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Original Research

Evaluation of *Cinnamomum verum* Essential Oils against Ochratoxin A-producing *Aspergillus parasiticus* in Stored Wheat, Maize and Rice

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Abstract

Eight essential oils: *Zingiber officinale*, *Curcuma longa*, *Eucalyptus globulus*, *Syzygium aromaticum, Nigella sativa*, *Elettaria cardamomum*, *Cinnamomum verum* and *Cuminum cyminum* were evaluated for antifungal activity against Ochratoxin A (OTA)-producing *Aspergillus parasiticus* (n = 3) using grains as a substrate. Minimum inhibitory concentration (MIC) and log reduction assay were used to evaluate the antifungal activity. Both the highest zone of inhibition (24.00±2.00 mm) and least minimum inhibitory concentration (1.04±0.45 µg/mL) were recorded for *C. verum* essential oils. By log reduction assay *S. aromaticum, C. verum* and *E. globulus* essential oils exhibited antifungal activity after 15 min of exposure. After 30, 60 and 90 min of exposure of *A. parasiticus* spores to selected essential oils, complete inactivation of fungal spores (6±0.00 log reduction) was observed. In stored grains, inoculated with *A. parasiticus*, the lowest OTA (8.16±0.05 ng/g) was observed at 10% moisture in broken rice. An increase in OTA production was noticed with an increase in moisture from 10-40% but this trend reversed at 50-70% moisture. No increase in OTA in un-inoculated and *C. verum* essential oil-treated inoculated grains was noticed. Gas chromatography-mass spectrometry (GC-MS) analysis indicated the highest percentage of oleic acid (23.7%) in *C. verum*. *Cinnamomum verum* (cinnamon) oil is an effective and safe antifungal agent against *A. parasiticus* in stored grains.

Keywords: antifungal, *Eucalyptus globulus*, grain preservation, plant essential oils, *Syzygium aromaticum*

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Introduction

A. parasiticus is a filamentous fungus having dark green conidiophores. This saprophytic fungus is widely distributed in nature. The fungus grows rapidly at the temperature range of 12 to 42ºC and a pH of 2.4-10.5. Microscopically the fungus appears as having rough, thick-walled and spherical conidia with short conidiophores and phialides arranged in a biseriate pattern on conidial heads [1]. Mycotoxins are the secondary metabolites of pathogenic fungi that exhibit pathological effects on both man and animals. *A. parasiticus* has the potential to produce secondary metabolites such as OTA and aflatoxin [2]. The International Agency for Research on Cancer has declared OTA a possible carcinogen for both humans and animals owing to its nephrotoxic, hepatotoxic and teratogenic effects [3].

Grains are susceptible to heavy metal intoxication as well as mold contamination that may lead to spoilage and /or mycotoxin production [4-6]. OTA contaminates maize, wheat and wheat bran [7]. Phytopathogenic fungi including OTA-producing *Aspergillus* have also been reported to occur in chili powder [8]. Moisture, temperature and storage conditions determine the extent of OTA production in cereals and feed. *A. parasiticus* can contaminate stored grains such as maize, coffee beans, and peanuts [9]. Higher moisture content in grains provides a favorable environment for the growth of molds such as *A. parasiticus* and *A. flavus* [10].

To minimize mold growth and toxin production, various control strategies can be adopted including the use of chemicals, gamma irradiation, bacteria, fungi and plant essential oils [11-12]. Plant essential oils can be extracted by hydro-distillation or fermentation and are widely used as anti-fungal and anti-toxigenic agents. Essential oils from *Z. officinale*, *C. longa*, *E. globulus*, *S. aromaticum, N. sativa*, *E. cardamomum*, *C. verum* and *C. cyminum* have been used for their antifungal properties. Moreover, cinnamon, lemongrass, clove and thyme oil can also inhibit *A. parasiticus* [12]. Likewise, ginger (*Z. officinale*) essential oils have significant antifungal activity against mycotoxigenic *A. parasiticus* [13]. Roselle (*Hibiscus sabdariffa)* and black cumin (*N. sativa*) essential oils have indicated metabolic effects on the aflatoxin biosynthesis in *Aspergillus* species [14]. Plant essential oils being botanical in origin have minimum impact on environmental health, hence supporting One Health.

Plant essential oils' antifungal potential can be evaluated by antimicrobial assays such as well diffusion assay, minimum inhibitory concentration (MIC) and log reduction. The OTA production potential of *A. parasiticus*, in cereals and cereal-derived products, can be determined by using chromatographic techniques such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) [15].

