



Original Research Article

Caesulia axillaris Roxb Leaf Oil-A Potential Alternate of Synthetic Pesticides for Protecting Seeds of Groundnut During Storage

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ABSTRACT

Keywords

Storage pest of groundnut, *Caesulia axillaris* oil, Synthetic fumigant

Samples of groundnut seeds were collected from stores and examined for their associated mycoflora and insects. Fifteen species of fungi and one insect-*Trogoderma granarium* were isolated from the samples. Volatile constituents extracted in the form of essential oils from higher plants were evaluated against the dominant fungi, *Aspergillus flavus* and *Aspergillus niger* as well as *Trogoderma granarium*. The oil of *Caesulia axillaris* (asteraceae) exhibited the greatest toxicity. The oil was found to be fungicidal and thermostable at its minimum inhibitory concentration (MIC) of 800ppm. The oil was characterized by the determination of its various physico-chemical properties. The oil protected the groundnut seeds completely for 6 months at 0.50 and 0.76 mL in containers of 500 mL capacity holding 400g seeds. It did not exhibit any adverse effect on seed germination, seedling growth and general health and morphology of plants. Thus, the oil of *Caesulia axillaris* showed potential as preservative for groundnut seeds against spoilage by fungi and insects during storage.

Introduction

Groundnuts are a cheap and rich source of fat, proteins and vitamins. They are grown on a large scale in almost all the tropical and subtropical countries of the world, the most important being India, China, USA and in West Africa. In India, post-harvest losses caused by the unscientific storage, insects, rodents, micro-organisms etc., account for about 10 per cent of total food grains. The major economic loss caused by grain infesting insects is not always the actual material they consume, but also the amount

contaminated by them and their excreta which make food unfit for human consumption. About 500 species of insects have been associated with stored grain products. Nearly 100 species of insect pests of stored products cause economic losses. The range of fungi found in groundnuts is large (Mc Donald, 1970; Pitt *et al.*,1993).The current study concerned storage of groundnut seeds in rural areas where poor storage practice leads to heavy deterioration caused by fungi and insects.

The fumigation plays a major role in insect pest elimination in stored products. Currently, phosphine and methyl bromide are the two common fumigants used for stored-product protection worldwide. Insect resistance to phosphine is a global issue now and control failures have been reported in field situations in some countries. Methyl bromide, a broad-spectrum fumigant, has been declared an ozone-depleting substance and therefore, is being phased out completely (Taylor,1989 and Collins *et al.*,2002). In view of the problems with the current fumigants, there is a global interest in alternative strategies including development of chemical substitutes, exploitation of controlled atmospheres and integration of physical methods (MBTOC,2002).

In order to develop endemic natural pesticide Essential oils were extracted through hydrodistillation from 32 plants and evaluated against the dominant fungi and insect species in the search for a renewable, Effectiveness of the most active oil was compared with that of synthetic fumigants.

Materials and Methods

Stored Seed collection

Samples of groundnuts that had been in storage for between 6-8 months were collected.

Twenty five places were visited for collection of stored seeds.

Storage pest analysis of groundnut seeds

The seeds were analysed for their mycoflora through agar plate (Muskett,1948) using czapek dox agar medium and standard blotter (De Tempe,1953) techniques. In agar plate technique, 100 seeds were equidistantly spread out on czapeks dox agar medium in

separate petri plates, each containing 5 seeds. In blotter test, the seeds were similarly plated on three layered moistened blotter pads in sterilized petriplates. The assay plates were then incubated at 28 ± 2 °C and observed daily upto 7 days for appearance of fungal isolates. Pure cultures of each isolates were maintained on a czapeks dox agar slants and identified.

In order to detect the internal seed mycoflora, the seeds were first surface sterilized with 0.1% sodium hypochlorite for five minutes washed with sterilized distilled water and then subjected to agar plate and standard blotter techniques for isolation of the fungi. Excess water was removed from the seed using folds of sterilized blotters. Drying the seeds in sterilized blotters before plating on agar plates helped to reduce bacterial and actinomycete contamination to a great extent. This enables superficial inoculums to be separated from the one which is deep seated (Neergaard,1977). The insects were examined by hand lens.

Fungal identifications were confirmed on the basis of colony characters and by examining the slide preparation under microscope. Keys and description given by Raper and Thom (1949), Gilman (1967), Raper and Fennell (1965), Booth (1971) and Ellis (1971,76) were followed.

