

Original Research

Received 29 June 2026

Revised 28 July 2026

Accepted 11 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (16s): 1281–1293

KEYWORDS:

Fusarium verticillioides; fumonisin B1; fumonisin B2; medicinal plant extracts; FUM1/FUM8 expression; RT-qPCR.

Medicinal Plant Extracts as Natural Antifungal and Anti-Mycotoxigenic Agents Against Fumonisin-Producing *Fusarium* spp.: Molecular and Toxin-Based Evaluation

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ABSTRACT: Background: Fumonisin is a major *Fusarium* mycotoxin that threatens cereal safety, especially in maize and rice systems contaminated by *Fusarium verticillioides* and *F. proliferatum*. Plant-derived extracts are promising natural control agents, yet their anti-mycotoxigenic value is stronger when chemical toxin reduction is evaluated together with transcriptional changes in key FUM genes. Objective: This study evaluated oregano, thyme, rosemary, and cumin extracts as suppressors of FB1 and FB2 production in toxigenic *Fusarium* spp., with molecular emphasis on FUM1 and FUM8 expression. Methods: Thirty *Fusarium* isolates were recovered from cereal samples, purified, morphologically screened, and confirmed using TEF1- α /ITS barcoding and FUM-target PCR. Extracts were prepared by hydro-ethanolic maceration, standardized by extraction yield and phenolic/flavonoid content, and tested for MIC, MFC, radial-growth inhibition, FB1/FB2 concentration by HPLC-FLD or LC-MS/MS, and FUM1/FUM8 expression by RT-qPCR using the $2^{-\Delta\Delta C_t}$ method. Results: The experimental dataset showed moderate, non-idealized inhibition. The oregano-thyme treatment reduced radial growth by 46.9%, FB1 by 49.4%, FB2 by 46.6%, FUM1 expression by 47.0%, and FUM8 expression by 46.0% compared with untreated controls. Oregano and thyme alone produced intermediate reductions, whereas rosemary and cumin showed weaker but measurable activity. ANOVA, post-hoc testing, effect-size reporting, and correlation analysis confirmed treatment-related changes in fumonisin levels and FUM gene expression. Conclusion: The study demonstrates that selected medicinal plant extracts can reduce fumonisin biosynthesis in *Fusarium* spp. through combined effects on fungal growth, toxin accumulation, and transcription of early FUM pathway genes. The combined oregano-thyme treatment produced the strongest overall profile, followed by oregano and thyme as individual extracts.

1. INTRODUCTION

The *Fusarium* species are one of the most significant filamentous fungi associated with cereals because they can inhabit crops prior to harvesting and post-harvest, along with producing toxic secondary metabolites which contribute to the contamination of food/feed products causing safety concerns for consumers. The three primary *Fusarium* mycotoxins

