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Preservation of *Arachis Hypogea* L. Food Seeds by *Cuminumcyminum* L. Essential Oil

Narendra Kumar*

Amity Institute of Biotechnology, Amity University Haryana, Gurgaon Manaser-122413, Haryana, India

Abstract

Samples of groundnut seeds were collected from stores and examined for their associated mycoflora and insects. Fifteen species of fungi were identified by blotter method and 12 species of fungi by agar plate method. *In vitro* volatile constituents extracted in the form of essential oils from 32 plant species were evaluated against the dominant fungi, *Aspergillus flavus* and *Aspergillus niger*. The 2 commercial fungicides was assessed for their antifungal activity against allisolated fungi.

The oil of *Cuminumcyminum* (*Apiaceae*) exhibited the greatest toxicity. The oil was found to be fungicidal and thermostable at its minimum inhibitory concentration (MIC) of 400 ppm. The oil was characterized by the determination of its various physico-chemical properties. *In vivo* studies depict that the oil as seed dressing agent and as a fumigant was able to preserve the groundnut food seeds completely for 6 months at 0.50 and 0.76 mL in containers of 500 mL capacity holding 400 g seeds with minimal changes in organoleptic behavior of food seeds during storage. It did not exhibit any adverse effect on seed germination, seedling growth and general health and morphology of plants. GC and GC-MS analysis of the oil revealed recognition of p-mentha-1, 4-dien-7-al (27.4%), γ-terpinene (12.8%), β-pinene (11.4%) and cuminaldehyde (16.1%) as major compounds.

Keywords: *Arachis hypogea* L; *Cuminumcyminum* seed oil; Storage deterioration

Introduction

Arachis hypogea (peanut, groundnut), an annual oil seed belonging to the Leguminosae family and the Papillionaceae subfamily, is a legume native to South America but now grown in diverse environments in six continents between latitudes 40 degrees N and 40 degrees S. *Arachis hypogea* can grow in a wide range of climatic conditions [1]. In a seed production programme, storage of seeds until the distribution during next season assumes paramount importance [2].

A large number of fungi have been reported on seeds of *Arachis hypogea* [3].The current study concerned storage of groundnut seeds in rural areas where poor storage practice leads to heavy deterioration caused by fungi and insects. Joel-Coats [4] highlighted that Synthetic pesticides brought a new order of insect control, but also a new college of risks. At present, only two fumigants are in common use, methyl bromide and phosphine. Methyl bromide has been identified as a major contributor to ozone depletion, which casts a doubt on its future use in pest control. There have been repeated indications that certain pests have developed resistance to phosphine and metylbromide, so its use is in much suspense. New questions have arisen regarding environmental quality, especially contamination of water air and soil by a host of chemicals some of which are pesticides or their degradation products. In view of the problems with the current fumigants, there is a global interest in alternative strategies including development of chemical substitutes. The interest has been shown inplant products, i.e., essential oils for fumigant action.

The *in vivo* efficacy of the *Cuminumcyminum* L seed oil as a seed-dressing agent and fumigant of higher plant origin in the preservation of food seeds of *Arachis hypogea* was compared synthetic fungicides and fumigants and its physic chemical properties and GC MS analysis were done in order to know major compounds.

Materials and Methods

Stored seed collection

Food samples of *Arachis hypogea* that had been in storage for between 6-8 months were collected. Twenty-five farmer places were visited for collection of stored food seeds.

Mycobiota of stored food seeds of *Arachis hypogea*

The mycobiota of stored food seeds of groundnut was studied through agar plate [5] using czapekdox agar medium and standard blotter [6] techniques. Fungal identifications were confirmed following keys and description given by Raper and Thom [7], Gilman [8], Raper and Fennell [9], Booth [10] and Ellis [11,12].

