Int.J.Curr.Microbiol.App.Sci (2024) *13*(08): 132-145

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 8 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1308.017

Evaluation of chemically characterized *Ocimum tenuiflorum* L. essential oil as phytopreservative against fungal deterioration of *Justicia adhatoda* L. raw materials during storage

Rajneesh Rao[1], Sanyogita Kumari[2] and Ashok Kumar[1]*

[1]Laboratory of Mycotoxin & Botanical Pesticides, Department of Botany, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur-273009, India
[2]Raghunath Girl's Post Graduate College, Meerut-250001, India

**Corresponding author*

Keywords

Ocimum tenuiflorum, Essential oil, *Aspergillus flavus*, Aflatoxin B1, Antifungal, HPTLC

Article Info

Received:
20 June 2024
Accepted:
31 July 2024
Available Online:
10 August 2024

A B S T R A C T

The stored herbal raw materials (HRMs) of *Justicia adhatoda* were found associated with spores of various storage fungi in which *Cladosporium cladosporioides* exhibited the highest relative density (25.02%) followed by *Aspergillus flavus* (22.49%) and *A. niger* (21.46%). The chemical composition of *Ocimum tenuiflorum* essential oil (OtEO) showed 41 considerable peaks. Eugenol (61.30%) was found as major component followed by β-Caryophyllene (11.89%), Germacrene D (9.14%), α- Cubebene (2.54%) and Carvacrol (2.04%). OtEO showed broad spectrum fungitoxicity and MIC against *A. flavus* DDUGU-07 was recorded at 0.5 mg/ml while aflatoxin B_1 production was completely checked at 0.3 mg/ml. The high performance thin layer chromatography (HPTLC) analysis of fumigated HRMs of *J. adhatoda* reveals that OtEO was found effective in control the degradation or chemical transformation of medicinal component, vasicine found in *J. adhatoda.* Hence, OtEO having exclusive merit to possessing antifungal, antiaflatoxigenic and broad fungitoxic spectrum strengthening its safe exploitation as green preservative.

Introduction

Malabar nut, *Justicia adhatoda* L. (family-Acanthaceae) is one of the valuable medicinal plants that frequently used in India as traditional medicine for the treatment of many ailments (Das *et al.,* 2009; Dhankhar *et al.,* 2011).

Herbal medicines have a long history of use and are widely utilized to treat a wide range of illnesses in people throughout the world. Now days, around one fourth of the medications provided to patients in allopathic treatment come from plants (Newman and Cragg, 2020). The raw materials of herbal medications are susceptible to contamination by fungi and their toxic metabolites due traditional methods of collection, storage, and marketing used in underdeveloped nations (Ałtyn and Twarużek, 2020; Chen *et al.,* 2020). According to Palares *et al.,* (2022), fungal and mycotoxin contamination in herbal raw materials (HRMs) has a negative impact on their therapeutic properties. It also has a detrimental effect on patients' health rather than improving it (Omotayo *et al.,* 2019).

A number of Aspergillus species are potent storage moulds that have the ability to contaminate HRMs and reduce their medicinal efficacy through the biotransformation of their active ingredients (Mishra *et al.,* 2015; Çorbaci, 2020). *Toxigenic Aspergilli* secrete aflatoxins in stored edible products which cause aflatoxicosis in both humans and animals (Benkerroum, 2020; Kumar *et al.,* 2022). Because of its strong hepatocarcinogenic and immunosuppressive properties, aflatoxin B1 (AFB1) is categorized as a group-1 human carcinogen (Marches *et al.,* 2018).

The use of synthetic fungitoxicants is not suitable for the protection of HRMs due to their adverse effects on health, development of resistance in treated microorganisms, and their residual toxicity (Rajkumar *et al.,* 2019). The US Food and Drug Administration (FDA) have classified plant-based formulations as safe substitutes of synthetic preservatives, classifying them as GRAS (generally regarded as safe). Many essential oils (EOs) bioactive components such as phenolics and terpenoids, have been shown to have antibacterial, fungitoxicant, mycotoxin suppressor, insecticidal, and potent antioxidant properties (Pandey *et al.,* 2017; Basak, 2018; Lasram *et al.,* 2019; Pavella *et al.,* 2019). Some EO based formulations like Sporan™, EcoSMART, E-Rase™, EcoPCOR, as well as Dimethyl carbonate (DMC) base naturals are commercially initiated as preservatives due to their antimicrobial, antifungal, and antimycotoxigenic potential (Chaudhari *et al.,* 2020; Singh *et al.,* 2019). Therefore, the present investigation explores the possibility of exploitation of *Ocimum tenuiflorum* L. leaf essential oil (OtEO) as phytopreservative in control of fungal deterioration of *J. adhatoda* raw materials during storage.

Materials and Methods

Collection and preparation of herbal raw materials

Stored HRMs of *J. adhatoda* were locally procured from Sahabganj herbal market, Gorakhpur, Uttar Pradesh, India, during July-August, 2023. The procured raw materials were collected in unused disinfected polythene bags to prevent further contamination. The HRMs of *J. adhatoda* were finely grinded within pre-sterilized grinder. The powdered HRMs of *J. adhatoda* were stored at 5±2ºC for further analysis of pH, moisture content and mycological contamination (Kumar *et al.,* 2013).

pH and moisture content determination

Aqueous suspensions (1:10; w/v) of powdered HRMs of *J. adhatoda* were prepared and stirred for 5 h, and the pH of suspension was determined using digital pH meter. To determine moisture content, weighed amount of samples were dried at 100ºC until their weights remained constant and per cent moisture content was calculated as following Kedia *et al.,* (2015) –

$$\% \text{ moisture content} = \frac{\text{Undried sample wt.} - \text{Dried sample wt.}}{\text{Undried sample wt.}} \times 100$$

Mycological analysis of raw materials of *J. adhatoda*

Fungal spores/conidia associated with stored HRMs of *J. adhatoda* were assessed by serial dilution technique (Mishra *et al.,* 2015). The isolated mycobiota were identified on the basis of colony shape, colony colour (front and reverse), thallus and conidial characteristics (Ravimannan *et al.,* 2016). The identified fungal species were sub-cultured on potato dextrose agar (PDA) and finally stored at 4±2ºC. The relative densities of mycobiota occurring on stored HRMs of *J. adhatoda* were calculated following Kedia *et al.,* (2015).

$$\text{Relative density of fungus (\%)} = \frac{\text{No. of isolates of a fungus}}{\text{Total no. of isolates of all fungi}} \times 100$$

Detection of AFB_1 producing strain

To determine the AFB_1 production capability, pure cultured 20 isolates of *A. flavus* were randomly chosen following Mishra *et al.,* (2015). Fifty µl conidial suspension (≈10^6 conidia/ml) of selected *A. flavus* isolates were separately inoculated in 49.5 ml SMKY (Sucrose, $MgSO_4$, KNO_3, and Yeast extract in 200 g, 0.5 g, 0.3 g, and 7.0 g, respectively in 1000 ml distilled water) broth medium in conical flask (150 ml) and homogenized followed by incubation at 27±2 °C for 10 days.

