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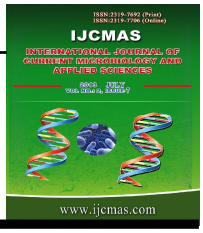

Original Research Article

Zingiber officinale Roscoe. Oil: A preservative of stored commodities against storage mycoflora

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A B S T R A C T

In this communication bioactivity of essential oil extracted from Ginger (*Zingiber officinale* Roscoe.) that belongs to family Zingiberaceae was evaluated chemically. The essential oil has been extracted from ginger. The chemical components were analyzed by GC-MS. The oil yield was 2.0±0.03%. Ginger rhizome oil was effective against a range of bio-deteriorating storage fungi. The MIC value was determined by volatile activity assay. The study reveals that essential oil had a wide spectrum of antifungal activities against *Aspergillus flavus, Penicillium expansum, Alternaria alternata* and *Fusarium oxysporum.* Minimum inhibitory concentration (MIC) of oil was found to be effective showing results at 500ppm of oil in respect to all fungi. At higher concentration of 1000ppm of oil was fungicidal in action. The toxicity of the oil did not change even after the storage of 6 months

Keywords

Zingiber officinale; essential oil; post-harvest fungi; GC-MS; MIC.

Introduction

Biodeterioration of various types of harvested and stored products due to ubiquitous presence of molds is a chronic problem in tropical and subtropical countries which gets aggravated due to hot and humid climate. The dominating group of fungi proliferating on agricultural commodities is *Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium* etc. that are able to produce toxic metabolites called mycotoxins. These fungi pose serious phytopathological and mycotoxicological risks at pre harvest and postharvest stages. *Fusarium* and *Alternaria* species are often classified as 'field fungi', because they require high growth and mycotoxin synthesis (>20%) whereas the 'storage fungi', mainly species of *Aspergillus* and *Penicillium*, can also grow well at lower moisture contents. Thus, *Fusarium* and *Alternaria* usually pose a high mycotoxicological risk at pre-harvest level or in freshly harvested products, whereas toxigenic species of *Aspergillus* and *Penicillium* represent a higher risk for food and feed products in storage or other kind of processing (Logrieco *et al.*, 2003).

Mycotoxins are well known for their hazardous effects. The primary disease associated with mycotoxins intake is

hepatocellular carcinoma (HCC, or liver cancer). This disease is the third-leading cause of cancer death globally (INCHEM, 2012) with about 550,000–600,000 new cases each year. Mycotoxins occurring the food commodities are secondary metabolites of filamentous fungi, which contaminate many types of food crops throughout the food chain (Reddy *et al.*, 2010).

Management of fungal contamination of harvested seeds /grains is based on physical (aeration, cooling and rapid drying) and chemical treatment with ammonia, food preservative even with pesticides (Atanda *et al*., 2007). Therefore, the use of herbal pesticide has become indispensible tool against the various synthetic pesticides causing health problems, environmental pollution, pathogen resistance to chemicals and consequent pest resurgence. Although different synthetic antimicrobials have been successfully commercialized in recent years they encounter major problem not only due to adverse side effects on consumers but also for the development of resistance by micro-organisms. (Tolouee *et al*., 2010).

An alternative way to resolve these problems utilization of regenerative resources such as plants and their products for newer strategies of pest pathogen management is the demand of this century. Plants are very rich source of bioactive organic chemicals. Essential oils are natural products extracted from plants and fruits, which have been illustrated to be inhibitory, against a wide range of food spoiling microbes, depending upon their concentration, method of testing and active constituents present (Fisher and Phillips, 2008). The main advantage of essential oils was that they could be used in any food and are considered generally recognized as safe.

Zingiber officinale Roscoe. (Family Zingiberaceae) is well known in Asia. The ginger has been listed in Generally Recognized as Safe (GRAS) document of USFDA and has antimicrobial and antimycotoxigenic effects (Tatsadjieu *et al.*, 2009) because of its aroma and taste has been used for culinary purposes from ages. It has also been reported in use for medicinal purposes for more than 2500 years. Ginger rhizome is widely used as an ingredient in food, pharmaceutical, cosmetic and other industries. Ginger is also known to possess antioxidant properties. Ginger contains a unique flavour derived from both nonvolatile and volatile oils.

