

ANTIMICROBIAL AND PESTICIDAL POTENTIALITY OF ESSENTIAL OILS OF SOME MEDICINAL PLANTS AGAINST DETERIORATION OF CUMIN SEEDS

El-Shoraky, Fathia S.* and Nahed M. M. Rashed**

* Institute of Plant Pathology, Agric. Res. Center, Giza, Egypt.

**Dept. of Medicinal and Aromatic Plants, Agric. Res. Center, Dokki, Giza, Egypt.

ABSTRACT

This experiment was carried out at Sakha Agricultural Research Station from June 2011 to May 2012 to study the problem of reduction of cumin (*Cuminum cyminum*) seed germination. Cumin seeds were obtained from different sources for conducting the study. Several microbial species including sixteen fungal taxa, some yeasts and bacteria, were isolated from tested cumin seeds. The results exhibited suppressive of *Fusarium* spp. and *Aspergillus* in seed borne fungi. The most storage fungi which attack stored seeds led to decrease the germination percent. Ten essential oils plants (peppermint, eucalyptus, rosemary, nigella, caraway, thyme, marjoram, clove, double jasmine and celery) were used as seed coating materials in two forms, one as commercial and the other was as extraction. All oils used increased germination percent as comparing with vitavax except caraway and clove oils, in addition nigella oil proved to be the highest germination % (42.88%) comparing with 38.33% for the control. At the same time, caraway oil gave the lowest mean percent of germination (4.44%). The treated cumin seeds stored for one year maintaining it in petri dishes under room conditions and examination of seeds associated fungi and bacteria and germination after an equal period. Generally, germination percent increased with increasing the period of coating seeds with essential oils. All oils significantly reduced the percent of fungi associated the seeds especially *Fusarium* and *Aspergillus*. At the end of the storage period some of treatments were deteriorated and the other were observed at the healthy shape (seeds coating with caraway, celery, eucalyptus, double jasmine, thyme and rosemary oils). Oils of nigella; peppermint and eucalyptus inhibited *Fusarium* percent (100% reduction), while caraway oil inhibited *Aspergillus* (100% reduction). The bacterial infection inhibited by marjoram; peppermint; nigella and clove. The deterioration by insects suppressed by caway; celery; eucalyptus and jasmine treatments. The fungicidal and insecticidal effect of caway may occurs from the highly percent of carvon (61.58%) and limonene (29.11%). While, the bactericidal and insecticidal effect of marjoram oil may come from α -terpineol (35.4%). This work gives highlights on the potential of using essential oils as seed coating before storage for the pest managements.

Keywords: Cumin (*Cuminum cyminum*); essential oils; funicidal; bactericidal and insecticidal

INTRODUCTION

Cumin *Cuminum cyminum* L. belongs to the family Apiaceae and is considered as annual herbal crop native of Egypt. It is extensively cultivated as a winter season crop. The plants are annual or perennial herbs and cultivated in different parts of the world for the recovery of their essential oil (Nasir & Ali, 1972). It is mainly cultivated in India, Egypt, Libya, Iran, Pakistan

and Mexico (Peter and Nybe 2002). Cumin oil is employed advantageously in many types of flavouring preparations particularly in curries and culinary preparations of oriental type. It is also used to an extent in soap perfumery and in flavoring beverages. Cumin aldehyde has a powerful odour and is used only in traces in compounding synthetic floral perfumes such as cassie. Cumin seeds are largely used as a condiment or spice in curries and pickles etc. Its seeds contain 3-4% volatile oil and about 15% fixed oil. Egyptian cumin oil contains 39.2% cumin aldehyde (Srinivas,1986).The cumin oil, shows antifungal activity, which could be linked to the cumin aldehyde content (Lawrence,1992). Cumin is an important medicinal and aromatic seeds in Egypt, and the world. It is used to flavor foods, for medical preparations, food industries and added to fragrances (Iacobellis, *et al.*, 2005).

Recently, the importance of aromatic plants is considerable due to their applications in folk medicine and their potential for commercial exploitations, which are used as aroma and flavor enhancers, cosmetics and in pharmaceuticals. Cumin (*Cuminum cyminum*), one of the most important medicinal and aromatic seeds in Egypt, and the world. It is used to flavor foods, for medical preparations, food industries and added to fragrances (Iacobellis, *et al.*, 2005). Following harvest and during storage, they are subjected to attack and damage by numerous fungi, i.e. *Aspergillus* spp., *Penicillium* spp. *Rhizopus* spp. and *Fusarium* spp. (Moharram *et al.*, 1989 and Regina and Roman, 1992). The incidence of infection and severity of damage by these fungi depend on storage temperature, seed moisture content, relative humidity, fungal species and their counts present at preharvest and mechanical damage of flowers (Prasad *et al.*, 1988 and Regina & Roman, 1992). Most storage fungi which attack stored medicinal and aromatic seeds produce toxins that cause food and feed hazards. (Campbell & Stoloff, 1974; El-Bazza *et al.*, 1996; Abou-Zeid *et al.*, 1997 and Ragab & El-Syied, 1998).

