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ADHATODA VASICA LEAF OIL-A POTENTIAL FUMIGANT PRESERVATIVE FOR GROUNDNUT DURING STORAGE

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Abstract

Samples of groundnut seeds were collected from stores in Eastern Uttar Pradesh,India 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 32 plant species were evaluated against the dominant fungi,Aspergillus flavus and Aspergillus niger as well as Trogoderma granarium.The oil of Adhatoda vasica exhibited the greatest toxicity. The oil was found to be fungicidal and thermostable at its minimum inhibitory concentration(MIC) of 400ppm.The oil was characterized by the determination of its various physico-chemical properties.The oil protected the groundnut seeds completely for 6 months at 0.25 and 0.38 mL in containers of 250 mL capacity holding 200g seeds.It did not exhibit any adverse effect on seed germination,seedling growth and general health and morphology of plants.Thus,the oil of Adhatoda vasica showed potential as preservative for groundnut seeds against spoilage by fungi and insects during storage.

***Key Words**; Storage pest of groundnut,Adhatoda vasica Nees oil,synthetic fumigant*

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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.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.

Rajendran(2002) highlighted that stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses .Taylor(1989)and Collins *et al*(2002) reported that 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 worldover.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. Inview 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). The interest has been shown in plant products, i.e., essential oils for fumigant action since it is believed that natural compounds from plant sources may have the advantage over conventional fumigants in terms of low mammalian toxicity , rapid degradation and local availability.

No systematic work has been done on post harvest storage pest of groundnut seed and less attention has been focused on plant products showing fumigant action against storage pest . In present investigation storage pest of stored seeds were studied. Essential oils were isolated from 32 plants and evaluated against the dominant fungi and insect species in the search for a renewable,natural protectants for groundnut seeds.Effectiveness of the most active oil was compared with that of synthetic fumigant aluminium phosphide and ethylene dibromide in terms of its effect on the mycoflora,seed germination,seedling growth and general health and morphology of groundnut after 6 months of storage. The comparative *in-vivo* efficacy of most potent oil and aluminium phosphide and ethylene dibromide at 1000ppm and 1500ppm were compared.

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.

Collection Place

Basti district-Ganeshpur,Kalwari,Makhauda,Chhawni Bazar,Walterganj

in Santkabir nagar district-Baghnagar,Mehdaul,Matiuli,Alinagar,Gagargarh,

in Siddhartha nagar district-Bansi,Itwa,Chandapar,Chilia,Birdpur

inGorakhpur district-Brhalganj,Golabazar,Kauriram,Kusmi,Pali

in Maharajganj district-Nautanwa,Sanduriya,Khucha,Paniyara,Nichlaul

Storage pest analysis of collected and stored 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,100seeds were equidistantly spread out on czapeks dox agar medium in separate petri plates,each containing 5 seeds.In blotter test,the seeds were similarily 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 hpochlorite 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 extant.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

The fungi isolated from seeds were tested for their pathogenic nature by studying the effects of culture filterates on seed germination and mortality.The fungal species were cultured in czapeks solutions for 15 days at28 ± 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 filterate 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 following Kumar and Tripathi(2004).

Isolation 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).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(0.005,0.01 and 0.02 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 *Adhatoda vasica* leaf 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 *Adhatoda vasica* leaf 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 200 to 600ppm were prepared by dissolving requsite amount of oil in 0.5ml acetone and then mixing with 9.5ml czapeks dox agar medium seperately.In control sets the petriplates having acetone and medium without oil were used.Fungal discs(5mm diam) abtained 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+-2C 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 *Adhatoda vasica* leaf oil and fumigation with synthetic fumigant-aluminium phosphide and ethylene dibromide

Fresh dried groundnut seeds were locally collected in presterilized polyethylene bags.Aliquots of 0.25ml(1000ppm) and 0.38 ml(1500ppm) of oil and ethylene dibromide were used separately with 200g of freshly dried groundnut seeds in presterilized gunny bags of 250ml capacity.Likewise samples of groundnut to be treated with oil or ethylene dibromide were stored separately in metal containers(tins)of 250ml capacity.

