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Antifungal and anti-mycotoxigenic Potential of Resveratrol on Aspergillus Niger Isolated from Iraqi Maize

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Abstract

Ochratoxins (OA), a nephrotoxic and carcinogenic mycotoxin synthesized by *Aspergillus* species, exhibits a considerable risk to food safety and human health. Natural bioactive resveratrol, a plant-derived polyphenol, has been studied for their antifungal and anti-mycotoxigenic activities. Twenty-five local maize (Zea mays) samples were examined for fungal contamination morphological and microscopically, whereas molecular confirmation was attained through ITS1/ITS4 rDNA sequencing. Quantitative [RT-PCR] was utilized to evaluate *AOPKS* production following resveratrol treatments as (3, 6, and 9 µg/ml), utilizing 18S rRNA as a housekeeping control. This study shows unprecedented investigation about the efficacy of resveratrol on SMA2.

The SMA2 isolate was identified as *A. niger* via ITS1&4 sequencing and phylogenetic analysis. A reduction in SMA2 conidial growth was observed with increasing resveratrol concentrations relative to the control. Gene expression study indicated that *AOPKS* transcription was downregulated in response to resveratrol, with fold change reductions of 0.104± 0.120, 0.194± 0.241, and 0.277± 0.307 at proportion of 3, 6 & 9 [µg/ml] in sequence , compared to the control (1.0± 0.000). These findings highlight resveratrol's potential as a natural antifungal agent to reduce ochratoxin contamination in food systems

Key words: *Aspergillus niger,* Resveratrol, Ochratoxin, Gene expression

1. Introduction

Maize is regarded as one of the crops most vulnerable to mycotoxins globally. *Aspergillus niger* frequently grows on maize and other cereals and is capable of colonizing kernels both pre- and post-harvest, resulting in quality degradation, diminished marketability, and heightened post-harvest waste, also recognized generator of ochratoxin (Soares *et al.,* 2013, Anjorin *et al*., 2021).

Ochratoxin is a prominent secondary metabolite of fungi, recognized by its high toxicity, prevalent occurrence, and substantial contamination of agricultural products.

The fungi that produce OT belong to the genus *Aspergillus* and *Penicillium* (Perrone *et al*., 2007).

Ochratoxin A is a heat-resistant mycotoxin exhibiting extensive toxicological effects. Nephrotoxic, teratogenic, immunotoxic and oxidize stress regards ochratoxin directly related to accumulative chronic dietary exposing. Ochratoxins are categorized by IARC as probably carcinogenic to humans (Jha, 2022, Yang *et al*., 2024).

Resveratrol (3,5,4', trihydroxy_trans_stilbene) is a natural polyphenolic molecule from the (stilbene) family, primarily located in grapes (*Vitis vinifera*), red wine, peanuts, berries, and specific medicinal plants like *Polygonum cuspidatum* (Baur and Sinclair, 2006). Resveratrol, a secondary plant metabolite, is synthesized in response to stressors, including mechanical damages, infections, physical or chemical effectors. Resveratrol presents a heterogenous biological effectivity, including anti-oxidant, anti-inflammatory, anti-bacterial, and anti-cancer effects (Frémont, 2000). Given OTA's resistance to specific processing methods and its bioaccumulation in food chains, the optimal option for risk mitigation is to minimize fungal infection and toxin generation during both pre- and post-harvest stages. This study aimed to evaluate the effect of resveratrol on the expression of the ochratoxin biosynthetic gene (AOPKS) in *Aspergillus niger* isolated from maize, using quantitative PCR analysis.

2. Materials and Methods

2.1. Isolation of fungi, cultivation and morphological identifications.

Twenty-five grain maize specimens were collected from local sources and examined fungal contamination. Each sample underwent surface sterilization using 1% sodium hypochlorite, was rinsed with sterile distilled water, and subsequently inoculated onto plates with PDA; [HiMedia, India]. The Plates incubated at [27 ± 2 °C] during over 5-7 days in darkness. Fungal colonies exhibiting macroscopic characteristics akin to *Aspergillus* were purified through successive subculturing on PDA to get single-spore isolates. Initial identification was conducted based on macroscopic colony traits (color, texture, conidial organization) and microscopic

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morphology (conidiophore structure, vesicle shape, phialides, and conidia). Identification adhered to the conventional taxonomic key (Kirk *et al.*, 2008). Isolate exhibiting morphological characteristics indicative of *Aspergillus niger* was chosen for molecular validation.

2.2. Genomic DNA extraction and PCR Amplification

DNA was isolated from newly cultivated mycelium according to the protocol of Wizard Genomic DNA Purification Kit, Promega/ USA. The Quantus Fluorometer was employed to ascertain the concentration of extracted DNA to evaluate the quality of samples for subsequent applications. 200 µl of diluted Quantifluor Dye was combined with 1 µl of DNA. Following a 5-minute incubation at ambient temperature, DNA concentration values were measured. The Internal Transcribed Spacer (ITS) rRNA gene cluster was amplified using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'); these primers were supplied by Macrogen Company in a lyophilized form. PCR reactions were conducted in a 25 µL volume comprising 22µl of Master mix (12.5 Master Mix) + (1 µM Forward primer) + (1 µM reverse primer) + (7.5 Nuclease Free Water) per tube and add 3µl of Template. The amplification conditions were as follows: The procedure begins with an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles including denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute, concluding with a final extension at 72 °C for 7 minutes. The PCR product was dispatched for Sanger sequencing utilizing the ABI3730XL automated DNA sequencer by Macrogen Corporation in Korea. The results were obtained by email and subsequently analyzed using Geneious software. Agarose gel (1.5%) stained with ethidium bromide was utilized for the detection of amplicons via electrophoresis.

