



Inhibitory effect of essential oils on growth and citrinin production by three strains of *Aspergillus terreus*

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Abstract

The screening of essential oils of castor (*Ricinus communis*), palmolive (*Elaeisguineensis*), groundnut (*Arachishypogaea*), coconut (*Cocosnucifera*), brassica (*Brassica oleracea*), sunflower (*Helianthus annuus*), Sesame (*Sesamumindicum*), Eucalyptus (*Eucalyptus globulus*), Neem (*Azadirachtaindica*), and Olive (*Oleaeuropaea*) for their effectiveness among the growth and Citrinin production by three strains of *Aspergillus terreus* was investigated. Neem oil, eucalyptus oil and olive oil were more effective inhibitors when compared to other oils. Based on the observations neem oil, eucalyptus oil and olive oil proved to be prospective bio-control agents for *Aspergillus terreus* and Citrinin production.

Keywords: *aspergillus terreus*, essential oils, growth, citrinin, HPLC

Introduction

Mycotoxins are a gaggle of structurally diverse secondary metabolites of fungi that occur as contaminants of grains. Mycotoxins possess high biological activity against other microorganisms (antibiotics), plants (phytotoxins) and animals (mycotoxins). Several secondary metabolites like fumagillin, Fusaric acid and mycophenolic acid elaborated by fungi are valuable therapeutic agents, while other metabolites may be potent toxins (Osweiler, 2000 [15]; CAST, 2003). Wicklow (1994) [27] feel that mycotoxins production by fungi might have evolved as an efficient associate in nursing-insect or as an anti-rodent agent. The character and amount of mycotoxins elaborated is entirely influenced by the interaction of certain fungi. Some of the oils are reported to protect the food commodities from mould infestation and deterioration (Narendra Kumar and Tripathi, 2002 [13] and PriyankaSingh *et al.*, 2009). Advantages of the use of oils are their low cost, excellent spreading and sticking properties to plant surfaces and no toxicity towards a man and are eco-friendly with nature. The use of oils in the control of plant diseases has been reported by Vandana Pandey *et al.* (2002) [25], Rattanapitigorn *et al.* (2006) [20], Achour *et al.* (2008) and Saban *et al.* (2009). Therefore, an attempt has been made to evaluate the efficiency of different oils against three strains of *Aspergillus terreus*.

Materials and Methods

The essential oils of Brassica, castor, coconut, eucalyptus, groundnut, neem, olive, Palmolive, sesame and sunflower were procured from companies located in Warangal, Telangana. The three fungal strains of *Aspergillus terreus* isolated from food and feeds and maintained on CYA medium and preserved at room temperature (Rao *et al.*, 2015) [11] were employed. The effect of oils on citrinin production and biomass growth of the fungi were done as per the methods described earlier by Rao *et al.* (2015) [11]

Toxin extraction and clean up

The possible production of mycotoxins of all isolates of *Aspergillus* species was grown on CYA broth of 7 days old cultures. The spore suspension of 10⁻⁵ dilution was inoculated for 15 days at 27±2 °C into 50 ml of CYA broth and incubated in dark for on shaker for 150 rpm. At the end of the incubation period, 25 ml of the broth was filtered through what man No.42 filter paper. The filtered samples were acidified with 6 N O-Phosphoric acid. Add twenty-five ml of ethyl acetate to 25 ml culture filtrate of *Aspergillus* species in 100 ml of the conical flask shake thoroughly and extracted twice with 25 ml of ethyl acetate. The ethyl acetate extract was evaporated by a water bath and the obtained compound was resuspended in 500 µl of methanol and preserve at 10 °C until use.

Detection of citrinin by High-performance Liquid Chromatography (HPLC)

Aspergillus terreus was assessed for production of citrinin. A volume of 10µl (1×10⁻⁶) spore suspension was grown in 100 ml of CYA broth at 28 °C for 15 days for citrinin production. Following the incubation period, cultures were harvested and citrinin extraction was performed using ethyl acetate twice to enhance the purity of the compound. Further purification was performed using immunoaffinity column (Vicam, USA) and quantified by HPLC (Rao *et al.*, 2016) [10]. The HPLC (JASCO-975), C-18 isocratic reverse phase column (300 × 3.9 mm, 15 µm) and mobile phase existed methanol: water: (20:80: v/v) at 0.7 ml/min at 210 nm under UV detector. A calibration curve was prepared to estimate the amount of CIT production by plotting the values on to a standard curve.

Statistical analysis: ANOVA was calculated to study the significant difference between more than two groups. By using the sample data statistic 'F' value is calculated and the value so obtained is compared with 'F' table value (F crit) at given levels of significance α and also 'P' (probability). If

$P \leq \alpha$ (0.01. 0.05. 0.001) then our study is significant otherwise not significant.