Sterile cotton swabs(0.25g),soaked with doses of oil or ethylene dibromide and wrapped in sterilized muslin cloth (0.50g) were placed at the bottom of each container of groundnut seed.Similarily,200g samples of groundnut were treated with phosphine from a 0.25(1000ppm) or 0.38g (1500ppm)of tablet(80 and 120mg equivalent phosphine) in 250ml 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 incidence 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 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.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 reducd stored sissoo 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,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.The insect-*Trogoderma granarium* caused 20%germination and 80% mortality.It is evident from table 2, that *A.niger* and *A.flavus* and insect-*Bruchus pisorum* caused high degree of mortality and reduction in germination.

Evaluation of essential oils against test organisms

The essential oil of *Adhatoda vasica* exhibited absolute toxicity at 500ppm inhibiting mycelial growth of both test fungi completely,while other oils at these concentrations showed moderate,lower level of fungitoxicity(Table 3).The leafl oil showed 100%repellency against test insect *Trogoderma granarium* with a dose of 0.02ml.Other oils at this concentration showed moderate or lower level of repellency(Table4).

The physicochemical properties of the *Adhatoda vasica* leaf oil are recorded in Table5.

Fungitoxic properties of *Adhatoda vasica* leaf oil

The MIC of the oil was found to be 400ppm against both the test fungi.The oil exhibited fungicidal nature at hyper MIC against both the test fungi(Table 6)while it was fungicidal in nature at 500ppm.The *Adhatoda vasica* leaf oil completely inhibited the mycelial growth of 10 fungi at 400ppm(Table7).

The oil its MIC(400ppm) 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 inoculums 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 8).

Preservation of seeds by *Adhatoda vasica* leaf oil and fumigants during storage

As evident from control sets in Table9, the groundnut seeds were associated with 15 fungal speciesviz.*Alternariaalternata,Aspergilluscandidus,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.

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 80mg dose in both containers.At 120mg ,it was effective.Ethylene dibromide at 0.25 and 0.38ml was ineffective.

With respect to germination capacity,the oil treated seeds showed 80-90%,phosphine70-75% and ethylene dibromide55-65%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.

DISCUSSION

Several other fungal species were isolated by different workers from groundnut seeds viz.,*Aspergillus candidus,A.chevalieri and A.ruber*(Mukherjee *at al.*,1992);*Mucor sp*(Swamy and Shambulingappa,1994);*Fusarium moniliforme,F.pallidoroseum,F.solani,Microsporum 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.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.

Rajak *et al*(1992) studied post harvest mycoflora of some forest trees of Madhya Pradesh and found blotter method to be the best as it yielded maximum number of fungi in comparison to agar plate method.Similarily in present investigation blotter method yielded 15 fungi and agar plate method yielded 12 fungi.

The review of literature reveals that seed germination has been affected by fungal infections and caused mortality in shisham.Vigayan and Rehill(1990) and Pathan *et al*(2007) reported that *Aspergillus flavus,A.niger,F.oxysporum* has inhibitory effect on seed germination of shisham seeds.Rajput *et al* (2010) recorded 50%germination and 93.3% mortality when infested with *F.solani*.In present investigation *A.niger,A.flavus* and insect-*Trogoderma granarium* caused significant reduction in germination and mortality of groundnut seeds.

In present investigation the MIC of *Adhatoda vasica* leaf oil was found to be 400ppm 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 1000ppm 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.

According to Wellman(1967) a fungicide must retain its fungitoxicity at the extreme of temperatures.The fungitoxicity 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*ppm(Tripathi and Kumar,2007).The leaf oil retained its fungitoxicity 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*ppm(Tripathi and Kumar,2007).. The fungal toxicity was not affected by storage upto 180 days during present investigation.So this show that the *Adhatoda vasica* leaf oil can be safely stored at any ambient temperature for long periods without loss in toxicity.

