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How an herbicide can affect plant diseases in a crop rotation program? A case study on Othello® OD

Bir herbisit, ürün rotasyon programındaki bitkilerin hastalıklarını nasıl etkileyebilir? Othello® OD üzerine bir vaka çalışması

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ABSTRACT

Herbicides can play an essential role in crop rotation programs' integrated management of plant diseases. *Trichoderma* species are major biological control fungi (BCF) used in agriculture. This study examined the effect of the herbicidal product Othello® OD on the *in vitro* growth of *T. asperelloides*, as well as some phytopathogenic fungi (*Bipolaris* sp., *Fusarium graminearum*, *F. oxysporum*, and *Rhizoctonia solani*). The poisoned food method was utilised with potato dextrose agar as the basal medium. The plates were then incubated at 26 °C in the dark. Results showed that all tested fungi were statistically similar in their sensitivity to the herbicide Othello® OD. Despite this, the significantly higher growth rate of *T. asperelloides* compared to the other tested fungi and its negligibly low sensitivity to Othello® OD suggests the potential of using the herbicidal product and *T. asperelloides* for managing essential diseases in a crop rotation program. The alignment of acetolactate synthase amino acid sequences using COBALT tool obtained a tree with 17 nodes separating the three fungal groups. The acetolactate enzymes of *Bipolaris oryzae* and *B. sorokiniana* were similar enough to form a unique group, indicating that they may be considered distinct species. In the second group, the enzyme of *Fusarium oxysporum* was more identical to that of *T. asperelloides* than *F. graminearum*. The third group only included enzymes from *R. solani* isolates.

INTRODUCTION

Integrated disease management (IDM) is the practice of preventing and managing diseases in crops by using a range of measures. IDM is essential for reducing the damage caused by pathogens that are not easily controlled by traditional methods and is a conscious choice when facing diseases that cannot be effectively managed with pesticides alone. IDM can help reduce pathogen inoculum potential, pesticide application rates, environmental pollution,

and production costs and manage fungicide resistance. It represents a new perspective in the fight against plant diseases, focusing on managing the most important diseases in a crop rotation program. This approach is innovative and proactive, preventing losses before they occur. In disease management, IDM represents the third viewpoint, following previous approaches that focused on controlling single diseases in a single crop or managing multiple diseases in a single crop (Pakdaman and Mohammadi Goltapeh 2018a, Singh 2001). The agricultural system based on this idea can leverage the benefits of both intensive and extensive culture systems. Herbicides and biological control agents (BCAs) such as *Trichoderma* spp. play crucial roles in this approach. Phytopathogenic fungi can colonise the residues of herbicide-treated weeds left among crop plants, providing material and energy for the production of pathogen inoculum. The antifungal activity of herbicides has been a fascinating subject of research, as evidenced by publications by Garcia et al. (2018) and Mehta et al. (2021). Various herbicides have been tested for their potential efficacy against *Trichoderma* species and phytopathogenic fungi, as demonstrated in studies by Abyawi et al. (2024) and Pakdaman et al. (2002, 2007a, 2007b). A herbicide with proper antifungal activity can suppress the growth and development of pathogens without harming beneficial microorganisms, such as *Trichoderma* spp. and *Bacillus thuringiensis*, which can control plant diseases and pests through various mechanisms such as competition for food and ecological niches and antibiosis, as well as parasitism. Additionally, they can activate plant defence pathways, bioremediate harmful chemicals, improve plant nutrition, and increase plant growth and yield. Unlike chemical inducers, beneficial microorganisms enhance plant development and productivity. *Trichoderma* spp. control phytopathogenic fungi, oomycetes, and nematodes and show potential as the biological control agents for insects and mites (Pakdaman and Mohammadi 2020). They not only help eliminate phytopathogenic microorganisms and herbivorous insects and mites but also have the potential to sanitise agrobiomes against livestock (such as parasitic nematodes and *Pythium insidiosum*), poultry, human pathogens, and their vectors (Pakdaman et al. 2013, Podder and Ghosh 2019, Zarrin et al. 2015). Therefore, these massively cultivable species have great importance from a third perspective. Considering the above, screening herbicides for their selective antifungal activities against phytopathogenic fungi, but biased toward *Trichoderma* species, would be an appropriate approach. One herbicide commonly used in wheat production fields is Othello® OD, which contains iodosulfuron, mesosulfuron, and diflufenican as active ingredients formulated with mefenpyrdiethyl as a safener produced by Bayer Crop Science in Germany (Anonymous 2019). This herbicide works by inhibiting the meristem cellular division of weeds by inhibiting acetolactate synthase (Loubet et al. 2023). It is applied post-emergence up to growth stage 30 of wheat to control both broad-leaf and grass weeds (Anonymous 2019). Therefore, the antifungal activity of the herbicidal product Othello® OD was investigated.

