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Fungi and Their Associated Mycotoxins Contaminating Tomato Fruits (*Solanum lycopersicum*) and Potential Control of Fruit Spoilage by Essential Oils

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ABSTRACT

Tomato fruits (*Solanum lycopersicum*) are among the most horticultural popular crops consumed worldwide. They are susceptible to fungal infection and subsequent contamination with mycotoxins. So the current work aimed to assess the occurrence of spoilage fungi and their associated mycotoxins in tomato fruits, and evaluation of antifungal activity of cinnamon and fennel essential oils (alone and in pair combination) in controlling the tomato spoilage caused by mycotoxigenic fungi. The obtained data resulted that, 1373 fungal isolates were detected. Nine fungal species were identified as *Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *A. parasiticus*, *Colletetrichum* sp., *Geotrichum candidum*, *Mucor* sp., *Penicillium expansum*, and *Rhizopus stolinifer*. *Alternaria alternata* isolates No. (2 & 29) from locations D & A produced Alternariol toxin, *A. parasiticus* isolate No. (1) from location A was aflatoxins producer, and *Penicillium expansum* isolate No. (1) from location B produced Citrinin. On the other hand, Cinnamaldehyde, (E) - compound was the major constituent detected in cinnamon oil, while Estragole compound was the main constituent in fennel oil. Cinnamon and fennel essential oils and their mixture reduced significantly the mycelial growth of the tested mycotoxigenic fungi at all concentrations used under *in vitro* and *in vivo* conditions compared with the untreated control. It could be concluded that cinnamon and fennel essential oils and their mixture are highly effective against tomato spoilage caused by mycotoxigenic fungi.

Keywords: Tomato fruits, Mycotoxigenic Fungi, Mycotoxins, Antifungal activity, Cinnamon essential oil, Fennel essential oil.

1. Introduction

Tomato (*Solanum lycopersicum*), is a member of the *Solanaceae* family and is a highly popular and widely cultivated vegetable worldwide. It is extensively consumed in various forms, both fresh and processed. Among all vegetables, tomatoes hold significant value as a crop and are consumed by a large number of people globally (Moneruzzaman *et al.,* 2008). The tomato possesses significant nutritional and health advantages, as well as considerable economic value (Raiola *et al.,* 2014). The tomato is abundant in essential nutrients like vitamins, carbohydrates, proteins, fats, and potassium (Talvas *et al.,* 2010). Similar to many other agricultural commodities, the occurrence of mold contamination in tomatoes initiates in the fields (Brackett 1988). The quality of tomatoes is significantly impacted by post-harvest handling, inadequate storage practices, transportation issues, and improper marketing. Fungal infections, caused by various species such as *Geotrichum candidum, Rhizopus stolonifer, Alternaria* sp. (causing black mold rot), and *Fusarium* sp. (causing Fusarium rot), are the main culprits behind tomato fruit rots. It is important to note that the consumption or inhalation of tomatoes contaminated with Fusarium species poses a health risk to humans, as these fungi produce mycotoxins that can lead to mycotoxicoses (Wagacha and Muthomi 2008). Approximately 25.80% of fresh tomato fruits are estimated to be lost during the post-harvest stage (Thirupathi *et al.,* 2006). Harvesting tomatoes without causing any damage is a challenging task. The extent of post-harvest losses is more

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pronounced in developing countries compared to developed nations (Enyiukwu 2014). The issue of fungal rot affecting tomatoes is not limited to Egypt but is a global concern. Various methods have been utilized to prevent and control post-harvest fungal spoilage. Natural products, including those derived from higher plants; offer promising alternatives to synthetic chemical fungicides, biological control agents, and physical methods. These natural products exhibit a relatively wide range of effectiveness, are cost-efficient, and are environmentally safe (Etebu *et al.,* 2013). This study aims to control the natural infection of tomato fruits postharvest caused by fungi using different doses of pure essential oils of cinnamon (*Cinnamomum zeylanicum* L.) and fennel (*Foeniculum vulgare*) which are widely consumed as spices (Anzlovar *et al.,* 2014), and are largely safe at low doses (Sinha *et al.,* 2014). Therefore, the objectives of this study were to assess the occurrence of spoilage fungi and their associated mycotoxins in tomato fruits, identify the chemical composition of cinnamon and fennel essential oils and evaluation of their antifungal activity (alone and in pair combination) in controlling the tomato spoilage caused by mycotoxigenic fungi.

2. Material and Methods

2.1. Collection of samples

Randomly ten fresh samples of tomato fruits were collected from each of six different locations in Egypt during (2022). Each sample was collected in sterile polyethylene bags and conveyed to the microbiology laboratory for analysis within 24 hours of collection.

2.2. Essential Oils

Pure essential oils of cinnamon (*Cinnamomum zeylanicum* L.) and fennel (*Foeniculum vulgare*) were purchased from the Medicinal and Aromatic Plants Research Center in Al-Qanatir Al-Khairiya, Egypt. All plant oils were stored in dark bottles at 4°C for further studies.

2.3. Culturing Procedure

Naturally infected samples were washed with distilled water and then, disinfected by immersing them in ethanol 70% for 2 min. Sterilized fruits were washed twice with sterile double–distilled water (5min each), and allowed to dry for 1 hr. in laminar flow. An appropriate size of infection site of spoilt tomatoes was carefully cut with of sterile blade. Sliced portions (0.5 cm) were then plated on a sterile Potato Dextrose Agar (PDA) medium complemented with 2% tetracycline to inhibit bacterial growth. Three replicates of each tested sample were inoculated with 4 pieces for each plate. Incubation was done at 28 ± 2°C for 5 days until fungal growth was noticed. The different isolates were sub-cultured on freshly prepared (PDA) to obtain their pure culture.

2.4. Identification Procedure

All developing fungal colonies were examined morphologically and microscopically and then identified according to Pitt and Hocking (1997); Raper and Fennel (1965); Samson (1979).

2.5. Determination of mycotoxins production

All isolated mycotoxigenic fungi (*Alternaria alternata*, *A. flavus, A. parasiticus,* and *Penicillium expansum*) were tested for mycotoxins production. Production of Alternariol toxin was done by culturing *Alterneria* sp. on rice medium according to Torres *et al.,* (1998), and was determined according to Logrieco *et al.,* (1990). All *A. flavus,* and *A. parasiticus* isolates were propagated as pure culture in 100 ml yeast extract sucrose (YES) medium to be tested for Aflatoxins production (Munimbazi and Bullerman 1998; Younos and Akl 2022), and was extracted and determined according to Kumar *et al.,* (2010); Rubert *et al.,* (2012), while Citrinin production was done by culturing pure culture of *Penicillium expansum* isolates on 100 ml yeast extract sucrose (YES) medium, and determined and quantified by HPLC according to Coton *et al.,* (2019).

2.6. Gas chromatography-mass spectrometry (GC/ MS) analysis of essential oils (EOs)

The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Samples were diluted with hexane (1:19, v/v). The GC was equipped with a DB-WAX column (30 m x 250 μm internal diameter and 0.25 μm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split 1:10 of, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280°C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50-550 and solvent delay 3 min. The components of the EOs were identified by matching their mass spectral fragmentation patterns with those reported in computerized MS- data bank spectral libraries NIST 98 and WlLEY 138 (Sparkman 2012).

2.7. In vitro antifungal activity of cinnamon and fennel oils (mycelial growth inhibition)

The antifungal effects of essential oils (cinnamon and fennel oils and their mixture) were determined against isolated fungi (*Alternaria alternata, Aspergillus flavus, A. parasiticus,* and *Penicillium expansum*) *in vitro*. The tested essential oils were dissolved in 0.1% sterile Tween 20 (1:1) individually and then mixed with sterilized Potato Dextrose Agar (PDA) medium at different concentrations (0.125, 0.25 & 0.50 % (v/v)) in separately sterilized Petri dishes. All Petri dishes were inoculated separately at the center with 5mm-disc inoculums 7-day old of each of the tested isolated fungi using a sterilized cork borer. Three plates were used as replicates for each treatment and then incubated at 28 ± 2°C. Colony diameter was measured after 7 d of the incubation period (Singh *et al.,* 2014). Medium-free of oil was used as a control. Growth inhibition percent was calculated according to Jabeen *et al.,* (2013) by using a formula:

$$\text{Growth inhibition (\%)} = (C - T) / C \times 100$$

Where C = growth in control, T = growth in treatment

2.8. In vivo assessment of antifungal activity of cinnamon and fennel oils on tomato fruits spoilage

The antifungal assay of the essential oils (cinnamon and fennel oils and their mixture) against spoilage tomato fruits caused by mycotoxigenic fungi (*Alternaria alternata, A. parasiticus* and *Penicillium expansum*) *in vivo* was conducted according to the method of Tian *et al.,* (2011); Tabaestani *et al.,* (2013). Healthy red tomato fruits were surface sterilized with ethanol for 1 minute and washed in five changes of distilled water. A 5 mm cork borer was punched to a depth of 4 mm into the healthy tomato fruits and the bored tissues were removed. A five mm diameter disc from the pure culture of each of the tested fungi was placed back into the bored hole on the tomato fruits individually. The wounds were sealed with prepared candle wax according to the method of Fawole and Oso (1998). The control experiment was set up in the same manner except that the sterile agar disc was used instead of inoculums. The essential oils treatments consisted of five minutes of immersion of the aforementioned fruits (inoculated wounded fruits) in tested essential oils individually (0.50% concentration) and in sterilized distilled water in case of control treatment. Fruits were then forced-air cooled to 5°C and placed in white plastic containers each containing 10 fruits, each moistened with-wet balls of absorbent cotton wool to create a humid environment and incubated at 28°C under sterile conditions for 15 days. Three replicates were used per treatment. The fruit decay development (disease severity) was expressed as diameter of rot lesion in mm on fruit respect to control.