CONCLUSION

Thus *Adhatoda vasica* leaf 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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Table1.Percent incidence of different fungi on the seeds of groundnut

Fungi recorded	Moist blotter method		Czapeks dox agar method	
	US	SS	US	SS
Alternaria alternate(Fr.)Keissler	2.4	1.2	3.2	-
Aspergillus candidus 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
Fusarium moniliforme 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
Penicillium glabrum(Wehmer) Westling	4.1	-	11.2	-
Rhizopus nigricans Ehr.	2.3	-	-	-
Trichoderma viride Pers.ex.Fr.	2.1	-	1.3	-
Trichothecium roseum(Persoon) Link ex	1.2	-	3.1	-

Insect –*Trogoderma granarium*

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

Fungal species	Percent germination	Percent mortality
Alternaria alternata	65.5	34.5
Aspergillus candidus	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
Fusarium moniliforme	49.5	50.5
F.oxysporum	35.4	64.6
F.solani	61.4	38.6
P.glabrum	65.9	34.1
Rhizopus nigricans	66.4	33.6
Trichoderma viride	85.3	14.7
Trichothecium roseum	67.4	32.6
Sterilized distilled water(control)	84.3	15.7
Insect- *Trogoderma granarium*	20.0	80.0

Table 3.Evaluation of essential oils of higher plants against *Aspergillus niger* and *A. flavus*

Plant species	Per cent inhibition of mycelia growth of test fungi at 500ppm		
	Family	*Aspergillus niger*	*A.flavus*
Adhatoda vasica Nees	Acanthaceae	100.00*	100.00*
Ageratum conyzoides L.	Asteraceae	76.5	64.2
A. houstonianum	Asteraceae	82.5	80.5
Anetum graveolens L.	Umbelliferae	39.0	33.0
Anisomeles ovate R.Br.	Lamiaceae	64.3	60.3
Artabotrys hexpetalous(Lamm)Merr.	Annonaceae	53.2	46.7
Azadirachta indica A. Juss.	Meliaceae	43.1	38.7
Caesulia oxillaris Roxb.	Asteraceae	49.1	47.1
Callestemon lanceolatus DC	Myrtaceae	38.3	48.2
Cannabis sativa L.	Cannabinaceae	12.0	9.5
Cinnamomum tamla Nees and Bbrem	Lauraceae	39.0	23.0
Citrus aurantifolia Christm	Rutaceae	38.2	29.3
*C.medica var limonia(*L.)	Rutaceae	47.9	59.3
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
Feronia elephantum Correa	Rutaceae	49.7	60.3
*F.limonia(*L.) Swingle	Rutaceae	50.8	65.4
*Hyptis suaveolens(*L.) Poit	Lamiaceae	47.2	27.4
Lantana camera L.	Verbenaceae	58.3	39.1
L.indica Roxb.	Verbenaceae	55.7	40.0
Mentha arvensis L.	Lamiaceae	53.9	38.6
M.piperata L.	Lamiaceae	63.3	50.3
M.spicata L.	Lamiaceae	60.3	48.2
Murraya koenighii(L.)Spreng	Rutaceae	25.8	40.1
Ocimum adscendens 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
Putranjiva roxburghii Wall	Euphorbiaceae	90	95
Tagetes erecta L.	Asteraceae	44.0	30.7
Thuja occidentalis L.	Cuppressaceae	24.0	46.3

