



Fumonisin Production and Virulence of *Fusarium fujikuroi* Species Complex Strains Obtained from Maize in Türkiye

Türkiye'deki Mısır Örneklerinden Elde Edilen *Fusarium fujikuroi* Tür Kompleksi İzolatlarının Fumonisin Üretimi ve Virülensliği

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ABSTRACT

Maize (*Zea mays* L.) is an important agricultural product with high dietary value for humans, a good feedstuff for livestock, and economic value for subsistence farmers in Türkiye and around the world. The many species of *Fusarium fujikuroi* species complex (FFSC) are of reduced quality and quantity, damaging roots, stems, leaves, or grains of rice, wheat, barley, and particularly maize, which are important cereals. The aim of this study is to investigate FB production ability by distinct species of FFSC obtained from various maize fields in Türkiye, assess their virulence for seedling and foliar diseases *in planta*, and revealing whether a phenotypic effect on the development of maize seedlings occurs due to the Fumonisin B (FB) levels of the species. For FB analysis, six characterized FFSC strains, including three for *F. verticillioides*, one for *F. proliferatum*, *F. thapsinum*, and *F. andyazi*, together with two undefined *Fusarium* strains, were cultured. All the FFSC strains, except one of them, were produced at various quantitative levels of either FB1 or both mycotoxins on the synthetic medium conditions. Pathogenicity test on the seedlings was completed with a known pathogenic *F. verticillioides* and a non-inoculated treatment as a control, in addition to all FFSC strains, on a sweet maize hybrid (cv. Vega F1) and flint type maize cultivar (cv. Karadeniz Yıldızı) under the growth chamber conditions. As a result of evaluating all plant growth and diseases, the plant ratio with atrophy and stalk rot disease severity was statistically significant at $P < 0.05$ and 0.01 , respectively, for only the hybrid maize cultivar. Even if there was no direct relationship between their ability to produce FB and the virulence or pathogenicity of FFSC strains, particularly those of *F. verticillioides*, utilizing a limited number of strains, such as *F. thapsinum*, and *F. andyazi*, which were tested for the first time, has been a remarkable result. Thus, it will be able to understand the association between mycotoxin production and virulence with more strains of these species in further study that is subjected to genetic and phenotypic approaches.

Keywords: *Fusarium fujikuroi* Species Complex, *fum1* Gene, Sweet Maize, Fumonisin B1, Stalk Rot.



TÜRKİYE'DEKİ MISIR ÖRNEKLERİNDEN ELDE EDİLEN *FUSARIUM FUJIKUROI* TÜR KOMPLEKSİ İZOLATLARININ FUMONİSİN ÜRETİMİ VE VİRÜLENSLİĞİ

ÖZ

Mısır bitkisi, Türkiye'de ve dünyada insanlar açısından besleyici bir gıda, hayvanlar için iyi bir yem ve çiftçiler için ekonomik değere sahip önemli bir tarımsal üründür. *Fusarium fujikuroi* tür kompleksi (FFSC) içinde yer alan birçok fungal tür, başta mısır olmak üzere çeltik, buğday, arpa gibi önemli tahıl bitkilerinin kök, sap, yaprak ya da tanelerinde zarar meydana getirerek verim ve kaliteyi azaltmaktadır. Bu çalışmanın amacı, Türkiye'nin çeşitli mısır tarlalarından elde edilen farklı FFSC türlerinin Fumonisin B (FB) üretim kapasitesini araştırmak, bitki üzerinde fide ve yaprak hastalıklarına karşı virülanslarını değerlendirmek ve bu türlerin FB düzeylerinin mısır fidelerinin gelişimi üzerinde fenotipik bir etkiye sahip olup olmadığını ortaya koymaktır. FB analizi için, FFSC'ye ait *F. verticillioides*'ten üç, *F. proliferatum*, *F. thapsinum* ve *F. andyazi*'den birer tane olmak üzere toplam altı karakterize edilmiş izolat (strain) ile tür düzeyinde tanımlanmamış iki *Fusarium* izolatı besi ortamında geliştirilmiştir. Bu FFSC izolatlarından sadece biri hariç, tümü sentetik besiyeri koşullarında ya sadece FB1 ya da her iki mikotoksini çeşitli miktarlarda ürettiği tespit edilmiştir. Fideler üzerinde yapılan patojenite testi, geliştirme odası koşullarında, tüm FFSC izolatları yanında, patojen olduğu bilinen bir *F. verticillioides* izolatı ve kontrol grubu olarak tohumla herhangi bir uygulamanın yapılmadığı uygulamalar şeklinde tatlı mısır hibridi (Vega F1) ve sert taneli mısır çeşidi (Karadeniz Yıldızı) ile yürütülmüştür. Tüm bitki büyüme ve hastalık değerlendirmeleri sonucunda, sadece hibrit mısır çeşidinde, atrofiye sahip bitki oranı ile sap çürüklüğü hastalığının şiddeti, sırasıyla %5 ve %1 düzeyinde istatistiksel olarak anlamlı bulunmuştur. FB üretme yetenekleri ile FFSC izolatlarının, özellikle de *F. verticillioides* izolatlarının virülansı veya patojenitesi arasında doğrudan bir ilişki olmasa da, *F. thapsinum* ve *F. andyazi* gibi tek bir izolat ile temsil edilen türler ilk kez test edildi ve bu nedenle, genetik ve fenotipik yaklaşımlara dayanan ilerdeki çalışmalarda bu türlerin daha fazla izolatı ile mikotoksin üretimi ve virülenslik arasındaki ilişkiyi anlamak mümkün olacaktır.