In comparison to synthetic compounds, the pesticidal compounds of plant origin are more effective and have little or no side effects on human beings (Kumar *et. al.* 1995). Green plants appear to be reservoir of biotoxicants and constitute inexhaustible source of number of pesticides (Swaminathan, 1978). Hooda and Srivastava (1998) have mentioned that natural fungicides are free from environmental toxicity as compared to synthetic compound. Natural compounds are less phytotoxic, easily biodegradable and more systematic (Saxena *et. al.*, 2005). Some essential oils like these for cassia; clove; nigella; oregano; cinnamon; lemongrass; thyme; bay; marjoram; sage and basil exhibited activity against seed borne fungi as *Aspergillus*; *Curvurlaria* ; *Chaetomium* and *Trichoderma* (Chatterjee,1990 and Vagi *et al.*, 2005) and some of them showed strong antimicrobial activity against certain pathogenic bacteria (EL-Kamali *et al.*, 1998 and Mejlholm & Dalgaard,2002)

Many plants produce volatile essential oils that are thought to function as defenses against pathogens and insect herbivores. These naturally occurring substances are known to have a wide range of biological activities, including toxicity and repellency to certain insect pests (Isman,

2000). Because of these toxic and/or repellent effects, certain essential oils historically have been used as pesticides against stored grain pests and biting flies (Burfield & Reekie, 2005, Isman, 2006 and Momol, *et al.* 2003). Recent work has examined the potential of essential oils to protect crops from pests such as thrips, whiteflies, aphids, caterpillars, and spider mites (Calmasur *et al.* 2006, Chiasson, *et al.* 2004, Choi, *et al.* 2003 and Hummelbrunner& Isman, 2001).

Aim of this work is terms of the most storage fungi which attack cumin seeds and produce aflatoxins that cause food and feed hazards. The most pathogens that led to decreased the germination and wilted the plants. Evaluate the efficacy of essential oils as pest protects ants and safe to humans. Improve the seeds quality and quantity by used the essential oils.

MATERIALS AND METHODS

This experiment was conducted from June 2011 to May 2012 at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate. To identify the problem of reduction of cumin (*Cuminum cyminum*) seed germination. The required quantities of dry seeds were obtained from different sources (some from medicinal and aromatic department, ARC and the other from seeds productions) for conducting this study. Two hundred grams seeds of each sample were kept in cloth bags. Totally 10 samples were prepared for various treatments.

Detection of seed borne fungi

The blotter test method of diagnosing seed diseases, as recommended by ISTA Rules (Anonymous, 1996), was used in this study. Seeds were placed at equal distances on three layers of moistened filter paper in sterile Petri dishes. Seeds were incubated for seven days in a germination chamber with light. Then these were examined after eight days using a stereoscopic and compound microscopes. The seeds infections were recorded and expressed in percentage. Isolation of every individual fungus was done by picking the spores and streaking in a plated potato dextrose agar (PDA). The mycelial growth of each fungus was taken and transferred aseptically in the PDA slants for identification. Each isolate was identified based on the different books and pamphlets on seed pathology and other literature on taxonomy of fungi (CMI. 1971; Neergaard, 1977; Booth, 1977; Ainsworth *et al.*, 1973; Nelson, *et al.*, 1983; Waghmare, 1996; Quimio & Hanlin, 1999; Sutton, 1980; and Watanabe, 2002).

Essentials oils

Ten essential oils plants were used in this study as pest botanical agents in two forms, five of them namely, peppermint (*Mentha piperita* ,L.), eucalyptus (*Eucalyptus globules* Labill), nigella (*Nigella sativa* ,L.), clove (*Syzygium aromaticum* ,L.) and double jasmine (*Jasminum sambac*, Soland), as commercial form and the others were extracted as shown in Table (A) . All oils were used at the rate of 1ml/kg seed. Vitavax 200 was used as recommended fungicide (at rate of 2g/kg seed) against seed and root rot pathogenic fungi (Nene & Thapliyal, 1993). Two hundred grams seeds of each

sample of one source of cumin seeds were treated with oils and vitavax 200 as comparing with the control (without any treating) .The treated cumin seeds stored for one year maintaining it in petri dishes under room conditions and examination of seeds associated fungi and bacteria and germination after an equal period and studying the performance of seeds at the end of storage.

Table (A): The botanical term of selected plants which used as essential oils in this experiment

Arabic name	English mane	Scientific name	Family	Parts used	Source	Form of used
النعناع	peppermint	<i>Mentha piperita</i> ,L.	Lamiaceae	herb	Commercial	Oil
كافور	eucalyptus	<i>Eucalyptus citriodora</i> , Labill	Myrtaceae	leaves	Commercial	Oil
حصالبان	rosemary	<i>Rosmarinus officinalis</i> ,L.	Lamiaceae	herb	Extracted	Oil
حبة البركة	nigella	<i>Nigella sativa</i> ,L.	Rannunculaceae	seeds	Commercial	Oil
كراوية	caraway	<i>Carum carvi</i> ,L.	Apiaceae	seeds	Extracted	Oil
زعتر	Thyme	<i>Thymus vulgaris</i> ,L.	Lamiaceae	herb	Extracted	Oil
بردقوش	marjoram	<i>Majorana hortensis</i> ,L.	Lamiaceae	herb	Extracted	Oil
قرنفل	Clove	<i>Syzygium aromaticum</i> ,L.	Myrtaceae	Calyxes	Commercial	Oil
فل	double jasmine	<i>Jasminum sambac</i> , Soland	Oleaceae	Flowers	Commercial	Oil
كرفس	Celery	<i>Apium graveolens</i> , Mill.	Apiaceae	seeds	Extracted	Oil

Essential oils extraction

The seeds from caraway (*Carum carvi*, L.) and celery (*Apium graveolens* ,Mill.) and herb of rosemary (*Rosmarinus officinalis* ,L.), thyme (*Thymus vulgaris* ,L.) and marjoram (*Majorana hortensis* L.) were dried in oven at 70°C until stability of the weight, and then ground into fine powder. The essential oils were extracted by hydro-distillation using an apparatus of Clevenger type. The extraction took 3.5 hours for mixing 200g of plants in 1600 ml of distilled water. After filtration the solvent is eliminated by pressure distillation reduced in rotary evaporator at 35°C and pure oil was stored at 4°C in obscurity till the beginning of analysis. Steam and essential oils were condensed and collected in the Florentine flask. The oils were dried over sodium sulphate , stored in clean brown glass bottles, and kept in a controlled temperature chamber at 20°C, until the time of their used (Guenther, 1961).
Germination (%)

The germination test was conducted as prescribed in ISTA Rules (Anonymous., 1996). Hundred seeds in four replications were kept for germination. They were placed equidistantly in circles in glass Petri dishes of 12 cm diameter, containing 3 moisted blotters. The Petri dishes were incubated for 7 days in an incubator at 25°C ± 1°C with 12 hrs light. The germination count on normal seedlings was taken at four equal periods. The germination was calculated on the basis of number of normal seedlings counted and expressed in percentage (Christensen & Kaufmann, 1965).