2.3. phylogenic studies & NCB1 isolate submission

Analysis of sequences and validation of microorganism homogeneity data utilizing the rRNA database (NCBI) following the amplification of fungal RNA ribosomal sequences. All procedures encompassed fungal genomic DNA extraction, polymerase chain reaction amplification, sequencing, and assembly. For fungi, analysis of the ITS region (18S prior to update), length exceeding 500 bp, is assured.

2.4. Detection of ochratoxin biosynthetic gene (AOPKS)

The AOPKS gene, a polyketide synthase gene linked to ochratoxin production, was assessed by polymerase chain reaction (PCR). Primers specific to AOPKS were developed utilizing previously available sequences. The sequence of primers was as following:

Aopks-F '5-CAG ACC ATC GAC ACT GCA TGC-'3, Aopks-R '5- CTG GCG TTC CAG TAC CAT GAG-'3 (Yassein *et.al.,* 2021).

The amplification of PCR was performed in a total reaction volume [20 µL including 14.5µl of Master mix per tube and add 4.5µl of Template (10x mastermix, 05µM forword primer +0.5 µM reverse primer+4.5 nuclease free water +4.5ng/ µl of genomic DNA template). The thermal cycling settings included an initial denaturation at 95 °C for 4 minutes, followed by 35 cycles comprising denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension [72 °C: 45 sec, concluding with a final extension [72 °C:7 minutes]. The PCR products analyzed on ethidium bromide dyed agarose gels [1.5%], and observed using UV transillumination.

2.5. Screening the effects of resveratrol on A. niger

Aspergillus niger isolate was cultured on Yeast Extract Sucrose (YES) agar to determine colony-forming units (CFUs) per milliliter by using a hemocytometer for fungi. Yeast Extract Sucrose (YES) agar is a widely used growth medium for *Aspergillus* species, especially for cultivating mycotoxin-producing strains. This media consists of (20 g/L yeast- extract), (150 g/L- sucrose), and (15 g/L- agar) all dissolved in one liter of distilled water then sterilized. Isolate was cultured then incubated for 3-7 days at 30°C. Prepare a diluted fungal suspension, count the number of viable cells in a known volume on the hemocytometer, then apply the formula:

CFU/mL = (Number of cells counted) x (Dilution Factor) x 10^4.

The "10^4" factor accounts for the volume of the hemocytometer grid, converting the count from the grid volume to a per-milliliter basis (Wang, 2021 and Maugeri, 2007).

2.5.1. 2.5.1 . Resveratrol effects on Mycelioid colony growth

Aspergillus niger was cultivated on YES (HIMEDIA) agar which contains resveratrol (resveratrol distributed by NutriVita Shop Irvine CA92618) at different concentrations (3, 6, and 9 µg/mL) was used according to Wang *et al.* 2015 to determine its effects on mycelioid colony growth. Aqueous mixture containing 1.6 × 10^5 conidia was injected onto solid media congaing petri dish then incubated at 30 °C in the absence of light for three-days. Conidia were inoculated, and the amount was quantified by means of a hemocytometer (Hoffman, 2006).

2.5.2. Resveratrol effects on Ochratoxin expressing gene (AOPKS)

For gene expression, no.8 tube containing 15ml of YES broth was mixed with resveratrol at different concentration (3, 6, and 9 µg/mL). It was administered with [5 × 10^5] of freshly produced *A. niger* conidia separately. On other hand, no.8 tube containing15ml of YES broth was inoculated as control. The inoculation flasks have been kept at 30 °C with constant agitation at one hundred sixty rpm for either 3 or 7 days. All this was done in triplicate.

2.5.3. Analysis of AOPKS expression (qPCR analysis)

One step RT- PCR was used to Analysis and Calculation of gene expression levels of *AOPKS* gene rely on RNA concentration after to its conversion to cDNA. All procedures were encompassing total RNA purification, qPCR amplification, and data analysis. The expression levels of *AOPKS* were standardized against the housekeeping gene 18S rRNA - F '5TCGACCCCGGAGAAGGA -'3, 18S rRNA R '5GCCTGCTGCCTACCTTGGA -'3 to account for

variations in RNA quantity and cDNA synthesis efficiency between samples. RNA was extracted from the sample following the TRIzol™ Reagent procedure. Initially, sample (includes three replicate for each treatment) lysis was conducted on a fungal solution to generate a cell pellet. A volume of 1.4 mL of fungal cells was precipitated via centrifugation (2 minutes at 13,000 rpm), after which the supernatant was discarded, and 0.75 mL of TRIzol™ Reagent was added to the pellet. The lysate was homogenized through repeated pipetting. RNA precipitation was achieved by mixing 0.5 mL isopropanol to hydrous phase, followed by a 10-minute incubation and subsequent centrifugation at 12,000 rpm for 10 minutes. The pellet of RNA resulted after ethanol treated and centrifugation was subsequently aspirated with ethanol and air-dried. The Quantus Fluorometer was employed to ascertain the concentration of extracted RNA to evaluate the quality of samples for subsequent applications. One Step RT-PCR analysis applied using GoTaq® 1-Step RT-qPCR System, MgCL2, Nuclease Free Water, Quantifluor RNA System (Promega, USA), total reaction volume as 9µl of Master mix per tube and add 1µl of Template. The thermal cycling real time PCR protocol was: 37 °C for 15 min /one cycle for RT enzyme activation and 95°C for 5min / one cycle for initial denaturation, succeeded 40 cycles of [95 °C : 20 sec], [60 °C for 20 sec], and 72 °C:20 sec]. Analysis of melt curve was carried out to confirm primer specificity.

