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ORIGINAL ARTICLE

Investigating the Inhibitory Effect of Turmeric Extract in Reducing Aflatoxin Production by *Aspergillus flavus* and *Aspergillus parasiticus*

Shokoufeh Sharokhi Rezaei[1], Leila Nouri [*1], Mansooreh Mazaheri [*2], Laleh Adlnasab[3]

[1]*Department of Food Science and Technology, Damghan Branch, Islamic Azad University, Damghan, Iran*

[2] *Department of Food Toxicology, Research Center of Food Technology and Agricultural Products, Standard Research Institute, Karaj, Iran, P.O. Box 31585-163*

[3]*Department of Chemistry and Petrochemistry, Research Center of Chemistry, Standard Research Institute, Karaj, Iran, P.O. Box 31585-163*

K E Y W O R D S

Aflatoxins;
Aspergillus flavus;
Aspergillus parasiticus,
Mycotoxins;
Pistachio;
Turmeric extract

A B S T R A C T

The consumption of pistachios (*Pistacia vera* L.) has been increasing due to their significant contribution to human health. Apart from being a valuable nutritional resource, they have been linked to potential chemical risks, including mycotoxins, which can lead to fungal contamination and secondary metabolism. In this study, after optimizing the extraction of turmeric extract based on the amount of total phenol and flavonoid compounds, treatments were applied using different concentrations of turmeric extract to assess its impact on the growth of *Aspergillus flavus* and *Aspergillus parasiticus*, and aflatoxin production. The treatments included various concentrations of turmeric extract and were implemented in a completely randomized design with three replications for each treatment. Results showed that the ultrasonic extraction method at 50 ºC for 60 minutes yielded the highest efficiency of turmeric extract. Specifically, the application of 300 µL turmeric extract significantly inhibited the mycelial growth of *Aspergillus flavus*, while 350 µL extract was more effective in inhibiting the growth of *Aspergillus parasiticus*. Furthermore, incubating both fungi with turmeric extract at 25°C for 3 days demonstrated a notable inhibitory effect on aflatoxin production. Additionally, the addition of turmeric extract to pistachios contaminated with both fungi effectively inhibited aflatoxin production. Therefore, turmeric extract, particularly at final concentrations of 1.77 mg/mL and 2.07 mg/mL, demonstrated significant potential as a natural antifungal agent for preventing aflatoxin production in pistachios.

Introduction

Aspergillus is a ubiquitous fungus capable of contaminating crops such as cereals, peanuts, oilseeds, spices, dried fruits, and tree nuts with aflatoxins (AFS). These toxins not only reduce crop quality but also pose severe health risks upon ingestion (Mahbobinejhad *et al*., 2019). Among AFS, Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2 (AFG2) are very important. *Aspergillus flavus* produces AFB1 and AFB2, while *Aspergillus parasiticus* synthesizes

[*]**Corresponding author**: Email address: nouri.le.ir@gmail.com, m-mazaheri@standard.ac.ir
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AFB1, AFB2, AFG1, and AFG2 (Ye *et al*., 2023). AFB1 has human mutagenic and carcinogenic effects between other aflatoxins (Nazhand, *et al*., 2020). They are produced in agricultural products before or after harvesting, during transportation, and storage (Massomo, 2020). Consuming food containing AFS is one of the causes of liver cancer in humans. To protect consumer health, maximum limits for AFT (AFB1+AFB2+AFG1+AFG2) and AFB1 have been set for different foods in many countries. According to the National Iranian standard, these limits are 8 $\mu g kg^{-1}$ for AFB1 and 10 $\mu g\ kg^{-1}$ for AFT in pistachio (INSO 5925, 2020)].

Regarding their carcinogenic effects, several studies have been conducted to find methods to reduce AFS bioavailability or detoxification in contaminated food and feed.

Some physical, chemical, and biological methods have been applied to decrease or detoxification of aflatoxin contamination of food and feed. The findings indicated that physical methods are not always practical to implement. (Khan *et al*., 2021). For example, binders can form complexes with toxins, thereby mainly decreasing their enteric absorption (Colovi´c, *et al*., 2019), but there are potential side effects of using the binders due to their capacity to adsorb nutrients such as vitamins and amino acids (Kihal *et al*., 2020). The application of fungicide as a chemical approach can lead to contamination of the environment and presents various health risks (Khan *et al*., 2021). Also, they are not practical for use after harvest. Therefore, it's necessary to create more environmentally friendly techniques for managing Aspergillus species contamination without the risks associated with chemical fungicides application (Khan *et al*., 2021).

The role of plant products as an antifungal has been well-known since antiquity. Using natural compounds with the potential to mitigate AFS-mediated adverse effects through different mechanisms can be an alternative method. It can complement the use of the existent ones or in the best of the cases that could represent an effective manner to inhibit AFS production. These active compounds can be contained either in aqueous or organic extracts or in essential oils (Habibi *et al*., 2022). The anti-fungal or anti-aflatoxigenic properties they possess may be attributed to one or multiple molecules, such as curcumin, thymol, eugenol, and carvacrol, which have shown effectiveness in inhibiting fungal growth and aflatoxin production.

Essential oils of some plants have shown antifungal activities against Aspergillus species by inhibiting mycelium growth, spore germination, and aflatoxin production (Kohiyama *et al*., 2015; Nerilo *et al*., 2016). Also, plants and their extracts may contain specific molecules that can lead to an effective inhibition of AFB1. Thus, the study of their active molecules may allow a better understanding of the mechanism of their biological action effect (Habibi *et al*., 2023).

Turmeric (*Curcuma longa*), also known as Curcuma longa, is a spice with well-established medicinal properties that have attracted attention from the medical and scientific communities as well as food enthusiasts. This is due to its high content of polyphenols, particularly curcumin, which has been found to assist in the treatment of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidemia (Negi, *et al*., 1999, Lam *et al*., 2016, Dai, *et al*., 2018).