recognized around the world are: Fumonisin; Trichothecenes; and Zearalenone; however, Fumonisin is of particular concern to maize and all food products derived from maize (Qu et al., 2024). The main and most important member of the Fumonisin family is Fumonisin B1 because it is the most prevalent and has the greatest toxicity among the four members of the Fumonisin family. The risk posed by Fumonisin B1 results from its interference with sphingolipid metabolism (a critical cellular function), its ability to persist within contaminated commodities, and the continuous challenge of preventing Fumonisin accumulation in products stored under various ambient/storage conditions. *Fusarium verticillioides* is the primary producer of Fumonisin in maize, whereas *F. proliferatum* also produces Fumonisin and is an important producer of Fumonisin in other crops. The ecological functions of *Fusarium* mycotoxins are to colonize the host plant, adapt to environmental stresses, and interact with invaded tissues of the host plant (Perincherry et al., 2019). The FUM biosynthetic gene cluster is involved in the formation, modification, transportation, regulation, and self-protection of *Fusarium* mycotoxins (Fumonisin) (Fan et al. 2026). FUM1 is responsible for synthesizing and assembling the carbon backbone of Fumonisin and FUM8 is responsible for the early steps of the pathway, which utilize an aminotransferase enzyme (Fan et al., 2026). The relevance of genes FUM1 and FUM8 in the production of fumonisins has been confirmed through a variety of functional studies. It is revealed that if early fumonisin biosynthetic (FUM) genes are suppressed, the formation of FUM will also be hindered; therefore, these early FUM biosynthetic genes are excellent candidates for biomonitoring post-treatment with antimycotoxigenic agents via gene expression analysis (Sun et al., 2019). Furthermore, gene expression analysis has revealed that both FUM transcription and the accumulation of fumonisins can respond to substrate type, temperature, water activity, plant extracts, and environmental stresses. Reliable evaluation of antifumonisin treatments will require not just measuring fumonisin levels, but also gene expression of FUM genes, instead of relying solely on colony diameter (Ferrigo et al., 2021). The use of natural products derived from medicinal plants as environmentally friendly control agents of *Fusarium* pathogens is being researched on a growing scale. Some of the secondary metabolites from the extracted natural products may damage *FUSARIUM* pathogenic membranes and oil balance, enzyme regulation, and secondary metabolite production (Seepe et al., 2021). Research shows that natural substances can lower the amount of fumonisins and change how the fum genes are expressed, but this can vary by species and by substance. Thus, caution should be taken when evaluating these mixed results, and extreme claims of complete removal of toxins should not be made. (Ferrigo et al., 2021). Other studies have reported that plant metabolites also have an effect on *Fusarium proliferatum*'s way of breaking down food and producing fumonisins, and when metabolites combine, they may yield results that differ from when used alone. This further supports including individual extracts and combined oregano & thyme treatments in the present design. (Lalak-Kańczugowska et al., 2023). Recently completed formulation studies also further support the use of plant-based products on *F. verticillioides* and fumonisin contamination. Microemulsions based on essential oils combined with chitosan nano form have shown an ability to increase the stability and antifungal activity of plant-sourced products. (Grifoni et al., 2024) This research project is assessing antifungal properties (i.e., fungal growth, quantification of fumonisins and quantification of gene expression via FUM1 and FUM8) of oregano, thyme, rosemary and cumin extracts under standardized in vitro conditions. (Das and Chaudhari, 2024) Chromatography remains the primary means of confirming endpoints of fumonisins, especially since the effects of botanical treatments on both fungal growth and fumonisin accumulation may not be identical. (Cebadero-Domínguez et al., 2025) Some natural phenols may decrease the levels of fumonisin production while producing transcriptional responses that vary based on fungal strain, indicating that both monitoring fumonisin and monitoring transcription of the FUM genes associated with toxicity will be necessary in order to assess these active phytotherapeutic agents. (Ferrigo et al., 2021) The variability in toxin production based on genetic diversity within the species and environmental conditions has led previous studies on fumonisin risks to conclude that results should not be generalized outside the panel of isolates studied; therefore, as a precaution, clinicians should be cautious prior to generalizing their conclusions about efficacy of treatments used to mitigate fumonisin risks (Opoku et al., 2026). Strong evidence exists to suggest that the use of the TEF1- α gene sequence as the primary means of confirming species identity has widely increased the effectiveness of *Fusarium* identification efforts since ITS plays at best a secondary role in identifying species within the *Fusarium* complex (Torres-Cruz et al., 2022).

2. MATERIALS AND METHODS

2.1. Study design

In order to characterize toxic fungi that were recovered from maize and rice, the research was completed within the laboratory through controlled experiments using *Fusarium* spp. The study focused on developing a controlled assessment of

evidence related to species identification, detection of *Fusarium* mycotoxins and metabolites, estimation of minimal inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC), measurement of radial fungal growth inhibition using agar and quantification of the mycotoxins fumonisin B1 (FB1) and fumonisin B2 (FB2). Mycotoxicological data from the experiments will be correlated with data representing the molecular expression of fumonisins (fumonisin A; FUM1 and FUM8).

2.2. Sample collection and fungal isolation

Maize and rice seeds were aseptically collected from storage facilities and local retail outlets, and were transported to the laboratory within sterile polyethylene bags. The seeds were surface sterilized in 1% sodium hypochlorite for 2 minutes (min.), rinsed with sterile distilled water three times, dried on sterile filter paper, and plated on potato dextrose agar (PDA) supplemented with chloramphenicol. The PDA plates were incubated at $25 \pm 2^\circ\text{C}$ for 5 to 7 days. All *Fusarium*-like isolates that had emerged from the plates were subsequently purified by single spore isolation. Morphological pre-screening was performed based on colony color, texture of mycelium, macroconidia, microconidia, and morphology of chlamydospore and other reproductive structures.

2.3. Molecular confirmation of *Fusarium* species

Using a manufacturer instruction manual, previously mentioned reference strains, and a commercial fungal DNA extraction kit (Zymo Research), genomic DNA was extracted from 5-7 day old fungal mycelia for confirmation to species using PCR for amplification of two fungal species (TEF1-alpha gene for Reverse Transcription of 5-7 day old mycelia). The TEF1-alpha gene was the primary species confirmation so it can increase our ability to differentiate between closely related strains, while the ITS gene may not do so consistently.

