

## **EFFECT OF INTERCROPPING OF PEA WITH SOME MEDICINAL PLANTS ON MICROBIAL COMMUNITY OF SOIL, DAMPING-OFF AND DOWNY MILDEW DISEASES, UNDER BEHEIRA GOVERNORATE CONDITIONS**

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### **ABSTRACT**

Isolation from diseased roots of pea plants collected from three locations at El Beheira governorate indicated that, the most frequently isolated fungi are *Fusarium solani* and *F. oxysporum* with the averages of 24.07% for both followed by *Alternaria solani* and *Rhizoctonia solani* with 14.81% and *F. moniliforme* with 9.26%, respectively. The disease severity of downy mildew, caused by *Peronospora viciae* was recorded during 2007/2008 and 2008/2009 at Etay Elbaroud, Badr and Kom Hamada provinces and the averages were 28.37, 22.24 and 13.27%, respectively. The essential oils of Cumin, Caraway and Anise showed the most reducing effect for the mycelial growth of *F. solani*, *F. moniliforme* and *Sclerotium rolfsii* *in vitro* with the averages of 70.99, 21.92 and 14.96%, respectively. The highest significant reduction of the spores number of *F. solani* and *F. moniliforme* was observed with the essential oils of *Cuminum cyminum*, *Carum carvi* and *Foeniculum vulgare*. In addition essential oils of *C. cyminum* and *C. carvi* reduced the number of sclerotia of *S. rolfsii*.

Intercropping of pea with medicinal plants was studied in field trials through two successive seasons. Cropping pattern side/side combination showed certain reduction of pea damping-off disease and disease severity of downy mildew than sole pea (check). Total damping-off was reduced using intercropping with *C. carvi*, *F. vulgare* and *Pimpinella anisum* compared with sole pea with averages of 39.19, 48.00 and 48.29% during 2007/2008 and 33.46, 39.33 and 40.33% during 2008/2009, respectively. Intercropping pea with medicinal plants *F. vulgare*, *C. carvi*, *Anethum graveolens* and *P. anisum* were more effective in decreasing disease severity of downy mildew with the averages of 20.20, 20.58, 21.27 and 22.83% during 2007/2008, respectively compared with check (sole pea).

Pea intercropped with some medicinal plants could regulate soil microbial community such as actinomycetes, bacteria and fungi effectively. The results showed that soil rizosphere was improved, and the fungal diversity differed. *Fusarium* spp. and *R. solani* were found only when pea intercropped with *Cuminum cyminum* and *Nigella sativa*. No familiar pathogens were found with intercropping with *C. carvi*, *F. vulgare* and *P. anisum*. The CFU of bacteria in case of intercropping with *C. carvi* and *N. sativa* was the highest during the two growth stages of pea, and that of actinomycetes in all six intercropping treatments was increased during the flowering stage of pea. The use of intercropping system in pea with medicinal plants as a mean for natural disease control is discussed.

Intercropping pea with *C. carvi* increased pea fresh and dry weight in addition to the weight of 100 seeds for both seasons (2007/2008 and 2008/2009) more than other intercropping treatments and sole pea. In contrast, the intercropping with *C. cyminum* and *N. sativa* had the least values.

**Keywords:** Pea, essential oils, medicinal plants, intercropping, soil microbial community, downy mildew.

## INTRODUCTION

Pea (*Pisum sativum* L.) is a major annual pulse crop of temperate region of the world and was originally cultivated in the Mediterranean basin Smartt (1990) and Sardana *et al.* (2007). Pea crop is prone to a number of diseases such as rhizoctonia seedling blight, bacterial blight, acochyta foot rot, downy mildew, powder mildew, pythium blight, *aphanomyces* root rot, wilt and root rot diseases caused *Fusarium* species (Hagedorn, 1991).

Pea is one of the most important winter vegetable crops in Egypt. It grows well all over the Egyptian provinces and cultivated for green pods and dry seeds. Pea, like other cultivated crop, is susceptible to several diseases such as damping-off and root-rot caused by *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, *F. moniliforme* and *Pythium debaryanum*. (Nour-Jehan 2003).

*Fusarium* wilt, near wilt and root rot diseases are a major cases, this pathogen has caused partial to complete loss of pea crop and is capable of causing such damage to other crops as well (Lestie and Summerell, 2006 and Smith, 2007).

Abdala *et al.* (1992) reported that *Sclerotium rolfsii* Sacc, *Rhizoctonia solani* Kuhn and *Macrophomina phaseolina*, (Tassi) Goid were the main soil borne pathogens responsible for causing damping-off and root rot diseases in grown pea plants.

Downy mildew has become the major disease of pea crop, especially in the northern governorates over the last decade causing significant losses in the yield of pea and its quality. Pea growers in Egypt has claimed that downy mildew diseases kills high numbers of their plants before flowering, in addition to the visible losses in the yield and its quality (Sahar 1995).

Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cown, 1999). The use of plant materials as fungicides is of a great importance and needs more attention (Boddle, 1982) and various plant products like gum, oils, resins etc. are used as fungicidal compounds. Some of the volatile oils, which often contain the principal aromatic and flavoring components of herbs and spices, have been recommended as plant based antimicrobials to retard microbial contamination and reduction in spoilage of food commodities (Malkhan, *et al.*, 2012). Ozan (2003) proved antagonistic properties of some essential oils such as Sage, Laurel, Dill, Cumin, Fennel and Thyme in controlling *Botrytis cinerea*.

In addition, increased frequency of chemical application leads to development of pathogen resistant strains and accumulation of residues in produce and resulting in risks to health, environment, and non-target organisms (Burkett-Cadena *et al.*, 2008).

Intercropping ideally allows for improved resource use and beneficial interactions between the crops. In the other words, light, water and nutrient are used by the crops instead to the weeds and some plants enhance the growing environment for their companion. Crop plant cultivar mixtures work to minimize the spread of plant diseases by reducing the quantity of susceptible host plants (Altieri, 1999 and Hauggaard *et al.*, 2007). Benefits of

intercropping include optimal use of resources, stabilization of yield, weed suppression, improved soil fertility conservation, and higher economic returns (Blaser *et al.*, 2007 and Kabura *et al.*, 2008). However, information on the role of intercropping on disease suppression is scanty.

Therefore, the objective of this study was to evaluate the efficacy of intercropping some medicinal plants with pea in the management of damping-off and downy mildew diseases moreover, the effect of intercropping medicinal plants on the total count of microorganisms in the rizosphere of pea plants. Also, to evaluate the effect of some essential oils *in vitro* on the linear growth and sporulation of *Fusarium solani*, *F. moniliforme* and sclerotial formation of *Sclerotium rolfsii*. The study aimed to reduce the use of fungicides and the environmental pollution using the alternative methods for minimizing damping-off and downy mildew severity on pea by intercropping with some medicinal plants.

## **MATERIALS AND METHODS**

### **1- Survey of pea root rot and downy mildew diseases in different locations:**

Pea rotten roots and wilted plants were collected from different localities that cultivated with pea at El-Beheira governorate namely Bader, Etay El-Baroud and Kom Hamada. Survey was conducted during seasons 2007 / 2008 and 2008 / 2009. 10 samples of naturally infected roots were collected from each field and the isolation, purification and identification of the different pathogenic fungi associated with samples were carried out.

#### **a- Sampling and isolation from infected seedlings and roots:**

Infected parts were cut into small pieces, washed thoroughly with running water to remove any adhering soil particles. These pieces were surface sterilized by immersing in 5% sodium hypochloride solution for 2 min., then washed several times in sterilized water and dried between sterilized filter papers. Four surface sterilized pieces were aseptically transferred on potato dextrose agar medium (PDA). Plates were incubated at 27C° for 3 – 7 days and observations were recorded (Christensen, 1957). Hyphal-tip of grown fungi were transferred individually to new PDA plates (Riker and Riker, 1936) and then identified according to their morphological and microscopical characters as described by (Gilman, 1957 and Jens *et al.*, 1991). Identification was confirmed by the Department of Mycology, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

Downy mildew severity on pea was surveyed on plants of different cultivars grown in fields at El-Beheira governorate during autumn seasons of 2007/2008 and 2008/2009 with three locations, each location was represented with five fields. Downy mildew severity was monitored weekly starting from the appearance of first symptoms. Twenty samples were randomly collected from each field and the disease severity was calculated, using 0-9 rating scale based on the percentage of leaf area affected (Warkentin, *et al.* 1996), where 0= no infection, 1= < 1%, 2= 1-5%, 3= 5-10%,

4= 10-20%, 5= 20-40%, 6= 40-60%, 7= 60-80%, 8= 80-90%. 9=> 90% of leaf area affected.

## 2- Effect of essential oils on the linear growth of the pathogens:

The effect of essential oils of Dill (*Anethum graveolens* L.), Caraway (*Carum carvi* L.), Cumin (*Cuminum cyminum*), Fennel (*Foeniculum vulgare* Mitter), Anise (*Pimpinella anisum* L.) and Black cumin (*Nigella sativa* L.) (Table 1) on the mycelial growth of the tested fungi namely *Fusarium solani*, *F. moniliforme* and *Sclerotium rolfsii* were carried out under laboratory conditions. The crude oils were added to autoclaved PDA medium at (10000 ppm) prior to pouring the media medium (Thakur *et al.*, 1989). Amended medium was poured into sterilized Petri dishes of 9 cm diameter at the rate of 15 ml/Petri dish and others without any essential oil were prepared and served as control. Petri dishes were inoculated with equal discs (4 mm in diameter) of each tested fungi and incubated at 27°C and the linear growths were measured as mentioned by (Atia, 1995). Inhibition percentage of mycelial growth is evaluated by comparing the colony diameter of poisoned plate (with essential oils) and non-poisoned plate and calculated using the formula given below (Das *et al.*, 2010).

$$\text{Mycelial inhibition} = \frac{\text{Mycelial growth}_{\text{control}} - \text{Mycelial growth}_{\text{treatment}}}{\text{Mycelial growth}_{\text{control}}} \times 100$$

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Mycelial growth<sub>control</sub>

