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Field Assessment of *Lamium album* in Reducing Mycotoxin Biosynthesis in Winter Wheat Infected by *Fusarium culmorum*

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Abstract: Fungicides play a crucial role in conventional agriculture for disease control, but their prolonged use raises health and environmental concerns. *Fusarium culmorum* (*F. culmorum*), a major wheat pathogen causing Fusarium head blight (FHB) and Fusarium crown rot (FCR), poses significant mycotoxigenic threats. The application of natural plant extracts has been proven to fight against phytopathogenic fungi. This study aimed to a field experiment that was carried out at the Field Experimental Station of the Institute of Plant Protection—National Research Institute in Winna Góra, Poland, during the 2022/2023 season to evaluate the potential of *Lamium album* (*L. album*) flower extract as a foliar spray against mycotoxigenic fungi in two winter wheat varieties: Arkadia and Julius. The supercritical carbon dioxide extraction method (SC-CO_2) was employed to obtain the *L. album* flower extract. Ergosterol (ERG) and mycotoxin accumulation in the harvested wheat grains were analyzed using chromatography-based methods. The results demonstrated a notable reduction in ERG content in the field plots treated with *L. album* flower extract, from 26.07 µg/g (control group) to 8.91 µg/g (extract-treated group) for Arkadia and from 70.02 µg/g (control group) to 30.20 µg/g (extract-treated group) for Julius. The treatment with *L. album* reduced mycotoxin biosynthesis in both varieties, with deoxynivalenol (DON) and zearalenone (ZEN) production significantly decreased. Additionally, Arkadia exhibited greater resistance to *Fusarium* infection, and the antifungal effect of *L. album* was more pronounced than in the Julius variety, which proved to be more sensitive. In conclusion, *L. album* flower extract exhibited promising antifungal effects in field experiments to fight against *F. culmorum* in winter wheat varieties, suggesting a potential alternative to synthetic fungicides. However, as complete prevention of mycotoxin contamination was not achieved, further research is warranted to optimize extract concentrations and conduct long-term analyses to consider this plant extract as a sustainable control agent.

Keywords: winter wheat; natural plant extract; plant protection efficiency; sustainable agriculture; in vivo antifungal activity

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1. Introduction

Cereal grains play a vital role in the global economy as staple food for majority of the world's population, being a rich source of essential nutrients, including carbohydrates, proteins, fibers, vitamins, and minerals [1]. They contribute significantly to food security, economic well-being, and poverty alleviation. In 2022, global wheat production alone reached 808.4 million metric tons, underscoring the critical importance of cereals in meeting dietary needs [2].

However, cereal cultivation faces substantial threats from mycotoxigenic fungi, particularly those within the *Fusarium* genus. *Fusarium culmorum*, specifically, is responsible for causing two significant diseases in wheat: Fusarium head blight (FHB) and Fusarium crown rot (FCR) [3,4]. FHB is far more critical promoted by warm and humid environments, develops from infection at anthesis and spreads until grain harvest. Conversely, FCR is prevalent worldwide in arid and semi-arid cropping regions [5]. The frequency and extent of FHB and FCR epidemics have escalated due to climate change, conservation agriculture, and rising wheat-maize crop rotations [3]. These diseases threaten global wheat production, contributing to yield and quality losses that can adversely impact food security. With an estimated 60% increase in global wheat production required over the next three decades to meet the demands of a growing population, the urgency of addressing these issues becomes apparent [3]. Furthermore, *F. culmorum* infections are invariably accompanied by the synthesis of various mycotoxins, notably zearalenone (ZEN) and its derivatives zearalenone-14-sulfate (ZEN-14S) and βeta-zearalenol (β-ZOL) and alpha-zearalenol (α-ZOL), as well as trichothecenes. These trichothecenes encompass type B trichothecenes such as deoxynivalenol (DON), acetyl-deoxynivalenol (3-ADON and 15-ADON), and nivalenol (NIV) [6,7], which pose severe risks to human health, livestock, food safety, and the economy [8].

To address these challenges caused by mycotoxigenic fungi in cereals, various studies explored good manufacturing practices and biological or chemical interventions to protect wheat from fungal infection and mycotoxin biosynthesis [9]. However, no foolproof technique protects cereals fully against *Fusarium* infections [10,11]. In addition, synthetic fungicides, such as benzimidazoles (thiabendazoles and carbendazim) and methyl bromide, have been extensively employed to combat these pathogens and protect crops effectively. Using these compounds has drawbacks such as pathogen resistance development, harm to non-target organisms, and potential toxicity for animals [12–14]. Seeking an alternative approach to control *Fusarium* pathogens with minimal risks and pursuing sustainable agriculture, the application of natural products derived from plants (plant extracts and essential oils) has gained prominence due to their various active compounds, such as alkaloids, phenols, and terpenes that have shown different biological properties, including antifungal activities [15,16].