MATERIALS AND METHODS

Fungal cultures

The stock cultures of the tested fungal species were received from the Laboratory of Phytopathology, Group of Plant Protection at the Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Khuzestan, Iran. The phytopathogenic fungi included *Bipolaris* sp. isolated from rice (*Oryza sativa* L.) leaf spot, *Fusarium graminearum* isolated from wheat (*Triticum aestivum* L.) with fusarium head blight disease, *F. oxysporum* isolated from tomato (*Solanum lycopersicum* L.) suffering from fusarium vascular wilt, *Rhizoctonia solani* isolated from potato (*Solanum tuberosum* L.) tuber, as well as a biological control fungus, *Trichoderma asperelloides* isolated from tarragon (*Artemisia dracunculus* L.),roots were selected for this study. The fungi were individually cultured on potato dextrose agar (PDA) plates under sterile conditions in a laminar flow hood. To culture the fungi, 5 mm discs were taken from the edge of each fungal colony using a sterile cork-borer, and a single disc was inversely placed in the centre of a PDA plate. The cultures were then incubated at 26 °C in the dark. Each fungus was cultured on three plates, resulting in preparations of 5-day cultures that were of the same age.

Preparation of herbicide-amended PDA plates

The herbicide studied, Othello® OD 12%, was a product from Bayer Crop Science in Germany. The recommended application rate for this product is 1.6 L ha^{-1}. To prepare the PDA media (39 g L^{-1}), flasks were autoclaved at 121 ºC, 1 bar, for 20 minutes and allowed to cool to approximately 45 °C. Herbicide product aliquots were added to each flask and mixed under sterile laminar flow conditions. The final concentrations of the herbicidal product were 62.80 µl L^{-1} (62.80 ppm, representing the highest application rate recommended for the product, 1.6 L ha^{-1}), 32.71 µl L^{-1} (32.71 ppm, half of the recommended rate), and 6.25 µl L^{-1} (6.25 ppm, 0.1 of the recommended rate). The media were distributed into 90 mm diameter plates, with PDA plates without herbicidal amendment used as controls. Three plates were prepared for each concentration per fungus. The plates were allowed to cool to room temperature under sterile conditions. To culture, a 5 mm disc from the edge of a 5-day-

old colony of each fungal species was inversely placed on an agar plate. The cultures were then incubated at 26 °C for 6 days, and the diameter of growing colonies was measured (in mm) with a ruler every 24 h. Three measurements were taken per colony, including the highest diameter, the perpendicular diameter, and the diameter between them. Thus, the poisoned food method (Sharvelle 1961) was used to study the effect of Othello® OD concentrations on the fungi.

Experimental design and statistical analysis of data

A factorial experiment using a completely randomised design was conducted with three replicates for each fungus at every herbicidal concentration tested. The study aimed to examine the main and interactive effects of two factors: fungal species (5 species) and herbicidal concentrations (4 concentrations) on fungal growth. Analysis of variance (ANOVA) was performed using SAS software (version 9.4), and mean comparisons were made using the Tukey test (Minimum Significant Difference, MSD = 0.01). A square root transformation was carried out to normalise the data distribution.

In silico analysis of phytoene desaturase and acetolactate synthase protein sequences

The amino acid sequences of the target enzymes inhibited by the active ingredients of Othello® OD, phytoene desaturase and acetolactate synthase were searched in the National Centre for Biotechnology Information (NCBI) database (available in https://www.ncbi.nlm.nih.gov), converted to FASTA format (Lipman and Pearson 1985). The COBALT (Constraint-Based Multiple Alignment Tool; available on the NCBI webpage) was applied to align the FASTA-formatted accessed sequences following the cobalt tree method (Papadopoulos and Agarwala 2007) under conditions of maximum sequence difference (Max Seq Difference) of 0.85, distance of Grishin (protein).