2.9. Statistical Analysis

Data obtained in this study were analyzed using software (IBM SPSS Statistics v.16. USA). Statistical significance was performed using a one-way Analysis of Variance (ANOVA) test. A value of $p<0.05$ was considered statistically significant. The least significant difference (LSD) was calculated at $P \leq 0.05$ according to Gomez and Gomez (1984).

3. Results

3.1. Total fungal isolates associated with tomato fruits

Since most fruits and vegetables such as tomato fruits contain high levels of water and nutrients, they serve as good substrates which support the growth of pathogenic microorganisms. Isolation of mycoflora associated with tomato fruitson Potato dextrose agar (PDA) medium from six different locations resulted that, 1373 fungal isolates were counted and recorded as shown in Table 1. On the other hand, data showed that location D had the highest total fungal isolates (333 isolates (24.25%)) followed by location E (275 isolates (20.03%)), location F (259 isolates (18.86 %)), location A (226 isolates (16.46 %)), and location B which recorded 162 isolates (11.80 %), while location C had the least fungal isolates (118 isolates (8.59%).

Table 1: Total fungal isolates associated with tomato fruits.

Samples		Locations						Total
		A	B	C	D	E	F	
1	T.C	13	29	8	18	22	35	125
	%	0.95	2.11	0.58	1.31	1.60	2.55	9.1
2	T.C	21	20	7	22	15	17	102
	%	1.53	1.46	0.51	1.60	1.09	1.24	7.43
3	T.C	20	16	13	20	9	28	106
	%	1.46	1.17	0.95	1.46	0.66	2.04	7.74
4	T.C	25	17	10	131	15	32	230
	%	1.82	1.24	0.73	9.54	1.09	2.33	16.75
5	T.C	24	14	10	13	34	36	131
	%	1.75	1.02	0.73	0.95	2.48	2.62	9.55
6	T.C	25	15	10	16	44	24	134
	%	1.82	1.09	0.73	1.17	3.20	1.75	9.76
7	T.C	19	5	20	30	17	22	113
	%	1.38	0.36	1.46	2.18	1.24	1.60	8.22
8	T.C	34	18	10	26	45	19	152
	%	2.48	1.31	0.73	1.89	3.28	1.38	11.07
9	T.C	32	21	19	30	35	15	152
	%	2.33	1.53	1.38	2.18	2.55	1.09	11.06
10	T.C	13	7	11	27	39	31	128
	%	0.95	0.51	0.80	1.97	2.84	2.26	9.33
Total		226	162	118	333	275	259	1373
%		16.46	11.80	8.59	24.25	20.03	18.86	100

T.C= Total count

3.2. Fungal frequencies associated with tomato fruits

Identification of fungal species associated with tomato fruits indicated that there are nine fungal species belonging to seven fungal genera were identified and recorded as shown in Table 2. These are *Alternaria alternata, Aspergillus niger, A. flavus, A. parasiticus, Colletetrichum* sp.*, Geotrichum candidum, Mucor* sp.*, Penicillium expansum,* and *Rhizopus stolinifer* On the other hand, *Geotrichum candidum* had the highest fungal frequency recorded 61.76%, followed by *Colletetrichum* sp. (29.86%), *Altarnaria alternate* (4.37%), *Mucor* sp. (2.62%), *A. parasiticus* (0.51%), *A. flavus* (0.44%), *A. niger* (0.22%), and *Penicillium expansum* (0.15%). Less fungal frequency was recorded with *Rhizopus stolinifer* which gave 0.07%.

3.3. Determination of mycotoxins production

Mycotoxins are secondary metabolites that are produced by fungi under suitable conditions. Determination of mycotoxins produced by mycotoxigenic fungi (*Alternaria alternata*, *Aspergillus flavus*, *A. parasiticus*, and *Penicillium expansum*) isolated from tomato fruits resulted that, *Penicillium expansum* (isolate No. 1) from location B produced the highest mycotoxin quantity (Citrinin), which recorded 1.880 µg/ml, while each of *Alternaria alternata* (isolates No. 2 & 29) from locations D & A produced Alternariol toxin with concentrations of 1.410 and 1.100µg/ml respectively. *A. parasiticus* (isolate No. 1) from location A produced least mycotoxin quantity (Aflatoxins), where it produced 0.014 µg/ml (0.002 AFB2 and 0.012 AFG2). None of *A. flavus* isolates was Aflatoxin producers as shown in Table 3

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Table 2: Fungal frequencies associated with tomato fruits

Locations		Fungi									Total
		Altarnaria alternata	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus parasiticus*	*Colletetrichum* sp.	*Geotrichum candidum*	*Mucor* sp.	*Penicillium expansum*	*Rhizopus stolinifer*	
A	**T.C**	36	NF	1	4	64	115	5	1	NF	226
	%	2.62	-	0.07	0.29	4.66	8.38	0.36	0.07	-	16.47
B	**T.C**	16	3	NF	NF	51	88	3	1	NF	162
	%	1.17	0.22	-	-	3.71	6.41	0.22	0.07	-	11.80
C	**T.C**	6	NF	NF	NF	20	91	0	NF	1	118
	%	0.44	-	-	-	1.46	6.63	0.00	-	0.07	8.59
D	**T.C**	2	NF	5	3	92	225	6	NF	NF	333
	%	0.15	-	0.36	0.22	6.70	16.39	0.44	-	-	24.25
E	**T.C**	NF	NF	NF	NF	126	135	14	NF	NF	275
	%	-	-	-	-	9.18	9.83	1.02	-	-	20.03
F	**T.C**	NF	NF	NF	NF	57	194	8	NF	NF	259
	%	-	-	-	-	4.15	14.13	0.58	-	-	18.86
Total	**T.C**	60	3	6	7	410	848	36	2	1	1373
	%	4.37	0.22	0.44	0.51	29.86	61.76	2.62	0.15	0.07	100

NF=Not Found

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Table 3: Determination of mycotoxins production by toxigenic fungi associated with tomato fruits

Locations	Toxigenic fungi	Isolate No.	Mycotoxins (µg/ml)						
			Alternariol	Aflatoxins					Citrinin
				AFB_1	AFG_1	AFB_2	AFG_2	Total Aflatoxins	
A	***Alternaria alternata***	29	1.100	-	-	-	-	-	-
	Aspergillus flavus	-	-	ND	ND	ND	ND	ND	-
	Aspergillus parasiticus	1	-	ND	ND	0.002	0.012	0.014	-
	Penicillium expansum	-	-	-	-	-	-	-	ND
B	***Alternaria alternata***	-	ND	-	-	-	-	-	-
	Aspergillus flavus	NF	-	-	-	-	-	-	-
	Aspergillus parasiticus	NF	-	-	-	-	-	-	-
	Penicillium expansum	1	-	-	-	-	-	-	1.880
C	***Alternaria alternata***	-	ND	-	-	-	-	-	-
	Aspergillus flavus	NF	-	-	-	-	-	-	-
	Aspergillus parasiticus	NF	-	-	-	-	-	-	-
	Penicillium expansum	NF	-	-	-	-	-	-	-
D	***Alternaria alternata***	2	1.410	-	-	-	-	-	-
	Aspergillus flavus	-	-	ND	ND	ND	ND	ND	-
	Aspergillus parasiticus	-	-	ND	ND	ND	ND	ND	-
	Penicillium expansum	NF	-	-	-	-	-	-	-
E	***Alternaria alternata***	NF	-	-	-	-	-	-	-
	Aspergillus flavus	NF	-	-	-	-	-	-	-
	Aspergillus parasiticus	NF	-	-	-	-	-	-	-
	Penicillium expansum	NF	-	-	-	-	-	-	-
F	***Alternaria alternata***	NF	-	-	-	-	-	-	-
	Aspergillus flavus	NF	-	-	-	-	-	-	-
	Aspergillus parasiticus	NF	-	-	-	-	-	-	-
	Penicillium expansum	NF	-	-	-	-	-	-	-

NF= Not Found, ND = Not Detected

3.4. The chemical compounds in cinnamon and fennel essential oils identified by GC-MS

The identification and quantification of cnnamon and fennel essential oils and their fractions were determined using GC/MS and summarized in Table 4 and Fig. 1. The obtained data indicated that seven compounds were detected in cinnamon essential oil, while Seventeen compounds were identified in fennel essential oil.

Table 4: The chemical compounds in cinnamon and fennel essential oils identified by GC-MS.

