EFFECT OF USING SOME BIOLOGICAL PRODUCTS OF (*NIGELLA SATIVA* L.) ON IMPROVING CUCUMBER PRODUCTIVITY AND THE MICROBIAL ACTIVITY ASSOCIATED WITH PLANT GROWTH

El-Sayed, Mahmoud A.M.^{*1} and Mohamed R. Hafez²

¹Department of Soil Fertility and Microbiology, Desert Research Center, El-Matareya, Cairo, Egypt

²Department of Plant Production, Desert Research Center, El-Matareya, Cairo, Egypt

^{*}E-mail: mahalyed@yahoo.com, mahalyeg30@gmail.com

wo field experiments were conducted to study the effect of black cumin (Nigella sativa L.) seed oil and seed cake on cucumber productivity (Prince cv.) and associated microorganisms. Seed cake was applied as soil addition before transplanting at rates of 0, 5, and 10 g per plant, while seed oil in 0, 0.5, 1and 1.5% solutions was applied as foliar application three times every 10 days beginning within 30 days of transplanting. Plants receiving the highest levels of seed cake (10 g) and seed oil (1.5%) showed high density of total bacterial counts; Azotobacter chroococcum, Azospirillum brasilense and Bacillus megaterium, and high rates of CO₂ evolution. However, the same treatments gave the lowest density of total fungal counts, including some pathogenic fungi (Fusarium, Rhizopus and Aspergillus niger). Both soil amendment and foliar application improved the growth characters of the cucumber, including the percentage of stand, the number of branches, plant height, the number of flowers and total chlorophyll as well as, the yield and fruit size.

Keywords: cucumber, biological products, *Nigella sativa*, microbial activity, growth, productivity

Cucumber (*Cucumis sativus* L.) is a widely cultivated plant in the gourd family Cucurbitaceae. The plant has a creeping vine, which bears cylindrical edible fruits. It could be cultivated year-round in desert lands by employing open field and green houses. Cucumber plants are susceptible to fungal diseases.

Organic fertilization is a very important technique in this respect. Significant attentions have been paid by both growers and agricultural authorities to replace synthetic chemical fertilizers with natural organic fertilizers, which appear to be safe for the environment and improve soil fertility (David, 2002).

Also, plant extracts have been demonstrated to increase the productivity of tomato (plant length, leaf area, the number of branches, fresh and dry weight/plant and chlorophyll content) as well as the number of flowers and fruits (El-Dougdoug et al., 2007). Powdery mildew (PM) of cucumber was reduced from 52 to 7.7% for plants treated with 0.5% black seed oil (BSO), to 18.6% for plants treated with rape seed oil, and to 20% for plants treated with paraffin oil. Therefore, it is important to give more attention to BSO and other plant oils as an alternative disease control method in organic food production (Hafez, 2008). The use of BSO reduced PM disease by about 52%.

The antifungal activity of essential oil of black cumin plants in combination with biocontrol bacteria *Azotobacter* and/or *Bacillus megaterium* and N-fertilizer were examined against four phytopathogenic fungi (El-Sayed, 2006). Also, Thoppil et al. (1998) studied the activity of essential oils from four varieties of *Nigella sativa* against *Fusarium semeticum*, *Aspergillus niger* and *Rhizopus stolonifer*. They observed that all essential oils exhibited activity; the essential oil from *Basilicum* var. *purpurascens* was the most potent.

The obtained data also revealed that plant treated with mixed inoculants of *Azotobacter chroococcum* and *Bacillus megaterium* in the presence of half dose of inorganic N-fertilizer allowed essential oils to have more inhibitory effect against the tested plant pathogenic fungi when compared with addition of full dose of N-fertilizer in combination with separate inoculation. This result was observed in the two seasons for all treatments (El-Sayed, 2006).

Powdery mildew disease is one of the most serious plant diseases, causing large yield losses in a number of crops (Kiss, 2003). The disease affects the leaves, stems and fruits of cucumber (*Cucumis sativus* L.) grown in greenhouses and in the field (Bettiol et al., 2008). Powdery mildew of cucumber is one of the most common foliage diseases, attacking cucumber plants in Egypt and other countries (Harfoush and Salama, 1992; Mosa, 1997; Reuveni et al., 1997 and Verhaar et al., 1997). Using water extract of black cumin (3.0%) led to elimination of TYLCV and produced virus-free tomato by tissue culture technique.

The main objective of the present work was to improve growth, yield and quality of cucumber under North Sinai conditions by addition of seed cake of *Nigella sativa* to the soil and by foliar spray with seed oil. It is also

speculated that these treatments may reduce fungal infection, release nutrients to plant, and stimulate CO_2 evolution.

MATERIALS AND METHODS

This study was carried out in the experimental farm of the Desert Research Center at El Sheikh Zowayed area, North Sinai Governorate, during the two summer growing seasons of 2009 and 2010.

Products from *Nigella sativa* (seed cake and seed oil) were produced under bioorganic agricultural conditions. The results of chemical analysis of seed cake and seed oil are given in table (1 & 2). Prince cv. seeds were sown in early or mid-April. The transplanting date was 25 days from sowing.

Table (1). Physical and chemical properties of essential oils of *N. sativa* untreated (A) and treated with *A. chroococcum* and *B. megaterium* (B).