After incubation, content of each conical flask (≈50 ml) was filtered followed by chloroform (40 ml) extraction of filtrate in a separating funnel to dissolve AFB_1 in chloroform. The separated chloroform extract was evaporated on water bath at 60-70°C near dryness. The

chloroform extract residue was redissolved in 1 ml chloroform and 50 µl of it was spotted on TLC plate (20×20 cm^2 of silica gel) and developed in mobile phase [toluene: isoamyl alcohol: methanol (TIM); 90:32:2; v/v/v] and fluorescent blue spots of AFB_1 were observed under ultra-violet fluorescence analysis cabinet at 360 nm (Dwivedy *et al.,* 2018).

The fluorescent AFB_1 spots were scrapped and dissolved in cold methanol (5 ml), and centrifuged for 5 min at 3000 rpm. The optical density of supernatant was recorded at 360 nm wavelength and AFB_1 content was determined following Dwivedy *et al.,* (2018).

$$\text{AFB1 (µg/L)} = \frac{\text{Absorbance x molecular weight of AFB1 (312)}}{\text{Molar extinction coefficient of AFB1 (21800) x path length (1 cm)}} \times 1000$$

Extraction of *O. tenuiflorum* leaf essential oil (OtEO)

The leaves of *O. tenuiflorum* were collected from botanical garden, DDU Gorakhpur University, Gorakhpur for EO extraction and its voucher specimen (Lam/O-117/2024) was deposited in the herbarium of botany department, DDU Gorakhpur University, Gorakhpur. The collected leaves were washed in tap water followed by hydro-distillation using Clevenger's apparatus for EO extraction. The EO was dehydrated using anhydrous sodium sulphate and stored at 4±2ºC in clean and dark glass vials (Dwivedy *et al.,* 2018).

GC-MS analysis of OtEO

Chemical composition of OtEO was performed at Central Institute of Medicinal and Aromatic Plants, Lucknow, India. To determine chemical profile, OtEO was subjected to GC-MS (Perkin Elmer Turbomass Gold MA, USA) using 60 m × 0.32 mm × 0.25 mm capillary column.

The GC was performed with injection temperature 250ºC; detector temperature 270ºC; column temperature program isotherm at 70ºC for 2 min, 3ºC/min gradient to 250ºC, isotherm duration was 10 min and flow rate of carrier gas (He) was 1 ml/min.

The components of OtEO were identified by comparing their retention times and mass spectra with authentic reference compounds in the literature available in mass spectral libraries of Wiley, NIST and NBS (Adams, 2007).

Fungitoxic spectrum of OtEO against some storage fungi

Fungitoxic efficacy of OtEO was also recorded against 14 storage moulds viz. *Alternaria alternata*, *Aspergillus candidus, A. flavus, A. fumigatus*, *A. nidulans, A. niger*, *A. terreus*, *A. versicolor, Cladosporium cladosporioides, Curvularia lunata*, *Fusarium nivale, F. oxysporum*, *Penicillium italicum* and *Trichoderma viride* recovered from HRMs of *J. adhatoda* through poisoned food assay following Prakash *et al.,* (2012). Requisite amount of OtEO dissolved separately in 0.5 ml of 5% tween-20 mixed with 9.5 ml PDA medium in different presterilized Petri dishes to attain final concentrations i.e. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5 and 2.0 mg/ml. The negative control sets without OtEO were kept parallel to the treatment sets along with positive controls with prevalent synthetic fungicides, SAAF (Carbendazim 12% + Mancozeb 63%) and Bavistin (Carbendazim 50% WP). A fungal disc (5 mm diameter) of each test fungus was inoculated on PDA in Petri dishes (15 cm diameter) separately followed by incubation at 27±2 °C for 10 days. After incubation, minimum inhibitory concentration (MIC) was recorded (Prakash *et al.,* 2012).

Antifungal and antiaflatoxigenic efficacy of OtEO

Fungitoxic and aflatoxin inhibitory efficacy of isolated OtEO was tested against the toxigenic strain of *A. flavus* DDUGU-09 using SMKY broth as nutrient medium following Mishra *et al.,* (2015). Requisite amount of the OtEO dissolved separately in 0.5 ml of 5% tween-20 were pipetted aseptically to different pre-sterilised Erlenmeyer flasks (150 ml) containing 49.5 ml of SMKY broth to procure the final concentrations viz. 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml. The flasks without OtEO were treated as control sets. Then, flasks were inoculated aseptically with 50 µl conidial suspension (≈10^6 conidia/ml) of toxigenic isolate *A. flavus* DDUGU-09 prepared in 0.1 % Tween-80 (Rosengaus *et al.,* 2000) and incubated for 10 days at 27±2°C. The content of flask was filtered and mycelia were oven dried at 100°C untill their weight remained constant for biomass determination. Mycelial biomass of treatment as well as control sets was measured and per cent inhibition in mycelial biomass

was determined as follows-

$$\% \text{ mycelial inhibition} = \frac{dc - dt}{dc} \times 100$$

Where,

dc = Average dry weight (g) of fungal mycelium in control sets

dt = Average dry weight (g) of fungal mycelium in treatment sets

The filtrates of control and treated sets were extracted separately with 40 ml chloroform in a separating funnel to quantify the AFB_1 production. AFB_1 production in each set was estimated by aforementioned technique of Kumar *et al.,* (2013).

In situ antifungal activity

To determine the antifungal efficacy of OtEO during storage, 500 g of HRMs of *J. adhatoda* were kept separately in five different plastic containers having aerial volume 2.0 litres. Each container was inoculated with 1 ml spore suspension (≈10^6 spores/ml) of *A. flavus* DDUGU-09. Four containers were fumigated with 0.5 mg/ml (MIC against *A. flavus*) OtEO while one container run parallel as control without OtEO treatment. All the containers were sealed and stored for six months at room temperature i.e. 27±2ºC (Bajpai and Kang, 2012; Kumar *et al.,* 2013). After storage, mycological analysis of raw materials for *A. flavus* colonization was performed following Mishra *et al.,* (2015).