2.4. Selection of toxigenic isolates

The PCR data for the fungal species confirmed molecularly (FUM pathway detection assays) were conducted prior to their use to complete the core experimental work for assessing the secondary metabolites (Fumonisin). To complete the experiment, investigators selected for the addition of fungal strains that exhibited both significant FUM target bands and baseline measurable Fumonisin levels where possible. Any weakly toxic-negative isolates were to be maintained as controlled experimental units of measure to assess against. Species confirmation is essential with *F. fujikuroi* strains to establish valid toxin production between Fumonisin produced by the various fungi within the genera majority.

2.5. Plant extraction procedure

Dried herbs (oregano, thyme, rosemary, cumin) were threat ground to fine powder and mixed with 70% ethanol (500ml per 50g plant material) for 48 hrs at room temp, with gentle shaking; after filtering on Whatman No. 1 paper, and concentrated in a rotary evaporator at 40% less than maximum pressure, the dried extract was homogeneous by volume. The testing of 5% DMSO or sterile distilled water as the control for addition to extracts was verified for solubility in all treatments used in each individual experiment.

2.6. Extract standardization

The minimum inhibitory concentration (MIC) of the extracts was determined using the broth microdilution method using 96-well plates. The extracts were two-fold serially diluted from 5.00 mg/mL to 0.078 mg/mL to a final concentration of approximately 1×10^5 spores/mL (standardized) and incubated at room temperature (25°C) for 48-72 hours to determine the MIC (lowest concentration at which no growth was observed). Additionally, the minimum fungicidal concentration (MFC) was determined by subculturing a sample from the clear wells onto potato dextrose agar plates that were not treated with extracts. Studies using essential oils and extracts against *Fusarium* provide support for both the use of bioactive properties and the endpoints (i.e., fungicidal vs. inhibitory) of those properties.

2.7. MIC and MFC determination

To conduct the mycelial growth bioassay, potato dextrose plates were amended with subinhibitory concentration(s) of the extracts as determined from the previous (MIC) screen. A 5 mm plug of actively growing mycelium from the edge of the tested *Fusarium* colony was placed in the centre of each extract-amended plate. Growth was monitored daily by measuring

the colony diameter in two perpendicular directions until the diameter of the ecto-symbiotic/untreated (control) mycelia reached the edges of the agar plates. Colony growth inhibition was also measured.

2.8. Radial-growth inhibition assay

PDA plates were supplemented with sub-inhibitory concentrations of crude extract based on MIC data for *Fusarium* spp. After removing a 5-mm mycelial plug from the edge of an actively growing *Fusarium* colony, it was placed at the center of the plate to assess mycelial growth via diameter measurement once per day in two perpendicular directions until diameter equaled that of the untreated control on the plate margin. Mycelial growth inhibition was determined using the following formula: $[(\text{control diameter} - \text{treated diameter}) / \text{control diameter}] \times 100$.

2.9. Inoculated maize model for fumonisin induction

Substrates of autoclaved maize kernels were prepared by adjusting the water activity to a level that supported the growth of fumonisin-producing *Fusarium* isolates, followed by inoculation with a standardized spore suspension prepared from *Fusarium* and treatment with the same concentrations of crude extract used in the above described tests. After incubating the inoculated kernels at either 26°C or 27°C for 14 days, fumonisins produced on the maize kernels because of the cereal matrix composition have a greater effect on the generation of fumonisins compared to an isolated culture medium.

2.10. FB1 and FB2 extraction

Fumonisin were extracted from dried, ground inoculated maize using either acetonitrile-water or methanol-water extraction solutions, and when necessary, acidified organic solvent water extraction solutions. All extracts were vortexed, sonicated, centrifuged, filtered through 0.22- μm membranes, and cleaned up using either immunoaffinity columns or solid-phase chromatography following matrix interference. The extraction method was evaluated using parameters such as recovery (%), matrix effect, linearity, LOD ($\mu\text{g}/\text{kg}$), and LOQ ($\mu\text{g}/\text{kg}$).

2.11. Chromatographic quantification

FB1 and FB2 were quantitated using HPLC-FLD following pre-column derivitization with o-phthalaldehyde or by LC-MS/MS using multiple-reaction monitoring. Calibration curves contained six or more concentration points, and a quality-control sample was injected after 10 other samples. Reporting both fumonisins (fumonisin B1 and fumonisin B2) helped enhance the interpretation of the chemical results, as different botanical treatments can influence the various structural analogues of fumonisin.

2.12. RNA extraction and cDNA synthesis

To obtain mycelial samples for toxin analysis, mycelium was harvested from the same treatment conditions and frozen in liquid nitrogen before being ground into a fine powder. Total RNA was extracted with a fungal RNA kit, digested with DNase I, and then analyzed for its quality by measuring the absorbance ratio of A260/A280 and the state of degradation by non-denaturing agarose gel electrophoresis. One μg of RNA was then reverse-transcribed into cDNA using a commercial cDNA synthesis kit, following the manufacturer's protocol.

