OCHRATOXIN DETOXIFICATION AND ANTIOXIDANT EFFICACY OF *AMARANTHUS VIRIDIS* AND *SORGHUM HALEPENSE*

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Abstract. The present study aims to investigate the antifungal, antioxidant, and ochratoxin degradation potential of *Amaranthus viridis* L. and *Sorghum halepense* L. Various concentrations (1-5%) of methanol extract of *A. viridis* and *S. halepense* leaves were tested in vitro against *Cladosporium cladosporioides* (Fresen.) G. A. de Vries and *Trichoderma viride* Pers. *A. viridis* leaf methanol extract caused a significant decline in the biomass of *C. cladosporioides* and *T. viride*. Methanolic extract from leaves of *S. halepense* also reduced the growth of both the tested fungi. The antioxidant activity of both experimental plants was also determined. Five concentrations (10-50%) were made in methanol, and Butylated hydroxytoluene was used as standard. Results from this assay revealed that both test plants have significant antiradical potential. The in vivo detoxification of ochratoxins by *A. viridis* extract against ochratoxin-producing *T. viride* and *C. cladosporioides* was also carried out. Peanuts and walnut were used as test samples from which *T. viride* and *C. cladosporioides* were isolated. Ochratoxins were extracted from the test fungi by solvent extraction method. Thin layer chromatography (TLC) was done for the detection of ochratoxin in both control and experimental samples followed by quantification through High-Performance Liquid Chromatography (HPLC). TLC analysis suggested that there was a remarkable reduction in ochratoxin level after the application of *A. viridis* in the walnut sample as compared to the control and maximum suppression was recorded in the walnut sample against *C. cladosporioides.* Similarly, HPLC analysis also revealed that the highest ochratoxin degradation was observed in the walnut sample against *C. cladosporioides*. This study concludes that botanical extracts can detoxify ochratoxins with little or no negative consequences.
Keywords: *antifungal, bioassay, detoxification, mycotoxin, secondary metabolites*

Introduction

Mycotoxins are secondary metabolites produced by broad categories of filamentous fungi. Common mycotoxin-producing fungal genera are; Aspergillus, Fusarium, Penicillium, Cladosporium, and Trichoderma (Angelina and Celeste, 2010). Ochratoxins belong to a family of structurally related, secondary fungal metabolites produced by various Claosporium, Penicillium, Trichoderma, and Aspergillus lines. Ochratoxins may be classified into three categories, amongst them, ochratoxin A is the most commonly occurring metabolite and the most dangerous nephrotoxic and carcinogenic agent (Rosa et al., 2008). Exposure to ochratoxin infected diet for an extended time frame leads to numerous health troubles including, kidney, and liver cancers and the weakening of the immune system. Ochratoxin leads to serious health issues for animals when consumed in agricultural products contaminated by such kinds of mycotoxins. Ochratoxin consumption often causes hepatotoxic, carcinogenic, genotoxic, immunotoxic, teratogenic, and neurotoxic health effects on animals (Lucchetta et al., 2010).

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Cladosporium species secretes mycotoxins such as cladosporin, isocladosporin, emodin, epi- and fagicladosporic acid, and ergot alkaloids. Besides these, over a dozen molecules were detected as secondary metabolites of Cladosporium species (Yano et al., 2003). The genus Trichoderma is broadly used as a biocontrol agent for fungal phytopathogens and the manufacturing of enzymes, and as a result, the capability of mycotoxin production of this genus is an inherent safety difficulty for the enterprise (Blumenthal, 2004). Mycotoxins reported from Trichoderma encompass the cytotoxic and immunosuppressive trichothecenes, gliotoxin, and ribosome-inactivating proteins (Nielsen et al., 2005).

Many foodstuffs and cereals are prone to fungal infection either inside the area or at some storage point. There are many factors affecting mycotoxin production in foodstuffs and stored grains, including environmental elements like temperature, water activity, pH, light, and the nature of substrate which ordinarily manipulate the mycotoxin production. Temperature and relative humidity range are two basic environmental elements; involved in mycotoxin manufacturing and the fungal boom. Agriculture zone use fungicides for fungal disease control that have significantly elevated agriculture productivity. However, excessive use of those chemical substances has ended in meal infection (Wu et al., 2014), bad environmental influences, and resistance disorders which have triggered drastic influences on food security and human health (Singh and Trivedi, 2017). Therefore, there is a dire need to explore natural sources of pathogen control (Kim et al., 2017). For the remedy of numerous diseases medicinal flora is used globally (Modak et al., 2007).

Sorghum halepense also known as Johnson grass belongs to the Gramineae family and is an herbaceous perennial plant located in dry areas, ditches, cultivated fields, and wastelands (Kim et al., 2017). This plant possesses the allelopathic potential and is supposed to have an antimicrobial ability due to the presence of p-hydroxybenzoic acid, tricin, salcolon, and luteolin (Baerson et al., 2008).