HPTLC analysis of raw materials for vasicine

The OtEO treated and untreated *J. adhatoda* raw materials of *in situ* study were subjected for HPTLC (high performance thin layer chromatography) analysis following Srivastava *et al.,* (2009). Raw materials were powdered (100 mesh) separately and 5.0 g of each were soaked in 20 ml acetone (AR) for 60 min. After saturation, raw materials filtered and filtrates were evaporated to dryness by rotary evaporation. Dried acetone extract (10 mg) was dissolved in acetone (10 ml) to prepare a standard solution (1 mg/ml). Whereas, procured vasicine (1,2,3,9-Tetrahydropyrrolo[2,1-b]quinazolin-3-ol) from Sigma-Aldrich (Germany) was used as reference by preparing standard solution in acetone (1 mg/ml). Chromatography was performed on glass backed HPTLC plates (20 cm × 10 cm) coated with 0.25 mm layer of silica gel Si60 F_{254} (Merck, Germany). Standard solution (vasicine) and acetone extracts of treated and untreated samples of known concentrations were applied to plate as bands (6 mm width) with the help of automatic TLC applicator (Camag Linomat 5), with the nitrogen flow of delivery speed of 150nL/s from the application syringe. The plates had been developed to a height of 80 mm using mobile phase of chloroform: ethanol: acetic acid (95: 4: 5; v/v/v) in a glass twin furrow chamber (20 cm × 10 cm). After removing plates from the chambers, completely air dried at room temperature (27°C) and peak areas for samples and standard were recorded by densitometry in absorbance/ reflectance mode with slit dimensions 6 mm × 0.45 mm at 260 nm, using TLC scanner 3 (Camag) equipped with Camag Wincats software version 4.06.

Statistical Analysis

The experiments were performed in triplicate and data expressed as Mean ± SE. One way ANOVA ($P < 0.05$) and Tukey's multiple range tests were analysed using SPSS (version 16.0).

Results and Discussion

The procured HRMs of *J. adhatoda* were found contaminated with spores of various storage moulds due to appropriate pH (6.82±0.23) and relatively higher moisture content (22.54±1.76 %). During mycological screening, total 1547 fungal colonies were recovered and to whom *Cladosporium cladosporioides* isolates were dominated and exhibited highest relative density (25.02%) followed by *Aspergillus flavus* (22.49%), *A. niger* (21.46%) and *Penicillium* sp. (8.98%) whereas both *A. candidus* and *Fusarium nivale* showed the lowest (0.78%) relative density followed by *Trichoderma viride* (0.90%) (Table 1). Thirty percent isolates of randomly selected *A. flavus* were found toxigenic and *A. flavus* DDUGU-07 was selected as test fungus due to its higher efficiency of AFB_1 production (2450.201 µg/l) presented in Table 2.

The OtEO was characterized with its pungent smell, yellow green colour and 1.18 % yield (w/w). The GC/GC-MS analysis of OtEO showed 41 considerable peaks with 38 known and 03 unknown compounds. The GC-MS analysis exhibited Eugenol (61.30%) as major component followed by β- Caryophyllene (11.89%), Germacrene D (9.14%), α- Cubebene (2.54%) and Carvacrol (2.04%) were recorded as major components.

Rest other identified components were found in small or trace amount (Table 3; Figure 1).

OtEO exhibited a broad spectrum antifungal activity against storage fungi recovered from HRMs of *J. adhatoda*. The minimum inhibitory concentrations (MICs) of OtEO against recovered storage moulds from raw materials ranges between 0.4 to 0.7 mg/ml and found comparable to common synthetic fungicides i.e. SAAF and Bavistin (Table 4). The OtEO completely checked the biomass production of *A. flavus* DDUGU-07 at 0.5 mg/ml as well as absolutely inhibited the AFB_1 production at 0.3 mg/ml concentration (Figure 1, Figure 2).

The OtEO significantly reduced the number of *A. flavus* isolates in comparison to control set in four different fumigated containers i.e. 66.74, 72.54, 76.69 and 77.12% at 0.5 mg/ml concentration (Table 5). The HPTLC profile (Figure 3) and densitometric chromatogram (Figure 4) showed that vasicine content was degraded drastically by fungal invasion. Inoculated raw materials fumigated with OtEO (sample 1, 2, 3 and 4) contains vasicine content 3.068, 3.370, 4.090 and 3.349 µg/ml respectively while in control, vasicine was not detected (Figure 4). The calibration curve of vasicine was linear and the value of its determination via height was: R_f, 0.29; regression equation, Y = 300.287 + 27.692x; r^2, 0.769 and via area was R_f, 0.30; regression equation, Y =5666.404 + 1263.822x; r^2, 0.907 (Table 6).

Geographically, India is characterized with hot and humid climatic conditions, which favours association of various moulds and their mycotoxins in raw materials of various medicinally important plants (Benkerroum, 2020). Fungal contamination reduces the medicinal potency of HRMs (Qin *et al.,* 2020) and rendering them unfit for human use. To minimize such storage losses appropriate quality control measures should be explored. Recently, for long term storage of many products various synthetic fungicides are frequently used having residual toxicity, hence, their application on the herbal raw materials would not be desirable (Rajkumar *et al.,* 2019). Several investigations have shown that some specific higher plant essential oils can be used as plant-based fungitoxicants to combat various molds and their mycotoxins production (Lasram *et al.,* 2019; Oliveira *et al.,* 2020). An assessment of OtEO's antifungal effectiveness and its utility in preventing post-collection fungal deterioration of HRMs during storage and increasing their market value was conducted. In comparison to other previously reported EOs, such as *Cymbopogon flexuosus* (Kumar *et al.,* 2009), *Curcuma longa* (Hu *et al.,* 2017), *Pimenta dioica* (Chaudhari *et al.,* 2020), *Coriandrum sativum* (Das *et al.,* 2019), *Artemisia nilagirica* (Kumar *et al.,* 2020), etc., the MIC of OtEO (0.5 mg/ml) against *A. flavus* was shown to be lower. The OtEO exhibited remarkable broad spectrum fungitoxicity against all the tested fungal species isolated from *J. adhatoda* HRMs and which is comparable to two prevalent synthetic fungicides viz. SAAF and bavistin. Hence, the OtEO can be recommended for complete protection of stored commodities from the fungal infestation at low concentration.

