http://dergipark.org.tr/trkjnat
Trakya University Journal of Natural Sciences, 23(1): 71-79, 2022
ISSN 2528-9691
DOI: 10.23902/trkjnat.1000196

Research Article

TERPINOLENE IS AN EFFECTIVE ESSENTIAL OIL COMPOUND TO PROTECT *Hordeum vulgare* L. FROM *Fusarium avenaceum* (Fr.) Sacc.

Zeynep DANISMAN[1], Sule INAN[2], Esma OZSOY[1], Emre YÖRÜK[1*], Tapani YLI-MATTILA[3]

[1] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, İstanbul Yeni Yüzyıl University, Cevizlibağ, İstanbul 34010, TÜRKİYE
[2] Department of Molecular Biology and Genetics, Faculty of Sciences, İstanbul University, Süleymaniye, İstanbul 34116, TÜRKİYE
[3] Molecular Plant Biology, Department of Life Technologies, University of Turku, FI-20014, Turku, FINLAND

Cite this article as:

Danisman Z., Inan S., Ozsoy S., Yörük E. & Yli-Mattila T. 2022. Terpinolene is an effective essential oil compound to protect *Hordeum vulgare* L. from *Fusarium avenaceum* (Fr.) Sacc. *Trakya Univ J Nat Sci*, 23(1): 71-79, DOI: 10.23902/trkjnat.1000196

Received: 24 September 2021, Accepted: 25 February 2022, Online First: 17 March 2022, Published: 15 April 2022

Edited by:
Mustafa Yamaç

***Corresponding Author:**
Emre Yoruk
emre.yoruk@yeniyuzyil.edu.tr

ORCID iDs of the authors:
ZD. orcid.org/0000-0002-4194-3924
SI. orcid.org/0000-0001-7921-0913
EO. orcid.org/0000-0002-3727-8466
EY. orcid.org/0000-0003-2770-0157
TYM. orcid.org/0000-0002-0336-880X

Key words:
Antifungal agent
Fusarium avenaceum
Hordeum vulgare L.
qRT-PCR
Terpinolene

Abstract: *Fusarium avenaceum* (Fr.) Sacc. is an important phytopathogen. Fight against *F. avenaceum* includes primarily fungicide usage. However, novel strategies are needed in a struggle with *F. avenaceum*. In this study, terpinolene was used against *F. avenaceum* as an antimicrobial agent, and the harmlessness of terpinolene was tested on two contrast barley genotypes, *Hordeum vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium. Firstly, minimum inhibitory concentration (MIC) and half inhibitory concentration (IC_{50}) were detected as 6 and 3 µg μL^{-1}. Quantitative real-time polymerase chain reaction (qRT-PCR) assay was informative about the effects of terpinolene on enniatin and zearalenone biosynthesis in *F. avenaceum*. Terpinolene leads to the potential decreased enniatin and zearalenone biosynthesis in *F. avenaceum*. However, no significant differences were recorded for gene expression of aurofusariun biosynthesis. In barley, electroconductivity (EC), catalase (CAT) activity, coupled restriction enzyme digestion-random amplification (CRED-RA), and qRT-PCR assays were tested. No significant ion leakage differences ($p>0.05$) were detected. Similarly, CAT activity and water loss rate (WLR) values in barley sets were not affected ($p>0.05$) by terpinolene treatment in majority of experiment sets. Relatively low levels of genomic template instability (75-85%) and epigenetics changes (11-20.6%) were recorded in barley due to terpinolene treatment. *WRKY6*, *WRKY24*, and *WRKY41* expressions were not significantly changed. The findings showed that terpinolene could be accepted as a potential plant protective agent against phytopathogenic fungi.

Özet: *Fusarium avenaceum* (Fr.) Sacc. önemli bir bitki patojenidir. *Fusarium avenaceum* ile mücadele temelde fungisit kullanımını içerir. Ancak yeni stratejilere ihtiyaç duyulmaktadır. Mevcut çalışmada iki zıt arpa genotipi olan *Hordeum vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium genotiplerinde terpinolenin zararsız etkileri ve *F. avenaceum*'a karşı antimikrobiyal etkileri araştırıldı. İlk olarak minimum inhibisyon konsantrasyonları (MİK) ve üremeyi baskılayan yarı konsantrasyon (IC_{50}) değerleri 6 and 3 µg μL^{-1} olarak belirlendi. Gerçek zamanlı polimeraz zincir reaksiyonu (kPZR) *F. avenaceum*'da zearalenone ve enniatin üretimi üzerinde terpinolenin etkisinin belirlenmesinde bilgi verici oldu. Terpinolene *F. avenaceum*'da enniatin ve zearalenone üretiminde potansiyel baskılamaya sebep oldu. Buna karşın aurofusariun biyosentez gen analtımında anlamlı farklılık görülmemiştir. Arpada elektrokonduktivite (EK), katalaz (KAT) aktivitesi, CRED-RA analizi ve kPZR analizleri test edildi. EK değerlerinde bilimsel olarak anlamlı ($p>0,05$) farklılık gözlemlenmedi. Benzer şekilde deney setlerinin çoğunda katalaz aktivitesi ve su kaybetme oranları (SKO) terpinolene uygulamasından etkilenmedi ($p>0,05$). Arpada terpinolene uygulamasına bağlı olarak göreceli olarak düşük genomik instabilite (%75-85) ve epigenetik değişimler (%11-20,6) kaydedildi. *WRKY6, WRKY24* ve *WRKY41* gen anlatımları anlamlı farklılık göstermedi. Bulgular terpinolenin fitopatojenik mantarlara karşı potansiyel bir bitki koruma ajanı olarak kabul edilebileceğini göstermektedir.

Introduction

Fusarium avenaceum (Fr.) Sacc. is a phytopathogenic fungus causing head blight and root rot diseases on small grain cereals (Yli-Mattila *et al.* 2004, 2018, Uhlig *et al.* 2007, Gräfenhan *et al.* 2013, Hietaniemi *et al.* 2016). This phytopathogenic species is widely distributed in temperate regions, especially in Europe, and produces several

significant mycotoxins such as moniliformin and enniatins (Jestoi *et al.* 2004, Uhlig *et al.* 2007). *Fusarium avenaceum* mainly infects wheat, barley and some other cereals, and diseases it causes can lead to serious economic losses (Yli-Mattila *et al.* 1997, Logrieco *et al.* 2002, Jestoi *et al.* 2004). The anamorphic phase of *F. avenaceum* has been reported previously (Cook 1967, Booth & Spooner 1984), however, no sexual stage has been determined despite the species has two mating type alleles (Kerényi *et al*. 2004). 13092 protein coding genes, 41.7 Mb genome size, 48.1% GC data was reported from genomic survey analysis (Lysøe *et al.* 2014, www.ncbi.nlm.nih.gov). Since *F. avenaceum* populations represent moderate and high levels of genetic diversity, struggle with *F. avenaceum* and management of the diseases caused by *F. avenaceum* needs comprehensive, efficient, and novel strategies.