Effect of storage fungi on *Arachis hypogea* food seeds

The fungi isolated from food seeds were tested in terms of seed germination and mortality. The fungal species were cultured in czapeks solutions for 15 days at 28 ± 2°C in stationary conditions. The cultures were filtered through whattman no-1 filter paper. Freshly harvested surface sterilized (0.1% sodium hypochlorite solution) and washed (sterilized water) seeds were soaked separately for 2 hr in 100 ml of each culture filterate of corresponding groundnut seed fungi in four replication of 25 seeds each. 25 treated seeds were placed in sterilized

***Corresponding author:** Narendra Kumar, Amity Institute of Biotechnology, Amity University Haryana, Gurgaon Manaser-122413, Haryana, India, Tel: 01242337015; E-mail: narendra.microbiology@rediffmail.com

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petridish containing three layers of moist blotters. The number of seeds germinated after 5 days interval for up to 20 days was observed. The controls were maintained by sowing surface sterilized seeds in sterilized blotters.

Isolation of essential oils fromhigher plants and evaluation of their toxicity against test fungi

The plant parts were surface sterilized by dipping in 70% ethanol and then washed repeatedly with sterilized double distilled water and hydrodistilled for isolation of volatile constituents separately for 6 hr in Clevenger's apparatus. After hydro distillation, immiscible oil was separated and dehydrated over anhydrous sodium sulphate. The toxicity of oil and fungicide copper oxychloride and carbendazimwas assessed by using the inverted petri plate technique of Bocher [13] and fungi toxicity measured following Dixit et al [14].

Physico-chemical properties of *Cuminumcyminum* seed oil

The oil was characterized by determination of its various physic-chemical properties viz., specificgravity, specificrotation, refractiveindex, acidvalue, saponification number, esternumber, phenolic content and solubility following Langenau [15].

Fungitoxic properties of *Cuminumcyminum* seed oil

The MIC of most effective oil was determined by poisoned food technique of Grover and Moore [16]. For studying nature, the oil treated discs of the fungi showing complete inhibition of their mycelia growth upto 7d were washed with sterile water and placed again on fresh solidified medium to observe the revival of mycelia growth. The fungi toxic spectrum of the oil was studied against various fungi isolated from groundnut seed samples. In addition, effect of temperature, autoclaving and storage on the fungi toxicity of oil was determined following Pandey et al [17].

Seed dressing

For seed dressing, a stock solution of Cumin oil was prepared by dissolving 50 µl of oil in 1 ml acetone, 200 g seed was filled in plastic containers and treated with 1ml stock solution of the oil, dressed by continuous shaking for 5min for proper coating. Likewise two preselected contact fungicides, copperoxychloride and carbendazim (500 mg/100 g seeds) were also run parallel for comparison purposes. For control set, the seeds were dressed in requsite amount of acetone in place of oil and fungicides. The containers were made airtight and kept at room temperature at 75 ± 5% humidity. Obervations for associated mycoflora were made after 6 months.

Fumigant bioassay

Fresh dried *Arachis hypogea* seeds kept for food purpose was locally collected in presterilized polyethylene bags. Aliquots of 0.50 ml (1000 ppm) and 0.76 ml (1500 ppm) of oil and ethylene dibromide were used separately with 400 g of freshly dried *Arachis hypogea* seeds in presterilized gunny bags of 500 ml capacity. Likewise, samples of *Arachis hypogea* to be treated with oil or ethylene dibromide were stored separately in metal containers (tins) of 500 ml capacity. Sterile cotton swabs (0.50 g) soaked with synthetic fumigants and oil and wrapped in sterilized muslin cloth (0.75 g) were placed at the bottom of each container of *Arachis hypogea* seed. Similarly, 400 g samples of groundnut were treated with phosphine from a 0.50 (1000 ppm) or 0.76 g (1500 ppm) of tablet (160 and 240 mg equivalent phosphine) in 500 ml containers and were stored in a cabinet in the Laboratory at room temperature for 6 months. Each set contained 5 replicates. Mycobiota associated with *Arachis hypogea* were then isolated by the agar plate technique and the standard blotter technique.

After 6 months storage, phytotoxicity of oil in terms of germination tests were carried out. One hundred seeds were selected randomly from each test lot and aseptically placed in presterilized petridishes containing three layers of moistened blotting paper. All sets were incubated at 28 ± 2°C in a dark chamber and germination was assessed from 2nd to the 9th day. The germinated seeds were allowed to grow for 9 days and radicle and plumule lengths were recorded on the 5th, 7th and 9thday. One hundred seed from each treatment and control sets were sown in 15 × 20 cm earthen pots (5 seeds in each pot) containing garden soil. The pots were irrigated at intervals of 4 days. After 45 days, the plants were observed for general health and morphology.