In the last 40 years, studies have shown that this substance can be used to treat many diseases such as cancer, diabetes, heart diseases, lung diseases, kidney and metabolic diseases, nervous system diseases, and other inflammatory diseases (Willenbacher, *et al*., 2019). Furthermore, a relatively low dose of the complex can provide health advantages for individuals without confirmed medical ailments (Jahanbani *et al*., 2021).

Liver cancer caused by AFB1 is characterized by the appearance of gamma-glutamyl transpeptidase in the liver tissue. Studies showed that the diet of 0.005% curcumin in mice has strongly reduced the

amount of this substance in the liver (Brown *et al*., 2009). The properties of curcumin are related to the chemistry and molecular structure of the complexes it forms. This valuable substance has a great ability to penetrate other molecules (Zhai *et al*., 2020). The antifungal effect of curcumin on *Aspergillus flavus* was evaluated and the results showed that curcumin decreased mycelial growth, sporulation, and pathogenicity of *Aspergillus flavus*. Also, some studies showed it can decrease AFB1 synthesis effectively in peanuts (Zhang *et al*., 2023). Curcumin is a molecule that modulates the biological activity of molecules. This activity is done through direct or indirect interactions using covalent bonds, hydrophobic (non-covalent), and hydrogen bonds. These properties make it interact with many molecules such as proteins and metal ions (Gupta, *et al.*, 2011). Phenyl curcumin rings can participate in π-π van der Waals interactions with cyclic amino acid chains. In the hydrophobic structure of curcumin, carbonyl, and phenolic functional groups located at the end and center of the molecule can participate in hydrogen bonds with target molecules. This structure causes a strong and direct electrostatic interaction that increases the desired free energy of the bond. The tautomerization of keto-enel creates a wide range of chemical functions for the curcumin molecule. The enol shape of the molecule makes it both a donor and acceptor of hydrogen bonds between the molecules. Its enolic form makes it a suitable chelator for positively charged metal ions that are often found in the active sites of target proteins (Baum and Ng, 2004]. The combination of hydrophobic interactions including π-π, strong hydrogen bonds, metal chelation, and covalent bonds allows curcumin to exhibit a wide range of mechanisms and interact with target proteins. According to numerous articles, the ability of curcumin to bind with many messenger molecules, such as inflammatory molecules, and proteins has been reported (Gupta, *et al*., 2011). The part of beta diketone unsaturated α and β, carbonyl and enolic groups of beta diketone, methoxy, and phenolic hydroxyl groups as well as phenyl rings are functional groups of curcumin that are suitable for interaction with macromolecules. (Mullaicharam and Maheswaran, 2012; Gupta, *et al*., 2011). According to the research conducted on the interactions of curcumin with some molecules, it has been reported that functional groups on curcumin are suitable for interaction with phenyl rings in molecules, and since AFS have a phenyl ring in its structure, curcumin is attached to them (Gupta, *et al*., 2011).

Nuts and dried fruits are widely cultivated and processed in Iran. Among various nuts, pistachios stand out due to their nutritional value (Sharifkhah *et al*., 2020; Nazoori *et al*., 2024). The cultivation of pistachio trees has become widespread in other regions of the world because of their high nutritional content and appealing taste. The commercial production of pistachios holds significant importance in the agricultural sector of countries like Iran (Norozi *et al*., 2019; Sharifkhah *et al*., 2020; Nazoori *et al*., 2022), contributing significantly to non-petroleum exports. While Iran leads globally in pistachio production and cultivated areas, challenges in exporting and AFS contamination hinder its position in the global market.

In this study, after examining various extraction methods of turmeric extracts, the best conditions for extraction were determined. Then, the optimal extract was used in the culture medium of *Aspergillus flavus*. Also, this study aims to explore whether turmeric extract can effectively reduce aflatoxin levels in pistachios.

This research was conducted from 2022 to 2023 at the Standards Research Institute.

Materials and Methods

Chemicals materials

AFS for the experiments was purchased from Sigma Company, Germany. The mixtures of AFS standard and working solutions (1 µg ml^{-1} for AFB1 and AFG1 and 0.4 µg ml^{-1} for AFB2 and AFG2) were prepared by dilution in methanol and stored in glass-

stoppered tubes at 0 °C. All solvents used for the AFS determination (methanol, acetonitrile, and deionized water) were HPLC grade from Merck Company. Sodium hydroxide, hydrochloric acid, sodium alginate, calcium chloride, sodium carbonate, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, acetonitrile, N hexane, cyclohexane, dipotassium hydrogen phosphate, folin reagent, gallic acid standard, quercetin standard, potassium bromide, and nitric acid were prepared from Merck Company. Also, Tryptic soy broth and sabouraud dextrose agar were prepared by Merk Company.

The strain of *Aspergillus flavus* (PTCC NO:5006) and *Aspergillus parasiticus* (PTCC NO: 5286) were obtained as a lyophilized powder from the Iranian Scientific and Industrial Research Organization.

The samples of turmeric were prepared from the market and approved by the pharmaceutical sciences faculty of Shahid Beheshti University. After washing and separating the impurities, they were dried and crushed by an electric grinder and turned into powder. The pistachio fruits of Khanjari variety was purchased from one of Damghan pistachio orchards and after drying, they were stored in a cold room at --20°C during the experiment period.

Extraction and determination of flavonoid compounds in the extracts

Method extraction of bioactive compounds of turmeric is very important to determine the efficacy and quality of the phytonutrient intermediate products. (List and Schmidt, 1989). To obtain the extraction of turmeric, two methods were used: maceration method and ultrasonic method at 25 ºC and 50 ºC with ethanol 70% and methanol 70% as solvents due to good solubility and compatibility with phenolic and flavonoid compounds. Then, the optimal times for extraction were determined in the intervals of 30, 60, 90, 120, 180, 240, and 360 minutes.