Anahtar Kelimeler: *Fusarium fujikuroi* Tür Kompleksi, *fum1* Geni, Tatlı Mısır, Fumonisin B1, Sap Çürüklüğü.



1. INTRODUCTION

Maize (*Zea mays* L.) is an important agricultural product with high dietary value for humans, a good feedstuff for livestock, and economic value for subsistence farmers in Türkiye and around the world. Its production is approximately 8.1 million tons per year, and a large amount of maize is grown on a total of 790,000 hectares in almost all regions of Türkiye, except East Anatolia (TUİK, 2025). Furthermore, different types of maize cultivars, mostly hybrids, are released in growing markets, and farmers are grown in these regions. However, in contrast to the use of new and smart growing technologies and high-yielding cultivars of maize, it is one of the main biotic factors, especially stalk and ear rot diseases caused by *Fusarium* species, limiting maize production at least 10-30% (Nelson et al., 1993; Munkvold and Desjardins, 1997; Reid et al., 1999; Logrieco et al., 2002; Tunalı et al., 2016; Oldenburg et al., 2017; Hudson et al., 2024). Moreover, these species can lead to contamination of mycotoxins such as fumonisins (FBs), moniliformin (MON), and beauvericin (BEA), which have hazardous effects on animals and humans through feed and food (Gelderblom et al., 1988; Bennett and Klich, 2003; Desjardins, 2006; IARC, 2012; Qu et al., 2022).

The section *Liseola* for *Fusarium* (asexual named) genus is one of the most comprehensive morphological species concepts, established by Wollenweber and Reinking (1935), and it has a lot of the *Fusarium* species identified as plant pathogens, mycotoxin contaminants, animal and human disease agents (Leslie, 1991; Marasas, 2001; Jain et al., 2011; O'Donnell et al., 2016; Al-Hatmi et al., 2019). In *Fusarium* taxonomy, the morphology of macroconidia is usually regarded as a primary characteristic for the definition of species in this genus. However, this criterion is not conducive to distinguishing the species at *Liseola*. Instead of macroconidia, the morphology and formation of microconidia are considerably different from those of the species of this section, and they do not form chlamydospores. Briefly, species of this section are formed both macroconidia and microconidia, which are endoconidia inside the hyphae or conidiophore, particularly chains and/or false heads in microconidia, and the conidia are also formed on monophialides or polyphialides (Toussoun and Nelson, 1975; Hawksworth et al., 1983; Nelson et al., 1983; Nelson, 1992). In further studies, some species, including *Liseola*, such as *F. dlamini*, *F. nygamai*, and *F. napiforme*, have been shown to produce chlamydospores (O'Donnell and Cigelnik, 1997). This has been a complication in classifying species in this section due to morphology. Molecular studies showed that the *Liseola* section was paraphyletic, and this clearly revealed complications of using their morphological characteristics to understand relationships between the species at various sections of the *Fusarium* because of their morphology often being discordant with the DNA sequence data (O'Donnell and Cigelnik, 1997; O'Donnell et al., 1998; O'Donnell et al., 1998; 2000). For this reason, the term 'species complex' has been introduced to

serve as a more acceptable approach in naming phylogenetic clades and to overcome the conflicts in classification based on the morphological species concept in the section *Liseola*.