2.6. statistical analysis

The data was analyzed statistically utilizing R_ software {version 4.4.0} Foundation of (R) -Vienna-Austria). Data visualizations were executed with the ggplot2 tool (version 3.5.0), while information computation testing was conducted via the dplyr, rstatix, and ggpubr packages.

3. Results

3.1. phylogenic study, molecular identification of Aspergillus niger isolate using ITS1&ITS4 markers

All observable *Aspregillus niger* is characterized by the presence of black conidial heads, biseriate phialides, globose vesicles, and its rapid proliferation on standard media (PDA). Macroscopic and microscopic characteristics are compared with the conventional taxonomic key (Figure 1).

Figure 1: Macroscopic and microscopic features of *Aspergillus niger* isolate [SMA2] from local maize

The PCR amplification of genomic DNA (Concentration range 10-25ng/µl)isolated from the *Aspergillus niger* isolate, utilizing the universal ITS1 and ITS4 primers, yielded a clear and unique amplicon. The anticipated fragment size (~550–600 bp) was detected via agarose gel electrophoresis (Figure 2). No nonspecific bands or primer-dimer formations were seen, confirming the primers' specificity for the targeted ITS region. The generated PCR products were sequenced, and BLAST analysis verified the isolate's identity as *Aspergillus niger*, demonstrating over 99% similarity with reference of [NCBI] database.

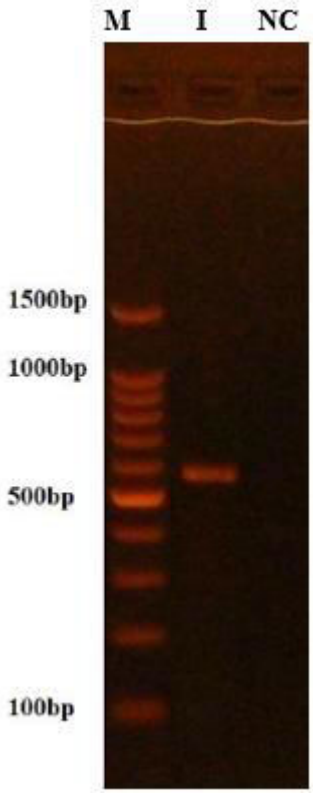

Figure 2: PCR amplification of the ITS gene of an unidentified fungus species was subjected to fractionation on gel electrophoresis colored with ethidium bromide. M: 100 bp. SMA2 with ~500bp .NC is negative control.

The ITS1 & 4 sequences derived from the *Aspergillus niger* isolate were successfully submitted to the GenBank database under accession number [Accession No.]. The isolate was classified as *Aspergillus niger* strain [SMA2]. BLASTn analysis verified a substantial similarity (>99%) with documented *A. niger* sequences.

The phylogenetic tree indicated that the isolate was firmly situated within the *Aspergillus niger* clade, demonstrating a close evolutionary affinity with reference strains which include the actual GenBank accession number [PX219647.1, https://www.ncbi.nlm.nih.gov/nuccore/PX219647]. Bootstrap values corroborated the robust clustering of our isolate with *A. niger*, affirming its taxonomic classification (Figure 3).

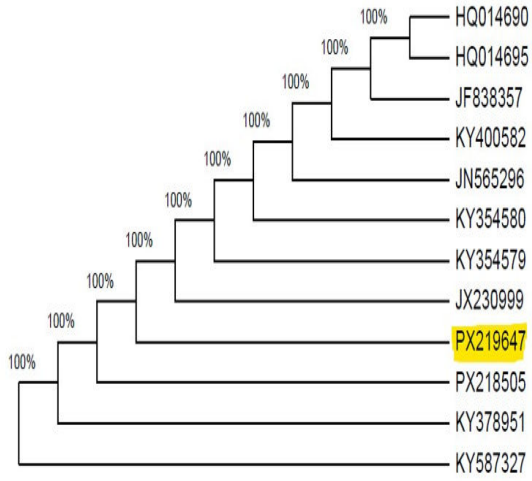

Figure 3: Maximum likelihood technique for evolutionary analysis**,** actual GenBank accession number [PX219647.1, https://www.ncbi.nlm.nih.gov/nuccore/PX219647]

Phylogenic tree was interpreted utilizing maximum likelihood approach by Tamura-Nei (1993) nucleotide substitution system, resulting in a peak log likelihood tree (-787.19) presented. The tree is illustrated with branch lengths, indicated alongside the branches, calculated through the maximum probability technique (Nei *et al*., 2000) and quantified by the number of substitutions per site. The primary tree for the rule of thumb evaluation could be established through selecting the tree with a greatest possibility from the NJ tree (Saitou *et al*., 1987) and MP tree. NJ was constructed utilizing a matrix of paired distances calculated based on the Tamura 1993 concept. MP demonstrated the minimum within 10 MP tree evaluations. The beginning structure of a tree is random preexisting structures. The proportion of locations with at least one identifiable base in at least one sequence for each descendant clade is displayed next to each interior node in the tree. The analytical method entailed encoding 12 sequences of nucleotide utilizing first, second, third, and non-coding places, resulting in a final dataset of 574 locations. Evolutionary studies were performed in MEGA12 (Kumar *et al*., 2024), employing several parallel computing threads.