Serial No.	RT	Constituent name	Formula	% Content	
				Cinnamon oil	Fennel oil
1	**16.789**	5-(Isothiocyanato)hept-1-en-6-yne	C8H9NS	0.58	-
2	**26.914**	Benzenemethanol	C7H8O	26.06	0.19
3	**27.081**	2-Propenal, 3-phenyl-	C9H8O	0.54	-
4	**32.001**	Cinnamaldehyde, (E)-	C9H8O	69.6	0.24
5	**35.307**	Eugenol	C10H12O2	3.1	-
6	**38.542**	Cinnamaldehyde Propylene Glycol Acetal	C12H14O2	0.04	-
7	**49.76**	Butanoic acid, 3-oxo-, phenylmethyl ester	C11H12O3	0.07	-
8	**4.366**	.alpha.-Pinene, (-)-	C10H16	-	0.30
9	**7.132**	.beta.-Myrcene	C10H16	-	0.14
10	**8.218**	Limonene	C10H16	-	1.23
11	**8.378**	dl-Limonene	C10H16	-	0.81
12	**13.37**	Fenchone	C10H16O	-	0.24
13	**13.619**	L-Fenchone	C10H16O	-	0.09
14	**13.744**	.alpha.-Thujone	C10H16O	-	0.06
15	**23.021**	Estragole	C10H12O	-	92.78
16	**23.14**	Z-Citral	C10H16O	-	1.38
17	**23.846**	E-Citral	C10H16O	-	1.93
18	**24.184**	.beta.-Gurjunene	C15H24	-	0.01
19	**25.484**	trans-Anethole	C10H12O	-	0.25
20	**29.977**	Benzaldehyde, 3-methoxy-	C8H8O2	-	0.14
21	**31.538**	2-Propenoic acid, 3-phenyl-, methyl ester	C10H10O2	-	0.02
22	**46.151**	trans-4-Methoxycinnamaldehyde	C10H10O2	-	0.18

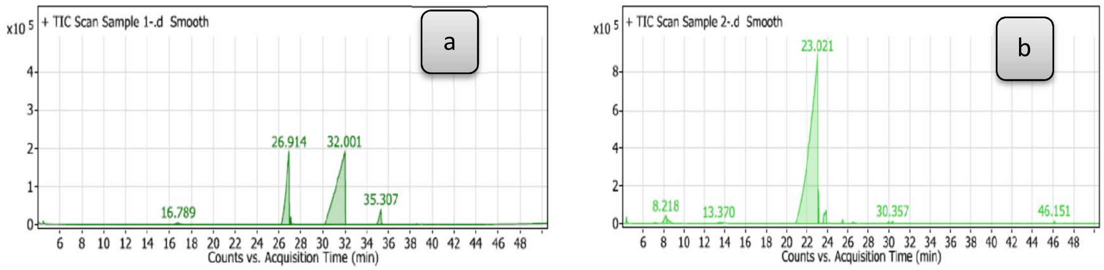

Fig. 1: Chromatograms of the chemical compounds identified by GC-MS of: a. Cinnamon oil, b. Fennel oil.

Cinnamaldehyde, (E)- compound was the major constituent in cinnamon oil, where it represents 69.6%, followed by Benzenemethanol (26.06%), Eugenol (3.1%), 5-(Isothiocyanato)hept-1-en-6-yne (0.58%), 2-Propenal, 3-phenyl- (0.54%), Butanoic acid, 3-oxo-, phenylmethyl ester (0.07%), and Cinnamaldehyde Propylene Glycol Acetal (0.04%). On the other hand, Estragole compound was the major constituent in fennel oil (92.78%), followed by Citral compound, which recorded 3.31% (1.93 % E-Citral and 1.38 % Z-Citral), Limonene compound, which represents 2.04% (1.23%Limonene and 0.81%dl-Limonene), Fenchone compound, which recorded 0.33% (0.24% Fenchone and 0.09% L-Fenchone), .alpha.-Pinene, (-)- (0.30%), trans-Anethole (0.25%), Cinnamaldehyde, (E)- (0.24%), Benzenemethanol (0.19%), trans-4-Methoxycinnamaldehyde (0.18%), each of .beta.-Myrcene and benzaldehyde, 3-methoxy- recorded (0.14%), alpha.-Thujone (0.06%), and 2-Propenoic acid and 3-phenyl-, methyl ester (0.02%), while .beta.-Gurjunene recorded (0.01%)

3.5. *In vitro* antifungal activity of cinnamon and fennel essential oils (mycelial growth inhibition)

The antifungal activity of cinnamon and fennel essential oils and their mixture were evaluated by employing various concentrations (0.125 %, 0.25 %, and 0.50 %) against the tested fungi (*Alternaria alternata*, *Aspergillus flavus*, *A. parasiticus,* and *Penicillium expansum*). Data in Table 5 and Fig. 2 indicated that cinnamon and fennel essential oils and their mixture reduced significantly ($P < 0.05$) the fungal growth of the tested fungi at all concentrations used compared with the untreated control. Also, the growth inhibition was increased by increasing the concentration used. On the other hand, cinnamon oil and its combination with fennel oil gave the highest antifungal activity against the tested fungi, in which cinnamon oil completely inhibited the fungal growth of *Alternaria alternata*, *A. parasiticus*, and *Penicillium expansum* at all concentrations used, while completely inhibited *A. flavus* growth at concentration of 0.50%, and gave 83.13 % and 68.75 % reduction at 0.25 % and 0.125 % respectively. The combination of cinnamon and fennel essential oils also completely inhibited the mycelial growth of *Alternaria alternata*, and *Penicillium expansum* at 0.25 %, and 0.50 %, and *A. flavus* at 0.50 %, while giving 91.33 %reduction of *A. parasiticus* growth at 0.50 %, and gave 86.67 % and 81.25 % reduction of *A. parasiticus* and *A. flavus* growth at 0.25 % respectively. At 0.125 %, the reduction of mycelial growth was recorded 82.00 %, 78.12 %, 72.50 %, and 71.43 % for *A. parasiticus*, *A. flavus*, *Penicillium expansum*, and *Alternaria alternata* respectively. Fennel oil at 0.50% reduced the mycelial growth of *Alternaria alternata*, *A. parasiticus, Penicillium expansum*, and *A. flavus* with reduction percent of 81.90 %, 80.00 %, 68.75 %, and 65.62 % respectively, while gave 74.29 %, 66.67 %, 46.87 %, and 31.25 % reduction at a concentration of 0.25 % and gave 62.86 %, 60.00 %, 43.75 %, and 15.63 % reduction at 0.125 % for *Alternaria alternata*, *A. parasiticus*, *A. flavus* and *Penicillium expansum* growth respectively.

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Table 5: *In vitro* antifungal activity of cinnamon and fennel essential oils (mycelial growth inhibition).

Essential oils	Conc. %	*Alternaria alternata*		*Aspergillus flavus*		*Aspergillus parasiticus*		*Penicillium expansum*		LSD 5%
		linear growth (mm)	R %	linear growth (mm)	R %	linear growth (mm)	R %	linear growth (mm)	R %	
Cinnamon oil	0.125 %	5.00 ± 0.10 a	100	25.00 ± 0.64 b	68.75	5.00 ±0.10 a	100	5.00 ± 0.17 a	100	1.0052A
	0.25 %	5.00 ± 0.20 a	100	13.50 ± 0.34 ab	83.13	5.00 ±0.15 a	100	5.00 ± 0.12 a	100	
	0.50 %	5.00 ± 0.15 a	100	5.00 ± 0.10 a	100	5.00 ±0.13 a	100	5.00 ± 0.01 a	100	
Fennel oil	0.125 %	19.50 ± 0.01 b	62.86	45.00 ± 0.71 c	43.75	30.00 ±0.60 c	60.00	67.50 ± 0.10 d	15.63	1.0052B
	0.25 %	13.50 ± 0.31 ab	74.29	42.50 ± 0.32 c	46.87	25.00 ±0.10 c	66.67	55.00 ± 0.15 c	31.25	
	0.50 %	9.50 ± 0.01 a	81.90	27.50 ± 0.01 b	65.62	15.00 ±0.50 b	80.00	25.00 ± 0.30 b	68.75	
The mixture of Cinnamon & Fennel oils	0.125 %	15.00 ± 0.20 b	71.43	17.50 ± 0.14 ab	78.12	13.50 ±0.41 b	82.00	22.00 ± 0.22 b	72.50	1.0052 A
	0.25 %	5.00 ± 0.10 a	100	15.00 ± 0.01 ab	81.25	10.00 ±0.32 ab	86.67	5.00 ± 0.10 a	100	
	0.50 %	5.00 ± 0.11 a	100	5.00 ± 0.10 a	100	6.50 ±0.10 a	91.33	5.00 ± 0.15 a	100	
Control		52.50 ± 0.64 c		80.00 ± 0.15 d		75.00 ± 0.22 d		80.00 ± 0.35 e		1.0052C
LSD 5%		1.1338 A		1.1338 B		1.1338 AB		1.1338 B		

Results are mean values of three replicates ± standard deviation. The different letters in each column indicate significant differences at P<05, R % = Reduction percent

