for FCR during February and March, which correlated with the T1 and T2 stages. There was abundant rainfall and high RH in February (14th day for ZGS25) and March (20th day for ZGS34) in the Trakya region, and high disease severity at these points. The application of fungicide to the infected wheat seed was also reasonably effective in reducing winter damage in the Trakya region.

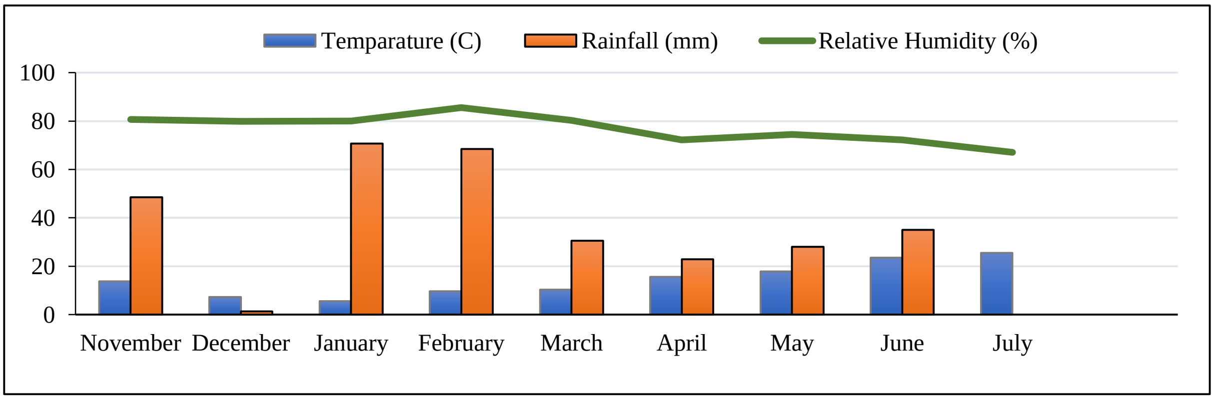


**Figure 1.** Monthly rainfall, high daytime temperature, and relative humidity at the meteorological station near the experimental site from November 10 to July 15, 2017 (Day of Year 314–365) and 2018 (Day of Year 1–196).

### 3.2. Field Experiment

This research offers the first comparison of FCR aggressiveness and control, with a focus on the wheat growth stage according to the Zadoks growth scale. The results of our study stand out in several important ways. First, in order to a fungicide application to be successful in reducing the disease severity of root and root collar infections, the fungicide must be applied to the seed. Secondly, depending on the developmental period of the wheat plant, the efficiency of the fungicide applied in the control of FCR may change, affecting yield. Thirdly, applying fungicide to the seed altered the efficiency of the fungicide used to control the FCR, demonstrating that it was effective in terms of FCR disease severity, sedimentation, and gluten. In addition, the application of fungicide to seeds was shown to have a significant effect in terms of reducing the winter damage rate of winter wheat (59.19%) (Figure 2). Brown lesions on the stem of the wheat up to the second node were registered in all plots (Figure 3).

