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THE EFFECTIVENESS OF PLANT ESSENTIAL OILS ON SEED AND SOIL BORNE FUNGAL PATHOGENS

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ABSTRACT

Field survey of seed borne fungi was carried out on some seed crops in the newly reclaimed soils. Two standard methods for seed health testing have been acted to determine the percentage of disease incidence of each crop.

Six essential oils suggested to have antifungal effect were tested.Carvone oil was more effective than thyme, caraway, peppermint, lavender and eucalyptus (blue gum) oils respectively. *In vivo* tests its minimum inhibitory concentration (MIC) was 50 ppm however, 400 ppm inhibited the five tested pathogens, Ascochyta pisi, *Cephalosporium maydis, Colletotrichum dematium, Fusarium oxysporum and Macrophomina phaseolina* .Thyme and caraway oils reduced the growth of tested fungi at 800 ppm. The highest concentration of treated oils induced reduction of disease severity, except of eucalyptus oil. seed germination was reduced following exposure to that tested oils at different exposure times.

Keywords: oily plant extracts, seed borne fungi.

INTRODUCTION

The modern studies are now being directed towards minimizing the fungicides applications, avoiding environmental pollution and increasing public health (Wilson and Wsniewski, 1992). For these reasons, there is a need to find alternatives to synthetic chemicals for the control of fungal diseases, which has been increasingly felt in agriculture researches. Beside biological and genetic engineering techniques, natural substances are being used which appear to have fungicidal properties. Literatures provide exampies of essential oils that have inhibited the development of fungal growth (Farag *et al.*, 1989). Effective results have been obtained with thyme oil (Maruzzela and Batler, 1959;Buchanan and Shepard,1981;;Paster,*et al.*,1995 Muller-Riebau *et al.*, 1995 ,Eloff, 1998 and El-Safwani and Aly, 2003), with mint oil (Maiti *et al.*, 1985 and Zambonelli *et al.*, 1996), Lavender oil (Zambonelli *et al.*, 1996 and Daferera *et al.*, 2003) and by testing eucalyptus oil (blue – gum) (Ismail, 1988 and Muller-Riebau *et al.*, 1995). The present work was devoted to study the fungicidal activity of tested oily crude plant extracts as a monoterpene compound (carvone) against certain seed borne fungi.

MATERIALS AND METHODS

Field Survey

Field survey to some seed borne fungi of selected crops, bean, chickpea, cucumber, lentil, lupine, maize grain, pea, soybean, sunflower, watermelon was carried out during the growing season of 2003 in newly reclaimed soils at West Delta. Four lines (4 replicates) each contain 25 hills were rundomisly chosen in each farm. Data were recorded as a percentage of disease incidences of each crop.

Seed Health Testing:

Seed samples of maize, bean, chickpea, lentil, lupine, pea, soybean, cucumber, watermelon and sunflower were collected during the season of 2003 from different farms through the field survey. Samples of 400 seeds each were tested using the two standard methods for seed health testing namely: Agar and Blotter method according to the International Seed Health Testing Association (ISTA, 1997). The fungal developing colonies were examined by using the stereo binocular microscope in case of the blotter method and the fungal developing colonies on the agar were examined by using the compound microscope.

Testedfungi:

Pure cultures of *Cephalosporium maydis* (Samara, Sabet and Hingorani), *Macrophomina phaseolina* (Maubi), *Fusarium oxysporum* Schlsht. ex. Fr., *Ascochyta pisi* Lib. and *colletotrichum dematium* (Pers.) Grove, which have been isolated earlier from seeds of maize, sunflower, lentil, pea and soybean respectively. Cultures were maintained on potato dextrose agar (Divco) slants at 5c° for further studies.