The present study was designed to evaluate the antifungal and antitoxigenic activity of *Z. officinale*, *C. longa*, *E. globulus*, *S. aromaticum, N. sativa*, *E. cardamomum*, *C. verum* and *C. cyminum* essential oils against *A. parasiticus* and OTA, respectively. The OTA inhibition potential of plant essential oils on cereal grains stored at various moisture levels was also evaluated after spiking them with fungus. The log reduction in fungus at different time intervals was also studied. The effective component analysis of essential oils was done by GC-MS assay.

Material and Methods

Revival of *Aspergillus parasiticus* Isolates

A. parasiticus isolates (n = 03) that were isolated from various types of poultry feed samples such as home-mixed, commercial and pelleted poultry feed and feed ingredients were collected from the Lahore District of the Punjab Province of Pakistan and characterized by a specie-specific polymerase chain reaction (PCR) were procured from the Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan. The isolates were revived from soil stocks by sprinkle plate method under sterile conditions. Sabouraud dextrose agar (SDA) plates were incubated at 25ºC for 3-5 days.

Macroscopic and Microscopic Identification

After incubation, macroscopic identification of purified fungus was done. Growth pattern, colony texture, colony margins and color on obverse side whereas ridges and pigmentation on the reverse side of plates were noted. For microscopic examination, the agar drop method was used. Briefly, fungal culture was inoculated on molten SDA drop at a glass slide. A sterile cotton swab dipped in sterile distilled water was placed in a glass Petri plate along with a cultured glass slide, incubated for 3-5 days and observed under a microscope at 4X, 10X and 40X for observing its microscopic characteristics.

Evaluation of Antifungal Pattern of Plant Essential Oils

A. parasiticus isolates were processed to evaluate the antifungal activity of various plant essential oils (n = 08) such as *Z. officinale*, *C. longa*, *E. globulus*, *S. aromaticum, N. sativa*, *E. cardamomum*, *C. verum*, and *C. cyminum* by well-diffusion method, MIC, and log reduction.

Fungal Inoculum Preparation

A. parasiticus inoculum was prepared by adjusting 10^6 spores/mL in normal saline. Fungal spores from each culture were picked aseptically and mixed in sterile normal saline followed by mixing using the

vortex. The spore counting was done using a sterile Neubauer chamber. For this, a cover slip was placed on an engraved area of the chamber and a volume of 10 µL of spore suspension was pipetted at the edge of the coverslip followed by an enumeration of fungal spores at 40X under a light microscope [16].

Well-Diffusion Assay

For the well-diffusion assay, wells were punctured in SDA plates by using a sterile well-borer well-borer after swabbing the fungal inoculum. Dimethyl sulfoxide (DMSO) in 1:1 ratio with plant essential oils was prepared in a micro-centrifuge tube followed by mixing using a vortex mixer and 20-50 µL was pipetted in each well. Plates were incubated at 25ºC for 3-5 d followed by measurement of zones of inhibition in millimeters using a measuring scale [17].

Minimum Inhibitory Concentration

The minimum inhibitory concentration of plant essential oils was determined against *A. parasiticus* (n = 03) isolates by micro-broth dilution method using 96-well flat bottom micro-titration plates. Briefly, a volume of 100 µL of SD broth was dispensed in 1 to 12 wells, followed by a serial 2-fold dilution of 100 µL of each essential oil (1:1 ratio of DMSO and essential oil) was performed and 100 µL volume was discarded from the 10th well. A volume of 100 µL of standard fungal suspension was pipetted in 1 to 11 wells each. At time zero, optical density (OD) was recorded at 530 nm wavelength followed by incubation at 25ºC for 3-5 days and the OD value was again recorded at 530 nm followed by calculation of results by subtracting initial OD from final OD value [18]. Plant essential oils that exhibited antifungal activity by well-diffusion assay and least MIC were further processed for log reduction.

Log Reduction of *A. parasiticus* Spores

A 1:1 ratio of standard spore inoculum of *A. parasiticus* and plant essentials oils was mixed in a glass tube and a further 1 mL volume was pipetted at different time intervals: 15, 30, 60 and 90 min followed by a serial 10-fold dilution in sterile peptone water. A volume of 100 µL was pipetted on SDA plates followed by spreading with a sterile glass spreader and incubation at 25ºC for 72 h. Results were recorded as colony-forming units and for each time interval, a percentage log reduction was calculated [19].

Evaluation of Antifungal Activity of *C. verum* Essential Oil in Stored Cereal Grains

Antifungal activity of effective essential oil such as *C. verum* was evaluated against OTA-producing *A. parasiticus* at various moisture levels such as 10, 20, 30, 40, 50, 60 and 70 percent on 15th, 30th, 45th and 60th d post-inoculation. Intact as well crushed grains of wheat, maize, and rice were selected in all experimental groups and 100 gm of each grain was sterilized by autoclaving in glass conical flasks followed by maintenance of moisture content with sterilized distilled water using a digital hygrometer [20].