In the ultrasonic method, turmeric powder was dissolved in ethanol 70% and methanol 70% separately. Then these solutions were subjected to ultrasonic radiation with frequencies of 100, 40, and 20 Hz at 25 °C and 50°C at different times including 30, 60, 90, 120, 180, 240, and 360 minutes (Igas *et al*., 2021). After the filtration and concentration of extracts, they were filtered and centrifuged for 10 minutes at a speed of 3000 rpm and the solvent was separated from the extracts using a rotary evaporator (Luis Miguel *et al*., 2023).

In the maceration method, according to Prasad and their colleague method, turmeric powder was dissolved separately in ethanol 70% and 100 ml of methanol 70% at 25°C and 50°C and at different times including 30, 60, 90, 120, 180, 240, and 360 minutes by stirring to investigate the effect of temperature and time on the extraction efficiency. Then the extracts were filtered and centrifuged at 3000 rpm for 10 minutes and the solvent was separated from the extracts using a rotary evaporator (Singh *et al*., 2022).

The total amount of phenolic compounds in the extracts produced by maceration and ultrasound methods at different temperatures and times was determined using the Folin-Ciocalto method (Vyvoda *et al*., 2011). Analysis of the results was done using the gallic acid standard curve calibration. Total flavonoid determination was done by the aluminum chloride color method (Cheng *et al*., 2002). Quercetin was used to draw a standard curve for flavonoid compounds.

Identification of compounds in the extracts

The total amount of phenolic and flavonoid compounds of the turmeric extract was used as a criterion to select the extract with the highest efficiency. To identify the compounds in it, the selected extract was injected into GC-MS (model 5973 manufactured by Hewlett-Packard, USA).

Preparation and activation of microorganisms

The activation of Fungal strains was performed according to the Clinical and Laboratory Standards

Institute (CLSI) guidelines (CLSI, 2006). The strain was cultured in Trypton Soy Broth (TSB) medium at 25 ºC for 7 to 14 days. Then, 2 to 3 colonies from the overnight culture of each strain were added to sterile physiological saline, and their turbidity was adjusted using the McFarland 0.5 standard for fungal testing. The absorbance of the fungal suspension was measured at a wavelength of 530 nm using the absorbance of the McFarland's 0.5 standard solution by a UV-Vis spectrophotometer (model T80Plus) to obtain a fungal suspension with a concentration of 1x107 (CFU mL^1) (/CLSI, 2006). After checking the toxigenicity of the fungus strain used, the absence of microbial contamination in the extracts was ensured.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC of the most effective extract was determined according to INSO 5875 (INSO 5875, 2003). This investigation was carried out for the fungi in the presence and absence of pistachios. For this purpose, spores of *Aspergillus flavus* ($1x10^4$ CFU mL^1) and *Aspergillus parasiticus* ($1x10^4$ CFU/mL) were spread on the surface of the culture medium using a sterile swab. The plates were incubated at 25°C for 24 hours to 48 hours. The culture medium containing the extracts and negative controls was incubated at the optimal temperature for the fungi (25°C) to observe possible growth .After determining the MFC of the extracts, the diameter of the fungal growth inhibition zones was measured using a Vernier caliper. *Aspergillus flavus* and *Aspergillus parasiticus* were incubated with 150 to 1000 µL of extract at 25°C, and after 3 to 5 days, the number and diameter of colonies were recorded.

Additionally, pistachio powder without aflatoxin contamination was sterilized in an autoclave for 15 minutes at 121°C and 15 psi to remove any contamination. Eight sterile laboratory tubes were prepared to test successive and different dilutions of the extract, with one tube as a positive control and one as a negative control. After adding 5 mL of sterile TSB culture medium to each tube, the extracted samples were added and homogenized. A fungal culture with a concentration of 0.5 McFarland was added to all test tubes except the negative control tube. 1 g of sterilized pistachio powder was added to each tube to investigate the effect of extracts

Investigation of the effect of turmeric extracts on aflatoxin production

In this study, the effect of turmeric extract on aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus* was evaluated both in the presence and absence of pistachios. Fungal cultures were incubated with 1 mL of turmeric extract at 25°C, and samples were collected at intervals time intervals including 2, 6, 24, 48, 72, 96, and 120 hours. Aflatoxin (AFS) determination was performed according to the Iranian National Standard No. 6872 (INSO 6872, 2010) using high-performance liquid chromatography (HPLC) with cleanup by immunoaffinity column after 14 days' incubation. The HPLC system used was equipped with a fluorescence detector set at an excitation wavelength of 360 nm and an emission wavelength of 420 nm for aflatoxin measurement.

The experiments were conducted in multiple stages. First, the analytical instruments were calibrated to ensure measurement accuracy. The baseline aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus* under standard conditions was determined. Subsequently, the impact of pistachio presence on the growth of both fungi and aflatoxin production was assessed. Following this, the effect of turmeric extract was examined, both in the absence and presence of pistachios. This approach allowed for a comprehensive analysis of the combined effects of pistachios and turmeric extract on aflatoxin production by both fungal species.

The study revealed that aflatoxin production can vary depending on the interaction between fungi, turmeric extract, and pistachios. The analyses showed

that the presence of turmeric extract can significantly alter aflatoxin levels, especially when combined with pistachios. These findings are crucial for understanding the potential of turmeric as a natural inhibitor of aflatoxin production in contaminated food products.

For the aflatoxin extraction process, 5 g of sodium chloride and 50 mL of each culture broth filtrate (with and without turmeric extract) were combined separately in a blender jar. They were mixed at high speed for 1 minute to extract the aflatoxins using a 200 mL methanol/water (80:20) solution. Then, 20 mL of each of these filtrated extracts and 130 mL of distilled water were mixed and passed through a glass-microfiber filter. Next, 70 mL of these solutions were passed into a 3 mL Aflatest® immunoaffinity column and allowed to elute at a rate of 1 to 2 drops per second. After washing the column twice with 1 mL of distilled water, aflatoxin residue was removed using 1 mL of HPLC-grade methanol. The AFS concentration was measured using an HPLC system equipped with a fluorescence detector set at 360 nm absorption and 450 nm emission with a Kobra cell for AFS derivatization (INSO 6872, 2010).