	Nig	ella sativa	seed oils (%)		
Fractions	A	В	Fractions	Α	В
Acidity % (as oleic acid)	3-35	2-95	α- Thujene	1.60	3.68
Peroxide value (m-equiv.O ₂ /kg sample)	48.33	29.83	α- pinene	0.87	0.84
Iodine value (Hanus)	130.40	130.41	Sapinene	0.87	0.84
Color units Yellow	35.00	35.00	ß- pinene p- cymene	2.50 46.90	4.18 50.15
Red Blue	17.30 11.00	17.30 11.00	d- limonene Longifolene	2.19 3.78	2.25 4.88
Fatty acids % Palmitic acid	13.31	13.25	Longipinene 4-Terpineol	1.17 3.15	1.22 2.43
(C16:0) Stearic acid (C	1.80	1.32	Thymoquinone	28.70	11.87
Oleic acid (C 18:1)	25.21	25.96	Carvacrol	0.00	0.43
18:2)	59.37	59.47	Total	97.52	97.37
(18:3n-2)	0.31	0.00			
Total faty acids %	100	100	1 / 4 1	1.0	• •

(A): Control (without inoculation) (B): Mixed (A. chroococcum and B. megaterium)

The experiment consisted of two factors: Soil application by adding some outputs of *Nigella sativa* (seed cake) with different doses (0, 5 and 10 g seed cake /plant) one week after transplanting, and by spraying solutions

containing different concentrations (0, 0.5, 1 and 1.5%) of oil extracted from *Nigella sativa* seed on the vegetative parts of the plant three times every ten days, the first spray after 30 days from transplanting.

		Amin	o acids	5			
Essential Amin	no acids	Non-Esse	ntial	Macronu	ıtrients	Micronut	trients
(%)		Amino ació	ls (%)	(%	5)	(ppr	n)
Threonine	3.56	Aspartic	2.82	Р	0.88	Fe	413
Valine	4.46	Serine	3.67	Mg	0.71	Mn	40
Methionine	4.98	Proline	1.69	K	0.58	Z	80
Isoleucine	3.63	Glycine	10.39	Ca	0.27		
Leucine	4.44	Alanine	10.92	Ν	0.19		
Phnylalanine	3.71	Cystine	0.036				
Lysine	4.35	Tyrosine	0.025				
Arginine	2.06	Glutamic	21.60				
Histidine	2.90						

Table (2). Physical and chemical properties of seed cake of *N. sativa*.

A split plots design with three replicates was used. The seed cake concentrations were assigned in the main plots, whereas the foliar spray by seed oil treatments were distributed in sub plots.

The experiment included 36 experimental units, 3 seed cake \times 4 foliar spray treatments \times 3 replicates. The experimental plot area was 10.5 m²; plant distance was 50 cm apart. The analysis of soil and irrigation water was performed according to Jackson (1958), Piper (1950) and Richards (1954), respectively, and are presented in tables (3, 4 & 5). Determination of antimicrobial activity of extracted oil was studied according to Jacobs and Gerstein (1960).

A drip irrigation system was used. The length of line was 10.5 m, and the width between lines was 1 m.

Seed cake was added to soil one week after transplanting. Cucumber plants were sprayed three times with an aqueous solution of seed oil during each growing season. The first spray was performed after 30 days from transplanting, whereas, the second was applied three times every 10 days then 30 days. All replicates received similar agricultural practices with regard to cultivation, fertilization, irrigation, and pest disease control.

Chicken manure stable compost was used as fertilizers at 20 m³ per feddan, 100 kg calcium super phosphate per feddan and 100 kg agricultural sulfur before planting. Ammonium sulfate at 300 kg, potassium sulfate at 150 kg and 15 kg phosphoric acid were divided into small quantities and added weekly in drip irrigation system during the growing seasons.

	Sherk		ayeu area.							
Donth	E.C.	Sol	uble anions	s So	oluble catio	ns	ом	0 C	Availahl	٥
(cm) PH	mmhos/	((meq./L.)		(meq./L.)			0.C.		CaCO ₃ %
(CIII)	cm	$\text{CO}_3^=\text{H}$	$I_2 CO_3^- Cl^- S$	$O_4^{=}Ca^+$	$^{+}Mg^{++}Na^{+}$	\mathbf{K}^+	/0	/0	1 205	
0 - 308.47	0.90	-	1.91 3.004	.744.0	8 0.82 4.500	0.25	0.52	0.30	7.00	2.80

 Table (3). Chemical analysis of the soil at North Sinai Research Station-El

 Sheikh Zowayed area.

 Table (4). Particle size distribution of the soil at North Sinai Research

 Station - El Sheikh Zowayed area.

Fine sand	Coarse sand	Silt	Clay	Texture
(%)	(%)	(%)	(%)	
31.96	58.16	7.43	2.25	Sandy

 Table (5). Chemical analysis of irrigation water at North Sinai Research

 Station -El Sheikh Zowayed area.

PH	EC	2.		Solub	le A	nions		Soluble cations					
				(n	neq./	L)		(meg	./L)				
	ppmmm	hos/cm	C03	H ₂ CO ₃	Cľ	$SO_4^{=}$	Ca++	Mg^+	Na^+	\mathbf{K}^{+}			
								+					
7.4	2491	3.892		2.72	8.7	33.38	7.0	10.5	25.3	2.01			
-													

1. Microbiological Determinations

Rhizosphere soil samples were collected from soil after germination at 30, 60 and 90 days from transplanting to estimate the number of different microbial types and total microbial counts on Bunt and Rovira agar medium (Bunt and Rovira, 1955), total fungal counts on Martin's medium (Allen, 1959), *Azotobacter* spp. on nitrogen deficient medium (Abd El Malek and Ishac, 1968), *Azospirillum* spp. on semi-solid malate medium (Dobereiner, 1978). *Bacillus* spp. was quantified on modified Bunt and Rovira medium (Abd El-Hafez, 1966) using the dilution frequency method. CO₂ evolution was measured according to Pramer and Schmidt (1994).

2. Determination of Antimicrobial Activity of Extracted Oil

The effect of the extracted oil of black cumin with concentrations of 500, 750, 1000 and 1250 ppm on the growth of pathogenic fungi was studied by measuring the inhibition zone and minimum inhibitory concentration as described by Sleigh and Timburg (1981).

Disc diffusion method was used for determining the antimicrobial activity of extracted oil against the pathogenic fungi (*Aspergillus niger*, *Fusarium semeticum and Rhizopus stolonifer*) as described by Jacobs and Gerstein (1960).

