

Control of seed born mycobiota associated with *Glycine max* L. Merr. seeds by a combination of traditional medicinal plants extracts

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Abstract: Seeds from soybean collected from different commercial markets were surveyed for seed-borne fungi. Ninety-eight fungal colonies were isolated all over three monthly isolations constituting twenty-two fungal species. The isolated fungi were belonging to the genera: *Alternaria*, *Aspergillus*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Macrophomina*, *Mucor*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, *Sclerotium*, *Stemphylium*, and *Verticillium*. Assay of the antifungal activity of four ethanolic extracts of clove, cinnamon, garlic, and mint was carried out against the most common fungal species (*Aspergillus flavus*, *A. niger*, *Fusarium solani*, *F. oxysporium*, and *F. moniliforme*), which significantly reduced the growth of tested fungi. Clove extract recorded the highest antimicrobial potentiality against the seed-borne fungal species, followed by cinnamon, garlic, and mint. Clove and cinnamon achieved minimum inhibitory concentration (MIC) values less than that of the reference antifungal drug fluconazole. Simultaneously, garlic and mint recorded MIC values equal to or higher than that of control. A combination of clove and cinnamon, clove and garlic, cinnamon and garlic, cinnamon, and mint extracts increased the mean relative activity percentage. They decreased MIC values below their values when used individually; this indicated synergistic interaction between the two extracts. A combination of clove with mint and garlic with mint recorded a decrease in the percentage of mean relative activity and an increase in MIC values than their values when used individually, indicating antagonistic interaction between them.

Introduction

Soybean (*Glycine max* L.) is an essential oil-producing leguminous plant (Wu *et al.*, 2017). Its high protein, starch, and oil content (Dhungana *et al.*, 2017). Recently, soy protein has been considered a green and renewable substitute for petroleum or animal-derived polymers in biomedical applications (Koshy *et al.*, 2015; Lligadas *et al.*, 2013). Soybean production accounts for approximately 6% of arable land worldwide. The expansion of soybean progresses much faster than other large grains or oils. Soybeans are being used by buyers as modern input (de la Casa *et al.*, 2018). They are predominantly used as intermediate food, feeding stuff, and industrial inputs, not as final consumer goods (Goldsmith, 2008). Just 2% soy protein is processed in soy products such as tofu, soy hamburger, or soy analogs directly by humans. A significant percentage of the remaining 98% is processed and fed to

cattle, including poultry and pigs, in soybean meals (SBM) (Rizzo and Baroni, 2018).

Seed-borne fungi can affect seed production and the food industry due to their effect on germination, growth parameters, and crop production (Ordon *et al.*, 2009). It causes seed and seedling diseases (Seethapathy *et al.*, 2016); it also causes biochemical changes (reduction in carbohydrate, protein, and oil content and production of mycotoxins (Ramirez *et al.*, 2018). Soybean seeds often become loaded with many fungal species (Anwar *et al.*, 2013). The Blotter method is frequently used to detect seed-borne fungi of soybean (Mancini *et al.*, 2016). *Aspergillus*,

Fusarium, and *Rhizoctonia* are mycotoxin-producing fungal genera isolated from soybean seeds. By the time, Filtrates of *Aspergillus niger* reduce the seed germination of soybean and decrease the root and shoot length (Jalander and Gachande, 2012).

Generally, the most common isolate fungal species in soybean cultivars is *Fusarium*, and the least isolated species is *Scopulariopsis* (Russo *et al.*, 2016). Fungicides are the traditional solution for the treatment of seed born fungi, but it causes many environmental hazards. Plants are the

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primary sources of naturally abundantly novel pesticides (Cantrell *et al.*, 2012). Treatment of seeds with many plant extracts was effective in reducing mycotoxin seed contamination (Ahmed *et al.*, 2013). Medicinal plant extracts influence the development of novel antifungal compounds. Compounds related to many phytochemical groups were assayed for antifungal activity, such as saponins, polyphenols, peptides, and essential oils (Vila *et al.*, 2013; Gupta and Birdi, 2017). Clove, cinnamon, garlic, and mint: essential oils (EOs) are safe antimicrobial compounds used as antifungal compounds (Mutlu-Ingok *et al.*, 2020). These are natural and complex plant secondary metabolites (Bakkali *et al.*, 2008) with broad-spectrum antimicrobial activity (Kalemba and Kunicka, 2005).