Effect of storage pest on groundnut seeds Health

The fungi isolated from seeds were tested for their pathogenic nature by studying the effects of culture filtrates on 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 whatman no-1 filter paper and the filterates were used to assay the toxin produced by assessing the percentage inhibition of seed germination and mortality of groundnut.

Freshly harvested surface sterilized (0.1% sodium hypochlorite solution) and washed (sterilized water) seeds were soaked separately for 2 hours in 100ml of each culture filtrate of corresponding groundnut seed fungi in four replication of 25 seeds each. 25 treated seeds were placed in sterilized petridish containing three layers of moist blotters. The number of seeds germinated after 5 days interval for upto 20 days was observed and the final percentage of germination and mortality was recorded till there was no further germination. The controls were maintained by sowing surface sterilized seeds in sterilized blotters.

The deterioration caused by insect was evaluated.

Extraction of essential oils from higher plants and evaluation of their toxicity against test fungi and insect

The plant parts of 32 higher plants collected were surface sterilized by dipping in 70% ethanol and then washed repeatedly with sterilized double distilled water. The surface sterilized leaves were macerated and hydrodistilled for isolation of volatile constituents separately for 6 hour in clevengers apparatus. After hydrodistillation immiscible oil was separated and dehydrated over anhydrous sodium sulphate separately to remove traces of moisture.

The toxicity of oil was assessed by using the inverted petri plate technique of Bocher(1938) at 0.050 ml oil(1000 ppm) of each plant against dominant fungi. The fungitoxicity of essential oils was measured

following Dixit *et al*(1978) and recorded in terms of per cent inhibition of mycelial growth.

The repellent activity of the oils against insect was studied following the method of Tripathi and Kumar(2007). Different amounts(0.01,0.02 and 0.03 ml) of the oil from each plant were applied separately into test sponge pieces and the test pieces were placed in one of the arms of Y tube olfactometer. Water soaked sponge pieces were placed in the other arms as controls. Twenty newly emerged adults of *Trogoderma granarium* obtained from a culture maintained in the laboratory were introduced into the basal arm of the Y olfactometer in 4 batches at interval of 5min to avoid mutual interference, if any. To compensate for possible minor asymmetry in the construction of olfactometer (made locally of corning tube) or in the experimental condition, the position of the test material(oil) and control(water) in the arms were alternated. The number of individuals in each arm were counted at the end of the test(after 30 min). The experiment was repeated five times for each set of tests.

Physico-chemical properties of *Caesulia axillaris* oil

The oil was characterized by determination of its specific gravity, specific rotation, refractive index, acid value, saponification number, ester number, phenolic content and solubility following the methods of Langenau(1948).

Fungitoxic properties of *Caesulia axillaris* oil

The MIC of most active oil was determined by poisoned food technique of Grover and Moore(1962). Different concentration of the oil ranging from 500 to 1000ppm were

prepared by dissolving requisite amount of oil in 0.5ml acetone and then mixing with 9.5ml czapeks dox agar medium separately. In control sets the petriplates having acetone and medium without oil were used. Fungal discs (5mm diam) obtained from periphery of seven d old culture of each of test 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 d and the average was used to calculate the percent inhibition of mycelia growth of test fungi separately. 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 fungitoxic spectrum of the oil was studied against various fungi isolated from groundnut seed samples. In addition 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 5 replicates.

Comparison of treatment with *Caesulia axillaris* oil and fumigation with synthetic fumigant-aluminium phosphide and ethylene dibromide

Fresh dried groundnut seeds were locally collected in presterilized polyethylene bags.

Aliquots of 0.50ml (1000ppm) and 0.76 ml (1500ppm) of oil and ethylene dibromide were used separately with 400g of freshly dried groundnut seeds in presterilized gunny bags of 500 ml capacity. Likewise samples of groundnut to be treated with oil or ethylene dibromide were stored separately in metal containers (tins) of 500 ml capacity.

Sterile cotton swabs (0.35g), soaked with doses of oil or ethylene dibromide and wrapped in sterilized muslin cloth (0.75g) were placed at the bottom of each container of groundnut seed. Similarly, 400g samples of groundnut were treated with phosphine from a 0.50 (1000ppm) or 0.76g (1500ppm) of tablet (160 and 240 mg equivalent phosphine) in 500ml containers and were stored in a cabinet in the Laboratory at room temperature for 6 months. Each set contained 5 replicates. Mycoflora associated with groundnut were then isolated by the agar plate technique of Muskett (1948) and the standard blotter technique of Tempe (1953). The insects were examined by hand lens.