Experimental Groups

Based on fungal inoculum and essential oil treatment, each type of grain was divided into three experimental groups: un-inoculated (only autoclaved grains), inoculated (sterilized grains with OTA producing *A. parasiticus*), and inoculated-treated group (sterilized grains inoculated with toxigenic *A. parasiticus* as well as treated with *C. verum* plant essential oil). Before maintaining the moisture content in each experimental flask, 1 mL standard spore inoculum was pipetted in inoculated and inoculated-treated group flasks followed by treatment with *C. verum* essential oil (2 µg/gm of grain) only in inoculated-treated group flasks. Moisture levels of 10, 20, 30, 40, 50, 60, and 70% were separately maintained for 3 experimental groups. All the experimental flasks of 3 groups were incubated at 25ºC for the duration of 60 d with periodic shaking on a shaking incubator. At 15, 30, 45 and 60 d post-inoculation, a sample of 25 gm from each experimental group flask was taken out followed by autoclaving and processed for quantification of OTA by TLC and HPLC as previously described with minor modifications [21].

Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was done for plant essential oils such as *E. globulus*, *S. aromaticum* and *C. verum* using GCMS-5977B (Agilent, USA) for active components analysis as described previously with minor modifications [22]. To perform GCMS, CARBOWAX capillary column along with helium as carrier gas was used. The injector was heated at 260ºC and the samples (essential oils *S. aromaticum*, *E. globulus* and *C. verum*) were injected at the rate of 1 µL per minute. In the test samples, active compounds were evaluated by the comparison of their retention time with that of the standard compound.

Statistical Analysis

Data obtained from well-diffusion assay, MIC, log reduction and all three experimental groups for plant essential oil were statistically analysed by one-way analysis of variance followed by Duncan's Multiple Range test using Statistical Package for Social Sciences (SPSS) software version 20.0.

Results

Macroscopic and Microscopic Identification

On macroscopic examination, *A. parasiticus* isolates were observed dark green from obverse side. Microscopically, a bunch of hyphae was observed having rough, thick-walled and spherical conidia with short conidiophores and phialides arranged in a biseriate pattern on conidial heads.

Activity of Plant Essential oils Against *A. parasiticus*

Essential oils of eight indigenous medicinal plants including *Z. officinale*, *C. longa*, *E. globulus*, *S. aromaticum, N. sativa*, *E. cardamomum*, *C. verum*, and *C. cyminum* were evaluated for antifungal activity against OTA-producing *A. parasiticus* by agar well diffusion method and the highest zone of inhibition was observed for *C. verum* (24.00±2.00 mm) followed by *S. aromaticum* (21.67±0.57 mm), and *E. globulus* (3.33±0.57 mm) (Fig. 1a). Antifungal activity was not exhibited by any of the other five tested plant essential oils against *A. parasiticus*. Statistically significant variations were recorded in antifungal activity of plant essential oils ($p<0.05$).

The MIC of plant essential oils which showed antifungal potential against OTA-producing *A. parasiticus* determined by micro-broth dilution method and the least mean MIC was recorded for *C. verum* (1.04±0.45 µg/mL) followed by *S. aromaticum* (1.30±0.5 µg/mL) and *E. globulus* (2.08±0.90 µg/mL) (Fig. 1b). However, these differences in MIC of the three essential oils against *A. parasiticus* were statistically not significant ($p>0.05$).