Table 4.Insect repellent activity of essential oils of some plants

Plant species	Family	%repellency against *Trogoderma granarium* at following amounts of oil(ml)		
		0.005	0.01	0.02
Adhatoda vasica Nees	Acanthaceae	60	75	100*
Ageratum conyzoides L.	Asteraceae	30	40	50
B. houstonianum	Asteraceae	35	60	70
Anetum graveolens L.	Umbelliferae	40	60	75
Anisomeles ovate R.Br.	Lamiaceae	30	55	65
Artabotrys hexpetalous(Lamm)Merr.	Annonaceae	35	40	50
Azadirachta indica A. Juss.	Meliaceae	35	60	70
Caesulia oxillaris Roxb.	Asteraceae	40	55	65
Callestemon lanceolatus DC	Myrtaceae	30	40	50
Cannabis sativa L.	Cannabinaceae	15	20	30
Cinnamomum tamla Nees and Bbrem	Lauraceae	30	50	60
Citrus aurantifolia Christm	Rutaceae	30	40	50
*C.medica var limonia(*L.)	Rutaceae	30	50	60
Eucalyptus citriodora Hook	Myrtaceae	40	50	60
E.globulus(l.) Herit	Myrtaceae	45	55	65
Eupatorium capillifolum (L.)	Asteraceae	30	40	50
Feronia elephantum Correa	Rutaceae	35	60	70
*F.limonia(*L.) Swingle	Rutaceae	40	50	60
*Hyptis suaveolens(*L.) Poit	Lamiaceae	30	45	60
Lantana camera L.	Verbenaceae	25	35	45
L.indica Roxb.	Verbenaceae	30	40	50
Mentha arvensis L.	Lamiaceae	20	35	45
M.piperata L.	Lamiaceae	25	35	50
M.spicata L.	Lamiaceae	25	30	50
Murraya koenighii(L.)Spreng	Rutaceae	10	20	30
Ocimum adscendens Willd	Lamiaceae	30	40	50
O.basilicum L.	Lamiaceae	30	35	45
O.canum Sims	Lamiaceae	30	45	60
O.sanctum L.	Lamiaceae	35	55	65
Putranjiva roxburghii Wall	Euphorbiaceae	60	75	95
Tagetes erecta L.	Asteraceae	40	70	80

Thuja occidentalis L.	Cuppressaceae	35	45	50

Table 5.Physicochemical properties of *Adhatoda vasica* Nees oil

arameters	**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 ether acetone and 90%ethanol in 1;1ratio but insoluble in water

Table 6. MIC of *Adhatoda vasica* Nees oil

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

*Fungicidal

Table 7.Fungitoxic spectrum of *Adhatoda vasica* Nees oil at sub lethal, lethal and hyperlethal doses

Fungal species	**Per cent inhibition of mycelial growth of isolated fungi**			
	Sublethal 200ppm	**Lethal 400ppm**	**Hyperlethal 600ppm**	**Hyperlethal 800ppm**
Alternaria alternata	45.6	80.0	100.0	100.0
Aspergillus candidus	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
Fusarium moniliforme	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
Rhizopus nigricans	54.0	100.0	100.0	100.0
Trichoderma viride	55.0	80.0	90.0	100.0
Trichothecium roseum	65.9	95.0	100.0	100.0

Table 8.Effect of physical factors on the fungitoxicity of *Adhatoda vasica* Nees 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 9. Mycoflora of 200g seed of groundnut treated with *Adhatoda vasica* Nees oil, Phosphine and ethylene dibromide after 6 months of storage in 250ml containers

Fungal species	control				treatment																							
					Adhatoda oil								Phosphine(mg)								Ethylene dibromide(ml)							
					0.25				0.38				80				120				0.25				0.38			
	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
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;G-gunny bags;T-tin containers
Detection method;A-agar plate technique;B-blotter tehnique
+;presence of fungi;-absence of fungi

Table 10. Germination of groundnut treated with *Adhatoda* oil,phosphine and ethylene dibromide after 6 months storage of 200g samples in 250ml containers

Period(days)	Germination%													
	control		*Adhatoda* oil				Phosphine(mg)				Ethylene dibromide(ml)			
			0.25		0.38		80		120		0.25		0.38	
	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