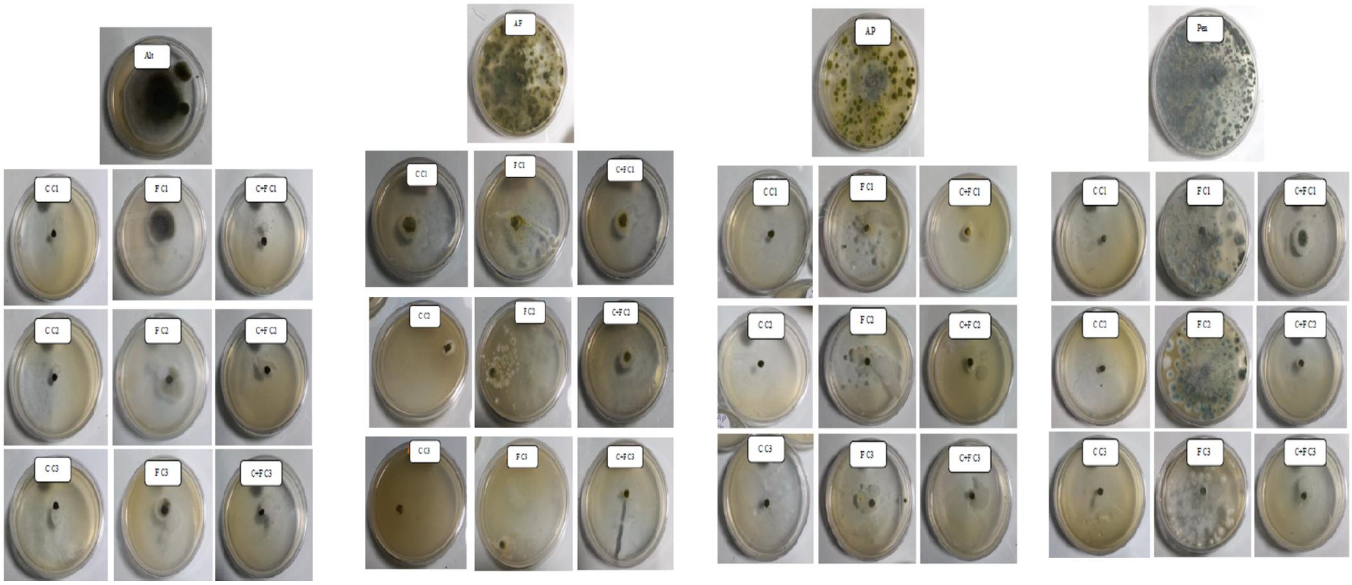

Fig. 2: Alt= *Alternaria alternata*, A.F= *Aspergillus flavus*, A.P= *A.parasiticus*, Pen= *Penicillium expansum*, CC1= Cinnamon oil (0.125 %), CC2 = Cinnamon oil (0.25 %), CC3= Cinnamon oil (0.50 %), FC1= Fennel oil (0.125 %), FC2= Fennel oil (0.25 %), FC3= Fennel oil (0.50 %), F+C C1= Mixture of Cinnamon and Fennel oils (1:1) (0.125 %), F+C C2= Mixture of Cinnamon and Fennel oils (1:1) (0.25 %), F+C C3= Mixture of Cinnamon and Fennel oils (1:1) (0.50 %).

3.6. In vivo assessment of antifungal activity of cinnamon and fennel essential oils on tomato fruits spoilage

The antifungal activity of cinnamon and fennel essential oils and their mixture were evaluated by employing 0.50 % conc. on tomato fruits spoilage caused by mycotoxigenic fungi (*Alternaria alternata*, *Aspergillus parasiticus,* and *Penicillium expansum*) *in vivo*. Data in Table 6 and Fig. 3 indicated that cinnamon and fennel essential oils and their mixture reduced significantly ($P < 0.05$) the fungal growth of the tested fungi on tomato fruits compared with untreated control.

Table 6: *In vivo* assessment of antifungal activity of cinnamon and fennel essential oils on tomato fruits spoilage.

Essential oils	Conc.	*Alternaria alternata*		*Aspergillus parasiticus*		*Penicillium expansum*		LSD 5%
		Linear growth (mm)	R %	Linear growth (mm)	R %	Linear growth (mm)	R %	
Cinnamon oil	**0.50 %**	12.00 ±0.10 [a]	65.71	15.00 ± 0.10 [a]	57.14	15.00 ± 0.17 [a]	64.71	0.1275 A
Fennel oil	**0.50 %**	17.50 ±0.22[a]	50.00	16.00 ± 0.35 [a]	54.29	21.00 ± 0.10 [a]	50.59	0.1751 A
The mixture of Cinnamon & Fennel oils	**0.50 %**	15.00 ±0.30 [a]	57.14	16.00 ± 0.20 [a]	54.29	20.00 ± 0.27 [a]	52.94	0.1997 A
Control		35.00 ±0.21 [b]		35.00 ± 0.10 [b]		42.50 ± 0.34 [b]		0.6164 B
LSD 5%		0.6538 A		0.5602 A		0.7528 A		

Results are mean values of three replicates ± standard deviation. The different letters in each column indicate significant differences at P<05. R % = Reduction percent

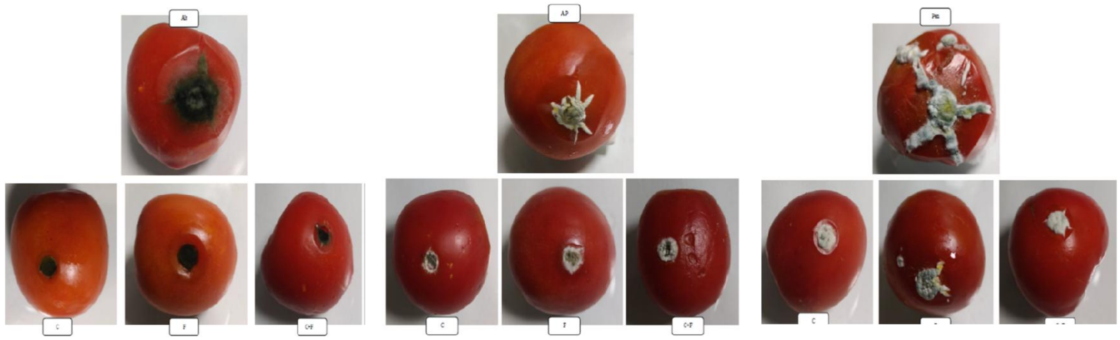

Fig. 3: Alt= *Alternaria alternata*, A.P= *A.parasiticus*, Pen= *Penicillium expansum*, C= Cinnamon oil (0.50 %), F= Fennel oil (0.50 %), F+C = Mixture of Cinnamon and Fennel oils (1:1) (0.50 %).

On the other hand, cinnamon oil recorded 65.71%, 64.71%, and 57.14 % reduction for *Alternaria alternata*, *Penicillium expansum*, and *A. parasiticus* growth on tomato fruits respectively; while fennel oil reduced the mycelial growth of *A. parasiticus*, *Penicillium expansum*, and *Alternaria alternata* on tomato fruits with 54.29 %, 50.59 % and 50.00 % reduction respectively. On the other hand, the combination of cinnamon and fennel essential oils recorded 57.14 %, 54.29 %, and 52.94 % reduction for *Alternaria alternata*, *A. parasiticus,* and *Penicillium expansum* growth on tomato fruits respectively.

4. Discussion

Tomato, scientifically known as *Solanum lycopersicum*, is widely considered the most popular vegetable globally. However, due to its high moisture content, tomatoes are prone to spoilage at a high rate. This is a concern because various harmful fungi that lead to diseases are extensively studied, as they negatively impact the productivity and quality of tomato fruits (Lamidi *et al.,* 2020). A total of 1373 fungal isolates belonging to nine different fungal species were obtained from tomato fruit samples from six different locations. The identified species included *Alternaria alternata, Aspergillus niger, A. flavus, A. parasiticus, Colletetrichum* sp*., Geotrichum candidum*, *Mucor* sp., *Penicillium expansum*, and *Rhizopus stolinifer*. Among these, *Geotrichum candidum* (61.76%), *Colletetrichum* sp. (29.86%), and *Altarnaria alternate* (4.37%) were the most predominant fungi. Similar results were detected by Maroutti and Valente (2009), who identified *A. flavus* and *A. parasiticus* in fresh tomato fruits and their by-products. In the study conducted by Rodrigues and Kakde (2019), each of *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Alternaria alternate*, *Colletotrichum gloeosporioides*, and *Rhizopus stolonifer* were frequently isolated from tomato fruits. Less frequently isolated fungi with varying frequencies included *Botrytis cinerea, Penicillium chrysogenum, Penicillium digitatum, Phoma* sp., *Cladosproium* sp., and *Geotrichum candidum*. Aborisade *et al.,* (2020) detected *Aspergillus niger*, *Aspergillus parasiticus*, yeast, *Fusarium* sp., and *Penicillium* sp. in spoiled tomato samples from Nigeria. Lamidi *et al.,* (2020) isolated *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp. from tomato fruits purchased at the Anyigba main market, with *Aspergillus* spp. being the most frequently encountered and *Fusarium* spp. being the least common. Kłapeć *et al.,* (2021) identified various fungal species, including *Acremonium* spp., *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *Phoma* spp., and *Trichoderma* spp. in tomato fruits. Sanzani *et al.,* (2021) reported that out of 71 fungal isolates from tomato fruits, 37% were from the Penicillium genus, 34% from Aspergillus, 18% from Alternaria, and 11% from other fungal genera such as Rhizopus and Fusarium. Slama *et al.,* (2022) identified *Alternaria alternata, Penicillium olsonii, Ulocladium atrum, Phytophthora nicotianae*, and *Aspergillus fumigatus* as the five fungi most commonly found in tomato fruits. These variations in fungal species can be attributed to climatic factors such as rainfall and temperature. Temperature and humidity play crucial roles in the growth of fungal pathogens on vegetable fruits and significantly impact the occurrence and severity of fungal diseases in plants (Rodrigues and Kakde 2019). Additionally, the high number of fungal colonies isolated from tomatoes could be due to physical damage and punctures that may occur during harvesting and post-harvest activities such as transportation, storage, marketing, and poor handling, which provide entry points for microbial contamination (Hayatu 2000).

