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Geographical Information System Used for Assessing Biodiversity of Entomopathogens in Egypt

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ABSTRACT

Occurrence of entomopathogenic agents in soil samples, collected from different sites (14) at Suez Governorate, Egypt, using *Tenebrio molitr* L. (Coleoptera:Tenebrionidae) bait method was studied. The agents were found to occur in 67.6% of the studied soil samples representing 190 isolates. Insect-pathogenic fungi were the most abundant entomopathogenic agents (50.0%) found in different districts (179 out of 358 samples), entomopathogenic nematodes existed only in four locations, representing 1.96%. Using Koch's postulates, mortality rates caused by the pathogenic isolates to *Tenebrio* larvae ranged between 17 and 92.6%. The genera *Beauveria*, *Metarhizium*, *Paecilomyces* and *Verticilium* were specific entomopathogens causing high mortality rates within a short period of time, while mortality rates caused by the nematodes were variable. Statistical analysis showed that correlation coefficient values for chemical and physical soil properties were poor and insignificant, except for calcium carbonate and magnesium (Mg^{++}) that showed high significant correlations. The overall indices for diversity values (richness, evenness, Shannon and Simposen indices) at Suez governorate were 15, 0.93, 2.51 and 0.90, respectively, demonstrating a great diversity. Certain pesticides commonly used in the region were tested to clarify their effect on the growth of the fungi *B. bassiana, M. anispoliae, Pacilomyces fumosoroseus* and the nematodes *Heterorhabditis* spp.

Key words: Entomopathogenic agents, Biodeversity, edaphic factors, GIS, Egypt.

INTRODUCTION

Control of pest insects with chemical pesticides has generated several problems including insecticide resistance, outbreaks of secondary pests normally held in check by natural enemies, safety risks for humans and domestic animals, contamination of ground water, decrease in biodiversity, and other environmental concerns. These problems and sustainability of programs based predominantly on conventional insecticides have stimulated increased interest in integrated pest management, for exploring a change in control tactics to find economically and environmentally sound alternatives for pest control. In the search for safer and more lasting methods, entomologists have turned their attention to the possibility of using insect pathogens, which show great promise as potential biological control agents (Inglis *et al*., 2001).

Effective microbial control agents that can fill the void of phased out chemicals exist, but their further development and implementation will require the following advances: improvements in the pathogens, their production, and formulation; better understanding of how they will fit into integrated systems and their interaction with the environment and other IPM components; greater appreciation for their full advantages (efficacy, safety, selectivity, etc.), not simply their comparison with chemical pesticides; and acceptance by growers and the general public.

Knowledge of the species composition and distribution of insect pathogens is essential for assessing the biocontrol potential of these pathogens in a specific agroecosystem. The availability of micro-organisms with entomopathogenic properties is very important for integrated and biological defence systems against insect phytophages. Microbiological control systems using pathogenic micro-organisms have increasingly been employed to control insect pests. The current importance of microbiological control, within the wider field of biological control, has been recognized through the creation of several international organizations and study groups (Inglis *et al*., 2001).
Insect host age, habitat and soil type, pesticide use, agricultural practices, and location, influence the natural distribution of biological control organisms (Vanninen 1996 and Chandler *et al.* 1997).

Aims of the present study are: I) search for new strains of entomopathogenic agents for development and use in biological control programs and to document their prevalence and biodiversity in different habitats and soil types in Egypt, II) study the effect of some edaphic factors on occurrence and distribution of such bioagents in soil, and III) study side effects of commonly used pesticides on survival and population density of prevailing agents.

MATERIALS AND METHODS

Study area

The research area was coterminous to the Suez Governorate, Egypt, situated east of Delta and north of the Suez Gulf. The area lies between 29° 58′ 0″ N and 32° 33′ 0″ E, The covered vegetation habitats included field crops, vegetables and fruit crops. The area consists of 14 districts; Alsokhna, Oyoun-Mousa, Portawfiq, Elgblayate, Amer, Elomda, Alraid, Alshalofa, Shandoura, Aldowlia, Alzeraien, Alshabab, Kabreet, and Genifa.