Results and Discussions

Biomass of three strains of *Aspergillus terreus* was recorded in the presence of Brassica, sunflower, castor, olive and palmolive oils at 1.5ug/ml in an ascending order. Bio mass and Citrinin production was statistically analyzed and presented in Table.2and 3, Tex Fig.1and 2).The use of oils in the control of fungal infections at the post-harvest stage of agricultural products is of recent development. The oils employed in disease control are mainly from petroleum by-products and to a much lesser extent, glyceride oils from plants and synthetic oils (Achour *et al.*, 2008; Saban *et al.*, 2009). Oils are cheap, excellent spreading and sticking properties to plant surface, non-toxic to man, and are eco-friendly. Vegetables and other oils are effective inhibitors of fungal infections (Bhattacharya, 1991). Rattanipitigorn *et al.* (2006), Achour *et al.* (2008), and Saban *et al.* (2009) have reported the efficacy of oils such as eugenol in the control of different pathogens studied by them. Vandana Pandey *et al.* (2002) [25] and Narendra Kumar and Tripathi (2002) [13] have also reported the efficacy of different oils such as Cumminium cyminum and Putrangiva in the protection of food products studied by them. Hence, it was considered

worthwhile to study the efficacy of different oils on growth and citrinin production by three strains of food and feed borne fungi of *A. terreus* and the results are presented in Table.1 reveals that of different oils tried, eucalyptus oil, castor oil, coconut, sesamum, and sunflower oil could check the mycotoxin production to a limited extent only. GSAKUMB-57D was comparatively more sensitive than other strains of *A. terreus* towards different oils tried. Eucalyptus, Neem, and olive oils influence on citrinin production at higher concentrations to a significant level. The pH was almost near to neutral. Similarly, Rao *et al.* (2015) [10] also reported Eucalyptus and Neem oils were reported inhibited citrinin production by *Penicillium verrucosum* and *P. nordicum* significant level. Velluti *et al.* (2003) [26] reported the inhibitory effect of cinnamon, clove, lemongrass, oregano, and palmorse essential oils on growth and fumonisin B, production by *Fusarium proliferatum* in maize grains. Out of different oils tried for the production of citrinin, eucalyptus oil olive oil and neem oils could prevent the production of Citrinin. Singh and Sameul and Tripathi (1994) [21] and Mishra *et al.* (2013) [12] have also recorded a significant decrease in fungal infestation by the treatment of seeds with oils. Jain *et al.* (1998a) [8] have also reported a significant decrease in the seed mycoflora when treated with neem oil.

Table 1: Effect of oils on growth and citrinin production by three strains of *Aspergillus terreus*

Name of the Compound	Conc (in ml)	GSAKUMB-AF			GSAKUMB-24			GSAKUMB-57D		
		Final pH	Dry weight (mg/ml)	Citrinin ($\mu\text{g/ml}$)	Final pH	Dry weight (mg/ml)	Citrinin ($\mu\text{g/ml}$)	Final pH	Dry weight (mg/ml)	Citrinin ($\mu\text{g/ml}$)
Brassica	1.00	7.7	2.6	0.070	7.3	2.1	0.064	7.5	2.02	0.060
	1.50	7.6	2.4	0.040	7.1	2.2	0.026	7.3	1.27	0.043
Castor	1.00	7.9	2.4	0.090	7.5	2.1	0.091	7.1	2.09	0.070
	1.50	7.7	1.8	0.040	7.1	1.3	0.046	7.3	1.6	0.040
Coconut	1.00	7.2	1.4	0.075	7.2	2.1	0.072	7.8	1.54	0.074
	1.50	7.4	1.1	0.043	6.9	1.2	0.054	7.0	1.3	0.045
Eucaliptus	1.00	7.2	0.5	0.019	7.6	0.6	0.018	7.8	0.66	0.016
	1.50	7.2	NG	ND	7.3	NG	ND	7.2	NG	ND
Groundnut	1.00	8.2	1.2	0.072	8.1	2.2	0.075	8.6	1.51	0.08
	1.50	8.4	1.1	0.054	7.4	1.3	0.053	8.3	1.49	0.055
Neem	1.00	8.4	2.0	0.015	8.0	1.5	0.019	9.1	1.2	0.013
	1.50	8.3	2.1	0.008	7.4	1.4	0.006	8.4	1.0	0.006
Olive	1.00	6.4	2.3	0.010	5.6	2.0	0.010	7.4	1.61	0.009
	1.50	6.3	1.4	0.008	6.9	1.5	0.008	5.6	0.94	0.008
Palmolive	1.00	8.2	2.2	0.080	8.9	2.3	0.082	8.5	1.89	0.070
	1.50	8.4	1.6	0.056	7.5	1.2	0.054	7.9	1.56	0.057
Sesamem	1.00	7.6	1.5	0.051	6.6	2.1	0.048	8.9	2.39	0.045
	1.50	7.7	1.4	0.039	6.6	2.2	0.041	9.0	2.2	0.036
Sunflower	1.00	8.7	2.5	0.050	8.1	2.1	0.043	8.6	1.57	0.056
	1.50	8.4	1.7	0.025	7.9	1.5	0.018	7.6	1.56	0.025
Control	-	6.7	1.03	0.060	6.8	1.10	0.050	7.6	0.89	0.050

Table 2: ANOVA of the effect of oils on citrinin production by three strains of *Aspergillus terreus*