Plant extracts are effective in controlling the development of *Fusarium* spp. both during the growing season and in storage [17]. The mode of action of plant-based compounds against fungi depends on various factors like fungal strain, the profile and purity of bioactive compounds, the type of solvent used for extraction, and the conditions in which these compounds are used [18,19]. In our previous research, extracts derived from *L. album* flowers have demonstrated efficacy in inhibiting the growth of *Fusarium* pathogens and reducing mycotoxin biosynthesis on PDA and in wheat seedlings [20]. Many authors have studied and confirmed the *in vitro* effect of various plant extracts against *Fusarium* spp. [16,19,21–24]. A growing number of plant extracts have been studied and tested as natural plant protection products [25], including Thyme Guard®, derived from *Thymus vulgaris*; Timorex Gold® obtained from *Melaleuca alternifolia*; Milsana®, sourced from *Reynoutria sachlinesis*; and Owel®, an extract obtained from *Macleaya cordata* [26,27]. Additionally, botanical solutions like NeemPro® derived from *Azadirachta indica* A. Juss [28] and NeemAzal® powder containing SilicoSec and azadarachtin (0.1%) [29] have demonstrated efficacy, especially in post-harvest disease prevention and maize seed treatments. These available products highlight the potential for developing plant extracts to combat diseases caused by pathogenic *Fusarium* species, as the plant-based fungicide availability is still low. Most of the studies were conducted in vitro, so the in vivo experiments under field conditions are required, as successful effect of plant extracts to inhibit fungal growth in vitro does not guarantee its effect in field conditions.

Furthermore, the choice of extraction technique significantly influences plant extracts' quality and inhibitory effect. Traditional methods, including maceration and distillation [24,30], often involve prolonged extraction times and large solvent quantities, raising

regulatory concerns. In contrast, the shift towards green solvent extraction, particularly supercritical fluid extraction (SFE) using CO_2 (SC-CO_2), addresses these limitations. The application of SFE for obtaining various natural compounds has been extensively evaluated [31–33], which aligns with sustainability goals as it utilizes non-toxic, non-flammable CO_2 under high-pressure and lower-temperature conditions. This method yields extracts with distinct chemical complexities compared to conventional techniques, making it a promising and environmentally-friendly choice for plant material extraction [31,34].

In the pursuit of sustainable agriculture, the main goal of this research was the assessment of antifungal potential of *L. album* flower extracts obtained through SC-CO_2 to protect winter wheat Arkadia and Julius cultivars against *F. culmorum* and associated mycotoxins biosynthesis in field conditions.

2. Materials and Methods

2.1. Plant Material and Extraction

Dried *L. album* flowers were purchased from a certified Polish company called Dary Natury in Podlaskie Voivodeship of Poland. Plant material was extracted using a dynamic-static extraction lab scale, as described by [19]. Multiple vessels were prepared, each containing ten grams of powdered *L. album* flowers, and then placed in an oven set at 50 °C/250 bar. The CO_2 flow rate was maintained at 4 mL/min, with methanol as a fixed co-solvent at 1 mL/min. Each extraction run lasted 180 min. The process continued until a sufficient volume of *L. album* extract was obtained. Subsequently, obtained extracts were evaporated in a vacuum evaporator (Buchi Labortechnik AG, Flawil, Switzerland) at 40 °C to eliminate methanol; dried extract was then reconstituted in a measured volume of distilled water (10:2, *v*/*v* ratio) and stored at −18 °C until required for the antifungal assays.

2.2. Studied Material

The studied material consisted of two commonly used winter wheat cultivars varying in susceptibility to *Fusarium*: a susceptible cultivar Julius and a resistant cultivar Arkadia. Both cultivars originated from the Plant Breeding Company in Poznań, Poland.

2.3. Fusarium Strain and Inoculum Preparation

Fusarium culmorum strain KF 846 was obtained from the collection of the Institute of Plant Genetics, Polish Academy of Sciences in Poznan, Poland. The pathogen was cultured in Petri dishes (9 cm diameter) containing potato dextrose agar medium (PDA, BioShop, Burlington, ON, Canada) and incubated in the dark at 28 °C for seven days to promote fungal growth. Upon maturation, fungal spores were harvested by washing the culture plate with sterile water, gently scraping the surface to dislodge the spores, and then filtering the suspension through sterile cheesecloth to remove any mycelial debris. The concentration of spores per milliliter was determined using a hemocytometer and adjusted to achieve a final concentration of 700,000 spores/mL.

2.4. Chemicals

Carbon dioxide (CO_2, SFE grade), contained in a dip tube cylinder, was purchased from Air Products (Poznan, Poland). Methanol for HPLC-super gradient was purchased from POCh (Gliwice, Poland). Acetonitrile, methanol, and water for LC-MS grade were acquired from POCh (Gliwice, Poland). Analytical standards purchased in ready-to-use solutions from Romer Labs (Tulln, Austria) included ZEN, DON, and 15+3-AcDON at 100 µg/mL. The β-ZOL concentration was 10 µg/mL. ZEN-14S (100 µg/mL) was purchased in Aokin (Berlin, Germany). ERG (a fungal growth indicator) was purchased from Sigma-Aldrich (Steinheim, Germany).