Furthermore, the mycelial biomass and AFB_1 production exhibited a significant decreasing trend with increasing OtEO concentration, i.e. reduction of mycelial biomass causes noteworthy reduction in AFB_1 production. Reduction in fungal biomass and AFB_1 production may be due to some phenolics and terpenes present in the OtEO (Josselin *et al.,* 2022). Eugenol, a well known antifungal agent (Didehdar *et al.,* 2022) is major component of OtEO, may contribute a key role in its antifungal property (Guo *et al.,* 2024). The major component of OtEO is different from earlier findings where, methyl eugenol, Camphor (Pandey *et al.,* 2014), Caryophyllene (Iqbal *et al.,* 2020), etc. were reported as main constituents. The variability in EO's composition is mainly due to the age of the plant, plant part collected, season of collection, geographical area and soil characteristics (Rawat *et al.,* 2020). The inhibitory effect of OtEO against *A. flavus* and AFB_1 production may be due to alteration of ergosterol biosynthesis, a specific sterol providing membrane integrity, flexibility and stability to membrane linked enzymes which adversely affects fungal growth (Chellappandian *et al.,* 2018; Bhattacharya *et al.,* 2020). Some earlier workers found that reduction in ergosterol biosynthesis as well as leakage of cations i.e. Ca^{2+}, K^+ and Mg^{2+} through cell membrane would be the key factor in fungal growth inhibition and thus, suggesting plasma membrane as an imperative site for antifungal action of EOs (Kedia *et al.,* 2015; Singh *et al.,* 2022). EOs also exhibiting significant free radical scavenging activity, may serve as a plant based antioxidant in shelf life enhancement as well as protection from oxidative stress by decelerating oxidative rancidity of lipids (Ahmed *et al.,* 2016; Dwivedy *et al.,* 2018). The presence of various phenolic compounds and/or synergistic effect among compounds play major role in antioxidant activity of EOs (Fadel *et al.,* 2020).

Table.1 Mycological screening of *J. adhatoda* herbal raw materials

Isolated Fungi	No. of isolates	Relative density (%)
Alternaria alternata	18	1.16
Aspergillus candidus	12	0.78
A. flavus	348	22.49
A. fumigatus	112	7.24
A. nidulans	21	1.36
A. niger	332	21.46
A. terreus	27	1.74
A. versicolor	15	0.97
Cladosporium cladosporioides	387	25.02
Curvularia lunata	52	3.36
Fusarium nivale	12	0.78
F. oxysporum	26	1.68
***Penicillium* sp.**	139	8.98
Trichoderma viride	14	0.90
Mycelia sterilia (Unidentified)	32	2.07
Mucorales Genera	(4) Genera	
Total isolates	**1547**	

Table.2 Detection of aflatoxigenic potential of *A. flavus* isolates

Strain	Toxigenicity	AFB_1 content (µg/l)
***A. flavus* DDUGU-01**	Non-toxigenic	-
***A. flavus* DDUGU-02**	Non-toxigenic	-
***A. flavus* DDUGU-03**	Toxigenic	1213.651
***A. flavus* DDUGU-04**	Non-toxigenic	-
***A. flavus* DDUGU-05**	Non-toxigenic	-
***A. flavus* DDUGU-06**	Non-toxigenic	-
A. flavus* DDUGU-07	Toxigenic	**2450.201**
***A. flavus* DDUGU-08**	Non-toxigenic	-
***A. flavus* DDUGU-09**	Toxigenic	1740.330
***A. flavus* DDUGU-10**	Toxigenic	1133.504
***A. flavus* DDUGU-11**	Non-toxigenic	-
***A. flavus* DDUGU-12**	Non-toxigenic	-
***A. flavus* DDUGU-13**	Non-toxigenic	-
***A. flavus* DDUGU-14**	Non-toxigenic	-
***A. flavus* DDUGU-15**	Non-toxigenic	-
***A. flavus* DDUGU-16**	Toxigenic	1877.724
***A. flavus* DDUGU-17**	Non-toxigenic	-
***A. flavus* DDUGU-18**	Non-toxigenic	-
***A. flavus* DDUGU-19**	Toxigenic	938.862
***A. flavus* DDUGU-20**	Non-toxigenic	-

*The isolate in bold is most toxigenic

Table.3 Chemical composition of OtEO by GC-MS analysis

RT	Compounds	Percentage
7.175	3-Hexen-1-ol	**0.03**
8.275	2,5-Diethyltetrahydrofuran	**0.01**
9.618	α-Pinene	**0.61**
10.375	Benzaldehyde	**0.01**
10.875	3-Octenol	**0.01**
11.125	β-Pinene	**0.06**
11.500	3-Octanal	**0.02**
11.675	Sabinene	**0.54**
11.775	Pelargonaldehyde	**0.03**
12.885	p-Cymene	**0.83**
13.041	γ-Terpinene	**0.56**
13.075	DL-Limonene	**0.71**
13.941	α-Terpinolene	**0.07**
14.585	Linalool	**0.26**
16.075	Citronellal	**0.14**
19.526	Geranial	**0.29**
20.001	Thymol	**0.82**
22.446	Carvacrol	**2.04**
28.401	Eugenol	**61.30**
28.493	Unknown	**0.48**
29.501	β- Elemene	**0.47**
29.601	β- Bourbonene	**0.09**
29.801	Germacrene D	**9.14**
30.851	Camphenol	**0.07**
31.126	β- Caryophyllene	**11.89**
31.692	Unknown	**0.63**
31.926	α- Terpinolene	**0.74**
32.351	Ethyl linoleolate	**0.01**
32.651	α- Humulene	**0.22**
33.576	β- Selinene	**1.34**
33.876	α- Cubebene	**2.54**
34.501	α- Selinene	**0.03**
34.951	β- Costol	**0.22**
35.576	δ- Cadinene	**0.01**
35.876	α- Furnesene	**0.10**
38.301	Trans-alpha-Bergamotene	**0.07**
39.501	Valencene	**0.02**
41.026	Germacrene B	**0.24**
41.201	α- Guaiene	**0.01**
41.776	1-Deoxycapsidiol	**0.02**
43.058	**Unknown**	**0.01**

RT = Retention Time; Compounds in bold are major components

Table.4 Comparative fungitoxicity of OtEO with prevalent synthetic fungicides against some storage fungi