Fusarium avenaceum is one of the major causal agents of Fusarium head blight (FHB) and root rot (RR) in barley, especially in Northern Europe and Asia. Barley is an economically important cereal worldwide. According to the Food and Agriculture Organization (FAO) data for the year 2019, Europe provided approximately 60% of the total barley production with 95.634.161 tons as the major barley production centre worldwide. FHB and RR are major diseases related in barley in humid and semi-humid regions. Infections result in reduced yield quality and quantity as well as mycotoxin contamination (Parry *et al.* 1995, Champeil *et al.* 2004, Yli-Mattila & Gagkaeva 2018, http://fao.org/faostat). Fight against *F. avenaceum* and related *Fusarium* species causing FHB and RR is based on several different strategies including fungicide treatment, disease-resistant cultivar development, and antagonistic microorganism usage (McMullen *et al.* 2008, Lori *et al.* 2009). Fungicide resistant strains, cultivars with a low levels of agronomic traits, and relatively high costs are some of the disadvantageous of these strategies (Chung *et al.* 2008, Arif *et al.* 2009, da Cruz *et al.* 2013, Talas & McDonald 2015, Dweba *et al.* 2017, Machado *et al.* 2018, Yang *et al.* 2018, Perczak *et al.* 2019). Thus, there is a need to develop novel strategies in combat with FHB and RR diseases in barley.

Essential oils (EOs) are volatile molecule-mixtures obtained from plants with different major and minor compounds (Perczak *et al.* 2019). Previous investigations have shown the presence of antimicrobial effects and potential usage in disease management of EOs (Fandohan *et al.* 2004, Marin *et al.* 2004, Velluti *et al.* 2004, Sumalan *et al.* 2013, Kumar *et al.* 2016, Sefer *et al.* 2017a, Yörük *et al.* 2018, Perczak *et al.* 2019). EOs may contain terpenoids and flavonoids showing strong antifungal effect. Therefore, considering the usage of EOs, single or in combination, might provide powerful insight into -FHB and RR management (Arif *et al.* 2009, Orhan *et al.* 2010, Daglia 2012, Goudjil *et al.* 2016, Gazdağlı *et al.* 2018a, Lingan 2018).

Plant derived EOs can be divided into two groups as major or minor compounds with their presence and quantity in plant extracts (Arif *et al*. 2009). Terpinolene (4-Isopropylidene-1-methylcyclohexene) is an EO easily obtained from several plants such as *Mentha* spp., *Thymus* spp. (Dorman & Deans 2000, Soković *et al.* 2009).

The potential antifungal effects of terpinolene on *F. avenaceum* and its phytoprotective effects on barley have not been previously investigated.

In the present study, the potential antifungal effects of this terpinolene on *F. avenaceum* and its harmless phytoprotective effects on barley were investigated.

Materials and Methods

Fungal material and minimum inhibitory concentration (MIC) determination

Fusarium avenaceum strain MFG118702 (= av48) from Russia representing the main group I (Yli-Mattila *et al.* 2018) was grown on potato dextrose agar (PDA) at 26 ± 2°C for 7 days. The concentrations of terpinolene were adjusted at distilled water: ethanol: diethyl ether (1:2:2 and V:V:V) solution mixtures. 0, 3, 6, 12, and 24 µg μL^{-1} terpinolene (Sigma, U.S.A.) including PDA media were used in MIC and half maximal inhibitory (IC_{50}) values were calculated via linear growth rate measurement at 4th and 7th days (Irzykowska *et al.* 2013). Each experiment was set up thrice.

qRT-PCR assays in F. avenaceum

Total RNA was extracted from the 100 mg fresh mycelium of 0 and 3 µg μL^{-1} time zero (T0) terpinolene treated fungal cultures via Trizol reagent (Invitrogen, U.S.A.). In T0 assay, terpinolene was amended to PDA medium before it was poured onto petri dishes three times and the cultures were exposed to terpinolene for seven days. The homogenization was carried out using sterile pestle, mortar, liquid nitrogen, and 1 mL Trizol reagent. Binding, washing, and eluting steps were done following the manufacturer's recommendations. In DNAseI treatment, 1 µL DNAseI (10 mg mL^{-1}), 1× reaction buffer, and 10 µg total RNA were mixed in 50 µL reaction volume and incubated at 37ºC for 10 min. 1µL of TE buffer (50 mM) was added to the mixture and samples were incubated at 70ºC for 5 min. The quality and quantity of total RNA were analysed via spectrophotometer (260/280 nm absorbance) and 0.8% agarose gels (1 hour for 60 V).

cDNA conversion was carried out by using a commercial kit (Takara, Japan). The reaction volume was 20 µL and it include 1× PrimeScript RT Master Mix, 2µg total RNA and distilled water. The mixture was first incubated at 37°C for 30 min and then at 80°C for 5 min. Samples were diluted with Easy dilution (for real time PCR) at 1:4 (V:V) and then stored until they would be used for qRT-PCR assays.

In gene expression analysis, enniatin biosynthesis (enniatin synthase: *esyn1*), zearalenone biosynthesis (polyketide synthase 4: *pks4*) and aurofusariun biosynthesis (polyketide synthase 13: *pks13*) related genes were used as target genes and *β-tubulin* as the housekeeping gene. The primers (Table 1) were designed using the Primer3 software. Sybr Green I based fluorophore dye and the QuantStudio 5.0 (Thermo, USA) systems were used in

qRT-PCR assays. The reaction mixes included cDNA corresponding to 50 ng RNA, 1× Sybr Green I mix (Takara, Japan), 1 pmol of each primer, and distilled water with a volume to complete the total volume to 20 µL. The cycling conditions were as 95ºC for 2 min (initial denaturation), 40 cycles of 95ºC for 15 s, 58ºC for 15 s, 72ºC for 30 s, and 72ºC for 2 min, and a common melting temperature scanning step. Five logs of dilution series were used in calculating the PCR efficiency values. The 2-ΔΔCT formula was used in evaluating the fold changes in gene expression (Livak & Schmittgen 2001). All experiments were carried out three times.

Plant material, Electroconductivity (EC) and Water loss rate (WLR)

Two contrasting barley genotypes, *Hordeum vulgare* L. cv. Premium (abiotic-biotic stress resistant) and *H. vulgare* L. cv. Cervoise (abiotic-biotic stress sensitive), were used. The seeds were kindly provided by the Aegean Agricultural Research Institute-Turkey. The potential adverse effects of terpinolene on barley were evaluated via EC and WLR tests at physiological levels.