2.5. Experimental Design and Procedure

The field experiment was carried out in the 2022/2023 growing season at the Field Experimental Station of the Institute of Plant Protection—National Research Institute in

Winna Góra, Poland (52°12′41.7″ N, 17°25′45.6″ E). The experiment was set up in a random block design with four repetitions on plots of 3.75 m^2 with two cultivars of winter wheat: Arkadia and Julius. On 6 June 2023, in the BBCH 63 phase (flowering), inoculation with a spore suspension of *F. culmorum* was performed. A backpack sprayer was used for inoculation, applying (at a working pressure of 0.2 MPa) 170 mL of a suspension with a concentration of 700,000 spores/mL to the plot once. The day after inoculation (7 June 2023), *L. album* extracts were applied using a backpack sprayer. In the case of plots where plant extracts were used, the flow rate of the working liquid was 125 mL/plot (at a working pressure of 0.3 MPa). At maturity (4 August 2023), grains were harvested with a plot harvester Wintersteiger, model Classic (Ried, Austria), and prepared for analysis.

2.6. Weight of 1000 Grains Evaluation

Grain samples were randomly selected for analysis. Each sample plot was divided into three batches, each comprising 200 grains. Subsequently, the individual batches were weighed. The average weight of 1000 grains were computed using the mean weight obtained from the three batches with the purpose of assessing the impact of *Fusarium* infection and the efficacy of *L. album* treatment on the grain quality. Results are expressed in grams.

2.7. Chemical Analyses

2.7.1. ERG Content in the Harvested Wheat Grains

Harvested wheat was ground into a fine powder and then analyzed for the ergosterol content following the method described by Waśkiewicz et al. (2014) [35]. Briefly, wheat samples (100 mg) were suspended in 2 mL of methanol in a culture tube, and 0.5 mL of 2 M aqueous sodium hydroxide was added. The mixture underwent three 10 s microwave irradiations (370 W) and was then neutralized with 1 mL of 1 M aqueous hydrochloric acid. After treatment, samples were subjected to extraction with n-pentane (3 × 4 mL), and collected extracts were evaporated to dryness using a stream of nitrogen. The resulting dry residues were reconstituted in 1 mL of methanol and filtered through a syringe filter of 0.2 µm mesh. Using a chromatographic system, ERG separation was carried out on a 3.9 mm Nova Pak C-18 (Waters, Milford, MA, USA), 4 mm column, employing methanol: acetonitrile (90:10, *v*/*v*) as the mobile phase at a 1.0 mL/min flow rate. Detection of ERG was performed using a Waters 2996 Photodiode Array Detector (Waters, Milford, MA, USA) set at 282 nm. ERG was quantified by measuring the peak areas at the retention time according to the relevant calibration curve. The limit of detection was 10 ng/g.

2.7.2. Mycotoxins in the Harvested Winter Wheat Grain

Mycotoxins were extracted by adding a mixture of 20 mL acetonitrile, water, and formic acid (79:20:1, *v*/*v*/*v*) to 5 g of fine powder of winter wheat grain samples. Extraction process involved vortexing for approximately 30 s and mixing on a horizontal shaker for 24 h, samples were centrifuged at 7500 rpm for 10 min. Afterwards, approximately 2 mL of mycotoxin extracts were filtered through a 0.20 µm nylon syringe filter and collected in vials for mycotoxin identification and quantification.

Mycotoxins detection and determination were analyzed with UHPLC-HESI-MS/MS. The analytes were separated on a non-porous C18 Cortecs chromatographic column (100 mm × 2.1 mm × 1.6 µm). The mobile phase consisted of water–methanol 90:10 (A) and methanol–water 90:10 (B); both phases had 5 mM ammonium formate and 0.2% formic acid. The following flow gradient (A/B ratio) was applied: 100:0 for 0–2 min; 75:25 for 2–3 min; 40:60 for 3–6 min; 0:100 for 6–20 min; 0:100 for 20–26 min; 100:0 for 26–28 min; 100:0 for 28–30. The flow rate was 0.3 mL/min, and sample volume (2 µL) was injected into the system [19].

The compounds were quantitatively analyzed using multiple reaction monitoring. All samples were analyzed in triplicate.

2.8. Statistical Analysis

In this study, statistical analyses were conducted using Analysis of Variance (one-way ANOVA model) to compare mean mycotoxin levels, ergosterol content, and thousand wheat grain weights among different treatment groups (control vs. extract) for both Arkadia and Julius wheat varieties. Post hoc analyses were performed using the Duncan multiple range test (Statistica 13.3. StatSoft, Tulsa, OK, USA) to identify specific pairs of groups with statistically significant differences. The significance level was set at $p < 0.05$, ensuring a robust statistical evaluation. The experiment was done in four replications.

