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Research Article

The Influence of Low Pesticide Doses on Fusarium Molds

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Abstract

The agricultural sector is a large consumer of synthetic chemical products, especially fertilizers and plant protection products. Therefore, an emerging concern nowadays is to reduce chemicals' use in agriculture. One of the approaches is to reduce the doses of plant protection products, as much as possible, while keeping the treatments' efficacy. The present work presents the antifungal action of three commercial plant protection products, tested at recommended as well as reduced doses, against important phytopathogenic molds of the *Fusarium* genus. *In vitro,* results have shown that two of the tested products could be used at reduced doses while keeping their antifungal activity. The commercial pesticide containing prothioconazole 53 g/L, spiroxamine 224 g/L, and tebuconazole 148 g/L mixture was able to inhibit completely the growth of three virulent *F. culmorum* strains, even when fungicide treatment was applied in 25% reduced dose. Lower efficacy was seen on *F. graminearum* strains, however, there were no significant differences ($p < 0.05$) between the commercially recommended dose and the 25% reduced dose. Another efficient pesticide in *Fusarium* control contains triadimenol 43 g/L, spiroxamine 250 g/L, and tebuconazole 167 g/L. Tested in a reduced dose (28.6% less than the commercial recommended dose) it completely inhibited the *F. graminearum* Fg183 (DSM 4527) strain and inhibited the growth of various *F. culmorum* strains with at least 97.50% efficacy. However, there are some fungal strains, such as the aggressive *F. graminearum* Fg96 strains that were less susceptible to pesticide treatments even at commercially recommended doses of fungicides.

Introduction

Fusariosis is an extremely damaging disease for cereals and many other economically important plants. The infection can be installed in all vegetation stages and can continue also during storage, creating significant losses or reducing the yield quality. However, in wheat, the most damaging are the infections that occur during flowering. The Fusarium Head Blight (FHB) is highly detrimental to all cereals and could be caused by potentially mycotoxigenic molds of the genus *Fusarium*. The most common *Fusarium* species on wheat are *F. graminearum* and *F. culmorum*, which belong to the *Discolor* section [1]. Other *Fusarium* species are also mentioned, such as *F. avenaceum,* a member of the Roseum section [2], as well as *F. poae* and *F. langsethiae*, both from the Sporotrichiella section [3].

The economic losses are not limited to yield reduction. In many cases, the quality is also depreciated, making the harvest unfit for human consumption or animal feed, due to mycotoxin contamination. These toxins are secondary metabolites produced by the fungi, that can be accumulated in the crop. If certain contamination levels are reached within the harvest, the crop quality is depreciated, making the yield unsuitable for consumption [4]. Most of the *Fusarium* spp. contaminants in cereals are mycotoxin producers. Moreover, the *Fusarium* spp. mycotoxins are considered among the most dangerous toxins for human and animal health [5]. These fungal species are capable of producing three of the most important classes of mycotoxins: fumonisins (FB1, FB2, FB3), zearalenone, and trichothecenes, such as deoxynivalenol, nivalenol, HT-2 and T-2 toxins, diactoxyscripenol and monoacetoxyscripenol. Such fungi can also produce emerging mycotoxins, like fusoproliferin, beauvericin, eniatins, moniliformin, or other mycotoxins such as fusaric acid, fusarin AD, gliotoxin, butenolith, which are relatively recent discovered and less studied [6].

Although ruminants and poultries are less sensitive than monogastric the deleterious effects of *Fusarium*

mycotoxins can still occur, damaging the liver and kidney functions. Fusariotoxicoses on farm animals highly reduce their performance. Various acute or chronic symptoms/effects occur, depending on the intoxication levels. Immunosuppressive, hepatotoxic, nephrotoxic, as well as alteration of the reproductive function are some of the most evident side effects [7]. In humans, the *Fusarium* mycotoxins induce acute toxicity, although, carcinogenic effects are also considered [8]. The main risks of counteracting food contamination the crop production and food processing or improper storage [9].

To prevent *Fusarium* spp. infections, several management practices can be considered, such as crop rotation, tolerant cultivars, and rational use of agrochemical inputs, as the most significant. There are many commercial plant protection products available on the market, some of which contain a mixture of different classes of active substances that make the product much more effective. Among the currently-used fungicides against FHB include tebuconazole, propiconazole, metconazole, picoxistrobin, trifloxystrobin, proquinazid, triadimenol, and many others [10]. However, studies have shown that strobilurin treatments, although effective against FHB, are inducing higher levels of DON mycotoxins [11]. Biological means can also improve plants' health and prevent fungal infections [10]. On the European market, several active ingredients of biological origin are allowed. Against fusariosis, the most effective microbial strains belonging to *Bacillus* and *Trichoderma* genera.