The present work was carried out to isolate, identify seed-borne fungi from soybean and assay the fungal inhibitory effect of ethanolic extract of some medicinal plant clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), garlic (*Allium sativum*), and mint (*Mentha piperita*).

Materials and Methods

Collection of seed samples

Soybean seeds were collected from four markets in the Giza governorate in Egypt. Seeds were subjected immediately to mycoflora isolation in the laboratory.

Isolation and identification of seed-borne fungi

For detecting colony-forming units (CFU/g), twenty grams of ground soybean were soaked in 180 mL sterile water containing 0.02% Tween-80, shook on a shaker at 50 rpm for 30 min. Serial dilutions (10^{-1} , 10^{-2} , 10^{-3}) were prepared, and 1 mL from dilution 10^{-3} was inoculated into each plate containing Czapek-Dox agar with 1% streptomycin to inhibit bacterial growth. The inoculated plates were incubated at 25°C for ten days, and the colony-forming units were counted. The isolated fungi were identified microscopically using the available references (El-Shafie, 1996; Raper and Fennell, 1965).

Preparation of plant extracts

Ethanolic extract 100 g of each clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), garlic (*Allium sativum*), and mint (*Mentha piperita*) were soaked in 70% ethanol for 72 h at room temperature to extract active ingredients. Concentration was 5% (w/v) of each extract, the extracts were then filtered using muslin or Whatman filter paper No. 1, and the filtrates were evaporated using a lyophilizer. The final lyophilized extracts were stored in labeled sterile screw-capped bottles and kept in the refrigerator until usage. An amount of freeze-dried plant extract in addition to reference antimycotic drug was dissolved in 2 mL sterile distilled water or 10% DMSO, sterilized by filtration through a 0.45-mm membrane filter, and then mixed with the amount of sterilized medium in order to give a final concentration of 15 µg/mL (Irobi and Daramola, 1993).

Determination of antifungal activity

The agar disc diffusion method was used to test the antifungal activity. Potato dextrose medium was inoculated with a swab of fungal spores added to the plates. Sterile paper discs (6 mm

in diameter) soaked with 10 µL of each of the plant extracts were placed on the plate's surface. Fluconazole was used as control; plates were incubated at 28°C for seven days, and the inhibition zone diameter around each disc was determined (Ranković *et al.*, 2010).

Minimum inhibitory concentration

Determination of minimum inhibitory concentration (MIC) was performed by a serial agar dilution plate technique, where solutions containing reconstituted plant extracts (6.25–100 mg/mL concentrations) were incorporated into Sabouraud Dextrose Agar medium (SDA). The medium was poured into plates and allowed to solidify, then inoculated with the test fungi and incubated at 25–27°C for seven days. Control plates, which contained no plant extracts, were also used. The MIC values of each plant extract were determined after seven days, this being the lowest concentration at which no visible growth was observed (Fabry *et al.*, 1996).

Preparation of fungal filtrates

The isolated fungal species were cultivated in 250 mL conical flasks containing 100 mL of potato dextrose broth and incubated at 25°C for 14 days. After incubation, cultures were filtered using Whatman no. 1 filter paper. Filtrates were stored at 4°C (Jalander and Gachande, 2012).

Blotter method

Sterilized blotter paper discs of 9 cm diameter were placed in sterile Petri plates (9 cm diameter) after being moistened with sterile distilled water. Seeds were transferred to the plates containing the moist blotter paper. Ten seeds per plate were placed at equidistance in a circle. Forty seeds from each sample were placed in the plates in four replications. The plates were incubated at $25 \pm 2^\circ\text{C}$ for seven days under alternate cycles of 12 h light and 12 h darkness for seven days (International Rules for Seed Testing 2019-ISTA Online-International Seed Testing Association, n.d.).