Leslie (1991; 1995) first developed a biological species classification with six asexual species termed the “*Gibberella fujikuroi* species complex” based on mating populations (MPs) of the species in the *Liseola* section. According to this description, the *G. fujikuroi* species complex comprises *Fusarium verticillioides* (Sacc.) Nirenberg (synonym *F. moniliforme* Sheldon; teleomorph: *Gibberella moniliformis* Winland) as *G. fujikuroi* mating population A (MP-A) with a major pathogen on maize and sorghum (Frederiksen, 1986; White, 1999), *F. sacchari* (E.J. Butler) W. Gams (teleomorph: *G. sacchari* Summerell et Leslie sp. nov.) as MP-B with a stem rot from sugarcane (Leslie et al., 2005a), *F. fujikuroi* Nirenberg (teleomorph: *G. fujikuroi* Sawada) as MP-C with bakanae disease in rice (Nirenberg, 1976), *F. proliferatum* (Mats.) Nirenberg (teleomorph: *G. fujikuroi* var. *intermedia* Kuhlman) as MP-D with phytopathogen on wheat, barley, rice and maize (Leslie, 1991), *F. subglutinans* (Wollenw. and Reinking) P.E. Nelson, Toussoun and Marasas (teleomorph: *G. subglutinans* (Wollenw. and Reinking) P.E. Nelson, Toussoun and Marasas) as MP-E with common pathogen on teosinte and maize (Nelson et al., 1983), and *F. thapsinum* Klittich, J.F. Leslie, P.E. Nelson and Marasas (teleomorph: *G. thapsina* Klittich, J.F. Leslie, P.E. Nelson and Marasas) as MP-F with a stalk rot from sorghum (Klittich et al., 1997). In subsequent studies, it has been reported that new mating populations added by classification as *F. nygamai* L.W. Burgess & Trimboli (MP-G), *F. circinatum* Nirenberg & O'Donnell (MP-H), *F. konzum* Zeller, Summerell & J.F. Leslie (MP-I), and *F. xylarioides* Steyaert (MP-J) (Klaasen and Nelson, 1996; Britz et al., 1999; Zeller et al., 2003; Geiser et al., 2005). The last expansion of the *G. fujikuroi* species complex revealed that MP-K and MP-L were included as *F. temperatum* and *F. tuiense*, respectively (Scaufaire et al., 2011; Lima et al., 2012). In a recent study, the *G. fujikuroi* species complex, which mostly corresponds to the *Fusarium* section *Liseola*, clustered into more than 60 distinct phylogenetic species, including twelve individual MPs (Yilmaz et al., 2021), and was preferred named as *Fusarium fujikuroi* species complex (FFSC) for its more inclusive and widespread use in both *Liseola* and other sections of *Fusarium*.

The members of FFSC are known to cause a lot of plant diseases, ranging from cereals to tropical trees, and also produce a large number of secondary metabolites, particularly fumonisin and its derivatives. Fumonisin B series, as derivatized FB1, FB2, and FB3, are of neurotoxic, immunotoxic, cytotoxic, and carcinogenic effects in humans and livestock, and *F. verticillioides* and *F. proliferatum* are predominant pathogens in the occurrence of maize diseases and are major producers of a large amount of the FBs (Munkvold and Desjardins, 1997; Rheeder et al., 2002; Foreman et al., 2004; Yu et al., 2021; Omotayo and Babalola, 2023). Out of two main pathogens, it has been reported that fifteen species in FFSC, such as *F. thapsinum*,

F. fujikuroi, *F. andiyazi*, *F. nygamai*, *F. dlamini*, produced at more and less levels of the FBs (Rheeder et al., 2002).

Secondary metabolism in fungal genus/species confers some biological advantages for their pathogenicity to host plants, which is mostly attributed to a virulent factor for fungi. In a considerable study, Proctor et al. (1995) reported that Deoxynivalenol (DON), as a virulence factor, contributed to an increase in severity and occurrence of scab disease in wheat. Similarly, FB accumulation in maize is closely related to disease severity in the field, and FB was indicated to play a role in virulence on maize seedlings, but was not necessary (Desjardins et al., 1995; Desjardins et al., 1998; Williams et al., 2006). In this study, we aimed to investigate FB production by distinct species of FFSC obtained from various maize fields in Türkiye, assess their virulence for seedling and foliar diseases *in planta*, and revealing whether a phenotypic effect on the development of maize seedlings occurs due to the FB levels of the species.

2. MATERIALS AND METHODS

2.1. Fungal Cultures

All fungal strains that belong to FFSC members were originally obtained from maize fields in various locations of Türkiye (Kansu, 2021), and they are given in Table 1. Fungal cultures that were long-term stored in 15% glycerol at -80 °C freezer were initially maintained on synthetic nutrient agar medium (Merck Life Science Product, Germany) and stored as a spore suspension in the micro tubes with 1.5mL at -20 °C in freezer, before using the experiments and analysis.

Table 1. The fungal species and strains belong to FFSC in this study

Accession Code	<i>Fusarium fujikuroi</i> Species Complex Species/Strains*	Locations	<i>fum1</i> PCR**
TR11-V137m	<i>Fusarium verticillioides</i>	Sinop/Merkez	+
TR11-V96	<i>Fusarium verticillioides</i>	İzmir/Menemen	+
TR11-V40	<i>Fusarium verticillioides</i>	Osmaniye/Sumbas	+
TR11-P26	<i>Fusarium proliferatum</i>	Konya/Karapınar	+
TR11-V134	<i>Fusarium thapsinum</i>	Ordu/Ünye	-
TR11-V11	<i>Fusarium andiyazi</i>	Adana/Yüreğir	-
TR13-228	<i>Fusarium</i> sp.	Mersin/Tarsus	no PCR
TR11-V147	<i>Fusarium</i> sp.	Giresun/Tirebolu	no PCR
TR11-V1***	<i>Fusarium verticillioides</i>	Adana/Seyhan	+

*Kansu, 2021; ** Species-specific PCR for *fum1* gene in *F. verticillioides* and *F. proliferatum*;

***Pathogenic strain for ear rot disease which was tested in fields experiment

2.2. Culture Preparation for Fumonisin Production

A five-day old cultures growth on SNA medium was used for the formation of fumonisins in culture. An agar plug from these cultures for each FFSC strain in this study was transferred to 9cm plastic plates, which included 20mL malt extract agar (MEA, Merck Life Science Product, Germany) medium that was suspended with 50g into 1L distilled water and autoclaved at 121 °C and 1 atm. pressure. MEA medium was used for the highest amount of fumonisin formation in culture, reported by Frisvard et al. (2007). All culture plates were incubated in a dark condition at 25 °C and 60% humidity for fifteen days.