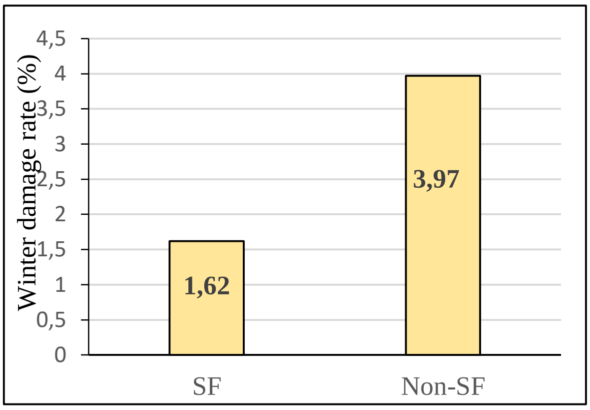


**Figure 2.** Winter damage (%) of wheat after seed fungicide/non-seed-fungicide application.

**Figure 3.** Disease severity of FCR in the non-SF plot.

The application of a fungicide against *Fusarium* spp. to seeds, with the active ingredients fludioxonil, fluquinconazole, difenoconazole, maneb, mancozeb, metalaxyl-M, tebuconazole, prothioconazole, triadimenol, triticonazole, and thiram alone or in a mixture, has been reported to increase the seedling emergence, dry weight, and grain yield of *Fusarium*-infected wheat (Dawson and Bateman 2000; Arslan and Baykal 2002; Martin 2005; Schaafsma and Tamburic-Ilincic 2005; Demirci and Maden 2006; May et al., 2010; Köycü, 2018; Correia et al., 2020). Thiabendazole, particularly when applied to cultivars sensitive to crown rot, protects seedlings against pathogen infection by reducing disease development, ultimately leading to increased productivity (Pariyar et al., 2014). It has been shown that the efficacy of seed fungicide application is limited to the early stages of the wheat growth cycle, but it will not maintain its efficiency much beyond the seedling stage (Balmas et al., 2006). Our research, however, shows that the application of fungicide to the seed played a significant role in reducing *F. culmorum* DS, and verifies the effectiveness of fungicide application in terms of FCR management, as greater disease severity developed in non-SF compared to SF plants.

Interactions between the seed fungicide application and fungicide timing for FCR were statistically significant for the DS, ZS, and WG (Table 2). Disease severity was visually scored for crown rot symptoms, and evaluated per subplot. Epoxiconazole plus prochloraz resulted in significant (P≤0.01) differences in the DS, ZS, and WG values in the different growth stages of SF/non-SF plots. The application of fungicide at the different growth stages of the wheat plants caused no significant effects on protein rate or gluten index. Non-SF plants showed greater disease severity than SF plants, which was significant but still lower. The application of fungicide at different growth stages reduced the infections of FCR compared to the check. A high disease severity (DS) of *Fusarium culmorum* was observed in SF (26.50%) and non-SF (31.33%) plants at the same stage. T1-T2 and T1-T2-T3 showed significantly lower DS values at all stages with epoxiconazole plus prochloraz application in SF compared to non-SF. A significant increase in ZS occurred in the T1-T2 of SF compared to the check. The amount of wet gluten varied between 27 and 31%, and the highest value was found in the T1 stage in SF.

The optimum fungicide application time for FCR management and for ensuring an increased yield at wheat harvest is T1 (ZGS 25), as this prevents high levels of infection in the stem elongation and booting stages. The effect of fungicide application timing on FCR was most clear in the T1-T2 (ZGS25-ZGS34) stage, with the treatment applied twice in wet and humid environments. The critical period for FCR development is between seed germination and spike emergence. The weather conditions during the generative period, in which protein reserves are accumulated, have a significant impact on wheat quality (Finlay et al., 2007). The Zeleny sedimentation value is the most important quality property, as it influences the quality of gluten proteins as affected by the environment (Grausgruber et al., 2000). *F. culmorum* reduces the water-binding capacity of gluten; the quality of proteins and the baking quality of flour during storage can be reduced, causing significant losses in bread quality (Wang et al., 2005; Schmidt et al., 2017). The effects of fungicide application on grain quality parameters may vary depending on the fungicide used. The sedimentation value was significantly lower in Fusarium-infected spikes of wheat, and almost all cultivars contained higher wet gluten levels (Spanic et al., 2021). Epoxiconazole plus fenpropimorph, prothioconazole plus spiroxamine, epoxiconazole plus pyraclostrobin, epoxiconazole plus dimoxystrobine, and cyprodinil plus propiconazole, when applied to septoria leaf blotch, did not significantly affect Zeleny sedimentation when considered across years and locations (El Jarroudi et al., 2015). An increase in Zeleny sedimentation rate was detected in *Fusarium culmorum*-infected spike grains given prothioconazole+trifloxystrobin, compared to a control

(Köycü, 2022). In our study, the Zeleny sedimentation values were affected by the stage of fungicide application and the number of applications in SF and non-SF plants. The Zeleny sedimentation value increased when fungicide was applied to the seed in the T1-T2 period by 52 mL (4%) compared to the check; this was found to be the most effective spray period in terms of reducing FCR disease severity, and >30 mL was considered a high-quality grain. In addition, the gluten contents at different growth stages of wheat grains subjected to fungicide application were determined by the application of SF or non-SF. As a result, monitoring Zeleny sedimentation and wet gluten values after fungicide application under high FCR pressure is important for evaluating wheat flour quality. We determined that epoxiconazole plus prochloraz was more effective when the seed fungicide was applied to seeds infected with *F. culmorum* under the field conditions found in the Trakya region.