Results

A comparison of total phenol and flavonoid extraction using 70% ethanol and methanol as solvents, alongside ultrasound and maceration methods, indicated that ethanol as a solvent combined with the ultrasound method was more efficient. Using the ultrasound method at 50°C for 60 minutes, the turmeric extract yielded 23.92% flavonoid compounds and 59.8% total phenol. In contrast, the best results with the maceration method at 50°C after 120 minutes were 21.19% for flavonoids and 48.27% for total phenol. Therefore, it was found that the ultrasonic method at a temperature of 50 C in 60 minutes was the most effective in extracting total phenol and flavonoid compounds in turmeric (Figs. 1 & 2).

The compounds of the extract of turmeric with the highest efficiency was determined using injection to GC-MS (Table1).

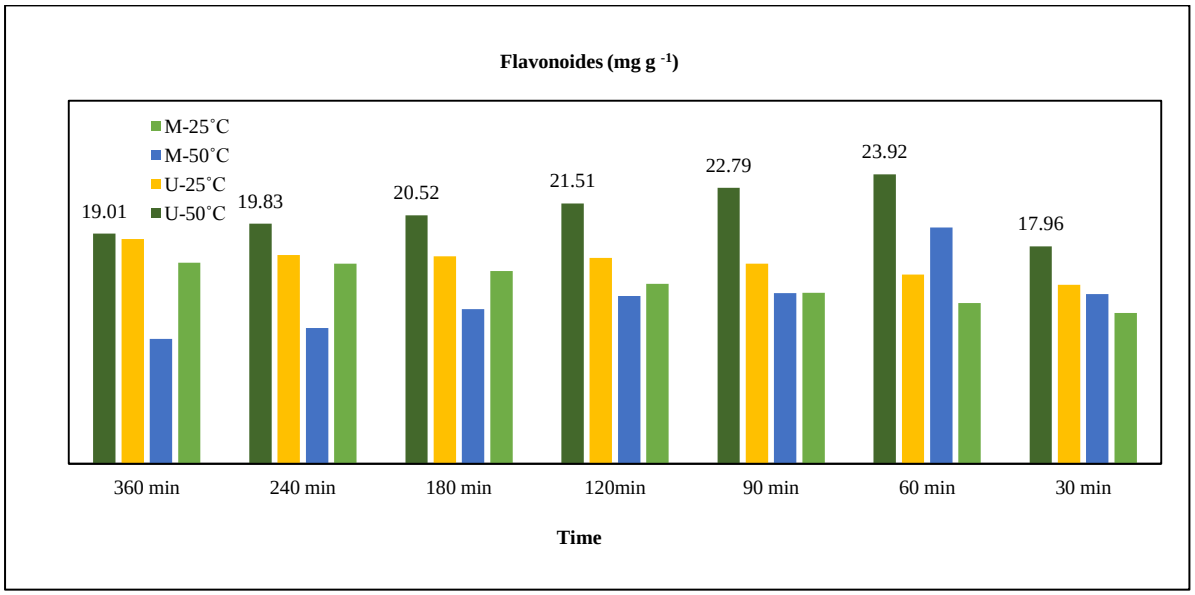

Fig.1. Total amount of flavonoids compounds of turmeric extract by ultrasonic (U) and maceration (M) methods.

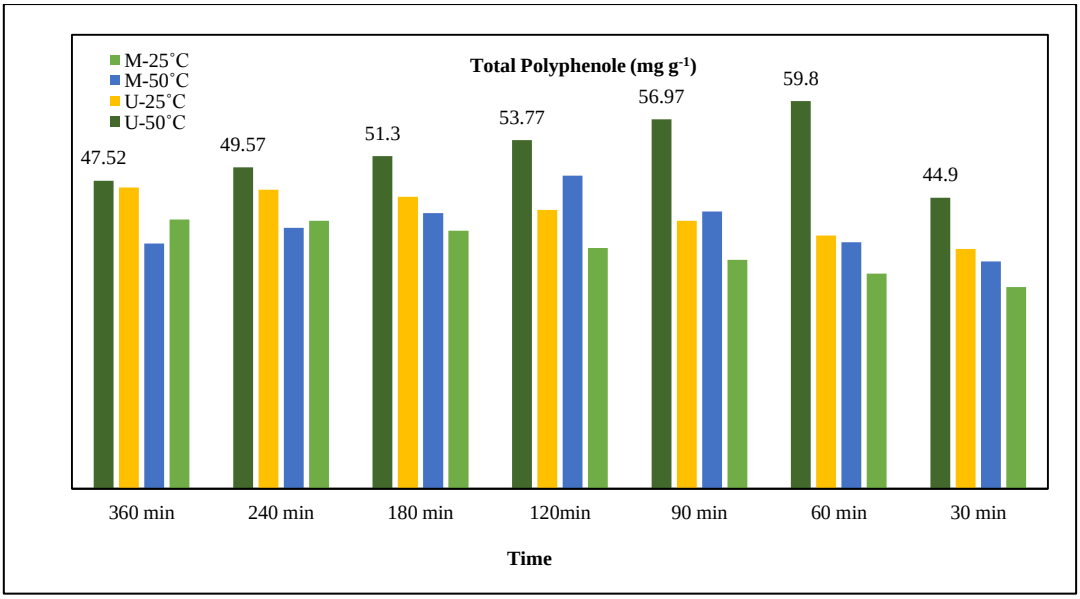

Fig. 2. The amount of total phenol of turmeric extract by ultrasonic (U) and maceration (M) methods.

Table 1. Active compounds of turmeric extract identified by GC-MS.