After 6 months storage, 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. The blotting papers were moistened with sterilized water at 2 day intervals. 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 9th day.

One hundred seed from each treatment and control sets were sown in 15x20cm 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.

Results

Storage pests

The most frequent genera were *Aspergillus* represented by seven species followed by

Fusarium (represented by three species). Highest percentage occurrence 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 occurrence 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 (Table 1) .

Twelve fungal species belonging to six genera were detected from unsterilized seeds plated over CDA medium. The most dominant genera were *Aspergillus* (represented by five species) followed by *Fusarium* (three species) and *Penicillium glabrum*. Highest percentage occurrence 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*, *Trichothecium 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. The blotter method seems to be superior to agar plate method.

During insect analysis only one insect-*Trogoderma granarium* was found to be present in all 25 collected samples .

Insects Damage symptoms

Damage distinctive, both adult and larvae fed on the inside of seeds. Feeding caused tiny, dot-like entrance holes. The feeding also caused larger, round exit holes with a diameter of 2.0 mm and excavated seed. Large populations reduced stored seed to little more than dust.

Storage pest deterioration of groundnut seed

The metabolites of most of the test fungi showed inhibitory effects on germination. The rating of fungi on the basis of 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.tamarii*, *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. The insect-*Trogoderma granarium* caused 20% germination and 80% mortality. It is evident from table 2, that *A.niger* and *A.flavus* and insect- *Trichoderma viride* caused high degree of mortality and reduction in germination

Description of active plant-*Caesulia axillaris* Roxb

Plant is prostrate or decumbent life form and develops adventitious roots at the base of

stems. Stem is hollow and glabrous. Leaves with alternate arrangement, linear lanceolate and pointed, 4 to 14cm long, 4 to 9 mm broad. The margin is remotely dentate: tapering at both ends, multicostate. The base is dilated enclosing the compound heads. Flowers bluish grouped in axillary sessile round heads, 4 to 14 mm in diameter. Flowers hermaphrodite, tubular, limb narrowly campanulate, deeply 5-fid, bracts two, opposite, enclosing the flower, adnate to and enclosing the achene. Achene in the laterally compressed bracts and without pappus. Compressed ribs prominent on sides, 2 to 3mm long, 1.4 mm broad.

Evaluation of essential oils against test organisms

The essential oil of *Caesulia axillaris* exhibited absolute toxicity at 1000ppm inhibiting mycelial growth of both test fungi completely, while other oils at these concentrations showed moderate, lower level of fungi toxicity. The leaf oil showed 100% repellency against test insect *Trogoderma granarium* with a dose of 0.03ml. Other oils at this concentration showed moderate or lower level of repellency.

The physicochemical properties of the *Caesulia axillaris* oil are recorded in Table 3.

Fungitoxic properties of *Caesulia axillaris* oil

The MIC of the oil was found to be 800ppm against both the test fungi. The oil exhibited fungicidal nature at MIC against both the test fungi (Table 4). The *Caesulia axillaris* oil completely inhibited the mycelial growth of 10 fungi at 800ppm (Table 5).

The oil its MIC (800 ppm) was able to inhibit the growth of all 10 discs (each of 5mm diam) as well as growth of single mycelia discs of 11mm diam, the maximum considered in this study. Thus fungitoxic potential of oil appeared to be retained heavy inoculum density. The highest temp (100C), autoclaving and storage upto 180 days, did not affect the toxicity of the oil against the test fungi and insect (Table 6).

Preservation of seeds by *Caesulia axillaris* oil and fumigants during storage

As evident from control sets in Table 7, the groundnut seeds were associated with 15 fungal species viz. *Alternaria alternata*, *Aspergillus candidus*, *A.flavus*, *A.niger*, *A.phoenicis*, *A.tamaritii*, *A.terreus*, *A.sydowi*, *Fusarium moniliforme*, *F.oxysporum*, *F.solani*, *P.glabrum*, *Rhizopus nigricans*, *Trichoderma viride*, *Trichothecium roseum* in both containers.

Trogoderma granarium was present in gunny bags but absent in sealed metal containers.

Seeds treated with oil were not associated with fungi or insects in either container. Phosphine was ineffective in control of the fungal species or *Trogoderma granarium* at an 160 mg dose in both containers. At 240 mg, it was effective. Ethylene dibromide at 0.50 and 0.76 ml was ineffective.