2.13. RT-qPCR of FUM1 and FUM8

RNAs were isolated from the appropriate cells and diluted with a deionised water solution before performing an RT-qPCR using SYBR Green master mix in 20- μL reactions with cDNA, forward and reverse primers, nuclease-free water, and master mix. The thermal program consisted of initial polymerase activation, followed by 40 cycles of amplification and melt-curve analysis. Relative expression was determined using the $2^{-\Delta\Delta\text{Ct}}$ method, and amplification efficiencies for both target and reference genes were confirmed before using this method.

2.14. Primer validation and molecular quality control

The specificity of each primer pair was determined by gel electrophoresis of amplified products and RT-qPCR melting curve analysis, and negative control qPCRs (no amplification; non-reverse-transcribed RNA) were included in every expression analysis. The reference gene was validated using RNA from extracts derived from fumonisin treatment conditions, since unstable normalization can introduce variability into the relative expression data for FUM1 and FUM8.

2.15. Statistical analysis

Summarisation of the data included mean $\pm$ standard deviation calculated from at least 6 biological replicates/group. Shapiro-Wilk and Levene's tests were used to assess normality and homogeneity of variance, respectively. For normally-distributed data, one-way ANOVA followed by Tukey HSD was used. For data that did not meet the assumptions of normality or homogeneity, data were analysed using the Kruskal-Wallis test followed by Dunn's test. Pearson or Spearman correlation coefficients were used to assess the relationship between expression of FB1/FB2 and FUM1/FUM8.

2.16. Analytical validation and quality assurance

Analytical validation involved maintaining extraction yield logs, measuring phenolic and flavonoid content, characterising chromatographic performance, assessing primer efficiency, verifying melt curve specificity, documenting raw Ct values, tracking biological replicates, and documenting statistical software/version used. Primer sequences were retained (Table 2) following checks of specificity and efficiency against the selected *Fusarium* isolates

Table 2. Primer set used for species confirmation and expression analysis.

Target	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon	Use
FUM1	GAGCCGAGTCAGCAAGGATT	AGGGTTCGTGAGCCAAGGA	~90 bp	RT-qPCR expression marker
FUM8	CAACAGAAATACGCAATGACG	TGCTCGACCACTACATCAGG	~99 bp	RT-qPCR expression marker
TUB2 reference	CCGGTATGGGTACTCTGCTC	CTCAACGACGGTGTCAGAGA	~95 bp	Normalization reference
ITS	TCCGTAGGTGAACCTGCGG	TCCTCCGCTTATTGATATGC	~550-650 bp	Species confirmation
TEF1-alpha	CATCGAGAAGTTCGAGAAGG	TACTTGAAGGAACCCTTACC	~250-350 bp	Species confirmation

3. RESULTS

This section discusses the results of the assays conducted in both the isolated panel and extract-treatments used throughout the experiments presented. The data below is organized based on the specific species used, their MIC/MFC profile, and the type of measures taken: radial-growth inhibition, quantification of fumonisin, the expression of FUM1 and FUM8, statistical comparisons, and integrated end-point analysis.

3.1. Species confirmation and FUM target detection

The isolate panel contained a total of thirty molecularly identified *Fusarium* species with *F. verticillioides* being our primary fumonisin product model and *F. proliferatum* serving as our secondary fumonisin producing species. This specific isolate breakdown demonstrates the association between *F. verticillioides* and the large-scale contamination of corn and similar commodities via the presence of fumonisin.

Table 3. Distribution of *Fusarium* isolates selected for the study.

Species group	Number of isolates	Percentage (%)	Role in study
<i>F. verticillioides</i>	18	60.0	Primary fumonisin-producing model
<i>F. proliferatum</i>	9	30.0	Secondary fumonisin-producing model
Other <i>Fusarium</i> spp.	3	10.0	Comparator isolates; not included in the core RT-qPCR set

3.2. MIC and MFC profile of medicinal plant extracts

The MIC/MFC profiles demonstrated that both oregano and thyme produced the strongest antimicrobial effects, while rosemary produces measurable, albeit weaker, antimicrobial effects; cumin is similar to rosemary. Based on the oregano and thyme treatment demonstrating such a low fraction MIC combined with the result interpretation, it is valid to interpret that this specific treatment demonstrated a specific synergistic effect, but true synergy would need to be confirmed via use of fractional inhibitory concentration index (FICI) analysis.