3. Results

3.1. Comparative Effect of L. album Flower Extract on the Grain Weight of the Harvested Winter Wheat Cultivars

The results presented in Table 1 depict the treatment effect of *L. album* on the weight of harvested wheat grains (1000 wheat kernels) for both Arkadia and Julius cultivars that have been artificially infected with *F. culmorum*.

Table 1. The effect of *L. album* on the weight of harvested wheat grains by comparing the studied cultivars.

Plot *	ARK_C	ARK_E	JUL_C	JUL_E
FUS 1A-405	43.30 ^{a,*} ± 0.18	43.65 ^{a} ± 0.09	38.65 ^{c} ± 0.10	41.45 ^{b} ± 0.17
FUS 1A-302	43.25 ^{a} ± 0.15	44.10 ^{a} ± 0.04	38.30 ^{c} ± 0.06	39.15 ^{c} ± 0.19
FUS 1A-204	44.00 ^{a} ± 0.21	44.40 ^{a} ± 0.03	37.80 ^{c} ± 0.19	38.2 ^{c} ± 0.31
FUS 1A-103	44.05 ^{a} ± 0.08	43.70 ^{a} ± 0.26	35.80 ^{d} ± 0.03	36.05 ^{d} ± 0.01

* According to Duncan's test, different letters correspond to significant differences ($p < 0.05$) between means. ARK_C: Arkadia control; ARK_E: Arkadia treated with extract; JUL_C: Julius control, JUL_E: Julius treated with extract. Plot *: symbolize the plot numbers used for the repetitions.

Regarding the Arkadia cultivar, the control group yielded wheat grains with an average weight of 43.25 to 44.05 g. The weight was marginally enhanced in the *L. album*-treated group, ranging from 43.65 to 44.40 g. The overall mean weight for the control group was 43.65 g. However, the extracts-treated group had a slightly higher average weight of 43.95 g. Within this cultivar, there were no significant differences observed between the control and extract-treated groups across all plot numbers, as indicated by the lack of variation in letter labels.

In contrast, for the Julius cultivar, notable differences in the grain weights were observed between the control and extract-treated groups. In general, the control group consistently showed lower mean grain weights (37.65 g) compared to the extract-treated group that showed a mean grain weights of 38.70 g. This trend was consistent across all plot numbers and was statistically significant, as denoted by the distinct letter labels assigned to the mean values.

In overall, the results suggest that the application of *L. album* flower extract has a positive effect on the weight of harvested wheat grains, particularly evident in the Julius cultivar.

3.2. ERG Level in the Harvested Wheat Grains

Analyzing ERG levels in harvested wheat grains from the studied cultivars, Arkadia, and Julius, infected with *F. culmorum* and treated with *L. album* extract, was crucial for assessing fungal biomass (a fungal growth indicator) and the extract's antifungal efficacy. Comparing ERG levels between treated and untreated samples helped in evaluating the extract's effectiveness in inhibiting fungal growth. Additionally, it provided insights into potential variations in susceptibility to *Fusarium* infection among different wheat cultivars. Therefore, the results showed that applying *L. album* as a natural antifungal agent against *F. culmorum* in winter wheat cultivars has led to a differential reduction in ERG content

for both cultivars (Table 2). In the case of the Arkadia cultivar, the control group exhibited ERG levels ranging from 18.64 to 35.92 μg/g, with an average of 26.07 μg/g. Conversely, samples treated with *L. album* extracts demonstrated a significant decrease in ERG content, ranging from 5.63 to 13.01 μg/g, with an average of 8.91 μg/g.

Table 2. The effect of *L. album* on ERG content [μg/g] of the two winter wheat varieties.

Plot *	ARK_C	ARK_E	JUL_C	JUL_E
FUS 1A-405	28.50 ^{f,*} ± 0.76	5.63 ^{i} ± 0.62	53.54 ^{c} ± 2.25	35.62 ^{e} ± 3.69
FUS 1A-302	21.25 ^{g} ± 0.97	13.01 ^{h} ± 2.67	46.61 ^{d} ± 2.76	29.26 ^{f} ± 1.17
FUS 1A-204	18.64 ^{g} ± 1.16	7.40 ^{i} ± 1.66	63.75 ^{b} ± 3.83	20.88 ^{g} ± 0.97
FUS 1A-103	35.92 ^{e} ± 4.35	9.60 ^{hi} ± 1.25	116.17 ^{a} ± 6.62	35.02 ^{e} ± 1.98
Mean	26.07	8.91	70.02	30.20

* According to Duncan's test, different letters correspond to significant differences ($p < 0.05$) between means. ARK_C: Arkadia control; ARK_E: Arkadia treated with extract; JUL_C: Julius control, JUL_E: Julius treated with extract. Plot *: symbolize the plot numbers used for the repetitions.