3. Plant Parameters

Five plants were randomly taken from each experimental plot after 50 days from transplanting. Data on vegetative growth, fruit yield, and fruit quality were recorded as following:

3.1. Vegetative growth

1. Percentage of stand

2. Fresh and dry weight (g)

3. Plant length (cm)

4. Total chlorophyll using chlorophyll meter according to A.O.A.C. (1990)

5. Number of flowers/ plant

3.2. Total yield and fruit quality

During each experimental season all fruits from each plot were harvested ten times and total fruits were collected. The yields per plant and per plot as well as per feddan were then calculated. The data that have been taken were as following:

1. Fruit length (cm)

2. Average fruit weight (g)

3. Fruit diameters (mm)

4. Total yield (g/plant or ton/fed)

5. Dry weight per fruit (g)

4. Statistical Analysis

Data were analyzed using ANOVA according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

1. Effect of Using Some Biological Products of *Nigella sativa* L. on Total Counts of Bacteria and Fungi

Data presented in table (6) show the effect of using seed cake and seed oil of *Nigella* at different levels on total bacterial counts during two seasons and at different stages of plant growth. With respect to adding seed oil at different rates, data show that increasing seed oil addition in the absence of seed cake increased total bacterial counts for all stages of plant growth.

In the presence of seed cake at 5 g with or without seed oil, total count also increased more than in case of seed cake absence. Increasing seed cake to 10 g /plant with adding seed oil levels at 1.5% gave the highest density of total bacterial counts at different stages of plant growth, reaching 99, 277, 259×10^5 CFU/g dry soil for 30, 60 and 90 days for the first season and 105, 297, 265×10^5 CFU/g dry soil at the same stages during the second season.

	Seeds oil	Total Bacte	erial coun	its (Cour	nts × 10°	CFU/g o	dry soil)	Average as total		total
Seeds		Se	ason 1		2	Season	2	micr	obial co	unts
dregs		30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day
	Zero	32	39	36	35	47	40	34	43	38
Control	0.5%	39	48	46	44	56	50	42	52	48
Control	1%	45	65	62	49	77	62	47	71	62
	1.5%	48	67	65	52	80	66	50	74	66
	Zero	37	70	65	50	90	81	44	80	73
5 a	0.5%	49	99	89	57	115	105	53	107	97
5 g	1%	60	131	110	69	135	120	65	133	115
	1.5%	65	135	115	71	140	127	68	138	121
	Zero	55	121	97	61	139	120	58	130	109
10 a	0.5%	83	215	210	89	256	227	86	236	219
10 g	1%	95	269	255	101	295	262	98	282	259
	1.5%	99	277	259	105	297	265	102	287	262
		-		2						

Table (6). Effect of using some biological products of Nigella sativa L. ontotal bacterial counts during 2009/2010 seasons.

*Initial total bacterial count was 65×10^{2} (CFU/g dry soil).

While the results presented in table (7) show the impact of using seed cake and seed oil of *Nigella* at different levels on total fungal counts during the two seasons and at different stages of plant growth. With respect to adding seed oil at different rates, data show that by increasing seed oil addition, in the absence of seed cake, fungi decreased at different stages of plant growth during two seasons.

 Table (7). Effect of using some biological products of Nigella sativa L. on total fungal counts during 2009/2010 seasons.

 Seads cil.
 Total fungal counts (Counts × 10² CEU/a day)

\searrow	Seeds oil	Total f	ungal co	ounts (C	ounts ×	10 ² CF	U/g dry	Ave	rage as	total
Seeds		5	Season	<u> </u>	<u>n)</u>	Season	2	fun	gal cou	nts
dregs		30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day
	Zero	70	80	84	60	70	80	65.0	75.0	82.0
Cont	0.5%	47	40	36	50	40	33	48.5	40.0	34.5
Cont	^{roi} 1%	38	32	28	40	35	31	39.0	33.5	29.5
	1.5%	33	22	20	37	31	28	35.0	26.5	24.0
	Zero	35	29	28	37	31	29	36.0	30.0	28.5
-	0.5%	23	18	16	25	20	15	24.0	19.0	15.5
5 g	⁵ 1%	20	16	14	22	17	13	21.0	16.5	13.5
	1.5%	16	13	11	15	12	10	15.5	12.5	10.5
	Zero	30	22	17	31	28	22	30.5	25.0	19.5
10	0.5%	16	14	11	22	16	11	19.0	15.0	11.0
10 g	^g 1%	13	10	8	14	11	9	13.5	10.5	8.5
	1.5%	11	8	5	10	5	3	10.5	6.5	4.0

*Initial total fungal count was 90 \times 10 ² (CFU/g dry soil).

In the presence of seed cake at 5 g with or without seed oil, total counts of fungi also decreased more than in case of absence of seed cake. Increasing seed cake to 10 g /plant with adding seed oil levels at 1.5% gave the lowest density of total fungal counts at different stages of plant growth and during the two growing seasons, reaching 11, 8, 5×10^2 CFU/g dry soil for 30, 60 and 90 days for the first season and to 10, 5, 3×10^2 CFU/g dry soil at the same stages during the second season.

The recorded data indicated that control treatment without using any biological products gave higher growth of fungi. This may be due to the production of siderphore compounds, which inhibit fungal spore germination via causing unavailability of nutrients (Jagnow, 1991) or due to the lyses of chitinaceous cell walls of pathogenic fungi by specific enzymes produced by bacteria (Nelson et al., 1986).

2. Effect of Using Some Biological Products of Nigella sativa L. on Azotobacter spp. and Azospirillum spp. Densities

Data in tables (8 and 9) show that when seed oil was applied in the absence of seed cake, the numbers of *Azotobacter* spp. and *Azospirillum* spp. increased at different stages of plant growth during the two seasons. In the presence of seed cake at 5 g with or without seed oil, *Azotobacter* spp. and *Azospirillum* spp. also increased more in case of seed cake absence.