Collection of soil samples

Soil samples were collected approximately from each of 100 feddan/ location. The locations and altitudes of the sampled soils were identified with coded numbers, using global positioning system (GPS) equipment (Garmin, etrex, Garmin Ltd), that used to view, edit, plot data and export data to geographical information system (GIS). GIS-generated base maps of the research area were developed by assistance of a GIS consultant. These initial maps were useful in visually planning for the study.

A total of 358 soil samples were collected throughout the period; August - December 2012 and January - March 2013. Samples were taken from the top 10-15 cm of the soil at each site using sterilized auger. The samples were placed in plastic bags. Each sample was separately mixed with two 500 gm/ soil. A sample of each was subjected for examination and analysis at the Plant Protection and Soil testing laboratories of Ismailia Agricultural Research Station, Egypt.

Isolation and identification of insect pathogens

Tenebrio molitor L. (Coleoptera:Tenebrionidae) was used as insect bait for trapping entompatheogenic and parasitic agents as well the nematodes from soil (Zimmermann 1986; Vanninen 1996 and Bidochka *et al.*, 1998). Fungi were isolated and identified using selective medium according to Samson *et al.*, (1988) and Goettel and Inglis (1997). Nematodes were collected and identified from the infected larvae using the technique of Woodring and Kaya (1988). Identification of obtained organisms was confirmed by the specialists of the Plant Pathology Institute, Agricultural Research Centre, Giza, Egypt.

Preliminary pathogenicity test (Koch's postulates)

Infections of isolates with no knowledge about their pathogenicity to the insect *T. molitor* larvae were bioassayed. The tested insect pathogens (fungi) were grown on PDA plate for 10–12 days and their spores were washed with 1mL sterile water into 1.5 mL tubes. Larvae of *T. molitor* were immersed into the spore suspension using forceps for about 3–5s, and then transferred into 9 cm diameter Petri dishes with moistened filter paper. Petri dishes were sealed with parafilm to maintain the humidity and were incubated at 20– 25°C in darkness. Infected larvae were inspected daily until death or pupation. Fungal structures growing out of the dead larvae were identified to confirm the fungus species that was inoculated. Nematodes were tested using the technique of Woodring and Kaya (1988). Ten larvae were treated for insect pathogens and the experiment was replicated three times to evaluate the pathogenicity of the entomopathogens.

Physical and chemical properties of soils

To determine the relationship between occurrence of the agents and chemical and physical properties of the soil; 250 gm samples were subjected to chemical analysis, using the methods adopted by Jackson (1967) and physical properties of soil were determined, using the method of Gee and Bauder, (1986).

Effect of pesticides on insect pathogens

Susceptibility of obtained agents to certain registered pesticides, commonly used in the experimental area was tested (Table 1). The pesticides were applied at their recommended concentrations and tested according to the method of Gardner and Storey (1985).

Table (1): Tested pesticides, their trade name, active ingredients contents and applied concentration

Pesticide	Trade name	Active ingredient	Recommended concentration g or ml/100 L water
Acaricides	Ortus	Fenpyroximate (5 % EC)	50 ml
	Vertemic	Abamectin (1.8 % EC)	40 ml
Fungicides	copper oxychloride	copper oxychloride (WP)	250 gm
	Kocid 101	copper hydroxide (77% WP)	250 gm
	Sulfur	Sulfur (WP)	250 gm
	Topsin M	Thiofannat methyl (70% WP)	100 gm
Herbicides	Sencor	Metribuzin (70% WP)	175 gm
	Gallant	Haoxyfop-methyl (12.5 EC)	750 ml
Insecticides	Actellic	Pirmifos methyl (50% EC)	375 ml
	Cidial	Phenthoate (50% EC)	150 ml
	KZ oil	Mineral oil (95% EC)	1500 ml
	Sumthion	Fenitrothion (50% EC)	375 ml
Nematicides	Furdan	Carbofuran (10% G)	2500 gm
	Vydate	Oxamyl (24% L)	500 ml

Nematodes were tested, using 10 ml of suspension of infective juvenile nematodes extract. Number of alive nematodes was counted before treatment and after 24 hours. Percentages of dead nematodes were also counted. Four replicates were used for each pesticide as well as an untreated check. Petri dishes of the tested fungi were incubated at 25°C and their linear growths were measured. Average diameters of colonies (mm) and percentages of decrease relative to the control were recorded. All data were computerized and statistically analyzed using complete random design.