Similarly, in the Julius cultivar the control group displayed ERG content ranging from 46.61 to 116.17 μg/g, with an average of 70.02 μg/g. The extract-treated group exhibited a noticeable reduction in ERG content, ranging from 20.88 to 35.62 μg/g, with an average of 30.20 μg/g. Consequently, the results affirm that *L. album* extracts effectively reduced ergosterol content in both wheat varieties, underscoring their antifungal properties.

Notably, the Arkadia cultivar demonstrated a more substantial reduction in ergosterol, marking a 65.82% decrease compared to the control group. In contrast, the Julius cultivar exhibited a 56.86% reduction in ERG relative to the control groups. These differential impacts of *L. album* extracts on ergosterol reduction, highlight the potential variations in cultivar response to fungal infection and the antifungal treatment. Specifically, Arkadia proved its resistance to *F. culmorum* compared to Julius, which exhibited greater susceptibility.

3.3. The Inhibitory Impact of L. album Flower Extracts on Mycotoxin Biosynthesis in the Investigated Winter Wheat Cultivars

The ability of *L. album* extracts to inhibit and reduce the mycotoxin accumulation in winter wheat cultivars artificially inoculated with *F. culmorum* was assessed. The presence of DON, 3- and 15-acetyl-deoxynivalenol (3- + 15-AcDON), ZEN, zearalenone-14-sulfate (ZEN-14S), α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL) was analyzed in both control and extract-treated groups (Table 3). The data were statistically analyzed using ANOVA and post-hoc tests (Duncan test) with a significance level of $p < 0.05$.

The produced mycotoxins were the same in both varieties. However, the levels of mycotoxins accumulated in the harvested grains were significantly different, with the control group exhibiting the highest concentration of mycotoxins compared to the *L. album*-treated samples. The accumulation of the mycotoxins in both cultivars was statistically different, with the Julius cultivar showing more mycotoxin accumulation compared to the Arkardia. Additionally, among the biosynthesized mycotoxins in both cultivars, ZEN-14S (285.71–668.97 μg/g) was the most produced, while β-ZOL (1.09–2.94 μg/g) and α-ZOL (0.26–3.53 μg/g) were the least produced. In the Arkadia cultivar the mycotoxin accumulation was as follows: ZEN-14S > ZEN > 3+15AcDON > DON > β-ZOL > α-ZOL. For the Julius cultivar it was different: ZEN-14S > ZEN > 3+15AcDON > DON > α-ZOL > β-ZOL. Furthermore, Figure 1 highlights the potential of *L. album* in reducing mycotoxins in both cultivars, with the Arkadia cultivar exhibiting a more substantial reduction in mycotoxin levels, ranging between 33.29 to 46.82%, compared to the Julius variety, where mycotoxins were reduced in the range from 22.34 to 40.66%, with DON and ZEN the most reduced mycotoxins in both varieties.

Table 3. Mycotoxins produced [μg/g] by *F. culmorum* KF 846 strain in the absence/presence of *L. album* extracts in Arkadia and Julius winter wheat cultivars.