Amaranthus viridis belongs to the family Amaranthaceae and is commonly known as green amaranth or domestically as “Karund”. This plant contains phytochemicals like flavonoids, quercetin, and rutin. Flavonoids have a biochemical impact that inhibits the interest of enzymes, and hormone regulation and feature antimicrobial, antioxidant and anticancer activities (Bagepalli et al., 2009). Therefore, the purpose of the present investigation was to manage the ochratoxin-producing fungi by the extracts of *A. viridis* and *S. halepense.*

Materials and methods

Collection and procurement of experimental material

The plants *A. viridis* and *S. halepense* at a vegetative stage were collected from the Raiwand site of Lahore. Test plants were surface sterilized, dried under shade, and ground to a fine powder. *T. viride* (FCBP-671) and *C. cladosporioides* (FCBP-976) were obtained from the First Fungal Culture Bank of Pakistan, Department of Plant Pathology, University of Punjab, Lahore. Test fungi were sub-cultured and maintained on 2% MEA (Malt Extract Agar) and kept at 4 °C in the refrigerator.

Antifungal bioassays

Twenty grams of both *A. viridis* and *S. halepense* were soaked separately in 100 mL of methanol for one week at room temperature. Methanol was selected for the

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maceration as methanol is considered the best solvent for the extraction (Osmić et al., 2019). Materials were filtered through an autoclaved muslin cloth. The volume of methanolic extract was reduced up to 2 g by evaporating at 35 °C in an electric oven and then diluted by adding the appropriate quantity of distilled water to make the final volume up to 100 mL. The stock extracts were stored at 4 °C. ME (2%) was prepared in 250 mL flasks and was autoclaved at 121 °C for 30 min. Various concentrations viz. 1%, 2%, 3%, 4%, and 5% w/v were made by adding an appropriate amount of test plant extracts in ME media.

The control treatment was without any plant extracts. Each concentration was endowed with a Chloromycetin capsule @50 mg 100 mL-1 of medium to avoid bacterial contamination. All the concentrations were replicated three times. Mycelial discs (5 mm in diameter) were prepared using a sterilized cork borer from the tip of the 7-day-old culture of *C. cladosporioide*s and *T. viride* was placed in the center of each flask. After 7 days, fungal growth was measured by filtering the solution of each concentration through pre-weighed Whattsman no. 1 filter paper (Khan et al., 2021). The test fungal biomass was measured by using *Equation 1:*

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100 \qquad \text{(Eq.1)}$$

Antioxidant activity/DPPH assay

The antioxidant activities of methanolic extracts of *A. viridis* and *S. halepense* were evaluated through the free radical scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical using the protocol of Saeed et al. (2021). For this purpose, 10 g of both plants were dipped in 50 mL of methanol and subjected to filtration. Three mL of 0.004% DPPH methanolic solution served as control was added into 10, 20, 30, 40, and 50 µL of sample extracts making 10%, 20%, 30%, 40%, and 50% of stock solutions and in the same way concentrations for standard “Butylated hydroxytoluene” were made. The mixture was thoroughly mixed and kept still in the dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer (UV-1700, Shimadzu Japan). Results were expressed as the percentage of inhibition of radical DPPH which can be related to the decrease in absorbance for the control value and was calculated by using *Equation 2:*

$$\text{\% age inhibition of DPPH} = \frac{[\text{Abs in control sample} - \text{Abs in experimental sample}]}{\text{Abs in control sample}} \times 100 \qquad \text{(Eq.2)}$$

Ochratoxin degradation assay

Ochratoxins were extracted from *T. viride* and *C. cladosporioides* by solvent extraction method. For this purpose, colony margins were scraped into a 250 mL Erlenmeyer flask containing 70 mL chloroform and 30 mL acetone and shaken on a rotary shaker for 30 min at 200 rpm (Iram et al., 2016; Yazdani et al., 2010). After that filtration was carried out of fungal crude extract from the solvent mixture (Nisa et al., 2014).

One hundred and fifty grams of powdered *A. viridis* was dipped in 350 mL of distilled water. Homogenate was filtered through muslin cloth and centrifuged at 14,000 rpm for 20 min. The centrifuged extract was again filtered with Whatman No. 1 filter paper to remove any other impurities. After filtration 145 mL of plant extract was obtained (Velazhahan et al., 2010).

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In vivo, ochratoxin detoxification assay was also conducted and for this 30 g of walnuts and peanuts were used as test samples (Iqbal et al., 2018). These test samples were separately autoclaved, ground, and kept in a 250 mL Erlenmeyer flask. The control group was spiked with 50 mL of extracted toxins from fungal strains while the experimental group (peanuts and walnuts) was spiked with 15 mL of fungal toxin and 35 mL of *A. viridis* methanolic extract. Separate flasks were made for each fungal strain. All flasks were incubated at 30 °C for 72 h. After incubation, filtration was carried out using a disposable microfilter (0.22 µm). The obtained filtrate was preserved for further quantitative analysis of ochratoxins using TLC and HPLC (Hierro et al., 2008).