**Table (1). Scientific, English, Arabic names of the plants, their oils and their families**

Scientific name	English name	Family	Arabic name
<i>Anethum graveolens</i>	Dill	Apiaceae	شبيب
<i>Carum carvi</i>	Caraway	Apiaceae	كراوية
<i>Cuminum cyminum</i>	Cumin	Apiaceae	كمون
<i>Foeniculum vulgare</i>	Fennel	Apiaceae	شمر
<i>Pimpinella anisum</i>	Anise	Apiaceae	ينسون
<i>Nigella sativa</i>	Black cumin	Ranunculaceae	حبة البركة

## 3- Effect of essential oils on sporulation and sclerotial formation:

The tested fungi, *F. solani*, *F. moniliforme* and *S. rolfsii* were plated on PDA medium amended with the different essential oils and incubated for 10 days at 27 C°

For *F. solani* and *F. moniliforme* spore suspensions were prepared by adding 10 ml sterilized water and the average number of micro and macro conidia were recorded by using haemocytometer slide (Hansen, 1926 ) and *S. rolfsii* were observed for their different numbers of sclerotia.

## 4- Field experiments:

These experiments were carried out in open field in Etay El-Baroud Agricultural Research Station at El-Beheira governorate during two successive growing seasons 2007/2008 and 2008/2009. The experiments were designed to study the effect of intercropping medical plants with pea cultivar Master B on damping-off and downy mildew. And also on the total count of microorganisms in the rhizosphere of pea roots.

Experimental unit comprised four plots each plot had 4 rows (each 7 miter long and 0.7 m wide =14.70 m<sup>2</sup>). Pea was sown in mid of October in 2007/2008 and 2008/2009, three seeds/ hill and hills were spaced at 30 cm apart in east of row while, the west side was sown with alternative medicinal plants, i.e. Dill (*A. graveolens* L.), Caraway (*C. carvi* L.), Cumin (*C. cyminum*), Fennel (*F. vulgare* Mitter), Anise (*P. anisum* L.) and Black cumin (*N. sativa* L.) control (as check) was sown with pea seed alone. In both seasons, the normal agricultural practices of growing pea including fertilization and irrigation were followed. The experiments design used was a randomized complete block design with four replications. Plants were left to natural infection with downy mildew during the growing seasons, plants were examined, and visually scored using 0-9 rating scale described above.

The recorded data were:

- The pre-emergence damping-off was assessed after 21 days from sowing.
- The post-emergence damping-off after 35 days from sowing.
- The fresh and dry weights were recorded after 50 days from sowing.

#### **4.1- Effect of intercropping medicinal plants on the total count of microorganisms in the rhizosphere of pea roots:**

The total numbers of cultivable bacteria, fungi and actinomycetes were determined according to colony forming units (CFU) on agar plates using dilution plate methods. Microorganisms were counted in soil at two stages of plant growth; vegetative stage 30 days after sowing, and flowering stage 60 days after sowing. The root system was gently shaken to collect excessive adhering soil particles. Ten grams from these particles were added to 9 milliliter sterilized distilled water in a 250 ml. conical flask. They were shaken thoroughly on a mechanical shaker for 15 minutes. This approximately gave a dilution of 10<sup>-1</sup> concentrations according to (Skinner *et al.*, 1952). In order to investigate the presence of fungal genera, serial dilutions of soil suspensions were conducted to 10<sup>-3</sup>. One ml of the latter suspension was placed in a sterilized Petri-dish before pouring Martin 's medium (Martin, 1950).

To count the total bacteria existing in the rhizosphere, soil extract yeast agar medium, modified after (Skinner *et al.*, 1952) was used, then one ml of the rhizosphere soil suspension was diluted till 10<sup>-6</sup>. One ml of the last dilution was plated on the soil extract yeast medium.

In order to count actinomycetes, Jensen 's medium was used as mentioned by (Allen, 1953). One ml of the rhizosphere soil suspension 10<sup>-5</sup> was placed in a sterilized Petri-dish before pouring Jensen 's medium, and incubated at 28°C for seven days.

#### **5- Statistical analyses:**

Data obtained were subjected to the statistical analysis according to the standard methods recommended by (Gomez and Gomez, 1984) using the computer program (costate). Means were compared using L.S.D. at the level 5% of probability.

## RESULTS AND DISCUSSION

### 1- Survey of pea root rot and downy mildew diseases in different location:

Results in Table (2) show that 108 isolates from soil borne fungi were isolated from rotten and wilted roots of pea plants cultivated at three locations from El-Beheira governorate. The collected isolates were identified and ranked in descending order as *Fusarium oxysporum* and *F. solani* (13) isolates which showed the highest frequency of 24.07 % for both followed by *Alternaria solani* and *R. solani* (8) isolates with frequency of 14.81% for both then *F. moniliforme* (5) isolates, *Pythium ultimum*, *Macrophomina phaseolina* (3) isolates and *S. rolfsii* (1) isolates with frequencies of 9.26, 5.56, 5.56 and 1.85%, respectively. The number of isolated fungi was the highest with samples of Badr followed by Kom-Hamada and Etay-El baroud.