**Table 2.** Means of DS-disease severity ratio, GP-Grain protein content, ZS-Zeleny sedimentation value, WG-wet gluten content, GI-gluten index within seed fungicide/non-seed fungicide experiment conducted to determine the effects of fungicide application timing on *Fusarium culmorum* crown rot

| | DS (%) | GP (%) | ZS (ml) | WG (%) | GI (%) |
|---|---|---|---|---|---|
| Seed Fungicide | | | | | |
| Check | 26.50b* | 13.16a | 50.33b-e | 30.00a-c | 94.66a |
| T1 | 15.23d-f | 13.06a | 51.00a-d | 31.33a | 92.66a |
| T2 | 17.83c-e | 13.30a | 51.67ab | 29.33a-d | 93.00a |
| T3 | 19.17cd | 12.70a | 49.00c-f | 27.67d | 94.33a |
| T1-T2 | 5.67h | 12.76a | 52.00a | 29.34a-d | 93.66a |
| T2-T3 | 11.00g | 13.16a | 49,67b-f | 29.67a-d | 95.00a |
| T1-T3 | 15.33d-f | 12.93a | 51.00a-d | 30.00a-c | 94.00a |
| T1-T2-T3 | 6.00h | 13.00a | 51.00a-d | 30.33ab | 95.00a |
| Non-Seed Fungicide | | | | | |
| Check | 31.33a | 12.76a | 48.33d-f | 28.00cd | 94.00a |
| T1 | 18.00cd | 13.00a | 48.00ef | 29.00b-d | 94.33a |
| T2 | 19.67c | 13.13a | 49.00c-f | 28,66b-d | 94.66a |
| T3 | 27.5ab | 13.10a | 51.33ab | 30.00a-c | 94.00a |
| T1-T2 | 13.67fg | 12.96a | 50.33a-d | 29.67a-d | 94.00a |
| T2-T3 | 14.00e-g | 12.86a | 49.00c-f | 28.66b-d | 94.00a |
| T1-T3 | 15.83c-f | 13.23a | 50.00a-f | 30.67ab | 95.00a |
| T1-T2-T3 | 13.83fg | 12.86a | 49.33b-f | 29.33a-d | 94.66a |

*Means followed by the same letter within a column are not significantly different according to the least significant difference (LSD) test at P≤0.01.

The FCR field treatment results summarize the effects of applying epoxiconazole plus prochloraz on GY (ton/ha), and TGW (g) concerning fungicide application time and number in the different growth stages of wheat (Figure 4). The differences between the means of seed fungicide application X timing interaction were not statistically significant for TGW and grain yield. There were significant (P≤0.01) differences in TGW and GY by fungicide application time and number. At all stages, the TGW was higher than in the check (31.18 g). TGW and TGW increase was higher than at the other stages at T2-T3 (39.13-25.53%) and T1-T2-T3 (39.49-26.69%). GY (ton/ha) ranged from 6.4 to 6.78, except for the check (6.47). The highest GY and GY increase was obtained when the fungicide was applied twice at the T1-T2 stage (6.89-6.49%). T3 exhibited lower TGW (36.21) and GY (6.47), according to the other stages. These results suggest that the fungicide application time and a number have differential effects on TGW and GY.