 Table (8). Effect of using some biological products of Nigella sativa L. on

 Azotobacter densities during 2009/2010 seasons.

s	eeds oil	Azotoba	<i>cter</i> Den	Average Azotobacter						
Seeds		S	eason 1	-		Season	2	-	counts	
aregs		30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day
	Zer) 11.40	27.60	20.70	16.70	33.40	23.50	14.1	30.5	22.1
Canta	0.5%	ú 13.60	38.70	24.50	22.40	47.30	29.30	18.0	43.0	26.9
Contr	01 1%	20.70	41.40	34.40	29.70	49.10	37.80	25.2	45.3	36.1
	1.5%	22.30	53.50	39.20	31.20	51.80	41.10	26.8	52.7	40.2
	Zere	24.40	55.20	41.40	31.30	58.60	44.60	27.9	56.9	43.0
5 ~	0.5%	39.70	62.40	45.60	41.60	84.30	72.20	40.7	73.4	58.9
5 g	1%	44.90	82.70	72.50	53.40	90.10	77.30	49.2	86.4	74.9
	1.5%	48.80	88.80	75.70	56.90	92.90	80.30	52.9	90.9	78.0
	Zer	49.20	62.40	52.30	50.50	65.80	53.20	49.9	64.1	52.8
10 ~	0.5%	52.40	82.80	76.00	56.40	98.50	80.10	54.4	90.7	78.1
10 g	1%	58.80	92.40	79.80	61.90	125.10	101.40	60.4	108.8	90.6
	1.5%	65.70	95.90	83.60	66.80	129.30	107.10	66.3	112.6	95.4

*Initial Azotobacter densities was 30×10^{2} (CFU/g dry soil).

See	ds oil	Azospiril	lum D	ensities	(Coun	ts × 10	⁴ CFU		Avora	
				g dry s	soil)			. A zogni	- ver ag	;e
Seeds		Se	S	eason	2	Azospu	Azospiritium counts			
dregs		20 dor	60	90	30	60	90	30	60	90
		50 day	day	day	day	day	day	day	day	day
	Zero	14.7	18.6	15.0	15.0	21.0	18.6	14.9	19.8	16.8
Control	0.5%	17.3	24.0	22.0	21.0	28.0	23.9	19.2	26.0	23.0
Control	1%	21.0	28.0	24.5	22.0	34.0	28.0	21.5	31.0	26.3
	1.5%	23.9	34.0	28.0	24.5	36.0	30.5	24.2	35.0	29.3
	Zero	21.1	28.0	24.5	22.4	31.0	26.2	21.8	29.5	25.4
5 a	0.5%	24.5	32.0	30.5	28.0	34.0	30.5	26.3	33.0	30.5
эg	1%	28.0	34.0	32.0	32.0	40.0	36.0	30.0	37.0	34.0
	1.5%	30.5	43.0	34.0	34.0	41.0	39.0	32.3	42.0	36.5
	Zero	28.0	34.0	30.5	26.2	34.0	28.0	27.1	34.0	29.3
10 a	0.5%	34.0	43.0	36.0	36.0	50.0	39.0	35.0	46.5	37.5
10 g	1%	41.5	60.0	43.0	41.5	59.0	41.0	41.5	59.5	42.0
	1.5%	43.0	92.0	64.0	43.0	84.0	50.0	43.0	88.0	57.0

Table (9). Effect of using some biological products of Nigella sativa L. onAzospirillum densities during 2009/2010 seasons.

*Initial Azospirilla Densities was 30×10^{2} (CFU/g dry soil).

Increasing seed cake to 10 g /plant with adding seed oil gave the highest density of *Azotobacter* spp. & *Azospirillum* spp. at different stages of plant growth, with the average densities reaching 66.3, 112.6 and 95.4 ×10⁴ CFU/g dry soil for 30, 60 and 90 days for *Azotobacter* spp. and reaching 43, 88 and 57×10^4 CFU/g dry soil for *Azotobic er spp.* When plants have been treated with 10 g seed cake combined with foliar application with seed oil at 1.5%, gave the highest density of *Azotobacter* spp. and *Azospirillum* spp. at different stages of plant growth, with the average of *Azotobacter* spp. and *Azospirillum* spp. and *Azospirillum* spp. at different stages of plant growth, with the average of *Azotobacter* spp. and *Azospirillum* spp. densities reaching 66.3, 112.6, 95.4, 43.0, 88.0 and 57.0×10^4 CFU/g dry soil, respectively, for 30, 60 and 90 days.

3. Effect of Using Some Biological Products of *Nigella sativa* L. on *Bacillus* spp. Densities

With respect to adding seed oil at different rates, data in table (10) show that increasing seed oil addition in the absence of seed cake increased *Bacillus* spp. densities at different stages of plant growth and during the two seasons.

In the presence of seed cake at 5 g with or without seed oil also increased *Bacillus* spp. densities more than observed in case of absence of seed cake. Increasing seed cake to 10 g /plant with the addition of 1.5% seed oil gave the highest density of *Bacillus* spp. at different stages of plant growth, with the average of *Bacillus* spp. densities reaching 98, 148 and 125 $\times 10^4$ CFU/g dry soil for 30, 60 and 90 days.

Seed	s oil	Bacillus	spp. d	ensities	s (count	$s \times 10^4$	CFU/g	Avera	ge of <i>Ba</i>	icillus.
Seeds dre	20	Se	eason 1	dry s	oil) S	eason	2	spp. counts		
Secus ure		30 day	60	90	30	60	90	30	60	90
	Zero	33	38	35	35	46	37	34	42	36
Control	0.5%	36	41	38	40	50	42	38	46	40
Control	1%	39	44	40	45	55	49	42	50	45
	1.5%	42	49	44	50	63	55	46	56	50
	Zero	40	70	55	50	80	60	45	75	58
5 a	0.5%	51	81	70	63	95	78	57	88	74
5 g	1%	57	95	78	70	105	85	64	100	82
	1.5%	63	105	95	85	115	100	74	110	98
	Zero	55	80	65	70	95	80	63	88	73
10 a	0.5%	64	95	85	90	150	105	77	123	95
10 g	1%	71	105	90	100	160	120	86	133	105
	1.5%	80	115	105	115	180	145	98	148	125

Table (10). Effect of using some biological products of *Nigella sativa* L. on *Bacillus* spp. densities during 2009/ 2010 seasons.