Seeds were germinated on moisture filter papers in Petri dishes for 7 days at 22ºC±2ºC (in dark). 2 leaved stage seedlings were transferred to plastic pots including soil and then plantlets were sprayed with 0 and 3 µg μL^{-1} terpinolene following the procedure reported by Nardemir *et al.* (2015). The spraying was carried out for five times a day for each plantlets with at least two seconds intervals., Plantlets were removed from soils on the 14^{th} day of development and EC (i) and WLR (ii) were calculated as reported by Gürel *et al.* (2016). Leaves were inserted into glass tubes including 5 mL distilled water and incubated at dark for 16 hours and then EC1 was measured. After the autoclaving process, EC2 was calculated, and EC was calculated according to the formula.

$$EC = [1-(EC1/EC2)]\times 100.$$

Similarly, in WLR assays, firstly, fresh weight (FW) leaves of 14-day-old plantletswere measured. Leaves were left on filter paper for 24h and dried at 65°C for 2h. Weight of the leaves 24-hour-left on filter papers (W_{24}) and dry weight (DW) values were recorded. WLR was determined according to the formula

$$WLR\ (gh^{-1}g^{-1}\ DW) = (FW-W_{24})/(DW\times 24).$$

The experiments were carried out three times.

Genetics and epigenetics assays

Coupled restriction enzyme digestion-random amplification (CRED-RA) method was used to detect genomic template stability changes and epigenetic alterations due to terpinolene treatment. For this purpose, firstly genomic DNA (gDNA) molecules were extracted from 7-day-old fungal cultures using a common sodium dodecyl sulphate (SDS) based protocol (Niu *et al.* 2008, Yörük *et al.* 2016). The quality and quantity of gDNA were analysed via spectrophotometer (Thermo, U.S.A.) and 0.8% agarose gel electrophoresis, respectively.

Table 1. Gene specific primers used in gene expression analysis.

Gene	Primer	Forward (5′-3′)	Reverse (5′-3′)	Size (bp)
β-tubulin	Tubf/r	agggtcattacaccgagggt	gtaccaccaccaagagagtgg	121
esyn1	Esyf/r	ttcaagggctggacgtctatgta	gtgaagaaagcaggctcaacgag	587
pks4	pks4f/r	cgtcttcgagaagatgacat	tgttctgcaagcactccga	208
pks13	pks13f/r	cagctcctgtgattccaaca	ggcacgatagccacctacat	124
α-actin	hvactF/R	ggcacactggtgtcatggt	gcgcctcatcaccaacata	90
WRKY6	hvwrky6F/R	cgaaggtcattgtgctgttg	ctgtacccatcgctcatctt	101
WRKY24	hvwrky24F/R	catgagcagagcaccatct	gacatcatccgcacctgtat	110
WRKY25	hvwrky25F/R	catcatggaggtccaagcaa	acccgacaatgtccttctgg	114
WRKY41	hvwrky41F/R	agtgaaggacagtgctgatg	ggtcttcctcgttctcttcc	104

Table 2. List of CRED-RA primers, band specifications, RP and PIC values.

			***H. vulgare* L. cv. Cervoise/*H. vulgare* L. cv. Premium**			
Primer	**Sequence (5′-3′)**	**% GC**	**Total Band Number**	**% Polymorphic band**	**RP**	**PIC**
OPA-2	tgccgagctg	70	4/4	0.25/0.5	0.32/0.32	0.152/0.329
OPA-3	agtcagccac	60	na	na	na	na
OPA-7	gaaacgggtg	60	5/4	0.4/0.5	1.02/1.02	0.48/0.468
OPB-6	tgctctgccc	70	4/4	0.25/0.5	0.32/0.64	0.152/0.277
OPB-9	tgggggactc	70	na	na	na	na
OPB-14	tccgctctgg	70	na	na	na	na
OPC-5	gatgaccgcc	70	9/9	0.444/0.333	1.64/0.66	0.277/0.301
OPC-7	gtcccgacga	70	6/5	0.333/0	0.32/0	0.239/0
OPG-13	ctctccgcca	70	10/10	0.5/0.4	1.02/1	0.42/0.339
OPG-16	agcgtcctcc	70	8/8	0.375/0.125	1.3/0.32	0.186/0.079
Mean			**6.57/6.28**	**0.364/0.336**	**0.848/0.56**	**0.27/0.256**

na: not amplified

The alterations in genomic template stability (GTS) and epigenetic potential of the genome were investigated via CRED-RA assay. Totally 10 decamer primers were used (Table 2). GTS assay [accompanied with resolution power (RP) and polymorphisms information content (PIC) assays] was evaluated with different Random Amplified Polymorphic DNA (RAPD) profiling in *MspI*-digested, *HapII*-digested, and non-digested sets. Digestion mixture preparation, PCR mixtures, and conditions were carried out as reported by Gazdağlı *et al.* (2018a). 1.7% agarose gels were used in lateral electrophoresis and amplicons were visualized under UV light. The GTS, PIC, RP, and epigenetic alterations were calculated as reported by Alkan *et al.* (2019) and Nardemir *et al.* (2015). All experiments were carried out three times.

Catalase (CAT) activity and qRT-PCR assays in barley

Total proteins were extracted from 1 g of fresh leaves. Samples were firstly homogenized with sterile pestle and mortar using liquid nitrogen. And then, the common protocol provided by Harris & Angal (1989) was followed in protein extraction. Protein concentrations were determined using a commercial colorimetric BCA assay kit (Atlas, Turkey). The kinetic intervals dependent spectrophotometric analysis at 240 nm was used in CAT activity analysis. Fold CAT activity changes were recorded for each cultivar (Cho & Park 2000, Panda *et al.* 2003).

qRT-PCR analysis in terpinolene treated and non-treated barley sets were used to reveal if there are physiological alterations due to terpinolene treatment in barley. For this purpose, α-actin was used as housekeeping gene and *WRKY6*, *WRKY24*, *WRKY25* and *WRKY41* genes coding common WRKY transcription factors were used as target genes. These genes have records on GenBank for cereal species. PCR cycling conditions were as described above. Primers used in barley gene expression assays were the same as primers used by Tufan *et al.* (2020). All experiments were carried out three times.

Statistical analysis

All experiments were done in thrice. The statistical analysis was carried out using GraphPad Prism 5.0 and R-Studio softwares by using Student's t-test, one-way analysis of variance (ANOVA) with Tukey's LSD post-test. The significance level was 0.05 and the mean with standard deviation for each set was determined by column statistics. Heatmap analysis for gene expression assays were carried out via http://heatmapper.ca online software accompanied with average linkage clustering and Euclidian distance measurement methods.

Results

In vitro growth capacity assays and qPCR analysis in F. avenaceum

Terpinolene repressed fungal biomass and macroconidium production *in vitro*. Every T0 concentration of terpinolene treatment except 3 µg µL^{-1} terpinolene lead to total inhibition in fungal biomass. 3 µg µL^{-1} terpinolene treatment lead to half inhibition in radial growth. Moreover, each concentration of terpinolene treatment resulted in no macroconidium formation. MIC and IC_{50} values were determined as PDA+6 µg µL^{-1} and PDA+3 µg µL^{-1} terpinolene on *F. avenaceum* MFG118702 strain, respectively. IC_{50} value (3 µg µL^{-1} terpinolene treatment) was used in further studies including qRT-PCR analysis, infection, and spray treatment.