Effect of culture filtrates on seed germination and seedling growth

Soybean seeds were sterilized with 0.1% mercuric chloride. Ten seeds were added to the culture filtrates from each fungal species and then incubated at 28°C for 24 h (ISTA, 1996). After seven days of incubation, the percent of germination, radicle, and plumule lengths were estimated (International Rules for Seed Testing 2019-ISTA Online-International Seed Testing Association, n.d.).

Statistical analysis

Data were analyzed, and treatments were compared using variance analysis with Duncan's multiple range test ($p < 0.01$) (Duncan, 1955).

Results

Fungi isolated during three surveys indicated that ninety-eight fungal colonies were isolated from soybean seeds. The total count constituted twenty-two fungal species. The isolated fungal species were: *Alternaria alternata*, *Aspergillus carneus*, *A. flavus*, *A. nidulans*, *A. niger*, *A. versicolor*,

Cephalosporium acremonium, *Chaetomium globosum*, *Cladosporium tenuissimum*, *Colletotrichum truncatum*, *Curvularia lunata*, *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Macrophomina phaseolina*, *Mucor indicus*, *Penicillium chrysogenum*, *Rhizoctonia solani*, *Rhizopus stolonifer*, *Sclerotium rolfsii*, *Stemphylium herbarium* and *Verticillium nubilum* (Tab. 1). Moreover, *Aspergillus niger* and *A. flavus* were the most common fungal species in soybean seeds, with a relative density of 20.41 and 25.51% of the total count, respectively (Tab. 1).

The effect of the isolated seed-borne fungal species on the growth parameters of soybean (Tab. 2) indicated general inhibition in seed germination when treated with culture filtrate of seed-borne fungi. However, the highest inhibition was recorded by *Aspergillus flavus*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Aspergillus niger*, *Fusarium moniliformis*, and *F. solani* with percent of inhibition of 71, 70, 68, 67, 67, and 65%, respectively. Regarding radicle length, it was found that *Fusarium solani*, *F. oxysporum*, *Aspergillus flavus*, *Fusarium moniliformis*, *Aspergillus niger*, and *Rhizoctonia solani* exhibited the highest reduction in radicle length to reach 2.00, 2.10,

2.33, 2.33, 2.42 and 2.81 cm, respectively, out of control (5.42 cm) (Tab. 2).

The plumule length was also affected by the seed-borne fungal species. It was found that fungal filtrates of *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *F.oxysporum*; *F. moniliforme* and *Mucor indicus* and *Rhizoctonia solani* recorded the highest plumule reduction (1.43, 1.54, 1.54, 1.65, 1.74, 1.95, and 1.19 cm, respectively) compared to control value (3.20 cm). Concerning growth parameter inhibition; It was found that *A. flavus*, *Rhizoctonia solani*, *A. niger*, *Fusarium oxysporum*, *F. moniliforme*, *F. solani*, *Colletotrichum truncatum*, *Curvularia lanata*, and *Cladosporium tenuissimum* recorded the highest inhibition (more than 48% inhibition compared to the control value) in all growth parameters of soybean (Tab. 2).

In the present study, some medicinal plant extracts were tested for inhibiting the growth of the seed-borne fungal species affecting the soybean plant's growth. It was found that the ethanolic extract of clove recorded the highest inhibition in fungal growth with mean relative activity (RA) of 131.66%, compared to fluconazole, the standard antifungal drug. The most sensitive fungal species to clove

TABLE 1

Total count and relative density of fungal species isolated from soybean seeds all over three isolations