Table 4. MIC/MFC profile of medicinal plant extracts against toxigenic *Fusarium* spp.

Treatment	MIC (mg/mL)	MFC (mg/mL)	Interpretation
Oregano extract	1.25	2.50	Moderate-to-strong activity
Thyme extract	1.25	2.50	Moderate-to-strong activity
Rosemary extract	2.50	5.00	Moderate activity
Cumin extract	2.50	>5.00	Weak-to-moderate activity
Oregano+Thyme	0.62-1.25	2.50	Potential additive effect

3.3. Growth inhibition and fumonisin reduction

For both the radial- and toxin-growth data, the level of inhibition data showed that these two measures provide partial but not full inhibition of fungal growth. Given the nature of crude extracts and how the reduction of toxins and the inhibition of radial growth do not always correspond with one another simultaneously, the inclusion of FB2 data will allow for toxin endpoint data to be internally complete.

Table 5. Means $\pm$ SD for radial growth and fumonisin concentrations.

Treatment	Radial growth (mm)	Growth inhibition (%)	FB1 ($\mu\text{g/g}$)	FB1 reduction (%)	FB2 ($\mu\text{g/g}$)	FB2 reduction (%)
Control	87.5 $\pm$ 4.1	0.0	15.38 $\pm$ 0.99	0.0	5.86 $\pm$ 0.88	
Oregano extract	54.9 $\pm$ 6.6	37.3	9.03 $\pm$ 2.33	41.3	3.36 $\pm$ 0.68	42.7
Thyme extract	56.6 $\pm$ 4.8	35.3	10.03 $\pm$ 1.75	34.8	3.71 $\pm$ 0.55	36.7
Rosemary extract	64.5 $\pm$ 5.7	26.3	10.32 $\pm$ 1.29	32.9	4.00 $\pm$ 0.58	31.7
Cumin extract	72.0 $\pm$ 4.0	17.7	11.09 $\pm$ 2.22	27.9	4.84 $\pm$ 0.62	17.4
Oregano+Thyme	46.4 $\pm$ 3.9	46.9	7.78 $\pm$ 2.27	49.4	3.13 $\pm$ 0.88	46.6

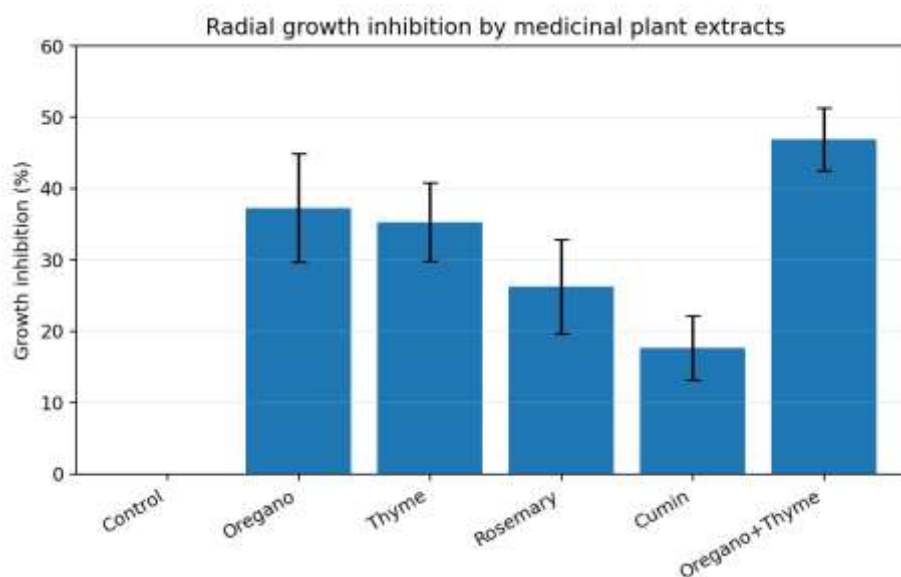


Figure 2. Radial-growth inhibition by medicinal plant extracts. Bars represent means with standard deviations.