Up to 3 µg µL^{-1} total RNA was isolated from 7-day-old cultures of *F. avenaceum* control and experiment sets. cDNAs were synthesized from 2 µg total RNAs.

Fold changes in the expression of genes related to apoptosis, oxidative stress, and toxin production processes were evaluated via qRT-PCR assays. Scientifically acceptable E values were recorded for each gene. Minimum and maximum mean±SD Cp values were detected as 19.63±1.34 and 26.28±1.57, respectively. Relative mRNA abundance of *pks4* (aurofusariun biosynthesis), *pks13* (zearalenone biosynthesis), and *esyn1* (enniatin synthesis) genes were recorded as 0.22±0.14, 1.54±1.03, and 0.23±0.08, respectively. Statistical analysis showed that there were significant differences ($p<0.05$) between the control and experiment sets for *pks4* and *esyn1* genes while no significant differences were present for the *pks13* gene in *F. avenaceum*. Fig. 1 shows the heat map profile of three target genes.

EC and WLR assays

7-day-old seedlings were subjected to dH_2O (control set) and 6 µg µL^{-1} terpinolene treatment (treated set) for 7 days. No significant changes between dH_2O and terpinolene-treated sets were detected for ion leakage assays ($p>0.05$). EC values (mean±SD) ranged from 61.72±6.01 to 76.13±9.04. Terpinolene treatment resulted in no physiological alterations in terms of WLR values in *H. vulgare* L cv. Premium ($p>0.05$, Fig. 2) but significant changes were detected in *H. vulgare* L. cv. Cervoise ($p<0.01$, **). WLR values were recorded between 0.1±0.02 gh^{-1}g^{-1} and 0.44±0.12 gh^{-1}g^{-1}.

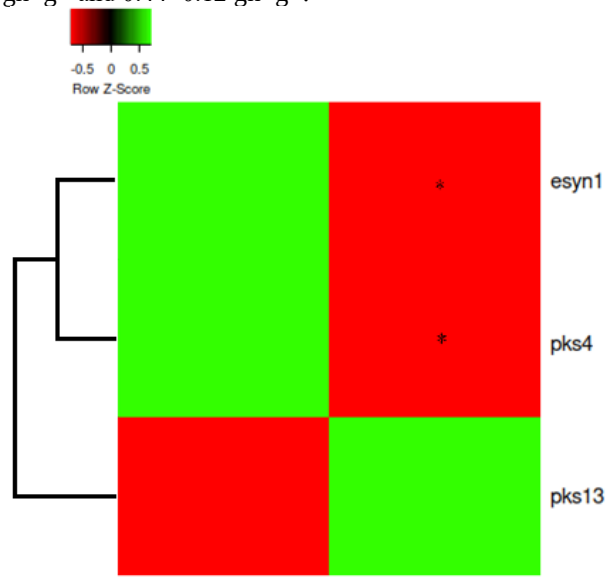

Fig. 1. Graphic for qRT-PCR analyses of *pks4*, *pks13* and *eysn1* genes in *F. avenaceum*. Asterisks indicate the presence of statistically significant differences between sets. Student's T-Test was used in comparison of *F. avenaceum* control (*FAC)* and *F. avenaceum* experiment (FAE) sets.

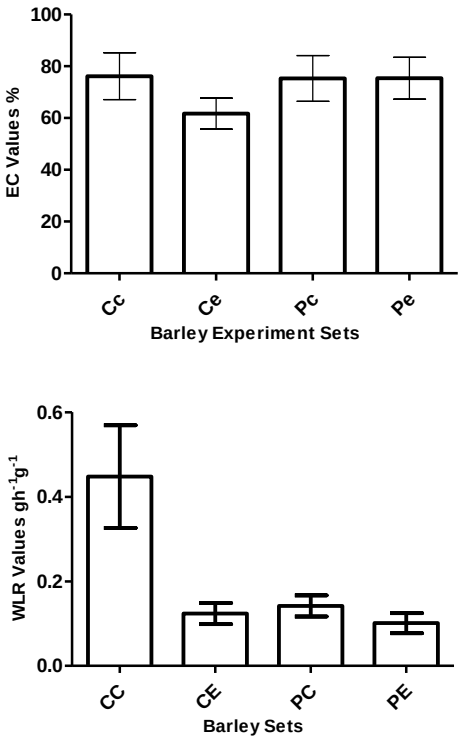

Fig. 2. Ion leakage and WLR patterns of H. vulgare L. cv. Cervoise, and H. vulgare L. cv. Premium control and experiment sets. Student's T-Test was used in comparison of control and experiment sets. "C" and "P" mean H. vulgare L. Cervoise and Premium, respectively. "c" and "e" mean control and experiment sets, respectively.

Genetics and epigenetics analysis in barley

CRED-RA assays were carried out using 10 decamer primers and each primer gave amplicons (Fig. 3). Uncut, *Hap*II-digested, and *Msp*I-digested samples for both cultivars yielded homomorphic and or idiomorphic profiles (Table 2). Maximum and minimum band numbers were 4 (OPA-2) and 10 (OPG-13), respectively. GTS was recorded as 75% and 85% for *H. vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium, respectively. Low (level of) RP values were recorded as 0.848 and 0.56 for *H. vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium, respectively. Similarly, low levels of polymorphisms were determined via PIC values as 0.27 and 0.25 for *H. vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium, respectively (Table 2). *Hap*II and *Msp*I related polymorphisms percent were also found at low levels for both cultivars. *Hap*II/*Msp*I related polymorphisms values for *H. vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium were detected as 20.6/12.5% and 15.7/11%, respectively.

*Hap*II and *Msp*I restriction endonucleases provided information about the potential epigenetic changes related to cytosine methylation. The potential epigenetic alterations related patterns were given in Table 2. The addition and loss of any band were recorded as a reason for the polymorphisms related to the epigenetic alterations. The mean of polymorphisms for *H. vulgare* L. cv. Cervoise was 20.6% and 12.5% for *Hap*II and *Msp*I digestion profiling. The values were 15.7% and 11.0% for *H. vulgare* L. cv. Premium. GTS values were recorded as 75% and 85% for *H. vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium, respectively.

*Hap*II and *Msp*I restriction endonucleases provided information about the potential epigenetic changes related to cytosine methylation. The potential epigenetic alterations related patterns were given in Table 2. The addition and loss of any band were recorded as a reason for the polymorphisms related to the epigenetic alterations. The mean of polymorphisms for *H. vulgare* L. cv. Cervoise was 20.6% and 12.5% for *Hap*II and *Msp*I digestion profiling. The values were 15.7% and 11.0% for *H. vulgare* L. cv. Premium. GTS values were recorded as 75% and 85% for *H. vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium, respectively.