*Initial total *Bacillus* count was 35×10^2 (CFU/g dry soil).

4. Effect of Using Some Biological Products of *Nigella sativa* L. on CO₂ Evolution

Table (11) describes the effect of using seed cake and seed oil of *Nigella* at different levels of microbial activity through CO₂ evolution (μ g / g dry soil / h) in the area of soil surrounding the roots of the plants during the two seasons and at different stages of plant growth.

Table (11). Effect of using some biological products of *Nigella sativa* L. on CO₂ evolution (μg/g dry soil/h) during 2009/2010 seasons.

	Seeds oil		CO ₂	evoluted	(µg/g dr	y soil/ h)		Avorago	of CO a	volutod
			Season	1		Season	2	Average	01 CO2 e	voluteu
Seeds di	regs	30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day
	Zero	13.50	14.30	13.80	14.10	15.60	15.12	13.8	14.95	14.5
Control	0.5%	14.66	15.60	15.10	15.30	16.20	16.10	15.0	15.90	15.6
Control	1%	15.20	16.10	15.80	16.10	16.70	16.14	15.7	16.40	16.0
	1.5%	16.10	16.90	16.70	16.90	17.30	17.10	16.5	17.10	16.9
	Zero	14.70	15.90	15.00	15.20	16.62	16.12	15.0	16.26	15.6
5 a	0.5%	15.10	17.10	16.10	16.12	18.10	17.10	15.6	17.60	16.6
5 g	1%	16.30	18.00	17.20	16.80	19.10	18.10	16.6	18.55	17.7
	1.5%	16.80	18.70	17.60	17.40	19.60	18.35	17.1	19.15	18.0
	Zero	15.70	17.61	16.10	16.10	18.00	17.31	15.9	17.81	16.7
10 -	0.5%	16.20	18.90	17.11	17.33	20.24	18.60	16.8	19.57	17.9
10 g	1%	16.80	19.30	17.70	17.60	21.10	19.10	17.2	20.20	18.4
	1.5%	17.10	20.00	18.10	18.80	21.52	20.00	18.0	20.76	19.1

* Initial CO₂ evolution is 12.10 μ g /100 g soil/24 h.

With regard to adding seed oil at different rates, the data show that increasing seed oil addition in the absence of seed cake increased the rate of

 CO_2 evolution (µg / g dry soil / h) at different stages of plant growth and during two seasons.

In the presence of seed cake at 5 g with or without seed oil, the rate of soil CO_2 evolution also increased (µg /g dry soil /h) compared to soils without seed cake. Increasing seed cake to 10 g /plant with adding oil seed gave the highest quantity of CO_2 activity (µg /g dry soil / h) at different stages of plant growth. The average of CO_2 exchange (µg /g dry soil /h) reached 18, 20.78, and 19.10 for 30, 60 and 90 days when plants were treated with 10 g seed cake combined with foliar application with seed oil at 1.5%.

5. Effect of Using Some Biological Products of *Nigella sativa* L. on Some Plant Pathogenic Fungi

The results in table (12) and Fig. (1) show that using solutions with concentrations of 500, 750, 1000 and 1250 ppm of oil from *Nigella sativa* seeds inhibited the growth of some pathogenic fungi to cucumber plant including *Fusarium, Rhizopus* and *A. niger*. The inhibition zones were the largest at concentrations of 1250 and 1000 ppm. The inhibition zones produced by concentrations of 750 and 500 ppm were smaller but still significant compared to control treatments.

6. Cucumber Growth and Yield Characteristics as Affected by *Nigella* sativa L. Treatments

6.1. Growth characteristics

It is clear from results presented in table (13) that soil application with seed cake of *Nigella sativa* at 10 g produced a significant increase in vegetative growth characters, i.e., percentage of stand, plant height, number of flowers/plant, fresh weight/plant and total chlorophyll, except dry matter percentage, which has given the highest values in the two experimental seasons as compared with control treatment. The results were true in both growing seasons.

The effect of foliar spray treatments on growth parameters showed the highest value with *Nigella sativa* oil at 1.5% treatment when compared with the other tested treatments in both seasons, while the control treatment showed the lowest value in both seasons. This result suggests that powdery mildew of cucumber (*Podosphaera xanthii*) was reduced by spraying *Nigella sativa* oil, which is in agreement with Hafez (2008). The combination of soil amendment with seed cake with foliar spray of oil gave a significant improvement on all growth characters in both tested seasons. These results indicated the important role of *Nigella sativa* oil and seed cake in improving growth characteristics of cucumber plants. Also seed cake application alone has positive effect on plant growth by stimulating the growth of rhizosphere microorganisms, which are playing fundamental role in minerals uptake by

plant. Regardless, the result agree with those obtained by Hassan and Vancura (1970) who detected that microbial products of *Azotobacter chroococcum*, *Azospirillum brasilense*, *Bacillus megaterium* had a positive effect on plant length and weight, due to excretion of some growth regulators as gibberellin like substances in microbial culture.

Table (12). Effect of using some biological products of Nigella sativa L. onsome plant pathogenic fungi during 2009/2010 seasons.