For TLC detection, 2 mL of filtrates were taken and allowed to evaporate and re-dissolve in a solvent mixture of toluene and acetic acid in the ratio of 0.5:0.5 up to 1 mL. Spotting of all filtrates (5 µL), as well as ochratoxin standard (5 µL), was carried out on a TLC plate and the plate was dipped in 40 mL of mobile phase (toluene: acetic acid: formalin; 24: 12: 4). TLC Plate was observed in UV light (365 nm) and violent colored spots indicated the presence of ochratoxins in both samples. Fluorescent intensities of samples or filtrates were compared and calculated using the Rf value with standard spot (Nisa et al., 2014). Rf value was calculated by using *Equation 3:*

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}} \quad \text{(Eq.3)}$$

Quantitative analysis of the control and experimental group was done by using High-Performance Liquid Chromatography. For this purpose, 10 µL of the preserved filtrate was analyzed on an HPLC system consisting of a reverse phase C18 column and a fluorescence detector. The mobile phase consisting of distilled water, methanol, and acetonitrile was made up to 200 mL in the ratio of 120:40:40. Flow rate of 1 mL^{-1} was applied and ochratoxin was detected at excitation and emission wavelengths of 230-340 nm (Hierro et al., 2008).

Statistical analysis

Data obtained in this study were statistically evaluated using analysis of variance (ANOVA) to determine variability among groups by Statistix 8.1 software at a significance level of $P \leq 0.05$.

Results and discussion

Antifungal activity

A. viridis methanolic leaf extract showed significant antifungal activity against *C. cladosporioides and T. viride* (*Figs. 1* and 2). All the applied concentrations (1-5%) of methanolic extract of leaves of *A. viridis* exhibited substantial antifungal activity against the test fungi. Shirazi et al. (2020) reported that *A. viridis* showed maximum inhibitory potential against the test fungal pathogen; therefore *A. viridis* can be utilized for the management of fungal diseases. Earlier, Iqbal et al. (2012) studied the phenolics, antioxidant and antimicrobial activities of leaf and seed extracts from *A. viridis*. The tested extracts showed considerable antimicrobial activity. Bruna et al. (2012) also

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reported the existence of antiphytopathogenic properties of organic extracts from the leaves of *A. viridis.*

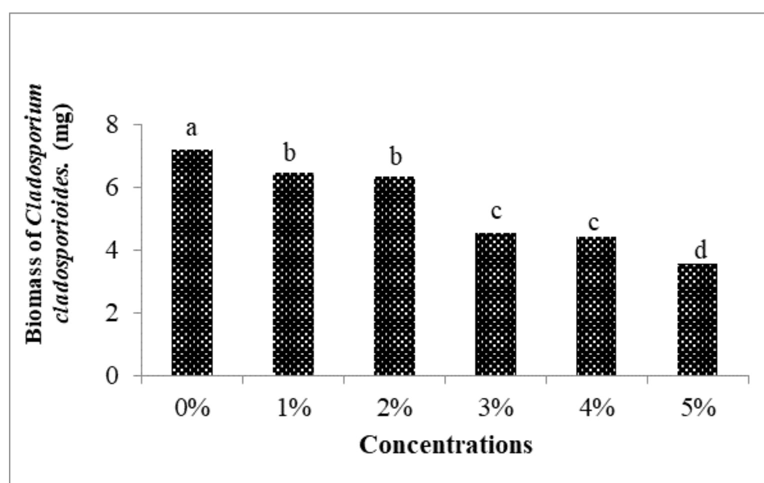

Figure 1. *Effect of methanolic leaf extract of Amaranthus viridis on in vitro growth of Cladosporium cladosporioides. Values with different letters show significant difference as determined by LSD at (P = 0.05)*

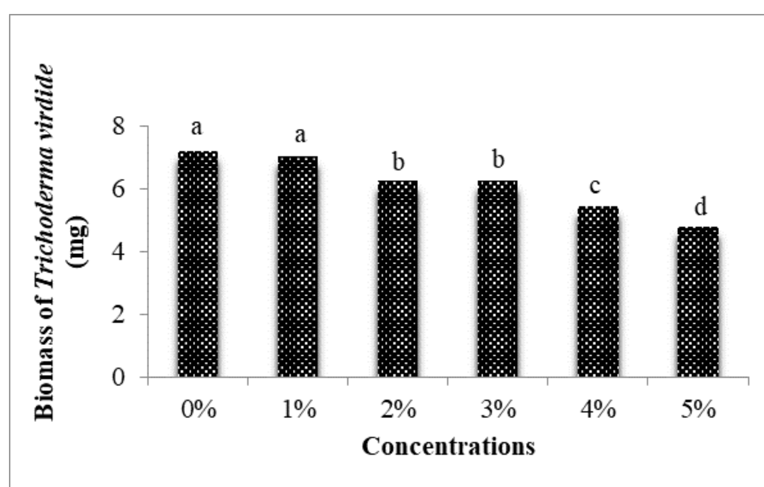

Figure 2. *Effect of methanolic leaf extract of Amaranthus viridis on in vitro growth of Trichoderma viride. Values with different letters show significant difference as determined by LSD at (P = 0.05)*

The methanolic leaf extract of *S. halepense* demonstrated strong antifungal activity against *C. cladosporioide*s and *T. viride* (*Figs. 3* and *4*). The tested extract of *S. halepense leaves* significantly inhibited the *in vitro* biomass of the test fungi. The existence of several phytoconstituents such as sterols is deliberated as a strong antimicrobial agent in *S. halepense* (Salazar-Lopez et al., 2018). Phenols and flavonoids have been documented in different parts of *S. halepense* playing a role in enhancing its allelopathic and antimicrobial probability (Huang et al., 2010).