**Table (2): Number of isolates (N) from rotten and wilted roots of pea and their frequency% (F) of three locations at El-Beheira governorate.**

Location Isolated fungi	Etay-El baroud		Badr		Kom Hamada		Total N	Average of frequency
	N	F%	N	F%	N	F%		
<i>A. solani</i>	2	14.29	4	16.67	2	12.50	8	14.81
<i>F. solani</i>	1	7.14	6	25.00	6	37.50	13	24.07
<i>F. oxysporum</i>	5	35.71	7	29.17	1	6.25	13	24.07
<i>F. moniliforme</i>	1	7.14	3	12.5	1	6.25	5	9.26
<i>M. phaseolina</i>	1	7.14	1	4.17	1	6.25	3	5.56
<i>P. ultimum</i>	1	7.14	1	4.17	1	6.25	3	5.56
<i>R. solani</i>	3	21.43	1	4.17	4	25.00	8	14.81
<i>S. rolfsii</i>	0	0.00	1	4.17	0	0.00	1	1.85
Total	14		24		16		54	

F%: Frequency% = {number of isolated fungus / Total} × 100

Downy mildew severity of pea was surveyed on plants of different cultivars grown in a commercial field at El-Beheira governorates, was represented by three locations under investigation during 2007/2008 and 2008/2009.

Data presented in Table (3) show that, values of disease severity during 2008/2009 were higher than those recorded during 2007/2008. Means of disease severity at the studied locations were significantly different and varied from 15.81 to 29.93% during 2<sup>nd</sup> season 2008/2009. But during the first season (2007/2008) means of disease severity incidence ranged from 10.72 to 26.8%.

**Table (3): Survey of downy mildew severity of pea in field of three locations at El-Beheira during 2007/2008 and 2008/2009.**

Province	Field	Disease severity				
		1st	Mean	2nd	Mean	Grand Mean
Etay-Elbaroud	F1	27.00	26.80	32.1	29.93	28.37
	F2	28.00		25.30		
	F3	25.00		28.50		
	F4	28.25		33.25		
	F5	25.75		30.50		
Badr	F1	20.00	19.74	27.70	24.74	22.24
	F2	15.00		18.00		
	F3	18.70		25.00		
	F4	25.00		23.01		
	F5	20.00		30.00		
Kom Hamada	F1	12.00	10.72	17.30	15.81	13.27
	F2	10.10		14.00		
	F3	8.75		19.00		
	F4	12.25		8.75		
	F5	10.50		20.00		
Grand mean			19.09		23.49	
L.S.D at 5% =						
Region				2.93		
Year				2.39		
Region x Year				Ns		

## **2- Inhibitory effect of essential oils of medicinal plants on linear growth of some pathogenic fungi *in vitro*:**

Data in Table (4) and Fig. (1) indicate that essential oils of *C. cyminum*, *C. carvi*, *P. anisum* and *A. graveolens* gave the highest reduction with averages of 70.99, 21.92, 14.96 and 13.83 %, respectively, whereas, the least effective essential oils in this respect were *F. vulgare* and *N. sativa* with the averages of 7.53 and 6.54%, respectively. These results are in agreement with that obtained by (Bauomy, 2003) who studied several concentrations of Egyptian plant essential oils *in vitro* and *in vivo* against *S. rolfisii* and appeared that anise, avocado, blue gum and thyme oils completely inhibited both mycelial growth and sclerotial germination. Behdani *et al.* (2012) showed that essential oils of anise, cumin, caraway and thyme have a significant effect against *B. cinerea*, on PDA culture, by using the volatile compound production method, essence of cumin, dill, anise, and caraway showed the most inhibition effect. Many other investigators emphasized that the antimicrobial effects of essential oils constitutes are depending on their hydrophobicity and partition in the microbial plasmatic membranes. The effect of specific ions due to their addition in/on plasmatic membrane had a great effect on proton motive force, intracellular, ATP content and all activity of microbial cell, including turgor pressure control, solute transport and metabolism regulation (Lanciotti *et al.*, 2004). The statistical analysis showed that significant differences between fungi, oils of medicinal plants, and the interaction between fungi x oils of medicinal plants were highly significant.

Table (4): Reduction percentage of mycelial growth of *F. solani*, *F. moniliforme* and *S. rolfii* on PDA medium mixed with some essential oils (*in vitro*).

Fungi	Oils of medicinal plants						
	Ck.	<i>A. graveolens</i>	<i>C. carvi</i>	<i>C. cuminum</i>	<i>F. vulgare</i>	<i>P. anisum</i>	<i>N. sativa</i>
<i>F. solani</i>	0.00	35.56	29.26	87.41	19.63	25.26	17.04
<i>F. moniliforme</i>	0.00	3.33	7.04	47.78	0.00	19.64	5.56
<i>S. rolfii</i>	0.00	2.59	29.45	77.78	0.00	0.00	0.00
Mean	0.00	13.83	21.92	70.99	6.54	14.96	7.53