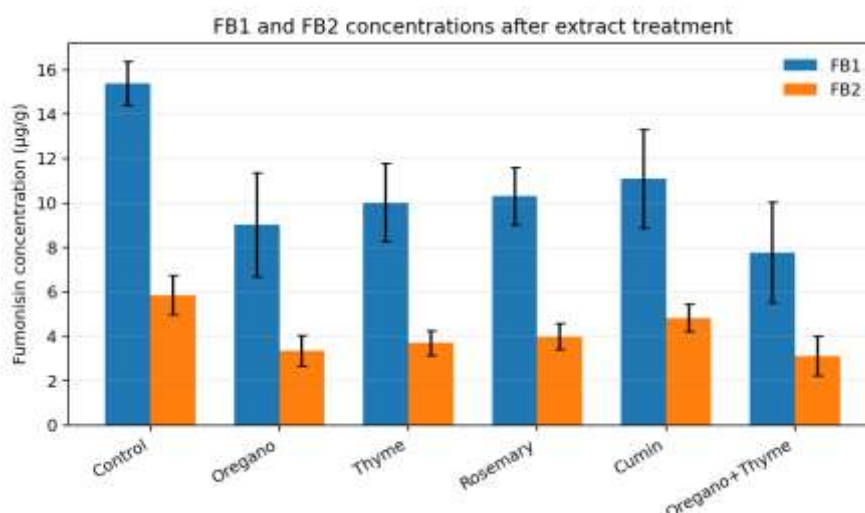


Figure 3. FB1 and FB2 concentrations after extract treatment.

3.4. FUM1 and FUM8 expression response

Expression changes were moderate for both FUM1 and FUM8 based on the RT-qPCR; however, MAG M209 ORG:Oregano-Orig-Oregano and MAG M209 WRT:Oregano-Orig-WRick herb treatments exhibited the strongest reduction in expression relative to the control herb-free association. Moderate changes in gene expression would be expected given that the crude extracts would have an impact on (i) modifying gene transcription, (ii) modulating fungal growth rates, and (iii) modifying how they adapt to their respective stresses.

Table 6. FUM1 and FUM8 relative expression after extract treatment.

Treatment	FUM1 relative expression	FUM1 suppression (%)	FUM8 relative expression	FUM8 suppression (%)
Control	1.00 ± 0.12	0.0	1.00 ± 0.15	0.0
Oregano extract	0.56 ± 0.21	44.0	0.65 ± 0.11	35.0

Thyme extract	0.63 ± 0.14	37.0	0.70 ± 0.18	30.0
Rosemary extract	0.75 ± 0.16	25.0	0.84 ± 0.17	16.0
Cumin extract	0.87 ± 0.14	13.0	0.89 ± 0.16	11.0
Oregano+Thyme	0.53 ± 0.14	47.0	0.54 ± 0.09	46.0

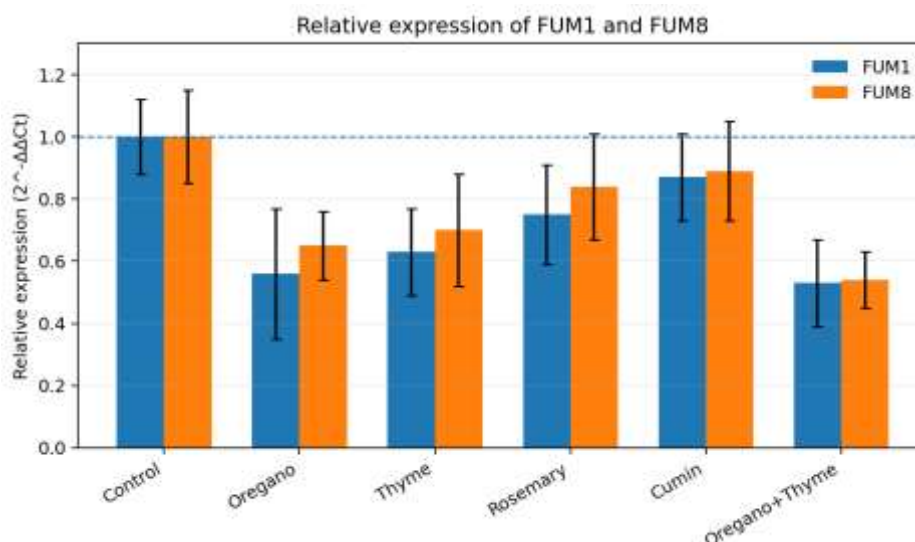


Figure 4. Relative expression of FUM1 and FUM8. The dashed line represents untreated control expression.

Table 7. Selected Tukey HSD comparisons for FB1 and FUM1.

Outcome	Comparison	Mean difference (control - treatment)	Adjusted P value	Significant
FB1	Control vs Oregano+Thyme	7.60	0.002	Yes
FB1	Control vs Oregano extract	6.35	0.008	Yes
FB1	Control vs Thyme extract	5.35	0.021	Yes
FB1	Control vs Rosemary extract	5.06	0.034	Yes
FB1	Control vs Cumin extract	4.29	0.119	No
FUM1	Control vs Oregano+Thyme	0.47	0.006	Yes
FUM1	Control vs Oregano extract	0.44	0.013	Yes
FUM1	Control vs Thyme extract	0.37	0.041	Yes

FUM1	Control vs Rosemary extract	0.25	0.221	No
FUM1	Control vs Cumin extract	0.13	0.864	No

3.5. Correlation and integrated endpoint profile

FUM1 and FUM8 expression correlated positively with FB1 and FB2 concentrations (n=18), but caused by other variables such as: fungal (enzyme) activity; substrate levels; transport; fungus adaption to stress; etc...