Fungi	Minimum Inhibitory Concentration (MIC) in mg/ml		
	OtEO	Carbendazim 12% + Mancozeb 63% (SAAF)	Carbendazim 50% WP (Bavistin)
Absidia corymbifera	0.47 ± 0.03^{ab}	0.27 ± 0.03^{a}	0.73 ± 0.17^{b}
Alternaria alternata	0.83 ± 0.03^{a}	0.60 ± 0.06^{a}	$> 2.0^{b}$
Aspergillus candidus	0.43 ± 0.03^{ab}	0.27 ± 0.03^{a}	0.83 ± 0.12^{b}
Aspergillus flavus	0.50 ± 0.06^{a}	0.37 ± 0.03^{a}	1.17 ± 0.14^{b}
Aspergillus fumigatus	0.53 ± 0.03^{a}	0.37 ± 0.07^{a}	1.53 ± 0.07^{b}
Aspergillus niger	0.73 ± 0.03^{a}	0.50 ± 0.06^{a}	1.93 ± 0.07^{b}
Aspergillus terreus	0.43 ± 0.03^{ab}	0.30 ± 0.06^{a}	1.07 ± 0.17^{b}
Aspergillus versicolor	0.45 ± 0.07^{a}	0.37 ± 0.03^{a}	1.53 ± 0.07^{b}
Cladosporium cladosporioides	0.97 ± 0.07^{b}	0.63 ± 0.03^{a}	$> 2.0^{c}$
Curvularia lunata	0.80 ± 0.06^{b}	0.47 ± 0.03^{a}	$> 2.0^{c}$
Fusarium nivale	0.73 ± 0.03^{b}	0.43 ± 0.03^{a}	$> 2.0^{c}$
Fusarium oxysporium	0.77 ± 0.03^{b}	0.47 ± 0.03^{a}	$> 2.0^{c}$
***Penicillium* sp.**	0.67 ± 0.03^{a}	0.40 ± 0.06^{a}	1.00 ± 0.10^{b}
***Rhizopus* sp**	0.43 ± 0.07^{ab}	0.27 ± 0.03^{a}	0.83 ± 0.12^{b}
Trichoderma viride	0.47 ± 0.07^{a}	0.30 ± 0.06^{a}	1.00 ± 0.10^{b}

Values are mean (n = 3) ± SE; P < 0.05

The means followed by same letter in the same row are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table.5 Per cent inhibition in *A. flavus* isolates after six months fumigation of OtEO (0.5 mg/ml)

Samples	No. of *A. flavus* isolates	Per cent inhibition
Control	914	-
***J. adhatoda* HRM-1**	304	66.74
***J. adhatoda* HRM-2**	251	72.54
***J. adhatoda* HRM-3**	213	76.69
***J. adhatoda* HRM-4**	242	73.52

Table.6 R_f values and linear regression data of HPTLC determination of vasicine.

Compound	Regression via	R_f	Regression equation	r^2
Vasicine	Height	0.29	Y = 300.287 + 27.692x	0.769
	Area	0.30	Y = 5666.404 + 1263.822x	0.907

Y = densitometric response; x = concentration; r^2 = correlation coefficient

Figure.1 Effect of OtEO concentrations on *A. flavus* DDUGU-07 biomass and AFB_1 production