RESULTS

The analysis of variance (ANOVA) of the fungal growth reduction percentage in different treatments indicated that the fungi were not different from each other ($F_{4,40}$=1.73^{ns}, P=0.1617, CV=9.56%). Therefore, all tested fungi showed similar growth reduction percentages based on Tukey test (alpha = 0.01) and were placed in a single Tukey group (Table 1). However, numerically, *F. graminearum*, *T. asperelloides* T-13, and *R. solani* were the most affected fungi, while *Bipolaris* was the least affected species. ANOVA analysis of the colonial diameters of the tested fungi grown in plates with varying concentrations of the herbicide Othello® OD revealed a highly significant impact of the herbicide concentrations on fungal growth ($F_{3,40}$=71.46***, P<0.0001, CV=9.56%). A Tukey test (alpha = 0.01) was conducted to compare the mean colonial growth inhibition (%) in different concentrations of Othello® OD, resulting in only three groups (Table 2). The interactions between the tested fungal species and herbicidal concentrations also had a significant impact on colonial growth inhibition ($F_{12,40}$=2.46**, P<0.01, CV=9.56%). A comparison of the mean colonial growth inhibition rates (%) influenced by these interactions grouped them into five Tukey groups (alpha=0.01; Table 3). The comparison of mean colonial diameters of the tested fungi by Tukey test (alpha = 0.01) led to four Tukey groups of highly significantly different colonial growth rates ($F_{4,40}$=5274.26***, P<0.0001, CV=3.29%) (Table 4). *T. asperelloides* T-13 was of the highest colonial growth and occupied the Tukey group "a", while *F. oxysporum* and, in particular, *Bipolaris* sp. were of the least growth and were allocated in the same Tukey group, "d".

Table 1. The growth reduction of the tested fungi on potato dextrose agar medium at 26 °C in the dark, compared by Tukey test, MSD (0.01) = 0.562

Fungus	Growth Reduction ± SE (%)
Trichoderma asperelloides T-13	11.506±2.204a*
Fusarium graminearum	12.203±2.009a
Rhizoctonia solani	11.012±3.464a
Fusarium oxysporum	7.617±1.749a
Bipolaris sp.	9.726±2.263a

*The similar letters within the same column are not statistically significant (Tukey test, P<0.0001); SE, indicated standard error

Table 2. Effect of different concentrations of Othello® OD amended to potato dextrose agar (used as the basal medium) on the fungal growth reduction at 26 °C in the dark, compared by Tukey test, MSD (0.01) = 0.524

Concentration (µl l^{-1})	Growth Reduction ± SE (%)
0	0±0^{c}
6.25	3.433±0.686^{b*}
32.71	14.665±1.665^{a}
62.80	13.140±1.408^{a}

*Different letters within the same column are statistically highly significant (Tukey test, P<0.0001). SE, indicated standard error

Table 3. The comparative interaction effects of fungal species and Othello® OD concentrations on fungal growth reduction at 26 °C in the dark, as revealed by Tukey test, MSD (0.01) =1.616

Fungus	Concentration (µl l^{-1})	Growth Reduction±SE (%)
Trichoderma asperelloides T-13	0.00	0±0.000^{c}
	6.25	4.969±0.801^{bc}
	32.71	10.076±0.570^{abc}
	62.80	19.473±1.789^{a}
Fusarium graminearum	0.00	0±0.000^{c}
	6.25	6.641±1.558^{abc}
	32.71	19.071±2.367^{a}
	62.80	10.899±0.707^{abc}
Rhizoctonia solani	0.00	0±0.000^{c}
	6.25	0±0.000^{c}
	32.71	13.315±3.865^{ab}
	62.80	19.722±5.277^{a}
Fusarium oxysporum	0.00	0±0.000^{c}
	6.25	2.772±0.681^{bc}
	32.71	10.022±3.026^{abc}
	62.80	10.058±3.082^{abc}
Bipolaris sp.	0.00	0±0.000^{c}
	6.25	2.784±0.555^{bc}
	32.71	13.218±3.499^{ab}
	62.80	13.175±3.575^{ab}