Catalase (CAT) activity and qRT-PCR assays in barley

Terpinolene treatment related oxidative stress presence was investigated on barley. CAT activity was measured via kinetic interval absorbance detection (Fig. 4). According to the fold changes in CAT activity (0.945±0.14 for *H. vulgare* L. cv. Cervoise, and 0.657±0.35 for *H. vulgare* L. cv. Premium), no significant differences were recorded between control and experimental sets ($p>0.05$). Similarly, *WRKY6*, *WRKY24* and *WRKY41* expression were not affected by terpinolene treatment and fold changes in *WRKY* expression were recorded as 0.42±0.31 and 3.29±0.61 in *H. vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium, respectively. *WRKY41* and *WRKY24* were co-clustered while *WRKY6* clustered alone in a heat map (Fig. 5). One Way ANOVA analysis and Tukey's post-test showed that no significant decrease and or increase were present in *H. vulgare* L. cv. Cervoise and *H. vulgare* L. cv. Premium experimental sets. The fold changes in gene expression values changed between 0.42±0.31and 3.29±0.61 (Table 3). Heatmap pattern revealed two distinct groups due to relative fold change expression (Fig. 4).

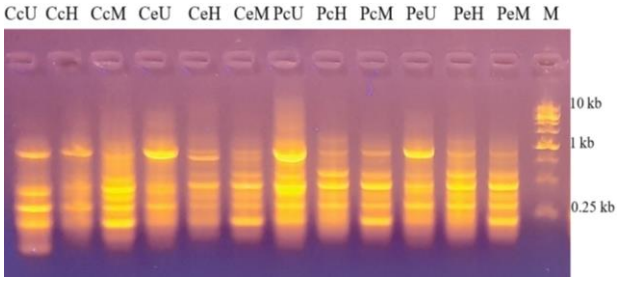

Fig. 3. CRED-RA profiles of two barley cultivars with OPG-13. "C" and "P" mean H. vulgare L. Cervoise and Premium, respectively. "c" and "e" mean control and experiment sets, respectively.

U: undigested, H: *Hap*II-digested, M: *Msp*II-digested, M: 1 kb marker (Thermo, USA).

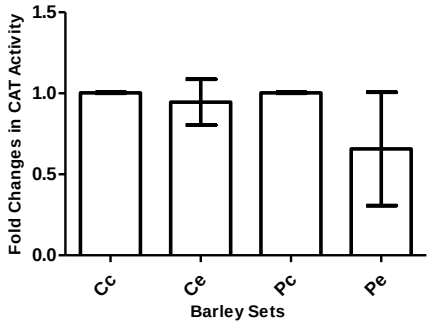

Fig. 4. CAT Activity patterns of *H. vulgare* L. cv. Cervoise, and *H. vulgare* L. cv. Premium control and experiment sets. *Student's T-Test was used in comparison of control and experiment sets*. ''C'' and ''P'' mean *H. vulgare* L. Cervoise and Premium, respectively. ''c'' and ''e'' mean control and experiment sets, respectively.

Table 3. Fold changes in gene expression for two barley cultivars.

Fold changes in Gene Expression		
Gene	***H. vulgare* L. cv. Cervoise**	***H. vulgare* L. cv. Premium**
WRKY6	3.29±0.61	1.76±0.51
WRKY24	0.72±0.63	0.42±0.31
WRKY25	2.17±1.37	1.24±0.07
WRKY41	1.64±0.04	0.88±0.20

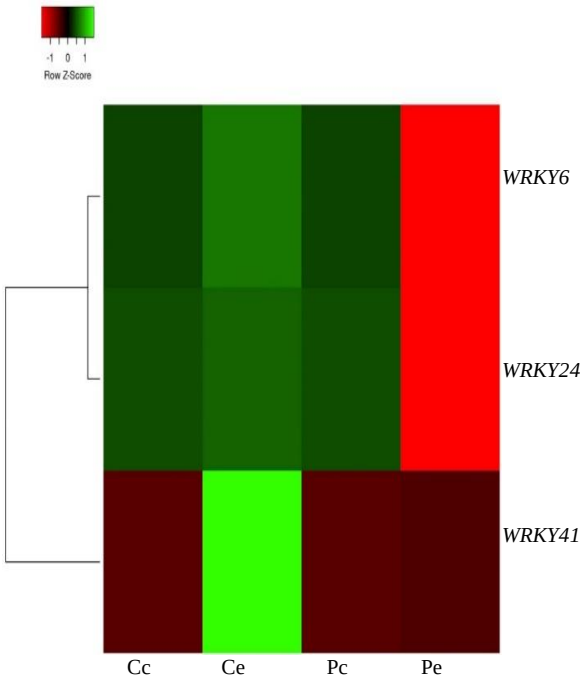

Fig. 5 Heatmap patterns of three target genes in *H. vulgare* L. cv. Cervoise (C) and *H. vulgare* L. cv. Premium (P) control (c) and experiment (e) sets. Student's T-Test and One way ANOVA tests were used in evaluation of fold changes in gene expression.

Discussion

The management *F. avenaceum* diseases includes several different strategies and the most common approach is fungicide treatment. Local and/or global reports revealed the presence of strains and isolates resistant to most preferred fungicides such as thiophanate methyl, carbendazim, tebuconazole, etc. worldwide (Kopacki & Wagner 2006, Chung *et al.* 2008, Lysøe *et al.* 2014, Ivic *et al.* 2015, Talas & McDonald, 2015, Yang *et al.* 2018, Chen *et al.* 2019). The major reasons for potential resistance development to these common fungicides may be unconsciousness usage of fungicides and the high level of genetic diversity within the populations of *Fusarium* spp. (Miedaner *et al.* 2008, Geiser *et al.* 2013, Lysøe *et al.* 2014, Pasquali & Migheli 2014). Therefore, we all need additional common strategies or approaches to fight against head blight and root rot. Previous reports published in the last two decades showed that plant derived EOs could be an alternative approach to fungicide treatment (Arif *et al.* 2009, Sumulan *et al.* 2013, Sefer *et al.* 2017b, Gazdağlı *et al.* 2018a, b).

EOs, produced by numerous plant species worldwide, are secondary metabolites with aromatic and volatile characteristics (Arif *et al.* 2009, Regnault-Roger *et al.* 2012). These secondary metabolites include various types of chemical compounds with minor or major quantities/percentages. Antifungal effects of EOs have been generally evaluated at phenotypic and physiological levels. These investigations have been supported by analytical methods. However, the majority of the studies included mixtures of EOs in comparison to a specific compound. Moreover, the studies including antifungal effects of specific compounds did not include in planta investigations (Arif *et al.* 2009, Orhan *et al.* 2010, Daglia 2012, Sefer *et al.* 2017a, b, Gazdağlı *et al.* 2018a, b, Yörük *et al.* 2018). Here, we described the potential antifungal effects of terpinolene on *F. avenaceum* and the of terpinolene on barley for the first time.