The benefits of using plant protection products are undeniable in agricultural performance, as they prevent crop losses and improve the yield and quality of the harvest [12]. However, at the European level, there is a current demand to reduce the amount of chemical pesticides used in agriculture [13]. This is due to the negative side effects of pesticide residues on human and animal health, as well as on the environment [12]. One of the proposed measures is to reduce the doses of the active substances if efficacy is maintained [14]. Therefore, this study aimed to evaluate, *in vitro*, the antifungal action against *Fusarium* molds of some commercial pesticides in reduced doses compared to the recommended dose.

Materials and methods

Three Commercial Fungicides (CF) were used in this study, encoded CF1-NP, CF2-F, and CF3-FP (Table 1). All these are chemical-based products available on the Romanian market and in other European countries. The CF1-NP product contains two active ingredients, while the other two (CF2-F and CF3-FP) fungicides contain three active substances. These three commercial pesticides were tested *in vitro*, in two doses. One of the tested doses was currently recommended to control fusariosis on cereals as presented on the product's label, while the other was a reduced dose, as presented in the following table (Table 1). In the field, the recommended dose of the CF can be applied in 200 to 400 L of water per ha. This was extrapolated, for the *in vitro* study, as 130 µl of fungicide solution per plate of 9 cm diameter.

The antifungal activity of these products was evaluated against five plant pathogenic *Fusarium* spp. molds. The phytopathogenic fungi used in the laboratory trials were *Fusarium culmorum* FC 46, FC 1056, and FC 1471 strains, and *F. graminearum* FG 96 and FG 183 strains. The FG 183 strain is a reference strain, available in the German Collection of Microorganisms and Cell Cultures GmbH, under accession number DSM 4527.

The *in vitro* testing was performed on Potato Dextrose Agar. The fungicides were suspended in sterile distilled water (SDW) and plated on the medium. One of the tested doses was the application dose, currently recommended on the product label, while the other dose was reduced to 25%, in the case of CF1-NP and CF2-F, or 28.57% in the case of CF3-FP. As the test was performed in Petri plates of 9 cm diameter, the amounts of CF were adapted from L/ha to µl/Plate (Table 1). To ensure proper distribution of the fungicide on the substrate, the CF was resuspended in SDW, and 130 µl of the resulting solution was uniformly dispersed on top of the PDA medium using a Drigalski spreader. After infusion, the fungi were inoculated on top of the agar layer as mycelial plugs of 5 mm diameter. Control plates of each fungal pathogen were prepared, where fungi were grown on PDA with no pesticides.

The incubation was carried out at 25°C, while the biometric measurements of the fungal colony diameter were taken after 5, 7, and 10 days from inoculation. The inhibition efficacy of the pesticides against the fungi was calculated according to Lahlali and Hirji [15]. To evaluate the CF efficacy on fungal inhibition, control plates with no fungicide treatments were prepared. These control plates allowed fungal development in normal growth conditions. The inhibitory activity of the CF treatments, in currently recommended and reduced doses, was compared to these controls.

Results and discussions

The mycelial growth of the fungi was comparatively measured after the incubation on PDA, and PDA supplemented with CF at recommended and reduced application dose for field treatments.

In the first 5 days of incubation, some of the fungal strains were completely inhibited by the presence of pesticides within the medium (Table 2). All *F. culmorum* strains were not able to grow in the presence of CF3-FP pesticide, at any of the tested doses, and on CF2-F pesticide in the recommended dose for field applications. Compared to the CF2-F and CF3-FP

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Table 1: Commercial fungicides.

Commercial fungicide (CF)	Active substance (a.s.)	Concentration (mg/m^3)	Application strategy	Dose (L/ha)	*In vitro* application (µl CF/ 130µl water/ Plate)
CF1 – NP	Trifloxystrobin 150 g/L Prothioconazole 175 g/L	2,7 mg/m^3 7,4 mg/m^3	C	0.7	0.45
			R	0.5	0.32
CF2 – F	Triadimenol 43 g/L Spiroxamine 250 g/L Tebuconazole 167 g/L	1,61 mg/m^3 0,6 mg/m^3 0,2 mg/m^3	C	0.7	0.45
			R	0.5	0.32
CF3 – FP	Prothioconazole 53 g/L Spiroxamine 224 g/L Tebuconazole 148 g/L	1,4 mg/m^3 0,2 mg/m^3 0,6 mg/m^3	C	0.8	0.51
			R	0.6	0.38

Legend: C = *currently recommended dose* or control; R = *Reduced dose*.
Note: The recommended dose is labeled on the commercial product, and it was used as a control in the current study. The reduced fungicide dose is 25% to 28.57% less than the recommended dose.