Isolated fungal species	Isolate 1	Isolate 2	Isolate 3	Count	
				Total count	Relative density (%)
<i>Alternaria alternata</i>	3	4	1	8	8.16
<i>Aspergillus carneus</i>	1	2	1	4	4.08
<i>A. flavus</i>	9	3	8	20	20.41
<i>A. nidulans</i>	2	0	2	4	4.08
<i>A. niger</i>	8	7	10	25	25.51
<i>A. versicolor</i>	0	1	1	2	2.04
<i>Cephalosporium acremonium</i>	1	0	0	1	1.02
<i>Chaetomium globosum</i>	1	1	0	2	2.04
<i>Cladosporium tenuissimum</i>	1	0	1	2	2.04
<i>Colletotrichum truncatum</i>	0	0	1	1	1.02
<i>Curvularia lunata</i>	2	1	0	3	3.06
<i>Fusarium solani</i>	2	1	1	4	4.08
<i>F. oxysporum</i>	1	1	0	2	2.04
<i>F. moniliforme</i>	2	0	0	2	2.04
<i>Macrophomina phaseolina</i>	0	1	0	1	1.02
<i>Mucor indicus</i>	1	0	0	1	1.02
<i>Penicillium chrysogenum</i>	5	2	2	9	9.18
<i>Rhizoctonia solani</i>	1	0	0	1	1.02
<i>Rhizopus stolonifer</i>	0	0	1	1	1.02
<i>Sclerotium rolfsii</i>	1	0	0	1	1.02
<i>Stemphylium herbarum</i>	2	1	0	3	3.06
<i>Verticillium nubilum</i>	0	0	1	1	1.02
Total count	43	25	30	98	100
Relative density	34.88	25.51	30.61		100%

TABLE 2

Effect of soybean seed-borne fungal filtrates on growth parameters of soybean seedlings

Fungal species	Germination inhibition (%)	Radicle length (cm)	Plumule length (cm)
Control	00.0 ^a	5.42 ^a	3.20 ^a
<i>Alternaria alternata</i>	39 ^f	5.14 ^a	3.11 ^a
<i>Aspergillus carneus</i>	29 ^e	5.32 ^a	3.15 ^a
<i>A. flavus</i>	71 ⁿ	2.33 ^a	1.43 ^a
<i>A. nidulans</i>	28 ^e	5.21 ^a	3.06 ^a
<i>A. niger</i>	67 ^{im}	2.42 ^a	1.54 ^a
<i>A. versicolor</i>	19 ^d	5.41 ^a	3.11 ^a
<i>Cephalosporium acremonium</i>	16 ^{bcd}	5.44 ^a	3.32 ^a
<i>Chaetomium globosum</i>	14 ^b	5.32 ^a	3.21 ^a
<i>Cladosporium tenuissimum</i>	49 ⁱ	4.80 ^a	2.32 ^a
<i>Colletotrichum truncatum</i>	60 ^j	3.42 ^a	2.39 ^a
<i>Curvularia lunata</i>	59 ^j	3.43 ^a	2.13 ^a
<i>Fusarium solani</i>	65 ^{ki}	2.00 ^a	1.54 ^a
<i>F. oxysporum</i>	68 ^m	2.10 ^a	1.65 ^a
<i>F. moniliforme</i>	67 ^{kim}	2.33 ^a	1.74 ^a
<i>Macrophomina phaseolina</i>	64 ^k	2.75 ^a	1.95 ^a
<i>Mucor indicus</i>	14 ^b	4.74 ^a	3.22 ^a
<i>Penicillium chrysogenum</i>	45 ^b	4.61 ^a	3.11 ^a
<i>Rhizoctonia solani</i>	70 ⁿ	2.81 ^a	1.91 ^a
<i>Rhizopus stolonifer</i>	35 ^f	4.91 ^a	3.11 ^a
<i>Sclerotium rolfsii</i>	28 ^e	4.43 ^a	3.33 ^a
<i>Stemphylium herbarum</i>	17 ^{cd}	4.32 ^a	3.42 ^a
<i>Verticillium nubilum</i>	15 ^{bc}	4.34 ^a	3.11 ^a
L.S.D at 0.01	2.2	2.19	2.04

Note: *Mean in each column followed by the same letter(s) did not differ at $P \leq 0.01$, according to Duncan's multiple range tests.

extract were *A. niger* (166.7 RA), *A. flavus* (133.3 RA), *F. moniliforme* (133.3 RA), *F. solani* (125 RA), and *F. oxysporum* (100 RA). Cinnamon came next to clove with a mean RA of 95.00%, garlic (65.02%), and mint (34.98%), respectively (Tab. 3).