Gas chromatography

Requisite amount (0.1 µL) of pure seed oil of *Cuminumcyminum* was subjected to GC and GC/MS analysis. The GC was composed of an Agilent Technology 6890 N_2 gas chromatograph data handling system equipped with a split-splitless injector (split ratio 50:1) and fitted with a FID using N_2 as the carrier gas at flow rate 1 mL/min. The column was HP-5 capillary column (30 m × 0.32 mm, 0.25 µm film thickness) and temperature program was used as follows: initial temperature of 60°C (hold: 2 min) programmed at a rate of 3°C /min to a final temperature of 220°C (hold: 5 min). Temperatures of the injector and FID were maintained at 210°C and 250°C, respectively.

Gas chromatography-mass spectrometry

The GC-MS analysis of seed oil of *Cuminumcyminum* was carried out using Perkin Elmer Clarus 500 gas chromatograph (Shelton, CT06484, USA) equipped with a split-splitless injector (split ratio 50:1) data handling system. The column was an RtxR-5 capillary column (60 m × 0.32 mm, 0.25 µm film thickness). Helium (He) was the carrier gas at a flow rate 1.0 mL/min. The GC was interfaced with (Perkin ElmerClarus 500) mass detector operating in the EI+ mode. The mass spectra were generally recorded over 40-500 amp that revealed the total ion current (TIC) chromatograms. Temperature program was usedas the same as described above for GC analysis. The temperatures of the injector, transfer line and ionsource were maintained at 210°C, 210°C and 200°C, respectively.

Results

Storage fungi on food seeds of *Arachis hypogea*

Fifteen fungal species were detected from food seeds of *Arachis hypogea* through blotter method. The most frequent genera were *Aspergillus* represented by seven species followed by *Fusarium* (represented by three species). Highest percentage incidences were *F. moniliforme* and *A. flavus* (7.4 each) followed by *Fusarium oxysporum* (6.3*) F. solani* (5.4) and *Penicillium glabrum* (4.1). Other species of fungi like *Alternaria alternata, Aspergillus candidus, A. phoenicus, A. tamarii, A. terreus, A. sydowi, Rhizopus nigricans*, *Trichothecium roseum, Trichoderma viride* occurred less frequently. Seven fungal species of three genera were detected from surface sterilized seeds using moist blotter method. The most dominant genera were *Aspergillus* (represented by three species). Highest percentage incidence was of *A. flavus* (3.9) followed by *A. niger* and *F. solani* (2.5 each). Other forms like *Alternaria alternata, Aspergillus sydowi, F. moniliforme* and *F. oxysporum* were infrequent (Table1).

Twelb fungal species belonging to six genera were detected from unsterilized seeds plated over CDA medium. The most dominant

Fungi recorded	Moist blotter method		Czapeksdox agar method	
	US	SS	US	SS
Alternariaalternate (Fr.) Keissler	2.4	1.2	3.2	-
Aspergilluscandidus Pers ex.	2.1	-	3.3	-
A.flavus Link	8.1	3.9	19.9	6.6
*A.niger*van Tieghem	3.7	2.5	14.1	3.5
A.phoenicis Link	1.2	-	-	-
A.tamarii Kita	1.3	-	3.2	-
A.terreus Thom	1.3	-	-	-
A.sydowi (Bainier and Sartory) Thom and Church	2.4	1.0	5.0	1.0
Fusariummoniliforme Sheldon	8.1	1.2	3.0	-
*F.oxysporum*von Schlechtendal	6.3	1.4	6.3	3.1
F.solani (Mart.) Sacc.	5.4	2.5	3.2	3.6
Penicilliumglabrum (Wehmer) Westling	4.1	-	11.2	-
Rhizopusnigricans Ehr.	2.3	-	-	-
Trichodermaviride Pers.ex.Fr.	2.1	-	1.3	-
Trichotheciumroseum (Persoon) Link ex	1.2	-	3.1	-

Insect–*Trogodermagranarium.*

Table1: Percent incidence of different fungi on the food seeds of *Arachis hypogea* L.