Crude Plant Oil Extract:

Five plant extracts and one monoterpene compound (carvone) were prepared for testing their antifungal activity. The tested plants were thyme, *Thymus vulgaris* L (leaves - *Labiatae*), Pepperment, *Mentha piperita* L. (Leaves- *Labiatae*), Lavender, (*Labiatae)*, Caraway, *Carum carvi* L. (Fruit - *Umbelliferae*) and *Eucalyptus globules* (Blue gum) (leaves - *Myrtaceae*). The extraction technique was carried out according to the modified method given by Meisner *et al.*, (1981). Samples 200g each of dried plant material were soaked for three days with 80% EtoH (2xo.84), the combined ethanol extract was filtered and concentrated under low pressure at (45c°) to small volume (100ml) and then it was partitioned with petroleum ether (3 x 300 ml). The petroleum ether extract was filtered, concentrated and evaporated for drying and then obtain the crude oily extract (Davidson and Parish, 1989).

Effect of essential oils on the growth of fungal pathogens:

Tested plant crude extract and carvone compound were tested by dissolving each in absolute ethyl alcohol, added to PDA media immediately before it was poured into the Petri dishes at a temperature of 40 - 45 c° and prepared in concentrations of 50, 100, 200, 400, 800 and 1600 ppm .All treatments were incubated at 25C°± 1. The linear growth of each tested fungus was measured. The controls received the same quantity of absolute ethyl alcohol mixed with PDA. Petri dishes were inoculated by placing a disk of mycelial felt (4 mm) diameter in its center for each tested fungus, taken from the margin of 7-days-old-cultures. Fungi toxicity was expressed in terms as percentage of mycelial growth inhibition and calculated according to the formula of Caccioni and Guizzardi (1994) as the follow:

$$\frac{dc - dt}{dc} x100$$

Where: Dc= average diameter of fungal colony of control.

Dt= average diameter of fungal colony of treatment which were repeated four times

Exposure time of seed to the essential oils:

Seed treatment using essential oil was carried out by exposing it for 24 h, 48h and 72 h. Lots of seeds (50g each) into wire-mesh baskets, which were suspended from the flask stopper by the hook. Seeds were fumigated after each treatment; seeds were placed on PDA in Petri dishes (10 seeds/plate).

RESULTS

Field Survey:

Field survey of certain seed-borne fungal diseases affecting some crops grown in West Delta was presented in Table 1. Data show that the maximum percentage of field infection in maize was caused by *Fusarium moniliforme* 22%, followed by *Drechslera* blight, *Cephalosporium* late wilt and *Diplodia* 20 %, 8 % and 8% respectively .Legumenious crops, wilt of chickpea induced by *Fusarium oxysporum* f.sp. *ciceri* was very hight 22 %, followed by *Rhizoctonia* root – rot and *Sclerotinia* white rot 16 % and 12 % in bean wilt in lentil 12 %.Watermelon and cucumber wilt was very severe 30 % and 23 % respectively, also sunflower was affected by charcoal rot 12 %.

Seed health testing:

Data Table (1) show the following :*Cephalosporium maydis, Drechslera heterostrophus, Diplodia maydis* and *Fusarium moniliforme* were detected in maize tested seed samples, whereas the infection percentage reached up to 6,15,9 and 15 % respectively . *Rhizoctonia solani* 10 % and *Sclerotinia sclerotiorum* 13.0 % on bean seeds whereas, Ascochyta rabiei(5.5%) and *Fusarium oxysporum* f. sp. *Ciceri* on checkpea .,*F.oxysporum* on lentil (4.0 –9.0 %),*Glumerella cingulata* (5.0 – 7.0 %) on lupin, *Ascochyta pisi* (1.0 –10.0 %) on pea, *Colletotricum dematium* (2.0 – 5.0%) and *Cercospora kikuchii* (2.0 – 3.0%) on soybean , *F.oxysporum* f.sp. *cucumerinum* on cucumber (4.0 – 31.0 %), *F.oxysporum* f. sp.*niveum* on watermelon (6.0-30.0%) and *Macrophomina phaseolina* on sunflower (1.0 – 7.0%).

