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In vivo efficacy of natural essential oil of *Syzygium aromaticum* L. bud for protecting the *Pisum sativum* L. seeds

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The mycological investigations on sixty samples of stored garden pea food seeds revealed presence of twelve and ten species of fungi by blotter and agar plate techniques respectively. The fungal species were associated with genera viz., *Alternaria*, *Aspergillus*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium* and *Rhizopus*. The fungal species viz., *Cladosporium herbarum (Pers.) Link and Penicillium italicum Wehmer did not show its appearance in agar plate method. It showed dominance of Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceous*, *Aspergillus terreus* in blotter method in comparison to agar plating. *Aspergillus tmarii*, *P. italicum and Rhizopus stolonifer* did not grow on sterilized seeds in Blotter method. Pathogenicity tests of dominant fungi caused biodeterioration in garden pea seeds. The antifungal testing of essential oils revealed *Syzygium aromaticum* L. bud oil to be fungitoxic at 500 ppm (0.025 mL). The minimum inhibitory concentration of the *S. aromaticum* oil was found to be 300 ppm against four fungi viz., *A. flavus*, *A. niger*, *A. ochraceous* and *A. terreus*. At this concentration the oil was found to be fungicidal and thermostable. The oil activity was not affected by physical factors and showed broad spectrum. *In vivo* study depicted that clove oil was more effective in comparison to EDCT. It controlled a maximum of 4 fungi while the clove oil showed no growth of fungi even after 6 months storage.

Keywords: Clove bud, Fungitoxicant oil, MIC, *Pisum sativum* L., Spectrum, *Syzygium aromaticum* L.

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Introduction

The garden pea (*Pisum sativum* L.) is most commonly used as protein source in India. The garden pea is small spherical seeds from the family Fabaceae locally known as Matar in Hindi. It has a high nutritional value with about 23-25% protein. Carbohydrate in the whole pea ranges from 56.6 to 74% and in the kernel from 62.8 to 78.6%. The pea contains low concentration of free sugars; smooth pea contains higher level of sucrose than the wrinkled pea[1]. The pea protein concentrate has low fat (1.5 to 7.0%) and ash (2.3 to 7.9%) content[2]. In India, fresh green peas are used in various dishes such as *aloomatar* (curried potatoes with peas) or *matar paneer* (cheese with peas), though it can be substituted with frozen peas as well. Fresh peas from the plant are also eaten raw being sweet. The pea storage is carried out for six to eight months after drying the harvested seeds. For its protection during storage, more than 90% of farmers are not taking any precautions but, proper storage may help the farmers to earn higher profit. Fungi and insects are responsible for about 20-30% of the seeds loss. Careless storage of pea food seeds in rural areas results in heavy losses due to fungi and insects, but detailed studies on such deterioration of stored food seeds of pea have not been made so far. The deteriorated seeds result in abnormal seedlings.

If the seed borne pathogens are present internally or externally, they may cause seed rot, seed necrosis, seed abortion, reduction or elimination of the germination capacity, etc. This also causes seedling damage by mold fungi which grow on the seed substratum producing mycotoxins which are hazardous to humans and animals[3]. The use of synthetic pesticides is currently the most widely adopted method for grain protection against stored-grain pests. However, the extensive use of these substances has led to the development of resistance in several species during the past decades[4]. Resistance, combined with consumer demand for residue-free

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food, encourages the development of alternative, reduced risk methods for stored-grain protection. Several plant derivatives have been successfully evaluated against storage insect-pests[5].

Different synthetic preservatives have been introduced in the management of postharvest losses of food grains but due to the recent reports on their adverse effects on human health and environment, most of these grey chemicals are not liked by health conscious consumers. In this context, plant-derived products have been recognized to play a significant role as green chemicals in the formulation of eco-friendly and safer alternatives to food preservations. Azadirachtin, pyrethrin, carvone, limonene, rotenone, allylisothiocynate etc. are some popular antimicrobial plant products commonly used to protect food commodities during storage[6].

Resistance development amongst post-harvest insect pests is a burning issue in the developing countries. Several studies have been carried out in India and other parts of the world on the resistance problem in *Tribolium castaneum* for insecticides such as phosphine, malathion, fenitrothion and pirimiphos-methyl[7,8]. Plant products have long been used as efficient pesticides even before the onset of synthetics. Plant based pesticides are recognized as safer alternatives for the pest management since they have been present in nature for millions of years without any ill effects and having broad spectrum of activity[9]. Botanical pesticides based on essential oils (EOs) are promising contenders for commercial stored food protection. Utilization of bioactive products from higher plants for pest control can be a biorational approach in an Integrated Pest Management program[10]. Most of them have low mammalian toxicity, work in low concentrations. Essential oils work both as insect and fungal growth regulators and inhibit their growth and metabolism without killing.