Ginger is found to be rich in bioactive compounds and various researches have been carried out to explore the beneficial properties of ginger and its extracts. (Grzanna and other, 2005).Essential oils of ginger are of interest because of its richness in various functional compounds mostly terpene, monoterpene and sesquiterpenes, which gives the oil its biological activity (Daferera and others, 2002).

Thus objective of this study was to analyze essential oil composition of ginger and to study the antifungal activity of essential oil against fungi causing storage rot. Effect of MIC of *Zingiber officinale* oil against mycelial growth of *Aspergillus flavus, Penicillum expansum, Alternaria alternata* and *Fusarium oxysporum* were studied. These are dominant fungi which are associated with food spoilage.

Materials and Methods

Survey of storage pathogens

A survey was done for isolation of storage pathogens from different grain shops and storage godowns at Lucknow (India). Cereals, pulses, nuts and spices were selected as stored commodities.

Plant preparation

Ginger rhizomes were purchased from a local market in Lucknow (India). Ginger was cleaned with distilled water to remove soil and dust, peeled and chopped.

Ginger oil extraction

The fresh ginger (5 kg) was hydro-distilled for 6-7 hrs. in a Clevenger type apparatus. The oils were dried over anhydrous sodium sulphate. To isolate the oil from the aqueous portion, the oil was extracted with solvent ether in a separating funnel. The ether was removed at reduced pressure which resulted in an oily residue that was added to the oil collected earlier. The essential oil obtained was kept in sealed glass tube at 4^0C until analysis.

GC-MS analysis

Gas Chromatographic analysis followed by mass spectra was carried out in Perkin Elmer Autosystem XL Packed mode. Column used for analysis was OV-1, 100% Methyl gum (10 feet). The conditions were as follows; Temperature programming from 4°C-220°C, hold at 75°C for 20 minute. Injection temperature 250°C and detector temperature was 255°C. Carrier gas was N_2 at a flow rate 14 ml/min.

The identification of individual compound is based on their retention time's relatives to those of authentic samples and matching spectral peaks availably with NIST mass spectral libraries.

In vitro Experiment

Antifungal activity for determination of Minimum Inhibitory Concentration (MIC)

Antifungal activity was tested against *Aspergillus flavus, Penicillium expansum, Alternaria alternata* and *Fusarium oxysporum* by volatile activity assay.

Volatile Activity Assay

Tests for volatile activity were carried out in 90mm Petriplates containing 20ml of solidified potato dextrose plate. A 5mm diameter disc of inoculum of the test species cut from the periphery of an actively growing culture on PDA plates was placed on the agar in each Petriplate and then Petriplates were kept in inverted position. In the lid of each Petriplate a sterilized cotton swab was placed on to which a different concentration of oil was poured. The Petriplates were sealed by parafilm to check the release of volatile oil. For each corresponding control an equal amount of water was poured on sterilized cotton swab. The Petriplate were kept at 28 ± 2^0C for 30 days. Fungi toxicity was expressed in terms of radial growth.

Efficacy of Ginger oil during storage

The effect of storage on the toxicity of the oil was determined by storing a stock of the oil in an air tight glass vials at room temperature .The fungal toxicity of the oil taken from the stock at regular six months interval was tested at the MIC of respective fungi by the volatile assay and observations on mycelial growth were recorded.

In vivo Experiment

Efficacy of ginger oil as a preservative of storage grains

The storage grains as pulses, nuts and spices were obtained from the market and brought to the laboratory. The efficacy of *Zingiber officinale* oil as a preservative of pulses, nuts and spices against fungal spoilage was determined as follows: 1kg of the commodities was placed separately in presterilized plastic containers of 2000cc capacity. Different amounts of ginger oil were soaked separately in sterilized cotton swabs as to obtain final concentrations of 200ppm, 500ppm, 1000ppm and 2000ppm with respect to the volume of the containers. One swab of each concentration was placed in sterilized perforated polythene bag which was introduced into each plastic container for each concentration was prepared. A control set was run parallel to each treatment set uniform unsoaked sterile cotton swabs. All the sets were stored at room temperature ranging between 20-40^0C and relative humidity between 57 to 87% for a period of six months. Thereafter, fungal infestation of the stored commodities of both treatments and controls was determined by serial dilution plate method.