Effect of essential oils on the mycelial growth of tested fungi:

Data presented in Table (2) show the effect of different concentrations of essential oils, on the growth of certain tested fungi. It was cleared that carvone oil was more effective than thyme, caraway, peppermint, lavender and eucalyptus tested oils. In particular, carvone oil reduced the growth of all tested fungi at the lowest concentration used (50 ppm); at 400 ppm the growth of all tested pathogens was inhibited. Both Thyme and caraway oils demonstrated its fungicidal activity against all the tested fungi at the concentration of 800 ppm except for *Ascochyta pisi* treated with caraway oil . Eucalyptus oil, had the least effect.

Effect of essential oils on seed germination

Data Table (3) indicate that seeds germination was reduced following exposure to essential oils :carvone (400 ppm), thyme (800ppm) ,caraway(800 ppm) ,peppermint ,lavender and eucalyptus (1600ppm) which are applied as effective concentrations . Extending the exposure time of tested seeds to essential oils up to 48 or 72 h also resulted in reduction in seeds germination at different dosages applied.

Table 1: Seed health testing of selected ten crops grown during 2003 growing season (400 seeds /sample).

Crops	Fungi	Percentages % of the isolated fungi	
		SHT	Field Infection
Bean	*Rhizoctonia solani*	1.0 – 10	3.0 -16
	Sclerotinia Sclertiorum	12 -13	10 –14
Chickpea	*Ascochyta rabiei*	2.5 – 5.5	2.0 –9.0
	Fusarium oxysporum f.sp.ciceri	12 - 15	9.0 –22
Cucumber	*Fusarium oxysporum f.sp cucumerinum*	4.0 –31	4.0 -23
Lentil	*Fusarium oxysporum*	4.0 –9 0	3.0 -12
Lupine	*Glomerella cingulata*	5.0 –7.0	2.0 –8.5
Maize	*Fusarium moniliforme*	10 –15	8.0 -22
	Drechslera heterostrophus	12 –15	10 -20
	Diplodia maydis	1.0 —9.0	2.0 –8.0
	Cephalosporium maydis	3.0 –6.0	2.0 –8.0
Pea	*Ascochyta pisi*	1.0 –10	1.0 –10.5
Soybean	*Colletotrichum dematium*	2.0 -5.0	1.0 -10
	Cercospora kikuchii	3.0 –3.0	1.0 –5.5
Sunflower	*Macrophomina phaseolina*	1.0 –7.0	1.0 -12
Watermelon	*Fusarium oxysporum f.sp.niveum*	6.0 –30	3.0 -30

Data expressed as infection percentage. SHT:seed health testing

Table 2: Effect of different concentrations of some essential oils on mycelial growth of some of the isolated fungi.

Tested fungi	Essential oils concentrations (ppm)*.						
	Tested oils	50	100	200	400	800	1600
Ascochyta pisi	Carvone	8	52 79	70	100	100	100
Cephalosporium maydis		17	70 76	100 81	100	100	100
Colletotrichum dematium		14 19	68	80 83	100	100	100
Fusarium oxysporum		20			100	100	100
Macrophomina phaseolina					100	100	100
Ascochyta pisi	Thyme	0.0	12	45	96	100	100
Cephalosporium maydis		0.0	18	41 32	95 76	100	100
Colletotrichum dematium		0.0	14 28	44	100	100	100
Fusarium oxysporum		0.0	58	71	100	100	100
Macrophomina phaseolina		0.0				100	100
Ascochyta pisi	Caraway	0.0 0.0	0.0	0.0	21	32	100
Cephalosporium maydis		0.0 0.0	26	53	100	100	100
Colletotrichum dematium		0.0	25	56	100 100	100	100
Fusarium oxysporum			50	78	100	100 100	100
Macrophomina phaseolina			21	52			100
Ascochyta pisi	Pepermint	0.0	16	23	34	65	100
Cephalosporium maydis		0.0 0.0	21	40	51	60	100
Colletotrichum dematium		0.0 0.0	0.0	15	22	57 100	100
Fusarium oxysporum			0.0	31	87	100	100
Macrophomina phaseolina			23	64	100		100
Ascochyta pisi	Lavender	0.0	0.0	18	35	56	100
Cephalosporium maydis		0.0 0.0	10	20	47	75	100 67
Colletotrichum dematium		0.0	0.0 0.0	0.0 0.0	13	30	100
Fusarium oxysporum		0. 0	0.0	0.0	24	81 100	100
Macrophomina phaseolina					62		
Ascochyta pisi		0.0	0.0	0.0	0.0	0.0	0.0
Cephalosporium maydis	Eucalyptus	0.0	0.0	0.0	0.0	0.0	0.0
Colletotrichum dematium	(Blue gum)	0.0	0.0 0.0	0.0 0.0	0.0 0.0	26	52
Fusarium oxysporum		0. 0 0.0	0.0	0.0	0.0	11	30
Macrophomina phaseolina						20	37