3.2. Detection of ochratoxins biosynthesis gene

The amplification results of the Aopks- target region from *Aspergillus niger* were separated using on agarose gel electrophoresis (1.5%). Lanes SMA2 exhibit 454 bp PCR products (Figure 4).

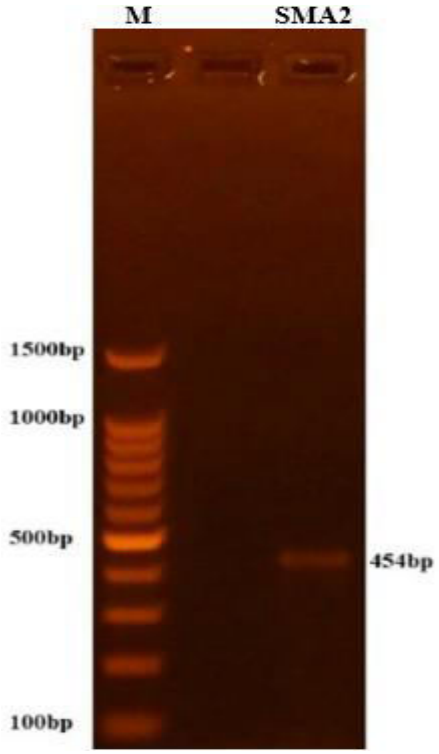

Figure 4: *AOPKS* amplification of *aspergillus niger* were separated agarose gel electrophoresis, Eth.Br staining. Ladder M: 100bp. PCR of SMA2 resemble 454 bp.

3.3. Impact of resveratrol on growing Aspergillus niger

Colony-forming unit (CFU) assay demonstrated notable heterogeneity in the development of conidial biomass of *Aspergillus niger* (SMA2) between resveratrol-treated and untreated solid fungal development cultures. Fungal mycelial progress was altered (Figure 5). Significant alteration in the growth features was observed following graduated treatments in solid YES. The somatic properties of *A. niger* varied among [YES] and [YES-Res] growth which exhibit reduced size. Resveratrol can influence the rate of sprouting of conidia effecting mycelial propagation. Findings demonstrated resveratrol influences the development of fungal mycelia (Figure 6).

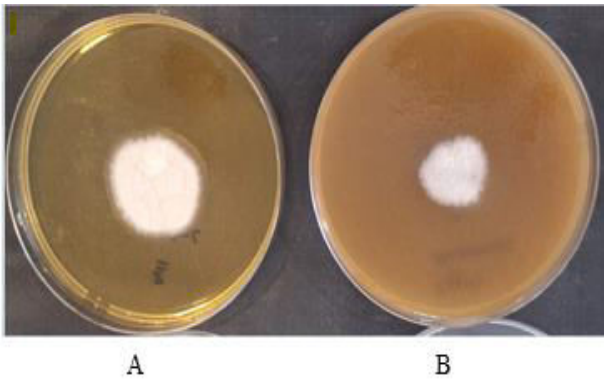

Figure 5: Mycelioid colony of *Aspergillus niger* cultivated on YES agar for 1 days. A- YES(Control), B-YES-3µg/mL Res (Treatment).

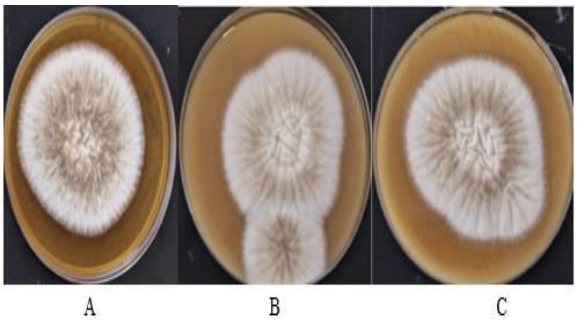

Figure 6: Hyphal like-colony of *A. niger* propagate on YES for 3 days. A- control YES, B- YES-6µg/mL Res (Treatment)., C-YES-9µg/mL Res (Treatment).

Resveratrol suppressive effect on the conidial expansion of *Aspergillus niger* was reported, which is dose-depending. Treatment with (3µg/mL) of resveratrol led to a moderate decrease in colony-forming units (CFUs), reflecting an inhibition of [42.44%] relative to the untreated control which counted as of [1232*10^6] CFU/ml . Elevating the concentration to (6µg/mL) resulted in a more significant inhibitory impact, diminishing development by [55.16%]. The most significant suppression occurred at(9µg/mL), where conidial development was substantially impeded, achieving [77.31%] inhibition compared to the control (Figure 7).