2.3. Fumonisin Extraction

For the extraction of fumonisins, a semiquantitative method based on agar plugs developed by Smedsgaard (1997) was used and five agar plugs (diameter= 6 mm) were cut as different locations included one center and four edges of colony for each strain by using a cork borer (No:6). Each tube weighed, and 750 µL of 75% methanol in water (75:25, v/v) was added for each tube. The plugs were extracted for 50 minutes using an ultrasonication bath (Isolab 10L, Germany). The extracts were filtered using an acro disk 0.45 µm syringe filter and used directly for analysis.

2.4. Chromatographic Analysis of Fumonisin

The extract was analyzed using a Shimadzu high-pressure liquid chromatography (HPLC) system equipped with an LC-20AT pump, SIL-20A autosampler, CTO-20A column oven, and RF-20A fluorescence detector. Chromatographic separation was performed on a reversed-phase C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of water and methanol under gradient elution at a flow rate of 1.0 mL/min. Pre-column derivatization with o-phthaldialdehyde (OPA) was applied before injection. Fluorescence detection was carried out at an excitation wavelength of 335 nm and an emission wavelength of 440 nm. The injection volume was 20 µL, and the column temperature was maintained at 30 °C (Visconti et al., 2001).

For the determination of the calibration curve, six different working solutions were prepared between 100-1000ng mL⁻¹ and 50-500ng mL⁻¹ for FB1 and FB2, respectively. R² was calculated as 0.998 for FB1 and 0.996 for FB2. The Recoveries, Limits of Detections (LODs), Limits of Quantifications (LOQs), and Retention Times (RT) for FB1 and FB2 were given in Table 2.

2.5. Seedling Test

Seedling test was performed to assess whether effect of plant development by several strains of FFSC that produce or non-produce fumonisins. A sweet maize hybrid “Vega F1” (May Seed Trade Company, Türkiye) and a certificated standard flint type maize cultivar “Karadeniz Yıldızı” (Black Sea Agriculture Research Institute, Türkiye) were surface disinfected for 10 min in 5.25% sodium hypochlorite (Biorad Hipo, USA), rinsed with sterile distilled water, and allowed to imbibe for 4 h in sterile water. Seeds were subjected to a heat shock treatment at 60°C for 5 minutes to eliminate internal fungal growth in the tissue (Bacon et al., 1994). Fungal inoculation for each FFSC strain was carried out by placing 40 seeds in a sterile plastic petri plate and inoculated with 10 ml of a conidial suspension containing 1×10^4 conidia mL^{-1} . As in inoculation, sterile distilled water at an equal volume was used for control seeds. Seeds were incubated in the darkness at 27 °C for a whole night and then placed onto the pots within the soil mixture (45% peat/55% perlite and soil mix), ten seeds for each pot. The pots were placed into a growth chamber at 27 °C for daily light with 18 hours and 18 °C for dark conditions with 6 hours, routinely irrigated by sterile distilled water every other day, and harvested on the 15th day after planting. The experiment was carried out to randomly block design with three replications and repeated twice under the same conditions (Glenn et al., 2008).

2.6. Data Analysis

Each FFSC strain was assessed by a total of 30 maize seedlings in three replicates. The number of survival plants, meaning germinated seeds (GSR), the number of plants with chlorotic and necrotic leaves (CNPR), the number of plants with atrophic leaves (APR), the number of plants with stem/stalk rot disease (SRD), and plant shoot length (PSL) were recorded for each plant of replicants in pots. All numbers of the plants assessed using observational data were converted to percentages as ratios of the total number of plants, except for the measured data on plant shoot length.

The SPSS v21 statistical package (IBM, Statistics, OMU Licensed for online users) was used to analyze differences in variances using one-way ANOVA at significant levels of 0.05 and 0.01% to assess the means of all FFSC strains. The variance homogeneity was analyzed using the Levene Test (Levene, 1960), and means were grouped by Duncan multiple range test (Duncan, 1955). The Arcsine transformation was applied to variance stabilization when the means failed the assumption of equal variance (Fisher, 1922), and the groups of the multiple range test were presented using non-transformed data.