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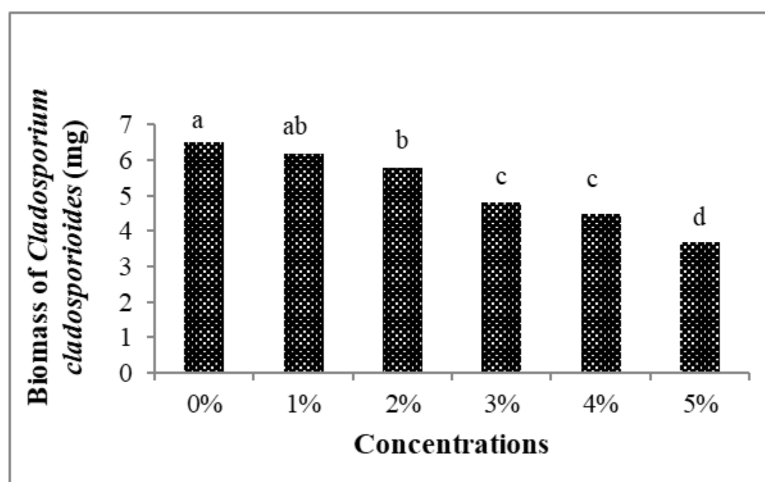

***Figure 3.** Effect of methanolic leaf extract of Sorghum halepense on in vitro growth of Cladosporium cladosporioides. Values with different letters show significant difference as determined by LSD at (P = 0.05)*

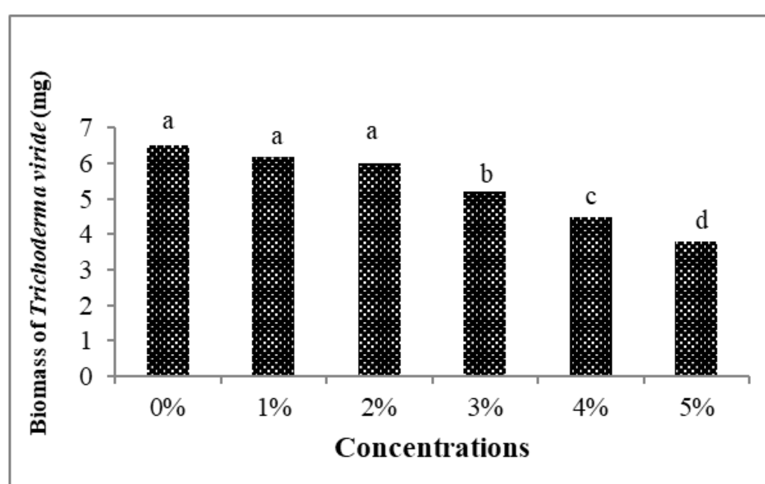

***Figure 4.** Effect of methanolic leaf extract of Sorghum halepense on in vitro growth of Trichoderma viride. Values with different letters show significant difference as determined by LSD at (P = 0.05)*

Radical scavenging activity/DPPH assay

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out spectrophotometrically to determine the antioxidant activity of *A. viridis* and *S. halepense*. Among both test plants, *A. viridis* showed significant antiradical potential in contrast to *S. halepense* which also showed antioxidant potency. In both test plants, the highest percentage inhibition was calculated at the concentration of 50% while the minimum potential was observed at a 10% of concentration. For *S. halepense*, maximum inhibition was recorded at the percentage of 61.94% while minimum activity was reported at 15.16%. On the other hand; In the case of *A. viridis* highest antiradical activity was given at 65.30% and minimum efficacy was investigated at 23.46% in comparison with standards that possess uppermost radical scavenging efficacy at 54.8%

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while lesser activity at 16.41%. From the results, it can be seen that antioxidant potential kept on increasing as concentrations were increased for both test plants. (*Figs. 5* and *6*). Ahmed et al. (2013) described the antioxidant analysis of methanol extracts of the dried leaves and seeds of *A. viridis* in which the highest DPPH activity was observed in seed extract. Akbar et al. (2020) studied the antioxidant activities of different parts of *A. viridis* prepared in n-hexane, chloroform, and ethyl acetate fractions. The results showed that the ethyl acetate leaf fraction exhibited better results as compared to other fractions. Khayal et al. (2019) evaluated the antioxidant activities of various extracts of *S. halepense.* Results indicated that ethyl acetate, n-hexane, and aqueous fractions showed excellent 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging potential. Shah et al. (2019) also examined the anti-oxidant potency of the *S. halepense* methanolic extract and its different fractions. The study revealed that aqueous fractions possess significant free radical scavenging properties in DPPH. The role of *S. halepense* as an antioxidant agent goes to the presence of bioactive polyphenols and dietary antioxidants as concluded from the analysis by Punia et al. 2021.