L.S.D at 5% = Fungi (F)  
1.57

Oils of medicinal plants (O)  
6.57

F x O  
1.07

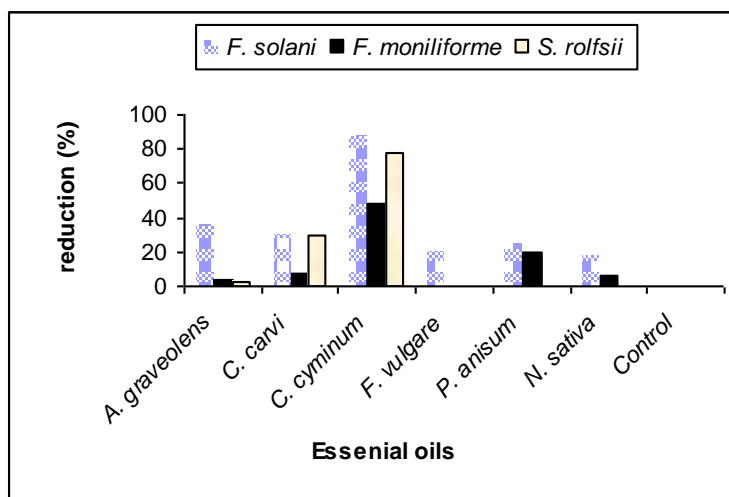
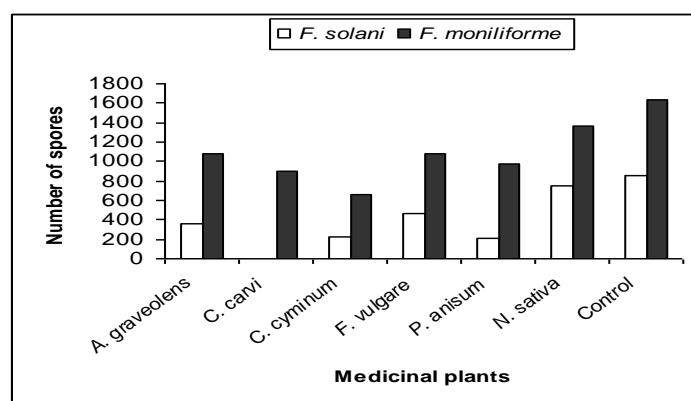


Figure (1): percentage of fungal growth reduction using different plant essential oils ( $10^4$  ppm) on PDA.

### 3- Effect of some essential oils on sporulation and sclerotia formation:

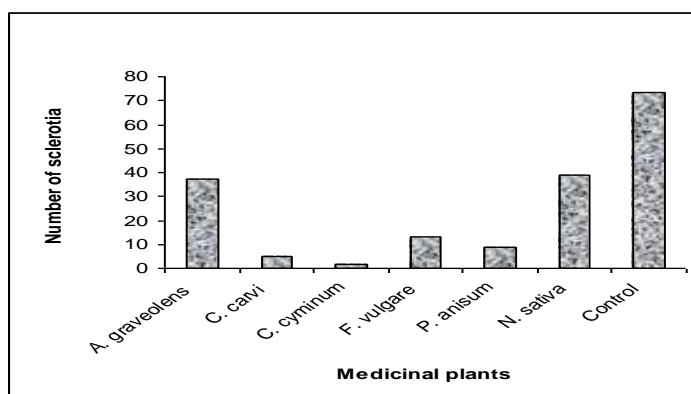
Data in Figure (2) show that all treatments of the tested essential oils of medicinal plants significantly decreased the sporulation of *F. solani* and *F. moniliforme* compared with check treatment. The highest significant reduction in spores number was observed with the essential oils of *C. cuminum* and *C. carvi*. El- Metwally *et al.*(2010) reported that, aminogren, cumin, carnation, garlic and rocket oils completely inhibited *Botrytis fabae* sporulation, these results may be due to the antioxidant materials, which found as a major content of these compounds.





**Figure (2): Reduction of Sporulation of *F. solani* and *F. moniliforme* on PDA medium mixed with some essential oils (*in vitro*).**

In addition, data in Figure (3) showed that essential oils of *C. cyminum* and *C. carvi* and *P. anisum* were more effective in reducing the sclerotia formation of *S. rolfsii*, while the data showed that the highest values of number of sclerotia formation was obtained with essential oils of *N. sativa* and *A. graveolens* compared with the other treatments. These results are in agreement with the findings of (Bauimy, 2003) who found that essential oils of anise, avocado, blue gum, cumin and sour orange leaves completely inhibited the sclerotia germination of *S. rolfsii*. The variation between the antifungal activity of the essential oils and other may be due to capability of this oil to: 1- penetrating the fungal cell and or 2- alternation in the fungal metabolism by mutagenic activity of its constitutes (phenolic esters, etc.).