Insect destroy (%)

At the end of experiment, it was expressed as percent of destroyed seeds resulting in insects feeding from the total seeds.

Statistical analysis

A complete randomized design was used in these experiments. The data collected from the experiment was analyzed statistically by Duncan's multiple range test for comparing means. Analysis was performed by the software Assistat-Statistical Attendance Silva & Azevedo, 2006 and Silva & Azevedo, 2009.

RESULTS

Seed borne fungi and bacteria of cumin and their effect on seeds germination:

The results obtained on various seeds quality parameters viz., germination; seed infection and associated fungi are presented in Table (1). According to the results the percent of germination influenced significantly by sample sources, the mean germination percentage ranged from 53.85 to 0.00%. The isolation results produced many genera of fungi as, *Fusarium*; *Aspergillus*; *Rhizopus*; *Geotrichum*; *Phoma*; *Chalara*; *Nigrospora*; *Mucor*; *Kunninghamela*; *Pythium*; *Tricothecium*; *Alternaria*; *Tritirachium*; *Epecoccum*; *Cladosporium*; *Penicillium* which their percent ranged from 69.23 to 23.08% for different samples. *Fusarium* and *Aspergillus* were the highly frequent fungi for all samples. The percent of fungi associated with seeds were increasing significantly with decreasing the percent of germination. The other isolation was bacteria which differed significantly from 36.37 to 0.00 %.

Table (1): Seed borne fungi and bacteria of cumin and their effect on seeds germination.

Sample No.	Germination %	Associated fungi	Fungi %	Bacteria %
1	0.00 f	<i>Fusarium</i> ; <i>Aspergillus</i> ; <i>Rhizopus</i>	43.75 e	0.00 f
2	0.00 f	<i>Rizopus</i> ; <i>Fusarium</i>	69.23 a	7.69 cd
3	0.00 f	<i>Geotrichum</i> ; <i>Fusarium</i> ; <i>Phoma</i> ; <i>Aspergillus</i>	59.10 c	36.37 a
4	0.00 f	<i>Aspergillus</i> ; <i>Fusarium</i> ; <i>Chalara</i>	37.50 f	3.13 e
5	47.37 b	<i>Aspergillus</i> ; <i>Phoma</i> ; <i>Nigrospora</i> ; <i>Mucor</i> ; <i>Fusarium</i> ; <i>Kunninghamela</i>	27.78 g	19.44 b
6	53.85 a	<i>Aspergillus</i> ; <i>Nigrospora</i> ; <i>Fusarium</i> ; <i>Pythium</i> ; <i>Rhizopus</i> ; <i>Tricothecium</i>	23.08 h	7.69 cd
7	28.21 c	<i>Fusarium</i> ; <i>Aspergillus</i> ; <i>Phoma</i> ; <i>Alternaria</i> ; <i>Tritirachium</i> ; <i>Geotrichum</i>	41.03 e	10.26 c
8	25.00 d	<i>Fusarium</i> ; <i>Phoma</i> ; <i>Aspergillus</i> ; <i>Epecoccum</i> ; <i>Pythium</i>	40.63 ef	9.38 cd
9	13.4 e	<i>Cladosporium</i> ; <i>Rhizopus</i> ; <i>Fusarium</i> ; <i>Aspergillus</i> ; <i>Penicillium</i> ; <i>Kunninghamela</i>	63.35 b	7.00 d
10	23.3 d	<i>Fusarium</i> ; <i>Aspergillus</i> ; <i>Alternaria</i> ; <i>Nigrospora</i> ;	47.32 d	16.63 b

Values in column followed by different letter are significantly different according to DMRT.

Essentials oils components:

Ten essential oils plants were used in two forms, one as commercial and the other was extracted. Chemical compositions of the extracted essential oils were shown in Table (2). Sixteen compounds were identified in rosemary

oil as (1,8 Cineol, β - terpineol, borneol, terpinen 4-ol , linalool , α -pinene, geraniol, limonene, carvone, bromylacetate, Linalylacetate, β – caryophyllene,geranylacetate,cavacrol,thymol, β -pinene).Twenty compounds were identified in caraway oil as (limonene, α -pinene, β -pinene,camphene, linalool, β -myrcene, α -terpineol, camphor, carvone, eugenol, citronellol, cuminaldehyde, Linalylacetate, cavacrol, thymol, γ -terpinene, terpinen 4-ol, β –caryophyllene, trans-carveol, cis-carveol).Eleven compounds were identified in thyme oil as (limonene, α -pinene, β -pinene, cineol, linalool, α -terpineol, camphor, cavacrol,thymol,borneol, methylchavicol). Forteen compounds were identified in marjoram oil as (α -terpineol, α -pinene, β -pinene,camphene,d-limonene, cymene, linalool, geraniol, carvone, eugenol, citronellol,cuminaldehyde, cavacrol,thymol).Nine compounds were identified in celery oil as (limonene, α -pinene, β -pinene, sabinene, apiole, myristicin, β –selinene, α - selinene, β –caryophyllene).