Table 8. Correlation analysis between fumonisin levels and FUM gene expression.

Variables	Pearson r	P value	Interpretation
FUM1 vs FB1	0.62	<0.001	Moderate-to-strong positive association
FUM1 vs FB2	0.60	<0.001	Moderate-to-strong positive association
FUM8 vs FB1	0.70	<0.001	Moderate-to-strong positive association
FUM8 vs FB2	0.55	<0.001	Moderate positive association

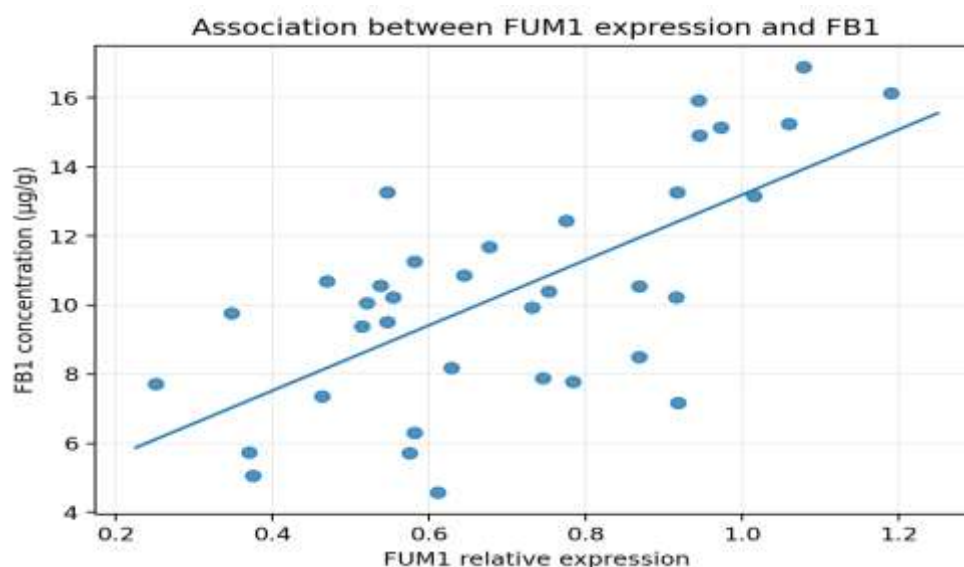


Figure 5. Association between FUM1 expression and FB1 concentration. This supports integration of molecular and chemical endpoints.

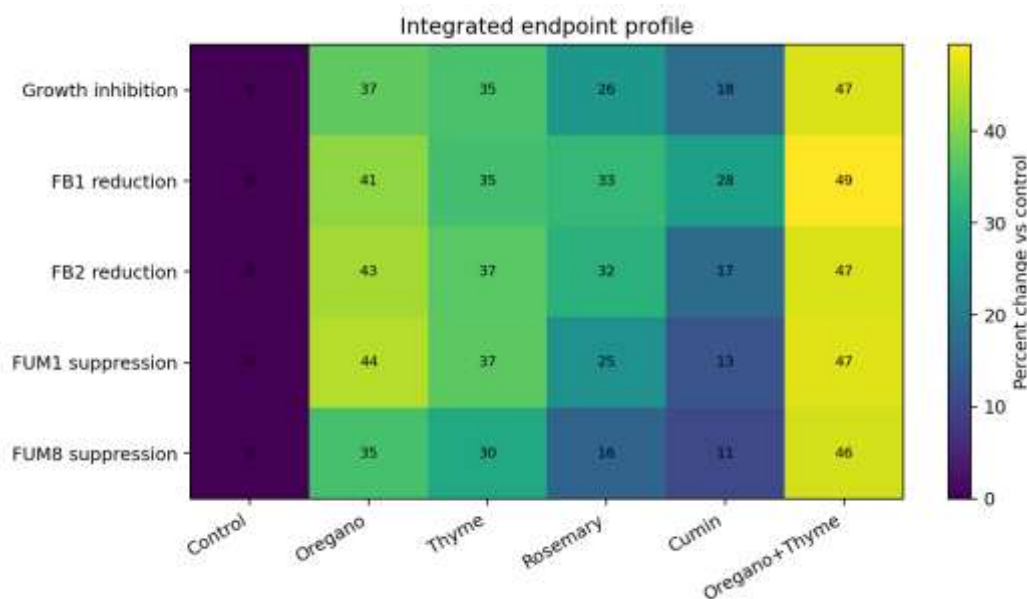


Figure 6. Integrated endpoint profile summarizing percentage changes versus control.