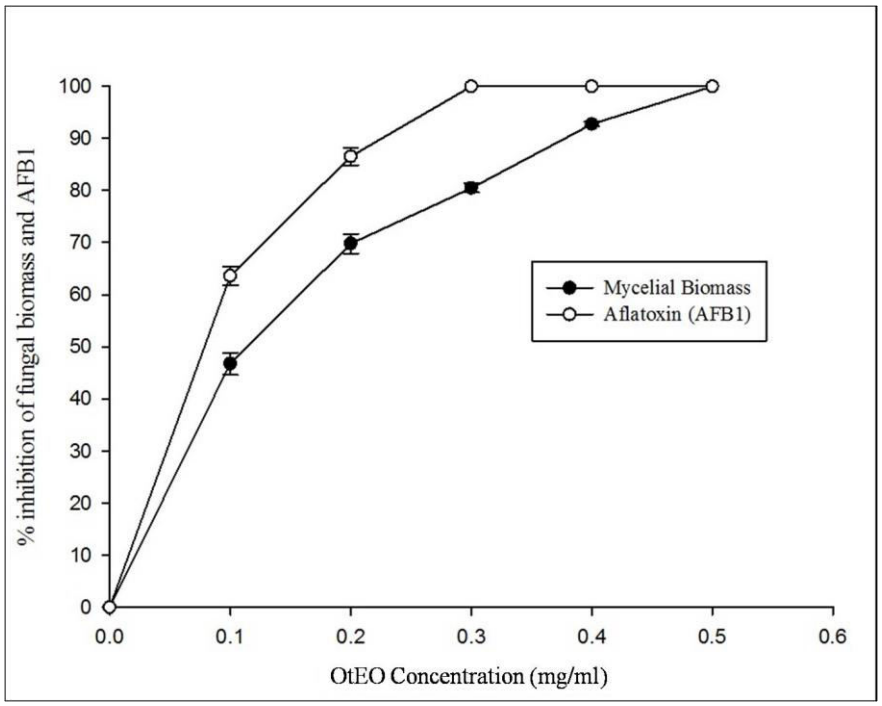


Figure.2 Intensity of AFB_1 with increasing concentration of OtEO

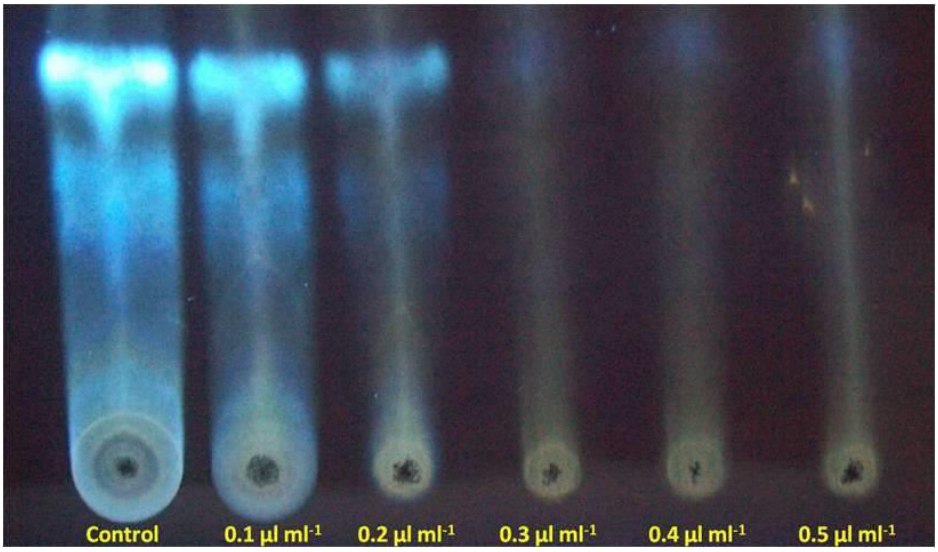
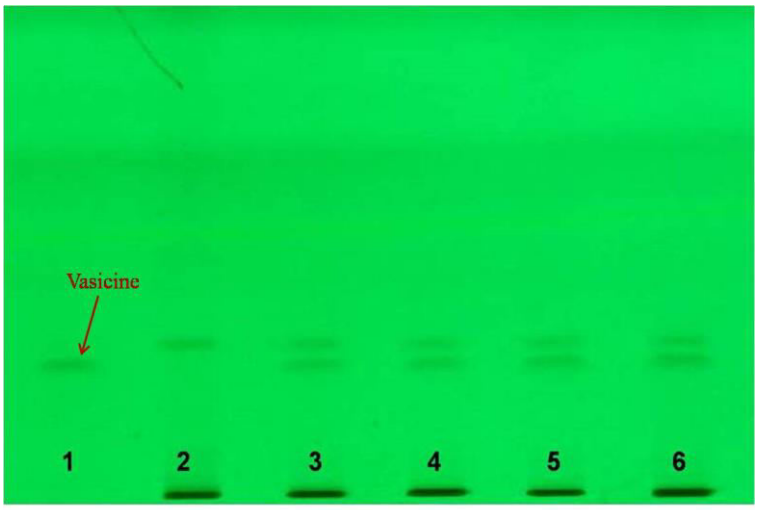
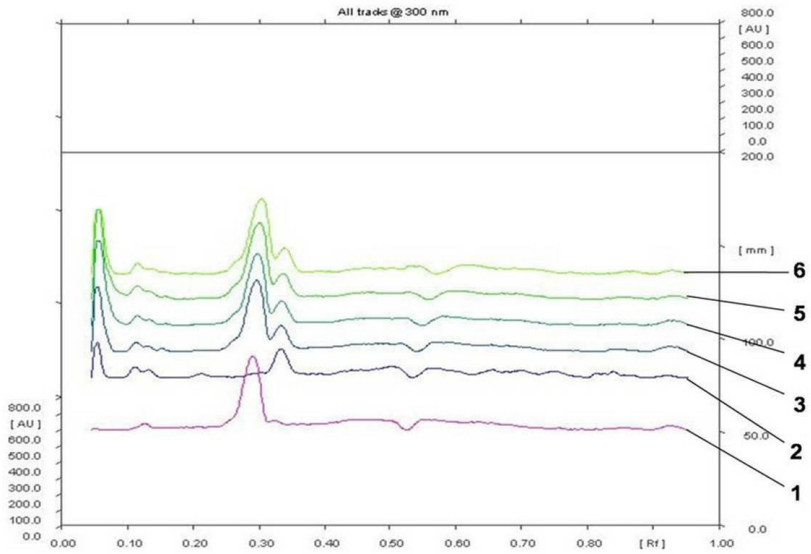


Figure.3 HPTLC profile of fumigated and non-fumigated *J. adhatoda* HRMs



1-Ref. (Vasicine); 2-Control (*J. adhatoda* HRM without OtEO fumigation);
3,4,5,6 - *J. adhatoda* HRM fumigated with OtEO

Figure.4 Densitometric scan (at 260 nm) showing vasicine peaks in samples



1-Ref. (Vasicine); 2-Control (*J. adhatoda* HRM without OtEO fumigation);
3,4,5,6 - *J. adhatoda* HRM fumigated with OtEO

To assess the practical applicability of OtEO, the HRMs of *J. adhatoda* were fumigated with OtEO. The significant reduction in number of *A. flavus* isolates indicates its suitability to control fungal contamination of raw herbal drugs. The protection of medicinal component (vasicine) by OtEO from fungal deterioration strengthens its exploitation as an appropriate fungitoxicant for the protection of herbal raw materials. The well known importance of OtEO in traditional medicine strengthens its exploitation as safe, green preservative to minimize mycological infestation in HRMs during storage (Kumar *et al.,* 2009; Nyarko *et al.,* 2002).

The findings of present investigation reveal that, HRMs of *J. adhatoda* get adversely contaminated with various moulds and their mycotoxins due to inappropriate handling and storage. The OtEO exhibited strong antifungal and antiaflatoxigenic and broad spectrum

fungitoxicity as well as long history of its use in traditional medicine strengthen its possible exploitation as an indigenous plant-based green preservative for post-harvest treatment of HRMs to enhance their shelf life.

Acknowledgement

Authors are thankful Head, Centre of Advanced Study (CAS) in Botany, Institute of Science, Banaras Hindu University, Varanasi; Head, Department of Botany, DDU Gorakhpur University, Gorakhpur and Prof. Sharad Srivastava, Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (NBRI), Lucknow for laboratory facilities.

Author Contributions

Rajneesh Kumar: Investigation, formal analysis, writing—original draft. Sanyogita Kumari: Validation, methodology, writing—reviewing. Ashok Kumar:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Adams, R. P. (2007). Identification of essential oil components by Gas Chromatography/Mass Spectrometry. Allured Publishing Corporation, Carol Stream, IL.

Ahmed, M., Pickova, J., Ahmad, T., Liaquat, M., Farid, A., and Jahangir, M. (2016). Oxidation of lipids in foods. Sarhad Journal of Agriculture. 32(3): 230-238. https://doi.org/10.17582/journal.sja/2016.32.3.23 0.238

Ałtyn, I., and Twarużek, M. (2020). Mycotoxin contamination concerns of herbs and medicinal plants. *Toxins. 12:* 182. http://doi.org/10.3390/toxins12030182.

Bajpai, V. K., and Kang, S. C. (2012). *In vitro* and *in vivo* inhibition of plant pathogenic fungi by essential oil and extracts of *Magnolia liliflora* Desr. Journal of Agriculture Science and Technology. 14: 845–856.

Basak, S. (2018). The use of fuzzy logic to determine the concentration betel leaf essential oil and its potency as a juice preservative. Food Chemistry. 240: 1113-1120. https://doi.org/10.1016/j.foodchem.2017.08.047

Benkerroum, N. (2020). Aflatoxins: producing-molds, structure, health issues and incidence in Southeast Asian and Sub-Saharan African countries. International Journal of Environmental of Research and Public Health. 17: 1215. https://doi.org/10.3390/ijerph17041215.

Bhattacharya, S., Sae-Tia, S., and Fries, B. C. (2020). Candidiasis and mechanisms of antifungal resistance. Antibiotics. 9: 312. https://doi.org/10.3390/antibiotics9060312.

Chaudhari, A. K., Singh, V. K., Dwivedi, A. K., Das, S., Upadhyay, N., Singh, A., Dkhar, M. S., Kayang, H., Prakash, B., and Dubey, N. K. (2020). Chemically characterised *Pimenta dioica* (L.) Merr. essential oil as a novel plant based antimicrobial against fungal and aflatoxin B_1 contamination of stored maize and its possible mode of action. Natural Product Research. 34(5): 745-749. https://doi.org/10.1080/14786419.2018.1499634

Chellappandian, M., Saravanan, M., Pandikumar, P., Harikrishnan, P., Thirugnanasambantham, K., Subramanian, S., Hairul-Islam, V. I., and Ignacimuthu, S. (2018). Traditionally practiced medicinal plant extracts inhibit the ergosterol biosynthesis of clinically isolated dermatophytic pathogens. Journal of Mycology and Medicine. 28, 143-149. https://doi.org/10.1016/j.mycmed.2017.11.001.