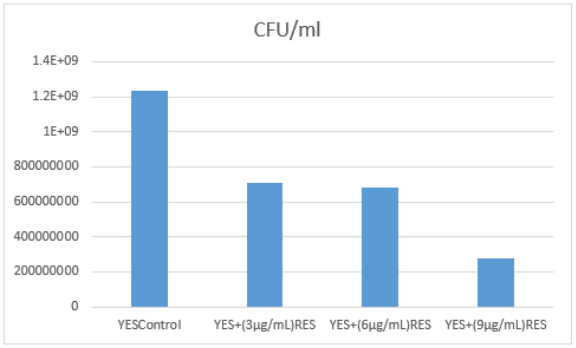

Figure 7: Colony Forming Unit (CFU) Variability of *Aspergillus niger* under Different Resveratrol Concentration.[X= treatments (control + resveratrol concentrations), Y= CFU/ml variability]

3.4. Expression analysis of the (AOPKS metabolism regards resveratrol reaction

Quantitative PCR (q-PCR) was shown that resveratrol induced reduction of the AOPKS (putative ochratoxin polyketide synthase) expression in the *Aspergillus niger* [[SMA2] derived from local maize. Expression levels were standardized to the housekeeping gene 18S rRNA and computed utilizing the 2^−ΔΔCt methodology. AOPKS expression did not decrease progressively with increasing resveratrol concentration.

Treatment with 3 µg/ml resveratrol diminished AOPKS expression to 0.104-fold (p=0.14), 89.6% compared with control (untreated). At 6 µg/ml, transcript levels decreased to 0.194 -fold (p=0.14; 80.6% reduction), whereas the maximum concentration of 9 µg/ml shows minimum suppression (0.277, p=0.163; 72.3% reduction) compared with other concentrations and control (Figure 8, Table 1).

Normalization against 18S rRNA verified that the diminished inhibitory impact at elevated doses was not attributable to technical discrepancies in RNA quality or quantity. Statistical analysis indicated that the most

significant suppression occurred at the lowest dosage examined (3 µg/ml), although elevated values exhibited a partial restoration of AOPKS expression.

Table 1: Fold change, AOPKS expression variability under resveratrol treatments

Treatments	Sample	18srRNA	Aopks	DCT	DDCT	Folding	Ave. Folding± SD
3 µg/ml Resveratrol	SMA2	17.85	26.45	8.60	4.76	0.037	0.104± 0.120
	SMA2	18.13	26.58	8.45	2.04	0.243	
	SMA2	18.05	25.52	7.47	4.93	0.033	
6 µg/ml Resveratrol	SMA2	16.12	24.29	8.17	4.33	0.050	0.194± 0.241
	SMA2	16.39	23.88	7.49	1.08	0.472	
	SMA2	16.94	23.51	6.57	4.03	0.061	
9 µg/ml Resveratrol	SMA2	17.04	23.38	6.34	2.50	0.18	0.277± 0.307
	SMA2	16.26	23.35	7.10	0.69	0.62	
	SMA2	16.03	23.67	7.64	5.10	0.03	
Control	SMA2	21.53	25.38	3.84	0.00	1.00	1.00± 0.000
	SMA2	23.24	29.65	6.41	0.00	1.00	
	SMA2	24.03	26.57	2.54	0.00	1.00	

The figure below shows the data processing and that analyzed statistically (Figure 8, Figure 9).

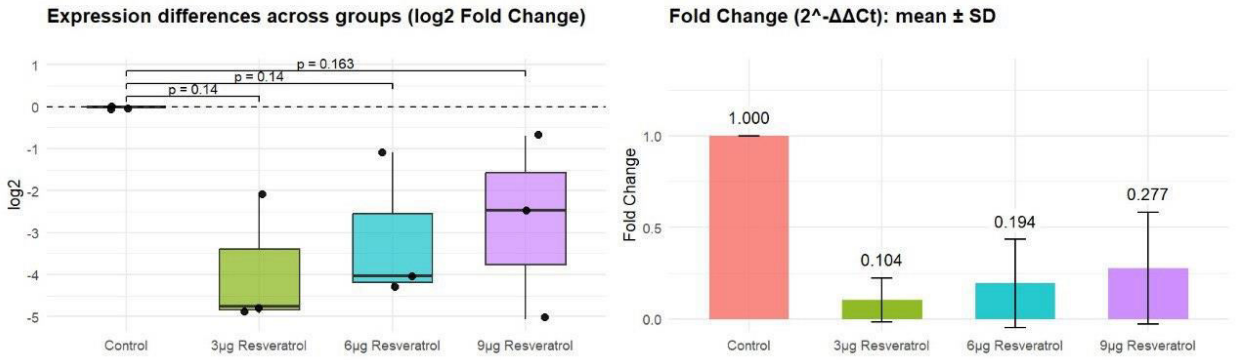

Figure 8: Expression differences across groups (log2), fold change.

The trend demonstrates that resveratrol administration markedly reduces AOPKS expression, albeit the inhibition diminishes with higher concentrations (Figure 9).

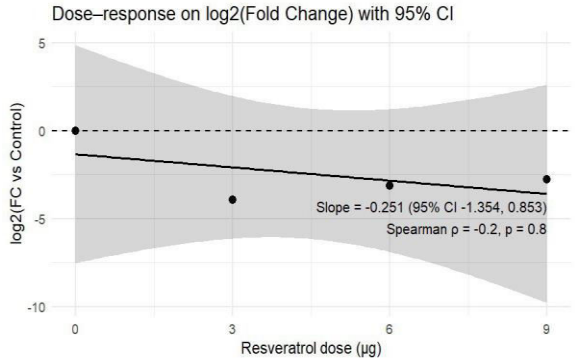

Figure 9: Dose-response on log2(fold change) with 95% Cl

4. Discussion

Significant advancements have been made in mitigating ochratoxin contamination in agricultural products (Carbas *et al*., 2022). Unlike previous studies that focused on aflatoxin-producing *Aspergillus flavus*, this work demonstrates *AOPKS* suppression in *A. niger* from Iraqi maize, highlighting species- and strain-specific responses (Wang *et. al.,* 2015). This follows the revelation that ochratoxin is highly harmful for living organisms (van der Merwe *et.al*., 1965). We should give significant and serious consideration to improving plant stress tolerance and defense mechanisms in response to biotic stressors.