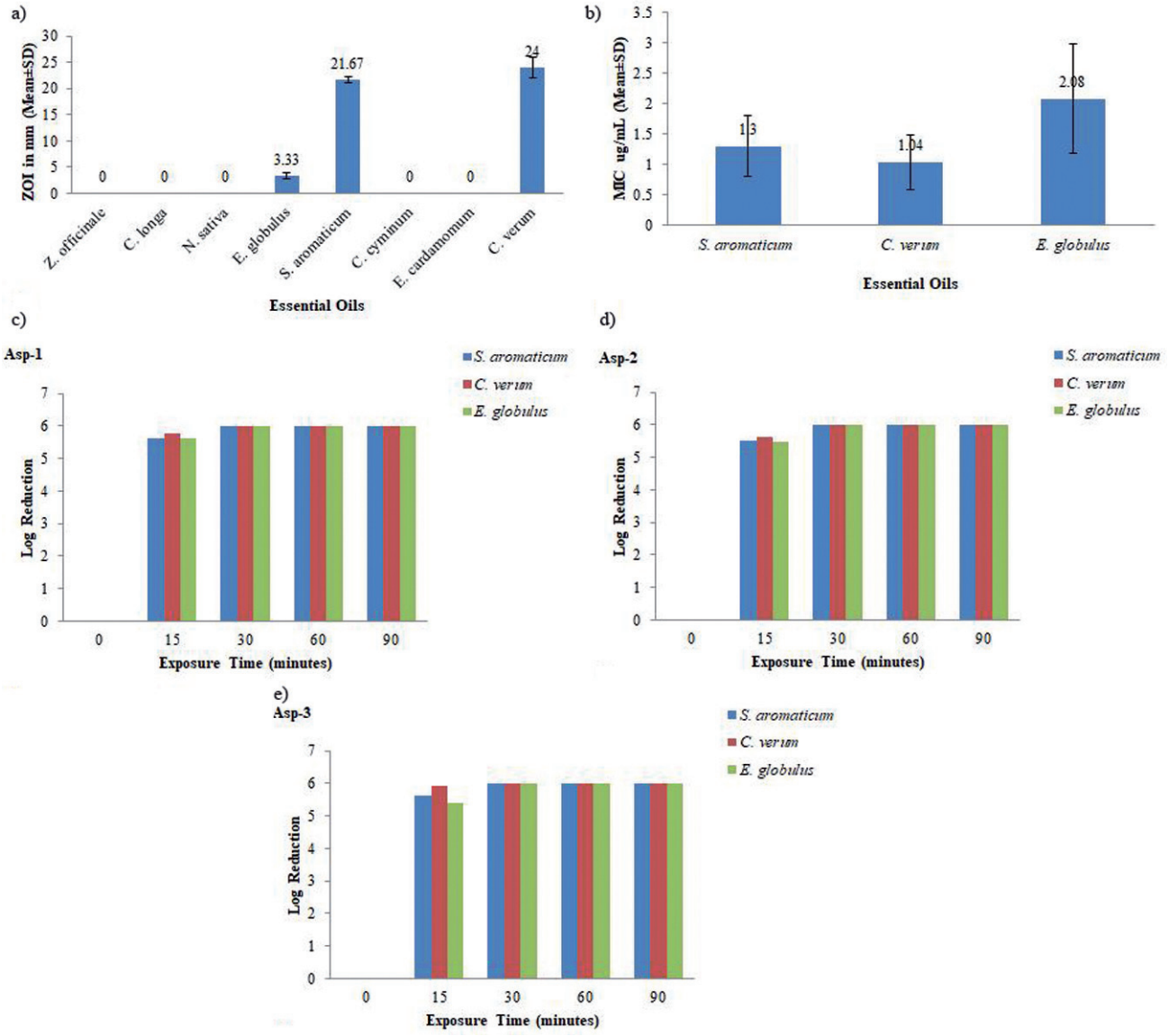

Fig. 1. Effect of essential oils on Ochratoxin-producing *Aspergillus parasiticus*. a) Mean Zone of inhibition of essential oils; b) Mean MIC values of essential oils; c) Log reduction of Asp-1; d) Log reduction of Asp-2; e) Log reduction of Asp-3.

Antifungal activity was also evaluated in terms of log reduction against OTA-producing *A. parasiticus* isolate-I, after 15 min of exposure a log reduction of 5.61±0.35, 5.77±0.00, and 5.60±0.00 was demonstrated by *S. aromaticum*, *C. verum* and *E. globulus* essential oils, respectively (Fig. 1c). In case of *A. parasiticus* isolate-II after 15 min of exposure a log reduction of 5.50±0.4, 5.61±0.00 and 5.45±0.00 was observed for *S. aromaticum*, *C. verum* and *E. globulus* essential oils, respectively (Fig. 1d). The log reduction values recorded for isolate-III after 15 min of exposure a log reduction of 5.60±0.3, 5.91±0.00 and 5.40±0.00 was demonstrated by *S. aromaticum*, *C. verum* and *E. globulus* essential oils, respectively (Fig. 1e). After 30, 60, and 90 min of exposure time of *A. parasiticus* spores to selected essential oils, complete inactivation of fungal spores (6±0.00 log reduction) was observed for three selected isolates (Figs 1 c, d, e). Differences observed among selected essential oils contact time were statistically not significant ($p>0.05$).