The results of this study suggest that the effective management of FCR depends on seed fungicide application, the timing of said application, and the number of repetitions. All of these factors have been shown to affect FCR disease severity and, together with wheat quality differences, may explain much of the recent fluctuation in the wet gluten and Zeleny sedimentation seen in the growth stages of wheat. Regarding the effects on wheat grain quality, fungicide application can have several positive outcomes. By reducing the severity of *Fusarium* crown rot, fungicides can help maintain the photosynthetic capacity of wheat plants, leading to improved grain filling and increased yield. This, in turn, can positively influence grain quality parameters such as Zeleny sedimentation and wet gluten. However, it's worth noting that the specific effects of fungicide application on grain quality can vary depending on several factors, including the disease pressure, timing and frequency of fungicide application, choice of fungicide, wheat variety, and environmental conditions. Different fungicides may have varying efficacy against *Fusarium culmorum* crown rot in the field, and their application must be carefully timed to achieve optimal results.

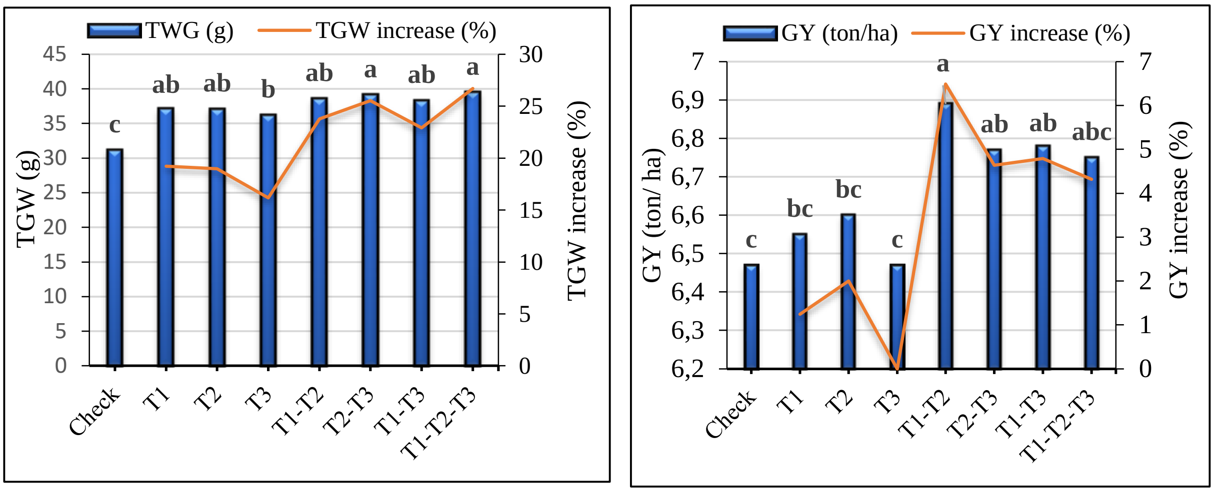


**Figure 4.** Thousand-grain weight (TGW), and grain yield (GY) of wheat grain after inoculation with *F. culmorum* in the harvest. Different letters above bars indicate significant (P≤0.01) differences according to LSD tests.

**3.3. Correlations Between DS, TGW, and GY**

Pearson's correlation coefficient between FCR disease severity and other parameters was calculated using the data from SF and Non-SF. In general, a higher correlation was observed in the SF application (Figure 5). FCR disease severity showed a significant (P≤0.01) negative correlation with TGW (r = 0.770) (r = 688) of both SF and non-SF applications respectively. In SF, DS/Yield were negatively correlated (r = 0.629) and GY/TGW were positively correlated (r = 0.406).

Significant positive relationships between TGW and yield (Samar et al., 2019). The results of the current study showed a significant positive relationship between TGW/GY when the fungicide was applicated to the seed. The high correlations were noted in the reduction between disease severity, TKW, and grain yield (Abdallah-Nekache et al., 2019).