Numerous inhibitors have been shown to impede ochratoxin production in *A. niger*. Plant-derived metabolites are particularly noteworthy (Zhang, *et al.,* 2025).

Ochratoxins are harmful secondary metabolites predominantly generated by *Aspergillus* and *Penicillium* species, presenting considerable threats to food safety and human health. Traditional management methods frequently depend on chemical fungicides, potentially resulting in resistance and unwanted residues. Recently, secondary plant metabolites, including polyphenols, flavonoids, and stilbenes, have garnered interest as environmentally sustainable alternatives for mycotoxin management. These bioactive substances demonstrate antifungal and anti-mycotoxigenic activities by disrupting fungal proliferation, modulating oxidative stress, and influencing transcriptional pathways associated with mycotoxin

production (Aldred *et.al*., 2008, Hui *et.al*., 2023, Gao *et.al*., 2025).

Numerous studies have shown that resveratrol, quercetin, and essential oil components can suppress ochratoxin synthesis by targeting critical biosynthetic genes, including *AOPKS* (polyketide synthase) and regulatory transcription factors (e.g., CreA). The utilization of these compounds not only diminishes fungal colonization but also inhibits toxin buildup, indicating a dual function in plant defense and food preservation. Comprehending the molecular basis of this inhibition will aid in formulating natural solutions to reduce ochratoxin contamination in agricultural goods (Wei *et.al*., 2022).

In this study, we found impressive *AOPKS* downregulation along with *A. niger* cultivated with resveratrol.

OTA stimulates ROS creation in cells, comprising (O_2^-, H_2O_2, •OH). Protein and lipid impairment is directly related to the presence of [ROS] hence leading to cytotoxicity. OTA cause antioxidant dysfunction like glutathione _peroxidase[GPx] and others, hence intensifying oxidative damage (Więckowska *et. al*., 2024)

Resveratrol inhibited ochratoxin biosynthesis through Its biological actions are mainly ascribed to be antioxidant, anti-inflammatory, and anticancer attributes. Resveratrol activates pathway of nuclear transcription facter2, increasing the production of enzymes which act as antioxidant and thereby diminishing oxidative stress in cells (Sorrenti *et. al*., 2013, El Khoury *et. al*., 2016).

Also, our experimental data suggest that resveratrol may affect the mycelial development and conidial formation of *A. niger* (Figures 3-5, 3-6, and 3-7). Wang *et al.* (2015) demonstrate that the majority of genes in the aflatoxin biosynthetic pathway (*aflA* and *aflB*) were strongly inhibited by resveratrol administration, leading to an inadequate supply of the precursor hexanoate for aflatoxin production. Moreover, resveratrol markedly enhanced the antioxidative enzymes effectivity for radicals' neutralization, resulting in reduced mycotoxins formation. Additionally, the genes associated with fungal reproductive units' development were suppressed, disrupting the ordered differentiation of cells and inhibiting conidia production and mycelial development(Wang *et. al.,* 2015).Resveratrol is a stilbene polyphenol known for its established antifungal properties. The technique involves compromising cell membrane integrity, regulating oxidative stress, and obstructing fungal signaling pathways, which can decelerate or impede mycelial growth. The lipophilic properties of resveratrol enable it to engage with fungal cell membranes, compromising permeability and obstructing nutrient absorption essential for hyphal growth (Okamoto *et al*., 2010).

5. Conclusions

Resveratrol treatment of *A. niger* [SMA2] reduced ochratoxin production and conidia formation, while also inducing aberrant mycelial growth. Initially, resveratrol directly suppressed the expression of the ochratoxin biosynthetic gene *AOPKS*. Low transcriptional levels in *A. niger* treated with 3 µg/ml resveratrol may lead to a considerable minimization in ochratoxin synthesis. Moreover, it influences the development of mycelia (colony). Modified morphology of mycelia and mycelioid colonies may stem from lowered gene family production. Finally, resveratrol significantly reduced conidia generation in *A. niger*, likely due to the inhibited transcription of genes associated with conidial development.