Data analysis

Co-stat software was used for statistical data processing. Biodiversity index was a non-parametric tool used to describe the relationship between species number and abundance. Four derived biodiversity indices were used for statistical analyses (Magurran, 2004).

Richness

S= number of species in the community

Shannon's diversity index

$$H = -\sum_{i=1}^{S} p_i \ln p_i$$

Simpson's diversity index

$$D = \frac{\Sigma\, n(n-1)}{N(N-1)}$$

Evenness (*J*) (pielou's index)

$$E_H = H / H_{max} = H / \ln S$$

Where:

Pi= Relative abundance of ith species

N= Total number of individuals of all kinds

N= number of individuals of ith species

Ln= log to base 2

RESULTS AND DISCUSSION

Occurrence and frequency rates

Insect-associated pathogens were found in 67.6% (242 out of 358) of the examined soil samples and included 190 isolates belonging to 13 fungi species, one entontompathogenic nematode (*Heterorhabditis* sp.) (Table 2). Insect-pathogenic fungi were the most abundant entomopathogenic agents distributed and found in different districts, representing 50.0% (179 out of 358), followed by bacteria species, existed in the fourteen surveyed locations, representing 15.46%, whereas, entomopathogenic nematodes existed only in four districts representing 1.96%.

Occurrence of the fungi varied from one site to another (Table 2). The most commonly isolated fungi species were *Fusarium* spp. (6.98%), *Aspergillus flavus* (6.70%) and *Paecilomyces* sp. (5.03%). The other fungi were *Alternaria* spp. (4.47%), *Cladosporium oxysporum* (3.91%), *Trichoderma* sp. (3.61%), *Penicillium* spp. (3.35%), *Beauveria bassiana* (2.63%) and *Metarhizium anisopliae* (2.3%). Occurrence of insect-associated pathogens were insignificantly different among locations of the Suez Governorate but were significantly different among insect pathogens, particularly the three fungal species (*Fusarium* spp. (6.98%), *A. flavus* (6.70%), *P. fumosoroseus* (5.03%) and bacteria species (x^2 = 102.39; P = 0.99).

Unidentified bacterium species were found frequently (18.42%), followed by the fungus species, while the nematodes were less frequent (3.16 %) in the isolates. However, each of the two fungal species; *A. flavus* and *Fusarium* spp., were the highest frequency isolated fungi species. They gave the same frequency percentages (10.0%), followed by *Alternaria* spp. and *P. fumosoroseus* (7.37 %), *C. oxysporum* (6.32%), *Trichoderma* sp. (5.79%). *Penicillium* spp. (5.26), *A. niger*, *B. bassiana*, *Stamphylium* sp. (4.74%), *Helminthosporium* sp. (4.21%) and *M. anaspoliae* (3.16%).

The nematodes appeared with less frequency (3.16 %) in the isolates. The two fungal species; *A. flavus* and *Fusarium* spp. occurred at the same frequency percentages (10.0%), followed by *Alternaria* spp., and *Paecilomyces fumosoroseus* (7.37%), *C. oxysporum* (6.32%), *Trichoderma* sp. (5.79%), *Penicillium* spp. (5.26%) *A. niger*, *B. bassiana*, *Stamphylium* sp. (0.74%), *Helminthosporium* sp. (4.21%) and *M. anisopliae* (3.16%). Total occurrence percentages are illustrated in Fig. (1), they ranged between 1.0 and 12.3% in all locations. Greatest occurrence was found in Genifa (12.29%), followed by Aldowlia (9.22%), Al-Zeraien (7.82%) and Shandora (7.26%). On the other hand, lowest occurrence was found at Al-Sokhna (1.12%) and Oyoun Mousa (1.68%) locations whereas, the variation ranged between 2.7 and 6.0 in the other locations.

Results in table (3), using Koch's postulates, showed that mortality rates caused by the pathogen isolates to *Tenebrio* larvae ranged from 17 to 92.6%. Isolates of the fungi *Beauveria*, *Metarhizium*, *Paecilomyces* and *Verticilium* were the most virulent isolates recovered and mortality of larvae started 2 to 3 days post infection. The nematodes showed mortality rate of up to 34.4%.