*Different letters within the same column are statistically highly significant (Tukey test, P<0.0001); SE, indicated standard error

Table 4. The growth rate of the tested fungi on potato dextrose agar medium at 26 °C in the dark, compared by Tukey test, MSD (0.01) = 1.6755

Fungus	Mean Colony Diameter ±SE (mm)
Trichoderma asperelloides T-13	75.5278±1.820^{a}
Fusarium graminearum	41.6944±0.706^{b}
Rhizoctonia solani	29.6250±0.665^{c}
Fusarium oxysporum	16.3750±0.429^{d}
Bipolaris sp.	15.6111±0.578^{d}

*Different letters within the same column are statistically highly significant (Tukey test, P<0.0001); SE, indicated standard error

The *in silico* BLAST search for the phytoene desaturase protein or gene in the genomes of *Rhizoctonia solani* and *T. asperelloides* available on the NCBI website yielded no results. Consequently, no information regarding the presence of phytoene desaturases in these fungi was found in the literature available online. The COBALT alignment for the amino acid sequence similarity of phytoene desaturase enzymes selected for species/ near to the tested fungi resulted in a cobalt tree with 9 nodes (Figure 1). The amino acid sequences of *B. sorokiniana* and *B. oryzae* phytoene desaturase formed a unique small cluster, while those of *F. graminearum* and *F. oxysporum* formed another small cluster. Both clusters were connected to each other by a single node, with the outgroup being the enzyme sequence from wheat (Figure 1). The cobalt alignment of acetolactate synthase sequences resulted in a cobalt tree with 17 nodes and the separation of three fungal groups. The acetolactate enzymes of two *Bipolaris* species., *B. oryzae* and *B. sorokiniana* were similar enough to form a unique group. In the second group, the enzyme of *F. oxysporum* was more similar to that of *T. asperelloides* than that of *F. graminearum*. The third group included enzymes only from *R. solani* isolates (Figure 2).

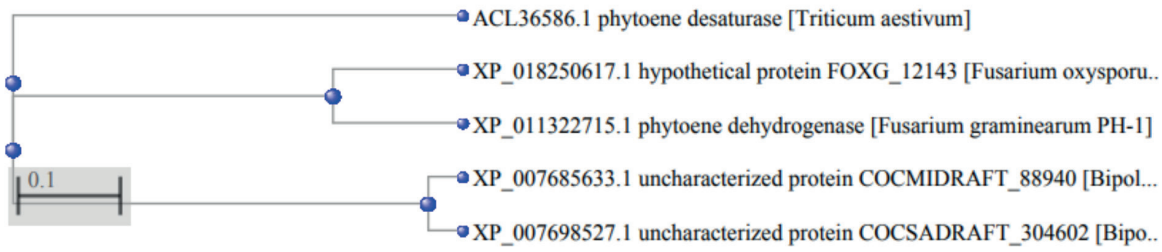

Figure 1. The comparison of phytoene desaturase amino acid sequences in the tested fungal species made by COBALT multiple alignment tool