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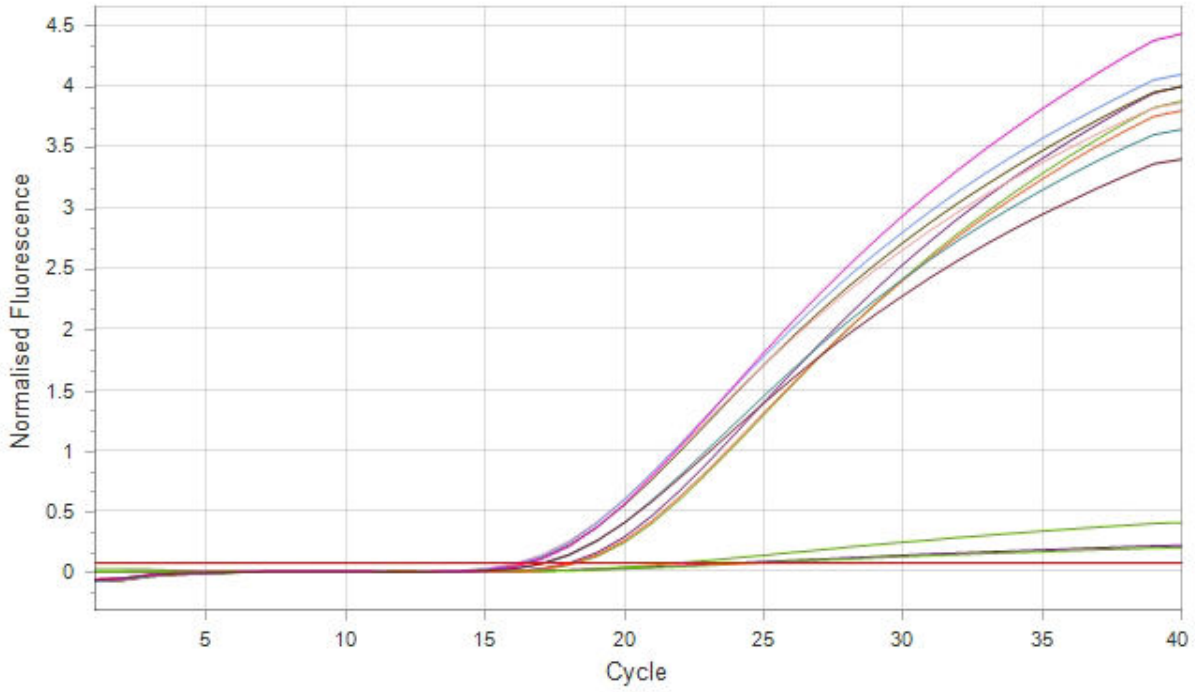
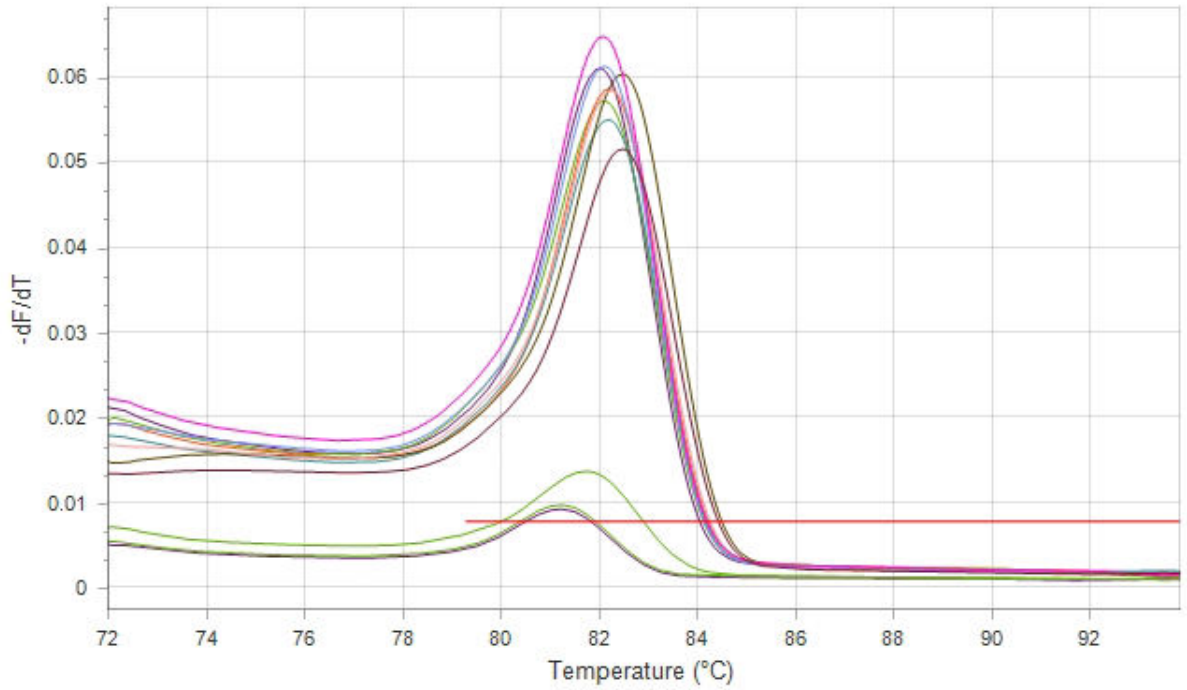
Appendix