Combination mixtures between clove with cinnamon, clove with garlic, and cinnamon with garlic recorded an increase in the mean relative activity compared to the fluconazole drug (control).

On the other hand, the combination between clove and mint recorded a decrease in the mean antifungal relative activity of 38.66% below that of clove alone. Moreover, garlic and mint recorded a decrease in the mean relative activity of 32.86% below that of garlic alone (Tab. 4).

Tab. 5 indicates that cinnamon extract achieved the highest effectiveness against tested fungi with the least MIC values of 12.5 mg/mL against all tested fungi, followed by clove extract that recorded MIC value equal to that of control against all tested fungi. Garlic and mint recorded the highest MIC values (100 mg/mL) against all tested fungal species.

The most effective combination mixture in inhibition of the tested plant parasitic fungi was clove-cinnamon (MIC

12.5 mg/mL), followed by a clove-garlic, clove-mint, and cinnamon-garlic, where MIC value of 50 mg/mL was achieved against all tested fungal species. MIC values were not detected in the case of a combination between cinnamon-mint and garlic-mint, which indicates the antagonistic action between the two oils (Tab. 6).

Discussion

During three surveys, identification and isolation of mycotoxigenic fungi revealed that ninety-eight fungal colonies were obtained from soybean seeds, in which the total count constituted twenty-two fungal species. Reliant to this particular result, (Marcenaro and Valkonen, 2016) isolated 133 fungal species from surface-sterilized bean seeds. It is essential to realize that type and number of the isolated fungi depend on variations of seeds moisture and seed development conditions, harvesting, and storage (Mordecai, 2012; Begum et al., 2013).

The commonly isolated soybean fungi were *Aspergillus*, *Fusarium*, *Macrophomina*, *Pythium*, *Phoma*, and *Phomopsis* (Riccioni and Petrović, 2000). The isolated

TABLE 3

Antifungal activity of some medicinal plant ethanolic extracts against the fungal species to soybean growth parameters

Treatment	Inhibition zone diameter (mm)										Mean R.A. (%)
	Aspergillus flavus		Aspergillus niger		Fusarium solani		Fusarium oxysporum		Fusarium moniliforme		
	Diameter (mm)	R.A.	Diameter (mm)	R.A.	Diameter (mm)	R.A.	Diameter (mm)	R.A.	Diameter (mm)	R.A.	
Fluconazole	3.0ab	100	3.0ab	100	4.0a	100	4.0a	100	3.0ab	100	100
Clove	4.0a	133.3	5.0a	166.7	5.0a	125	4.0a	100	4.0a	133.3	131.66
Cinnamon	3.0ab	100	3.0ab	100	4.0a	100	3.0ab	75	3.0ab	100	95
Garlic	2.0ab	66.7	2.0ab	66.7	3.0a	75	2.0ab	50	2.0ab	66.7	65.02
Mint	1.0b	33.3	1.0b	33.3	2.0a	50	1.0a	25	1.0b	33.3	34.98
L.S.D at 0.01	2.0		2.5		2.9		2.0		2.0		

Note: *R.A: relative activity (%).

**Mean in each column followed by the same letter(s) did not differ at $P \leq 0.01$, according to Duncan's multiple range tests.