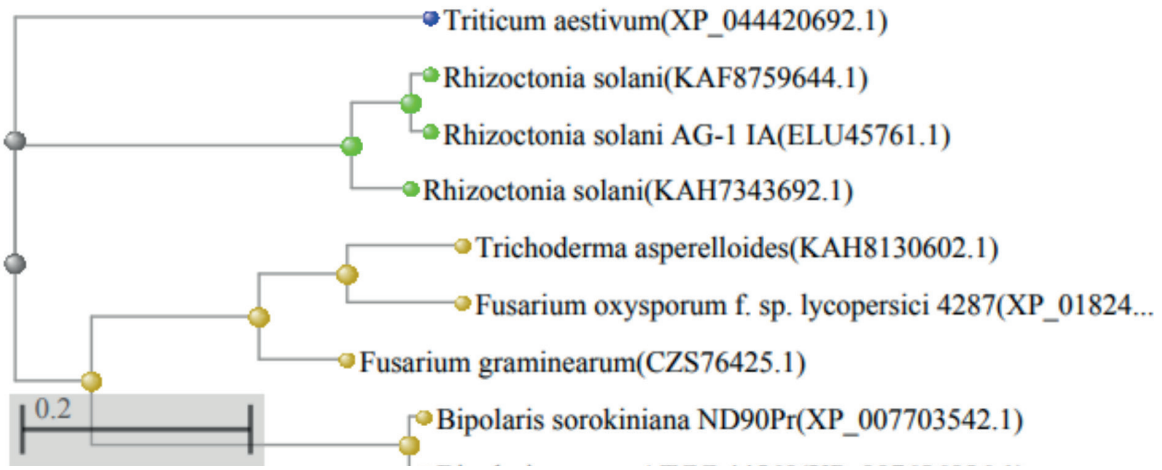

Figure 2. The comparison of acetolactate synthase sequences in the tested fungal species made by COBALT multiple alignment tool. The green nodes belong to basidiomycetous species, and the golden nodes belong to ascomycetous fungi, while the blue node belongs to a plant (bread wheat) included as an outgroup

DISCUSSION

The fungi selected and used in the study, except for *Bipolaris* sp., *F. graminearum*, *F. oxysporum*, and *R. solani*, are all known as the top 10 fungal pathogens based on scientific or economic importance throughout the world (Dean et al. 2012). This research investigated the antifungal potential of the herbicidal product Othello® OD. Since the formulation includes multiple materials that may interact with each other, and different formulations may have varying antifungal potentials, the whole formulation, Othello® OD, was applied in this study. However, the active ingredients were used to discuss the obtained results. A significantly higher growth rate of *T. asperelloides* T-13 was found compared to the tested pathogenic fungi, even under the recommended rates of the herbicidal product Othello® OD application (Table 4), which was a promising finding. In a previous study on another herbicidal product from Bayer Crop Science, Atlantis® OD42, we observed that despite the product reducing *T. asperelloides* growth rate more severely than that of the mycotoxigenic fungus *F. graminearum*, *T. asperelloides* successfully overgrew after a prolonged time of confrontation at the temperature optimised for *F. graminearum* (26 °C) rather than the optimum temperature for the growth of *T. asperelloides*, 28 °C (Pakdaman and Elahifard 2025). The least colonial growths were recorded with *Bipolaris* sp. and *F. oxysporum* (Table 4), suggesting that *T. asperelloides* T-13, as a biological control agent, could compete effectively with both phytopathogenic fungi. Othello® OD was found to have some antifungal activity against certain tested fungi. However, *Bipolaris* sp., *F. graminearum* and *F. oxysporum* did not show a statistically significant reduction in their colonial growth in the presence of all tested concentrations of Othello® OD (Table 3). Othello® OD is formulated as an oil dispersion and contains three active ingredients: diflufenican (50 g L^{-1}), mesosulfuron methyl (7.5 g L^{-1}), and iodosulfuron methyl sodium (2.5 g L^{-1}). The formulation also includes mefenpyr-diethyl as a safener (22.5 g L^{-1}). Diflufenican is a pyridinecarboxamide herbicide that inhibits carotenoid biosynthesis at the phytoene desaturase step. Phytoene desaturase is a bifunctional enzyme with phytoene synthase and lycopene cyclase activities. It is the first enzyme in the carotenoid biosynthesis pathway, catalysing the condensation of two geranylgeranyl diphosphate (GGPP) molecules to produce the colourless carotene-phytoene, the precursor to different