Clove commonly called as *Lavang* is an aromatic flower bud of the plant *S. aromaticum* L. which belongs to the family Myrtaceae. Chemically, clove has eugenol, eugenyl acetate, caryophyllene and α-humulene, β-elemene, α-cadinene, and ledol[11].

Mycological analysis of stored pea food seeds was conducted in order to find out the dominant fungal species responsible for its biodeterioration. The EO extracted from the locally available clove bud was evaluated for its fungitoxicity and its chemical analysis was also done. Experiments were conducted for its MIC, nature, spectrum, and storage. The efficacy of clove bud EO was compared with commercial pesticide- EDCT (safety grains) for pea food seed protection up to 6 months of storage.

Materials and Methods

Collection of garden pea food seed samples

The 60 samples of garden pea food seed kept in storage (3 to 8 months) were collected (Feb-April, 2020 at random from places of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj and Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur. From this all 10 selected spots from each centres/spots six samples of garden pea food seed (500 g) were collected and kept separately in pre-sterilized polyethylene bags after labelling the name of district, tehsil and place. Thus all 60 samples collected were brought to Laboratory for analysis of their associated mycoflora.

Mycological analysis

The mycological flora identification analyses of 60 pea food seeds samples was conducted both through agar plate[12] using Czapek's Dox agar medium (each containing 15 mL medium) and standard blotter[13] techniques. The pea food seeds (100 seeds from each of the 60 samples) were equidistantly placed on Czapek's Dox medium in separate Petri plates in agar plate technique. Each plate had 5 seeds. In blotter test, seeds were similarly plated on three layered moistened blotter pads in sterilized Petri plates. These assay plates were then incubated at 25±2°C and observed daily from 7 to15 days for observing of developing fungal colonies. Fungal colonies from pea food seeds were sub-cultured for isolation of pure colony. The microscopic and macroscopic observations of pure colony were recorded and fungi identified taking help of literature[14-17]. Their morphological and cultural characteristics were also compared by authentic cultures maintained in the Mycology Lab, Department of Botany, University of Gorakhpur and Amity Institute of Biotechnology, Amity University, Haryana. For studying internal seed-borne pathogens of pea food seeds they were first surface sterilized with aqueous 0.1% NaOCl for 5 minutes. This was followed by washing with double distilled water. These were then subjected to agar plate and standard blotter techniques for isolating the fungi. Each fungal species (%) associated with seeds samples of pea was estimated.

Evaluation of deterioration caused by dominant fungi

The deterioration caused by dominant fungal species viz., *A. flavus*, *A. niger* with respect to weight loss, % seed germination, carbohydrate and protein content were evaluated. For this purpose, freshly harvested sterilized Rachna variety, pea food seeds were taken in pre sterilized polyethylene bags (50 g seeds/bag) and inoculated by one disc (5 mm diam) of different fungal species separately and stored at room temperature for 21 days.

The analysis of carbohydrate content of each treated food seeds for fungi was done following the Anthrone method[18]. The dehydration of carbohydrates was done by conc. H_2SO_4 for furfural. The Furfural condenses with anthrone (10-Keto-9, 10 dihydroanthracene) to develop a blue-green (coloured) complex that is measured at 630 nm calorimetrically. Estimation of protein content was done following Lowry *et al.*[19] using bovine serum albumin as standard. At 650 nm, optical density of each specimen was estimated.

Isolation of essential oil from *S. aromaticum* bud

The extraction of EO was done from 200 g dried and ground clove by hydro distillation process in 2 L water in a Clevenger's apparatus at 90±2°C for up to 5-9 h. The extracted oil was dried over anhydrous sodium sulphate. It was then stored under sterile condition at a temperature of 4°C for further investigation. For developing fungitoxic fumigant activity of clove, EO was evaluated following Inverted petri plate technique[20] at 500 ppm (0.025 mL).