Antifungal Activity of *C. verum* Against OTA Production

The efficacy of *C. verum* essential oil against OTA produced by *A. parasiticus* at different moisture contents (10, 20, 30, 40, 50, 60 and 70%) was evaluated. On the 15th d of the experiment, the level of OTA was determined in wheat, maize, and rice (intact and broken) in all experimental groups. There was no increase in the quantity of OTA in the un-inoculated and inoculated-treated groups. The *C. verum* essential oil inhibited the growth of *A. parasiticus* at all moisture contents. However, a rise in OTA was observed in the inoculated group. In the inoculated group, the highest amount of OTA (12.52±0.11 ng/g) was determined in broken wheat at a 40% moisture level. At 15 d post-inoculation, a direct relation was observed in OTA production and moisture up to 40 % contents, with an increase in moisture from 10 to 40% there was an increase in OTA production. However, from 50 to 70% moisture content, a decrease in OTA by *A. parasiticus* was detected. The lowest OTA was quantified at 10% moisture, which was 2.01±0.12 ng/g in broken rice. Statistically significant differences were observed in OTA in inoculated wheat, maize, and rice (intact and broken) at all moisture levels and the OTA in inoculated-treated and un-inoculated groups ($p<0.05$). The values of OTA produced by *A. parasiticus* in the un-inoculated and inoculated-treated groups of intact and broken rice showed non-significant differences ($p>0.05$) (Fig. 2a).

On 30th d of the experiment, *C. verum* inhibited the growth of *A. parasiticus* at all moisture contents. Statistically, values of OTA produced by *A. parasiticus* in un-inoculated and treated groups of intact and broken rice showed non-significant differences ($p>0.05$). In inoculated group, the lowest OTA was quantified at 10% moisture, which was 3.53±0.10 ng/g in intact rice. The highest amount of OTA determined was 19.65±0.50 ng/g in broken wheat at a 40% moisture level. On 30 d post-inoculation, a direct relation was observed in OTA production and moisture up to 40% contents, with an increase in moisture from 10 to 40% there was an increase in OTA production (Fig. 2b). However, from 50 to 70% moisture content, a decrease in OTA by *A. parasiticus* was detected.

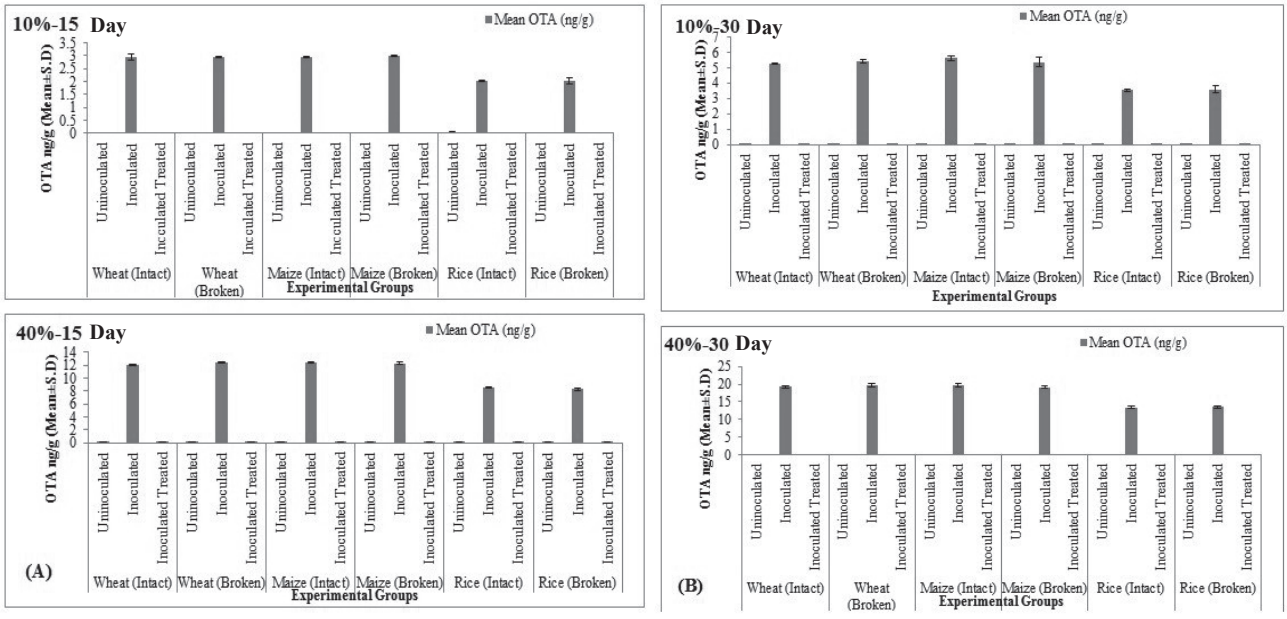

Fig. 2 a) Cinnamon efficacy against *Aspergillus parasiticus* in relation to ochratoxin A (ng/g) production at 15th day post inoculation (10% and 40% moisture levels). b) Cinnamon efficacy against *Aspergillus parasiticus* in relation to ochratoxin A (ng/g) production at 30th day post inoculation (10% and 40% moisture levels).