Table(2):Chemical composition of essential oil fractionated by GC technique

components	rosemary	caraway	thyme	marjoram	celery
α -pinene	5.8	0.16	1.19	1.16	0.40
β -pinene	0.24	0.14	1.81	0.38	3.30
camphene	-	0.12	-	0.80	-
d-limonene	-	-	-	7.88	-
limonene	2.95	29.11	23.01	-	39.4
cymene	-	-	-	6.71	-
cineol	-	-	0.37	-	-
1,8 Cineol	29.52	-	-	-	-
linalool	7.38	0.03	1.68	15.81	-
sabinene	-	-	-	-	0.10
β -myrcene	-	3.97	-	-	-
α -terpineol	-	0.03	0.37	35.40	-
β - terpineol	15.27	-	-	-	-
geraniol	5.13	-	-	1.94	-
camphor	-	0.02	1.68	-	-
carvone	2.01	61.58	-	4.33	-
eugenol	-	0.04	-	0.81	-
apiole	-	-	-	-	1.5
citronellol	-	0.05	-	0.26	-
cuminaldehyde	-	0.02	-	0.22	-
ethylcinnamate	-	-	-	-	-
Linalylacetate	1.12	0.07	-	-	-
bromylacetate	1.22	-	-	-	-
myristicin	-	-	-	-	1.3
β –selinene	-	-	-	-	15.4
α - selinene	-	-	-	-	2.5
geranylacetate	0.74	-	-	-	-
cavacrol	0.44	0.10	0.83	1.73	-
thymol	1.02	0.12	34.50	0.19	-
borneol	9.7	-	0.83	-	-
γ -terpinene	-	0.07	-	-	-
terpinen 4-ol	7.37	0.09	-	-	-
methylchavicol	-	-	2.81	-	-
β –caryophyllene	1.59	0.06	-	-	0.7
trans-carveol	-	0.27	-	-	-
cis-carveol	-	0.10	-	-	-
known	91.5	96.15	42.69	77.62	64.7
unknown	8.5	3.85	30.92	22.38	35.3

Efficacy of essential oils:

Results obtained from various seeds quality parameters viz., germination and seed infection are presented in Table (3). This data (the means of four equal periods) revealed that all oils significantly decreased the seeds of germination percent as comparing with the control (38.33%) except for nigella oil which obtained the highest significant seeds of germination percent (42.88%). At the same time, caraway oil had the lowest mean percent of germination (4.44%). All oils significantly reduced the percent of fungi associated with seeds which showed significant antimicrobial activity especially against both *Fusarium* and *Aspergillus* as comparing with the control. The peppermint oil had the lowest significant mean value (7.43%) of *Fusarium* while vitavax and caraway oil had the lowest significant mean value (2.93 and 6.7% ,respectively) of *Aspergillus*

Table (3): Effect of seeds coating with essential oils on germination and percent of fungi associated with cumin seeds.

Oils		Germination %	Associated fungi %	
			<i>Fusarium</i>	<i>Aspergillus</i>
Extraction	thyme	27.75 e	32.17 c	11.67 h
	marjoram	29.08 de	25.73 d	30.37 e
	celery	34.63 c	15.53 e	11.63 h
	rosemary	31.4 d	17.07 e	22.87 g
	caraway	4.44 i	48.93 b	6.7 i
Commercial	peppermint	26.11 ef	7.43 g	43.5 c
	eucalyptus	20.95 g	11.0 f	29.97 e
	nigella	42.88 a	22.93 d	58.17 b
	double jasmine	23.43 fg	17.6 e	38.53 d
	clove	13.48 h	31.6 c	26.1 f
Vitavax		21.7 g	24.57 d	2.93 j
Control		38.33 b	62.53 a	61.6 a

Values in column followed by different letter are significantly different according to DMRT.

Examination of seeds coating with essential oils associated fungi and bacteria and insect destroy% at the end of the experiment

Data observed in Table (4) showed that seeds coating with essential oils significantly decreased *Fusarium*, *Aspergillus*, bacteria and insect destroy % as comparing with the control. The highest reduction % of *Fusarium* reach to 100% for the seeds coating with peppermint , eucalyptus and nigella oils. The same trend for *Aspergillus* except for nigella oil which was uneffective against *Aspergillus* (0.00% reduction). The lowest bacteria% obtained from seeds coating with marjoram, nigella and clove oils. Seeds coating with celery ,caraway, eucalyptus and jasmine oils recorded highly significant reduction of insect destroy% (100%).

Table (4): Examination of seeds coating with essential oils associated fungi and bacteria and insect destroy% at the end of the experiment.

Oils	<i>Fusarium</i>		<i>Aspergillus</i>		Bacteria%		Insect destroy %		
	%	reduction	%	reduction	%	reduction	%	reduction	
Extraction	thyme	15.2 b	84.8	2.2 fg	98.8	8.7 b	78.25	5 gh	95
	marjoram	6.1 e	93.9	4.1 ef	95.9	0.0 e	100	60 d	40
	Celery	6.1 e	93.9	14.3 d	85.7	6.1 cd	84.75	0.00 h	100
	rosemary	11.8 c	88.2	21.6 c	78.4	7.8 bc	80.25	10 f	90
	caraway	10.2cd	89.8	0.0 g	100	6.7 bc	83.25	0.00 h	100
Commercial	peppermint	0.0 f	100	29.8 b	70.2	4.3 d	89.25	40 e	60
	eucalyptus	0.0 f	100	5.3 e	94.7	7.9 bc	80.25	0.00 h	100
	nigella	0.0 f	100	100 a	0.00	0.0 e	100	80 b	20
	Double jasmine	9.1 d	90.9	23.6 c	76.4	1.8 e	95.5	0.00 h	100
	Clove	5.9 e	94.1	5.9 e	94.1	0.0 e	100	70 c	30
Vitavax	4.3 e	95.7	2.1 fg	97.9	0.0 e	100	0.00 h	100	
Control	100 a	0.00	100 a	0.00	40.0 a	0.00	100 a	0.00	

Values in column followed by different letter are significantly different according to DMRT.

Development of germination seeds coating with essential oils at four equal periods and rot percent for three times:

Fig. (1) Shows (A) *Aspergillus* % (B) *Fusarium*% and (C) germination% . Comparing with the control seeds coating with essential oils decreased *Aspergillus* % in the three time except for peppermint, marjoram and eucalyptus oils in the first time , thyme, rosemary and double jasmine oils in the second one and nigella in the third time .Meanwhile , all oils treatment in the third time decreased *Fusarium*% comparing with the control . Germination percent increased with increasing the period of coating seeds with essential oils, however (G4) recorded the highest germination % . Nigella oil recorded the highest germination % for all periods.