Active compound	Rt(min)	Active compound	Rt(min)
D-2,3-Butane diol	1.21	Nopol (terpene)	1.08
o-cymene	0.34	α-Curcumene	30.71
Terpinolene	0.64	α-bergamotene	0.55
α-thujone	0.58	Zingiberene	0.48
Cis-sabinol	0.33	Tumerone	7.14
Ciminaldehyde	0.5	Longiverbenone	0.66
Thymol	0.71	Trans-Chrysanthenyl acetate	1.18
phenol	0.57	Caryophyllene oxide	1.77
Dihydrocurcumene	1.21	α-terpinene	0.48
Hemellitol	0.38	Germacrone	6.08
Cembrene	0.42	Elemol	0.93
α-guaiene	0.96	Geranyllinalool	0.86
Longipinene	0.37	Terpinyl formate	1.51
β-bisabolene	1.75	Cis-sesquisabinene hydrate	2.51
γ-gurjunene	0.54	Corymbolone	1.51
β-Curcumene	1.08	α-lonone	0.59
Cuparene	0.47	p-Cymen-2-ol	2.49
Cedr-8-ene	3.87	7,3`-Dimethyoy-3-hydroxyflavone	2.09
β-Cedrene	4.59	Pinane	2.05
Cis-p-mentha-2,8-diene-1-ol	2.1	Farnesol	2.1
Cis-p-mentha-6,8-diene-2ol	0.39	5β, 7βH, 10 α-eudesm-11-en-1a-ol	0.89
Cis-Lanceol	1.05	Phytol	0.38
Geranyl-α-terpinene	0.64	Stigmasterol	0.46
β-Himachalene	2.26	β-sitosterol	0.22
Spathulenol	0.51		

The results of investigating the impact of turmeric extract on mycelial growth of *Aspergillus flavus* and *Aspergillus parasiticus* showed that turmeric extract inhibited the vegetative growth and conidial germination of both of them. After incubating with 150 µL of turmeric extract, for 5 days at 25ºC, the highest number of colonies was observed for *Aspergillus flavus* (44 colonies), with a colony diameter of 12 mm, but by using 300 µL of the extract after 3 days, only one colony was observed. So, the application of 300 µL turmeric extracts significantly inhibited the mycelial growth of *Aspergillus. flavus* ($P < 0.05$) (Fig. 3).

It should be noted that in this figure, turmeric extract volumes are presented in µL. These correspond to final concentrations of approximately 0.88, 1.18, 1.47, and 1.77 mg/mL in the culture medium for 150, 200, 250, and 300 µL respectively.

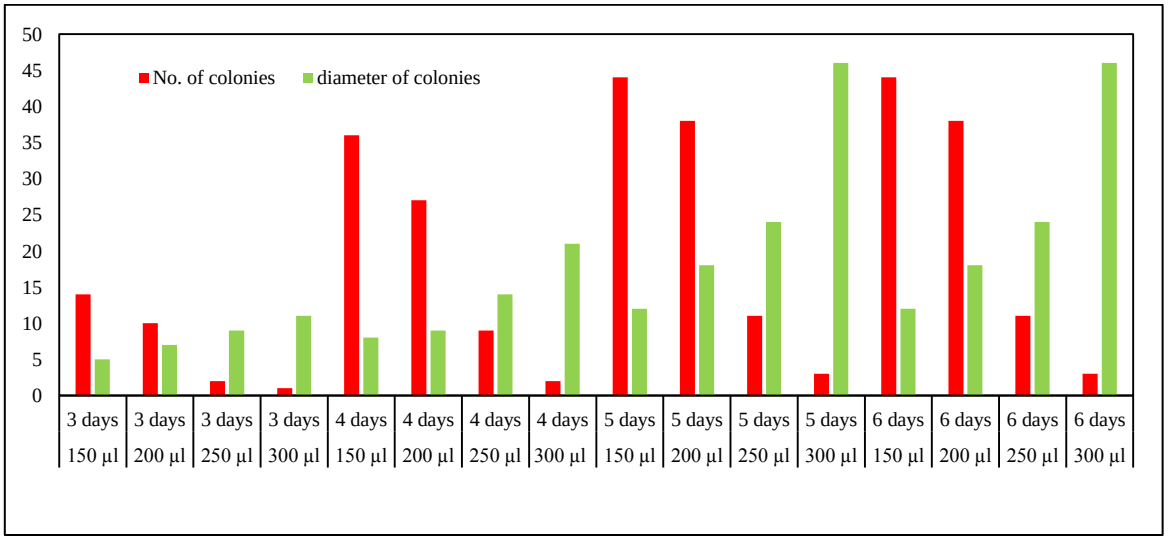

Fig. 3. Number and diameter of colonies (mm) after incubation of *Aspergillus flavus* with different volume of turmeric extract

About Aspergillus parasiticus, the highest number of colonies was observed after incubating with 150 µL of turmeric extract (1.77 mg/mL), for 5 days at 25 ºC (28 colonies), with a colony diameter of 14 mm, but by using 350 µL of the extract (2.07 mg/mL) after 3 days, only 1 colony were observed. So, the application of 350 µL turmeric extracts significantly inhibited the mycelial growth of *Aspergillus parasiticus* ($P < 0.05$) (Fig. 4).