**Figure (3): Reduction of sclerotia formation of *S. rolfsii* on PDA medium mixed with some essential oils (*in vitro*).**

**2- Field experiments:****1 – Effect of intercropping pea with some medicinal plants:****a – On the number of colony forming units (CFU) of microorganisms in the soil:**

Data in Table (5) appeared that under field conditions all medicinal plants intercropped with pea increased the number of colony forming units (CFU) of the actinomycetes compared with sole pea treatment (check) and the excess was sharply in case of *A. graveolens*, *C. carvi* and *P. anisum*. CFU of bacteria was decreased significantly, as compared with control at both pea growth stages. The same trend cleared in case of fungi especially with *A. graveolens*, *F. vulgare* and *P. anisum*. These results indicated that some medicinal plants might restrain the growth of some soil borne microorganisms, and increase others, which were beneficial to a healthy soil environment for pea. These results are in agreement with the finding of (Chuan *et al.*, 2009) who showed that medicinal plants; *Atractylodes lancea* and *E. pekinensis* had the strongest inhibitory effect on mould compared with Ck (mono-cropping peanut), and the CFU of mould in the treatments intercropped with *Atractylodes lancea* and *E. pekinensis* was decreased by 53.87% and 29.59%, respectively. Alternatively, medicinal plants may influence some other component of the soil microflora, which in turn affects the fungus. The statistical analysis showed that the differences between Microbial colony, Medicinal plants, interval (both period) and interaction between Microbial colony x Medicinal plants x interval were significant.

**Table (5). Effect of medicinal plants intercropped with pea on the microbial population of the soil under field conditions.**

Microbial colony	Intervals	Medicinal plants						
		Ck.	<i>A. graveolens</i>	<i>C. carvi</i>	<i>C. cyminum</i>	<i>F. vulgare</i>	<i>P. anisum</i>	<i>N. sativa</i>
Actinomycetes	a	3	10	10	3	7	13	3
	b	2	12	22	5	10	15	6
Bacteria	a	74	6	40	10	15	4	31
	b	70	12	48	15	17	3	30
Fungi	a	5	2	2	5	1	1	5
	b	6	1	2	2	1	1	5
Total		26.67	7.16	20.67	6.67	8.50	6.17	13.33

L.S.D. 5% = Medicinal plants (M) - Microbial colony (C) - Intervals (I) - M x I - M x C  
 1.47                      3.28                      0.43                      2.69                      1.36  
 = I x C - M x C x I  
 2.76                      0.42

a: vegetative stage      b: flowering stage

In this study, eight of fungal genera were identified in the treatments, including common soil borne fungi; *Aspergillus* spp. and *Alternaria* spp. Data in Table (6) show that the treatment of sole pea (control) had the common soil borne fungi; *Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp in addition to the pathogenic fungi; *Fusarium* spp. and *Rhizoctonia solani*. In case of the intercropping treatments *Fusarium* spp. was isolated from the rhizosphere of the soil of the intercropping with *C. cyminum* only and *R. solani* with the

intercropping with *C. cyminum* and *N. sativa*. While two pathogens of pea in addition to *Alternaria* spp. were found in the mono-cropping soil, those pathogens did not exist in *C. carvi* and *F. vulgare* intercropping treatments. Ismail (2005) reported that the reduction in the dry weight of tested fungi with intercropping might be due to some of these root had inhibitory substances such as phenolic substances.

**Table (6). Effect of medicinal plants intercropped with pea on genera of fungi in soils guiding with morphological colony identification.**

Fungi	Medicinal plants						
	Ck.	<i>A. graveolens</i>	<i>C. carvi</i>	<i>C. cyminum</i>	<i>F. vulgare</i>	<i>P. anisum</i>	<i>N. sativa</i>
<i>Asperigillus</i> spp.	+	+	+		+	+	+
<i>Penicillium</i> spp	+						
<i>Trichoderma</i> spp.			+		+		
<i>Fusarium</i> spp	+			+			
<i>R. solani</i> .	+			+			+
<i>Epicocum</i> spp.					+	+	
<i>Rhizopus</i> spp.						+	+
<i>Alternaria</i> spp.	+	+		+			+

**b – On pea damping off and disease severity of downy mildew:**

This experiment was conducted during the successive seasons (2007/2008) and (2008/2009) to study the effect of intercropping pea with some medicinal plants (*A. graveolens*, *C. carvi*, *C. cyminum*, *F. vulgare*, *P. anisum* and *N. sativa*) on wilt , root rot and downy mildew and some growth characters. In this respect, (Table 7) show that the intercropping in the first season 2007/2008 incited a significant reduction in pre-emergence damping-off of pea intercropped with *C. carvi*, *N. sativa* and *F. vulgare* with averages of 32.76, 44.15 and 44.29%, respectively. Also a significant reduction was cleared in case of the post emergence with the treatments intercropped with *P. anisum*, *A. graveolens* and sole pea with averages of 2.00, 2.58 and 3.00%, respectively. Decreasing percentage of infection with wilt and root rot under intercropping conditions might be due to root exudates of various used crop which caused change in total count microflora in rhizosphere region (Botros, 1988; Ismail, 1994 and Chuan *et al.*, 2009). Similar results were obtained by (El Gindy, 2003) who reported that intercropping four faba bean cultivars individually with each of fenugreek, coriander, anise, and caraway under field conditions decreased the incidence of chocolate spot than control because of the effect of medicinal plants odors with superiority of fenugreek and coriander as medical plants. Also Sahar (2006) reported that intercropping faba bean with each of onion, garlic and caraway reduced significantly chocolate spot severity caused by *B. fabae* under greenhouse and field conditions. In the second season, similar trend of results was detected with minor variation in the treatments rank. Healthy survival pea plants were increased in case of pea intercropped with *C. carvi*, *P. anisum*, *F. vulgare* and *A. graveolens* in first season 2007/2008 with the average of 60.81, 52.00, 51.71 and 51.14%, respectively. On the other hand, the same

four medicinal plants had the same arrangement in the second season with averages of 66.53, 60.67, and 66.53%, respectively. The Statistical analysis showed that the differences between the intercropping treatments were significant for pre and post emergence damping-off and for survival percentage