genera were *Aspergillus* (represented by five species) followed by *Fusarium* (three species) and *Penicillium glabrum*. Highest percentage incidence was of *A. flavus* (19.9) followed by *A. niger* (14.1), *Penicillium glabrum* (11.2) *F. oxysporum* (6.3) and *A. sydowi* (5.0). Other fungi like *Alternaria alternata, Aspergillus candidus, A. tamarii, F. moniliforme, F. solani, Trichoderma viride, Trichithecium roseum* were less common. Five fungal species of two genera were isolated from surface sterilized seeds using CDA medium. The fungi recorded to be internally seed borne were *A. flavus, A. niger, A. sydowi, F. oxysporum and F. solani* (Table 1). In present investigation, it was observed that in agar plate method fast growing fungi suppressed the development of other fungi making their detection difficult. Slow growing forms like *Penicillium, Trichothecium* and *Trichoderma* were better isolated in blotter method as compared to agar method.

Fungaldeterioration of food seed of *Arachis hypogea*

The metabolites of most of the test fungi showed inhibitory effects on germination. The rating of fungi based on inhibitory effects on germination put *A. niger* as highly potent. The other fungi in order of potentials for inhibiting seed germination were *A. flavus, A. tamari, F. moniliforme, A. phoenicus F. solani, F. oxysporum, Alternaria alternata, Aspergillus candidus, Penicillium glabrum, Rhizopus nigricans, Trichothecium roseum.* The metabolite of *A. sydowi* and *Trichoderma viride* showed promotive effect on the germination of seeds of groundnut as compared to control. It is evident from Table 2, that *A. niger* and *A. flavus* caused high degree of mortality and reduction in germination.

Evaluation of essential oils/synthetic fungicide against test organisms

The essential oil of *Cuminum cyminum* exhibited absolute toxicity at 500 ppm inhibiting mycelial growth of both test fungi completely, while other oils at these concentrations showed moderate, lower level of fungitoxicity (Table 3).The synthetic fungicide was also found effective in second order after this. The physicochemical properties of the *Cuminum cyminum* seed oil are recorded in Table 4. The cumin oil has characteristic pale yellow colour having 0.63% (v/w) yield on dry weight basis.

Fungitoxic properties of *Cuminum cyminum* seed oil

The MIC of the oil was found to be 400 ppm against both the test fungi. The oil exhibited fungicidal nature at hyper MIC against both the test fungi (Table 5) while it was fungicidal in nature at 500 ppm. The *Cuminum cyminum* seed oil completely inhibited the mycelial growth of 10 fungi at 400 ppm (Table 6) and 14 fungi at 600 ppm. The oil it's MIC (400 ppm) was able to inhibit the growth of all 10 discs (each of 5 mm diam) as well as growth of single mycelia discs of 11 mm diam, the maximum considered in this study. Thus, fungitoxic potential of oil appeared to be retained heavy inoculums density. The highest temperature (100°C), autoclaving and storage upto 180 days did not affect the toxicity of the oil against the test fungi and insect (Table 7).

In vivo preservation

It is evident from Table 8, table that cumin seed oil completely protected food seeds upto 120 days when seed dressed. The copper oxychloride protected for 60 days and carbandazim protected for 30 days from fungus infestation when seed dressed**.**

As evident from control sets in Table 9, the groundnut food seeds were associated with 15 fungalspeciesviz. *Alternaria alternata, Aspergillus candidus, A. flavus, A. niger, A. phoenicis, A. tamarii, A. terreus, A. sydowi, Fusarium moniliforme, F. oxysporum, F. solani, P.glabrum, Rhizopus nigricans, Trichoderma viride, Trichothecium roseum* in both containers.

Food seed stored with oil as preservative had better smell and taste when compared to ones stored with synthetic fungicides and fumigants.

Seeds treated with oil were not associated with fungi in either container. Phosphine was ineffective in control of the fungal species at an 80 mg dose in both containers. At 120 mg, it was effective. Ethylenedibromide at 0.25 and 0.38 ml was ineffective.

With respect to germination capacity, the oil treated seeds showed 80-90%, phosphine70-75% and ethylene dibromide 55-65% germination. The seeds of control set ,however exhibited only 45-50% seed germination (Table 10).The oil had no adverse effect on seed germination, seedling growth and general health of plants when compared with control and synthetic fumigants.