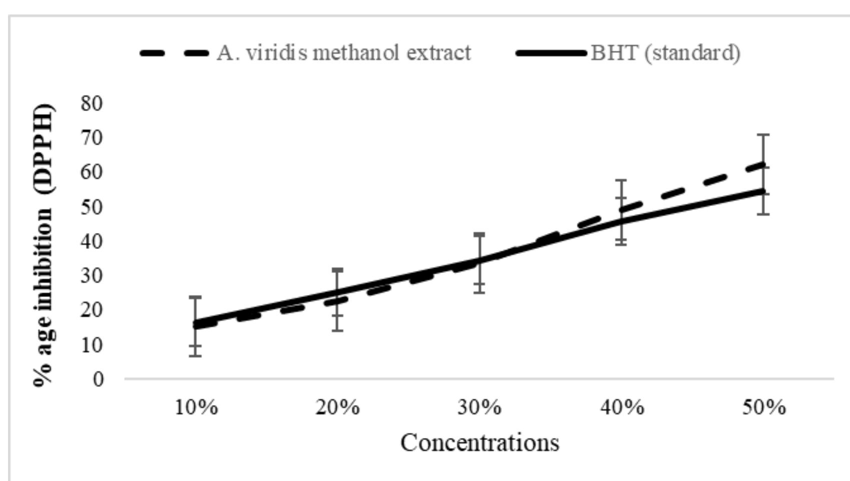

Figure 5. *Percentage yield and antioxidant activity of different methanolic concentrations of A. viridis at (P = 0.05)*

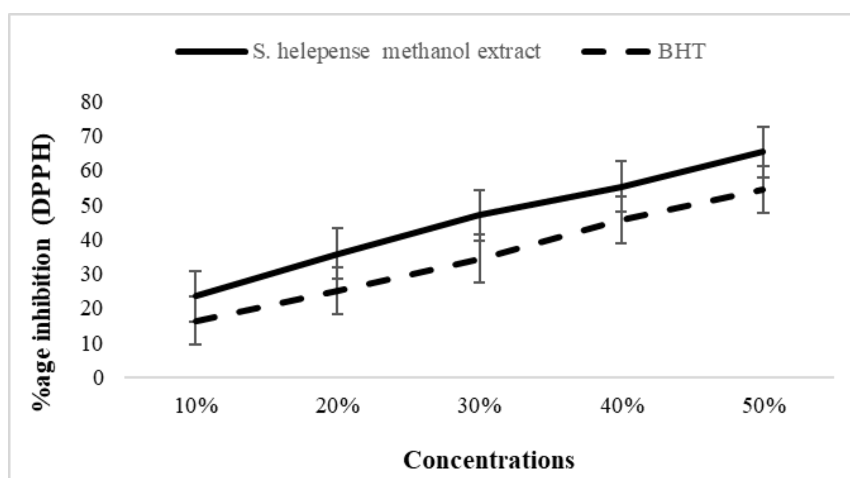

Figure 6. *Percentage yield and antioxidant activity of different methanolic concentrations of S. halepense at (P = 0.05)*

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Qualitative detection of ochratoxins by TLC after detoxification with A. viridis

Thin layer chromatography (TLC) is a rapid, inexpensive, and convenient method that can be used for screening mycotoxins. The dried powder of walnuts and peanuts was used for the qualitative detection of ochratoxin degradation by *A. viridis* methanolic extract. As depicted in *Table 1*; in control treatments walnuts shows the presence of ochratoxins in lesser amounts against *T. viride* while toxin level is almost up to the standard in samples for peanuts. On the other hand; after detoxification of all samples with *A. viridis* extract; the amount of ochratoxins produced by *C. cladosporioides* seems to be extremely reduced in walnuts and peanuts (*Tables 1* and *2*). *A. viridis* extract also shows retardation to a lesser extent in ochratoxin level that was produced *by T. viride* in walnuts and peanuts. The results clearly stated that *A. viridis* proved to be significant in reducing the occurrence of ochratoxins in *C. cladosporioides* and it can be used as the best suitable bio-control agent for inhibiting the growth of *C. cladosporioides.*

Table 1. *Detection of ochratoxins in control samples using TLC*

Sample labels	Retention factor Rf		
	Distance travelled by compound = Ds (cm)	**Distance travelled by solvent front = Df (cm)**	**Rf = Ds/Df**
C. cladosporioides (Walnuts)	3.3	10	0.33
T. viride (Walnuts)	0.6	10	0.06
C. cladosporioides (Peanuts)	4	10	0.4
T. viride (Peanuts)	4.1	10	0.41
Standard	5.8	10	0.58

Table 2. *Detection and estimation of ochratoxins in experimental samples using TLC*

Sample labels	Retention factor Rf		
	Distance travelled by compound = Ds (cm)	**Distance travelled by solvent front = Df (cm)**	**Rf = Ds/Df**
C. cladosporioides (Walnuts)	2.2	8	0.27
T. viride (Walnuts)	3.5	8	0.43
C. cladosporioides (Peanuts)	2.3	8	0.28
T. viride (Peanuts)	5.3	8	0.66
Standard	5.7	8	0.71

For the elimination of infectious diseases caused due to release of mycotoxins, natural plant-based products proved to be an exceptional alternative to synthetic pesticides as they are eco-friendly. This is because plants possess phytochemicals which are secondary metabolites that contribute to defending plants from pathogenic attacks and harsh ecological situations (Prakash et al., 2020). These phytochemicals have antimicrobial potential which attributes to their significant role in protecting animals and humans against many microbial diseases (Redondo-Blanco et al., 2019). Natural products originating from plants are extensively used in foodstuffs as a preservative of their sensory features, ensuring quality and protection during the shelf life (Negi, 2012). The antioxidant and antimicrobial efficiency of natural product constituents are

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determined by their chemical structure, active ingredients, concentration, and extraction technique (Vilela et al., 2016). The mode of action of these natural preservatives is the inhibition of infectious agent's development by certain oxidative enzymatic reactions taking place in the foodstuffs (Singh et al., 2010).