Data in Table (7) also show that intercropping pea with *C. carvi* and *F. vulgare* plants was more effective in decreasing percentage of disease severity of downy mildew with an average of 20.23% for both in the first season, followed by intercropping with *P. anisum* and *A. graveolens* with the averages of 22.27 and 22.90%, respectively. Also, the same trend cleared in the second season where the intercropping with *F. vulgare* was more effective in reducing disease severity with an average of 20.20% followed by *C. carvi*, *A. graveolens* and *P. anisum* with averages of 20.58, 21.27 and 22.83%, respectively. These results are in agreement with results obtained by (Omar *et al.*, 1993) who found that greatest reduction of chocolate spot disease severity was observed when broad bean was intercropped with caraway followed by broad bean + anise, broad bean + cumin and then broad bean + coriander. Reduction on the disease severity of downy mildew under intercropping conditions might be due to changes in the total count of microflora in phelosphere compared with total count of microflora in phelosphere region in sole cropping. In case of intercropping systems, there are two types of plants, one of them a host to the pathogenic fungi and the other non host, and this lead to reducing total count of spores of the pathogenic fungi and cannot reach to inocula potential caused a disease (Ismail, 1994 and Ismail *et al.*, 2000). Potential active chemical constituents of caraway and peppermint are reported by several workers in different fields. Caraway fruits contain 1-6% of essential oils consisting of about 30 compounds, from which carvone and limonene represent the main portions, about 95%, it is well documented that fruit medicinal and aromatic plants release. (Sedlakova *et al.*, 2003)

**Table (7): Effect of pea intercropping with some medicinal plants on its infection with some damping-off and downy mildew diseases.**

Treatments	Damping-off						Survival		Disease severity of downy mildew	
	Pre		Post		Total damping-off		1st	2nd	1st	2nd
	1st	2nd	1st	2nd	1st	2nd				
Sole pea	47.71	38.67	3.00	2.57	50.77	41.24	49.72	58.00	25.92	24.67
Pea + <i>A. graveolens</i>	46.29	37.34	2.58	3.00	48.87	40.34	51.14	59.67	22.90	21.27
Pea + <i>C. carvi</i>	32.76	25.41	6.43	8.05	39.19	33.46	60.81	66.53	20.23	20.58
Pea + <i>C. cyminum</i>	49.57	41.17	4.15	4.15	53.72	45.32	46.29	54.01	26.00	26.10
Pea + <i>F. vulgare</i>	44.29	35.00	4.00	5.33	48.29	40.33	51.71	60.67	20.23	20.20
Pea + <i>P. anisum</i>	46.00	37.00	2.00	2.33	48.00	39.33	52.00	60.67	22.27	22.83
Pea + <i>N. sativa</i>	44.15	34.83	6.14	7.17	50.29	42.00	49.72	58.01	25.73	25.63
L.S.D at 5%	5.67	3.53	1.92	2.62			2.85	6.34	3.33	4.22

**c - On some pea growth characters:**

Results in Table (8) were confirmed from experiments in seasons 2007/2008 and 2008/2009 concerning fresh weights, the results of the first season revealed variations among the treatments where pea fresh weight was increased significantly in case of intercropping with *C. carvi*, *A. graveolens* and *P. anisum* compared with sole pea. When the plants intercropped with *C. carvi* the average fresh weight was 8.04 (g). In contrast, the lowest fresh weight was found in pea intercropped with *C. cyminum* and *N. sativa*. These results are close to that obtained in the second season, where the highest value of fresh weight was found in pea + *C. carvi* with an average of 7.96 (g) followed by pea + *F. vulgare*, pea+ *A. graveolens* and pea+ *P. anisum* with averages of 7.91, 7.28 and 7.21(g), respectively. Dry weight of pea in first season showed the highest values with pea intercropped with either *C. carvi* or *A. graveolens*, with the averages of 4.10, 4.03 and in the second season with average of 4.50, and 4.43 (g), respectively. The highest values of 100 seeds were observed with pea intercropped with *C. carvi*, *F. vulgare* and *A. graveolens* with averages of 21.31, 19.91 and 19.11 (g), respectively in the first season. In the second season the same trend was noticed where pea intercropped with *C. carvi*, *A. graveolens* and *F. vulgare* had the highest weight of 100 seeds with the averages of 22.71, 22.11 and 20.44 (g), respectively. Statistical analysis showed that the differences between the intercropping treatments were significant for fresh and dry weight and weight of 100 seeds (g).