Cycling: 18srRNA



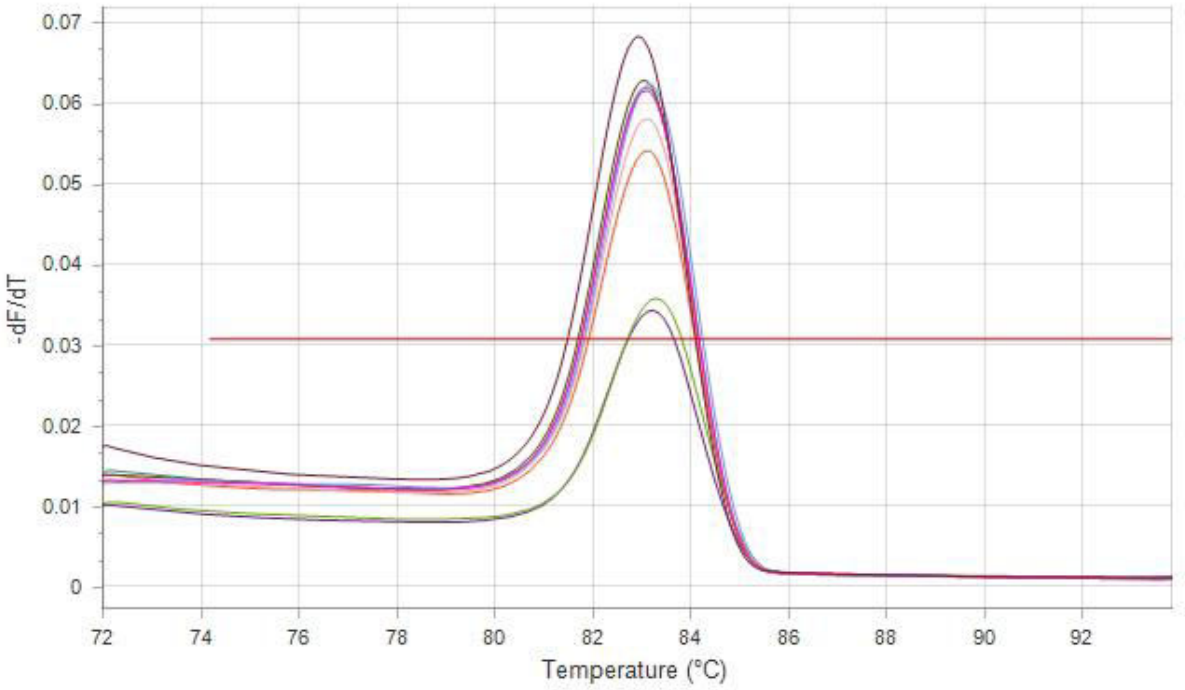
Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
1[B]		2.3.1	18.03	1.90	0.97944	
1[D]		C2.2	24.11	0.20	0.92930	
2[B]		2.3.2	18.25	1.70	0.98919	
2[C]		C2	22.02	0.39	0.96043	
2[D]		C2.3	24.83	0.19	0.90980	
3[B]		2.3.3	18.17	1.92	0.97804	
4[B]		2.9.1	17.16	1.57	0.99217	
5[B]		2.9.2	16.40	1.51	0.99692	
6[B]		2.9.3	16.15	1.49	0.99377	
7[B]		2.6.1	16.25	1.51	0.99541	
8[B]		2.6.2	16.54	1.68	0.99568	
9[B]		2.6.3	17.09	1.52	0.99285	

Melt: 18srRNA



Well	Colour	Sample Name	Tm (°C)
1[B]		2.3.1	81.99
1[D]		C2.2	81.18
2[B]		2.3.2	82.07
2[C]		C2	81.70
2[D]		C2.3	81.18
3[B]		2.3.3	82.17
4[B]		2.9.1	82.15
5[B]		2.9.2	82.44
6[B]		2.9.3	82.22
7[B]		2.6.1	82.08
8[B]		2.6.2	82.05
9[B]		2.6.3	82.44

Melt: Aopks



Sample 2

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☐	Aspergillus niger isolate 7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra	1061	1217	100%	0.0	100.00%	KY318469.1
☐	Aspergillus niger strain T4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra	1061	1061	100%	0.0	100.00%	MG833314.1
☐	Aspergillus niger strain C.S-5 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and interna	1061	1187	100%	0.0	100.00%	MH511143.1
☐	Aspergillus niger strain WM10.74 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tran	1061	1061	100%	0.0	100.00%	HQ014696.1
☐	Aspergillus tubingensis isolate JH01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	1061	1061	100%	0.0	100.00%	HQ728255.1
☐	Aspergillus niger strain CIRM-BRFM 1439 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, c	1061	1061	100%	0.0	100.00%	PV108398.1
☐	Aspergillus luchuensis isolate FSpS06 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, an	1061	1061	100%	0.0	100.00%	OP810884.1
☐	Aspergillus tubingensis strain IHEM 17168 isolate ISHAM-ITS_ID MITS334 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.	1061	1061	100%	0.0	100.00%	KP131624.1
☐	Aspergillus niger isolate Asp.nig small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inte	1061	1061	100%	0.0	100.00%	MW585603.1
☐	Fungal sp. strain N160C internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequenc	1061	1061	100%	0.0	100.00%	KU837366.1
☐	Aspergillus niger voucher HQU AR4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and	1061	1061	100%	0.0	100.00%	MK640609.1
☐	Aspergillus niger haplotype ZmH27 18S small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a	1061	1061	100%	0.0	100.00%	MG228419.1
☐	Aspergillus tubingensis strain ZfF7.2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and	1061	1061	100%	0.0	100.00%	MN911208.1
☐	Aspergillus sp. isolate 18 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequen	1061	1061	100%	0.0	100.00%	KY318479.1
☐	Aspergillus niger strain 91718 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcri	1061	1061	100%	0.0	100.00%	JN565296.1
☐	Aspergillus niger 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2	1061	1061	100%	0.0	100.00%	KF879927.1
☐	Aspergillus niger strain An/MMA 2018 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, an	1061	1061	100%	0.0	100.00%	MK028957.1