Statistical Analysis

Experiments were carried out in triplicates. Data were expressed as means of five replicates. Statistical analysis was performed with Microsoft excel 2007. Difference on statistical analysis of data were considered Significant at $P<0.05$. ANOVA was made based on the diameter of the radial growth among fungal isolates.

Result and Discussion

Nineteen fungal species were isolated from the 10 different surveyed sites (grain shop/storage godown) at Lucknow (India). Four major species group responsible for deterioration of stored commodities due to mycotoxin production were observed. *Aspergillus flavus, Penicillium expansum, Alternaria alternata* and *Fusarium oxysporum* (Table 1).

The chemical components of oils

In present study 81.25 % of the compounds were identified in oil. The chemical composition of oil was given in Table 2 and Figure 1. The main monoterpenene compound was camphene which was 23.9% followed by sesquiterpene zingiberene 12.2%, whereas 1, 8- cineole which is an oxygenated ether compound was reported as major component (27.9%). Other hydrocarbons were α- pinene 7.2%, α- seliene 0.9%, α-farnesene 1.1%, ar- curcumene 0.8%, Camphor 0.4%, Octanel 0.1%, Tricyclene 0.1%. The main oxygenated compounds were Geranial 2.8, Neral 0.9%, Elemol 0.9 %, Zingiberenol 0.9%, Nerolidol 1%, sabinene 0.05%.(Table 2).

Antifungal test of ginger oil against mycelial growth of test fungi

The data obtained from the *in vitro* experiment which is done by vapour toxicity method indicated that essential oil displayed a variable degree of antifungal

Table.1 Fungi isolated from different storage commodities.

Fungi isolated	Cereals	Pulses	nuts	spices
1.Alternaria alternata	+	+	+	+
2.Aspergillus tenuissima	-	-	-	-
3.Aspergillus fumigatus	-	-	-	+
4.Aspergillus flavus	+	+	+	+
5.Aspergillus ochraceous	-	+	-	-
6.Aspergillus nidulans	-	-	+	-
7.Aspergillus fischeri	-	-	-	+
8.Aspergllus niger	+	-	-	+
9.Cladosporium cladosporoides	+	-	-	-
10.Cladosporium herbarum	-	+	-	-
11.Fusarium oxysporum	+	+	+	+
12.Penicillium expansum	+	+	+	+
13.Penicilluim citrinum	-	-	+	-
14.Penicillium oxalicum	+	-	-	-
15.Penicillium italicum	-	-	+	-
16.Penicillium chrysogenum	-	+	-	+
17.Penicillium funiculosum	-	+	+	+
18. Rhizopus arrhizus	-	+	-	-
19.Rhizopus nigricans	+	-	+	-
20.S. racemosum	-	-	-	+
21.Tricothecium roseum	-	-	+	-
22.Yeast like fungi	+	-	-	-

Table.2 GC-MS of Ginger oil

S.no.	**Retention time**	**area**	**Compound**	**percentage**
1.	4.580	0.09	Tricyclene	0.1
2.	7.748	0.01	Octanel	0.1
3.	19.241	0.09	Camphor	0.4
4.	21.237	7.96	α -pinene	7.2
5.	22.087	31.40	Camphene	23.9
6.	23.699	3.94	Geranial	2.8
7.	24.836	0.06	Neral	0.9
8.	26.271	47.47	1,8- cineole	27.9
9.	29.844	0.10	sabinene	0.05
10.	29.844	0.09	ar-curcumene	0.8
11.	33.661	0.25	α- farnesene	1.1
12.	34.830	0.07	α- selinene	0.9
13.	36.707	5.81	α- Zingiberene	12.2
14.	38.027	0.46	Elemol	3.4
15.	48.229	0.16	Zingiberenol	0.9
16.	48.920	0.10	Nerolidol	1.0