Fungitoxicity evaluation of clove EO

The MIC of clove EO was tested following the poisoned food technique[21,22]. Various concentrations of the EO ranging from 300 to 600 ppm (because 100 and 200 ppm were less effective) were carried out through dissolving requisite amount of oil in 0.5 mL acetone. This was then followed by mixing with 9.5 mL Czapeks-dox agar medium separately. For control sets, the Petri plates with acetone and medium without the clove EO were used. The Fungal discs (5 mm diam) were cut by cork borer from the peripheral region of seven days old culture of each of test fungi such as *A. flavus* and *A. niger*. Then these were aseptically placed in the control and treatment sets separately. These were all kept at 28±2°C for 6 days. The diameters of fungal colony of treatment/control sets of *A. flavus*, *A. niger* on the 7th day were taken in mutually perpendicular directions separately. The overall set up was repeated twice and each contained 3 replicates.

For determining the nature of antifungal properties of the clove EO, treated fungal disc of *Aspergillus flavus*, *A. niger* showing complete inhibition of mycelial growth up to the 7th day were washed with sterile water. They were placed again on fresh solidified medium to see the revival of mycelial growth. The fungi toxic spectrum of the clove EO was studied against fungal species isolated from stored food seeds samples of pea. The effect of autoclaving and storage period of 6 months on the fungitoxicity of clove EO was determined[22]. The experiment was repeated twice each having 3 replicates.

Comparative study of clove EO with EDCT

Freshly collected Rachna variety mature pea food seeds were firstly sun-dried, then placed in presterilized airtight tin containers. For testing *in vivo* preservative potential of clove essential oil and EDCT tin containers and polyethylene bags respectively were selected. These are used mainly by the farmers of this area for pea storage. About 400 g of pea food seeds were taken in 500 mL size tin containers and polyethylene bags. For the seeds, treatment with two separate doses of oil were prepared; one taking 0.50 mL (1,000 ppm) and another 0.76 mL (1,500 ppm) in airtight tin containers and polyethylene bags of 500 mL capacity containing 400 g seeds separately. Sterile cotton swabs weighing 0.50 g were soaked with the EO then wrapped in sterilized muslin cloth (0.75 g). These were kept at mid-point of each container of pea food seeds. Similarly, 400 g samples of pea food seeds were treated with EDCT 0.50 mL (1,000 ppm) or 0.76 mL (1,500 ppm) in 500 mL containers and were stored in a cabinet in the laboratory at room temperature for 6 months. Each set contained five replicates. The mycological analyses were done following agar plate technique and the standard blotter technique.

For control sets, Rachna variety of pea food seeds were dressed with requisite amount of only acetone in place of clove EO and EDCT. Both containers (tin and polyethylene) were sealed airtight. These were incubated at room temperature at 75±5% humidity. The mycological study was done after 6 months of storage. After 6 months of storage, germination tests were performed by selecting 100 seeds randomly from each test lot. These were placed aseptically in presterilized petri dishes having three layers of moistened blotting paper. At 3 day intervals,

these were regularly moistened with sterile water and incubated at 28± 2°C in a dark chamber. The germination % was assessed from the 3rd to 15th day.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

Freshly extracted (0.1 µL) of clove bud essential oil was subjected to GC and GC-MS analysis. The identification of compound was done by calculating their Kovats indices[23] mass spectra available in literature[24] and taking help of GC-MS computer database (NIST 98 and Wiley-5).

Results and Discussion

The fungal biota analysis of the pea food seeds collected irrespective of variety, at random from grocery stores of Gurgaon and Gorakhpur revealed presence of 12 fungal species (Fig. 1). During analysis, twelve species of fungi were identified by blotter method and 10 species of fungi by agar plate method viz., *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. niger* van Tieghem, *A. ochraceous* Wilhelm, *Aspergillus tmarii* Kita, *A. terreus* Thom, *Chaetomium globosum* Kunze, *Cladosporium herbarum* (Pers.) Link, *Curvularia lunata* (Wakker) Boedijn, *F. oxysporum* von Schlechtendal, *Penicillium italicum* Wehmer, *Rhizopus stolonifer* Vuillemin (Table 1). The fungal species viz., *Cladosporium herbarum* (Pers.) Link and *Penicillium italicum* Wehmer were not be found present in agar plate method of study. The blotter method test was found superior in isolation of more number of fungal species over agar plate. *A. tmarii*, *P. italicum* and *Rhizopus stolonifera* did not show its presence on sterilized seeds upon blotter method of study. *Alternaria alternata*, *A. tmarii*, *Cladosporium herbarum*, *P. italicum* and *R. stolonifer* did not show its growth in sterilized seeds in agar plate method of study (Table 1). The fungal species which shows dominance on the basis of per cent occurrence were viz., *A. flavus* 65.3±0.07, *A. niger* 60.4±0.23, *A. ochraceous* 23.0±0.16, *A. terreus* 11.0±0.16 in blotter

Fig. 1 — A look at fungi appearing on the pea food seeds.