**Table (8): Effect of pea intercropped with some medicinal plants on some pea growth characters.**

Treatments	Fresh plant /weight (gm)		Dry plant /weight (gm)		Weight of 100 seeds (gm)	
	1st	2nd	1st	2nd	1st	2nd
<b>Sole pea</b>	6.37	6.94	3.80	4.20	16.99	18.99
<b>Pea + <i>A. graveolens</i></b>	7.55	7.28	4.03	4.43	19.11	20.44
<b>Pea + <i>C. carvi</i></b>	8.04	7.96	4.10	4.50	21.31	22.71
<b>Pea + <i>C. cyminum</i></b>	5.00	5.60	2.45	2.85	16.09	17.61
<b>Pea + <i>F. vulgare</i></b>	6.93	7.91	3.69	4.09	19.91	22.11
<b>Pea + <i>P. anisum</i></b>	7.44	7.21	3.79	4.19	16.26	18.39
<b>Pea + <i>N. sativa</i></b>	5.01	5.68	3.08	3.48	11.92	13.32
<b>L.S.D at 5%</b>	0.71	0.77	0.73	0.73	1.91	2.25

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### تأثير تحميل البسلة مع بعض النباتات الطبية على المحتوى الميكروبي للتربة لموت البادرات ومرض البياض الزغبي تحت ظروف محافظة البحيرة

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أوضح العزل من جذور نباتات البسلة المصابة التي تم جمعها من ثلاثة مراكز مختلفة من محافظة البحيرة، أن أكثر الفطريات المعزولة تعددا كانت فيوزاريوم سولاني وفيوزاريوم اوكسيسبورام بنسبة 24,07% لكل منهما يليها ألترناريا سولاني وريزوتونيا سولاني بنسبة 14,81% لكل منهما وكذلك فيوزاريوم منيليفورم بنسبة 9,26% تم حصر وتطور مرض البياض الزغبي في ثلاثة مراكز من محافظة البحيرة خلال الموسمين 2007/2008 و 2008/2009 وتم تسجيل شدة الإصابة في مركز ايتاي البارود و بدر و كوم حمادة بمتوسط عام 28,37 و 22,24 و 13,27% على التوالي.

تم دراسة تأثير ستة زيوت من النباتات الطبية معمليا على الفطريات الممرضة فيوزاريوم سولاني و فيوزاريوم منيليفورم وسكليروشيم رولفيزياي و وجد أن جميع الزيوت محل الدراسة ( الشبت، الكراوية، الكمون، والشمر، الينسون، وحبّة البركة) لها تأثير مثبط بدرجات مختلفة، و أن زيت الكمون والكراوية و الينسون لهم تأثير خافض للنمو المسليومي لهذه الفطريات بنسبة 14,96- 21,92- 70,99 % على الترتيب

وجد أن أعلى تأثير للزيوت المستخدمة في تقليل عدد الجراثيم للفطريات فيوزاريوم سولاني و فيوزاريوم منيليفورم كان زيت الكمون والكراوية و الشمر على الترتيب. بالإضافة للتأثير خافض لهذه الزيوت على تكوين الاسكلورشيات للفطر سكليروشيم رولفيزياي.

تم دراسة تأثير تحميل النباتات الطبية الشبت، الكراوية، الكمون، الشمر، الينسون، وحبّة البركة مع نبات البسلة في تجارب حقليّة بمزرعة محطة البحوث الزراعية بإيتاي البارود- محافظة البحيرة لمدة عامين متتاليين. وجد أن نظام التحميل المستخدم ريشة/ريشة، قد قلل من نسبة الإصابة بموت البادرات وشدة الإصابة بالبياض الزغبي. وجد أن موت البادرات يقل عند تحميل البسلة+الكراوية و البسلة+الشمر و البسلة+الينسون مقارنة بنمو البسلة منفردة بنسبة 39.19



و 48.55 و 48.29 % على الترتيب خلال الموسم الزراعى 2008/2007 وقلل من نسبة الإصابة بنسبة 33,46 و 39,33 و 40,33 % على الترتيب خلال الموسم الزراعى 2009/2008. تحت ظروف التجربة الحقلية وجد أن تحميل البسلة مع النباتات الطبية يؤدي الى تغير المحتوى الميكروبي في التربة المحيطة بجذور البسلة (الريزوسفير) بإختلاف النباتات الطبية المحملة. وقد عزلت الفطريات الممرضة الفيوزاريوم- والريزوكتونا من التربة المحيطة بجذور البسلة المحملة مع الكمون وحب البركة ولم تعزل من التربة المحيطة بجذور البسلة المحملة مع الكراوية والشمر و الشبت و الينسون أثناء مرحلة التزهير. وكذلك نقص عدد مستعمرات البكتريا في حالة التحميل مع الينسون والشبت. وجدت زيادة الاكتينوميسيتات المعزولة مع البسلة المحملة مع كل النبات الطبية في مرحلة التزهير خاصة مع التحميل مع الكراوية والينسون. تحميل البسلة + الكراوية يزيد من الوزن الرطب والجاف لنباتات البسلة وكذلك وزن المائة بذرة لها في كلا الموسمين 2008/2007 و 2009/2008 مقارنة بنمو البسلة بفردة بدون تحميل. وعلى العكس من ذلك وجد أن اقل مستوى من الوزن الرطب والجاف وكذلك وزن المائة بذرة كان عند تحميل البسلة مع الكمون وحب البركة.

#### **قام بتحكيم البحث**

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