carotenoids. Phytoene desaturase introduces up to five conjugated bonds into a phytoene backbone to produce dehydrogenated coloured carotenoids (Gmoser et al. 2017). Unlike photoautotrophic organisms, carotenoids are not essential for fungi. Carotenoids, as terpenoid pigments and antioxidants, protect heterotrophic organisms against reactive oxygen species from photosensitised reactions (Sandmann 2022). Secondary carotenoids like astaxanthin and canthaxanthin accumulate in response to environmental stress. Carotenoids can enhance the fluidity of the cell membrane under extreme temperatures, high light, or when lipids are more unsaturated. They act as antioxidants against reactive oxygen species (ROS) and help alleviate cell membrane damage. Carotenoids and other antioxidants work together to extend the survival time of fungi (Gmoser et al. 2017). Therefore, diflufenican does not kill fungi or significantly suppress their vegetative growth. It can act as a synergistic agent to enhance the antifungal effects of other stressors, including other antifungal chemicals. Inhibiting the enzyme may lead to the accumulation of GGPP involved in primary metabolism (Singkaravanit et al. 2009). The other two active ingredients belong to the sulfonyl urea group of herbicides and inhibit acetolactate synthase (ALS), also known as acetohydroxy acid synthase (AHAS). ALS catalyses the first common step in the biosynthesis of three branched-chain amino acids such as valine, leucine and isoleucine (Shao et al. 2022). Thus, the exposure of plants to ALS-inhibitors inhibits the production of the branched-chain amino acids (Majd et al. 2019). BCAAs are synthesized in bacteria, plants, and fungi, but not in animals. Because it does not occur in animals, BCAA synthesis has been successfully targeted for antifungal agents, antimicrobials, and herbicides (Amorim Franco and Blanchard 2017). Although BCAAs are structurally similar amino acids containing aliphatic side chains, their propensities for being found in protein structures are quite different. The small discrepancies in the size, hydrophobicity and the degree as well as the position of side chain branching explain the reason for their interchangeability in proteins, so while L-valine and L-isoleucine are overrepresented in beta-sheets, L-leucine is primarily found in alpha-helices, loops, and leucine zippers (Brosnan and Brosnan 2006). The functional R groups of all three BCAAs are branched (hence their name), small, and hydrophobic, rendering them critical components of most proteins (Chou and Fasman 1973, Dill 1990). BCAAs are building blocks for all life-forms (Neinast et al. 2018) and account for approximately 18% of amino acids and 63% of hydrophobic amino acids in protein across many life-forms (Moura et al. 2013). BCAAs possess important and, to some extent, special physiological and biological functions (Liang et al. 2021). With fungi, BCAAs and the intermediates of their biosynthesis pathway are involved in mycelial growth, fruitification (especially under oxygen deficiency conditions), mycelial and conidial morphogenesis, microsclerotial formation, appressorial penetration, fungal pathogenicity and virulence (Du et al. 2013, Liu et al. 2015, Luo et al. 2020, Shao et al. 2022, Zain ul Arifeen et al. 2021). So, the antifungal activity of Othello® OD can result from ALS inhibition by its sulfonyl urea herbicidal active ingredients. This is consistent with the heightened antifungal activity of Atlantis® OD42, which contains a greater sulfonylurea content (42%) compared to Othello® OD (Pakdaman and Mohammadi 2018b, Pakdaman and Elahifard 2025). Explaining the differences in the relative sensitivity of the tested fungi (though statistically not significant), the higher sensitivity of *T. asperelloides* T-13 may be due to the absence of carotenoids in the fungus. Our attempt to find phytoene desaturase (PDS) protein and gene in the genomic data of *T. asperelloides* available in the NCBI database (https://www.ncbi.nlm.nih.gov) was fruitless. So, the absence of carotenoid antioxidants can result in intrinsically higher sensitivity to the oxidative stress imposed by sulfonylurea compounds in Othello® OD. The pigments of *T. asperelloides* are not as effective as carotenoids and melanins of other tested fungi in protecting the fungal cell against the oxidative stress imposed by sulfonylurea herbicides (Table 3). Both *T. asperelloides* and *T. viride* belong to the section of *Trichoderma* (Liu et al. 2020). *T. viride* produces several non-carotenoid orange-red (chrysophanol) and yellow (emodin, pachybasin), as well as other pigments, including hydroxyanthraquinones such as T22 azaphilone, 1-hydroxy-3-methyl-anthraquinone, 2,4,5,7-tetrahydroxyanthraquinone, and 1,3,6,8-tetrahydroxyanthraquinone (Lagashetti et al. 2019). Like *T. asperelloides*, *R. solani* did not have the PDS protein and gene. However, *R. solani* appeared less sensitive than *T. asperelloides* T-13. *F. graminearum* was not significantly affected by Othello® OD. *F. graminearum* produces blue (6-O-demethyl-5-deoxybostrycoidin anthrone), green (5-deoxybostrycoidin anthrone), purple (purpurfusarin), red (aureofusarin, 5-deoxybostrycoidin, and rubrofusarin) and yellow (6-O-demethyl-5-deoxybostrycoidin) pigments (Lagashetti et al. 2019) in addition to orange (neurosporaxanthin) and pinkish-red (torulene) carotenoids (Jin et al. 2010). Despite the well-known role of carotenoids as antioxidants, aurofusarin, the non-carotenoid polyketide pigment of the naphthoquinone group, appears to be the predominant pigment (Cambaza 2018) and the only pigment of *F. graminearum* produced under oxidative stress (Medentsev et al. 2005). So, it seems that even when the biosynthesis of torulene and neurosporaxanthin is inhibited by diflufenican, a phytoene desaturase inhibitor in Othello®