Figure.1 Gas chromatogram of *Zingiber officinale* essential oil

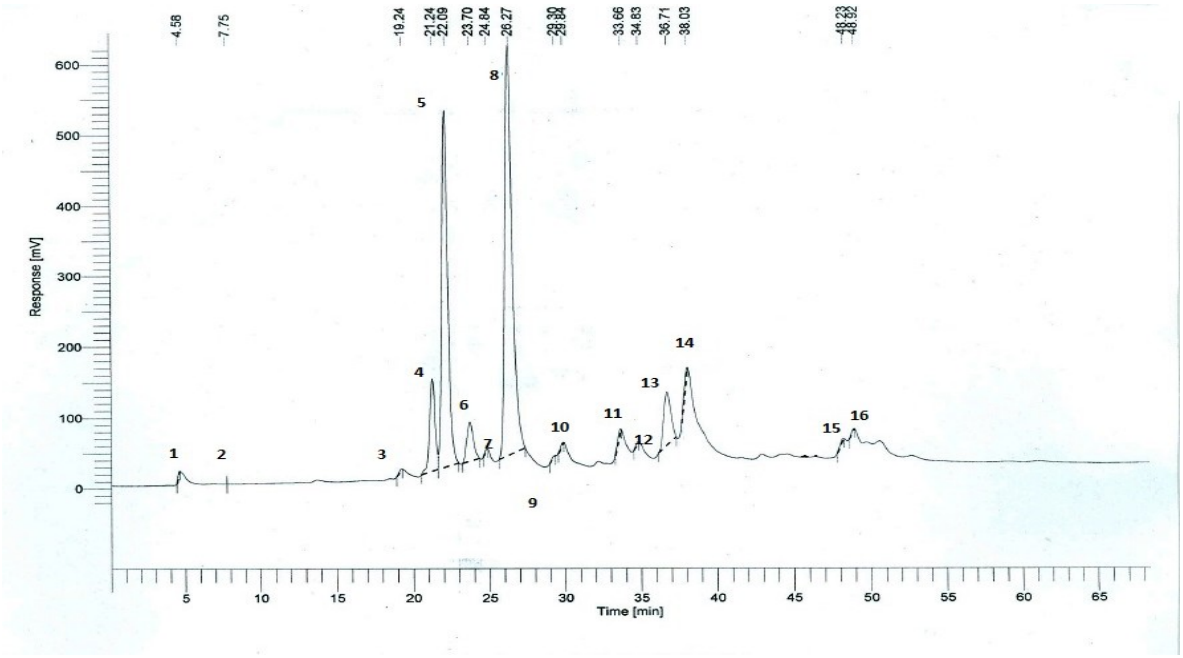
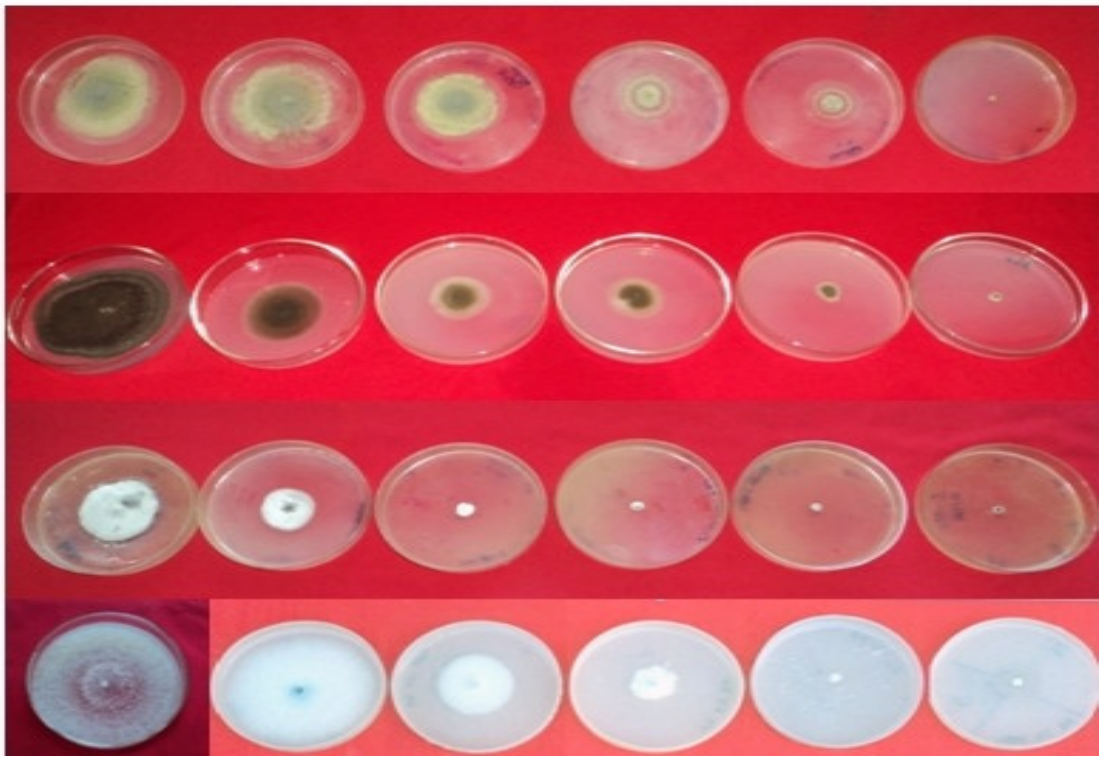