Qualitative estimation of ochratoxins by HPLC after detoxification with A. viridis

HPLC method has been used beforehand for the quantitative assessment of ochratoxins in foodstuff. For this purpose, selected food samples were passed through filtration and immuno-affinity columns for cleaning purposes and then they were subjected to HPLC analysis followed by fluorescence detection with 0.01–0.30 mg/kg as quantification limit depending on the type of sample (Skarkova et al., 2013).

Results from *in vitro* analysis revealed that in the case of peanuts with *C. cladosporioides* (control sample) three peaks were observed at a height of 46.54%, 12.78%, and 3.45% respectively compared with a standard which gives a peak at 37.22% which means that a considerable level of ochratoxins known to be present in the control sample. No reduction in ochratoxin level produced by *C. cladosporioides* was observed when treated with *A. viridis*. On another hand in the case of peanuts and *T. viride*, three peaks were obtained at a height of 17.62%, 7.10%, and 1.82% compared with standard showing peaks up to 73.46%. However, a substantial reduction in toxin level was found in experimental (peanuts + *T. viride* + *A viridis*) contrast with one peak at height of 8.92% in contrast with a peak of the standard at 91.08% (*Figs. A1–A5*).

On the other hand, when walnuts were treated with *C. cladosporioides*, two peaks were known to exist at heights of 25.47% and 13.67% in contrast with the standard peak (60.86%). While *A. viridis* was found to be an excellent source in inhibiting the release of ochratoxin produced by *C. cladosporioides* as results gave just one peak at 2.44% height which is an extremely lesser concentration when compared with the standard peak at height of 97.56%. Besides in the control sample for *T. viride* low level of ochratoxins was present when noted peak height of 22.90% in contrast with the standard peak of 77.10%. On the contrary significant retardation in toxin level was eminent by seeing peak height of 12.75% compared with the standard peak at 87.25% (*Figs. A6–A9*). Jadhav and Biradar (2016) reported that petroleum ether, ethanol, methanol, and distilled water extracts of *A. spinosous* also possess remarkable antifungal activity and hence can be used to control the release of ochratoxins from pathogenic fungal specimens. Peter and Gandhi (2017) reviewed that Amaranthus species encompass a wide range of bioactive compounds like alkaloids, flavonoids, glycosides, phenolic acids, steroids, saponins, amino acids, vitamins, minerals, terpenoids, lipids, betaine, catechuic tannins, and carotenoids that counts for its antifungal potential. Phenolic compounds act as reducing agents, triggering an antioxidant response. Flavonoids can remove free radicals, and inhibit cyclooxygenase and lipoxygenase enzymes. These enzymes are responsible for the evolution of oxidative odorousness in foodstuffs (Embuscado, 2015). Terpenoids possess antimicrobial activities and have the potential to use as natural preservatives in the food manufacturing industry (Lyu et al., 2019). The bioactive phytochemicals in plants have been used as substances to inhibit fungal development and mycotoxin infection (Mathuria and Verma, 2007). Stakheev et al. (2022) suggested the presence of inhibitors in natural entities that can suppress the biosynthesis of different mycotoxins without suppressing the growth and development of their producers (fungi).

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Conclusion

This study can be concluded that both test plants possess antifungal and antioxidant activities against ochratoxin-producing fungal strains. The production of bioactive components from such indigenous resources and their utilization as potential natural food preservatives could be of high economic value as they play a significant role in the suppression of toxic compounds that are released from pathogenic fungal strains and hence in return protect the agricultural commodities from fatal fungal attacks.

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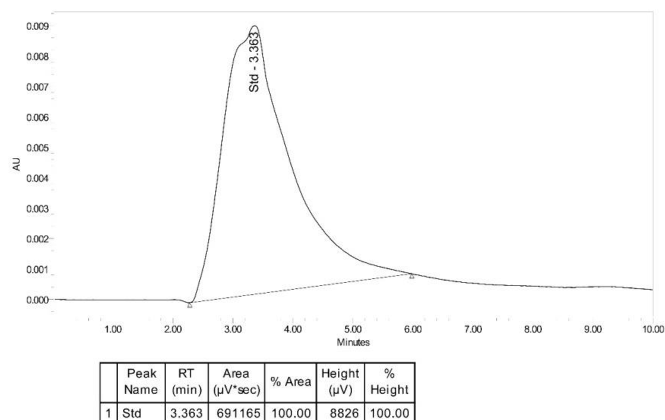
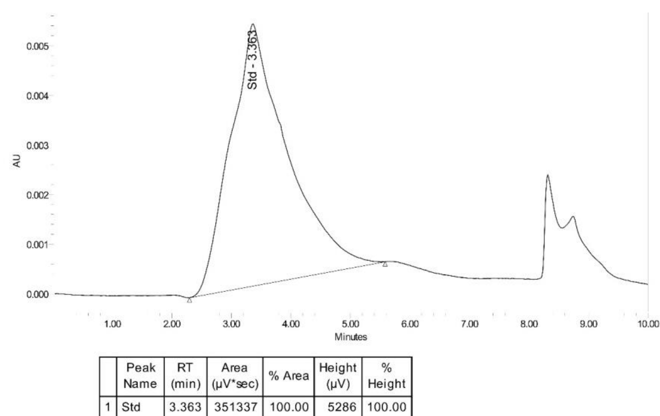
APPENDIX



