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Rapid communication

Preservative potential of cumin essential oil for *Pisum sativum* L. during storage

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Abstract: The samples of stored seeds of pea (*Pisum sativum* L.) were collected from 30 farmer markets. The mycobiota analysis showed presence of 15 fungal species and one species of insect *Callosobruchus chinensis.* The fungi such as *Aspergillus flavus, A. niger, A. ochraceous, A. terreus* were found to be dominant based on percent frequency of each in blotter method in unsterilized and sterilized seeds 18.9–7.9, 15.0–3.9, 12.2–3.7, 10.1–1.7, respectively, and in agar plate technique 17.9–8.3, 15.1–9.5, 12.8–5, 7.9.7–6.7, respectively. These species showed reduction in terms of weight loss, germination and protein content in pathogenicity testing. Essential oil extracted by hydrodistillation from fruits of *Cuminum cyminum* L. was evaluated against the most common occurring funi such as *A. flavus* and *A. niger* as well as the insect species *C. chinensis* and the oil exhibited high toxicity. The oil killed the tested fungi and showed thermostable nature at its minimum inhibitory concentration (MIC) of 400 ppm. The oil safely preserved pea seeds up to 120 days at 0.50 (1,000 ppm) and 0.76 ml (1,500 ppm) in polyethylene and jute bags of 500 ml capacity containing 400 g seeds separately. There were no changes in organoleptic appereance of food seeds during storage. The oil has beneficial effect on number of visible nodule formation and shoot and root dry biomass of 15-day-old plants in comparison to control sets. The cumin oil was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Key words: *Cuminum cyminum*, fruitoil, fungal and insect infestation of pea, *Pisum sativum*, synthetic fumigant

Introduction

Loss of food commodities due to pest infestations is a major reason of food crisis particularly in tropical countries (Prakash *et al*. 2013). Burt (2004) mentioned that in spite of the modern improvements in foodhygiene, it has been estimated that as many as 30% people in the industrialised countries suffer from foodborne diseases each year, and in 2,000 at least 2 mln people died of diarrhoeal diseases wordwide. Therefore, new methods are still needed for the reducing or inhibiting foodborne pathogens, possibly in combination with the existing methods (Holley and Patel 2005; Valero and Frances 2006). Especially the industrialised societies appear to experience the trend towards green consumption, desiring fewer synthetic food additives and products with a low impact on the environment (Burt 2004; Sacchetti *et al.* 2005). The versatile compositions of essential oils, broad spectrum of antimicrobial potentials and low level of toxicity (Bakkali *et al*. 2008) have enhanced their widespread use in perfumes, pharmaceutical and cosmetic industries as well as food preservatives and additives. They are capable of inhibiting food borne pathogens and extend the shelf-life of processed food (Smith-Palmer *et al*. 1998). Spices are aromatic or pungent vegetable substances used in small quantities to enrich, alter or mask the flavour of food (Al-Mofleh 2010). They prolong the storage life of foods by preventing rancidity and oxidation of lipids (Kelenand Tepe 2008). Pea is an important nutritious leguminous vegetable found in cool season regions as cold-hardy crop and is widely cultivated throughout the world. It is a rich source of protein (25%), amino acids, sugars (12%), carbohydrate, vitamins A and C, calcium and phosphorus, apart from having a small quantity of iron.

Therefore the presented study aimed at investigating the antifungal and insect repellent potential, and chemical composition of the essential oil of *Cuminum cyminum* L., and its application as botanical fumigants in storage of *Pisum sativum* L. (pea) seeds. The effect of the essential oil of *C. cyminum* on number of visible nodule formation, shoot and root dry biomass of 15-day-old plants were also studied.