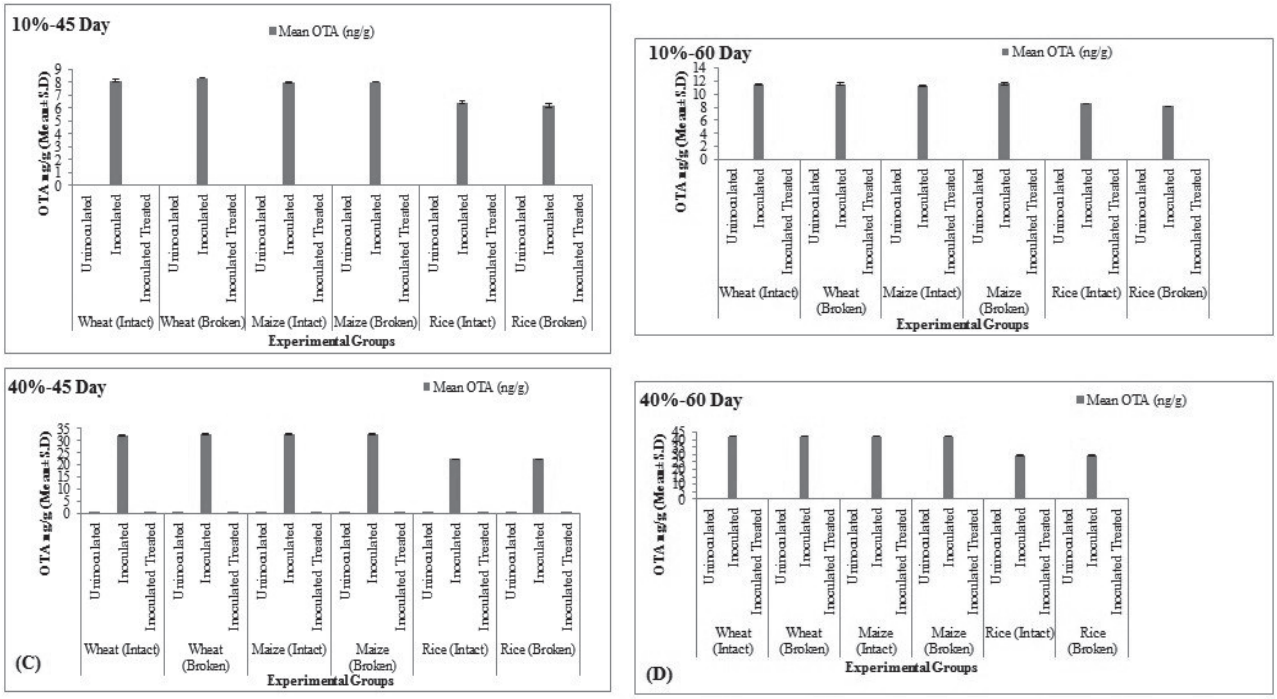

Fig. 2 c) Cinnamon efficacy against *Aspergillus parasiticus* in relation to ochratoxin A (ng/g) production at 45th day post inoculation (10% and 40% moisture levels). d) Cinnamon efficacy against *Aspergillus parasiticus* in relation to ochratoxin A (ng/g) production at 60th day post inoculation (10% and 40% moisture levels).

On 45th d post-inoculation, the level of OTA was determined in wheat, maize and rice (intact and broken) in all experimental groups. In inoculated group, the highest amount of OTA determined at 40% moisture level was 32.76±0.33 ng/g in broken wheat. The *A. parasiticus* produced OTA in increasing fashion up to 40% moisture contents, with an increase in moisture from 10 to 40% there was an increase in OTA production (Fig. 2c). A decrease in OTA by *A. parasiticus* was detected as moisture increased from 50 to 70%. The lowest quantity of OTA was found in broken rice having 10% moisture, which was 6.21±0.12 ng/g.

On the 60th d of the experiment, the level of OTA was determined in wheat, maize and rice (intact and broken) in all experimental groups. In inoculated group, the highest amount of OTA determined was 42.35±0.31 ng/g in intact wheat at a 40% moisture level. A direct relation was observed in OTA production and moisture up to 40% contents, with an increase in moisture from 10 to 40% there was an increase in OTA production (Fig. 2c). However, from 50 to 70% moisture content, a decrease in OTA production by *A. parasiticus* was detected. The lowest OTA was quantified at 10% moisture, which was 8.16±0.05 ng/g in broken rice.