Table 2: Mycelial growth of the fungal strains grown in fungicidal conditions compared to untreated controls after 5 days of incubation.

Fungal strains	Control	CF1-NP		CF2-F		CF3-FP	
		C	R	C	R	C	R
Fusarium culmorum Fc46	2.70 ± 0.00^c	0.46 ± 0.06^b	0.58 ± 0.06^b	0.00 ± 0.00^a	0.10 ± 0.10^a	0.00 ± 0.00^a	0.00 ± 0.00^a
F. culmorum Fc 1056	3.10 ± 0.10^c	0.58 ± 0.15^b	0.65 ± 0.10^b	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
F. culmorum Fc 1471	2.75 ± 0.10^d	0.53 ± 0.10^c	0.55 ± 0.06^c	0.00 ± 0.00^a	0.12 ± 0.03^b	0.00 ± 0.00^a	0.00 ± 0.00^a
Fusarium graminearum Fg96	3.38 ± 0.15^d	0.96 ± 0.21^b	1.43 ± 0.15^c	0.48 ± 0.15^a	1.18 ± 0.45^{bc}	1.30 ± 0.26^{bc}	1.51 ± 0.25^c
F. graminearum Fg183	2.11 ± 0.12^d	0.90 ± 0.10^c	1.05 ± 0.10^c	0.00 ± 0.00^a	0.00 ± 0.00^a	0.15 ± 0.10^a	0.65 ± 0.10^b

Legend: C = control, currently recommended dose for field application; R = reduced dose.
Note: All data are presented in cm, as mean values ± standard deviation. Different letters attributed to the same fungi indicate a significant difference between the experimental variants regarding their growth inhibition ($p < 0.05$).

pesticides that are based on three active ingredients, the CF1-NP (based on trifloxystrobin 150 g/L and prothioconazole 175 g/L) was less effective. However, it substantially reduced the fungal growth, even in a reduced dose, compared to the control, where no fungicide was added to the substrate.

F. graminearum Fg183 strain showed no growth in the first 5 days of incubation in the presence of CF2-F pesticide, in both recommended and reduced application doses. The other two fungicides CF1-NP and CF3-FP were not able to completely inhibit the mycelial growth, not even at the recommended dose. Among the tested fungicides, CF1-NP was the less effective. However, after the first 5 days of incubation, the reduced dose of CF1-NP inhibited the mycelial growth of Fg183 to half of the growth developed in the control plates.

Among the studied fungal strains, Fg96 was less affected. However, even at a reduced application dose, the tested fungicides inhibited mycelial growth to less than half of the growth in the control plates.

Seven days after incubation the fungal strains continued to the same growing trend. Moreover, those strains completely inhibited by the presence of the fungicide maintained no growth after seven days of incubation on PDA supplemented with pesticides (Table 3).

Ten days after inoculation, new biometric measurements were made on the mycelial growth, and the efficacy of fungal growth inhibition was calculated. According to our data, the CF1-NP pesticide at the recommended application dose was less effective in fungal inhibition compared to the other two products, even when CF2-F and CF3-FP were tested at reduced doses (Table 4).

Tested against various *F.culmorum* strains (FC 46, FC 1056, and FC 1471), the CF3-FP in both tested doses, and CF2-F in the currently recommended dose completely inhibited mycelial growth, compared to the untreated fungal control (Figure 1a-1c), while CF2-F in reduced dose inhibited the mycelial growth with at least 97.5% efficacy (Table 1).

The *Fusarium graminearum* Fg96 was the most tolerant strain to all pesticides used. The Fg96 developed mycelial growth in all pesticide-treated variants both in low dose, as well as in the recommended application dose (Figure 2).

Similar tests are mentioned to be performed on *Fusarium* spp. fungi isolated from infected wheat grains. Among the tested fungicides triazoles (prothioconazole 250 g/L and tebuconazole 251,2 g/L) and strobilurins (azoxystrobin 250 g/l and fluoxastrobin 480 g/l) were used in different concentrations. The fungi were grown on PDA supplemented with up to 100 mg/l commercial pesticides. Among the tested fungicides, prothioconazole was the most effective in reducing fungal growth, while tebuconazole proved to be efficient only in high concentration, against *Fusarium crookwellense, F. tricinctum* and *F. culmorum*. Isolates of *F. tricinctum* and *F. graminearum* were very responsive to fluoxastrobin treatment, but in general, the strobilurins showed a lower influence on the tested fusaria [16].