Materials and Methods

Analysis of fungi and insects

The collection of stored seeds was done from 30 farmer markets. Agar plate method using Czapek Dox Agar of Sigma-Aldrich (a semi-synthetic solid medium, containing sucrose as C-source and nitrate as the sole source of nitrogen, Muskett, 1948) and standard blotter method (De

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Tempe 1953) were used to study the seed-borne mycoflora of examined seeds. In agar plate technique, 100 seeds were equally distributed on Petri dishes, each containing a growth medium Czapek Dox Agar and 5 seeds. In a blotter test, 5 seeds were placed on a pair of sterile white blotter papers of 8.5 cm diam., soaked first in sterile distilled water and then placed in a presterilized Petri dish (90 × 15 mm). The dishes were incubated at 25±2°C and on 7th day of incubation, a microscope analyses of seed borne fungi were performed. For internal seed mycoflora examination, the seeds were first surface sterilized with 0.1% sodium hypochlorite for 5 min, washed with sterilized distilled water and then subjected to agar plate and standard blotter techniques for isolation of the fungi. Identifications of fungal species were confirmed following Booth (1971), Ellis (1971, 1976), Gilman (1967), Raper and Thom (1949), Raper and Fennell (1965).

The percent frequency was calculated by using following formulae:

$$\text{Frequency (\%)} = \frac{N}{T} \times 100$$

where: N – number of plates in which individual fungal species occurred; T – total number of plates studied.

Culture of insects

The cultures of *C. chinensis* were established from infested stored pea seeds collected from 30 farmer markets. The insects were identified following Drees and Jackman (1999) and Beck and Blumer (2007) and cultures were maintained subsequently on insecticide free newly harvested pea (*P. sativum*) seeds under laboratory conditions (25±2°C) in darkness to obtain same aged insects.

Effect of pests on stored pea seeds

The deterioration of pea seeds caused by occurrence of dominant fungal species such as *Aspergillus flavus, A. niger, A. ochraceous, A. terreus* in regards to weight loss and seed germination was evaluated. For this purpose freshly harvested and sterilized pea seeds were taken in presterilized polyetylene bags (200 g seeds/bag) and inoculated separately with two discs (5 mm diam.) of different fungal species. Likewise five insects of *C. chinensis* were kept separately in presterilized polyetylene bags containg 200 g pea seeds. The inoculated pea seed samples were stored for 20 days under laboratory conditions at room temperature. The experiments were repetaed in five replications. The protein content was studied following Lowry *et al.* (1951) using bovine serum albumin as standard. The optical density of each specimen was measured at 650 nm.

Extraction of volatile constituents and evaluation of their toxicity against tested fungi and insect species

Essential oil from cumin fruits was extracted separately using Clevenger's apparatus (Clevenger 1928) at 90±2°C for 6–8 h. The obtained essential oil was dried over anhydrous sodium sulphate and then stored at 4°C in clean glass vials. The toxicity of cumin fruit oil was assessed following Tripathi and Kumar (2007). The fungitoxicity was measured following Dixit *et al.* (1978) and recorded as a percent inhibition of mycelial growth. The repellent activity of the cumin oil against *C. chinensis* was studied following the method of Tripathi and Kumar (2007) with slight modification.

Fungitoxic properties of *Cuminum cyminum* seed oil

For minimum inhibitory concentration (MIC) of fruit oil of *Cumin* detection a poisoned food technique of Grover and Moore (1962) was followed. Different concentrations of the oil ranging from 200 to 600 ppm were prepared by dissolving required amount of oil in 0.5 ml acetone and then mixing with 9.5 ml Czapek Dox agar medium seperately. The Petri plates contained acetone and medium without oil were the control sets. Fungal discs (5 mm diam.) obtained from periphery of 7-day-old culture of each of tested fungi were aseptically inoculated in each of the treatment and control sets. All these sets were incubated at 28±2°C for 6 days. Diameters of fungal colony of treatment/control sets were measured in mutually perpendicular directions on the 7th day and the average was used to calculate the percent inhibition of mycelia growth of tested fungi separately. The oil treated discs of the fungi showing complete inhibition of their mycelia growth up to 7 days were washed with sterile water and placed again on fresh solidified medium to observe the recovery of mycelia growth. The fungitoxic spectrum of the cumin oil was studied against various fungi isolated from pea seed samples. In addition an effect of temperature, autoclaving and storage on the fungitoxicity of oil was determined following Pandey *et al.* (1982). Each experiment was repeated twice and contained five replicates.