ARK_C						
Plot *	**DON**	**3- and 15-AcDON**	**ZEN**	**ZEN-14S**	**α-ZOL**	**β-ZOL**
FUS 1A-405	68.54 ^{f,*} ± 1.45	106.56 ^{e} ± 5.93	175.74 ^{c} ± 3.80	550.30 ^{d} ± 22.29	0.57 ^{g} ± 0.03	1.75 ^{ef} ± 0.04
FUS 1A-302	77.61 ^{e} ± 2.93	104.20 ^{e} ± 10.78	143.78 ^{e} ± 4.52	400.46 ^{e} ± 17.49	0.54 ^{g} ± 0.04	1.86 ^{de} ± 0.04
FUS 1A-204	45.91 ^{hi} ± 4.39	82.96 ^{f} ± 5.29	105.06 ^{i} ± 8.54	338.01 ^{f} ± 43.19	0.27 ^{g} ± 0.02	1.03 ^{gh} ± 0.19
FUS 1A-103	52.04 ^{h} ± 7.81	111.64 ^{e} ± 17.29	134.92 ^{fg} ± 1.80	524.45 ^{d} ± 24.89	0.46 ^{g} ± 0.03	2.84 ^{bc} ± 0.18
Mean	61.02	101.34	139.88	453.30	0.46	1.87
ARK_E						
Plot *	**DON**	**3- and 15-AcDON**	**ZEN**	**ZEN-14S**	**α-ZOL**	**β-ZOL**
FUS 1A-405a	33.14 ^{j} ± 3.77	55.41 ^{g} ± 3.99	65.38 ^{l} ± 4.42	392.24 ^{e} ± 22.95	0.42 ^{g} ± 0.05	0.39 ^{i} ± 0.05
FUS 1A-302a	43.40 ^{i} ± 3.11	79.04 ^{f} ± 1.49	97.52 ^{ij} ± 4.05	215.79 ^{g} ± 7.60	0.24 ^{g} ± 0.03	1.10 ^{gh} ± 0.12
FUS 1A-204a	29.83 ^{jk} ± 2.44	54.86 ^{g} ± 4.40	67.48 ^{l} ± 4.61	131.50 ^{h} ± 26.86	0.14 ^{g} ± 0.03	0.89 ^{hi} ± 0.23
FUS 1A-103a	23.45 ^{k} ± 2.09	79.90 ^{f} ± 3.89	80.87 ^{k} ± 1.39	403.30 ^{e} ± 11.98	0.30 ^{g} ± 0.06	1.96 ^{de} ± 0.29
Mean	32.46	67.30	77.81	285.71	0.27	1.09
JUL_C						
Plot *	**DON**	**3- and 15-AcDON**	**ZEN**	**ZEN-14S**	**α-ZOL**	**β-ZOL**
FUS 2A-405	98.37 ^{bc} ± 3.41	161.44 ^{b} ± 9.82	186.91 ^{b} ± 9.72	733.00 ^{b} ± 11.18	2.16 ^{e} ± 0.17	1.85 ^{de} ± 0.35
FUS 2A-302	84.77 ^{d} ± 4.45	128.91 ^{d} ± 3.06	142.19 ^{ef} ± 5.26	510.65 ^{d} ± 58.76	4.04 ^{b} ± 0.53	2.90 ^{b} ± 0.13
FUS 2A-204	104.84 ^{b} ± 3.87	145.63 ^{c} ± 5.43	188.80 ^{b} ± 3.50	805.38 ^{a} ± 9.01	5.24 ^{a} ± 0.70	2.86 ^{bc} ± 0.63
FUS 2A-103	135.04 ^{a} ± 3.22	195.30 ^{a} ± 6.88	222.23 ^{a} ± 6.45	626.84 ^{c} ± 48.48	2.67 ^{d} ± 0.24	4.15 ^{a} ± 0.53
Mean	105.76	157.82	185.03	668.97	3.53	2.94
JUL_E						
Plot *	**DON**	**3- and 15-AcDON**	**ZEN**	**ZEN-14S**	**α-ZOL**	**β-ZOL**
FUS 2A-405a	45.31 ^{hi} ± 3.91	127.82 ^{d} ± 2.49	128.90 ^{g} ± 2.69	520.88 ^{d} ± 13.73	1.16 ^{f} ± 0.38	1.29 ^{fgh} ± 0.20
FUS 2A-302a	61.88 ^{g} ± 5.42	78.89 ^{f} ± 2.94	94.02 ^{j} ± 3.80	340.10 ^{f} ± 40.91	3.36 ^{c} ± 0.49	2.33 ^{cd} ± 0.37
FUS 2A-204a	49.14 ^{hi} ± 4.03	106.04 ^{e} ± 5.93	118.23 ^{h} ± 3.07	381.46 ^{ef} ± 32.81	4.35 ^{b} ± 0.19	1.48 ^{efg} ± 0.43
FUS 2A-103a	94.68 ^{c} ± 3.59	154.44 ^{bc} ± 5.59	160.01 ^{d} ± 2.46	609.29 ^{c} ± 12.52	1.64 ^{f} ± 0.26	3.28 ^{b} ± 0.33
Mean	62.75	116.80	125.29	462.93	2.62	2.10

* According to Duncan's test, different letters correspond to significant differences ($p < 0.05$) between means. Analyzed mycotoxins: deoxynivalenol (DON), 3- and 15-acetyl deoxynivalenol (3- and 15-AcDON), zearalenone (ZEN), zearalenone-14-sulfate (ZEN-14S), alpha-zearalenol (α-ZOL), and beta-zearalenol (β-ZOL). ARK_C: Arkadia control; ARK_E: Arkadia treated with extract; JUL_C: Julius control, JUL_E: Julius treated with extract. Plot *: symbolize the plot numbers used for the repetitions.