There was no increase in the quantity of OTA in un-inoculated and inoculated-treated groups. Cinnamon inhibited the growth of *A. parasiticus* at all moisture levels. However, a rise in OTA production was noticed in the inoculated group. Statistically, values of OTA produced by *A. parasiticus* in the un-inoculated and inoculated-treated groups of intact and broken rice showed non-significant differences ($p>0.05$). Significant differences were observed in OTA in inoculated wheat, maize and rice (intact and broken) at all moisture levels. Significant differences were also observed in inoculated-treated and un-inoculated groups ($p<0.05$).

Ochratoxin A Quantification

Grain samples of 3 experimental groups were processed for TLC and HPLC. Three samples having toxin recovered from *A. parasiticus* in chloroform solvent were positive for OTA production. HPLC was performed for OTA standard and grain samples.

Gas Chromatography-Mass Spectrometry Analysis of Plant Essential Oils

Out of eight essential oils, only three EO's, *E. globulus*, *S. aromaticum* and *C. verum,* exhibited antifungal activity and were processed for GC-MS for qualitative and quantitative fatty acid analysis (Table 1). The highest percentage in *E. globulus*, *S. aromaticum* and *C. verum* was of benzene (29.9%), eugenic acid (53.7) and oleic acid (23.7%), respectively.

Discussion

Aspergillus group of fungi produce highly toxic compounds such as OTA, aflatoxins and various other toxic metabolites which mostly contaminate agricultural products and cause indirect damage to human and livestock health. Essential oils are being increasingly used to cope with the fungal contamination of grains. The results obtained in the present study indicated that antifungal activity of *S. aromaticum* (clove), *C. verum* (cinnamon) and *E. globulus* (eucalyptus) essential oils are quite promising against OTA-producing *A. parasiticus*. In this study, the highest zone of inhibition was observed for *C. verum* (24.00±2.00 mm) followed by *S. aromaticum* (21.67±0.57 mm) and *E. globulus* (3.33±0.57 mm). Moreover, significant variations were recorded in the antifungal activity of plant essential oils ($p<0.05$). The present study's results demonstrating antifungal activity of *C. verum, S. aromaticum,* and *E. globulus* are consistent with Císarová, et al. (2016) who have also reported antifungal activity of these plant essential oils against *A. parasiticus* [23]. Similarly, Vilela and colleagues have reported antifungal activity of *E. globulus* against *A. parasiticus* and *A. flavus* and concluded that *E. globulus* oil inhibited fungal growth at the concentration of 1.3492 µL [24].

In the present study, the least minimum inhibitory concentration was recorded for *C. verum* essential oil at the concentration of 1.04±0.45 µg/mL followed by *S. aromaticum* at the concentration of 1.30±0.5 µg/mL and *E. globulus* essential oil at the concentration of 2.08±0.90 µg/mL suggesting that the cinnamon oil can be used as an effective antifungal against OTA-producing *A. parasiticus*. This result is consistent with Bocate et al. (2021) who reported that plant essential oils can inhibit the growth of mycotoxigenic fungi. They reported that garlic essential oil reduced the growth of *A. parasiticus* [25]. Likewise, the present study's result of cinnamon oil as an effective antifungal against *A. parasiticus*, corroborates a previous study reporting *Cinnamomum* essential oil having significant antifungal activity against *A. parasiticus* [26].

Cinnamomum verum, S. aromaticum and *E. globulus* essential oils exhibited significant antifungal activity after 15 min of exposure time by log reduction assay against OTA-producing *A. parasiticus* isolates. Moreover, after 30, 60 and 90 -min exposure time of *A. parasiticus* spores to selected plant essential oils, complete inactivation of fungal spores (6±0.00 log reduction) was observed for all the three isolates of *A. parasiticus*. The present study's results indicated statistically non-significant differences ($p>0.05$) among selected essential oils concerning contact time. The present study results of the log reduction are consistent with Hossain et al. (2019) findings who have reported antifungal activity of various essential oils against *A. parasiticus*. The same study reported a growth growth reduction of 51-77% in *A. parasiticus* with the use of essential oils [27]. In another study, poultry feed was inoculated with *A. parasiticus* and trans-cinnamaldehyde (an essential component of cinnamon oil). The trans-cinnamaldehyde exhibited

Table 1. Fatty acid profile of Eucalyptus globulus, Syzygium aromaticum and Cinnamomum verum by Gas chromatography-mass spectrometry analysis.