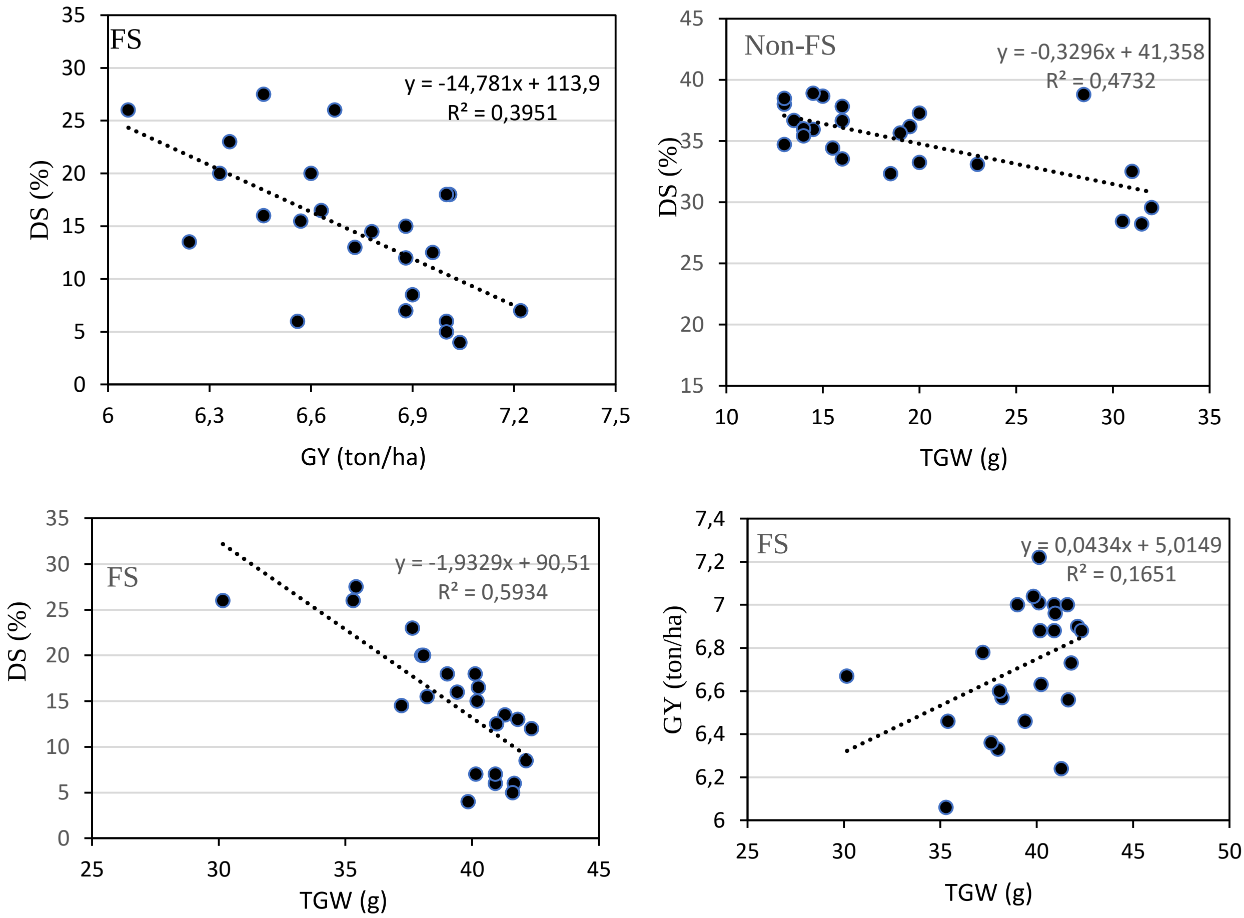


**Figure 5.** Correlation coefficients for FCR disease severity (DS), thousand-grain weight (TGW), and GY for seed fungicide (SF) and non-seed-fungicide (non-SF) (P≤0.01).

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | N.D.K. | F.S. |
|---|---|---|
| C | 80 | 20 |
| D | 80 | 20 |
| S | 90 | 10 |
| DCP | 40 | 60 |
| DAI | 90 | 10 |
| L | 80 | 20 |
| W | 80 | 20 |
| CR | 80 | 20 |
| SR | 90 | 10 |
| PM | 80 | 20 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because there was no study on animals or humans.

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