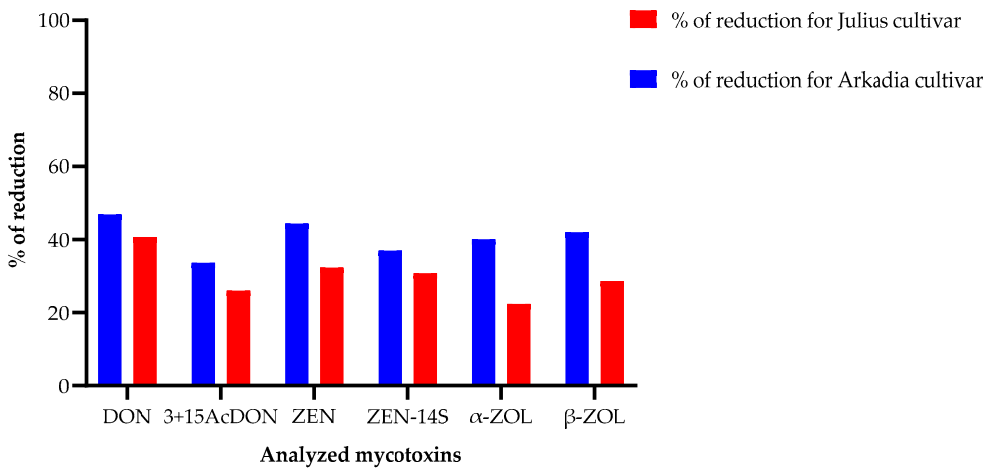

Figure 1. Comparative effect of *L. album* on mycotoxin reduction in winter wheat cultivars Arkadia and Julius. Data are expressed as % of mycotoxins reduction. Analyzed mycotoxins: deoxynivalenol (DON), 3- and 15-acetyl deoxynivalenol (3- and 15-AcDON), zearalenone (ZEN), zearalenone-14-sulfate (ZEN-14S), and beta-zearalenol (β-ZOL) and alpha-zearalenol (α-ZOL).

4. Discussion

Winter wheat is a major global crop [36] but is vulnerable to *Fusarium* infections, particularly FHB, which poses significant threats to grain yield, germination, quality, and safety [6]. Controlling *Fusarium* diseases in wheat is crucial due to the absence of complete resistance in any wheat variety, the long-term survival of the pathogen in soil, and the production of harmful mycotoxins in wheat tissues, which are harmful to consumers. To mitigate these issues and reduce reliance on chemical pesticides, there is growing interest in using natural plant extracts as protective agents. Many plants and their extracts have demonstrated antifungal properties [19,22,23,37,38].

While there is a growing interest in biological control methods, significant emphasis is placed on understanding and screening different plant species and natural compounds that can control fungal growth and mycotoxins in vitro. However, the expanding knowledge in this area should be used more extensively for practical applications in planta, pre-harvest, post-harvest, as well as during storage and food processing. To our knowledge, no previous research under field conditions has investigated the antifungal effect of *L. album* flower extracts. Additionally, studies of other natural plant extracts against *Fusarium* infection in winter wheat cultivars are still limited, and there is an increasing demand for organic produce [39]. The current study builds upon our prior research, conducted under controlled conditions, which has demonstrated the protective efficacy of *L. album* against artificially inoculated *F. culmorum* in wheat seedlings [40]. The present results confirm the capability of *L. album* to inhibit fungal growth under field conditions, as evidenced by the ERG and mycotoxins reduction in the harvested wheat grains and a moderate difference in thousand-kernel weight.

Specifically, Arkadia exhibited a higher weight, measuring 43.65 g in the control group and 43.97 g in the extract-treated group. Conversely, Julius cultivars displayed lower weights, with 37.65 g in the control group and 38.70 g in the extract-treated group. Some studies emphasize significant varietal differences, while others report no notable distinctions. For instance, a study assessing new winter wheat cultivars for organic farming found no discernible difference in 1000-kernel weight between Arkadia (41.6 g) and Julius (41.2 g) despite variations in other morphological features and canopy parameters influencing their competitive abilities [41]. This trend is further supported by an investigation examining wheat varieties' response to seed cleaning and fungicide treatment following FHB infection. The study noted variations in thousand-kernel weight attributed to wheat variety, with SY Wolf (28.6 g) and Everest (28.4 g) displaying higher weights compared to WB Grainfield

(27.2 g) [42]. This means that 1000-kernel weight alone may not be a decisive factor in determining the effect of plant extract, resistance, or yield potential of wheat varieties.

The observed decrease in ERG levels, a crucial component of fungal membranes, represents a significant finding in our study. The application of *L. album* extract resulted in a substantial decrease in ergosterol content, with a 65.82% reduction for the Arkadia cultivar and 56.86% for the Julius cultivar, indicating a potential disruption in fungal cell membranes, contributing to the inhibitory effect on *F. culmorum* growth in both cultivars. The inhibitory effect of *L. album* extract on *F. culmorum* growth in both cultivars could be attributed to its active compounds, such as verbascoside; isoscutellarein derivatives; flavonoids (quercetin, quercetin-3-O-glucoside, rutin, isoquercitrin, kaempferol-3-O-glucoside); and phenolic acids (protocatechuic, chlorogenic, vanillic, and caffeic), along with phenylpropanoid glycoside ester derivatives (lamalboside, acteoside, and isoacteoside) that have been identified with various biological activities [43–47]. The detrimental impact on ERG content indicates a significant disruption or blockage in biosynthesis within the plasma membrane of *F. culmorum*. This finding is consistent with previous studies that reported decreased ERG content in *Fusarium*-infected samples after applying plant extracts. For instance, the *Solanum torvum* Swartz leaf extract reduced ERG production in *Fusarium verticillioides* [48]. Additionally, thymol, a component of plant extracts, has been shown to inhibit the growth of *F. graminearum*, resulting in a reduction of ERG levels [49]. Similarly, extracts derived from *Melissa officinalis* demonstrated a concentration-dependent reduction in ERG content [19]. Furthermore, assessing ergosterol levels in harvested wheat grains after *F. culmorum* infection is crucial for identifying the susceptibility and resistance of studied cultivars to *Fusarium* infection. The observed reduction in ergosterol aligns with the antifungal activity of *L. album* extract, emphasizing its efficacy in impeding *F. culmorum* growth.