* Data presented as inhibition percentage .

Table 3: The effect of different concentrations of essential oils on crops seed germination.

Seed	Exposure time	Effective essential oils concentrations (ppm)						
		carvone (400ppm)*	thyme (800ppm)	caraway (800ppm)	peppermint (1600ppm)	lavender (1600ppm)	eucalyptus (blue-gum)	check
Bean	24h.	65	77	85	83	95	94	95
	48h.	63	76	81	79	93	93	95
	72h.	60	74	80	78	90	91	96
Chickpea	24h.	69	76	87	80	94	92	94
	48h.	66	75	85	80	92	90	95
	72h.	64	71	83	77	92	87	96
Cucumber	24h.	70	74	83	84	93	92	92
	48h.	69	70	81	80	92	92	95
	72h.	67	69	80	79	91	91	96
Lentil	24h.	71	75	89	83	95	93	93
	48h.	69	74	86	81	91	92	95
	72h.	68	71	84	80	90	91	96
Lupine	24h.	69	73	87	73	94	91	96
	48h.	68	70	81	71	93	90	96
	72h.	65	69	80	69	90	89	96
Maize	24h.	74	84	88	87	93	94	94
	48h.	73	83	86	85	93	93	95
	72h.	69	83	81	85	90	88	96
Pea	24h.	67	75	90	80	90	93	95
	48h.	65	70	87	79	90	90	95
	72h.	63	69	85	79	89	89	96
Soybean	24h.	76	81	85	83	90	94	94
	48h.	74	80	84	81	88	93	94
	72h.	70	79	83	80	86	92	95
Sunflower	24h.	75	75	88	81	93	93	95
	48h.	73	70	86	80	91	91	95
	72h.	70	70	84	77	90	88	94
Watermelon	24h.	77	79	82	85	90	92	93
	48h.	77	77	81	83	90	90	93
	72h.	72	75	80	79	90	87	96

* Data presented as percentage of seed germination.

DISCUSSION

The recent work revealed that certain natural and chemical materials proved to be as inhibitory agents. Plant extracts, oils, chemicals and their constituents present in some species are known to have antifungal properties. Study was undertaken to screen six essential oils that suggest having fungal inhibitory effect.

Among the essential oil tested carvone, inhibitory effect was observed at lower concentration (50 ppm) than thyme, caraway, peppermint, lavender and eucalyptus oils respectively and completely prevented fungal growth at a rate of 400 ppm for all tested fungi. On the other hand, Thyme and caraway showed inhibition at rate of 800 ppm for all tested fungi except of *Ascochyta pisi* in a case of caraway treatment. These findings support the work of Muller-Riebau *et al.*, (1995) who characterized thymol and carvacrol as a highly fungitoxic and there is a relationship between the chemical structure and its antifungal effect which might be due to the presence of a phenolic OH – group and it can easily form hydrogen bonds with active sites of enzymes. This finding is in close agreement with that found by Maruzzela and Batler (1959); Arras *et al.*, (1995) Zambonelli *et al.*, (1996); Daferera *et*