Figure.2 Effect of *Zingiber officinale* essential oil on radial growth of (1) *Aspergillus flavus* (2) *Penicillium expansum(3) Alternaria alternata* (4) *Fusarium oxysporum* using Volatile activity assay at concentration range from 25ppm to 500ppm



Control 25ppm 50ppm 100ppm 200ppm 500ppm

activity on different tested fungus (Table 3). It was observed that increasing concentrations of ginger oil have suppressed the fungal growth. At 100 ppm concentration the growth was reduced more than half than that of control in all test fungi. At 500ppm concentration of oil growth of all the test fungi was completely inhibited. The highest antifungal dose was 200ppm in case of *Penicillium expansum* was occupied 7.6 mm radial diameter, whereas at same dose *Aspergillus flavus* has shown 12.6mm radial growth only. 500ppm was minimum inhibitory concentration with respect to all fungi during *in vitro* experiment.

During *In vivo* trials we have taken pulses, nuts and spices due to its high nutritional value and economic importance among all stored commodities. Starting concentration ranges of oil were 200ppm, 500ppm, 1000ppm. 2000ppm concentration, although 200ppm has displayed notable reduction in radial growth during *in Vitro*, therefore this was the start up dose for *in vivo* trials (Figure 2).

When the fungal population from untreated commodities and treated commodities at 200ppm, 500ppm, 1000ppm and 2000ppm of oil respectively were compared (CFU/g) after six months of storage, in treated commodities a drastic decrease in CFU was recorded (Table 4)

A perusal of table 1 shows that various saprophytic as well as parasitic fungi were found associated with the stored products examined. As this study was designated to find out the possibility of utilizing volatile constituents of *Zingiber officinale* as preservatives of food commodities against fungal deterioration, *Aspergillus flavus*, *Penicillium expansum*, *Alternaria alternata* and *Fusarium oxysporum* were selected as the test organisms since they were found to be the most common bio-deteriogens during the survey of selected sites.

Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts (Bakkali, 2007).

In present study, monoterpenes hydrocarbons ($C_{10}H_{16}$) were responsible for the fungicidal activity of ginger oil. Among which, camphene account for 23.9% which was the major component followed by zingiberene 12.2%. In literature, many variations have been found in chemical composition of ginger oil. Agrawal *et al*., (2001) reported curcumene as the major constituent in the fresh ginger rhizomes, while Menut *et al*., (1994) identified citral as the main constituent of ginger oil.