3. RESULTS AND DISCUSSION

A fumonisin analysis by HPLC was conducted with an agar plug culture of FFSC strains from maize samples in different regions of Türkiye in this study. For the analysis, three for *F. verticillioides*, one for *F. proliferatum*, *F. thapsinum*, and *F. andyazi*, and two undefined *Fusarium* strains at the species level belonging to FFSC, were cultured in MEA agar medium, and all the FFSC strains, except one of them, were produced at various quantitative levels of either FB1 or both mycotoxins on the synthetic medium conditions (Table 2). Frisvard et al. (2007) reported that the MEA medium, particularly when made from Merck products, allowed for the highest concentration of FB1, FB2, and FB3 production compared with other synthetic agar media under culture conditions. It has been stated that the MEA medium is suitable for obtaining fast and reliable results in FB production and screening of FFSC members or other FB producers, and the results of this study confirm this.

Table 2. The amounts of FB1 and FB2 extracted from culture plates of FFSC strains

Accession Code	FFSC Species/Strains	Mycelial Weight (g)	FB1 $\mu\text{g kg}^{-1}$	FB2 $\mu\text{g kg}^{-1}$	Total FB $\mu\text{g kg}^{-1}$
TR11-V137m	<i>F. verticillioides</i>	0.999	4100.95	2294.23	6395.18
TR11-V96	<i>F. verticillioides</i>	0.914	2841.03	1855.86	4696.89
TR11-V40	<i>F. verticillioides</i>	0.910	97.17	n.d	97.17
TR11-P26	<i>F. proliferatum</i>	0.938	4902.75	3018.97	7921.72
TR11-V134	<i>F. thapsinum</i>	0.969	1952.38	964.99	2717.37
TR11-V11	<i>F. andyazi</i>	1.017	n.d	n.d	n.d
TR13-228	<i>Fusarium</i> sp.	0.851	4222.99	2052.63	6275.62
TR11-V147	<i>Fusarium</i> sp.	1.001	642.81	517.02	1159.83
LOD ($\mu\text{g kg}^{-1}$)			63.0	74.0	
LOQ ($\mu\text{g kg}^{-1}$)			192.0	218.0	
Recovery (%)			95	91	
RT (minutes)			3.5	4.0	

FFSC: *Fusarium fujikuroi* species complex, FB: Fumonisin B series, n.d.: not detected (>LOD)

Total amount of FB production by FFSC strains in this study ranged from 97.17 $\mu\text{g kg}^{-1}$ to 7921.72 $\mu\text{g kg}^{-1}$. The highest amount of total FB was detected at 7921.72 $\mu\text{g kg}^{-1}$ in *F. proliferatum*, while the lowest amount, 97.19 $\mu\text{g kg}^{-1}$, was found in *F. verticillioides* and was not produced in *F. andyazi* (Table 2 and Figure 1). Many reports have emphasized that *F. verticillioides* and *F. proliferatum* are the primary sources of FB in maize plants (Munkvold and Desjardins, 1997; Marin et al., 1999; Rheeder et al., 2002; Scott, 2012; Anumudu et al., 2024). Contrary to *F. verticil-*

lioides, *F. proliferatum* can colonize a broad range of hosts in addition to maize and bananas, as diverse as palm trees, asparagus, pine trees, particularly wheat and barley (Desjardins et al., 2007). This indicates that substrates such as MEA medium can provide an opportunity for colonization, and thus, the formation of FB is significantly more than that of *F. verticillioides*.