The identified constituents with their respective percentages and Kovat's indices are recorded in Table 11. GC and GC-MS analysis

Fungal species	Percent germination	Percent mortality
Alternariaalternata	65.5	34.5
Aspergilluscandidus	65.6	34.4
A.flavus	24.2	75.8
A.niger	6.0	94.0
A.phoenicis	58.6	41.4
A.tamarii	40.2	59.8
A.terreus	40.6	59.4
A.sydowi	89.4	10.6
Fusariummoniliforme	49.5	50.5
F.oxysporum	35.4	64.6
F.solani	61.4	38.6
P.glabrum	65.9	34.1
Rhizopusnigricans	66.4	33.6
Trichodermaviride	85.3	14.7
Trichotheciumroseum	67.4	32.6
Sterilized distilled water (control)	84.3	15.7

Table 2: Effect of culture filterate of fungi on seed germination and seedling mortality of groundnut.

Plant species/commercial fungicides	Percent inhibition of mycelia growth of test fungi at 500ppm		
	Family	*Aspergillusniger*	*A.flavus*
*Adhatodavasica*Nees	Acanthaceae	95.0	100.00*
Ageratum conyzoides L.	Asteraceae	76.5	64.2
A. houstonianum	Asteraceae	82.5	80.5
Anetumgraveolens L.	Umbelliferae	39.0	33.0
Anisomeles ovate R.Br.	Lamiaceae	64.3	60.3
Artabotryshexpetalous(Lamm) Merr.	Annonaceae	53.2	46.7
Azadirachtaindica A. Juss.	Meliaceae	43.1	38.7
*Caesuliaoxillaris*Roxb.	Asteraceae	49.1	47.1
Callestemonlanceolatus DC	Myrtaceae	38.3	48.2
Cannabis sativa L.	Cannabinaceae	12.0	9.5
*Cinnamomumtamla*Nees and Bbrem	Lauraceae	39.0	23.0
*Citrus aurantifolia*Christm	Rutaceae	38.2	29.3
*Cuminumcyminum(*L.)	***Apiaceae***,	100.0*	100.0*
*Eucalyptus citriodora*Hook	Myrtaceae	49.1	35.8
E.globulus(l.) Herit	Myrtaceae	60.0	34.9
Eupatorium capillifolum(L.)	Asteraceae	40.0	30.9
*Feroniaelephantum*Correa	Rutaceae	49.7	60.3
F.limonia(L.) Swingle	Rutaceae	50.8	65.4
Hyptissuaveolens(L.) Poit	Lamiaceae	47.2	27.4
Lantana camera L.	Verbenaceae	58.3	39.1
*L.indica*Roxb.	Verbenaceae	55.7	40.0
*Menthaarvensis*L.	Lamiaceae	53.9	38.6
*M.piperata*L.	Lamiaceae	63.3	50.3
*M.spicata*L.	Lamiaceae	60.3	48.2
Murrayakoenighii(L.)Spreng	Rutaceae	25.8	40.1
*Ocimumadscendens*Willd	Lamiaceae	53.0	52.4
*O.basilicum*L.	Lamiaceae	40.1	50.1
*O.canum*Sims	Lamiaceae	50.1	75.0
*O.sanctum*L.	Lamiaceae	49.1	52.3
*Putranjivaroxburghii*Wall	Euphorbiaceae	90	95
*Tageteserecta*L.	Asteraceae	44.0	30.7
*Thujaoccidentalis*L.	Cuppressaceae	24.0	46.3
Copper oxychloride	*Synthetic fungicide	94.0	90.0
Carbondazim	*Synthetic fungicide	84.0	96.1

Table 3: Evaluation of essential oils of higher plants/fungicides against *Aspergillusniger* and *A. flavus.*

Parameters	Values
Specific gravity	0.922
Specific rotation	+10
Refractive index	1.405
Acid value	3.45
Saponification number	153.49
Ester number	150.04
Phenolic content	Nil
Solubility	Completely miscible with petroleum etheracetone and 90%ethanol in 1;1ratio but insoluble in water

Table 4: Physicochemical properties of *Cuminumcyminum* seed oil.