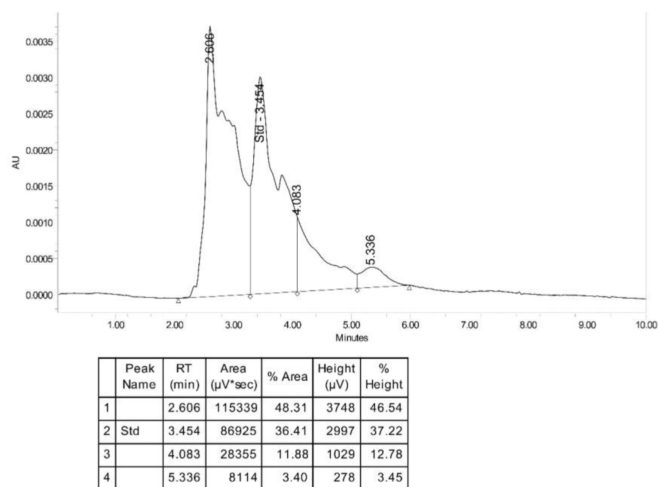
	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	Std	3.363	691165	100.00	8826	100.00

Figure A1. *Standard (10.13 ug/ml Biopure ochratoxin A) Peak obtained by HPLC*



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	Std	3.363	351337	100.00	5286	100.00

Figure A2. *HPLC chromatogram showing ochratoxin detoxification in control treatment (Peanuts + C. cladosporioides)*



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1		2.606	115339	48.31	3748	46.54
2	Std	3.454	86925	36.41	2997	37.22
3		4.083	28355	11.88	1029	12.78
4		5.336	8114	3.40	278	3.45

Figure A3. *HPLC chromatogram HPLC chromatogram showing ochratoxin detoxification in experimental treatment (Peanuts + C. cladosporioides + A. viridis)*

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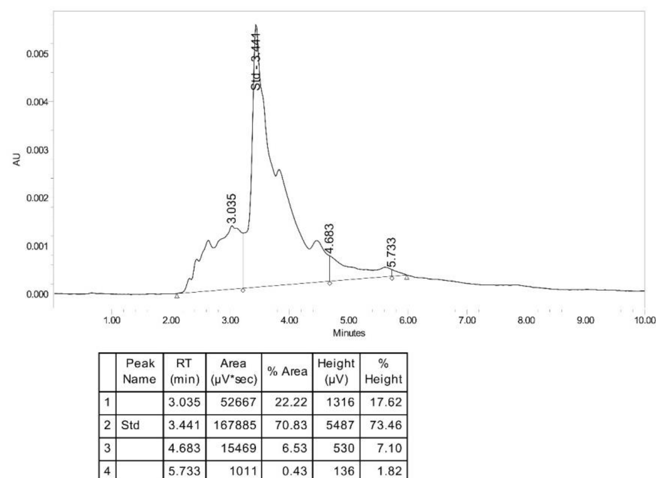
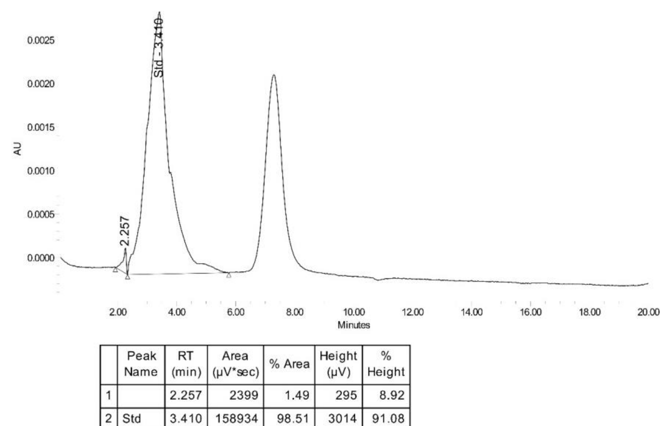


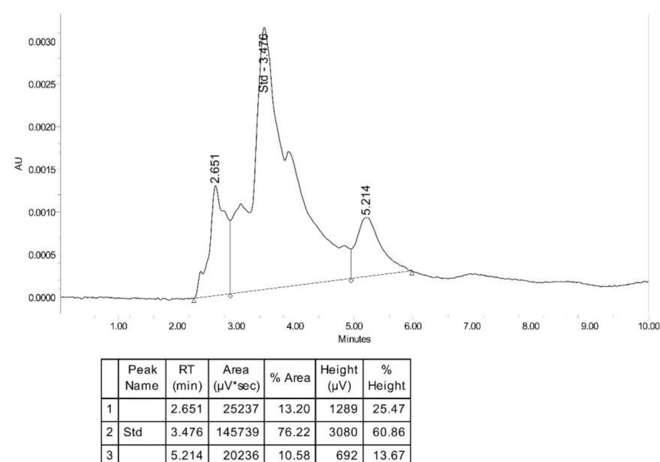
	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1		3.035	52667	22.22	1316	17.62
2	Std	3.441	167885	70.83	5487	73.46
3		4.683	15469	6.53	530	7.10
4		5.733	1011	0.43	136	1.82