In previous studies, plant derived extracts containing terpinolene have been shown to be a potential antimicrobial agent against several fungal species (Tabanca *et al.* 2007, Soković *et al.* 2009). The potential antifungal effects of terpinolene have been investigated on *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, *F. graminearum, and F. culmorum* (Wm.G.Sm.) Sacc., and minimum and maximum MIC values were recorded as 349.17 mg L^{-1} to 16 g L^{-1} (Agus *et al.* 2018, Gazdagli *et al.* 2018b). In comparison to MIC values related to fungicides, almost all MIC values of plant derived essential oil were relatively higher in previous studies (Dorman & Deans 2000, Chung *et al.* 2008, Arif *et al.* 2009, Ivic *et al.* 2015, Chen *et al.* 2019). The low amount of need for fungicides could be accepted as it is advantage of fungicides. However, side effects of fungicides and resistant development in fungal populations worldwide led scientist to find novel chemicals to be used in fight with fungal diseases. For this purpose, plant derived EOs seem to be promising agents to be used in plant disease management. In this study, relatively medium level of MIC value was detected for terpinolene resistance in *F. avenaceum*. This value could also be accepted as a relatively low level of MIC value in comparison to kaempferol and 2, 4-dimethylpyrrole (Sefer

et al. 2017a, b). Thus, the potential antifungal usage of this terpinolene could be promising in future studies.

In addition to tests on lethal and sublethal doses in *F. avenaceum*, the potential mycotoxin biosynthesis inhibiting potential of terpinolene was also investigated in this study. Enniatin synthesis related *esyn1* gene expression was significantly downregulated due to terpinolene treatment. This result presented similar data with previous studies including deoxynivalenol and fumonisin biosynthesis inhibition (Jurado *et al.* 2008, Yaguchi *et al.* 2009, Gazdağlı *et al.* 2018a, Yörük 2018, Yörük *et al.* 2018). In comparison to these investigations, terpinolene had a strong effect on mycotoxin inhibiting activity and, this is the first report to show that terpinolene could inhibit enniatin biosynthesis at transcription level.

The current study includes *planta* tests in addition to *in vitro* antimicrobial tests, in comparison to previous studies. Detailed analysis related to physiological tests, genomic instability, and transcriptomic changes tests were carried out in terpinolene treated and non-treated barley sets. Ion leakage was not affected by terpinolene treatment in two contrast barley genotypes ($p>0.05$). CAT activity, similarly, was not significantly changed via terpinolene treatment in experiment sets. Moreover, abiotic stress related four *WRKY* genes (Tufan *et al.* 2020) were not significantly changed between control and experiment sets. These four genes are deposited under GenBank with several accession numbers for at least two cereal species. The epigenetic status of two barley genotypes was decreased. However, in comparison to previous studies, a narrower range of change was detected in this study (Nardemir *et al.* 2015, Gazdağlı *et al.* 2018a, Yörük 2018). According to the findings obtained from this study, it can be concluded that terpinolene does not affect barley's physiology, transcriptomics, epigenetics, and oxidative stress levels. Thus, terpinolene could be used in fight against *Fusarium* spp. diseases. Daily usage of terpinolene up to 3 $\mu g\ \mu L^{-1}$ concentrations with sprayed, liposomal or capsulized forms could be used for *in vitro* or in field investigations. Further studies may include terpinolene treatment in *in vitro* or *in vivo* studies to fight against *Fusarium* related diseases. The present findings will be supported by plant tissue culture and mammalian cell line tests in near future investigations related to terpinolene and other EOs.

Acknowledgement

The authors are grateful to Dr. Ayşe Feyza Turan Dülger (İstanbul-Turkey) for providing *H. vulgare* L. primers.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Author Contributions: Concept: T.Y-M., E.Y., Desing: T.Y-M., E.Y., Execution: T.Y-M., E.Y., Material supplying: Z.D., S.I., E.O., Data acquisition: Z.D., S.I., E.O., Data analysis/interpretation: Z.D., S.I., E.O., Writing: T.Y-M., E.Y., Critical review: T.Y-M., E.Y.

Conflict of Interest: The authors have no conflicts of interest to declare.

Funding: The study was supported by Research Fund of the İstanbul Yeni Yüzyil University (Grant numbers 2020-02).

References

1. Agus, H.H., Sarp, C. & Cemiloglu, M. 2018. Oxidative stress and mitochondrial impairment mediated apoptotic cell death induced by terpinolene in *Schizosaccharomyces pombe*. *Toxicology Research*, 7: 848-858.
2. Alkan, M., Göre, M.E., Bayraktar, H. & Özer, G. 2019. Genetic variation of *Fusarium* spp. isolates associated with root and crown rot of winter wheat using retrotransposon-based iPBS assays. *Uluslar Tarım Yaban Hayatı Bilim Dergisi*, 5: 250-259.
3. Arif, T., Bhosale, J.D., Kumar, N., Mandal, T.K., Bendre, R.S., Lavekar, G.S. & Dabur, R. 2009. Natural products–antifungal agents derived from plants. *Journal of Asian Natural Products Research*, 11: 621-638.
4. Booth, C. & Spooner, B.M. 1984. *Gibberella avenacea*, teleomorph of *Fusarium avenaceum*, from stems of *Pteridium aquilinum*. *Transactions of the British Mycological Society*, 82(1): 178-180.
5. Champeil, A., Fourbet, J.F., Doré, T. & Rossignol, L. 2004. Influence of cropping system on fusarium head blight and mycotoxin levels in winter wheat. *Journal of Crop Protection*, 23: 531-537.
6. Chen, H., Wu, Q., Zhang, G., Wu, J., Zhu, F., Yang, H. & Zhuang, Y. 2019. Carbendazim-resistance of *Gibberella zeae* associated with fusarium head blight and its management in Jiangsu Province. China. *Journal of Crop Protection*, 124: 104866.
7. Cho, U.H. & Park, J.O. 2000. Mercury-induced oxidative stress in tomato seedlings. *Plant Science*, 156: 1-9.
8. Chung, W.H., Ishii, H., Nishimura, K., Ohshima, M., Iwama, T. & Yoshimatsu, H. 2008, Genetic analysis and PCR-based identification of major *Fusarium* species causing head blight on wheat in Japan. *The Journal of Plant Pathology*, 74: 364-374.
9. Cook, R.J. 1967. *Gibberella avenacea* sp. n., perfect stage of *Fusarium roseum f. sp. cerealis* Avenaceum'. *Phytopathology*, 57(7): 732-736.
10. da Cruz Cabral, L., Pinto, V.F. & Patriarca, A. 2013. Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. *International Journal of Food Microbiology*, 166: 1-14.
11. Daglia, M. 2012. Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, 23: 174-181.

12. Dorman, H.D. & Deans, S.G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88: 308-316.

13. Dweba, C.C., Figlan, S., Shimelis, H.A., Motaung, T.E., Sydenham, S., Mwadzingeni, L. & Tsilo, T.J. 2017. Fusarium head blight of wheat: pathogenesis and control strategies. *Journal of Crop Protection*, 91: 114-122.