Melchett [17] considers that the negative impact of pesticides on biodiversity is underestimated. The decline of

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Table 3: Mycelial growth of the fungal strains grown in fungicidal conditions compared to untreated controls after 7 days of incubation.

Fungal strains	Control	CF1-NP		CF2-F		CF3-FP	
		C	R	C	R	C	R
Fusarium culmorum Fc46	4.00 ± 0.00^{d}	0.66 ± 0.06^{b}	0.96 ± 0.06^{c}	0.00 ± 0.00^{a}	0.10 ± 0.10^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
F. culmorum Fc 1056	4.00 ± 0.00^{d}	0.66 ± 0.06^{b}	0.96 ± 0.06^{c}	0.00 ± 0.00^{a}	0.10 ± 0.10^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
F. culmorum Fc 1471	4.00 ± 0.00^{d}	0.90 ± 0.10^{c}	0.90 ± 0.00^{c}	0.00 ± 0.00^{a}	0.12 ± 0.03^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Fusarium graminearum Fg96	4.00 ± 0.00^{c}	1.38 ± 0.25^{b}	1.61 ± 0.67^{b}	0.68 ± 0.15^{a}	1.35 ± 0.14^{b}	1.46 ± 0.31^{b}	2.05 ± 0.36^{b}
F. graminearum Fg183	2.90 ± 0.20^{e}	0.95 ± 0.10^{c}	1.53 ± 0.12^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.23 ± 0.06^{b}	0.86 ± 0.21^{c}

Legend: C = control, currently recommended dose for field application; R = reduced dose.
Note: All data are presented in cm, as mean values ± standard deviation. Different letters attributed to the same fungi indicate a significant difference between the experimental variants regarding their growth inhibition ($p < 0.05$).

Table 4: Efficacy of fungal growth inhibition using different CF in reduced and currently recommended dose (after 10 days of incubation).

Fungal strains	CF1-NP		CF2-F		CF3-FP	
	C	R	C	R	C	R
	Efficacy (%) in fungal growth inhibition					
Fusarium culmorum Fc46	72.92 ± 1.91^{c*}	59.17 ± 1.44^{d*}	100 ± 0.00^{a*}	97.50 ± 0.00^{b*}	100 ± 0.00^{a*}	100 ± 0.00^{a*}
F. culmorum Fc 1056	65.00 ± 2.17^{c*}	57.92 ± 2.89^{d*}	100 ± 0.00^{a*}	98.75 ± 0.00^{b*}	100 ± 0.00^{a*}	100 ± 0.00^{a*}
F. culmorum Fc 1471	60,83 ± 2.89^{b*}	53.75 ± 5.45^{b*}	100 ± 0.00^{a*}	99.17 ± 1.44^{a*}	100 ± 0.00^{a*}	100 ± 0.00^{a*}
Fusarium graminearum Fg96	41.67 ± 5.05^{b**}	32.08 ± 6.41^{b**}	66.67 ± 5.91^{a**}	40.00 ± 13.05^{b**}	46.67 ± 3.82^{b***}	32.92 ± 7.64^{b***}
F. graminearum Fg183	46.67 ± 3.63^{d**}	40.17 ± 5.16^{d**}	100 ± 0.00^{a*}	100 ± 0.00^{a*}	83.53 ± 4.88^{b**}	71.96 ± 4.23^{c**}

Legend: C = control, currently recommended dose for field application; R = reduced dose.
Note: All data are presented in percentages (%), as mean values ± standard deviation.
Different letters (*a* to *d*) attributed to the same fungi indicate a significant difference between the experimental variants regarding their inhibition ($p < 0.05$).
Different symbols (* to ***) attributed within the same treatment indicate a significant difference between the experimental variants regarding their fungal inhibitory activity ($p < 0.05$).