This is the first detailed study of insect pathogens in the soil environment in Suez Governorate region as the present work in the fourteen locations yielded thirteen fungi species and one nematode. Distribution and occurrence of the insect pathogenic species was insignificantly among the studied

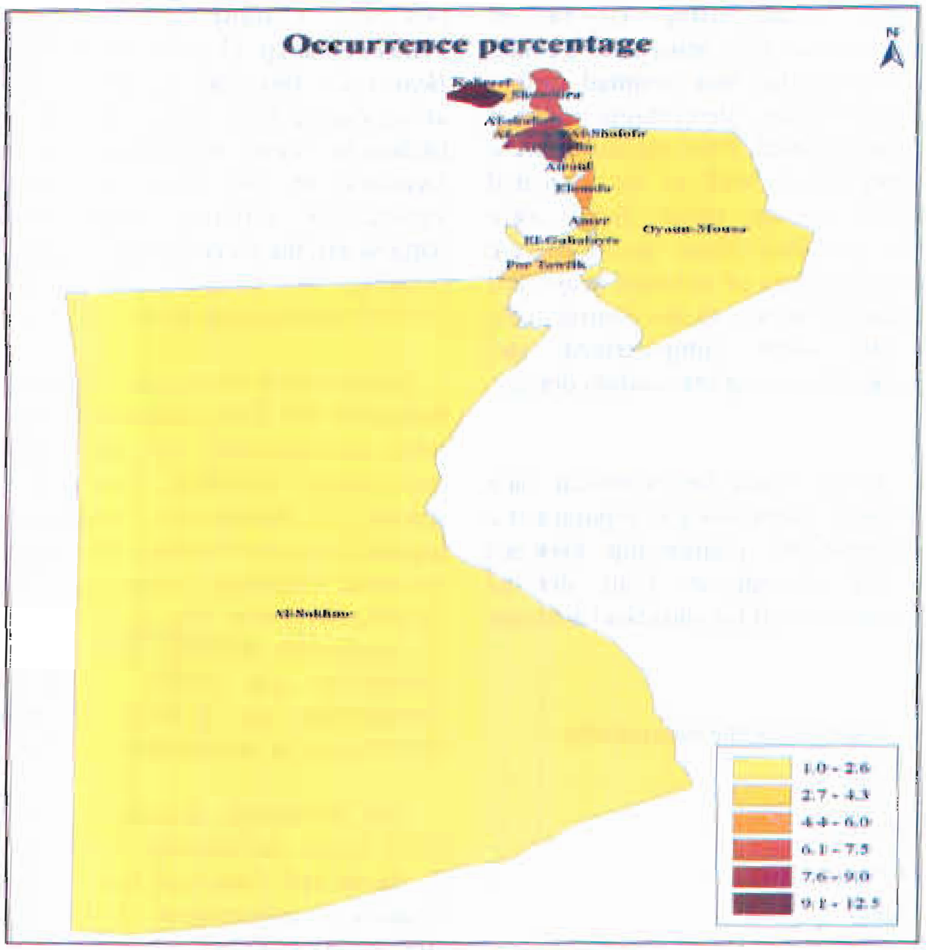

Fig. (1): Total percentage of entomopathogens occurrence.