Comparison of efficacy of cumin oil with synthetic fumigant

Fresh seeds of pea were collected locally; sun dried and kept in presterilized polyethylene bags. For testing *in vivo* preservative potential of oil and synthetic fumigants two jute bags and polyethylene bags were selected which farmers are using in this area for pea storage. Since cumin oil and ethylene dibromide are in liquid form two doses were prepared; one by taking 0.50 ml (1,000 ppm) and other 0.76 ml (1,500 ppm) in jute and polyethylene bags of 500 ml capacity containg 400 g seeds separately.

Sterile cotton swabs (0.50 g), soaked with doses of oil or ethylene dibromide and wrapped in sterilized muslin cloth (0.75 g) were placed at the bottom of each container of pea seeds. Similarily, 400 g samples of pea seeds were treated with phosphine from 0.50 g (1,000 ppm) or 0.76 g (1,500 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 120 days. Each set contained five replicates. Mycofloral analyses were done following agar plate technique of Muskett (1948) and the standard blotter technique of De Tempe (1953). The insects were examined by hand lens.

After 120 days of storage, germination tests were carried out. The randomly selected 100 seeds from each test lot were aseptically placed in presterilized Petri dishes containing three layers of moistened blotting paper. The blotting papers were moistened with sterilized water at 2-day intervals. The observation for number of visible nodules, shoot and root biomass were recorded on 15th day.

Gas chromatography (GC)

The required amount (0.1 μl) of pure fruit oil of *C. cyminum* was subjected to GC and gas chromatography-mass spectrometry (GC-MS) analysis. The GC was composed of an Agilent Technology 6890 N gas chromatograph data handling system equipped with a split-splitless injector (split ratio 50 : 1) and fitted with a flame ionization detector (FID) using N2 as the carrier gas at flow rate 1 ml · min^{-1}. The column was HP-5 capillary column (30 m × 0.32 mm, 0.25 μm film thickness) and a temperature program was used as follows: initial temperature of 60°C (hold – 2 min) programmed at a rate of 3°C · min^{-1} 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 (GC-MS)

The GC-MS analysis of fruit oil of *C. cyminum* 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 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^{-1}. The GC was interfaced with (Perkin ElmerClarus 500) mass detector operating in the EI+ mode. The mass spectra were generally recorded over 40–500 amu that revealed the total ion current (TIC) chromatograms. The temperature program was the same as described above for GC analysis. The temperatures of the injector, transfer line and ion source were maintained at 210°C, 210°C and 200°C, respectively.

The oil components were identified by calculating their Kovats indices (Davies 1990), comparing mass spectra with those reported in the literature (Jenninga *et al*. 1980; Adams 1995) and in the GC-MS computer database (NIST 98 and Wiley-5). Furthermore, the identity of some of the oil components was confirmed by GC analysis by coinjection with authentic substances.

Statistical analysis

The experimental results were expressed as mean ±SD (standard deviation) of five parallel measurements. Antifungal activity, MIC, spectrum, effect of physical factors on the cumin oil were obtained by calculating an average of five replicates.

Results and Discussion

Fifteen fungal species were isolated from the seed samples of *P. sativum* collected from the 30 farmer markets. The results presented in the Table 1 indicate that *A. flavus*, *A. niger*, *A. ochraceous* and *A. terreus* were the dominant species based of percent frequency of their occurrence. It is also evident that the most frequent isolated genera was *Aspergillus* represented by seven species.

The insect analysis showed that only one insect species i.e. *C. chinensis* was detected in all 30 collected samples.