Tomatoes are highly vulnerable to fungal colonization due to their soft outer skin and high moisture content (Moss 1984). Furthermore, some of these fungi can produce mycotoxins that pose risks to both human and animal health (Sanzani *et al.,* 2019). The contamination of food by mycotoxin-producing fungi, particularly those from the Alternaria, Aspergillus, Fusarium, and Penicillium genera, is a major global concern that threatens food safety and human well-being (Alizadeh *et al.,* 2022). Determination of the mycotoxins produced by mycotoxigenic fungi (*Alternaria alternata*, *A. flavus, A. parasiticus*, and *Penicillium expansum*) isolated from tomato fruits resulted that, *Alternaria alternata* isolates No. (2 & 29) from locations D & A produced Alternariol toxin at levels of 1.410 µg/ml and 1.100 µg/ml respectively, and *A. parasiticus* isolate No. (1) from location A was found to produce aflatoxins (0.014 µg/ml (0.002 AFB2 and 0.012 AFG2). Furthermore, *Penicillium expansum* isolate (No. 1) from location B produced citrinin at a concentration of 1.880 µg/ml. Similar findings were reported by Harwig *et al.,* (1979), who identified the production of citrinin by a *Penicillium expansum* isolate from tomatoes at levels up to 0.76 µg/g. Andersen and Frisvad (2004) also mentioned that *P. expansum* isolated from tomato fruits can produce citrinin in pure culture. Moreover, Muhammad *et al.,* (2004) detected aflatoxins in rotten tomatoes from various markets in Sokoto, Nigeria, indicating the association of *A. flavus* with aflatoxin production in tomato fruits. Kalyoncu *et al.,* (2005) found that among the species isolated from tomatoes, *Aspergillus flavus* and *A. parasiticus* were potential mycotoxin producers, specifically aflatoxins. Maroutti and Valente (2009) reported the identification of *A. flavus* and *A. parasiticus*, as well as the presence of aflatoxins, in fresh tomatoes and tomato by-products such as pulp, paste, purée, ketchup, dehydrated tomatoes, and dried tomatoes preserved in oil. In Lebanon, Habib *et al.,* (2021) assessed the ability of forty-nine Alternaria strains isolated from diseased tomato samples to produce mycotoxins such as alternariol monomethyl ether (AME), alternariol (AOH), altenuene (ALT), and tenuazonic acid (TeA). They found that the majority of tested strains produced AOH, AME, and ALT. Kłapeć *et al.,* (2021) reported a prevalence of 60.0% for total aflatoxin in forty-five tomato samples in Poland, with a recorded rate of 20.0% for aflatoxin B1. In Iran, Alizadeh *et al.,* (2022) found that *Alternaria* spp. were the main mycotoxin-producing fungi in tomato samples. These fungi were responsible for the production of alternariol, alternariol monomethyl ether, and tenuazonic acid. The accumulation of mycotoxins in agricultural food products is influenced by various environmental conditions that impact both pre-harvest mold development and subsequent mycotoxin production (Magan *et al.,* 2011). Intrinsic factors of tomatoes, such as pH levels (4.2–4.3) and water activity (aw) (Suleiman *et al.,* 2017), create conditions favorable for fungal growth and mycotoxin production (Cabral *et al.,* 2019). Certain mycotoxin-producing fungi, including *Penicillium patulum, Alternaria citri*, and specific species of Aspergillus, can thrive in low water activity conditions (0.84–0.64) (Suleiman *et al.,* 2017). This can raise concerns regarding the production of mycotoxins in tomatoes.

Gas-chromatography mass-spectrometry (GC-MS) is a widely used analytical technique for identifying phyto-constituents in plant materials (Ahmad *et al.,* 2018). Using GC-MS, the essential oils of cinnamon and fennel were analyzed, resulting in the detection of seven compounds in cinnamon oil and seventeen compounds in fennel oil. The major constituent in cinnamon oil was cinnamaldehyde, accounting for 69.6% of the oil, while in fennel oil; the concentration of cinnamaldehyde was 0.24%. Previous studies conducted in different regions have also identified cinnamaldehyde as the main volatile substance in cinnamon essential oil (Farias *et al.,* 2020; Gotmare and Tambe 2019; Li *et al.,* 2021; Valková *et al.,* 2022). On the other hand, the major constituent in fennel oil was found to be estragole, comprising 92.78% of the oil. This finding is consistent with studies conducted by multiple researchers (Abd-Elhafeez *et al.,* 2023; Afifi *et al.,* 2021; Wodnicka *et al.,* 2019). However, in China, He and Huang (2011) reported that fennel essential oil had relatively high concentrations of α-phellandrene, α-pentene, and fenchone, while estragole and (E)-anethole were present in low amounts. On the other hand, various compounds were also identified in cinnamon and fennel essential oils such as benzene methanol, which was detected in both oils, with concentrations of 26.06% and 0.19% for cinnamon and fennel oils, respectively. Cinnamon oil also contained eugenol (3.1%), 5-(isothiocyanate)hept-1-en-6-yne (0.58%), 2-propenal, 3-phenyl- (0.54%), butanoic acid, 3-oxo-, phenylmethyl ester (0.07%), and cinnamaldehyde propylene glycol acetal (0.04%). These findings align with previous studies. For instance, El-Baroty *et al.,* (2010) reported that cinnamon essential oil in Egypt primarily consisted of 2-propenal, 3-phenyl, and eugenol. In Turkey, Kaskatepe *et al.,* (2016) identified cinnamaldehyde propylene glycol acetal in cinnamon essential oil. In Slovakia, Valková *et al.,* (2022) reported that the main component of cinnamon essential oil was eugenol. While other compounds were detected in fennel oil in the current study, including citral (3.31%), limonene (2.04%), fenchone (0.33%), α-pinene, (-)- (0.30%), trans-anethole (0.25%), trans-4-methoxycinnamaldehyde (0.18%), β-myrcene (0.14%), benzaldehyde, 3-methoxy-(3-anisaldehyde) (0.14%), α-thujone (0.06%), 2-propenoic acid, 3-phenyl-, methyl ester (t-methyl cinnamate) (0.02%), and β-gurjunene (0.01%). These compounds identified in the current study were similar to those found in previous studies. In Morocco, Khalid *et al.,* (2015) reported that the analysis of essential oils obtained from *Foeniculum vulgare* Mill seeds revealed the presence of various compounds. These include trans-anethole, estragole, α-pinene, limonene, α-thujone and fenchone. In Saudi Arabia, Alam *et al.,* (2019) discovered that the main compounds in the methanolic extract of *F. vulgare* were trans-anethole, fenchone, benzaldehyde-3-methoxy, and D-Limonene. In Egypt, Afifi *et al.,* (2021) identified trans-anethole , fenchone, limonene, p-anisaldehyde, myrcene, and α-pinene in *F. vulgare* fruit oil in Fayoum and Minia. While Abd-Elhafeez *et al.,* (2023) conducted GC/MS analysis of fennel essential oil and detected estragole, trans-anethole, D-limonene, fenchone, β-myrcene, and Z-citral. These quantitative and qualitative variations in the chemical composition of essential oils seen in different countries can be attributed to various factors, including agro-climatic factors such as temperature, humidity, climate conditions, and the time of plant collection (Shahat *et al.,* 2011). Geography also plays a role (Diaz-Maroto *et al.,* 2006; Raal *et al.,* 2011), as well as the cultivated variety and the method of extraction (Hammouda *et al.,* 2013). Furthermore, the analysis conditions of essential oils can have a controversial effect on their composition (Diaz-Maroto *et al.,* 2006).

The use of plant products, particularly essential oils, has shown great promise in the development of safer antifungal agents (Varma and Dubey 2001). Evaluation of the antifungal activity of cinnamon and fennel essential oils and their mixture against various fungi (*Alternaria alternata*, *Aspergillus flavus*, *A. parasiticus*, and *Penicillium expansum*) using different concentrations (0.125%, 0.25%, and 0.50%) resulted that, cinnamon and fennel essential oils and their mixture reduced significantly the fungal growth of the tested fungi at all concentrations used compared with untreated control. The inhibitory effect on fungal growth increased with increasing the concentrations of the oils. On the other hand, cinnamon oil and its combination with fennel oil exhibited the highest antifungal activity. Where cinnamon oil completely inhibited the growth of *Alternaria alternata*, *A. parasiticus*, and *Penicillium expansum* at all concentrations and inhibited *A. flavus* growth at a concentration of 0.50%. The combination of both oils also completely inhibited the growth of *Alternaria alternata* and *Penicillium expansum* at concentrations of 0.25% and 0.50%, and *A. flavus* at 0.50%. These findings align with previous studies, such as Juglal *et al.,* (2002), who found that cinnamon and turmeric oils were effective against mycotoxin-producing moulds (*Aspergillus parasiticus* and *Fusarium moniliforme*), where cinnamon oil completely suppressed the growth of *A. parasiticus*. Wodnicka *et al.,* (2019) evaluated the fennel essential oil cultivated in Poland and Egypt and found that it exhibited varied activity against tested strains of pathogenic fungi (*Altarnaria alternate*, *Fusarium avenceum*, *F. culmorum*, *F. graminarum* and *Penicillium ochrochloron*). Devecioglu *et al.,* (2022) reported that cinnamon essential oil showed antifungal activity against *Penicillium carneum, Aspergillus flavus*, and *A. niger*. Naz *et al.,* (2022) evaluated the essential oils of eight medicinal plants for their antifungal activity against mycotoxigenic fungi (*Aspergillus parasiticus*) and found that *Cinnamomum verum* exhibited the highest antifungal activity. Finally, Abd-Elhafeez *et al.,* (2023) found that fennel essential oil exhibited a significant antifungal effect against *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium sphaerospermm*, *Mucor racemosus*, *Penicillium chrysogenum*, and *Rhizopus arrhizus*.