Dose of oil in ppm	*Aspergillusniger*	*A.flavus*
200	30	40
300	70	80
400	100*	100*
500	100	100
600	100	100

*Fungicidal

Table 5: Minimum inhibitory concentration of*Cuminumcyminum* seed oil.

Fungal species	Per cent inhibition of mycelial growth of isolated fungi			
	Sublethal 200 ppm	Lethal 400 ppm	Hyperlethal 600 ppm	Hyperlethal 800 ppm
Alternariaalternata	45.6	80.0	100.0	100.0
Aspergilluscandidus	49.6	89.0	100.0	100.0
A.flavus	50.0	100.0	100.0	100.0
A.niger	30.0	100.0	100.0	100.0
A.phoenicis	40.0	100.0	100.0	100.0
A.tamarii	48.0	100.0	100.0	100.0
A.terreus	59.0	100.0	100.0	100.0
A.sydowi	55.6	100.0	100.0	100.0
Fusariummoniliforme	40.0	100.0	100.0	100.0
F.oxysporum	42.0	79.6	100.0	100.0
F.solani	40.0	100.0	100.0	100.0
P.glabrum	59.0	100.0	100.0	100.0
Rhizopusnigricans	54.0	100.0	100.0	100.0
Trichodermaviride	55.0	80.0	90.0	100.0
Trichotheciumroseum	65.9	95.0	100.0	100.0

Table 6: Spectrum of *Cuminumcyminum* seed oil at different doses.

Physical factors	Per cent inhibition of mycelial growth at its MIC
Temperature (°C) Time of treatment-60min 40°C 60°C 80°C 100°C	 100 100 100 100
Autoclaving (15l bs/sq inch pressure at 120°C) For 15 min	100
Storage in days 15 30 45 60 75 90 105 120 135 150 165 180	 100 100 100 100 100 100 100 100 100 100 100

Table 7: Effect of physical factors on the fungitoxicityof *Cuminumcyminum* seed oil.

Period of incubation in days	Appearance of fungal species		
	Cumin oil	Copper oxychloride	Carbendazim
30	-	-	-
60	-	-	+
90	-	+	+
120	-	+	+
150	+	+	+
180	+	+	+

Table 8: *In vivo* efficacy of cumin oil and commercial fungicides in preservation of food seeds of *Arachis hypogea*

of the oil revealed recognition of p-mentha-1, 4-dien-7-al (27.4%), γ-terpinene (12.8%), β-pinene (11.4%) and cuminaldehyde (16.1%) as major compounds.

Discussion

Several other fungal species were isolated by different workers from groundnut seeds viz., *Aspergilluscandidus, A. chevalieri and A. ruber* [18]; *Mucorsp* [19]; *Fusarium moniliforme, F. pallidoroseum,*

Fungal species	control				treatment																							
					Cuminumcyminum oil								Phosphine(mg)								Ethylene dibromide(ml)							
					0.50				0.76				160				240				0.50				0.76			
	A		B		A		B		A		B		A		B		A		B		A		B		A		B	
	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T
Alternariaalternata	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Aspergilluscandidus	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
A.flavus	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
A.niger	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
A.phoenicis	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
A.tamarii	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
A.terreus	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
A.sydowi	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Fusariummoniliforme	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
F.oxysporum	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
F.solani	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
P.glabrum	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Rhizopusnigricans	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Trichodermaviride	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Trichotheciumroseum	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+

Storage system; G-gunny bags; T-tin containers

Detection method; A-agar plate technique; B-blotter tehnique

+; presence of fungi; -absence of fungi

Table 9: Food seed mycoflora of 400 g seed of *Arachis hypogea* L. treated with *Cuminumcyminum* seed oil, Phosphine and ethylene dibromide after 6 months of storage in 500 ml containers.