TABLE 4

The combined effect of some plant ethanolic extracts on the fungal species on soybean growth parameters

Treatment	Inhibition zone diameter (mm)										Mean R.A.
	Aspergillus flavus		Aspergillus niger		Fusarium solani		Fusarium oxysporum		Fusarium moniliforme		
	Diameter (mm)	R.A.	Diameter (mm)	R.A.	Diameter (mm)	R.A.	Diameter (mm)	R.A.	Diameter (mm)	R.A.	
Fluconazole	3.0ab	100	3.0ab	100	4.0a	100	4.0a	100	3.0ab	100	100
Clove+cinnamon	5.0a	166.6	4.8a	160	4.6a	115	4.5a	112.5	4.7a	156.6	142.14
Clove+garlic	4.0ab	133.3	4.8a	160	4.6a	115	4.6a	115	4.6a	153.3	135.32
Clove +mint	3.0ab	100	3.2a	106.6	3.3a	82.5	3.1ab	77.5	3.0ab	100	93.32
Cinnamon+garlic	4.0ab	133.3	4.1a	136.6	4.2a	105	4.3a	107.5	4.0ab	133.3	123.14
Cinnamon +mint	4.0ab	133.3	4.2a	140	4.4a	110	4.4a	110	4.0ab	133.3	125.32
Garlic +mint	1.0b	33.3	1.1b	36.6	1.1b	27.5	1.2b	30	1.0b	33.3	32.14
L.S.D. (0.05)	2.25		1.83		2.05		2.05		2.25		

Note: *R.A: relative activity (%).

Mean in each column followed by the same letter(s) did not differ at $p \leq 0.01$, according to Duncan's multiple range tests.

TABLE 5

Minimum inhibitory concentration (MIC) of some plant extracts on the inhibitors fungal species to soybean growth parameters

Treatment	MIC value (mg/mL)				
	Aspergillus flavus	Aspergillus niger	Fusarium solani	Fusarium oxysporum	Fusarium moniliforme
Fluconazole	50	50	50	50	50
Clove	25	25	25	25	25
Cinnamon	12.5	12.5	12.5	12.5	12.5
Garlic	100	100	100	100	100
Mint	100	100	100	100	100

seed-borne fungal species' effect on the growth parameters of soybean displayed general inhibition in seed germination when treated with culture filtrate of seed-borne fungi.

Regarding radicle length, it was found that *Fusarium solani*, *F. oxysporum*, *Aspergillus flavus*, *Fusarium*

moniliformis, *Aspergillus niger*, and *Rhizoctonia solani* exhibited the highest reduction in radicle length. The plumule length was also affected by the seed-borne fungal species. It was found that fungal filtrates of *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *F.oxysporum*,

TABLE 6

Minimum inhibitory concentration (MIC) of combined ethanolic plant extracts on the fungal species to soybean growth parameters

Treatment	MIC value (mg/mL)				
	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Fusarium solani</i>	<i>Fusarium oxysporum</i>	<i>Fusarium moniliforme</i>
Fluconazole	50	50	50	50	50
Clove + cinnamon	12.5	12.5	12.5	12.5	12.5
Clove + garlic	50	50	50	50	50
Clove + mint	50	50	50	50	50
Cinnamon + garlic	50	50	50	50	50
Cinnamon + mint	nd	nd	nd	nd	nd
Garlic + mint	nd	nd	nd	nd	nd

Note: *Nd: not detected.

F. moniliforme, and *Mucor indicus* and *Rhizoctonia solani* recorded the highest plumule reduction.

In this respect, many microorganisms can produce toxins in growth media. These toxins include amino acids, cyclic peptides, aromatic phenols, and terpenoids. Enzymes of pathogenic fungi are involved in the hydrolysis of plant tissues (Bills and Gloer, 2016).

Aspergillus, *Fusarium*, *Penicillium*, and *Rhizoctonia* are commonly known to produce toxins (Horn *et al.*, 1993) that reduce the plant's growth parameters. Moreover, toxic metabolites of *Aspergillus* species reduced seed germination; root-shoot elongation and the filtrate of *A. niger*, *A. flavus*, and *A. nidulans* were the most inhibitory species to all growth parameters of plants (Pangrikar *et al.*, 2009). Culture filtrate of *Aspergillus* significantly reduced root length and shoot length of cereals (Jalander and Gachande, 2012).