Figure A4. *HPLC chromatogram showing ochratoxin detoxification in control treatment (Peanuts + T. viride)*



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1		2.257	2399	1.49	295	8.92
2	Std	3.410	158934	98.51	3014	91.08

Figure A5. *HPLC chromatogram showing ochratoxin detoxification in experimental treatment (Peanuts + T. viride + A. viridis)*



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1		2.651	25237	13.20	1289	25.47
2	Std	3.476	145739	76.22	3080	60.86
3		5.214	20236	10.58	692	13.67

Figure A6. *HPLC chromatogram showing ochratoxin detoxification in control treatment (Walnuts + C. cladosporioides)*

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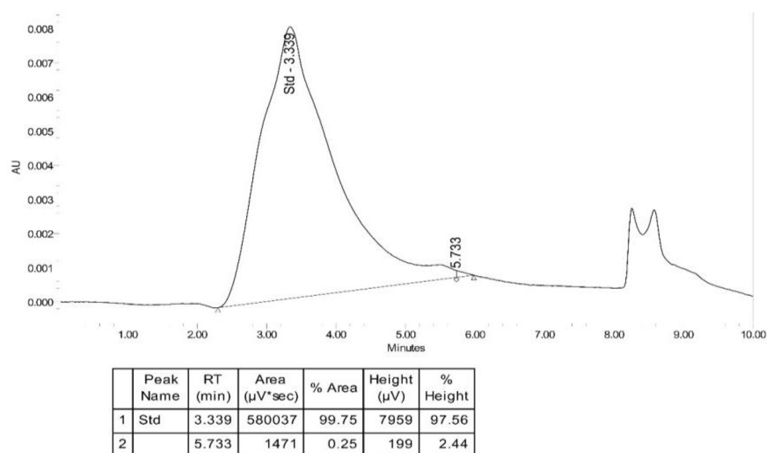
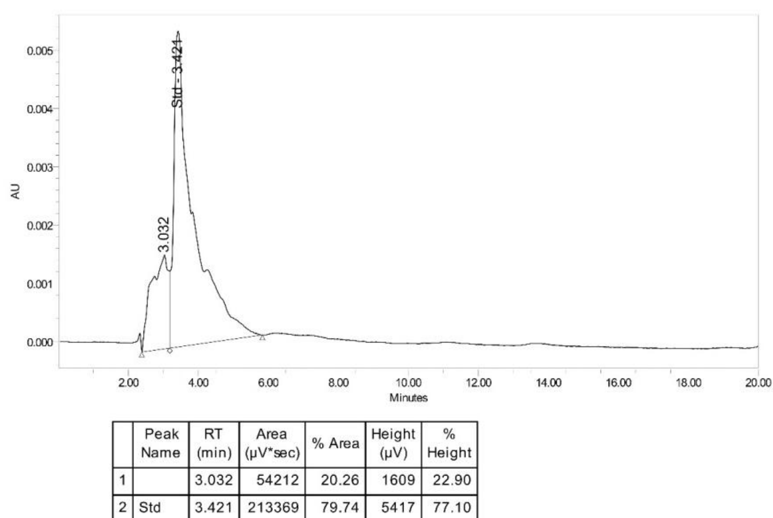


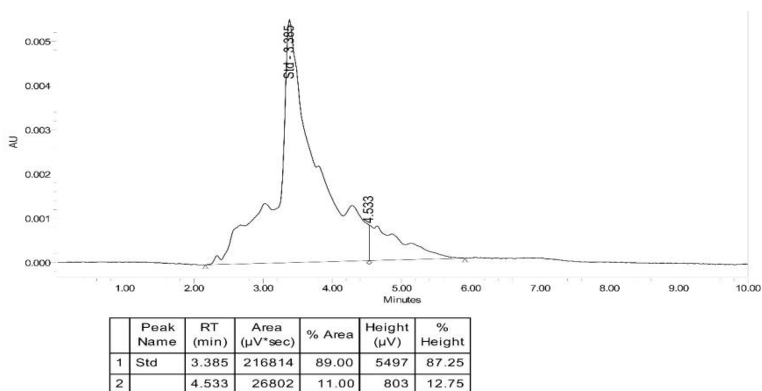
	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	Std	3.339	580037	99.75	7959	97.56
2		5.733	1471	0.25	199	2.44

Figure A7. *HPLC chromatogram showing ochratoxin detoxification in experimental treatment (Walnuts + C. cladosporioides + A. viridis)*



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1		3.032	54212	20.26	1609	22.90
2	Std	3.421	213369	79.74	5417	77.10

Figure A8. *HPLC chromatogram showing ochratoxin detoxification in control treatment (Walnuts + T. viride)*



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	Std	3.385	216814	89.00	5497	87.25
2		4.533	26802	11.00	803	12.75

Figure A9. *HPLC chromatogram showing ochratoxin detoxification in experimental treatment (Walnuts + T. viride + A. viridis)*

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