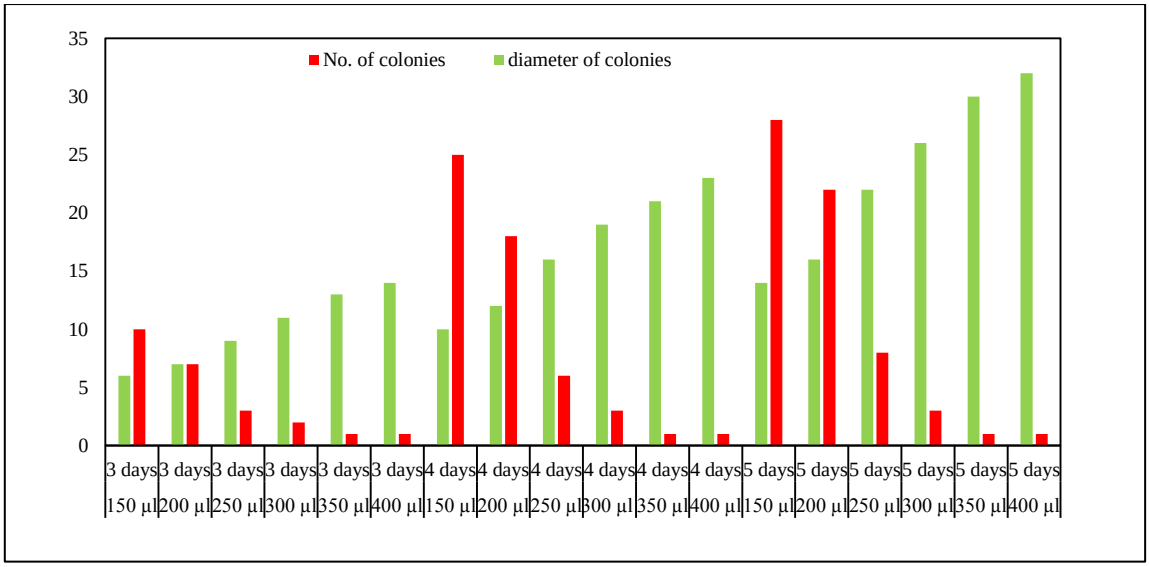

Fig. 4. Number and diameter of colonies (mm) after incubation of *Aspergillus parasiticus* with different volume of turmeric extract

This assay showed that the addition of turmeric extract notably inhibited *Aspergillus flavus* and *Aspergillus parasiticus* infections and decreased AFS biosynthesis. Adding pistachios fruit samples to culture medium include each of fungi increased AFS production, but AFS production decreases in the presence on pistachios by adding turmeric extract.

The results showed that the production of AFB1 by *Aspergillus flavus* decreased 81% in the presence of turmeric extract (Fig. 5). AFB2 production was inconsiderable for all treatments of *Aspergillus flavus.* In case of pistachios added to culture medium *of Aspergillus flavus*, after adding turmeric extract, AFB1 decreased 77% compared to without turmeric extract (Fig. 5).

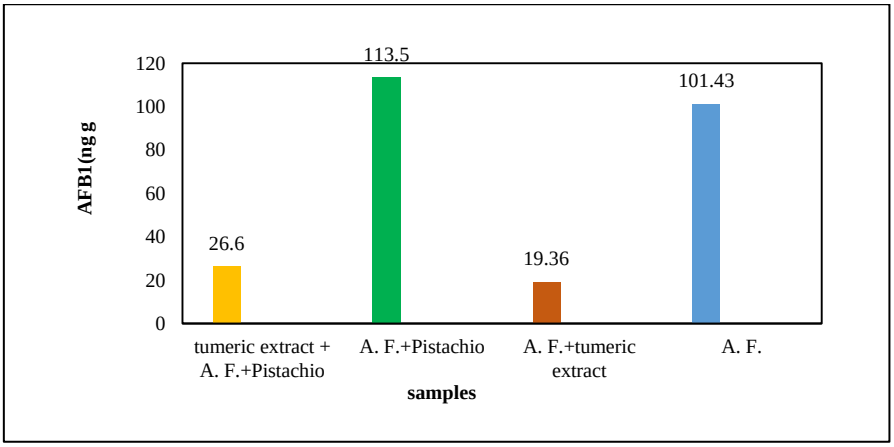

Fig. 5. AFB1 production by *Aspergillus flavus*, +tumeric extract, *Aspergillus flavus* +Pistachio and *Aspergillus flavus* +Pistachio+ tumeric extract

Similar results were observed in the production of aflatoxins for in case of *Aspergillus parasiticus*. The results showed that the production of AFB1 by *Aspergillus. parasiticus* decreased by 64% in the presence of turmeric extract. In case of pistachios samples added to culture medium *of Aspergillus. parasiticus*, after adding turmeric extract, AFB1 decreased 66% compared to without turmeric extract. The content of AFB2, AFG1, and AFG2 showed lower concentration in presence turmeric extract in the both in the absence and presence of pistachios (Figs. 6- 9).

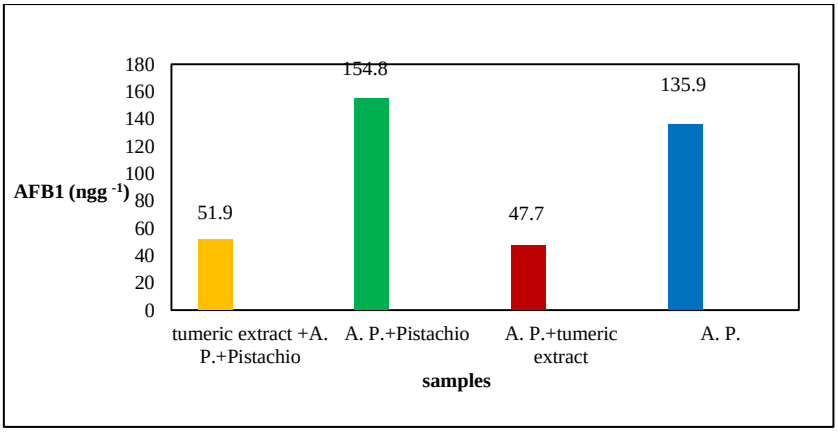

Fig. 6. AFB1 production by *Aspergillus parasiticus* +tumeric extract, *Aspergillus parasiticus* +Pistachio and *Aspergillus parasiticus* +Pistachio+ tumeric extract