Although ERG is a reliable predictor of fungal development, it is not a dependable indicator of mycotoxin contamination [19], as not all fungi synthesize mycotoxins. Conversely, mycotoxins can persist even in the absence of fungal viability. In the present study, the application of *L. album* extract (obtained by SC-CO_2) in the form of foliar spray exhibited mycotoxins reduction (DON, 3- + 15-AcDON, ZEN, α-ZOL, β-ZOL, and ZEN-14S) by 22.34 to 46.82% relative to control samples and a significant reduction was observed in DON and ZEN levels in both varieties. These findings are in line with other studies, demonstrating the effect of the extracts from medicinal and aromatic plants on *Fusarium* species [37,50–52]. Abbas and Yli-Mattila (2022) showed that methanolic extract of the medicinal plant *Zanthoxylum bungeanum* successfully decreased *F. graminearum* growth and abrogated DON production in wheat heads [51]. Similarly, mustard-based extracts reduced fungal infection in wheat grains and decreased DON accumulation under growth chamber conditions but were ineffective under field conditions [52]. Furthermore, the natural extracts from neem seeds demonstrated more potent inhibition of mycotoxin production of DON, 15AcDON, 3AcDON, and ZEN [38].

The α-ZOL (0.26–3.53 μg/g) and β-ZOL (1.09–2.94 μg/g) were the least produced mycotoxins, while ZEN-14S (285.71–668.97 μg/g) was the highest among the produced mycotoxins in both cultivars (Table 3). This also gives insight into the types of mycotoxins and their levels that were biosynthesized by the inoculated strain of *F. culmorum*, and it confirmed that natural plant extracts may have an inhibitory effect to several mycotoxins at once. ZEN-14S, α-ZOL, and β-ZOL are the modified forms of ZEN identified in this study. They are currently unregulated but may increase the risk of human animal exposure because of possible high incidence in cereals and cereal-based products, serving as staple food in several regions [53]. Veršilovskis et al. (2019) mentioned that ZEN-14S is approximately 60 times more estrogenic than ZEN and can be readily hydrolyzed to ZEN in the gastrointestinal tract, thereby increasing exposure to ZEN [54]. Furthermore, a high ZEN-14S/ZEN ratio in the malted wheat suggests that *Fusarium* can convert ZEN into a phase II metabolite through sulfation reactions [55]. Ayed et al. (2011) evaluated the cytotoxicity and genotoxicity of α-ZOL and β-ZOL in vivo, in mouse bone marrow cells and in vitro, in cultured HeLa cells, and compared it with ZEN. Their results showed that

ZEN and α-ZOL exhibited the same range of genotoxicity and cytotoxicity; both were more genotoxic and cytotoxic than β-ZOL [56].

In the present study, Arkadia was found to be more resistant to *F. culmorum* infection as it exhibited a lower ergosterol content and mycotoxin accumulation than the Julius cultivar, which was more sensitive and exhibited a high amount of ergosterol and mycotoxin accumulation as the fifth category for classifying wheat resistance (resistance to mycotoxins) [57]. This supports the notion that inherent differences may influence the susceptibility to fungal infection and the extract's effectiveness in combating *Fusarium.* Our findings align with the existing literature, reinforcing the significant varietal differences in susceptibility to *Fusarium* infection and mycotoxin accumulation in winter wheat cultivars [41,57,58].

5. Conclusions

Biological control and the search for new natural antifungal agents are gaining more and more interest. The potential of *L. album* flower extract in combatting *F. culmorum* in winter wheat cultivars was proven by reduced ERG, DON, and ZEN in the harvested wheat grains of both winter wheat cultivars Arkadia and Julius, compared to the controls. These results highlight the extract's promising role in promoting sustainable and resilient agricultural practices, contributing to the broader global discourse on eco-friendly disease management strategies. Furthermore, different mycotoxins were identified in the control and extract-treated groups. The results showed the most minor production of α-ZOL and β-ZOL, with ZEN-14S being the most abundant mycotoxin in both cultivars. This underscores the importance of considering multiple mycotoxins and their modified forms in evaluating the efficacy of natural plant extracts. To our knowledge, this is the first report showing the in vivo possibility of using *L. album* as a natural antifungal agent for some devastating winter wheat plant diseases. This study contributes valuable insights in using *L. album* flower extracts for sustainable and eco-friendly *Fusarium* control in winter wheat. The observed cultivar differentiation highlights the need for tailored approaches, and our findings pave the way for further exploration of natural plant extracts in promoting sustainable wheat production and valorizing locally available plants.

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