Source of variation	Sum of squares	Df	Mean Square	F- value	P-value	R ²	Result
GSAKUMB-AF							
Between columns	2.410e-005	2	1.205e-005	32.94	< 0.0001	0.6342	S
Between rows	0.04159	19	0.002189				
Random	1.390e-005	38	3.658e-007				
Total	0.04163	59					
GSAKUMB-24							
Between columns	2.890e-005	2	1.445e-005	60.34	< 0.0001	0.7605	S
Between rows	0.04207	19	0.002214				
Random	9.100e-006	38	2.395e-007				
Total	0.04211	59					
GSAKUMB-57D							

Between columns	3.610e-005	2	1.805e-005	361.0	< 0.0001	0.9500	S
Between rows	0.03621	19	1.805e-005				
Random	1.900e-006	38	5.000e-008				
Total	0.03624	59					

P > 0.05 - Not Significant (NS); P < 0.05 Significant (S); R²- Coefficient of determination

Table 3: ANOVA of the effect of oils on biomass by three strains of *Aspergillus terreus*

Source of variation	Sum of squares	Df	Mean Square	F- value	P-value	R ²	Result
GSAKUMB-AF							
Between columns	0.3610	2	0.1805	361.0	< 0.0001	0.9859	S
Between rows	26.66	19	1.403				
Random	0.01900	38	0.0005000				
Total	27.04	59					
GSAKUMB-24							
Between columns	0.3611	2	0.1805	343.0	< 0.0001	0.9713	S
Between rows	12.85	18	0.7140				
Random	0.01895	36	0.0005263				
Total	13.23	56					
GSAKUMB-57D							
Between columns	0.1919	2	0.09596	42.44	< 0.0001	0.9748	S
Between rows	10.57	18	0.5872				
Random	0.08140	36	0.002261				
Total	10.84	56					

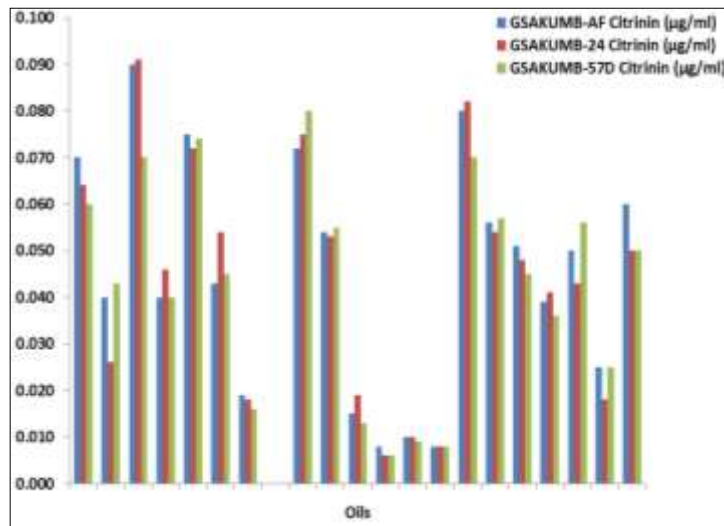


Fig 1: Effect of Oils on citrinin production by three strains of *Aspergillus terreus*

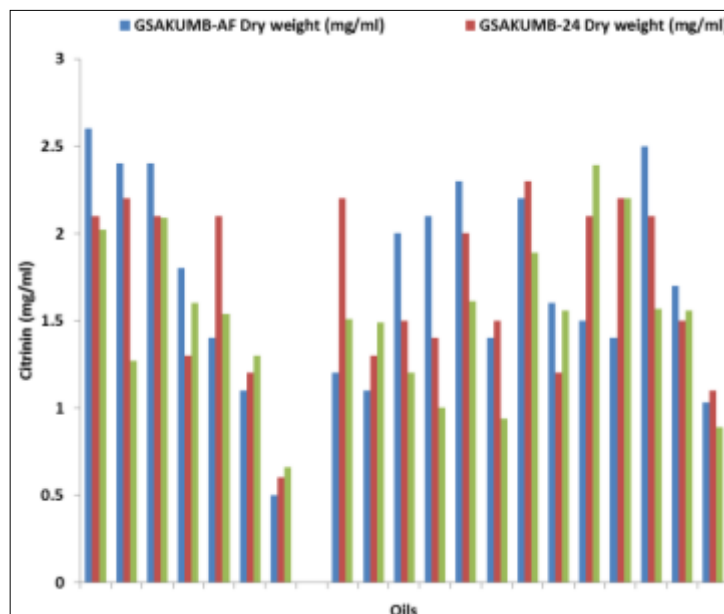


Fig 2: Effect of Oils on Biomass by three strains of *Aspergillus terreus*

Conclusion

From the present investigations, it can be concluded that the use of oils such as eucalyptus oil, neem oil, and olive oil has the potential to suppress citrinin production and inhibit the growth of fungi.

Acknowledgments

This research has been supported by University Grants Commission (NFST), New Delhi and the Head Department of Microbiology, Kakatiya University, Warangal

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