Mycotoxigenic fungi pose a threat to agricultural crops and food safety (Who 2018). While fungicides can control fungal diseases, they have adverse effects on human health (Juroszek and Von Tiedemann 2011). Therefore, natural alternatives are reliable sources for plant disease control (Mehta and Sharma 2016). Essential oils (EOs) are important alternatives that play a crucial role in plant protection and food preservation by managing plant pathogenic fungi and improving crop safety and quality (Bhavaniramya *et al.,* 2019). Evaluation of the antifungal activity of cinnamon and fennel essential oils, as well as their mixture (at a concentration of 0.50%), on mycotoxigenic fungi (*Alternaria alternata*, *Aspergillus parasiticus*, and *Penicillium expansum*) causing tomato fruit spoilage *in vivo* resulted that, significant reductions in fungal growth compared to the untreated control. It was also noted that there were only synergistic effects and no antagonistic effects when the essential oils were combined. These findings are consistent with previous studies, such as Abd-El-Aziz (2003), who reported that cinnamon and camphor oils effectively reduced decay incidence in tomato fruits artificially inoculated with *Alternaria alternata*. Similarly, Mostafa *et al.,* (2007) tested the effectiveness of fennel oil in controlling tomato fruit rot diseases caused by *Alternaria tenuis* and *Stemphylium botryosum* during storage at different temperatures and found that fennel oil significantly reduced the incidence of fruit rot diseases caused by the tested fungi. Abdolahi *et al.,* (2010) observed that the essential oils of fennel, ajowan, and caraway exhibited inhibitory effects on tomato fruit infections caused by *Alternaria alternate* and *Penicillium digitatum*, where Fennel and ajowan oils were the most effective, and the inhibitory effects of the essential oils were dose-dependent. Raafat *et al.,* (2016) tested the effect of cinnamon and spearmint oils on cherry tomato fruits with spoilage pathogens (*Alternaria alternate* and *Botrytis cinerea*) and observed that, a higher efficacy against *A. alternaria* compared to *B. cinerea* for cinnamon and spearmint oil. Kalleli *et al.,* (2019) reported that treatment with fennel oil suppressed Fusarium wilt in tomato plants after two weeks from artificial inoculation with *Fusarium oxysporum*. The variation in the antifungal activity of cinnamon and fennel essential oils may be attributed to factors such as the specific properties and metabolic capabilities of the pathogenic strains, as well as differences in the chemical composition of the oils. The antifungal properties of essential oils may be linked to the presence of specific bioactive substances in their composition (Bakkali *et al.,* 2008). Where Kazemi *et al.,* (2012) reported that the antifungal activity of fennel oil is associated with compounds like estragole, trans-anethole, fenchone, and limonene. Valková *et al.,* (2022) suggested that (E)-cinnamaldehyde and eugenol might be responsible for the antifungal effects of cinnamon essential oil. Moreover, the synergistic effects observed when combining cinnamon and fennel essential oils could be attributed to the combined action of all their components. Where Bocquet *et al.,* (2018) confirmed that, the interactions between the essential oil components significantly enhance their biological activity. This synergistic effect would be beneficial in protecting fruits and vegetables during postharvest, as it reduces the likelihood of pathogens developing resistance to the individual components (Bagamboula *et al.,* 2004). Researches have also demonstrated that the overall antifungal activity of essential oils is greater than the individual effects of even the most potent compounds (Terzi *et al.,* 2007).

5. Conclusion

The current study demonstrated that, various types of fungi were associated with tomato fruits, including mycotoxigenic fungi which produced several mycotoxins that caused potential health hazards to humans. On the other hand, based on *in vitro* and *in vivo* assays, it can be concluded that, a significant impact of cinnamon and fennel essential oils as well as their combination on inhibiting the growth of mycotoxigenic fungi was observed in tomato fruits. Moreover, the findings revealed that the cinnamon oil alone and its combination with fennel oil which generated synergistic and additive effects resulted in higher antifungal activity against the tested fungi. Therefore, the application of various plant essential oils can serve as an eco-friendly approach for managing diseases in tomato fruit production, as these oils are characterized by their environmental safety, economic feasibility, absence of residual issues, and cost-effectiveness, making them valuable alternatives to synthetic products.

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Code Availability Not applicable.

Declarations

Ethical approval Not applicable.

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Consent for publication All authors consent to publication.

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References

Abd-El-Aziz, S., 2003. Studies on some phytopathogenic microorganisms causing tomato fruit rot. Ph.D. Thesis, Botany Department, Faculty of Science, Zagazig University.

Abd-Elhafeez, E.M.A., B.R. Ramadan, S.H.M. Abou-El-Hawa, and M.R.A. Rashwan, 2023. Chemical Composition and Antimicrobial Activity of Anise and Fennel Essential Oils. Assiut j. agri. sci., 54 (2): 127-140. http://dx.doi.org/10.21608/ajas.2023.177714.1211

Abdolahi, A., A. Hassani, Y. Ghosta, T. Javadi, and M.H. Meshkatalsadat, 2010. Essential oils as control agents of postharvest Alternaria and Penicillium rots on tomato fruits. J. Food Saf. 30: 341–352. https://doi.org/10.1111/j.1745-4565.2009.00211

Aborisade, W.T., AT Ajao, and AF Jimoh, 2020. Effect of Heat Treatment on Aflatoxin Contents of Tomatoes Samples in Ilorin, Kwara State, Nigeria. Int J. Life Sci. Biotechnol. 3(2): 18-26. http://dx.doi.org/10.38001/ijlsb.664750

Afifi, S.M., A. El-Mahis, A.G. Heiss, and M.A. Farag, 2021. Gas Chromatography−Mass Spectrometry-Based classification of 12 fennel (*Foeniculum vulgare* Miller) varieties based on their aroma profiles and Estragole levels as analyzed using chemometric tools. ACS Omega, 6: 5775−5785.http://dx.doi.org/10.1021/acsomega.0c06188

Ahmad, B.S., T. Talou, Z. Saad, A. Hijazi, M. Cerny, H. Kanaan, A. Chokr, and O. Merah, 2018. Fennel oil and by-products seed characterization and their potential applications. Ind. Crops Prod., 111:92–98.https://doi.org/10.1016/j.indcrop.2017.10.008

Alam, P., M.S. Abdel-Kader, M.H. Alqarni, H.H. Zaatout, S.R. Ahamad, and F. Shakeel, 2019. Chemical composition of fennel seed extract and determination of fenchone in commercial formulations by GC–MS method. J. Food Sci. Technol. 56(5):2395–2403. https://doi.org/10.1007/s13197-019-03695-9.

Alizadeh, A., G. Shakeri, M. Marhamati, and A. Afshari, 2022. A systematic review on the diversity and importance of mycotoxins in tomato and derived products. CABI Reviews, 17: No. 015.http://dx.doi.org/10.1079/cabireviews202217015

Andersen, B., and J.C. Frisvad, 2004. Natural Occurrence of Fungi and Fungal Metabolites in Moldy Tomatoes, J. Agric. Food Chem. 52: 7507-7513. http://dx.doi.org/10.1021/jf048727k

Anzlovar, S., D. Barievic, J.A. Avgustin, and J.D. Koce, 2014. Essential oil of common thyme as a natural antimicrobial food additive. Food Technol. Biotechnol. 52: 263-268.

Bagamboula, C.F., M. Uyttendaele, and J. Debevere, 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and r-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol. 21: 33–42. http://dx.doi.org/10.1016/S0740-0020(03)00046-7

Bakkali, F., S. Averbeck, D. Averbeck, and M. Idaomar, 2008. Biological effects of essential oil - A review. Food Chem. Toxicol. 46: 446–475. http://dx.doi.org/10.1016/j.fct.2007.09.106

Bhavaniramya, S., S. Vishnupriya, M.S. Al-Aboody, R. Vijayakumar, and D. Baskaran, 2019. Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain Oil. Sci. Technol. 2: 49–55. http://dx.doi.org/10.1016/j.gaost.2019.03.001

Bocquet, L., C. Rivière, C. Dermont, J. Samaillie, J.L. Hilbert, P. Halama, A. Siah, and S. Sahpaz, 2018. Antifungal activity of hop extracts and compounds against the wheat pathogen *Zymoseptoria tritici*. Ind. Crop. Prod. 122: 290–297. http://dx.doi.org/10.1016/j.indcrop.2018.05.061

Brackett, R.E., 1988. Changes in the microflora of packaged fresh tomatoes. J. Food Qual. 2: 89-105. http://dx.doi.org/10.1111/j.1745-4557.1988.tb00870.x

Cabral, L., A. Rodríguez, J. Delgado, and A. Patriarca, 2019. Understanding the effect of postharvest tomato temperatures on two toxigenic *Alternaria* spp. strains: Growth, mycotoxins and cell-wall integrity-related gene expression. J. Sci. Food Agric. 99(15):6689–95. https://doi.org/10.1002/jsfa.9950

Coton, M., A. Auffret, E. Poirier, S. Debaets, E. Coton, and P. Dantigny, 2019. Production and migration of ochratoxin A and citrinin in Comté cheese byan isolate of *Penicillium verrucosum* selected among *Penicillium* spp. mycotoxin producers in YES medium. Food Microbiol. 82: 551–559. https://doi.org/10.1016/j.fm.2019.03.026

Devecioglu, D., M. Turker, and F. Karbancioglu-Guler, 2022. Antifungal activities of different essential oils and their electrospun nanofibers against *Aspergillus* and *Penicillium* species isolated from bread. ACS Omega 7: 37943−37953. http://dx.doi.org/10.1021/acsomega.2c05105

Diaz-Maroto, M.S., S. Perez-Coello, J. Esteban, and J. Sanz, 2006. Comparison of the volatile composition of wild fennel samples (*Foeniculum vulgare* Mill). from central Spain. J. Agric. Food Chem. 54: 6814–6818. http://dx.doi.org/10.1021/jf0609532

El-Baroty, G.S., H.H. Abd El-Baky, R.S. Farag, and M.A. Saleh, 2010. Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. Afr. J. Biochem. Res. 4(6): 167 – 174.