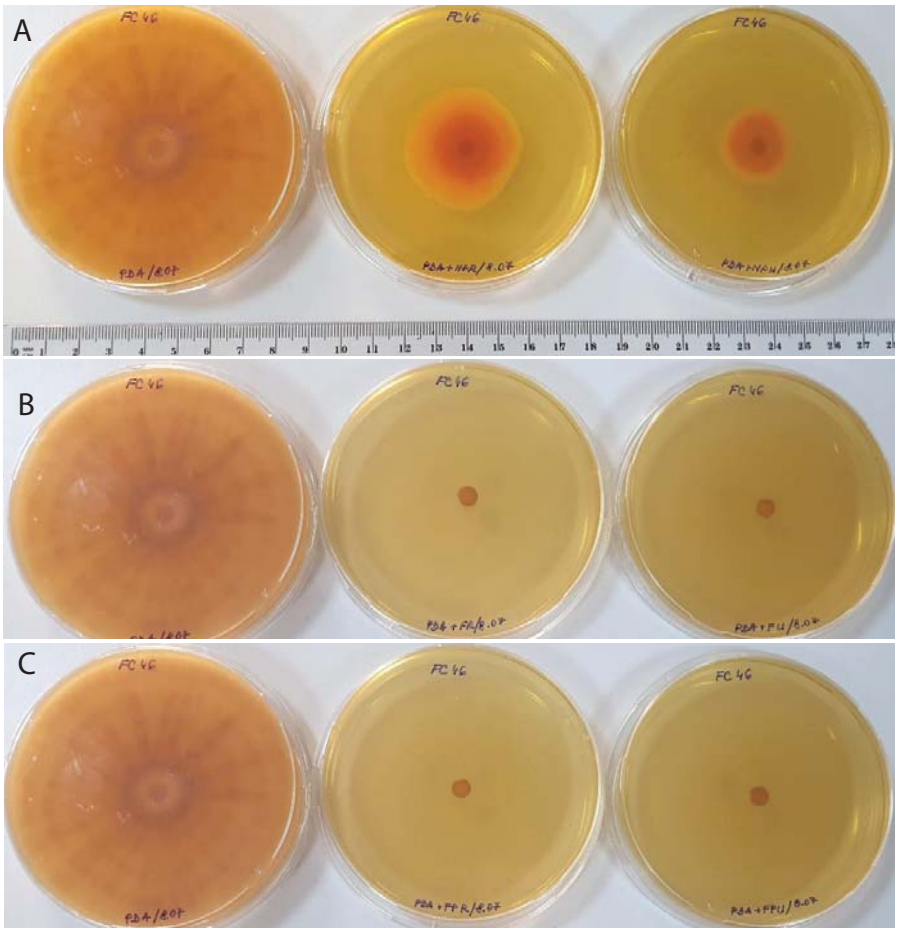

Figure 1: *Fusarium culmorum* Fc46 fungal growth in control plates (left) compared to CF1-NP (a), CF2-F (b), and CF3-FP (c) pesticide treatments in reduced (center) and currently recommended dose (right) (after 10 days of incubation).

Figure 2: *Fusarium graminearum* Fg96 fungal growth in control plates (left) compared to CF1-NP treatments in reduced (center) and currently recommended dose (right), after 10 days of incubation.

bee, butterfly, and partridges' populations is associated with pesticide use [17]. Direct and indirect exposure can counteract various health issues depending on the pesticide type [12]. Organophosphates and carbamates affect the nervous system, while others irritate the skin or eyes [18,19]. Acute toxic effects occur immediately after exposure and have more evident effects, on both humans and animals [12]. More problematic those, are the chronic health issues, which appear after long-term, low-dose exposure to pesticides, and include various diseases and disorders, including cancers, reproductive dysfunctionalities, neurobehavioral disorders, impaired immune function, and allergic sensitization reactions [12]. Therefore, tightening pesticide regulation, reducing synthetic pesticide use, as well as biopesticide implementation should be considered. This follows the current integrated pest management strategies and the European strategies for plant protection [13].

Conclusion

Studying the *in vitro* efficacy of three commercial fungicides against various *Fusarium* pathogens, it was revealed that *F. culmorum* and *F. graminearum* fungi were more sensitive to the CF2-F and CF3-FP pesticides, containing mixtures of three active ingredients (spiroxamine, tebuconazole, and triadimenol or prothioconazole, respectively), compared to CF1-NP pesticide, containing trifloxystrobin and prothioconazole. Although against various *F. culmorum* strains (FC46, FC 1056, and FC 1471), both CF2-F and CF3-FP pesticides provided a high inhibition efficacy when used in reduced dose (25% less PC3-FP, and 28.6% less PC2-F) they have a low efficacy against some *F. graminearum* pathogens (Fg 96 strain especially). Therefore, in the context of pesticide reduction in agriculture, the use of low pesticide doses cannot be considered a viable solution for plant protection. Other integrated pest management strategies should be considered.

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