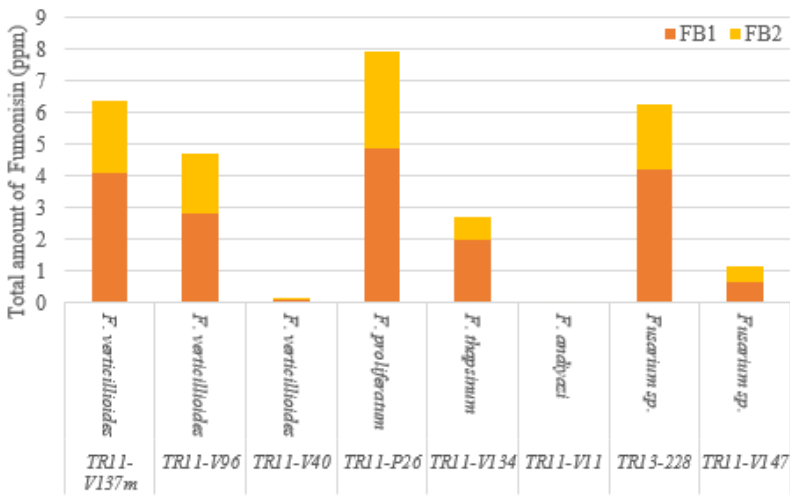


Figure 1. The results of total FB amounts in the FFSC strains by HPLC analysis

The biosynthesis of FB production in *Fusarium* was extensively detailed on many of the FFSC strains and isolates based on *fum1*, which encodes a polyketide synthase, and *fum21* related to Zn(II)2Cys6DNA-binding domain (Proctor et al., 1999; Brown et al., 2007; Alexander, 2009; Proctor et al., 2013). Four strains of *F. verticillioides*, including one strain (TR11-V1) as a reference strain for pathogenicity on maize seedlings in this study, were characterized on the *fum1* gene in a previous study (Table 1), and the amount of FB produced by these strains was determined at above 4 ppm, which is limited for FB to dietary intake of mycotoxins by EFSA (2014). However, a strain of *F. verticillioides* was found to produce a substantially lower amount of FB1 at 97.17 $\mu\text{g kg}^{-1}$ and none of FB2, respectively. Although the strain possessed a *fum1* gene, which is a key component in fumonisin biosynthesis, it produced considerably less FB1 and FB2 than other *F. verticillioides* strains. Glenn et al. (2008) revealed that *F. verticillioides* strains were unable to produce FB due to their lack of most of the gene cluster that encodes the necessary enzymes for FB biosynthesis. Similarly, it has been reported that five of twenty-two different *F. verticillioides* isolates obtained from maize plants in Italy did not produce FB, whereas they possessed the *fum1* gene (Covarelli et al., 2012).

Fusarium andiyazi TR11-V11 strain was found to be a non-producer of both FB among the FFSC in this study (Table 2, Figure 1). At the same time, it has been stated that the strain reported as the first-time isolated from maize seeds in Türkiye had no *fum1* gene locus, which was associated with FB biosynthesis in a previous study (Kansu, 2021) (Table 1). Although *F. andiyazi* was first reported and described as a pathogen in the sorghum plant, it mainly causes Bakanae disease that responds to fast elongation of the seedlings and weak rice growth (Sun and Snyder, 1978; Marasas et al., 2001). Zhang et al. (2014) and Venturini et al. (2017) have reported that this species also causes ear or seed rot disease on maize in further studies. However, a few strains or isolates of *F. andiyazi* associated with maize were found to produce FBs at trace levels or not at all in nature (Rheeder et al., 2002; Alberts et al., 2016; Dong et al., 2023). These results support the production of none of the FB by the *F. andiyazi* strain in this study.

Fusarium thapsinum, as another member of FFSC, was first described in sorghum (*Sorghum bicolor* L.), and recently reported as a frequent stalk rot and rarely ear rot pathogen on maize in various regions of the world (Klittich et al., 1997; Rahjoo et al., 2008; J. Zhang et al., 2021). The strain used here was produced in 1952.38 $\mu\text{g kg}^{-1}$ and 964.99 $\mu\text{g kg}^{-1}$ for FB1 and FB2, respectively. It has been reported that *F. thapsinum* produced in nothing, or trace amounts of FB, in nature and culture conditions (Rheeder et al., 2002; Leslie et al., 2005b; Busman et al., 2012). Although it did not contain the *fum1* gene, which is a key factor for FB biosynthesis, it was interestingly able to produce FB1 and FB2, albeit in lower amounts than those of *F. verticillioides* and *F. proliferatum* strains. Gil-Serna et al. (2020) reported that the *fum21* gene is a promoter element recognized to activate transcription of fumonisin biosynthesis genes, and its involvement in regulating fumonisin biosynthesis is evident. For the FB amounts of both *F. thapsinum* (V11-V134) and *F. verticillioides* (V11-V40) in this study, *fum21* is crucial to investigate, and thus can help understand the high and low FB levels for these strains.

Pathogenicity test on the seedlings was completed with an inoculated pathogenic *F. verticillioides* (TR11-V1) and a non-inoculated treatment as a control, in addition to all FFSC strains, on a sweet maize hybrid and flint type maize cultivar under the growth chamber conditions. Except for the plant ratio with atrophy and stem/stalk rot disease, all evaluated plant parameters for FFSC strains were not found to be statistically significant in both maize cultivars (Tables 3 and 4). On the other hand, the plant ratio of atrophy (%) and stalk rot disease severity (%) were statistically significant at 0.05% and 0.01%, respectively, for only the hybrid maize cultivar. Even if no statistical comparison was made between the maize cultivars, the standard maize cultivar “Karadeniz Yıldızı” exhibited noticeably more plant growth than the hybrid maize cultivar “Vega-F1”, when compared for both the means of FFSC strains and non-inoculated control in terms of shoot length of the plants. Contrary to plant shoot length, it was shown that less means on chlorosis/

necrosis, atrophy, and stem rot disease, compared to the hybrid maize cultivar (Tables 3 and 4).

Table 3. Pathogenicity of FFSC strains from Türkiye on cv. Vega-F1

FFSC Accession Codes	cv. VEGA F1						
	GSR	PSL	CNPR	APR	*	SRD	**
TR11-V137m	73,3	23,5	13,3	16,7	ab	36,7	bcd
TR11-V96	76,7	24,7	30,0	23,3	b	40,0	bcd
TR11-V40	93,3	21,9	43,3	46,7	bc	46,7	d
TR11-P26	93,3	32,2	13,3	40,0	bc	13,3	ab
TR11-V134	80,0	29,4	23,3	26,7	b	16,7	abc
TR11-V11	86,7	29,7	10,0	40,0	bc	23,3	a-d
TR13-228	90,0	21,2	33,3	20,0	ab	46,7	d
TR11-V147	90,0	28,5	3,3	63,3	c	33,3	bcd
TR11-V1	93,3	26,7	40,0	43,3	bc	43,3	cd
CONTROL	100,0	29,2	3,3	3,3	a	0,0	a
Means/Std. Deviation	87,7±2,4	26,7±1,1	21,3±4,1	32,3±4,0		30,0±3,6	