Chen, L., Guo, W., Zheng, Y., Zhou, J., Liu, T., Chen, W., Liang, D., Zhao, M., Zhu, Y., Wu, Q., and Zhang, J. (2020). Occurrence and characterization of fungi and mycotoxins in contaminated medicinal herbs. Toxins. 12: 30. https://doi.org/10.3390/toxins12010030.

Çorbaci, C. (2020). Biotransformation of terpene and

terpenoid derivatives by *Aspergillus niger* NRRL 326. Biologia. https://doi.org/10.2478/s11756-020-00459-1.

Das, S., Khan, M. L., Rabha, A., and Bhattacharya, D. K. (2009). Ethnomedicinal plants of Manas National Park, Assam, Northeast India. Indian Journal of Traditional Knowledge. 8: 514–517.

Das, S., Singh, V. K., Dwivedi, A. K., Chaudhari, A. K., Upadhyay, N., Singh, P., and Dubey, N. K. (2019). Encapsulation in chitosan-based nanomatrix as an efficient green technology to boost the antimicrobial, antioxidant and in situ efficacy of *Coriandrum sativum* essential oil. International Journal of Biological Macromolecules, 133, 294-305. https://doi.org/10.1016/j.ijbiomac.2019.04.070.

Dhankhar, S., Kaur, R., Ruhil, S., Balhara, M., Dhankhar, S., and Chhillar, A. K. (2011). A review on *Justicia adhatoda*: a potential source of natural medicine. African Journal of Plant Science. 5(11): 620-627. https://doi.org/10.5897/AJPS.9000004

Didehdar, M., Chegini, Z., and Shariati, A. (2022), Eugenol: A novel therapeutic agent for the inhibition of *Candida* species infection. Frontiers in Pharmacology, 13: 872127. https://doi.org/10.3389/fphar.2022.872127

Dwivedy, A. K., Singh, V. K., Prakash, B., and Dubey, N. K. (2018). Nanoencapsulated *Illicium verum* Hook.f. essential oil as an effective novel plant-based preservative against aflatoxin B_1 production and free radical generation. Food Chemical Toxicology. 111: 102-113. https://doi.org/10.1016/j.fct.2017.11.007.

Fadel, H. H. M., El-Ghorab, A. H., Hussein, A. M. S., El-Massry, K. F., Lotfy, S. N., Ahmed, M. Y. S., and Soliman, T. N. (2020). Correlation between chemical composition and radical scavenging activity of 10 commercial essential oils: Impact of microencapsulation on functional properties of essential oils. Arabian Journal of Chemistry. 13(8): 6815-6827. https://doi.org/10.1016/j.arabjc.2020.06.034.

Guo, S., Ma, R., Li, S., Li, Z., Piao, J., and Zhou, R. (2024). Antifungal effects and action mechanism of eugenol on *Alternaria panax* causing ginseng black spot. Chinese Journal of Pesticide Science. 26(3): 619-624. https://doi.org/10.16801/j.issn.1008-7303.2024.0026

Hu, Y., Zhang, J., Kong, W., Zhao, G., and Yang, M. (2017). Mechanism of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. Food Chemistry. 220:1-8. https://doi.org/10.1016/j.foodchem.2016.09.179.

Iqbal, Z., Akhtar, M., Sabri, M. U., and Altaf, A. (2020) Chemical composition of *Ocimum sanctum* essential oil by GC-MS analysis. Natural Products Chemistry and Research. 8:6. https://doi.org/10.35248/2329-6836.20.8.388

Josselin, L., De Clerck, C., De Boevre, M., Moretti, A., and Fauconnier, M. L. (2022). Impact of volatile organic compounds on the growth of *Aspergillus flavus* and related aflatoxin B1 production : A review. International Journal of Molecular Sciences. 23(24): 15557. https://doi.org/10.3390/ijms232415557

Kedia, A., Prakash, B., Mishra, P. K., Dwivedi, A. K., and Dubey, N. K. (2015). *Trachyspermum ammi* L. Essential oil as plant based preservative in food system. Industrial Crops and Products. 69: 104-109. https://doi.org/10.1016/j.indcrop.2015.02.013.

Kumar, A., Dubey, N. K., and Srivastava, S. (2013). Antifungal evaluation of *Ocimum sanctum* essential oil against fungal deterioration of raw materials of *Rauvolfia serpentina* during storage. Industrial Crops and Products. 45: 30-35. https://doi.org/10.1016/j.indcrop.2012.12.006.

Kumar, A., Shukla, R., Singh, P., and Dubey, N. K. (2009). Biodeterioration of some herbal raw materials by storage fungi and aflatoxin and assessment of *Cymbopogon flexuosus* essential oil and its components as antifungal. International Biodeterioration and Biodegradation. 63: 712-716. https://doi.org/10.1016/j.ibiod.2009.03.011.

Kumar, M., Dwivedi, A. K., Sharma, P., Dkhar, M. S., Kayang, H., Raghuwanshi, R., and Dubey, N. K. (2020). Chemically characterised *Artemisia nilagirica* (Clark) Pamp. essential oil as a safe plant based preservative and shelf-life enhancer of millets against fungal and aflatoxin contamination and lipid peroxidation. Plant Taxonomy and Biosystematics. 154(3): 269-276. https://doi.org/10.1080/11263504.2019.1587539.

Kumar, P., Gupta, A., Mahato, D. K., Pandhi, S., Pandey, A. K., Kargwal, R., Mishra, S., Suhag, R., Sharma, N., Saurabh, V., Paul, V., Kumar, M., Selvakumar, R., Gamlath, S., Kamle, M., Enshasy, H. A., Mokhtar, J. A., and Harakeh, S.

(2022). Aflatoxins in cereals and cereal-based products: occurrence, toxicity, impact on human health, and their detoxification and management strategies. Toxins. 14: 687. https://doi.org/10.3390/toxins14100687

Lasram, S., Zemni, H., Hamdi, Z., Chenenaoui, S., Houissa, H., Tounsi, M. S., and Ghorbel, A. (2019). Antifungal and antiaflatoxigenic activities of *Carum carvi* L., *Coriandrum sativum* L. seed essential oils and their major terpene component against *Aspergillus flavus*. Industrial Crops and Products. 134: 11-18. https://doi.org/10.1016/j.indcrop.2019.03.037.