Sr. No.	Plant essential oil	Fatty acids	Retention time (RT)	Percentage (%)
01	*E. globulus*	Bicyclo[4.1.0]heptane	14.4	0.33
		Benzene	15.1	1.9
		Benzene	15.3	29.9
		Cyclohexano	15.4	3.8
		γ-Terpinene	16	2.55
		Cyclohexene	16.5	5.22
		Benzene	16.6	3
		Trans-4-methoxy thujane	18.1	3
		Anisole, 2-isopropyl-5-methyl-	18.5	8
		Bicyclo[3.1.0]hexane, 6-isopropylidene-1-methyl-	19.1	17.7
		3-Cyclohexene-1-methanol,α,α,4-trimethyl-, acetate	19.2	3.8
		2,4-Octadienoic acid, 3-methyl	20.7	0.5
		Octana	21.2	7.7
		Methoxycitronellal	22	2
		Longifolene	22.8	0.4

Table 1. Continued.

02	*S. aromaticum*	Stearic acid	19.6	2.80
		Palmitic acid	20.5	13.6
		Eugenic acid	21.3	53.7
		Linoleic acid	25.6	4.03
		Oleic acid	26.5	18.5
03	*C. verum*	Cyclohexane	6.1	3.4
		Benzaldehyde	13.8	0.12
		p-Cymene	15.3	1.7
		N-α,N-ω-Di-cbz-L-arginine	15.4	0.05
		Cyclohexene	16.6	0.14
		3-Deoxy-L-ribose-2,5-dibenzoate	16.7	0.13
		Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-	17	0.68
		Benzenemethanol, 4-(1,1-dimethylethyl)-	18.5	0.11
		Acetaldehyde	19	1.4
		Bicyclo[3.1.0]hexane, 6-isopropylidene-1-methyl	19.1	0.65
		Linoleic acid	19.9	7.7
		Cinnamaldehyde	20.1	10.33
		9,12-Octadecadienoyl chloride	20.6	1.7
		Oleic acid	20.90	23.7
		10-Octadecenoic acid	20.94	14
		2-Propenoic acid	21.9	2.55
		9-Methoxybicyclo[6.1.0]nona-2,4,6-triene	22.1	9.3
		Benzene	22.3	7.65
		Octadecanoic acid	24.4	7.99
		Palmitic acid	25.8	4.2
		Tetradecanoic acid	29.2	0.67

a reduction in *A. parasiticus* growth as well up to a 60% reduction in toxin production consistent with the present study's findings [28].

In the grains inoculated with *A. parasiticus* at various moisture levels, a direct relation was observed in OTA production and moisture up to 40% moisture contents but at the higher moisture content (50 to 70%), a decrease in OTA by *A. parasiticus* was detected. These results are consistent with Moosavian et al. (2017) who have reported that *A. parasiticus* growth pattern was maximum at 27% moisture level on maize substrate [10].

One limitation of the present study was that all *A. parasiticus* isolates used were sourced from poultry feed samples collected from Lahore District of the Punjab Province of Pakistan. Future studies should evaluate plant essential oil's activity against *A. parasiticus* collected from other sources and other provinces of the country. However, the present study's findings indicate that plant essential oils can be an alternative to antifungal drugs.

Cinnamon oil inhibitory effects monitored by various anti-microbial assays such as well-diffusion, micro-broth dilution, and log reduction, as well as experimentation on grains by maintaining various moisture levels against emerging OTA-producing *A. parasiticus* suggest that *C. verum* oil can be an effective alternative antifungal agent. The use of plant essential oils such as cinnamon oil has an added advantage of being eco-friendly hence supporting the One Health Concept.

Conclusion

Mycotoxin contamination in foods and feed is a major concern of food regulatory authorities authorities as well as a public health concern. Besides,

spoiling of stored cereals or grains leads to significant economic losses. Owing to its promising antifungal activity against OTA-producing *A. parasiticus,* the *Cinnamomum verum* (cinnamon) essential oil can be an alternative to synthetic food preservatives.

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Author's Contributions

Gull Naz performed the experiments. Aftab Ahmad Anjum supervised the research and provided the necessary chemicals / reagents. Muhammad Nawaz and Sanaullah Iqbal served on the student's supervisory committee. Tehreem Ali assisted in the research work. Rabia Manzoor, Shahan Azeem and Aftab Ahmad Anjum wrote the manuscript. Shahan Azeem critically reviewed the manuscript and finalized the draft. All authors approved the final draft.

Conflict of Interest

The authors have declared no conflict of interest.

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