GSR: Germinated Seed Ratio (%), PSL: Plant Shoot Length (cm), CNPR: Chlorosis&Necrosis Plant Ratio (%), APR: Plant Ratio with Atrophy (%), SRD:Stem/Stalk Rot Disease (%). Std: Standard deviation; cv. VEGA F1: sweet corn hybrid cultivar

* There is a significant difference between the groups at $P<0.05$; ** $P<0.01$

Table 4. Pathogenicity of FFSC strains from Türkiye on cv. Karadeniz Yıldızı

FFSC Accession Codes	cv. Karadeniz Yıldızı				
	GSR	PSL	CNPR	APR	SRD
TR11-V137m	96,7	57,2	0,0	13,3	0,0
TR11-V96	100,0	60,8	6,7	13,3	0,0
TR11-V40	93,3	58,3	6,7	3,3	6,7
TR11-P26	83,3	57,3	0,0	10,0	0,0
TR11-V134	86,7	59,2	0,0	16,7	10,0
TR11-V11	83,3	57,7	0,0	6,7	3,3
TR13-228	83,3	58,7	13,3	30,0	3,3
TR11-V147	86,7	58,2	0,0	0,0	6,7
TR11-V1	93,3	62	3,3	23,3	3,3
CONTROL	100,0	59,0	0,0	3,3	0,0
Means/Std. Deviation	90,7±2,0	58,8±0,9	3,0±1,3	12,0±2,6	3,3±1,3

GSR: Germinated Seed Ratio (%), PSL: Plant Shoot Length (cm), CNPR: Chlorosis&Necrosis Plant Ratio (%), APR: Plant Ratio with Atrophy (%), SRD:Stem/Stalk Rot; Disease (%). Std: Standard deviation; cv. Karadeniz Yıldızı standard maize cultivar

The “Vega-F1” cultivar is a sweet corn genotype, and it has higher amounts of sucrose than “Karadeniz Yıldızı”. It has been stated that the high sucrose or sugar content in maize tissues can lead to an increase in fungal colonization and, thus, the main causal pathogen of maize, such as *F. verticillioides*, accumulates toxins at high levels, such as FB (Berger and Wolf, 1974; Styer and Cantliffe, 1984). Desjardins et al. (1995) demonstrated that various maize cultivars, including sweet, flint, dent, and popping maize types, responded with different virulence in terms of germinating seeds, rotting seedlings, and elongation of shoots, and higher sensitivity to reaction against *Gibberella fujikuroi*, particularly in sweet corn type.

The atrophic plant ratio due to FFSC strains in sweet corn type plants ranged from 3.3% to 63.3%, while the FFSC strains in this study were grouped into three statistical levels compared to the non-inoculated control (Table 3). The highest atrophy was seen at 63.3% in *Fusarium* sp. (TR11-V147), which produced FB with above 1 ppm. The second group was included between 40% and 46.7% in subsequently *F. verticillioides* TR11-V40, which had low production of FB1, *F. verticillioides* TR11-V1 as a known pathogen control, *F. proliferatum* TR11-P26, and *F. andiyazi* TR11-V11, while the last group was four strains between 16.7% and 26.7 in two *F. verticillioides* (TR11-V137m and V96), *F. thapsinum* (TR11-V134), and *Fusarium* sp. (TR13-228). Similarly, the chlorotic and necrotic plant ratio varied between 3.3% and 43.3%, even if there were no statistically significant differences between FFSC strains (Table 3, Figures 2 and 3). Chlorosis/necrosis was found relatively higher in *F. verticillioides* strains than in other species of FFSC. However, there was no direct evidence on the presence of chlorosis/necrosis among produced and low-produced FB strains of *F. verticillioides*, such as TR11-V40 and TR11-V137m. The foliar disease symptoms, including both atrophy and chlorosis/necrosis observations, were not directly related to FB production of FFSC strains. Glenn et al. (2008) have reported that *F. verticillioides* strains from various plants, locations, and distinct fumonisin genotypes exhibited different foliar disease symptoms with atrophy and necrotic lesions on leaves in sweet maize seedlings, and especially the non-produced FB strains had foliar disease symptoms at a lower incidence (1%) compared with produced FB (ranged 43% and 93%). In addition, these researchers have found that dent-type cultivars were more resistant to foliar diseases than sweet corn. Our results suggest that all *F. verticillioides* strains caused both foliar disease symptoms at various levels of virulence on leaves of sweet corn. Moreover, they have also reduced the plant shoot length compared with the control (Table 3). Contrary to Glenn’s results, only a TR11-V40 strain, which was a low producer of FB in *F. verticillioides*, resulted in high atrophy, chlorosis/necrosis lesions, and reduced plant length for foliar disease symptoms. The fact that this strain resulted in almost near foliar disease severity with pathogenic control *F. verticillioides* strain TR11-V1 needs to be highlighted in terms of its FB production ability by quantifying on other substrates again. Out of the *F. verticillioides* in FFSC, all other species exhibited a wide range of foliar disease symptoms on plants

at 3.3% and 63.3%, and all species also produced FB in various quantities, except for *F. andiyazi*. Even if there was no close correspondence between foliar diseases and FB production for these species, it needs to be examined with a larger number of representative isolates of each species.