Table 1 — Per cent frequency of isolated mycobiota from of stored seeds of pea (*Pisum sativum* L.)

Fungi recorded	Blotter method		Agar plate method	
	US	SS	US	SS
Alternaria alternata (Fr.) Keissler	2.1±0.04	1.0±0.02	3.1±0.03	-
Aspergillus flavus Link	65.3±0.07	30.1±0.04	56.0±0.16	27.9±0.05
Aspergillus niger van Tieghem	60.4±0.23	24.7±0.06	54.0±0.25	26.8±0.04
Aspergillus ochraceous Wilhelm	23.0±0.16	10.5±0.03	20.0±0.25	11.7±0.13
Aspergillus tmarii Kita	3.0±0.05	-	1.1±0.03	-
Aspergillus terreus Thom	11.0±0.16	3.4±0.10	10.6±0.23	4.6±0.15
Chaetomium globosum Kunze	4.6±0.06	2.1±0.04	5.0±0.03	0.9±0.03
Cladosporium herbarum (Pers.) Link	5.0±0.05	1.0±0.04	-	-
Curvularia lunata (Wakker) Boedijn	4.1±0.05	2.1±0.04	5.0±0.03	1.0±0.04
Fusarium oxysporum von Schlechtendal	5.1±0.11	5.0±0.23	5.0±0.31	4.7±0.23
Penicillium italicum Wehmer	2.2±0.03	-	-	-
Rhizopus stolonifer Vuillemin	1.3±0.03	-	0.2±0.02	-

-:Absence of fungal species; US: Unsterilized seeds; SS: Sterilized seeds
Values given are mean of three replicates; SD=Standard Deviation

method of study. The agar plate method showed *A. flavus* 56.0±0.16, *A. niger* 54.0±0.25, *A. ochraceous* 20.0±0.25, *A. terreus* 10.6±0.23 respectively (Table 1).

Time to time researchers have studied pea seed fungal biota and reported fungal infestations on pea seeds viz., *Ascochyta pinodes*, *Macrophomina phaseolina*, *Phoma medicaginis*, *Fusarium oxysporum*[25]; *A. flavus*, *A. niger*, *Fusarium moniliforme (Gibberella moniliformis)*, *F. roseum*, *M. phaseolina*, *Mucor globosus*, *Helminthosporium tetramera* (*Cochliobolus spicifer*) and *Rhizopus nigricans*[26]; *Fusarium* spp., *Alternaria* spp., *M. phaseolina*, *Phytophthora megasperma*, *Rhizoctonia solani and Sclerotium rolfsii*[27]; *Alternaria*, *Aspergillus*, *Rhizopus* spp.[28]; *Alternaria* spp., *Fusarium* spp., *Stemphylium* spp., *Ulocladium* spp., *Botrytis cinerea*, *Epicoccum nigrum* and *Phoma pinodella*[29]; *Phoma* spp., *Aspergillus* spp., *Fusarium* spp., *Alternaria alternata* and *Alternaria solani*[30]; *A. alternata*, *Fusarium oxysporum*, *Alternaria tenuis*, *Fusarium poae*, *A. niger*, *A. tmarii*, *Fusarium solani*, *Rhizoctonia solani*, *Acromoinum strictum and Stemphylium botryosum*, *Cladosporium herbarum*, *Chaetomium globosum*, *Fusarium equiseti* and *Sclerotinia sclerotiorum*[31]. The variation in fungal species may be due to different climatic conditions and storage periods.

The fungal species viz., C. herbarum, *P. italicum* did not grow on agar plate. This may be because of lack of fungal nutritional requirements. The number of fungal species got reduced in surface sterilized seeds that projects a high dose of the fungi were placed on seed coat. The sterile pea food seeds depicted less per cent occurrence of fungal colonies. Fungal species viz., *A. tmarii*, *P. italicum and R. stolonifer* could not grow on sterilized seeds in Blotter method of study (Table 1).