Performance of seeds at the end of the experiment:

Fig (2) shows the performance of seeds at the end of the experiment. However, at the end of the year we were observed that some of treatments were deteriorated and the other were observed at the healthy shape. Seeds coating with caraway, celery, eucalyptus, double jasmine, thyme and rosemary oils recorded the highly healthy shape comparing with the control .

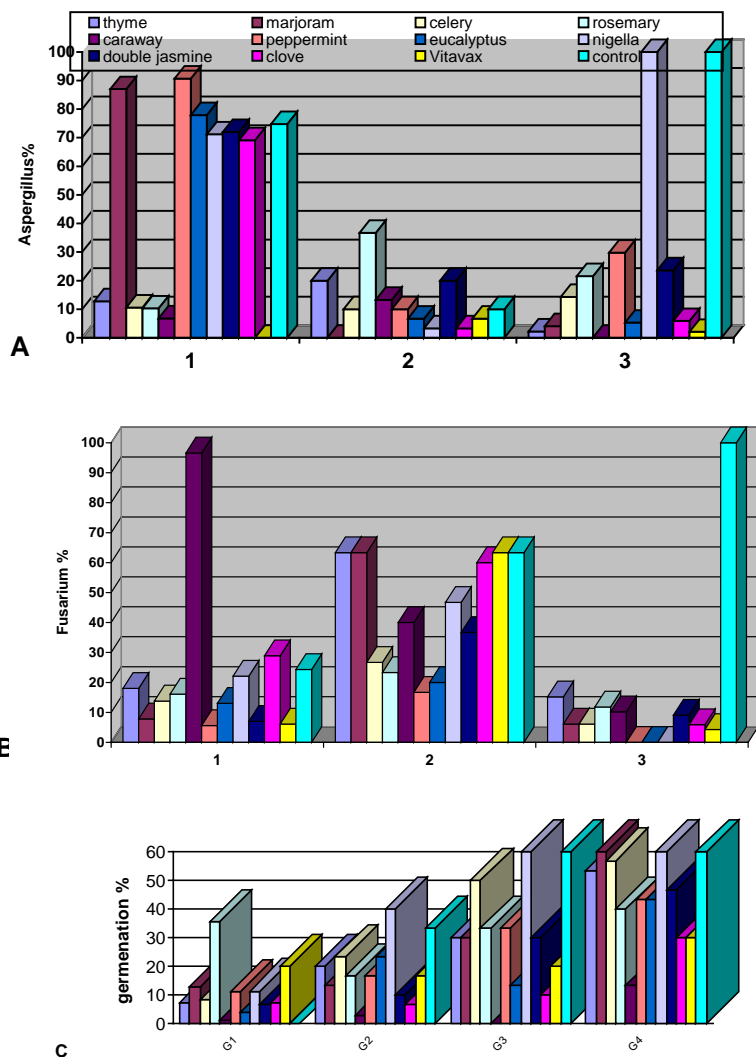


Fig (1): Effect of seeds coating with essential oils on (A) percent of *Aspergillus* and (B) percent of *Fusarium* for three times 1,2 and 3 and(C) germination percent at four equal periods (G1,G2,G3 and G4).

Fig.(2): Performance of seeds at the end of experiment, (Ros) *Rosmarinus officinalis* L., (Car) *Carum carvi* L., (Thy) *Thymus vulgaris* L., (Mar) *Majorana hortensis* L.; (Cel) *Apium graveolens* Mill. (Min) *Mentha piperita* L., (Euc) *Eucalyptus citriodora* Labill, (Nig) *Nigella sativa* L., (Clo) *Syngium aromaticum* (L.) Morrill&perry. (Jas) *Jasminum sambac* (Soland); (Vit) vitavax and (Con) control.

DISCUSSION

Seeds in the field as well as in ill storage conditions interact with several microbes which deteriorate the seeds, both qualitatively and quantitatively (Christensen & Kaufman, 1969). The microorganisms thrive on the seeds at the expense of easily digestible components. The successful invasion or colonization, however, depends largely upon the efficiency of microorganisms to degrade complex molecules into simpler forms (Bilgrami & Verma, 1978). Damage to the cumin seeds has been reported to be caused by fungi associated with them. Fungi like *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Fusarium* spp. causes discoloration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to seeds (Moharram *et al.*, 1989 ; Regina & Raman, 1992 and Chavan & Kakde, 2008). Fungi growing on stored grains, can reduce the germination rate along with loss in the quantum of carbohydrate, protein and total oil content, induces moisture content, free fatty acid content enhancing other biochemical changes of grains (Bhattacharya, 2002). Such seeds are not fit for human consumption and are also rejected at the industrial level. Christensen and Kaufmann (1965), working with the fungi associated with seeds, classified them into two groups, i.e., "field fungi," which must contaminate seeds in the field during harvest, and "storage fungi," which must be contaminate seeds during transit and storage. We cannot specify when and how the contamination occurred on these seeds, but most of these fungi can live under both seed and soil conditions. There must be a few fungi only limited to living in seed, which infect only seeds and complete their life cycle on the seeds. Many organisms may be introduced into soil by sowing, but on the other hand, some organisms penetrate, contaminate, and colonize plant tissues directly or indirectly in various growth stages, repeatedly. Among these fungi, some influence seed quality and reduce germinability. Aflatoxin and toxic substances are produced from toxicogenic fungi and affect the health of animals and human beings (Moharram *et al.*, 1989 and Regina and Roman, 1992); therefore, the study of seed fungi is very important as it concerns our health. Therefore, in first part of the study includes investigation of the most dominant seed borne fungi. This study revealed that there are sixteen genus of fungi associated with cumin seeds, and they frequent percent is related to reduce germinability, however reach to 0.00%. The most frequent genus was *Aspergillus* spp., and *Fusarium* spp