With respect to germination capacity, the oil treated seeds showed 80-90%, phosphine 70-75% and ethylene dibromide 55-65% germination. The seeds of control set, however, exhibited only 45-50% seed germination (Table 8). The oil had no adverse effect on seed germination, seedling growth and general health of plants when compared with control and synthetic fumigants.

Several other fungal species were isolated by different workers from groundnut seeds viz., *Aspergillus candidus*, *A.chevalieri* and *A.ruber* (Mukherjee *et al.*,1992);*Mucor sp* (Swamy and Shambulingappa,1994); *Fusarium moniliforme*, *F.pallidoroseum*, *F.solani*,*Microsporium phaseolina* and *Verticillium alboatrum* (Javed *et al.*,1987)but in present investigation 15 fungal species viz. *Alternaria alternata*, *Aspergillus candidus*, *A.flavus*, *A.niger*, *A.phoenicis*, *A.tamaritii*,*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.

In present investigation the MIC of *Caesulia axillaris* oil was found to be 800ppm against both *Aspergillus niger* and *A.flavus*. The previous literature revealed that there is a marked variation in the MIC of different plant oils against *Aspergillus niger*-thus *Ocimum adscendens* Willd 200ppm (Asthana and Singh, 1981), *Cymbopogon flexuosus* (Steud.) Wats 400ppm (Dixit,1991), *Syzygium aromaticum* (L.) Merrill and Perry 200ppm (Khan,1993), *Cedrus deodara* (Roxb.ex Lambert) G.Don 1000 ppm and *Trachyspermum ammi* (L.) Sprague 500ppm(Singh and Tripathi,1999); *Putranjiva roxburghii* Wall 400ppm (Tripathi and Kumar,2007).The variation in the MIC of different plant oils may be due to the presence of different chemical constituents.

Table.1 Percent occurrence of fungi on the food seeds of groundnut

Fungi recorded	Moist blotter method		Czapeks dox agar method	
	US	SS	US	SS
<i>Alternaria alternate</i> (Fr.)Keissler	2.4	1.2	3.2	-
<i>Aspergillus candidus</i> Pers ex.	2.1	-	3.3	-
<i>A.flavus</i> Link	8.1	3.9	19.9	6.6
<i>A.niger</i> van Tieghem	3.7	2.5	14.1	3.5
<i>A.phoenicis</i> Link	1.2	-	-	-
<i>A.tamaritii</i> Kita	1.3	-	3.2	-
<i>A.terreus</i> Thom	1.3	-	-	-
<i>A.sydowi</i> (Bainier and Sartory) Thom and Church	2.4	1.0	5.0	1.0
<i>Fusarium moniliforme</i> Sheldon	8.1	1.2	3.0	-
<i>F.oxysporum</i> von Schlechtendal	6.3	1.4	6.3	3.1
<i>F.solani</i> (Mart.)Sacc.	5.4	2.5	3.2	3.6
<i>Penicillium glabrum</i> (Wehmer) Westling	4.1	-	11.2	-
<i>Rhizopus nigricans</i> Ehr.	2.3	-	-	-
<i>Trichoderma viride</i> Pers.ex.Fr.	2.1	-	1.3	-
<i>Trichothecium roseum</i> (Persoon) Link ex	1.2	-	3.1	-

Insect –*Trogoderma granarium*

Table.2 Effect of isolated fungi and insect- *Trogoderma granarium* on seed germination and seedling mortality of groundnut

Fungal species	Percent germination	Percent mortality
<i>Alternaria alternata</i>	65.5	34.5
<i>Aspergillus candidus</i>	65.6	34.4
<i>A.flavus</i>	24.2	75.8
<i>A.niger</i>	6.0	94.0
<i>A.phoenicis</i>	58.6	41.4
<i>A.tamarii</i>	40.2	59.8
<i>A.terreus</i>	40.6	59.4
<i>A.sydowi</i>	89.4	10.6
<i>Fusarium moniliforme</i>	49.5	50.5
<i>F.oxysporum</i>	35.4	64.6
<i>F.solani</i>	61.4	38.6
<i>P.glabrum</i>	65.9	34.1
<i>Rhizopus nigricans</i>	66.4	33.6
<i>Trichoderma viride</i>	85.3	14.7
<i>Trichothecium roseum</i>	67.4	32.6
Sterilized distilled water(control)	84.3	15.7
Insect- <i>Trogoderma granarium</i>	20.0	80.0