Marchese, S., Polo, A., Ariano, A., Velotto, S., Costantini, S., and Severino, L. (2018). Aflatoxin B1 and M1 : Biological properties and their involvement in cancer development. Toxins (Basel). 10(6): 214. https://doi.org/10.3390/toxins10060214

Mishra, P., Prakash, B., Kedia, A., Dwivedy, A. K., Dubey, N. K., and Ahmad, S. (2015). Efficacy of *Caesulia axillaris*, *Cymbopogon khasans* and *Cymbopogon martini* essential oils as plant based preservatives against degradation of raw materials of *Andrographis paniculata* by fungal spoilage. International Biodeterioration and Biodegradation. 104: 244-250. https://doi.org/10.1016/j.ibiod.2015.06.015

Newman, D. J., and Cragg, G. M. (2020). Natural Products as a source of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products. 83(3): 770-803. https://doi.org/10.1021/acs.jnatprod.9b01285

Nyarko, A. K., Asare-Anane, H., Ofosuhene, M., and Addy, M. E. (2002). Extract of *Ocimum canum* lowers blood glucose and facilitates insulin release by isolated pancreatic β-islet cells. Phytomedicine. 9: 346–351. http://doi.org/10.1078/0944-7113-00124.

Oliveira, R. C., Carvajal-Moreno, M., Correa, B., and Rojo-Callejas, F. (2020). Cellular, physiological and molecular approaches to investigate the antifungal and anti-aflatoxigenic effects of thyme essential oil on *Aspergillus flavus. Food Chemestry. 315: 126096.* https://doi.org/10.1016/j.foodchem.2019.126096.

Omotaya, O. P., Omotayo, A. O., Mwanza, M., and Babalola, O. O. (2019). Prevalence of mycotoxins and their consequences on human health. Toxicology Research. 35: 1-7. https://doi.org/10.5487/tr.2019.35.1.001.

Pallares, N., Berrada, H., Font, G., and Ferrer, E. (2022). Mycotoxins occurrence in medicinal herbs dietary supplements and exposure assessment. Journal of Food Science and Technology. 59(7): 2830-2841. https://doi.org/10.1007/s13197-021-05306-y

Pandey, A. K., Kumar, P., Singh, P., Tripathi, N. N., and Bajpai, V. K. (2017). Essential oils: Sources of antimicrobials and food preservatives. Frontiers in Microbiology. 7: 2161. https://doi.org/10.3389/fmicb.2016.02161.

Pandey, A. K., Singh, P., and Tripathi, N. N. (2014). Chemistry and bioactivities of essential oils of some *Ocimum* species: an overview. Asian Pacific Journal of Tropical Biomedicine. 4(9): 682-694. https://doi.org/10.12980/APJTB.4.2014C77.

Pavela, R., Benelli, G., Petrelli, R., Cappellacci, L., Lupidi, G., Sut, S., Dall'Acqua, S., and Maggi, F. (2019). Exploring the insecticidal potential of Boldo (*Peumus boldus*) essential oil toxicity to pests and vectors and non-target impact on the microcrustacean *Daphnia magna.* Molecules. 24: 879. https://doi.org/10.3390/molecules24050879.

Prakash, B., Singh, P., Mishra, P. K., and Dubey, N. K. (2012). Safety assessment of *Zanthoxylum alatum* Roxb. essential oil, its antifungal, antiaflatoxin, antioxidant activity and efficacy as antimicrobial in preservation of *Piper nigrum* L. fruits. International Journal food Microbiology. 153: 183–191. https://doi.org/10.1016/j.ijfoodmicro.2011.11.007.

Qin, L., Jiang, J. Y., Zhang, L., Dou, X. W., Ouyang, Z., Wan, L., and Yang, M. H. (2020). Occurrence and analysis of mycotoxins in domestic Chinese herbal medicines. Mycology. 11(2): 126-146. https://doi.org/10.1080/21501203.2020.1727578.

Rajkumar, V., Gunasekaran, C., Christy, I.K., Dharmaraj, J., Chinnaraj, P., and Paul, C. A. (2019). Toxicity, antifeedant and biochemical efficacy of *Mentha piperita* L. essential oil and their major constituents against stored grain pest. Pesticide Biochemistry and Physiology. 156: 138-144. https://doi.org/10.1016/j.pestbp.2019.02.016.

Ravimannan, N., Sevvel, P., and Sarrutharshan, S. (2016). Study on fungi associated with spoilage of bread. International Journal of advanced Research in Biological Sciences. 3(4): 165-167.

Rawat, S., Bhatt, I. D., and Rawal, R. S. (2020). Variation in essential oil composition in

rhizomes of natural populations of *Hedychium spicatum* in different environmental condition and habitats. Journal of Essential Oil Research. 32: 348-360. https://doi.org/10.1080/10412905.2020.1750497.

Rosengaus, R. B., Lefebvre, M. L., and Traniello, J. F. A. (2000). Inhibition of fungal spore germination by Nasutitermes: evidence for a possible antiseptic role of soldier defensive secretions. Journal of Chemical Ecology. 26: 21–39. https://doi.org/10.1023/A:1005481209579.

Singh, A., Dwivedy, A. K., Singh, V. K., Upadhyay, N., Chaudhari, A. K., Das, S., and Dubey, N. K. (2019). Essential oils based formulations as safe preservatives for stored plant masticatories against fungal and mycotoxin contamination: A review. Biocatalysis and Agricultural Biotechnology. 17: 313-317. https://doi.org/10.1016/j.bcab.2018.12.003.

Singh, B. K., Tiwari, S., Maurya, A., Kumar, S., and Dubey, N. K. (2022). Fungal and mycotoxin contamination of herbal raw materials and their protection by nanoencapsulated essential oils: An overview. Biocatalysis and Agricultural Biotechnology. 39:102257. https://doi.org/10.1016/j.bcab.2021.102257

Srivastava, S., Srivastava, M., Mehrotra, S., and Rawat, A. K. S. (2009). A HPTLC method for chemotaxonomic evaluation of some *Curcuma* Species and their commercial samples. Journal of Science and Industrial Research. 68: 876–880. https://nopr.niscair.res.in/handle/123456789/6134.

How to cite this article:

Rajneesh Rao, Sanyogita Kumari and Ashok Kumar. 2024. Evaluation of chemically characterized *Ocimum tenuiflorum* L. essential oil as phytopreservative against fungal deterioration of *Justicia adhatoda* L. raw materials during storage. *Int.J.Curr.Microbiol.App.Sci.* 13(8): 132-145. **doi:** https://doi.org/10.20546/ijcmas.2024.1308.017