Concentrations of		Diameter of Inhibition zone (cm)										
			Α		В							
Fungi	500	750	1000	1250	500	750	1000	1250				
Fusarium	0.0	0.0	1.20	1.25	1.23	1.77	2.50	3.80				
Rhizopus	0.0	0.0	0.40	0.81	1.50	2.68	3.17	4.03				
A. niger	0.0	0.0	1.10	1.30	2.07	3.97	4.67	5.83				
			3 3			1 5						

A= (Control) without inoculation B = Mixed (A. chroococcum and B. megaterium)



Fig. (1). Effect of using some biological products of *Nigella sativa* L. on some plant pathogenic fungi.

6.2. Yield and its component

It is evident from table (14) that seed cake gave significant increase in total yield and its components, *i.e.* fruit length, average fruit weight fruit diameter and total yield per plant (g) and per fed (ton). The highest values of yield and its components were obtained by application of seed cake at rate of 10 g around plant root, except dry matter (%). Control treatment showed a significant increase in fruit dry matter (%) in both growing seasons.

Foliar spray effect with *Nigella sativa* oil at 1.5% produced the highest value of fruit length, average fruit weight, fruit diameter, dry matter (%) and total yield per plant and per feddan. These results may be due to the role of *Nigella sativa* oil in increasing cell division, as suggested by El-Dougdoug et al. (2007).

The interaction treatment between seed cake at rate of 10 g per plant and *Nigella sativa* oil at 1.5% showed the highest values on total yield and its components, except the dry matter (%) character. These results suggest a role of *Nigella sativa* oil and seed cake in reducing powdery mildew of cucumber and improving soil conditions around the root zone (Harfoush and Salama, 1992; Mosa, 1997; Reuveni et al., 1997 and Verhaar et al., 1997).

Table (13). Effect of using some biological products of (*Nigella sativa* L.)on growth characters of cucumber plant during 2009/2010seasons

Treatments		Percentage of		Fresh weight		Plant length		Number of		Total		Dry matter	
Seeds	Seeds	stand		g/plant		(cm)		flowers/plant		chlorophyll		(%/plant)	
dregs	oil	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
{ontrol	Zero	32,33	30,67	127,67	210,33	67,00	113,00	4,67	8,67	25,00	30,33	26,83	28,90
	0.5%	34,33	32,33	167,67	244,67	75,00	128,67	12,33	18,00	26,77	34,67	61,03	63,13
	1%	41,00	39,00	149,67	266,00	76,67	141,00	6,00	7,00	31,00	37,00	66,43	64,80
	1.5%	43,00	41,67	167,67	350,67	71,33	144,33	15,00	16,00	28,93	36,23	67,87	65,87
5 g	Zero	35,67	32,67	159,33	173,67	62,33	85,67	9,00	14,67	25,60	29,23	19,30	19,87
	0.5%	41,67	36,00	163,67	261,33	82,00	115,33	11,67	18,00	27,77	31,07	27,23	28,33
	1%	49,67	40,00	179,33	283,33	73,00	113,67	10,00	11,67	30,30	34,67	38,13	39,23
	1.5%	54,67	52,00	267,67	294,67	66,00	130,00	17,00	20,67	28,70	30,33	53,33	54,33
10 g	Zero	40,00	40,67	108,33	304,00	74,33	111,00	13,33	18,00	25,83	26,83	22,37	23,33
	0.5%	49,33	49,33	134,00	320,00	75,67	129,33	16,33	12,67	28,90	34,53	41,90	42,97
	1%	58,00	55,67	149,00	361,67	76,33	136,00	17,33	13,00	30,17	35,40	55,20	55,13
	1.5%	60,33	57,33	245,00	459,67	86,33	145,33	20,33	21,33	29,67	35,60	67,10	66,87
LSD		1,21	0,88	1,93	4,14	1,39	2,61	0,90	1,20	0,41	0,89	0,56	0,99
Control		37,67	35,92	153,17	267,92	72,50	131,75	9,50	12,42	27,93	34,56	55,54	55,68
5 g		45,42	40,17	192,50	253,25	70,83	111,17	11,92	16,25	28,09	31,33	34,50	35,44
10 g		51,92	50,75	159,08	361,33	78,17	130,42	16,83	16,25	28,64	33,09	46,64	47,08
LSD		0,75	0,68	1,35	1,75	0,93	1,61	0,50	0,65	0,28	0,60	0,09	0,43
Control		27,00	26,00	98,83	172,00	50,92	77,42	6,75	10,33	19,11	21,60	17,13	18,03
0.5% oil		31,33	29,42	116,33	206,50	58,17	93,33	10,08	12,17	20,86	25,07	32,54	33,61
1% oil		37,17	33,67	119,50	227,75	56,50	97,67	8,33	7,92	22,87	26,77	39,94	39,79
1.5% oil		39,50	37,75	170,08	276,25	55,92	104,92	13,08	14,50	21,83	25,54	47,08	46,77
LSD		0,70	0,51	1,11	2,39	0,80	1,51	0,52	0,69	0,24	0,51	0,33	0.51

The recorded data indicate that control treatment gave higher fungal counts than seed cake and seed oil of *Nigella sativa* treatments, which contain beneficial bacteria, e.g. *Azotobacter chroococcum*, *Azospirillum brasilense*, *Bacillus megaterium*. Similarly, Kavimandan and Gaur (1971) found that *Bacillus megatherium* can decompose organic phosphates and thereby improve crop yield. This may be due to the production of siderphores compounds, which inhibit fungal spore germination and increase availability of nutrients to plant and improve growth (Jagnow, 1991). Also,

the highest total yield of cucumber plants, which were treated with seed cake and seed oil of *Nigella sativa* than other treatments, may be due to reductions in fruit dropping (Wilocox, 1994) and/or increases in the supply of growth hormones produced by microorganisms (Amara, 1994).

Table (14). Effect of using some biological products of (*Nigella sativa* L.)on yield and its component of cucumber plant during 2009/2010 seasons.