al., (2003) and El Safwani and Aly (2003) who mentioned that thyme and carvacrol led to a complete inhibition of *F.moniliforme, R.solani, S.sclerotiorum, Ph.capsici, B.fabae* .The present work revealed that some certain natural and chemical materials proved to be as inhibitory agents. Plant extracts, oils, chemicals and their constituents present in various species are known to have antifungal properties which cause degeneration of the fungal mould. Our study was undertaken to screen six essential oils that can inhibit fungal growth. The present study also confirmed the antifungal activity at mint oil documented by Zambonelli *et al.*, (1996) who indicated that a higher concentration of mint oil was more effective i.e. at 1600 ppm. It can completely inhibited the growth of *F.solani, P.ultimum* var. *ultimum, R.solani* and *C.lindemuthianum*. Similar results were obtained by Yegen,*et al.*, (1992); Meena, and Mariappan, (1993) and Singh, *et al.*, (1994) who found that essential oil of *Mentha arvensis* inhibit the mycelial growth and spore germination of the seed borne mycoflora. Although lavender oil appeared to be less active than the other oils tested did reveal a certain moderate antifungal activity, due to linalool are in line with Zabonelli *et al.*, (1996) and Daferera *et al.*, (2003) who explained that linalool and linalyl acetate were the active ingrediat of lavander oil which presented less inhibitory activity at 1000 ug/ml. The weak activity of eucalyptus oil (blue –gum) on mycelial growth was reported by Muller-Riebau *et al.*, (1995). On the other side, it has been reported that four plant extracted oils encluded eucalyptus resulted in completely suppression of growth of *A.flavus* and aflatoxin production Michail *et al.*, (1994). Ceruti *et al.*, (1982) indicated that three kinds of eucalyptus oils inhibited the growth of many fungi i.e., *Fusarium oxysporum*. Pattnaik *et al.*, (1996) investigated that eucalyptus oil was effective against *Fusarium oxysporum, F.solani, Helminthosporium compactum, Macrophomina phasolina, Sclerotium rolfsii* and other post –harvest fungi. In addition, the results concluded that the essential oils act as antimicrobial agents due to main characters: the first is their natural origin to be safety and the environment, and the second that it is difficult for the pathogens to develop resistance to such a mixture of oil components.

All the essential oils tested caused, to different extents, a reduction in seed germination. The mode of action in preventing germination is not known, but it is clear that the oils cannot be used for seed preservation but only to preserve seeds used for human or animal consumption.

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التأثير الايجابى للزيوت النباتية الطيارة علـى الممرضـات الفطريـة لقاطنـات البذوروالتربة

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تم الحصر الحقلى لبعض الفطريات المحمولة ببذور بعض المحاصـيل المنزرعـة بـالا راضى الجديدة المستصلحة غرب الدلتا، وقد استخدمت طريقتين نموذجيتين فى اختبارات صـحة وسلامة البذور لتقدير مدى حدوث تلك الامراض الناجمة عن تلك الفطريات على كل محصول .

تتجه التقنية الحديثة الان نحو استخدام المركبات الطبيعية كمثبطات فطرية وبذلك تم تقييم ستة من الزيوت الطيارة لهذه الصفات وأظهر زيت الكارفون اعلى تثبيطـا للفطريـات المختبـرة مقارنة ببقية الزيوت المختبرة مثل زيت الزعتر و الكراوية والنعناع الفلفلـى واللافنـدر وزيـت الكافور على التوالى ، حيث اظهر زيت الكارفون اقل تركيز مثبط عند (٥٠ جزء فى المليـون) بينما كان اعلى تركيز مثبط له عند (٤٠٠ جزء فى المليون) وذلك للاجناس الفطريـة المختبـرة اسكوكيتة بيزى وسيفالوسبوريوم مايدز وكوليتـوتريكم ديمـاتيم و فيوزاريـوم اوكسيسـبورم و ماكروفومينا فاسولينا. وأعطى كلا من زيت الزعتر والكراوية تأثيرا مثبطا للنمو الفطـرى عنـد تركيز (٨٠٠ جزء فى المليون) وباستثناء زيت الكافور فان التركيزات الاعلـى لبـاقى الخمـس زيوت المختبرة كان لها نشاطا مقللا ومثبطا لتلك النموات الفطرية من قاطنات البـذور. اظهـرت اختبارات تعرض البذور للمعاملة لفترات أطول بالزيوت الطيارة نقصا فى نسبة الانبات.