14. Fandohan, P., Gbenou, J.D., Gnonlonfin, B., Hell, K., Marasas, W.F.O. & Wingfield, M.J. 2004. Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. *Journal of Agricultural and Food Chemistry*, 52**:** 6824-6829.

15. Gazdağlı, A., Sefer, Ö., Yörük, E., Varol, G., Teker, T. & Albayrak, G. 2018a. Investigation of camphor effects on *Fusarium graminearum* and *F. culmorum* at different molecular levels. *Pathogens*, 7: 90.

16. Gazdağlı, A., Sefer, Ö., Yörük, E., Varol, G.İ. & Albayrak, G. 2018b. Investigation on the terpinolene effects in *Fusarium graminearum* and *F. culmorum* (at different molecular levels), pp. 33-42. In: Aina, A.T. & Demirçalı, A (eds) *Academic studies in Science and Mathematics*, Gece Kitaplığı, Türkiye, 65 pp. ISBN: 978-605-288-613-7.

17. Geiser, D.M., Aoki, T., Bacon, C.W., Baker, S.E., Bhattacharyya, M.K., Brandt, M.E. *et al.* 2013. One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. *Phytopathology*, 103: 400-408.

18. Goudjil, M., Ladjel, S., Bencheikh, S.E., Hammoya, F., Bensaci, M.B., Zighmi, S. & Mehani, M. 2016. Bioactivity of *Artemisia herba alba* essential oil against plant pathogenic fungi. *Der Pharma Chemica*, 8: 46-52.

19. Gräfenhan, T., Patrick, S.K., Roscoe, M., Trelka, R., Gaba, D., Chan, J.M., McKendry, T., Clear, R.M. & Tittlemier, S.A. 2013. *Fusarium* damage in cereal grains from Western Canada. 1. Phylogenetic analysis of moniliformin-producing fusarium species and their natural occurrence in mycotoxin-contaminated wheat, oats, and rye. *Journal of Agricultural and Food Chemistry*, 61: 5425-5437.

20. Gürel, F., Öztürk, N.Z., Yörük, E., Uçarlı, C. & Poyraz, N. 2016. Comparison of expression patterns of selected drought-responsive genes in barley *(Hordeum vulgare L.)* under shock-dehydration and slow drought treatments. *Plant Growth Regulation*, 80: 183-193.

21. Harris, E.L.V. & Angal, S. 1989. *Protein purification methods*, IRL Press at Oxford University Press, Oxford, 336 pp.

22. Irzykowska, L., Bocianowski, J. & Baturo-Cieśniewska, A. 2013. Association of mating-type with mycelium growth rate and genetic variability of *Fusarium culmorum*. *Central European Journal of Biology*, 8: 701-711.

23. Hietaniemi, V., Ramo, S., Yli-Mattila, T., Jestoi, M., Peltonen, S., Kartio, M., Sievilainen, E., Koivisto, T. & Parikka, P. 2016. Updated survey of *Fusarium* species and toxins in finnish cereal grains. *Food Additives & Contaminants: Part A*, 33: 831-848.

24. Ivic, D., Sever, Z. & Kuzmanovska, B. 2015. In vitro sensitivity of *fusarium graminearum*, *F. avenaceum* and *F. verticillioides* to carbendazim, tebuconazole, flutriafol, metconazole and prochloraz. *Pesticides and Phytomedicine*, 26: 35-42.

25. Jestoi, M., Rokka, M., Yli-Mattila, T., Parikka, P., Rizzo, A. & Peltonen, K. 2004. Presence and concentrations of the *Fusarium*-related mycotoxins beauvericin, enniatins and moniliformin in finnish grain samples. *Food Additives and Contaminants*, 21: 794-802.

26. Jurado, M., Marín, P., Magan, N. & González-Jaén, M.T. 2008. Relationship between solute and matric potential stress, temperature, growth, and FUM1 gene expression in two *Fusarium verticillioides* strains from Spain. *Applied and Environmental Microbiology Journal*, 74: 2032-2036.

27. Kerényi, Z., Moretti, A., Waalwijk, C., Oláh, B. & Hornok, L. 2004. Mating type sequences in asexually reproducing *Fusarium* species. *Applied and Environmental Microbiology Journal*, 70: 4419-4423.

28. Kopacki, M. & Wagner, A. 2006. Effect of some fungicides on mycelium growth of *Fusarium avenaceum* (Fr.) sacc. pathogenic to chrysanthemum (Dendranthema grandiflora Tzvelev). *Agronomy Research*, 4: 237-240.

29. Kumar, P., Mishra, S., Kumar, A. & Sharma, A.K. 2016. Antifungal efficacy of plant essential oils against stored grain fungi of *Fusarium* spp. *The Journal of Food Science and Technology*, 53: 3725-3734.

30. Lingan, K. 2018. Antifungal activity of *Artemisia nilagirica* essential oil from the western ghats nilgiris against food borne fungi. *Applied Biochemistry and Microbiology*, 2: 2-6.

31. Livak, J.K. & Schmittgen, T.D. 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25: 402-408.

32. Logrieco, A., Rizzo, A., Ferracane, R. & Ritieni, A. 2002. Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. *Applied and Environmental Microbiology Journal*, 68: 82-85.

33. Lori, G.A., Sisterna, M.N., Sarandón, S.J., Rizzo, I. & Chidichimo, H. 2009. Fusarium head blight in wheat: impact of tillage and other agronomic practices under natural infection. *Journal of Crop Protection*, 28: 495-502.

34. Lysøe, E., Harris, L.J., Walkowiak, S., Subramaniam, R., Divon, H.H., Riiser, E.S., Llorens, C., Gabaldón, T., Kistler, H.C., Jonkers, W., Kolseth, A.K., Nielsen, K.F., Thrane, U. & Frandsen, R.J. 2014. The genome of the generalist plant pathogen *Fusarium avenaceum* is enriched with genes involved in redox, signaling and secondary metabolism. *PLoS One*, 9: e112703.

35. Machado, A.K., Brown, N.A., Urban, M., Kanyuka, K. & Hammond-Kosack, K.E. 2018. RNAi as an emerging approach to control fusarium head blight disease and mycotoxin contamination in cereals. *Pest Management Science*, 74: 790-799.

36. Marin, S., Velluti, A., Ramos, A.J. & Sanchis, V. 2004. Effect of essential oils on zearalenone and deoxynivalenol production by *Fusarium graminearum* in non-sterilized maize grain. *Food Microbiology*, 21: 313-318.

37. McMullen, M., Halley, S., Schatz, B., Meyer, S., Jordahl, J. & Ransom, J. 2008. Integrated strategies for fusarium head blight management in the United States. *Cereal Research Communications*, 36: 563-568.

38. Miedaner, T., Cumagun, C.J.R. & Chakraborty, S. 2008. Population genetics of three important head blight pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*. *Journal of Phytopathology*, 156: 129-139.