Period (days)	Germination%													
	control		*Adhatoda* oil				Phosphine (mg)				Ethylene dibromide (ml)			
			0.50		0.76		160		240		0.50		0.76	
	G	T	G	T	G	T	G	T	G	T	G	T	G	T
2	15	15	15	15	15	15	15	15	15	15	15	15	20	15
3	25	25	50	50	50	50	40	50	40	35	30	35	35	30
4	45	45	75	85	75	80	65	70	65	65	60	60	50	35
5	45	50	80	90	80	85	70	75	70	70	65	65	55	60
7	45	50	80	90	80	85	70	75	70	70	65	65	55	60

G;Gunny bags

T;Tin containers

Table 10: Seed germination of *Arachis hypogea* L. (groundnut) treated with *Cuminumcyminum* oil, phosphine and ethylene dibromide after 6 months storage of 400 g samples in 500 ml containers.

Components	Kovat's indices	% Content
p-mentha-1,4-dien-7-al (27.4%),	1280	27.4%
γ-terpinene	1068	12.8%
β-pinene	977	11.4%
cuminaldehyde	1239	16.1%

Table 11: Chemical composition of *Cuminumcyminum* seed essential oil.

F. solani, Microsporum phaseolina and Verticilliumal boatrum; *Macrophominaphaseolina, Rhizoctonia solani, Fusarium solani*, *F. oxysporum*, *Aspergillus flavus* and *A. niger* [3] but in present investigation 15 fungal species viz.*Alternaria alternata, Aspergillus candidus, A. flavus, A. niger, A. phoenicis, A. tamarii, A. terreus, A. sydowi, Fusarium moniliforme, F. oxysporum, F. solani, P. glabrum, Rhizopus nigricans, Trichoderma viride, Trichothecium roseum* were isolated. The variation in fungal species may be due to different climatic conditions, isolation periods and different storage containers.

Shaziz et al [3] isolated higher number of fungi by blotter method was used as compared to agar plate and deep-freezing method. Surface sterilization of seeds reduced the incidence of *A. flavus* and *A. niger*. Similarily in present investigation higher number of species were isolated in blotter method and surface sterilization reduced the number of species.

In present investigation, the MIC of *Cuminum cyminum* seed oil was found to be 400 ppm against both *Aspergillus niger* and *A. flavus*. There is a marked variation in the MIC of different plant oils against *Aspergillus niger*-thus *Ocimumadscendens* Willd 200 ppm [20], *Cymbopogon flexuosus* (Steud.) Wats 400 ppm [21], *Syzygium aromaticum* (L.) Merrill and Perry 200 ppm [22], *Cedrusdeodara* (Roxb.ex Lambert) G. Don 1000 ppm and *Trachyspermumammi* (L.) Sprague 500ppm [23]; *Putranjivarox burghii* Wall 400 ppm [24]. The variation in the MIC of different plant oils may be due to the presence of different chemical constituents.

Wellman [25] mentioned that a fungicide must retain its fungitoxicity at the extreme of temperatures. The fungitoxicity of leaf oil of *Adhatoda vasica* was found to be thermostable upto 100 C like *Ageratum conyzoides* [26]; *Nardosta chysjatamansi* [27]; *Putranjivarox burghii* ppm [24]. The cumin seed oil retained its fungitoxicity on autoclaving (15 lbs/square inch pressure).This quality of oil will facilitate the isolation of their constituents in active state.

Wellman [25] highlighted that a fungicide should be able to retain its activity during long period of its storage. The fungitoxic factor in the oil of *Adenocalyma allicea* was lost within 21 d of storage [28] while persisted for long period in the oil of *Ageratum conyzoides* [26]; *Trachyspermumammi* [23] and *Putranjivarox burghii* ppm [24]. The fungal toxicity was not affected by storage upto 180 days during present investigation. Therefore, this shows that the *Cuminum cyminum* seed oil can be safely stored at any ambient temperature for long periods without loss in toxicity.

Many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternates as they are known to have minimal environmental impact and danger to consumer in contrast to synthetic fungicides [29,30].

Cumin oil was more effective than commercial pesticides during *in vivo* both during seed dressing and fumigation studies. Seed fumigation method was more effective than seed dressing method, protected seeds of *Arachis hypogea* kept for food purpose up to 180 days from fungal infestationincreased its shelf life.

Conclusion

The study revealed that Cumin oil was more fungi toxicants than tested fungicides, thereby indicating the possibility of its exploitation as an antifungal agent for protection of food seeds of groundnut during storage. This may be a fumigant for future as alternate of synthetic pesticides.

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