Enyiukwu, D.N., A.N. Awurum, , and J.A. Nwaneri, 2014. Efficacy of plant derived pesticides in the control of myco-induced post-harvest rots of tubers and agricultural products. Net J. Agric. Sci. 2(1): 30-45.

Etebu, E., A.B. Nwauzoma, and D.D.S. Bawo, 2013. Postharvest spoilage of tomato (*Lycopersicon esculentum* Mill.. and control strategies in Nigeria. J. Biol. Agric. Health. 3: 51- 61.

Farias, A.P.P., O.D.S. Monteiro, J.K.R. da Silva, P.L.B. Figueiredo, A.A.C. Rodrigues, I.N. Monteiro , and J.G.S. Maia, 2020. Chemical composition and biological activities of two chemotype-oils from *Cinnamomum verum J. Presl* growing in North Brazil. J. Food Sci. Technol. 57: 3176 – 3183. http://dx.doi.org/10.1007/s13197-020-04288-7

Fawole, M.O., and B.A. Oso, 1998. Laboratory Manual of Microbiology. Ibadan: *Spectrum Books Limited*, 26-31.

Gomez, K.A., and A.A. Gomez, 1984. Statistical procedures for Agricultural Research. Interscience Publication. New York.

Gotmare, S., and E. Tambe, 2019. Identification of Chemical Constituents of Cinnamon Bark Oil by GCMS and Comparative Study Garnered from Five Different Countries. Glob. j. sci. front. res., C Biological Science 19 (1): Version 1.0.

Habib W, M Masiello, R El Ghorayeb, E Gerges, A Susca, G Meca, JM Quiles, AF Logrieco, and A Moretti, 2021. Mycotoxin Profile and Phylogeny of Pathogenic Alternaria Species Isolated from Symptomatic Tomato Plants in Lebanon. Toxins 13: 513. https://doi.org/10.3390/toxins13080513.

Hammouda, F.M., M.A. Saleh, N.S. Abdel-Azim, K.A. Shams, S.I. Ismail, A.A. Shahat, and I.A. Saleh, 2013. Evaluation of the essential oil of *Foeniculum vulgare* Mill (fennel. fruits extracted by three different extraction methods by GC/MS. Afr. J. Tradit. Complement. Altern. Med. 11: 277-279. http://dx.doi.org/10.4314/ajtcam.v11i2.8

Harwig, J., P.M. Scott, D.R. Stoltz, and B.J. Blanchfield, 1979. Toxins of Molds from Decaying Tomato Fruit. Appl. Environ. Microbiol. 38 (2): 267-274. http://dx.doi.org/10.1128/aem.38.2.267-274.1979

Hayatu, M., 2000. Post-harvest physiological studies of some selected members of family *Solanaceae*. M. Sc Thesis (unpublished). Dept. of Biological Sciences, Bayero University, Kano., 25.

He, W., and B.A. Huang, 2011. A review of chemistry and bioactivities of a medicinal spice: *Foeniculum vulgare*, J. Med. Plant Res. 5: 3595–3600. https://doi.org/10.5897/JMPR.9000022

Jabeen, N., M. Ahmed, S. Shaukat, and I. Salam, 2013. Allelopathic effects of weeds on Wheat (*Triticum Aestivum* L.) germination and growth. Pak. J. Bot. 45(3): 807-811. https://www.researchgate.net/publication/332031606

Juglal, S., R. Govinden, and B. Odhav, 2002. Spice oils for the control of co-occurring mycotoxin producing fungi. J. Food Prot. 65: 683–687. http://dx.doi.org/10.4315/0362-028X-65.4.683

Juroszek, P., and A. Von Tiedemann, 2011. Potential strategies and future requirements for plant disease management under a changing climate. Plant Pathol. J. 60: 100–112. http://dx.doi.org/10.1111/j.1365-3059.2010.02410.x

Kalleli, F., I.B. Salem, N. Boughalleb-M'hamdi, and M. M'hamdi, 2019. *In vitro* and *in vivo* efficiency of fennel essential oil against tomato Fusarium wilt and its promotion effect in plant growth. Int. J. Agric. Environ. Biores. 4: 180–199. http://dx.doi.org/10.35410/IJAEB.2019.4417

Kalyoncu, F., A.U. Tamer, and M. Oskay, 2005. Determination of fungi associated with tomatoes (*Lycopersicum esculentum* M.) and tomato pastes. Plant Pathol. J. 4 (2): 146-149. http://dx.doi.org/10.3923/ppj.2005.146.149

Kaskatepe, B., M.E. Kiymaci, S. Suzuk, E.S. Aslan, S. Cesur, and S. Yildiz, 2016. Antibacterialeffects of cinnamon oil against carbapenem resistant nosocomial *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates. Ind. Crops Prod. 81:191-194. https://doi.org/10.1016/j.indcrop.2015.11.058

Kazemi, M., E. Mousavi, and H. Kharestani, 2012. Chemical compositions and antimicrobial activities of essential oils of *Varthemia persica, Foeniculum vulgare* and *Ferula lycia.* Curr. Res. Bacteriol., 5:42–52. http://dx.doi.org/10.3923/crb.2012.42.52

Khalid, S., B. Mohamed, R. Mhamed, B.E.D. Tariq, J. Fatima, N. Laila, and E. Lhoussaine, 2015. Antifungal potential of the seed and leaf *Foeniculum vulgare* Mill essential oil in liquid and vapor phase against phytopathogenic fungi. J. Appl. Pharm. Sci. 5 (11): 050-054. http://dx.doi.org/10.7324/JAPS.2015.501108

Kłapeć, T., A. Wójcik-Fatla, E. Farian, K. Kowalczyk , G. Cholewa, A. Cholewa, and J. Dutkiewicz, 2021. Levels of filamentous fungi and selected mycotoxins in leafy and fruit vegetables and analysis of their potential health risk for consumers. Ann. Agric. Environ. Med. 28 (4): 585–594. http://dx.doi.org/10.26444/aaem/143031

Kumar, A., R. Shukla, P. Singh, and N. Dubey, 2010. Chemical composition, antifungal and anti aflatoxigenic activities of *Ocimum Sanctum* l. essential oil and its safety assessment as plant based antimicrobial. Food Chem. Toxicol. 48:539-54. https://doi.org/10.1016/j.fct.2009.11.028

Lamidi, Y., G.A. Agieni, and O. Abiodun, 2020. Isolation and identification of fungi associated with Tomato (*Lycopersicon Esculentum* M.. rot. Sumerianz j. agric. vet. 3 (5): 54-56. https://doi.org/10.9790/2380-0907018789

Li, C., Y. Luo, W. Zhang, Q. Cai , X. Wu, Z. Tan, and L.A. Zhang, 2021. Comparative study on chemical compositions and biological activities of four essential oils: *Cymbopogon citratus* (DC.. Stapf*, Cinnamomum cassia* (L.) Presl, *Salvia japonica* Thunb. and *Rosa rugosa* Thunb. J. Ethnopharmacol. 280: 114472. http://dx.doi.org/10.1016/j.jep.2021.114472

Logrieco, A., A. Bottalico, M. Solfrizzo, and G. Mule, 1990. Incidence of *Alternaria* species in grains from Mediterranean countries and their ability to produce mycotoxins. Mycologia, 82: 501-505. https://doi.org/10.1080/00275514.1990.12025914

Magan, N., A. Medina-Vaya, and D. Aldred, 2011. Possible climate-change effects on mycotoxin contamination of food crops pre- and postharvest. Plant Pathol. J. 60: 150–163. http://dx.doi.org/10.1111/j.1365-3059.2010.02412.x

Maroutti, L.R., and L.M. Valente, 2009. Survey of aflatoxins in tomato products. Ciênc. Tecnol. Aliment. 29: 431–434. http://dx.doi.org/10.1590/S0101-20612009000200031

Mehta, S., and K. Sharma, 2016. Natural resources: An ecofriendly and safer alternate to control plant diseases. Int. J. Pharm. Sci. Res. 7: 4327. https://doi.org/10.13040/IJPSR.0975-8232.7(11).4327-40

Moneruzzaman, K.M., A. Hossain, W. Sani, and M. Saifuddin, 2008. Effect of stages of maturity and ripening conditions on the biochemical characteristics of tomato. Am. J. Biochem. Biotechnol. 4:336-344. https://doi.org/10.3844/ajbbsp.2008.336.344

Moss, M.O., 1984. Conditions and factors influencing mycotoxin formation in the field and during the storage of food. Chem. Ind. (London) 15(15): 533–536.