The second part of the study includes ecofriendly management of seed-borne fungi by essential oils of some medicinal plants. Essential oils are highly concentrated substances extracted from various parts of aromatic plants and trees. They are usually captured by steam distillation. We can find them in processed food, perfumes, health products, cleaning products, air fresheners, candles, cosmetics, chewing gum, candy, soft drinks, liqueurs, toothpaste, mouthwash, and many other sources. It is highly volatile and will evaporate if left in the open air so they are frequently combined with carrier oils in order to stabilize them (Iacobellis, *et al.*, 2005). Essential oils are used against plant pests and diseases. Most of the research to date has been

done testing the growth of fungi in the laboratory under ideal conditions. The difficulty may be to apply the oils effectively under commercial conditions. Essential oils are often fungistatic rather than fungicidal. This means that they stop the growth of the fungi while it is exposed to the oil, but once the oil is removed the fungi can continue to grow Hooda and Srivastava (1998). Sydney Postharvest Laboratory is currently trying to develop a method for applying the oils as a vapour at a low concentration during storage of fresh produce. Application of the oil as a vapour at a continuous, low concentration should prevent tainting of the product. Thin skinned products, not surprisingly are more prone to tainting than those with thicker skins. This work highlights the potential for using essential oils for postharvest disease control of fresh fruit and vegetables. Essential oils which have been registered as Food Additives are much easier to register for postharvest use than new synthetic pesticides. Application of these oils via the vapour phase should also make their use more expensive than dipping. In this work we used the essential oils as seed coating and without observing differences between oils application either in extracted or commercial phase. Where, the extracted oils had two kinds of oils one is essential and other is stable. At the same time, these commercial oils some of them had the two kinds and the other were combined with carrier oils in order to stabilize them. We wished to continue our work in an effort to determine the optimum concentration of oil for maximum control of the pathogens with acceptable levels of product. We used the synthetic fungicide as recommended treatment in controlling seed born pathogens. Our work revealed that some of oils were effective which the highest reduction % of *Fusarium* reach to 100% for the seeds coating with peppermint , eucalyptus and nigella oils and the others were suppressive like nigella oil which was ineffective against *Asperigillus* (0.00% reduction).The stabilize of oils effect even the end of experiment indicate that it had a fungicidal effect.

Still, widespread commercialization of plant essential oils as crop protectants has lagged, often because critical data on their efficacy and optimal application methods are lacking (Isman, 1997 and Ujváry, 2002). Although applications of essential oils as commercial crop protectants have been limited to date, many of these essential oils are safe to humans and currently are used widely in the food and cosmetic industries. Further, with their low mammalian toxicity and low potential for other negative environmental effects (Ebbon, 2002 and Isman, 2000), the use of essential oils as crop protectants is highly appealing. The effects of individual oils or their constituents on specific target pests can differ depending on their particular mode of action (Isman, 2000). They are rapid degradation; less persistence in environment may be applied shortly before harvest without leaving excessive residues. This characteristic, although desirable in some respects, it needs more frequent applications. When we left our treatments under the storage conditions for long time (more than one year), the most of them saved by it's healthy shaped. This means that we can use it as safety treatment for long time. The essential oils may act very quickly to stop feeding by pest insects (Chiasson, *et al.* 2004). They may not cause death for hours or days, but they often cause immediate paralysis or cessation of

feeding. The essential oil of bergemont, anise, sage, tea tree, geranium, mint, and thyme, hyssop, rosemary, thyme, and white clover can be used to control certain pests on plants. They have been shown to reduce the number of eggs laid and the amount of feeding damage by certain insects (Calmasur *et al.* 2006).

REFERENCES

- Abou-Zeid,A.; Metwally, M. and Badria, F. 1997. Physiological and hepatotoxic studies on fungal aflatoxins isolated from Egyptian Amita-Shrivastava., Jain, P.C. and Shrivastava, A. 1992. Seed mycoflora of some spices. *J. Food Sci. and Technol.*, 29: 228-230.
- Ainsworth,G. C.; F. K. Sparrow, and A. S. Sussman (eds). 1973. *Fungi: An advanced treatise IVA*. Academic Res. Inc. London Ltd.
- ANONYMOUS, 1996, International rules for seed testing. *Seed Science and Technology*, 24:1-335.
- Bhattacharya, K. and Raha, S. (2002). Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. *Mycopathologia*. 155: 135–141.
- Bilgrami, K.S. and Verma, R.N. (1978). *Physiology of fungi*. Vikas publishing House Pvt. Ltd., New Delhi. Pp. 597.
- Booth,C. 1977. The genus *Fusarium*. Commonwealth Mycological Institute, England.
- Burfield, T., and Reekie, S. L. 2005. Mosquitoes, malaria and essential oils. *Int. J. Aromatherapy* 15:30-41.
- Calmasur, O., Aslan, I., and Sahin, F. 2006. Insecticidal and acaricidal effect of three Lamiaceae plant essential oils against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Ind. Crop. Prod.* 23:140-146.
- Campbell. T.C. and Stoloff, L. 1974. Implication of mycotoxins for human health. *J. Agric. Food Chem.*, 22: 1006-1015
- Chatterjee, D. 1990. Inhibition of fungul growth and infection in maize grains by spice oils. *Let. Appl. Microbiol.* 11: 148-151.
- Chavan, A. M. and Kakde, R. B. (2008). Studies on abnormal oilseeds mycoflora from Marathwada region. *Bionano Frontier* 2 (2): 101-104.
- Chiasson, H., Vincent, C., and Bostanian, N. J. 2004. Insecticidal properties of a *Chenopodium*- based botanical. *J. Econ. Entomol.* 97:1378-1383.
- Choi, W. I., Lee, E. H., Choi, B. R., Park, H. M., and Ahn, Y. J. 2003. Toxicity of plant essential oils to *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 96:1479-1484.
- Christensen, C. M. and Kaufmann, H. H. 1965. Deterioration of stored grains by fungi. *Annu. Rev. Phytopathol.* 3:69–84.
- Christensen, C.M. and Kaufman, H.H. (1969). *Grain storage. The role of fungi in quality losses*. Univ. Minnesota, Press Minneapolis.
- Commonwealth Mycological Institute (CMI.). 1971. *Description of pathogenic fungi and bacteria*. CMI, Kew, Surrey, England. PP. 91-95.