Table 1. Frequency occurrence of different fungi on the seeds of *Pisum sativum*

Detected fungi	Moist blotter method		On Czapek Dox Agar medium	
	US	SS	US	SS
Alternaria alternata(Fr.) Keissler	1.9	1.2	3.0	–
Aspergillus candidus Pers ex.	1.0	–	2.1	–
A. flavus Link	18.9	7.9	17.9	8.3
A. niger van Tieghem	15.0	3.9	15.1	9.5
A. ochraceous Wilhelm	12.2	3.7	12.8	5.7
A. tamarii Kita	1.1	–	2.1	–
A. terreus Thom	10.1	1.7	9.7	6.7
A. sydowi (Bainier and Sartory) Thom and Church	2.1	1.3	4.1	1.1
Fusarium moniliforme Sheldon	1.0	1.1	3.0	–
F. oxysporum von Schlechtendal	1.1	1.2	1.0	1.3
F. solani (Mart.) Sacc.	1.3	2.1	2.1	1.2
Penicillium glabrum (Wehmer) Westling	3.0	–	0.5	–
Rhizopus nigricans Ehr.	1.2	–	–	–
Trichoderma viride Pers.ex.Fr.	2.0	–	0.2	–
Trichothecium roseum (Persoon) Link ex	1.5	–	0.7	–

US – unsterilized seeds; SS – sterilized seeds
"–" fungus not reported
*values are given as per cent of mean of 30 analyzed samples
Insect – *Callsobruchus chinensis*

Wilman *et al.* (2014) reported that *Alternaria* spp. were the most common fungi isolated from pea seeds, followed by *Fusarium* spp., *Stemphylium* spp., *Ulocladium* spp., *Botrytis cinerea* Pers., *Epicoccum nigrum* Link., and *Phoma pinodella* L.K. Jones. The highest percentage of infected seeds (55%) was recorded for cultivar Ezop. The presence of a large number of fungi was found in 2012 for cultivars Santana, Tarchalska, Medal, Cysterski, Mentor, Lasso, and Ezop. Fodder cultivars displayed a lower infection level than edible cultivars. The final cunclusion was that *Alternaria* spp. occurred the most frequently in pea seeds in Poland and *Fusarium* spp. were likely the most dangerous in regards to their established mycotoxigenic abilities. *Aspergillus nidulans, A. niger, A. fumigates, A. flavus, Alternaria alternate, Acremonium* sp., *Chaetomium* sp., *Rhizopus nigricans, Fusarium moniliforme, F. solani, F. oxysporum, Macrophomina phaseolina, Botrytis* sp., *Cladosporium, Helminthosporium* sp., *Curvularia, Drechslera, Humicola, Penicillium chrysogenum, Mucor racemosus, Mucor hiemalis, Phoma, Monilia sitophila, Pythium, Trichoderma viridae, T. hamatum, Nigrospora oryzae, Rhizoctonia bataticola, Sclerotium* sp. and *Verticillium* sp. etc., were reported in 2013 (Ghangaokar and Kshirsagar 2013) but in present investigation 15 fungal species such as *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 igricans, Trichoderma viride, Trichothecium roseum* were isolated. The variation in fungal species may be due to different climatic conditions, isolation periods and different storage containers.

Based on the data in the Table 2, *A. flavus*, *A. niger* and the insect species – *C. chinensis* played an important role in seed weight loss and seed germination. The protein contect in *A. flavus* inoculated seeds was 10%, *A. niger* 13% while insect inoculated 10% while control set without fungus or insect inoculation showed 25% protein content. The data in the Table 2 shows, that fungus and insect have capability to reduce protein content. On account of wide occurrence and their pathogenicity these were selected as test organisms.

The fruit oil of *C. cyminum* exhibited absolute toxicity at 500 ppm inhibiting completely mycelial growth of both tested fungi (Table 3). This also showed 100% repellency against the examined insect *C. chinensis* with a dose of 0.02 ml.

The inimum inhibitory concentration of the oil was found to be 400 ppm against both the test fungi *A. flavus* and *A. niger.* The oil exhibited fungicidal nature at hyper MIC against both tested fungi (Table 4) while it was fungicidal in nature at 500 ppm. The *Cuminum cyminum* oil completely inhibited the mycelial growth of 10 fungi at 400 ppm (Table 5).

The oil at minimum inhibitory concentration (400 ppm) was able to inhibit the growth of all 10 discs (each of 5 mm diam.) as well as growth of a single mycelium disc 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 up to 120 days, did not affect the toxicity of the oil against the tested fungi and insect (Table 6).