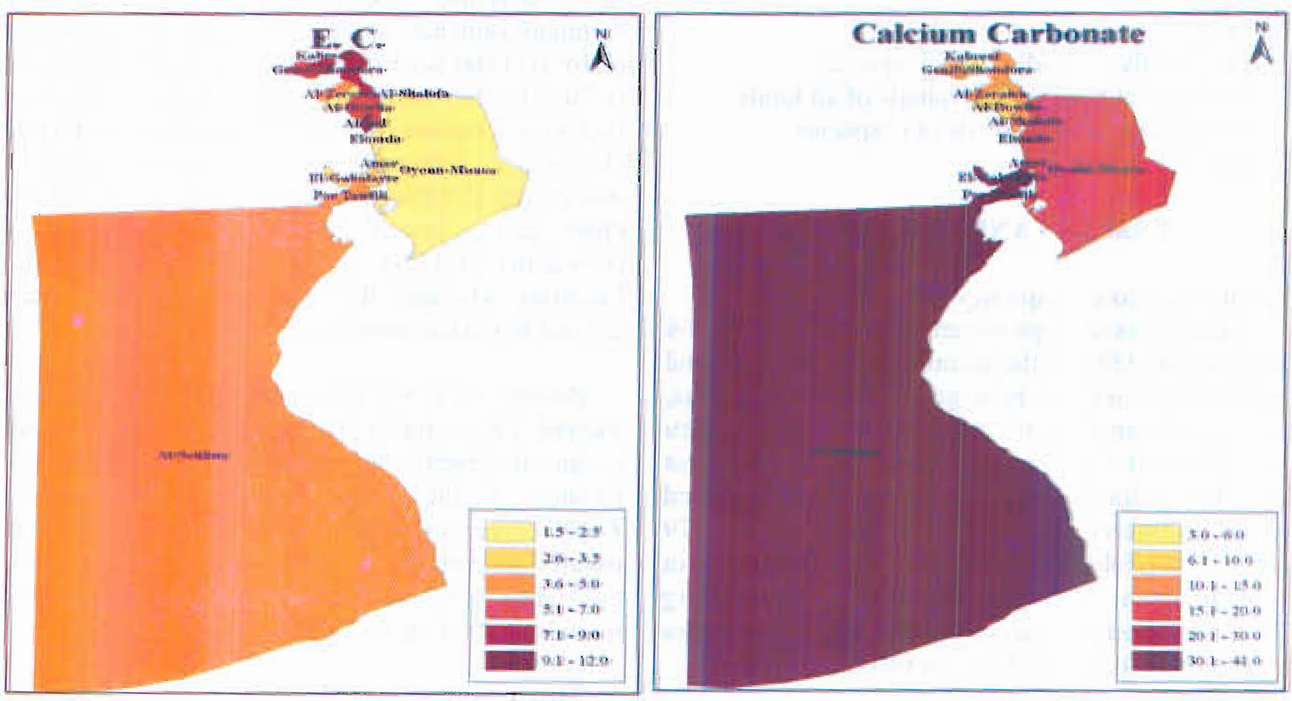

Fig. (2): Concentration of electrical conductivity (E.C.) and Calcium Carbonate in soil of searched area.

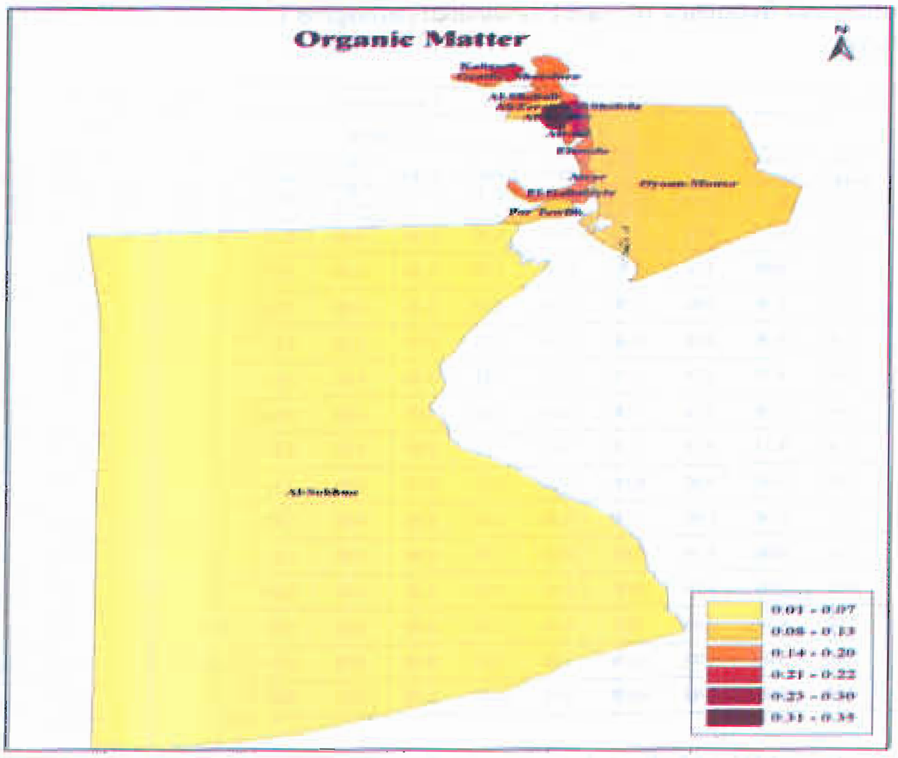

Fig. (3): Percentage of organic matter (O. M.) in soil samples of searched area.