- Ebbon, G. P. 2002. Environmental and health aspects of agricultural spray oils. Pages 232- 246 in: *Spray Oils Beyond 2000*. G. A. C. Beattie, D. M. Watson, M. L. Stevens, D. J. Rae, and R. N. Spooner-Hart, eds. University of Western Sydney Press, Sydney, NSW.
- El-Bazza, Z.F.; Mahmoud, M.T.; Roushdy, H.M.; Farrag, H.A. and El-Tablawy, S.Y. 1996. Fungal growth and mycotoxigenic production in certain medicinal herbs subjected to prolonged cold storage and possible control by gamma irradiation. *Egypt. J. Pharmaceutical Sci.*, 37: 85-95.
- El-Kamali, H. H., Ahmed, A. H., Mohamed, A. S., Yehia, A. A. M., El-Tayeb, I. and Ali, A. A. 1998. Antibacterial properties of essential oils from *Nigella sativa* seeds, *Cymbopogon citrates* leaves and *Pulicaria undulata* aerial parts. *Fitoterapia* 69: 77-78.
- Guenther, E. 1961. "The Essential Oils". Van Nostrand Comp. Inc. New York, Vol. III, pp.448.
- Hooda, K.S. and Srivastava, M.P. (1998). Biochemical response of scented rice as influenced by fungitoxicant and neem products in relation to rice blast. *Indian J. Pl. Pathol.* 16: 64-66.
- Hummelbrunner, L.A., and Isman, M.B. 2001. Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). *J. Agric. Food Chem.* 49:715-720.
- Iacobellis, N. S., Lo Cantore, P., Capasso, F., & Senatore, F. (2005). Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *Journal of Agricultural and Food Chemistry*, 53(1), 57–61.
- Lawrence, B.M., 1992. Progress in essential oils. *Perfumer and Flavourist*, 17: 42-44.
- lalitha.v,kiran.b.andraveesha.k.a.(2011)antifungal and antibacterial potentiality of six essential oils extracted from plant source. *inter. j. e sci. and tech.* 3(4):3029-3038
- Isman, M. B. 1997. Neem and other botanical insecticides: Barriers to commercialization. *Phytoparasitica* 25:339-344.
- Isman, M. B. 2000. Plant essential oils for pest and disease management. *Crop Prot.* 19:603-608.
- Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* 51:45-66.
- Kumar, A., Roy, S.K., Saxena, D.C. and Saxena, A.R. (1995). In vitro control of *E. coli* by herbal treatment. *Neo Botanica.* 3:1-2.
- Mejlholm, O. and Dalgaard, P. 2002. Antimicrobial effect of essential oils on the seafood spoilage microorganism *Photobacterium phosphoreum* in liquid media and fish products. *Lett. Appl. Microbiol.* 34: 27-31.
- Moharram, A.M.; Abdel-Mallek, A.Y. and Abdel-Hafez, A.I. 1989. Mycoflora of anise and fennel seeds in Egypt. *J. Basic Microbiol.*, 29: 427-435.
- Momol, M. T., Olson, S. M., Funderburk, J. E., and Marois, J. J. 2003. Integrated management of tomato spotted wilt on tomato. (Abstr.) *Phytopathology* 93:S115.

- Nasir, E and S.I. Ali. 1972. Flora of West Pakistan, Stewart Herbarium, Gordon College Rawalpindi, No 20.
- Neergaard,P. 1977. Seed pathology. Halsted Press Book. New York: John Wiley and Sons.
- Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. Fusarium Species. An Illustrated Manual for Identification. Pennsylvania State University Press, University Park. 193 pp.
- Nene, Y.L. and Thapliyal, P.N. (1993). Fungicides in plant diseases control. Oxford and IPH. Publishing Co. Pvt. Ltd., New Delhi pp.531.
- Prasad, B.K.; Shanker, U.; Narayan, N.; Dayal, S. and Kishor. A. 1988. Physiochemical changes in food reserve of coriander seed due to storage moulds. Indian Phytopathol., 41: 386-388.
- Peter K.V., Nybe E.V. 2002. Dominating global markets. The Hin-du Survey of Indian Agricul.: 87–99.
- Quimio, H. T. and R. T. Hanlin. 1999. Illustrated genera and species of plant pathogenic fungi in the tropics. College of Agriculture, UP Los Banos. College. Laguna.
- Ragab, W.S. and El-Syied, S. I. 1998. Aflatoxin production on some food grains as affected by moisture content and autoclaving. Assuit. J. Agric. Sci., 29: 1-9.
- Regina, M., and Roman, T. 1992. Biochemical changes in stored caraway seeds due to fungi. Indian Phytopathol., 45: 380-384.
- Saxena, A.R., Sahni, R.K.,Yadav, H.L., Upadhyay, S.K. and Saxena, M. (2005). Antifungal activity of some higher plants against *Fusarium oxysporum* f.sp. pisi. J. liv. World. 12: 32-39.
- Silva, F. de A. S. e. & Azevedo, C. A. V. de.(2006) A New Version of The Assistat-Statistical Assistance Software. In: WORLD CONGRESS ON COMPUTERS IN AGRICULTURE, 4, Orlando-FL-USA: Anais... Orlando : American Society of Agricultural and Biological Engineers, . p.393-396
- Silva, F. de A. S. e. & Azevedo, C. A. V. de.(2009) Principal Components Analysis in the Software Assistat-Statistical Attendance. In:WORLD CONGRESS ON COMPUTERS IN AGRICULTURE, 7, Reno-NV-USA: American Society of Agricultural and Biological Engineers, .
- Srinivas,S.R., 1986. Atlas of essential oils. New York: Bronx, pp: 36.
- Sutton, B. C. 1980.The coelomycetes fungi imperfecti with pycnidia, acervuli and stromata. CMI, Kew, Surrey, England.
- Swaminathan,M.S.(1978). Inaugural adress, Fruit, Bot. conference, Meerut, India, pp. 1-31.
- Sydney Postharvest Laboratory Information Sheet. www.postharvest.com.au
spl@postharvest.com.au
- Ujváry, I. 2002. Transforming natural products into natural pesticides - xperience and expectations. Phytoparasitica 30:439-442.
- Vagi, E., Simandi, B., Suhajda, A. and Hethelyi, E. 2005. Essential oil composition and antimicrobial activity of *Origanum majorana* L. extracts obtained with ethyl alcohol and supercritical carbon dioxide. Food Res. Int. 38: 51-57.