It may be mentioned that fungal flora of seed reduces its quality in terms of nutritional value and germination failure. It is evident from Table 2, *A. flavus*, *A. niger* caused maximum biodeterioration of food seeds in terms of weight loss, seed germination, carbohydrate and protein content. Germination in *A. flavus* inoculated seeds showed 49%, *A. niger* 52%, while control set without fungus showed 94-97% seed germination. The seeds inoculated with fungi showed 32.3, 28.7% carbohydrate content while control set showed 60.5%. It is evident from Table 2 that fungal inoculated pea seeds after 21 days of storage had 11.5, 10.9% protein content while control seeds had 22.5% protein content. This may be due to its potential of causing biodeterioration.

For preserving seeds, storage conditions and containers are significant. The fluctuation in fungal species occurs because of various isolation timings and even storage containers. The isolated species differ and the possible reason may be different climatic conditions. The recovery of clove bud EO through Clevenger's apparatus at 90±2°C up to 5-9 h of extraction by hydrodistillation was 2.54±0.021%. This is now well recorded that plant based EOs are an alternate of synthetic pesticides, the reason being minimal danger to consumers and adverse impact on the environment[32]. The clove EO exhibited the greatest toxicity against dominant fungi *Aspergillus flavus* and *Aspergillus niger* at 500 ppm (0.025 mL). Its MIC (clove EO) was found to be 300 ppm against four fungi Viz., *A. flavus*, *A. niger*, *A. ochraceous* and *A. terreus*. The clove EO was found to be fungicidal and thermostable at its MIC of 400 ppm.

The clove EO inhibited all 12 fungal species viz., *A. alternata*, *A. flavus*, *A. niger*, *A. ochraceous*, *A. tmarii*, *A. terreus*, *Botrytis cinerea*, *Chaetomium globosum*, *C. herbarum*, *Curvularia lunata*, *F. oxysporum*, *P. italicum*, *R. stolonifer* at 500 ppm isolated from garden pea food seeds (Table 3).

The clove EO activity was not affected by autoclaving at 15 lb/psi at 120°C. This showed fungitoxicity even after storage of oil up to 6 months.

The present investigation depicted that MIC of clove oil was found to be 300 ppm against four fungi viz., *A. flavus*, *A. niger*, *A. ochraceous* and *A. terreus* isolated from food seeds of garden pea. The previous investigations have reported a marked fluctuation in the MIC of various oils when tested against *A. niger*, 500 ppm in *Adhatoda vasica*[33], 400 ppm in *Cuminum*[23]; 400 ppm in *Tinospora cordifolia*[34]. The variation in MIC occurs in plant oils mainly because of chemical profile and physical parameters variations and time of isolation of oils.

Table 2 — Studies on potential of fungal species causing biodeterioration of garden pea food seeds after a storage period of 21 days

Fungal species	Weight loss (g)		Germination (%)		Carbohydrate (%)		Protein (%)	
	C	T	C	T	C	T	C	T
Aspergillus flavus	-	0.191	94	49	60.5	32.3	22.5	11.5
Aspergillus niger	-	0.189	97	52	60.5	28.7	22.5	10.9

C: Control; T: Treatment; - Nil

Table 3 — Fungitoxic potential of clove oil against fungi isolated from stored garden pea food seeds at various concentrations

Fungal species	Per cent inhibition of mycelial growth of isolated fungi (%)		
	500 ppm	700 ppm	900 ppm
Alternaria alternata (Fr.) Keissler	100±0.37	100±0.49	100±0.29
Aspergillus flavus Link	100±0.14	100±0.08	100±0.49
*A. niger*van Tieghem	100±0.13	100±0.07	100±0.47
A. ochraceous Wilhelm	100±0.49	100±0.24	100±0.57
Aspergillus tamari Kita	100±0.53	100±0.56	100±0.17
A. terreus Thom	100±0.39	100±0.27	100±0.38
Chaetomium globosum Kunze	100±0.47	100±0.44	100±0.48
Cladosporium herbarum (Pers.) Link	100±0.39	100±0.37	100±0.46
Curvularialunata (Wakker) Boedijn	100±0.57	100±0.37	100±0.38
F. oxysporum von Schlechtendal	100±0.23	100±0.09	100±0.17
Penicillium italicum Wehmer	100±0.48	100±0.47	100±0.51
Rhizopus stolonifer Vuillemin	100±0.39	100±0.17	100±0.47

Values given are mean of three replicates; SD=Standard Deviation

Table 4 — Fungal Biota analysis of 6 months stored garden pea food seeds treated with clove oil and EDCT (safety grains)