Table.3 Physicochemical properties of *Caesulia axillaris* oil

Parameters	Values
Specific gravity	0.901
Specific rotation	+10
Refractive index	1.385
Acid value	3.40
Saponification number	153.41
Ester number	150.01
Phenolic content	Nil
Solubility	Completely miscible with petroleum ether acetone and 90% ethanol in 1;1ratio but insoluble in water

Table.4 MIC of *Caesulia axillaris* oil

Dose of oil in ppm	<i>Aspergillus niger</i>	<i>A.flavus</i>
400	30	40
600	70	80
800	100*	100*
1000	100	100
1200	100	100

*Fungicidal

Table.5 Fungitoxic spectrum of *Caesulia axillaris* oil at sub lethal, lethal and hyperlethal doses

Fungal species	Per cent inhibition of mycelial growth of isolated fungi			
	Sublethal 600ppm	Lethal 800ppm	Hyperlethal 1000ppm	Hyperlethal 1100ppm
<i>Alternaria alternata</i>	45.6	80.0	100.0	100.0
<i>Aspergillus candidus</i>	49.6	89.0	100.0	100.0
<i>A.flavus</i>	50.0	100.0	100.0	100.0
<i>A.niger</i>	30.0	100.0	100.0	100.0
<i>A.phoenicis</i>	40.0	100.0	100.0	100.0
<i>A.tamarii</i>	48.0	100.0	100.0	100.0
<i>A.terreus</i>	59.0	100.0	100.0	100.0
<i>A.sydowi</i>	55.6	100.0	100.0	100.0
<i>Fusarium moniliforme</i>	40.0	100.0	100.0	100.0
<i>F.oxysporum</i>	42.0	79.6	100.0	100.0
<i>F.solani</i>	40.0	100.0	100.0	100.0
<i>P.glabrum</i>	59.0	100.0	100.0	100.0
<i>Rhizopus nigricans</i>	54.0	100.0	100.0	100.0
<i>Trichoderma viride</i>	55.0	80.0	90.0	90.0
<i>Trichothecium roseum</i>	65.9	95.0	100.0	100.0

Table.6 Effect of physical factors on the fungitoxicity of *Caesulia axillaris* oil

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

Table.7 Mycoflora of 400g seed of groundnut treated with *Caesulia axillaris* oil, Phosphine and ethylene dibromide after 6 months of storage in 500ml containers

Fungal species	control		treatment																								
			Caesulia 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	C	T	C	T	C	T	C	T	C	T									
<i>Alternaria alternata</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>Aspergillus candidus</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>A.flavus</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
<i>A.niger</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>A.phoenicis</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>A.tamaraii</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
<i>A.terreus</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>A.sydowi</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>Fusarium moniliforme</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>F.oxysporum</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>F.solani</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>P.glabrum</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>Rhizopus nigricans</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>Trichoderma viride</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>Trichothecium roseum</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+

Storage system;G-gunny bags;T-tin containers
 Detection method;A-agar plate technique;B-blotter technique
 +;presence of fungi;-absence of fungi

Table.8 Germination of groundnut treated with *Caesulia axillaris* oil,phosphine and ethylene dibromide after 6 months storage of 200g samples in 250ml containers

Period(days)	Germination%													
	control		Caesulia 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

According to Wellman(1967) a fungicide must retain its fungi toxicity at the extreme of temperatures. The fungi toxicity of leaf oil of *Adhatoda vasica* was found to be thermostable up to 100C like *Ageratum conyzoides*(Dixit *et al.*,1995); *Nardostachys jatamansi* (Mishra *et al.*,1995); *Putranjiva roxburghii* (Tripathi and Kumar,2007).The caesulia oil retained its fungi toxicity on autoclaving(15lbs/square inch pressure).This quality of oil will facilitate the isolation of their constituents in active state.

A fungicide should be able to retain its activity during long period of its storage(Wellman,1967).The fungitoxic factor in the oil of *Adenocalyma allicea* was lost within 21 d of storage (Chaturvedi,1979) while persisted for long period in the oil of *Ageratum conyzoides* (Dixit *et al.*,1995) ; *Trachyspermum ammi* (Singh and Tripathi,1999)and *Putranjiva roxburghii* (Tripathi and Kumar,2007).

The fungal toxicity was not affected by storage upto 180 days during present investigation. So this show that the *Caesulia axillaris* oil can be safely stored at any ambient temperature for long periods without loss in toxicity.

Thus *Caesulia axillaris* oil shows potential as a potent fumigant preservative for the management of post harvest infestation of seeds of groundnut on the basis of its strong fungal toxicity at low MIC, insect repellency and long shelf life.

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