Waghmare, B. M. (1996). Studies on seed-borne species of Fusarium (Link) from different Plant seeds. Ph.D. Thesis,
Watanabe, T, 2002. Pictorial Atlas of Soil and Seed Fungi, Morphologies of Cultured Fungi and Key to Species . CRC press Boca Raton London New York Washington, D.C. 506 pp.

**قدرة الزيوت الطيارة لبعض النباتات الطبية على مقاومة الافات المرضية و
الحشرية التي تسبب تلف بذور الكمون
فتحية سليمان الشراكي* و ناهد مصطفى محمد راشد**
* معهد بحوث امراض النباتات - مركز البحوث الزراعية الجيزة
** قسم بحوث النباتات الطبية والعطرية - معهد بحوث البساتين - مركز البحوث الزراعية**

أجريت هذه التجربة بمحطة البحوث الزراعية بسخا في المدة من وذلك للتعرف على مشكلة انبات
بذور الكمون حيث تم الحصول على عينات لبذور الكمون من مصادر مختلفة في بداية موسم الزراعة . وتم
القيام بعزل و تعريف الكائنات المصاحبة لتلك البذور و قد اسفرت النتائج المتحصل عليها وجود ستة عشر
جنس من الفطريات بالاضافة لبعض انواع من البكتريا. و قد كانت اعلى الفطريات في نسبة العزل الفطرين
فيوزاريوم و اسبرجلس. و قد اظهرت النتائج وجود ارتباط عكسي بين نسبة الاصابة بالفطريات ونسبة انبات
البذور. تم استخدام عشرة زيوت طيارة لنباتات طبية مختلفة وهي (النعناع، الكافور، حصالبان، حبة البركة
، الكراوية، زعتر، بردقوش، قرنفل، فلفل، كرفس) و قد اظهرت نتائج المعاملة قدرة تلك الزيوت على مقاومة
الفطريات و البكتريا المصاحبة للبذور. كل الزيوت المستخدمة زادت من نسبة الانبات عند مقارنتها بمبيد
الفينافاكس عدا زيت الكراوية و القرنفل بالاضافة لتفوق زيت حبة البركة (42,88%) عند مقارنة
بالكنترول(38,33%) وفي نفس الوقت اظهرت الكراوية اقل نسبة انبات (4,44%). تركت تلك المعاملات
مخزنة في اطق بترى تحت ظروف المعمل وتم تقييم قدرة الزيوت في التأثير على الانبات و نسبة الاصابة
بالافات و تم التقييم على فترات متساوية و قد تبين ازدياد نسبة الانبات كلما طالت فترة تخزين البذور المعاملة
بالزيوت ونقص نسبة الاصابة بالفطرين فيوزاريوم و اسبرجلس. و في نهاية فترة التخزين التي تعدت السنة
لوحظ اصابة بعض المعاملات دون الاخرى بحشرات المخازن بينما احتفظت الاخرى بحالتها الجيدة وهي
البذور المعاملة بزيت الكراوية و الكرفس و الكافور و الفلفل و الزعتر و حصالبان . و قد اشارت النتائج بعد تلك
الفترة ان كل الزيوت ادت الى انخفاض نسبة الاصابة بالفطريات و البكتريا و الحشرات. و ان زيوت حبة
البركة و النعناع و الكافور قد ادت الى تثبيط نمو الفطر فيوزاريوم في حين ادى زيت الكراوية الى تثبيط نمو
فطر الاسبرجلس. اما زيوت البردقوش و النعناع و حبة البركة و القرنفل فقد عملت كمانع لنمو البكتريا. ادت
الاصابة الحشرية الى حدوث تلف واضح في البذور في حين اظهرت زيوت الكراوية و الكرفس و الكافور و
الفلفل القدرة على منع الاصابة الحشرية. و نتيجة للتركيب الكيماوى للزيوت المستخلصة فانه قد يرجع التأثير
ضد الفطريات و الحشرات في زيت الكراوية الى وجود مركبات
(61,58%) و الليمونين(29,11%) بنسب عالية. اما التأثير المبيد للبكتريا و الحشرات في زيت البردقوش
فقد يرجع لوجود مركب الالفا تريبينيول بنسبة عالية (35,40%). . يعتبر هذا العمل بداية مباشرة لاستخدام
الزيوت العطرية في مكافحة الافات المرضية و الحشرية التي تسبب تلف للبذور اثناء التخزين عن طريق
تغليف البذور قبل التخزين .

قام بتحكيم البحث

**كلية الزراعة – جامعة المنصورة
كلية الزراعة – جامعة كفر الشيخ**

**أ.د / محمد الششتاوى عبد ربه
أ.د / السيد فهمى مشعل**