Treatments		Fruit length		Average fruit		Total vield(g)		Total vield		Fruit diameter		Dry matter	
Seeds dregs	Seeds oil	(cm)		weight (g/ fruit)		/plant		(ton)/fed		(mm)		(%)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
control	Zero	12,40	11,97	35,83	36,50	398,33	376,67	39,83	36,33	23,00	25,00	4,57	4,37
	0.5%	12,70	12,30	70,23	72,63	406,33	403,33	40,83	39,00	26,67	30,33	8,17	8,33
	1%	15,67	14,20	57,30	54,53	505,00	490,00	50,50	48,00	25,67	27,00	8,57	8,43
	1.5%	16,53	14,97	80,20	80,97	606,67	585,00	60,67	56,83	26,67	30,67	11,07	10,40
5 g	Zero	14,33	14,20	45,67	46,30	405,00	405,00	40,50	40,50	31,00	31,67	6,37	6,47
	0.5%	13,10	13,80	46,20	47,30	613,33	621,67	69,30	65,67	29,67	36,33	7,73	7,23
	1%	13,40	12,47	44,70	45,17	693,00	670,00	61,33	62,17	33,67	31,67	3,93	3,50
	1.5%	15,10	14,70	69,63	65,00	798,67	793,33	79,87	77,00	34,33	41,00	7,67	8,03
10 g	Zero	14,10	15,00	59,57	60,23	500,00	491,67	50,00	48,17	32,67	31,33	3,73	3,97
	0.5%	15,10	13,87	65,20	64,23	696,67	693,33	69,67	67,67	34,00	32,67	7,60	7,43
	1%	16,33	16,40	45,33	45,17	794,33	793,33	79,43	72,83	35,67	41,33	5,03	4,83
	1.5%	17,33	19,33	106,07	105,63	893,33	890,00	89,33	74,67	36,67	45,67	7,97	8,63
LSD		0,43	0,47	1,26	1,15	7,27	14,58	0,73	1,01	0,67	1,38	0,54	0,32
Control		14,33	13,36	60,89	61,16	479,08	463,75	47,96	45,04	25,50	28,25	8,09	7,88
5 g		13,98	13,79	51,55	50,94	627,50	622,50	62,75	61,33	32,17	35,17	6,43	6,31
10 g		15,72	16,15	69,04	68,82	721,08	717,08	72,11	65,83	34,75	37,75	6,08	6,22
LSD		0,30	0,21	0,52	0,39	3,71	4,05	0,39	0,26	0,44	0,45	0,19	0,17
Control		10,21	10,29	35,27	35,76	325,83	318,33	32,58	31,25	21,67	22,00	3,67	3,70
0.5% oil		10,23	9,99	45,41	46,04	429,08	429,58	44,95	43,08	22,58	24,83	5,88	5,75
1% oil		11,35	10,77	36,83	36,22	498,08	488,33	47,82	45,75	23,75	25,00	4,38	4,19
1.5% oil		12,24	12,25	63,98	62,90	574,67	567,08	57,47	52,13	24,42	29,33	6,68	6,77
LSD		0,25	0,27	0,73	0,66	4,20	8,42	0,42	0,58	0,38	0,80	0,31	0,19

CONCLUSION

From the results obtained, it can be concluded that using *Nigella* sativa L., as seed cake in rates of 10 g/plant as soil application and 1.5% seed oil as foliar spray to cucumber plants, improved growth and productivity of cucumber and reduced fungal diseases under North Sinia conditions.

REFERENCES

Abd El-Hafez, A.M. (1966). Some studies on acid producing microorganisms in soil and rhizosphere with special reference to phosphate dissolvers. Ph.D. Thesis, Fac. Agric., Ain Shams Univ., Cairo, Egypt.

- Abd El-Malek, Y. and Y.Z. Ishac (1968). Evaluation of methods used in counting *Azotobacter*. J. Appl. Bacteriol., 31:267-275.
- Allen, I.N. (1959). In "Experiments in Soil Bacteriology". Burgess Publishing Co., Minneapolis, Minnesota.
- Amara, A.T. Mervat (1994). Wheat response to rhizosspheric bacterial inoculation under field condition. Desert Inst. Bull., Egypt, 44(2):333–355.
- A.O.A.C. (1990). In "Official Methods of Analysis Association of Officinal Analytical Chemists". 16th ed. Washington, D.C., USA.
- Bettiol, W., S.A.S. Harllen and C.R. Ronielli (2008). Effectiveness of whey against zucchini squash and cucumber powdery mildew. Sci. Hortic., 117:82-84.
- Bunt, J.S. and A.O. Rovira, (1955). Microbiological studies of some subontoretic soil. Journal of Soil Science, 6:119-126.
- David, G. (2002). Tree Fruit Production with Organic Farming Methods. Center for Sustaining Agriculture and Natural Resources. Washington State University, Wenatchee, USA.
- Dobereiner, J. (1978). Influence of environmental factors on the occurrence of *Spirillum lipoferum* in soil and roots. Ecol. Bull. (Stockholm), 26:343-352.
- El-Dougdoug, Kh.A., Hanaa H.A. Gomaa and Rehab A. Daoud (2007). Elimination of some viruses infecting tomato plants by phytoantivirus. Research Journal of Agriculture and Biological Sciences, 3(6):994-1001, INSInet Publication.
- El-Sayed, M.A.M. (2006). Effect of biofertilizers application on the productivity of *Nigella saliva* cultivated in desert sandy soils and efficiency of produced seeds against some pathogenic microorganisms. Ph.D. Thesis, Fac. Agric., Moshtohor, Benha Univ., Egypt.
- Hafez, Y.M. (2008). Effectiveness of the antifungal black seed oil against powdery mildews of cucumber (*Podosphaera xanthii*) and barley (*Blumeria graminis* f. sp. *hordei*). Acta Biologica Szegediensisal, 52(1):17-25.
- Harfoush, D.I. and D.A. Salama (1992). Induction of systemic resistance to powdery mildew in cucumber leaves by seed soaking application with cobalt. Ann. Agric. Sci., Mansoura Univ., 17:3555-3565.
- Hassan, A. and V. Vancura (1970). Formation of biologically active substances by rhizosphere bacteria and their effect on plant growth. Microbil. Prague, 11:468-478.
- Jackson, M.L. (1958). In "Soil Chemical Analysis". Prentice-Hall-Inc., Englewood Cliffs, N.J., USA.