Some medicinal plant extracts (clove, cinnamon, garlic, and mint) were tested for inhibiting the seed-borne fungal species' growth. Ethanolic extract of clove recorded the highest growth inhibition. Combination mixtures between clove with cinnamon, clove with garlic, and cinnamon with garlic recorded an increase in the mean relative activity compared to fluconazole drug (control) synergistic interaction between them than a single treatment.

On the other hand, the combination between clove and mint recorded a decrease in the mean antifungal relative activity below that of clove alone, indicating antagonistic interaction between clove and mint extracts. Moreover, garlic and mint recorded a decrease in the mean relative activity below that of garlic alone, indicating antagonism.

Cinnamon extract achieved the highest effectiveness against tested fungi with the least MIC values against all tested fungi, followed by clove extract that recorded MIC value equal to that of control against all tested fungi. Garlic and mint recorded the highest MIC values against all tested fungal species.

The most effective combination mixture was detected between clove and cinnamon, followed by a combination between clove and garlic, clove and mint, and cinnamon and garlic. MIC values were not detected in the case of a combination between cinnamon plus mint and garlic plus mint, indicating the presence of antagonism between them.

Plants synthesize secondary metabolites (phenols, phenolic acids, and others), which enable them to act as a biocontrol agent in reducing severe crop loss. Secondary metabolites of plants have shown potential antifungal and antibacterial activity (Compean and Ynalvez, 2014; Ribera and Zuñiga, 2012; Ranković *et al.*, 2010).

A compelling research study by Noreen *et al.* (2018) evaluated the antibacterial activity of medicinal plant extracts against pathogenic bacteria involved in urinary tract infections (UTI), and they recorded that clove with cinnamon extracts showed the best antibacterial activity against UTI.

Also, Lukovic *et al.* (2018) investigated the antifungal activity of two essential oils, cinnamon and clove, when evaluated against *Cladobotryum dendroides*. They found that clove essential oil showed more potent activity than cinnamon against both tested fungi. However, cinnamon oil was more toxic to *Lecanicillium fungicola* than to *Cladobotryum dendroides*.

As well reported that clove oil has intense antifungal activity against many fungal species, including *Fusarium moniliforme*, *Fusarium oxysporum*, *Aspergillus* sp., *Mucor* sp., *Trichophyton rubrum*, and *Microsporium gypseum* (Rana *et al.*, 2011). The MIC of clove was assayed against *Fusarium moniliforme*, *Fusarium oxysporum*, *Aspergillus* sp., *Mucor* sp., *Trichophyton rubrum* *Microsporium gypseum*. The MIC values were found to be from 9–12 mg/mL. *Mucor* sp., *M. gypsum*, and *T. rubrum* were inhibited at 9 mg/mL of clove oil, followed by *Aspergillus* sp. and *F. oxysporum* at 10 mg/mL and *F. moniliforme* at 12 mg/mL. *In vitro*, clove oil exhibited an inhibitory effect against the mycelial growth of *Fusarium oxysporum*, *F. solani*, *F. semitectum*, and *Rhizoctonia solani*. Complete growth inhibition was observed in *Fusarium oxysporum* and *Rhizoctonia solani* when clove oil was applied at a concentration of 4% (Thabet and Khalifa, 2018).

Medicinal and aromatic secondary metabolites are found in the structure of ketones, esters, alcohols, terpenes, phenols, aldehydes amines, and sulfides, terpineol, thujanol, myrcenol, neral, thujone, camphor, and carvone are strongly associated with their potential biocontrol and pathogenic microorganism eradications (Bakkali *et al.*, 2008).

The chemical composition of EO plants varies according to the form and geographic location of the plant species and the weather, ripening stage, and the method of EO obtaining (Nazzaro *et al.*, 2017). Another important aspect is the plant's portion, where EO is derived from flowers, stems, leaves, and buds (Ben El Hadj Ali *et al.*, 2015). To our expertise, and though its antibacterial and antioxidant activities are well known, it is substantial to study the antifungal and antioxidant activity of EOs thoroughly.