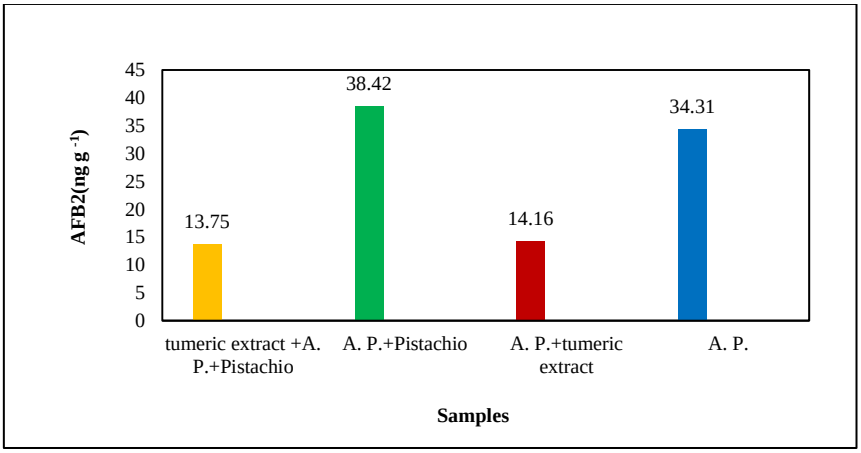

Fig .7. AFB2 production by *Aspergillus parasiticus* +tumeric extract, *Aspergillus parasiticus* +Pistachio and *Aspergillus parasiticus* +Pistachio+ tumeric extract

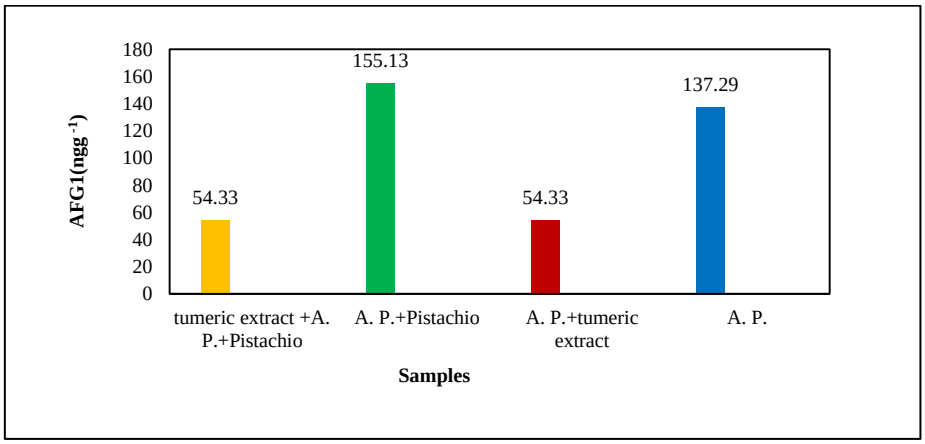

Fig. 8. AFG1 production by *Aspergillus parasiticus* +tumeric extract, *Aspergillus parasiticus* +Pistachio and *Aspergillus parasiticus* +Pistachio+ tumeric extract.

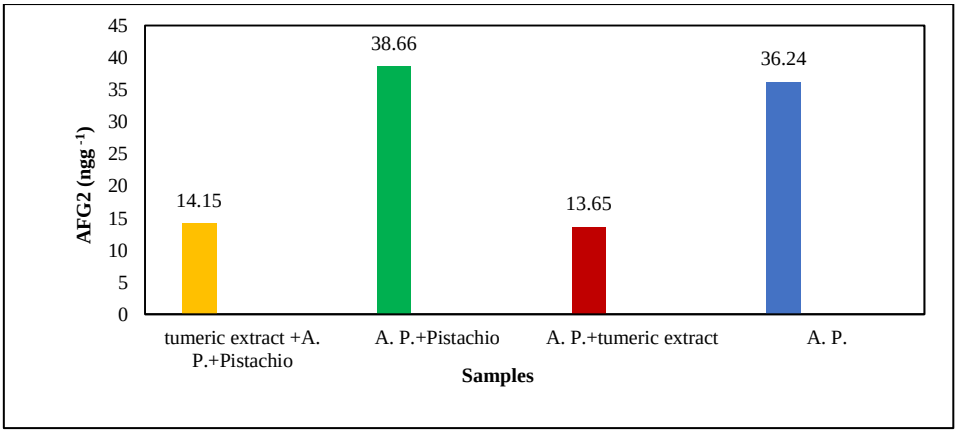

Fig. 9. AFG2 production by *Aspergillus parasiticus* +tumeric extract, *Aspergillus parasiticus* +Pistachio and *Aspergillus parasiticus* +Pistachio+ tumeric extract

Comparing the results shows that turmeric extract has more inhibiting effect for AFB1 production by *Aspergillus flavus.*

So, adding turmeric extract significantly reduces aflatoxin contamination in fungus and pistachios.

Fig. 10 shows the AFB1 chromatograms produced by *Aspergillus flavus*. Also, chromatogram of AFB1, AFB2, AFG1, and AFG2 in the presence of *Aspergillus parasiticus* are shown in Fig. 11.

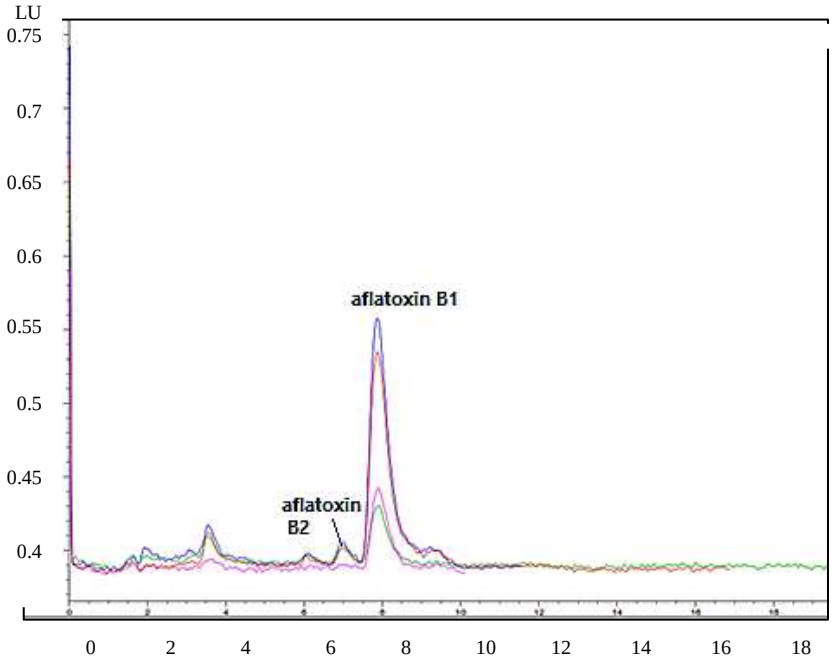

Fig 10. -AFS peak for *Aspergillus flavus* + pistachio, - AFS peak for *Aspergillus flavus* - *AFS for turmeric extract+ Aspergillus flavus + pistachio,* - AFS for turmeric extract+ *Aspergillus flavus* (Ex=365 nm, Em=435 nm)