OD, the production of aurofusarin compensates for the lack of carotenoid antioxidants required for the neutralization of the oxidative stress imposed by the sulfonylurea herbicides in Othello® OD. Similar to *F. graminearum*, *F. oxysporum* produces a broad range of pigments that can protect its cells from the damage caused by the oxidative stress imposed by sulfonylurea compounds in Othello® OD under the conditions of carotenoid biosynthesis inhibition by diflufenican. Orange [2-(1-hydroxyethyl)-3,8-dihydroxy-6-methoxy anthraquinone, and neurosporaxanthin], purple (uncharacterized naphthoquinones), red-orange (beta-carotene, and O-methylanhydrofusarubin), red (bikaverin, bostrycoidin, O-methylfusarubin, and norjavanicin), and yellow [2-acetyl-3,8-dihydroxy-6-methoxy anthraquinone, 2,7-dimethoxy-6-(acetoxyethyl)juglone, O-methylanhydrofusarubin, O-methyljavanicin, O-methyl-6-hydroxynorjavanicin, and nectriafurone] metabolites have been reported as the pigments produced by *Fusarium oxysporum* (Lagashetti et al. 2019). Additionally, *in silico* cobalt alignment analysis indicates some differences in the sequence of phytoene desaturase between two *Fusarium* spp. (Figure 1). That may lead to the difference in their reaction to Othello® OD. As wheat phytoene desaturase is inhibited by diflufenican (hence, it is protected by the use of the safener) it seems that the enzyme of *F. graminearum,* and especially that of *F. oxysporum* are intrinsically more sensitive to diflufenican than the more distant phytoene desaturases of *B. oryzae*, and *B. sorokiniana*. However, both *Fusarium* spp. and *Bipolaris* sp. were not significantly affected by Othello® OD, indicating that the inhibition of phytoene desaturase and the suppressed biosynthesis of carotenoids by diflufenican had been compensated, so the ROS were effectively detoxified by a mechanism not mediated by carotenoid antioxidants. However, the major difference between two species may also result from their differences in the sequence of acetolactate synthase (Figure 2). Interestingly, the acetolactate synthase sequence of resistant *F. oxysporum* was more similar to that of the most sensitive species, *T. asperelloides*, than that of *F. graminearum*. This indicates the high importance of especially non-carotenoid pigments of *F. oxysporum* in neutralising oxidative stress caused by sulfonyl urea herbicides in Othello® OD. Melanins abundantly produced by *Bipolaris* sp. (Li et al. 2024) and, to a lesser extent, by *R. solani* were effective against the oxidative stress imposed by sulfonyl urea compounds. So, the highly melanised *Bipolaris* sp. did not show any significant reduction in its colonial growth in the presence of all concentrations of Othello® OD. In contrast, the less melanised *R. solani* indicated only less sensitivity. Melanin seems to protect fungi against microbial attack and toxic activity of xenobiotics (Tseng et al. 2011) via the reduction of their toxicity or absorption, especially for the substances affecting the stability of fungal cell walls and plasma membrane and respiration (Singh et al. 2005). Melanin significantly contributes to fungal tolerance to biocides and photosensitizers (Pascoe and Maillard 2021). In addition to less melanin content, the lack of phytoene desaturase and, thus, the absence of carotenoids in *R. solani* can result in further liability for the fungus compared to the resistant *Bipolaris* sp. Interestingly, both phytoene desaturase and acetolactate synthase sequences in *Bipolaris* spp. located in the groups distinct from other fungi (Figures 1 and 2). These differences may partially explain the lower sensitivity of *Bipolaris* sp. to the antifungal activity of Othello® OD. The study led to different conclusions of importance in different scientific disciplines. From the standpoint of Phytopathology, *T. asperelloides* was the tested fungus most affected by Othello® OD, however thanks to its higher growth rate compared to the resistant and less affected phytopathogenic fungi even in the recommended concentration of the herbicidal product, it seems possible to successfully plan integrated disease management programs based on *Trichoderma* and Othello® OD in the production of wheat and other crops in crop rotation programs. The experiment confirmed the possibility of the third viewpoint application in the management of the diseases of a crop rotation program. In the pharmacology and agrochemical industries, the antifungal potential of branched-chain amino acid synthesis inhibitors such as sulfonyl urea compounds was confirmed once more, and it was indicated that diflufenican and similar inhibitors of phytoene desaturase as well as the inhibitors of fungal pigments could be applied in the enhancement of fungicidal activity of the commercial fungicides. Furthermore, if we consider fungi as laboratory models, such inhibitors can also be used in the improvement of other pesticides and drugs. Also, this study indicated that the pigments of *T. asperelloides* had less antioxidative activity compared to carotenoid and non-carotenoid pigments in *F. graminearum* and *F. oxysporum*. Melanin also led to high resistance. The cobalt multiple alignment tool available in the Analyse choice freely available on the website of the National Library of Medicine, National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov) was found useful in further understanding the reasons behind the observed variation in the sensitivity of the tested fungi to the active ingredient(s) of an agrochemical product such as Othello® OD. The tool was also useful for comparing the reactions of fungi to Oxadiazon® (Abyawi et al. 2024). As far as the authors know, these are the first global records that have successfully used the cobalt multiple alignment tool to analyse fungi reaction to the agrochemicals.