Cumin is the seed of the herb *C. cyminum*, that is small umbelliferous plant. The seeds come as paired or separated carpels, and are 3–6 mm (1/8–1/4 in) long. They have a striped pattern of nine ridges and oil canals, and are hairy, brownish in colour, boat-shaped, tapering at each extremity, with tiny stalks attached.

The fruit oil of *C. cyminum* howed minimum inhibitory concentration at 400 ppm against both *A. niger* and *A. flavus.* The previous literature revealed that there is a marked variation in the MIC of different plant oils against *A. niger* thus *Ocimum adscendens* Willd. 200 ppm (Asthana and Singh 1981), *Cymbopogon flexuosus* (Steud.) Wats 400 ppm (Dixit 1991), *Syzygium aromaticum* (L.) Merrill and Perry 200 ppm (Khan 1993), *Cedrus deodara* (Roxb. ex Lambert) G. Don 1,000 ppm and *Trachyspermum ammi* (L.) Sprague 500 ppm (Singh and Tripathi 1999). The variation in the MIC of different plant oils may be due to the presence of different chemical constituents.

Wellman (1967) highlighted that a fungicide must retain its fungitoxicity at the extreme of temperatures. The fungi toxicity of seed oil of *Putrnjiva* was found to be thermostable up to 100°C like *Ageratum conyzoides* L. (Dixit *et al*. 1995); *Nardostachys jatamansi* (D. Don) DC (Mishra *et al*. 1995) and *Adhatoda vasica* Nees oil (Kumar 2014). The seed oil retained its fungitoxicity despite autoclaving (15l bs/square inch pressure). This quality of oil will facilitate the isolation of their constituents in active state.

Wellman (1967) reported 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 days of storage (Chaturvedi 1979) while persisted for long period in the oil of *A. conyzoides* (Dixit *et al.* 1995); *T. ammi* (Singh and Tripathi 1999) and *Adhatoda vasica* (Kumar 2014). The fungal toxicity was not affected by storage up to 120 days during present investigation. These results indicate that *C. cyminum* seed oil can be safely stored at any ambient temperature for long periods without loss in toxicity.

The pea seeds were associated with 15 fungal species such as *A. 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 (Table 7).

Calosobruchus chinensis was present in jute bags but absent in polyethylene bags. The pea seeds treated with oil were not associated with fungi or insects in either container. Phosphine was ineffective in control of the fungal species or *C. chinensis* at 160 mg dose in both containers, however, it was at 240 mg. Ethylene dibromide at 0.50 and 0.76 ml was ineffective.

The data in the Table 8 suggest that cumin oil have promoting effect in terms of number of nodule formation and formed 13–15, aluminium phosphide formed 10–12 and ethylene dibromide 9–10 while the control sets showed 7–8 nodules.

It is evident from the Table 9, that cumin oil have promoting effect in terms of shoot dry mass and formed 0.19±0.009, aluminium phosphide formed 0.16±0.009 and ethylene dibromide 0.15±0.009 g · $plant^{-1}$ in comparison to the control sets which showed 0.10±0.009 (g · $plant^{-1}$), respectively.

Table 2. Fungal and insect species regarding weight loss, germination and protein content of pea – *Pisum sativum* – seeds after 20 days storage

Fungal and insect species	Weight loss [g]		Germination [%]		Protein [%]	
	control	treatment	control	treatment	control	treatment
Aspergillus flavus	nil	0.186	85.43	46.57	25	10
A. niger	–	0.179	87.43	50.30	25	13
A. ochraceous	–	0.130	80.00	73.33	25	23
A. terreus	–	0.050	90.00	76.24	25	23
Insect – *Callosobruchus chinensis*	–	0.184	90.00	46.23	25	10

Table 3. Evaluation of cumin oil and synthetic fungicides against *Aspergillus niger* and *A. flavus*