Mostafa, M.A., K.K. Sabet, and M.H. Abou Hatem, 2007. Controlling tomato fruit rots caused by *Alternaria tenuis* and *Stemphylium botryosum* during storage using some plant oils and extracts.Pl. Pathol. Dept. Fac. of Agric. Cairo Univ. J. Agric. Sci. Mansoura Univ. 32 (5): 3497 – 3513. http://dx.doi.org/10.21608/jpp.2007.208139

Muhammad, S., K. Shehu, and N.A. Amusa, 2004. Survey of the market diseases and aflatoxin contamination of tomato (*Lycopersicon esculentum* MILL. fruits in Sokoto, northwestern Nigeria. Nutr. Food Sci. 34(2): 72-76. http://dx.doi.org/10.1108/00346650410529032

Munimbazi, C., and L. Bullerman, 1998. High Performance Liquid Choromatographic method for the determination of moniliformin in corn. J. AOAC Int. 81: 999-10. https://doi.org/10.1093/jaoac/81.5.999

Naz, G., A.A. Anjum, M. Nawaz, S. Iqbal, S. Azeem, T. Ali, and R. Manzoor, 2023. Evaluation of *Cinnamomum verum* Essential Oils against Ochratoxin A-producing *Aspergillus parasiticus* in Stored Wheat, Maize and Rice, Pol. J. Environ. Stud. 32 (1): 667-676. http://dx.doi.org/10.15244/pjoes/155084

Wodnicka, A., E. Huzar, M. Dzięcioł, and M. Krawczyk, 2019. Comparison of the composition and fungicidal activity of essential oils from fennel fruits cultivated in Poland and Egypt, Pol. J. Chem. Technol. 21 (2): 38-42. http://dx.doi.org/10.2478/pjct-2019-0018

Pitt. J.I., and A.D. Hocking, 1997. Fungi and Food Spoilage. Blackic Academic and Professional, London. http://dx.doi.org/10.1007/978-1-4615-6391-4

Raafat, S.M., M.I. Abou-Zaid, M.R. Tohamy, and H.E. Arisha, 2016. Impact of some plant essential oil treatments on controlling cherry tomatoes spoilage, improvement shelf life and quality attributes during storage. Zagazig J. Agric. Res. 43 (3). http://dx.doi.org/10.21608/zjar.2016.101014

Raal, A., A. Orav, and E. Arak, 2011. Essential oil composition of *Foeniculum vulgare* Mill.fruits from pharmacies in different countries. Nat. Prod. Res. 26: 1173-1178. http://dx.doi.org/10.1080/14786419.2010.535154

Raiola, A., M.M. Rigano, R. Calafiore, L. Frusciante, and A. Barone, 2014. Enhancing the health-promoting effects of tomato fruit for biofortified food. Mediators Inflamm. Article ID 139873. http://dx.doi.org/10.1155/2014/139873

Raper, K.B., and D.I. Fennel, 1965. The Genus Aspergillus. Baltimore, Williams and Wilkins,686. https://doi.org/10.2307/3756932

Rodrigues, B.B., and U.B. Kakde, 2019. Post-harvest fungi associated with *Solanum lycopersicum* (Tomato) fruits collected from different markets of Mumbai. Int. Multidiscip. Res. J. 09 (01).

Rubert, J., C. Soler, and J. Mañes, 2012. Application of an HPLC–MS/MS method for mycotoxin analysis in commercial baby foods. Food Chem. 133(1):176-183. http://dx.doi.org/10.1016/j.foodchem.2011.12.035

Samson, R.A., 1979. The Genus *Aspergillus* Described Since 1965. Stud. Mycol. 18: 80.

Sanzani, S.M., F. Djenane, O. Incerti, N. Admane, A. Mincuzzi, and A. Ippolito, 2021. Mycotoxigenic fungi contaminating greenhouse-grown tomato fruit and their alternative control. Eur. J. Plant Pathol. 160: 287–300. http://dx.doi.org/10.1007/s10658-021-02240-9

Sanzani, S.M., T. Gallone, F. Garganese, A.G. Caruso, M. Amenduni, and A. Ippolito, 2019. Contamination of fresh and dried tomato by Alternaria toxins in southern Italy. Food Addit. Contam :Part A 36 (5): 789–799. https://doi.org/10.1080/19440049.2019.1588998.

Shahat, A.A., A.Y. Ibrahim, S.F. Hendawy, E.A. Omer, F.M. Hammouda, F.H. Abdel-Rahman, and M.A. Saleh, 2011. Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. Molecules, 16: 1366–1377. http://dx.doi.org/10.3390/molecules16021366

Singh, S., S. Srivastava, J. Mishra, R. Raaj, and A. Sina, 2014. Evaluation of some plant extract against predominant seed mycoflora of Mungbean *Vigna Radiata* (L.. Wilczek seed. Life sci. leafl. 51: 83- 89.

Sinha, S., M. Jothiramajayam, and M. Ghosh, 2014. Evaluation of toxicity of essential oils palmarosa, citronella, lemongrass and vetiver in human lymphocytes. Food Chem. Toxicol. 68: 71-77. http://dx.doi.org/10.1016/j.fct.2014.02.036

Slama, A., F. Mezni, F. Ayari, and A. Khaldi, 2022. Phytopathogenic /Mycotoxigenic Fungi Infecting *Solanum lycopersicum* Fruits (Market Storage Level). European j. biol. biotechnol. 3 (5). http://dx.doi.org/10.24018/ejbio.2022.3.5.400

Sparkman, D., 2012. Informatics and Mass Spectral Data bases in the Evaluation of Environmental Mass Spectral Data. ILM Publications

Suleiman, M.S., L.C. Nuntah, H.L. Muhammad, S.C. Mailafiya, H.A. Makun, and A.N. Saidu, 2017. Fungi and aflatoxin occurrence in fresh and dried vegetables marketed in Minna, Niger State, Nigeria. J. plant biochem. physiol. 5 (1): 1–4. http://dx.doi.org/10.4172/2329-9029.1000176

Tabaestani, H., N. Sedaghat, E. Pooya, and A. Alipour, 2013. Shelf life improvement and postharvest quality of cherry tomato (*Solanum lycopersium* L.) fruit using basil mucilage edible coating and cumin essential oil. Int. j. agron.plant prod. 4 (9): 2346- 2353.

Talvas, J., C. Caris-veyrat, L. Guy, M. Rambeau, B. Lyan, R. Minet-Quinard, J.A. Lobaccaro, M. Vasson, S. George, and A. Mazur, 2010. Differential effects of lycopene consumed in tomato paste and lycopene in the form of purified extract on target genes of cancer prostatic cells. Am. J. Clin. Nutr. 91: 1716-1724. http://dx.doi.org/10.3945/ajcn.2009.28666

Terzi, V., C. Morcia, P. Faccioli, G. Valè, G. Tacconi, and M. Malnati, 2007. *In vitro* antifungal activity of the tea tree (*Melaleuca alternifolia*. essential oil and its major components against plant pathogens. Lett. Appl. Microbiol. 44: 613–618. http://dx.doi.org/10.1111/j.1472-765X.2007.02128.x

Thirupathi, V., S. Sasikala, and Z. John Kennedy, 2006. Preservation of fruits and vegetables by wax coating, 1-10. In: Science Technology Enterpreeur. (Mittal, H.K. *et al.,* (eds. NSTEDS, DST. Delhi.

Tian, J., X. Ban, H. Zeng, B. Huang, J. He, and Y. Wang, 2011. *In vitro* and *in vivo* activity of essential oil from dill (*Anethumgraveolens* L.. against fungal spoilage of cherry tomatoes. Food Control, 22: 1992-1999.http://dx.doi.org/10.1016/j.foodcont.2011.05.018

Torres, A., H. González, M. Etcheverry, S. Resnik, and S. Chulze, 1998. Production of alternariol and alternariol mono-methyl ether by isolates of *Alternaria* spp. from Argentinian maize, Food Addit. Contam. 15 (1): 56-60. http://dx.doi.org/10.1080/02652039809374598

Valková, V., H. Dˇ úranová, L. Galoviˇcová, N.L. Vukovic, M. Vukic, P.Ł. Kowalczewski, and M. Kaˇcániová, 2022. Application of three types of cinnamon essential oils as natural antifungal preservatives in Wheat bread. Appl. Sci. 12: 10888. http://dx.doi.org/10.3390/app122110888

Varma, J., and N.K. Dubey, 2001. Efficacy of essential oils of *Caesulia axillaris* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. Int. J. Food Microbiol. 68: 207-210. http://dx.doi.org/10.1016/S0168-1605(01)00506-2

Wagacha, J.M., and J.W. Muthomi, 2008. Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. Int. J. Food Microbiol. 124: 1-12. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.01.008

WHO , 2018. Mycotoxins. WHO Factsheets, https://www.who.int/newsroom/fact-sheets/detail/mycotoxins

Younos, M.A., and E.M. Akl, 2022. Evaluation of enzymatic phenolic extract from Garden Cress seed meal against aflatoxigenic fungi isolated from Eggplant fruits. Egypt J. Chem. 65(4): 287–299. https://doi.org/10.21608/EJCHEM.2021.95601.4487