Fungi recorded	Clove oil		EDCT	
	US	SS	US	SS
Alternaria alternata (Fr.) Keissler	-	-	4.9±0.06	-
Aspergillus flavus Link	-	-	15.3±0.03	-
Aspergillus niger van Tieghem	-	-	16.9±0.14	4.7±0.12
Aspergillus ochraceous Wilhelm	-	-	-	-
Aspergillus tmarii Kita	-	-	9.7±0.03	4.8±0.07
Aspergillus terreus Thom	-	-	-	-
Chaetomium globosum Kunze	-	-	14.8±0.05	1.4±0.03
Cladosporium herbarum (Pers.) Link	-	-	-	-
Curvularia lunata (Wakker) Boedijn	-	-	6.2 ±0.23	1.4±0.07
Fusarium. oxysporum von Schlechtendal	-	-	-	-
Penicillium italicum Wehmer	-	-	9.3±0.02	1.2±0.04
Rhizopus stolonifer Vuillemin	-	-	8.6±0.11	1.3±0.04

-:Absence of fungal species; US: Unsterilized seeds; SS: Sterilized seeds

Values given are mean of three replicates; SD=Standard Deviation

A fungicide should show fungitoxicity even after autoclaving. Similarly, the clove oil retained fungitoxicity against isolated fungi of food seeds of garden pea even after autoclaving (15l bs/psi) as reported by various workers in plants viz., *A. vasica*[35]; *Cuminum cyminum*[23] and *T. cordifolia*[34]. A fungicide must retain its power for a long storage period. Time to time workers studied storage period for fungi toxic activity in various plant oils which was not altered even longer periods of storage in oils of *A. vasica*[35]; *C. cyminum*[23] and *T. cordifolia*[34]. The antifungal toxicity of clove EO was not affected by storage up to 6 months. The study showed that clove EO was more effective in comparison to EDCT. It is evident from Table 4, that EDCT controlled a maximum of 4 fungi while the clove EO showed complete pea food seed protection showing growth of no fungi even after 6 months of storage. It shows its potential efficacy as the Seed Protectant as it is able to protect against all 12 fungi (Table 4). Hence, it appears that clove EO increases the pea food seed's shelf life. The garden pea food seeds which served as control showed proliferation of all 12 fungal species after 6 months of storage. The present study depicted that clove EO was more fungitoxic than EDCT used in the present experiments. The antifungal property of clove EO suggests exploiting it as an ideal protectant of pea food seeds during storage.

After 6 months of storage, germination tests revealed 89-94% seed germination in clove EO treated sets, EDCT treated sets revealed 59-64% while control set revealed poor seed germination of 49-54% from seeds from both containers. The clove EO showed no adverse effect on seedling growth and general health and morphology of plants. Thus, clove EO projected great potential as protective agent for pea food seeds against spoilage by fungi during storage.

Table 5 — Chemical profile of clove essential oil

Constituent	Retention time	% area
α-Farnesene	50.002	0.98
Germacrene D	35.044	0.61
Elixene	31.724	0.48
Cubenol	30.724	0.56
Eugenyl acetate	24.923	8.34
α-Guaiene	24.025	0.72
β-Caryophyllene	21.546	12.67
Eugenol	19.789	75.63
Linalool	15.070	0.74
γ-Terpinene	14.593	0.69
Eucalyptol	13.908	0.49
α-Phellandrene	9.884	0.41
β-Pinene	8.899	0.48
β-Phellandrene	7.605	0.74
α-humulene	37.001	1.63
β-elemene	35.002	0.03
α-cadinene	7.799	0.06
Ledol	8.991	0.07

It is evident from Table 5, that clove EO contains 75.63% eugenol, 12.67% caryophyllene, 8.34% eugenyl acetate and 1.63% α-humulene as major components while others depict lower levels of percentage.

Conclusion

The present study revealed that clove bud essential oil was more fungitoxic than the tested synthetic pesticides-EDCT (safety grain). It completely protected garden pea seeds. This indicates its potential for its exploitation as seed protectant of pea food seeds for storage. Clove is an indigenous plant which grows abundantly in South India. Essential oil may be easily extracted or procured from market. This will be a source of a renewable fungi toxicant. It will be helpful in keeping the garden pea food seeds stored safely without damage for six months. This protects pea food seeds without any toxic effect on human beings and the environment.

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