Oil/synthetic fungicide	Plant part/used synthetic pesticide	Per cent inhibition of mycelia growth of test fungi at 500 ppm	
		Aspergillus niger	*A. flavus*
*Cuminum cyminum**	fruit (plant part used in hydrodistillation)	100±0.46	100±0.27
Copper oxychloride**	synthetic fungicide (Yogi Dye Chem Industries Satyam Complex, M. G. Road, Ghatkopar East, Mumbai – 400077, Maharashtra)	90.0±0.37	94.0±0.24
Carbondazim**	synthetic fungicide (Canary Agro Chemicals Pvt. Ltd., New Delhi)	96.1±0.28	84.0±0.34

*oil; **synthetic fungicide

Table 4. Minimum inhibitory concentration (MIC) of *Cuminum cyminum* fruit oil

Dose of oil in ppm	*Aspergillus niger*	*A. flavus*
200	30±0.23	40±0.24
300	70±0.21	80±0.22
400	100±0.23	100±0.21
500	100±0.01*	100±0.01*
600	100±0.02	100±0.03

*fungicidal

Table 5. Fungitoxic spectrum of *Cuminum cyminum* seed oil at sub lethal, lethal and hyperlethal doses against fungi isolated from pea seeds

Fungal species	Per cent inhibition of mycelial growth of isolated fungi			
	sublethal 200 ppm	lethal 400 ppm	hyperlethal 600 ppm	hyperlethal 800 ppm
Alternaria alternata	35.0±0.2	72.4±0.1	100.0±0.1	100.0±0.1
Aspergillus candidus	48.7±0.1	87.1±0.2	100.0±0.1	100.0±0.1
A. flavus	53.3±0.2	100.0±0.1	100.0±0.1	100.0±0.1
A. niger	50.7±0.3	100.0±0.1	100.0±0.1	100.0±0.1
A. phoenicis	42.2±0.2	100.0±0.1	100.0±0.2	100.0±0.1
A. tamarii	47.9±0.2	100.0±0.1	100.0±0.1	100.0±0.1
A. terreus	58.3±0.3	100.0±0.1	100.0±0.1	100.0±0.2
A. sydowi	54.0±0.1	100.0±0.1	100.0±0.1	100.0±0.2
Fusarium moniliforme	41.3±0.3	100.0±0.3	100.0±0.1	100.0±0.1
F. oxysporum	41.1±0.3	79.3±0.3	100.0±0.1	100.0±0.1
F. solani	41.3±0.2	100.0±0.1	100.0±0.1	100.0±0.2
P. glabrum	58.7±0.1	100.0±0.2	100.0±0.2	100.0±0.1
Rhizopus nigricans	53.2±0.2	100.0±0.2	100.0±0.1	100.0±0.1
Trichoderma viride	54.3±0.3	80.1±0.2	90.4±0.3	100.0±0.1
Trichothecium roseum	55.0±0.1	95.0±0.2	100.0±0.1	100.0±0.1

Data are mean of five replicates (±standard error)

Table 6. Physical factors regarding fungitoxicity of *Cuminum cyminum* oil

Physical factors	Per cent inhibition of mycelial growth at its MIC
Treatment by 60 min in the following temperatures [°C]	
40	100
60	100
80	100
100	100
Autoclaving (15l bs/sq inch pressure at 120°C) for 15 min	100
Storage in days	
15	100
30	100
45	100
60	100
75	100
90	100
105	100
120	100

MIC – minimum inhibitory concentration

Table 7. Mycobiota of 400 g seed of pea treated with *Cuminum cyminum* oil, phosphine and ethylene dibromide after 120 days of storage in 500 ml containers

Fungal species	Control				Treatment																							
					Cuminum cyminum fruit oil [ml]								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	
	J	P	J	P	J	P	J	P	J	P	J	P	J	P	J	P	J	P	J	P	J	P	J	P	J	P	J	P
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	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+

Storage system: J – jute bags; P – polyethylene bags
Detection method: A – agar plate technique; B – blotter tehnique
"+" presence of fungi; "–" absence of fungi