In other previous report on ginger oil (Singh *et al*., 2005a, b, c), α-zingiberene was found to be the major constituent. These differences in the chemical composition of the oil from the same plant/plant part could be due to the environmental, developmental, genetic or some other factors. Yield and composition of oil differ widely with the production conditions (Blair *et al*., 2001), variety, cultivars or population (Galambosi and Peura, 1996) and on climatic and soil factors.

Some ginger compounds such as α-pinene, borneol, camphene and linalool are

Table.3 Antifungal activity of ginger oil against fungi

Conc.	**Radial diameter (mm)**			
	A. flavus	***P.expansum***	***A. alternata***	***F.oxysporum***
Control	83±2.34	63.8±1.65	84.6±1.66	89±0.40
25ppm	70.2±2.13	53.4±1.44	63±2.16	83.2±0.75
50ppm	46.6±4.52	32.2±2.52	42.2±0.85	54.4±0.65
100ppm	23.6± 2.53	13±1.73	22.6±1.61	31.2±0.75
200ppm	12.6±2.10	7.6±0.65	11±0.91	10.4±0.65
500ppm	5	5	5	5
Here, it is notable that 5mm is the diameter of fungal plug				

Table.4 Fungi isolated from treated and untreated grains after six months of storage

Commodities	**Appearance of fungi(log_{10}CFU/g)**				
	Untreated commodities	**Treated stored commodities**			
		200ppm	**500ppm**	**1000ppm**	**2000ppm**
Pulses	7.5×10^{6}	3.5×10^{4}	1.0×10^{2}	1.5×10^{1}	-
Nuts	2×10^{5}	3.5×10^{2}	1.0×10^{1}	-	-
Spices	2.5×10^{3}	1.5×10	-	-	-

responsible for its antimicrobial activities. (Nychas and Skandamis, 2003). Here, we are reporting 1, 8- cineole as major component responsible for antimycotic activity.

This cytotoxic property is of great importance in the applications of essential oils not only against certain human or animal pathogens or parasites but also for the preservation of agricultural or marine products. Ginger essential oil is indeed effective against several mycotoxin producing fungi of stored commodities. It appears that the fungicidal/ fungitoxic nature of the oil is due to monoterpenes camphene and zingiberene. The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism is known as the MIC. The determination of the MIC of oil is necessary for prescribing its appropriate dose. Clearly, unnecessarily high doses of oil increase wastage and may cause considerable harm to the quality of the commodity treated. A perusal of the MIC's of most of the oils shows a range between 1000 to 5000ppm. It is noteworthy that in some instances, the oil of a plant investigated by different workers has shown variation in the MIC (Singh and Handique, 1997 and Pandey *et al*., 1982). Such variation may be due to the use of different test fungi or different technique adopted. However, in the present work the MIC of ginger oil 500ppm was effective against all test fungi (*Aspergillus flavus, Penicillium expansum, Alternaria alternata* and *Fusarium oxysporum*).

A fungitoxicant may act as a fungistat or a fungicide inhibiting the growth of fungus temporarily or permanently respectively (Sharma and Tripathi, 2007). In our *in vivo* experiment it is clear that ginger oil has shown fungistatic nature at 500ppm, but at higher dose it becomes fungicidal (1000ppm). A fungicide should be able to retain its activity over a long period of shelf life. There was a notable decrease in fungal CFU of treated and untreated commodities (Table 4).

Based upon the present study it could be concluded that volatile oil from *Zingiber officinale* and its major component 1,8-cineole, camphene and Zingiberene possess fungitoxic activity worth exploiting for the management of spoilage of stored commodities due to mycotoxin producing fungi. Several plant essential oils are marketed as fungicides for organic farming (Dayan 2009) these include E-Rase™ from jojoba (*Simmondsia californica*) oil , Sporan ™ from rosemary (*Rosamarinus officinalis*) oil, Promax™ from thyme (*T. vulgaris*) oil, Trigoly™ from neem (*A . indica*) oil and GC-3™ being a mixture of cotton seed (*Gossypium hirsutum*) oil and garlic (*Allium sativum*) oil. Notable thing is that Ginger oil can serve as natural fungicide or possibly as a template for the synthesis of novel fungicides should be commercialized as for treatment of Postharvest pathogens like other plant oils.

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