- Jacobs, M.B. and M.J. Gerstein (1960). In 'Hand Book of Microbiology''. De. Van Nostrand, Co., Inc., New York, p. 139-202.
- Jagnow, G. (1991). Microbial interaction in the cereal rhizosphere. Proc. of the 32nd Symposium of the British Ecological Sco. with the Association of Applied Biolgists, Univ. of Cambridge, 113–137.
- Kavimandan, S.K. and A.C. Gaur (1971). Effect of seed inoculation with *Pseudomonas* sp. on phosphate uptake and yield of maize. Cuar. Sci., 40:439–440.
- Kiss, L. (2003). A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Manage Sci., 59:475-483.
- Mosa, A.A. (1997). Effect of foliar application of phosphate on cucumber powdery mildew. Annals Agric. Sci., Ain Shams Univ., Cairo, 42:241-55.
- Nelson, E.B., Wei Lian Chao, J.M. Norton, G.T. Nash, and G.H. Harman (1986). Attachment of *Enterobacter cloaceaee* to hyphae of *Pythium ultima* possible role in the biological of *Pythium* preemergence damping-off. Phytopath., 76:327-335.
- Piper, C.S. (1950). In "Soil and Plant Analysis". Univ. Adelaide. Interscience Publishers. Inc. New York, p. 258-275.
- Pramer, D. and E.L. Schmidt (1994). In "Experimental Soil Microbiology". Burgerss Publishing Co., Minnesota, USA, p. 235 -237.
- Reuveni, M, V. Agapov and R. Reuveni (1997). A foliar spray of micronutrient solutions induced local and systemic protection against powdery mildew (*Sphaerotheca fuliginea*) on cucumber plants. Europen J. Plant Patho., 103:581-585.
- Richards, L.F. (1954). In "Diagnosis and Improvement of Saline and Alkaline Soils". Agric., Hand Book, USA.
- Sleigh, J.D. and M.C. Timburg (1981). In "Notes on Medical Bacteriology". Churchill, Livingstone, 43 pp.
- Snedecor, G.W., and W.G. Cochran (1989). In "Statistical Methods". 8th ed. Iowa State Univ. press, Iowa, USA.
- Thoppil, J.E., A. Tajo and J. Minija (1998). Antibacterial and antifungal activity of four varieties of *Ocimum basilicum*. Fitoterapia, 69(2):191-192.
- Verhaar, M.A, K.K. Ostergaard, T. Hijwegen and J.C. Zadoks (1997). Preventative and curative applications of *Verticillium lecanii* for biological control of cucumber powdery mildew. Biocontrol Sci. Technol., 7:543-551.
- Wilocox, G.E. (1994). Effect of potassium on tomato growth and production. Proc. Hort. Sci., 85:484-489.

أثر إستخدام بعض نواتج حبة البركة المنتجة حيويًا على تحسين إنتاجية الخيار وأثرها على النشاط الميكروبي المصاحب لنمو النبات

محمود علي مجد السيد^{ا*} **ومجد رائف حافظ^٢** ^اقسم خصوبة وميكروبولوجيا الأراضي، مركز بحوث الصحراء، القاهرة، مصر ^٦قسم الإنتاج النباتي، مركز بحوث الصحراء، القاهرة، مصر

أقيمت تجربتان حقليتان خلال موسمي الزراعة الصيفي ٢٠٠٩- ٢٠١٠ بمحطة تجارب مركز بحوث الصحراء بمنطقة الشيخ زويد بشمال سيناء، لدراسة تأثير بعض نواتج حبة البركة المنتجة حيويًا وذلك بغرض تحسين إنتاجية الخيار صنف البرنس. وقد أستخدم تفل بذور حبة البركة بمعدلات صفر، ٥، ١٠ جرام كإضافة أرضية حول منطقة جذور النبات. بينما أستخدم زيت حبة البركة كرش ورقي بمعدلات صفر، ٥، ١٠، ١٠، ١٠ رشًا على النباتات بعد الشتل ثلاث مرات كل ١٠ أيام.

وقد تم دراسة تأثير هذه المعاملات على نشاط بعض الميكروبات النافعة والممرضة للنبات وأثر ذلك على صفات النمو والإنتاج والجودة لمحصول الخيار. وكان من أهم النتائج المتحصل عليها، أن استخدام تفل بذور حبة البركة بمعدل ١٠ جم حول جذور كل نبات من شتلات الخيار وكذلك الرش بزيت حبة البركة بمعدل ١٠ / أعطى أعلى كثافة لأعداد البكتريا الكلية وأقل كثافة لأعداد بعض الفطريات الممرضة لنبات الخيار بالتربة المحيطة بجذور النباتات، وكذلك أعلى كثافة لأعداد كل من بكتريا الأزوتوباكتر، ألأزوسبريلليم، باسلس ميجاتيريم بالإضافة الى تقييم النشاط الميكروبي بالتربة من خلال تقدير ثاني أكسيد الكربون، وأيضا دراسة تأثير زيت بذور حبة البركة على تثبيط نشاط بعض الفطريات الممرضة لنبات الخيار.

وبدراسة بعض صفات النمو والإنتاج وصفات الجودة لمحصول الخيار فقد أظهرت نفس المعاملة أعلى النتائج في صفات طول النبات، نسبة البقاء، الوزن الطازج للنبات، عدد الأزهار والكلورفيل الكلي. وأظهرت نفس المعاملة أعلى قيم في إنتاجية النبات والفدان، طول وقطر الثمرة والوزن الطازج بينما أعطت معاملة الكنترول أعلى قيمة لصفة النسبة المئوية للمادة الجافة للنبات والثمار.