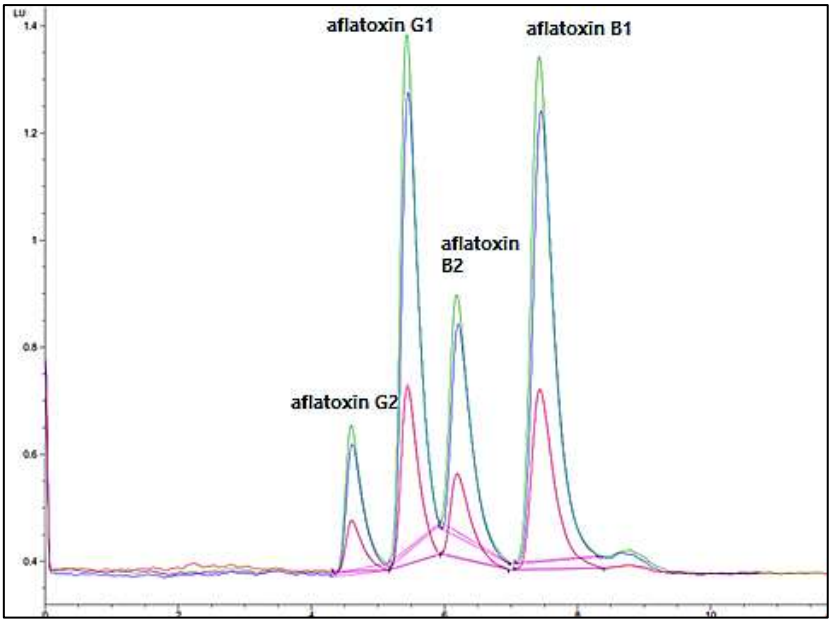

Fig. 11. -AFS peak for *Aspergillus parasiticus* + pistachio, - AFS peak for *Aspergillus parasiticus* - AFS for turmeric extract+ *Aspergillus parasiticus* + pistachio, - AFS for turmeric extract+ *Aspergillus parasiticus* (Ex=365 nm, Em=435 nm)

Discussion

This study confirmed that the application of turmeric extract significantly suppressed the biosynthesis of all four aflatoxins (AFB1, AFB2, AFG1, and AFG2) produced by *Aspergillus parasiticus*. A similar result was also observed in inhibiting the production of AFB1 and AFB2 by *Aspergillus flavus*. Additionally, the contamination of aflatoxins in pistachios was markedly reduced. The content of AFB1, AFB2, AFG1, and AFG2 in infected pistachios was considerably lower compared to the control samples. These findings further emphasize that turmeric extract is highly effective in reducing overall AFS contamination in pistachios, leading to a significant decrease in toxin levels in comparison to untreated controls ($p < 0.05$).

The inclusion of both *Aspergillus flavus* and *Aspergillus parasiticus* in this study provided a comprehensive analysis of turmeric extract's antifungal properties, given their different profiles of aflatoxin production.

AFS contamination in agriculture products has become a major food safety problem; the toxic effects of AFS pose serious health risks to the population. Furthermore, AFS contamination cause economic losses by limiting exports of products such as pistachios. So, there is a need to develop novel preservation techniques to control the growth of fungi in products, prevent AFS production, and decrease it. The results suggest that turmeric extract possesses antifungal properties that can effectively suppress AFS production in *Aspergillus flavus* and *Aspergillus parasiticus*. This inhibition may be attributed to the bioactive compounds present in turmeric, particularly curcumin, which has been shown to interfere with fungal growth and toxin synthesis pathways. Further studies are warranted to elucidate the specific mechanisms by which turmeric inhibits AFS biosynthesis and to optimize its application in agricultural and food safety practices. Considering the effect of turmeric extract, studies indicate that such extracts have commercialization potential as natural preservatives. For example, Gul and Bakht demonstrated that turmeric extract at 1% to 2% concentrations effectively reduced microbial contamination and prolonged the shelf life of food products by up to 90 days. Additionally, they found that adding turmeric extract significantly decreased

bacterial and fungal loads, supporting its potential for industrial application as a preservative in food storage. These findings suggest that turmeric extract could be commercialized for use during pistachio storage and preservation to inhibit aflatoxin development effectively (Gul and Bakht, 2015) .

Conclusions

The results of this study suggest that turmeric extract, obtained using the ultrasonic method by ethanol (70%) at 50°C for 60 minutes, demonstrates potential as a natural inhibitor of AFS production by Aspergillus *flavus* and Aspergillus *parasiticus*. Specifically, a concentration of 300 µL of turmeric extract (1.77 mg/mL) effectively inhibited the mycelial growth of Aspergillus *flavus*, while a concentration of 350 µL (2.07 mg/mL) was more effective for inhibiting Aspergillus *parasiticus*. This extract could control AFS production on infected pistachios in the presence of these fungi. In other words, turmeric extract could be used as an antifungal agent to control AFS contamination in foods such as pistachios. Incorporating turmeric into agricultural practices or food processing may offer a sustainable strategy to mitigate aflatoxin contamination in crops and enhance food safety.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors have read and agreed to the published version of the manuscript.

CRediT authorship contribution statement

Shokoofeh Sharokhi: Data curation, Development or design of methodology, Investigation

Leila Nouri: Supervision, Project administration

Mansooreh Mazaheri: Conducting a research, Investigation, Supervision, Writing - Original Draft, Project administration

Laleh Adlnasab: Development or design of methodology

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