39. Nardemir, G., Aga, G., Arslan, E. & Erturk, F.A. 2015. Determination of genetic and epigenetic effects of glyphosate on *Triticum aestivum* with RAPD and CRED-RA techniques. *Theoretical and Experimental Plant Physiology*, 27: 131-139.

40. Niu, C., Kebede, H., Auld, D.L., Woodward, J.E., Burow, G. & Wright, R.J. 2008. A safe inexpensive method to isolate high quality plant and fungal DNA in an open laboratory environment. *African Journal of. Biotechnology*, 7: 2818-2822.

41. Orhan, D.D., Özçelik, B., Özgen, S. & Ergun, F. 2010. Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiological Research*, 165: 496-504.

42. Panda, S.K., Chaudhury, I. & Khan, M.H. 2003. Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. *Plant Biology*, 46: 289-294.

43. Parry, D.W., Jenkins, P. & McLeod, L. 1995. Fusarium ear blight (scab) in small grain cereals -a review. *Plant Pathology*, 44: 207-238.

44. Pasquali, M. & Migheli, Q. 2014. Genetic approaches to chemotype determination in type B-trichothecene producing Fusaria. *The International Journal of Food Microbiology*, 189: 164-182.

45. Perczak, A., Gwiazdowska, D., Marchwińska, K., Juś, K., Gwiazdowski, R. & Waśkiewicz, A. 2019. Antifungal activity of selected essential oils against *Fusarium culmorum* and *F. graminearum* and their secondary metabolites in wheat seeds. *Archives of Microbiology*, 201: 1085-1097.

46. Regnault-Roger, C., Vincent, C. & Arnason, J.T. 2012. Essential oils in insect control: low-risk products in a high-stakes world. *The Annual Review of Entomology*, 57: 405-424.

47. Sefer, Ö., Yörük, E., Develi, E.S., Sezer, A.S. & Konukcu, Z. 2017a. Investigation of the effects of 2, 4-Dimethylpyrrole on *Fusarium culmorum* causal agent of head blight disease. *International Journal of Secondary Metabolite*, 4: 103-113.

48. Sefer, Ö., Yörük, E., Develi, E.S., Zümrüt, I.M., Sezer, A.S. & Konukçu, Z. 2017b. Effects of Kaempferol on Growth and Toxin Production of Head Blight Agent *Fusarium culmorum*. *Ziraat Fakültesi Dergisi,* 12: 24-33.

49. Soković, M.D., Vukojević, J., Marin, P.D., Brkić, D.D., Vajs, V. & Van, Griensven, L.J. 2009. Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. *Molecules*, 14: 238-249.

50. Sumalan, R.M., Alexa, E. & Poiana, M.A. 2013. Assessment of inhibitory potential of essential oils on natural mycoflora and *Fusarium* mycotoxins production in wheat. *Chemistry Central Journal*, 7: 32.

51. Tabanca, N., Demirci, B., Crockett, S.L., Başer, K.H.C. & Wedge, D.E. 2007. Chemical composition and antifungal activity of *Arnica longifolia*, *Aster hesperius*, and *Chrysothamnus nauseosus* essential oils. *Journal of Agricultural and Food Chemistry*, 55: 8430-8435.

52. Talas, F. & McDonald, B.A. 2015. Significant variation in sensitivity to a DMI fungicide in field populations of *Fusarium graminearum*. *Plant Pathology*, 64: 664-670.

53. Tufan, F., Kuluş, E.N., Sefer, Ö., Eraslan, M., Sefalı, S., Güngör, Ö. & Yörük, E. 2020. Determining WRKY transcription factors related to salinity stress response in wheat (*Triticum aestivum* L.) and barley (*Hordeum* vulgare L.). *Anadolu Tarım Bilim Dergisi,* 35: 1-7.

54. Uhlig, S., Jestoi, M. & Parikka, P. 2007. *Fusarium avenaceum*-The North European situation. *The International Journal of Food Microbiology*, 119: 17-24.

55. Velluti, A., Sanchis, V., Ramos, A.J., Turon, C. & Marin, S. 2004. Impact of essential oils on growth rate, zearalenone and deoxynivalenol production by *Fusarium graminearum* under different temperature and water activity conditions in maize grain. *Journal of Applied Microbiology*, 96: 716-724.

56. Yaguchi, A., Yoshinari, T., Tsuyuki, R., Takahashi, H., Nakajima, T., Sugita-Konishi, Y., Nagasawa, H. & Sakuda, S. 2009. Isolation and identification of precocenes and piperitone from essential oils as specific inhibitors of trichothecene production by *Fusarium graminearum*. *Journal of Agricultural* and *Food Chemistry*, 57: 846-851.

57. Yang, Y., Li M,, Duan, Y., Li T., Shi, Y., Zhao, D. & Zhou, Z. 2018. A new point mutation in β2 -tubulin confers resistance to carbendazim in *Fusarium asiaticum*. *Pesticide Biochemistry and Physiology*, 145: 15-21.

58. Yli-Mattila, T., Mironenko, N.V., Alekhina, I.A., Hannukkala, A. & Bulat, S.A. 1997. Universally primed polymerase chain reaction analysis of *Fusarium avenaceum* isolated from wheat and barley in Finland. *Agricultural and Food Science*, 6: 25-36.

59. Yli-Mattila, T., Paavanen-Huhtala, S., Parikka, P., Konstantinova, P. & Gagkaeva, T. 2004. Molecular and morphological diversity of *fusarium* species in Finland and northwestern Russia. *The European Journal of Plant Pathology*, 110: 573-585.

60. Yli-Mattila, T. & Gagkaeva, T.Y. 2018. Fungi: Applications of fungi and their management strategies, pp. 293-317. In: Deshmukh, S.K., Misra, J.K., Tewari, J.P. & Papp T. (eds). *Fusarium toxins in cereals in northern Europe and Asia*, CRC Press, Florida, 496 pp.

61. Yli-Mattila, T., Hussien, T., Gavrilova, O. & Gagkaeva, T. 2018. Morphological and molecular variation between *Fusarium avenaceum*, *Fusarium arthrosporioides* and *Fusarium anguioides* strains, *Pathogens*, 7(4): 94; https://doi.org/10.3390/pathogens7040094

62. Yörük, E., Tunali, B., Kansu, B., Ölmez, F., Uz, G., Zümrüt, I.M., Sarıkaya, A. & Meyva, G. 2016. Characterization of high-level deoxynivalenol producer *Fusarium graminearum* and *F. culmorum* isolates caused head blight and crown rot diseases in Turkey. *The Journal of Plant Diseases and Protection*, 123: 177-186.

63. Yörük, E. 2018. Tetraconazole leads to alterations in *Fusarium graminearum* at different molecular levels. *Applied Ecology and Environmental Research*, 16: 6155-6167.

64. Yörük, E., Sefer, Ö., Sezer, A.S., Konukcu, Z. & Develi, E.S. 2018. Investigation of the effects of eugenol on *Fusarium culmorum*. *Journal of The Institute of Science and Technology*, 8: 215-221.