Besides all these facts, specific techniques are needed to conserve the biological activities of food processing facilities. EOs can be quickly degraded by oxygen and variations in temperatures and volatile at room temperature (Borugã *et al.*, 2014; Zhavah *et al.*, 2015). Methods are therefore crucial for improving the stability and operation of EOs, and modern technologies such as encapsulation, the use of edible coatings, and active packaging can solve these difficulties (Prakash and Kiran, 2016).

In food items with some novel uses such as encapsulation, edible films, and edible coating, EOs may be used directly. The technology for encapsulation improves the stability of oxidation, heat stability, and anti-microbial and antioxidant EOs (Campos *et al.*, 2011). The attaching of EOs to edible film and coating can also boost heat stability and breakage resistance, minimize swelling and solubility and increase and/or increase product antioxidant and anti-microbial activities (Du *et al.*, 2011).

The number of studies on other biological activities of EOs has increased significantly nowadays due to growing data on microorganism -cidal or -static effects of EOs. Regarding the mechanism of action, EOs could disrupt a bacterial and fungal culture cell membrane (Raveau *et al.*, 2020; Khorshidian *et al.*, 2018). The antimicrobial activity of EOs is so potent that the cell membrane can easily be disrupted and even more permeable. Also, they disrupt processes of ion transportation and interact with membrane proteins and other intracellular compounds (Kalemba and Kunicka, 2005; Nazzaro *et al.*, 2017). Also, EOs have an adverse effect on the enzymes by interfering with their active sites. After EOs implementation, electrolyte loss was measured as assessed by K^+ , Ca^{2+} , and Na^+ concentration ions levels (Mutlu-Ingok *et al.*, 2020).

In short, the interaction of EO and the cell system with the plasma membrane and disruption of mitochondrial function were found to be antimicrobial effects (Hu *et al.*, 2017). A distortion between ATP concentrations intracellular and extracellular ultimately contributes to cell death (Hu *et al.*, 2017). Besides, the antimicrobial impacts of EOs may be associated with the usability of EOs by the cell membrane of microorganisms (diffusion coefficient, zeta potential, and droplet size of EOs) (Mutlu-Ingok *et al.*, 2020). While in many works of literature, the antimicrobial mechanisms of EOs have been well described, the antimycotoxigenic mechanism is not yet known. In recent works, however, several approaches were documented. Antimycotoxigenic EOs were linked in one study with their effect on the aflatoxin biosynthesis (Bluma *et al.*, 2008).

Conclusion

Because of their low cost, availability, and wide range of biological activities, essential oils -and their components- are

valuable. Another advantage is that they do not disturb the taste and scent and, therefore, enhance the foodstuff's shelf life while they are being used in adequate proportions. It is essential to find from health and economic aspects. The efficient, safe, and cost-effective anti-fungal agents to control both growth and the production of mycotoxins. However, different results were observed in various studies because of differences in crops used in antimicrobial analyses, geographical origin, harvest time, part of the plant-derived from EO, and methods of extracting and analyzing. These parameters are critical considerations while working with EO, as they influence their germicidal activities. While EOs antibacterial and antioxidant capabilities are well documented, there are still limited antifungal and anti-mycotoxin activity studies. In terms of organoleptic properties of the food, the use of essential oil mixtures, which are designed according to food characteristics, might be seen as a new particular perspective. Since EOs are unstable under environmental stresses, such as light and temperature, new technologies might protect and improve their biological characteristics. However, further research should concentrate on the synergistic influence and mechanism action between different essential oils and/or components. A further recommendation is that the anti-fungal action against polycultures should also be investigated in addition to these monoculture studies.

Lastly, new techniques to improve the EOs stability and decrease the required concentration to ensure food safety with minimal sensorial changes could be an interesting research scope to explore deeply.

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