Table 8. Number of visible nodules in 15-day-old plants of pea treated with *Cuminum cyminum* oil and synthetic fumigants after 120 days of storage of 400 g samples in 500 ml containers

Period [days]	Number of visible nodules of 15-day-old plants of pea													
	control		cumin oil [ml]				phosphine [mg]				ethylene dibromide [ml]			
			0.50		0.76		160		240		0.50		0.76	
	J	P	J	P	J	P	J	P	J	P	J	P	J	P
15	7±2	8±2	13±2	14±2	15±2	15±2	10±2	11±2	11±2	12±2	9±2	9±2	10±2	10±2

J – jute bags; P – polyethylene bags

Table 9. Shoot dry mass of 15-day-old plants of pea treated with *Cuminum cyminum* oil and synthetic fumigants after 120 days storage of 400 g samples in 500 ml containers

Period [days]	Shoot dry mass pea [g · plant^{-1}]													
	control		cumin oil [ml]				phosphine [mg]				ethylene dibromide [ml]			
			0.50		0.76		160		240		0.50		0.76	
	J	P	J	P	J	P	J	P	J	P	J	P	J	P
15	0.10 ±0.009	0.11 ±0.009	0.18 ±0.009	0.18 ±0.009	0.19 ±0.009	0.19 ±0.009	0.15 ±0.009	0.16 ±0.009	0.16 ±0.009	0.16 ±0.009	0.14 ±0.009	0.15 ±0.009	0.15 ±0.009	0.15 ±0.009

J – jute bags; P – polyethylene bags

Table 10. Root dry mass of 15-day-old plants of pea treated with *Cuminum cyminum* oil and synthetic fumigants after 120 days storage of 400 g samples in 500 ml containers

Period [days]	Root dry mass pea [g · plant^{-1}]													
	control		cumin oil [ml]				phosphine [mg]				ethylene dibromide [ml]			
			0.50		0.76		160		240		0.50		0.76	
	J	P	J	P	J	P	J	P	J	P	J	P	J	P
15	0.8 ±0.007	0.9 ±0.007	0.17 ±0.007	0.18 ±0.007	0.18 ±0.007	0.18 ±0.007	0.14 ±0.007	0.15 ±0.007	0.15 ±0.007	0.15 ±0.007	0.13 ±0.007	0.14 ±0.007	0.14 ±0.007	0.14 ±0.007

J – jute bags; P – polyethylene bags

Table 11. Chemical composition of *Cuminum cyminum* essential oil

Component	KI	Composition [%]
tricyclene	924	0.1
alpha-pinene	935	0.6
sabinene	973	0.5
β-pinene	976	11.4
myrcene	990	0.9
β-phellandrene	1,010	1.3
o-cymene	1,025	3.1
p-cymeneb	1,035	5.7
limonene	1,038	3.1
β-phellandrene	1,038	2.2
γ-terpinene	1,067	12.8
cumin aldehyde	1,238	16.1
cumin alcohol	124	10.4
p-mentha-1,3-dien-7-al	1,255	8.7
(E)-2-decenal	1,261	0.2
p-mentha-1,4-dien-7-al	1,281	27.4
perillaldehyde	1,290	0.6
perilla alcohol	1,298	0.3
eugenol	1,354	0.7
geranyl acetateb	13,787	1.7
caryophyllene	1,413	1.3

KI = Kovats index

The Table 10 reveals that cumin oil have promoting effect in terms of root dry mass and formed 0.18±0.007, aluminium phosphide formed 0.15±0.007 and ethylene dibromide 0.14±0.007 (g · plant^{-1}) in comparison to the control sets which showed 0.08±0.007 (g · plant^{-1}) respectively.

The seed stored with oil as preservative had better smell and taste when compared to ones stored with synthetic fumigants. The identified constituents with their respective percentages and Kovat's indices are recorded in the Table 11. Gas chromatography (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.

Conclusions

The study revealed that cumin oil has more fungitoxic potential than tested synthetic pesticides, thereby indicating the possibility of its exploitation as an agent for protection of seeds of pea during storage. This may be a fumigant as alternate of synthetic pesticides.

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