Figure 2. Pathogenic effects of FFSC strains that produce and non-produce FB in maize cultivars

Fusarium stem/stalk rot is one of the most important diseases in maize, and it was reported to reduce the yield by 10-20% (Kommedahl and Windels, 1981; Yang, 2010). As the predominant species, *Fusarium verticillioides*, *F. graminearum*, *F. proliferatum*, *F. subglutinans*, *F. flocciferum*, *F. temperetum* and *F. oxysporum* were responsible for the disease as pathogens (Gherbawy et al., 2002; Shin et al., 2014; Douriet-Angulo et al., 2019; Jambhulkar et al., 2024). Here, we found that all fungal strains have had a pathogenic effect on stem/stalk rot disease, and the disease severity reached 46.7%, particularly in the sweet corn cultivar ($P < 0.01$, Table 3, Figure 3). The first group, which had less virulence on maize, included *F. proliferatum*, *F. andiyazi*, and *F. thapsinum* with an untreated control (ranging from 0 to 23.3%), while the second group was mainly in *F. verticillioides* strains, and undefined *Fusarium* spp. as a highly virulent phenotype (above 23.3%). Although the *F. verticillioides* was found to be highly virulent for stem/stalk rot disease in this study, there was no correspondence between FB production and pathogenicity/virulence of *F. verticillioides* strains, and other species of FFSC. Covarelli et al. (2012) have reported that there was no correlation between FB production

and pathogenicity of distinct *F. verticillioides* strains obtained from Italy, and also non-producing FB strains of *F. verticillioides* actually could have a potential for FB production and restored themselves under particular environmental conditions.



Figure 3. Chlorosis, necrosis, and atrophy symptoms of leaves, and stem/stalk rot diseases in Vega-F1 hybrid maize plants treated with FFSC strains; **A.** Atrophic leaves in TR11-V96 *F. verticillioides*, **B.** Chlorotic leaves in TR11-V134 *F. thapsinum*, **C.** Necrotic symptoms on leaves in pathogen TR11-V1 *F. verticillioides*; **D.** Stem/stalk rot disease symptoms with highly virulence strain TR13-228 *Fusarium* sp., **E.** TR11-V40 *F. verticillioides*, **F.** Weak virulence strain TR11-P26 *F. proliferatum*, **G.** Pathogenic control TR11-V1.

4. CONCLUSION

Fusarium fujikuroi species complex is a comprehensive and includes more important plant pathogenic fungi that cause ear rot and stem/stalk rot diseases, such as *F. verticillioides*, *F. proliferatum*, *F. thapsinum*, *F. andiyazi*, and other species. This study provides an evaluation of the relationship between FB production and pathogenicity/virulence for the mentioned above species of FFSC members *in vitro*. FFSC strains exhibited high quantitative diversity in FB1 and FB2 production, with *F. proliferatum* identified as the most significant producer in culture conditions. For the FB biosynthesis, the presence of the *fum1* gene is a key indicator, yet its presence did not always result in high toxin levels, as observed in some *F. verticillioides* strains. Furthermore, the ability of *F. thapsinum* to produce FB despite lacking *fum1* suggested that the *fum21* gene plays a crucial regulatory role in activating biosynthesis pathways. The possible involvement of other FUM-cluster genes, such as *fum21*, should be investigated in future studies. On the other hand, maize cultivars responded differently to infection; sweet corn genotypes (like “Vega-F1”) were significantly more susceptible to fungal colonization, atrophy, and stalk rot than flint types. This increased sensitivity was likely linked to higher sucrose content, which facilitates fungal growth and toxin accumulation.

A critical finding provided that there was no direct relationship between their ability to produce FB and the virulence or pathogenicity of FFSC strains. Strains with low or no FB production are still capable of causing severe disease symptoms, such as atrophy and stem rot, particularly in susceptible cultivars. These results, obtained using a limited number of strains from different species in FFSC, particularly *F. thapsinum*, *F. proliferatum*, and *F. andiyazi*, may lead to clearer and more detailed findings in future studies that utilize a larger number of strains from one or more species to establish the relationship between FB production and disease development in maize.

Conflict of Interest

The authors declared that they have no conflict of interest.

Ethics

This study does not require the ethics committee approval.

Author Contribution Rates

Design of Study: BK (70%), HÖ (30%)

Data Acquisition: BK (50%), HÖ (50%)

Data Analysis: BK (60%), HÖ (40%)

Writing up: BK (65%), HÖ (35%)

Submission and Revision: BK (75%), HÖ (25%)

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