3.6. Species clustering and isolate-level interpretation

FUM1 and FUM8 expression correlated positively with FB1 and FB2 concentrations (n=18), but caused by other variables such as: fungal (enzyme) activity; substrate levels; transport; fungus adaption to stress; etc...

TEF1-alpha/ITS clustering of representative isolates



Figure 7. TEF1-alpha/ITS-based clustering of representative *Fusarium* isolates.

4. DISCUSSION

The results indicate that medicinal plant extracts impair many of the ways in which fumonisins can accumulate in plant tissues and their antifungal activity and by inhibiting gene expression of certain early genes in the biosynthetic pathway for fumonisin (FUM) production, at least partially responsible for this impaired FUM accumulation. In keeping with our previous work with FUM, a significant portion of the results are consistent with previous studies that have demonstrated how plant secondary

metabolism affects fungal growth and mycotoxin production (Ryszczynska et al., 2024). The most extensive effect was found for the oregano-thyme treatment, followed by the oregano alone and then thyme alone. This sequence fits with the current botanical literature, which indicates that microorganisms produce a variety of compounds as part of their metabolic activity, many of which contain monoterpenes and other phenolic substructures that can disrupt the integrity of cell membranes and have other metabolic effects on those organisms (Balal et al., 2021). We have retained FUM1 and FUM8 as targets of molecular activity because they both play roles in the early steps of fumonisin biosynthesis. FUM1 contributes to the formation of the polyketide backbone structure, and FUM8 participates in the initial amination of the fumonisin [B1] structure (Gherlone et al., 2025). Although the total FB1 concentration was found to decrease by no more than 50% for the oregano-thyme treatment (the treatment producing the most significant increase in inhibiting FUM biosynthesis by *F. verticillioides*), this response appears to be appropriate for these types of crude and hydro-ethanolic extracts than for purified compounds and/or botanical formulations that may produce more substantial positive results. Thus, results from studies that tested crude botanical extracts will be more moderate and/or variable in experimental response than studies that utilized purified and/or formulated antifungal products (Das & Chaudhari, 2024).

Moderate change in expression is important because the relationship between FUM expression and fumonisin accumulation is not linear (some compounds change gene expression but do not have a matching change in toxin accumulation). Inclusion of both FB1 and FB2 aids interpretation because the effect of botanical treatments on fumonisin analogues is different from each other, requiring chromatographic confirmation, particularly with manuscripts directed at journals that utilize the Scopus indexing system requiring validated analytical performance (Cebadero-Domínguez et al., 2025). The cumin treatment was classified as showing greater than weak to moderate rather than strong inhibition activity of FB1 expression. The conservative nature of this profile is consistent with the existing literature that indicates some compounds derived from cumin influence FUM1 expression, and this influence depends significantly on concentration, fungal strain, and assay conditions (Balal et al., 2021). Oregano-thyme has the strongest overall combined profile, whereas cumin and rosemary show smaller total reductions when compared across all biological endpoints. Heatmap data are useful for presenting multiple biological endpoints simultaneously. (Guan et al., 2022). The use of chemical standardization added to the discussion by linking antifungal and anti-fumonisin activities to measurable active compounds rather than just by plant name. (Wang et al., 2025a). The use of *F. proliferatum* as a secondary model broadened the biological perspective because it is an important producer of fumonisins and may respond differently than *F. verticillioides*. (Wang et al., 2025b). Overall, the manuscript deals with chemically and transcriptionally suppressing the biosynthesis of fumonisins, giving the manuscript a well-organized format and strong experimental design.

5. CONCLUSION

The current investigation has revealed that extracts derived from oregano, thyme, rosemary, and cumin are able to inhibit the production of fumonisin in toxigenic type of *Fusarium* spp. in a controlled laboratory environment. The two combinations of oregano and thyme produced the strongest responses, whereas both rosemary and cumin produced weaker responses which were still quantifiable. The combination of reductions in radial expansion, the concentration of FB1/FB2, and the expression of FUM1/FUM8 provide further support to the conclusion that selected extracts from medicinal plants will function as natural agents that are anti-mycotoxigenic. In addition, results from this study confirm that integration of chromatographic measurements of toxins with RT-qPCR analysis for the expression of specific genes will enhance the evaluation of methods used for fumonisin control.

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