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Author's Contributions

Authors declare that each author's contribution is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Herbisitler, bitki hastalıklarının ürün rotasyonu programlarının entegre yönetiminde önemli bir rol oynayabilir. *Trichoderma* türleri, tarımda kullanılan başlıca biyolojik kontrol funguslarıdır (BCF). Bu çalışma, Othello® OD herbisitinin *T. asperelloides*'in yanı sıra bazı fitopatojenik fungusların (*Bipolaris* sp., *Fusarium graminearum*, *F. oxysporum* ve *Rhizoctonia* solani) *in vitro* büyümesi üzerindeki etkisini incelemiştir. *In vitro* da herbisitin fungusların büyümesi üzerine etkisinin belirlenmesi çalışmasında, temel besiyeri olarak patates dekstroz agar kullanılmıştır. Daha sonra petriler karanlıkta 26 °C'de inkübe edilmiştir. Sonuçlar, test edilen tüm fungusların Othello® OD herbisitine duyarlılıklarının istatistiksel olarak benzer olduğunu göstermiştir. Buna rağmen, *T. asperelloides*'in diğer test edilen funguslara kıyasla önemli ölçüde daha yüksek büyüme hızı ve Othello® OD'ye karşı ihmal edilebilir derecede düşük duyarlılığı, bu herbisitin ve *T. asperelloides*'in bir ürün rotasyonu programında temel hastalıkların yönetimi için kullanılma potansiyelini göstermektedir. COBALT aracı kullanılarak asetolaktat sentetaz amino asit dizilerinin hizalanması, üç fungus grubunu ayıran 17 düğüme sahip bir ağaç ortaya çıkarmıştır. *Bipolaris oryzae* ve *B. sorokiniana*'nın asetolaktat enzimleri, tek bir grup oluşturacak kadar benzerdir ve bu da bunların ayrı türler olarak kabul edilebileceğini göstermiştir. İkinci grupta, *Fusarium oxysporum*'un enzimi, *F. graminearum*'dan ziyade *T. asperelloides*'in enzimine daha benzerdir. Üçüncü grupta ise yalnızca *R. solani* izolatlarından elde edilen enzimler yer almıştır.

Anahtar kelimeler: asetolaktat sentetaz, *Bipolaris*, COBALT aracı, *Fusarium*, *Rhizoctonia*, *Trichoderma*

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