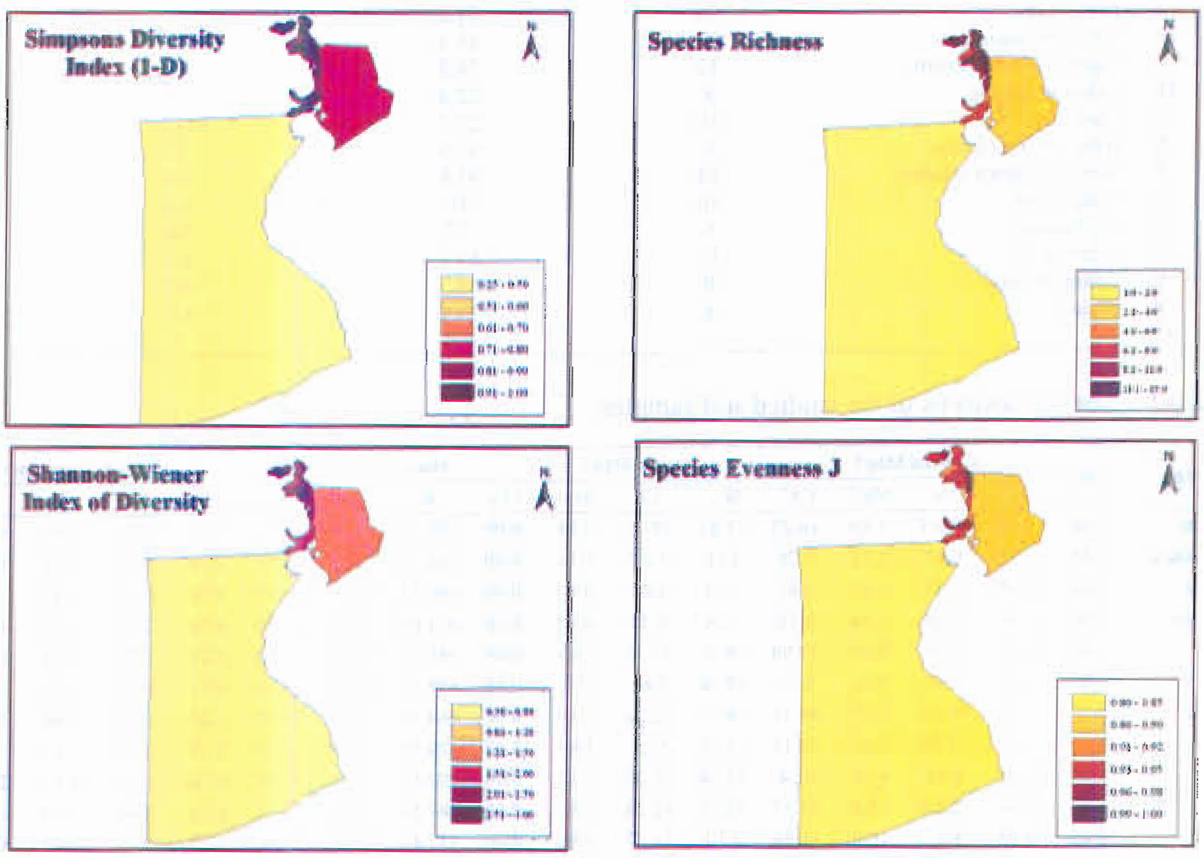

Fig. (4): Species diversity at different locations of Ismailia governorate, Egypt.

Table (2): Distribution and frequency of insect-associated pathogens in the soils of different locations at Suez Governorate, Egypt

Organism	Frequency % ᵃ														% Frequency (F)
	Locations														
	Al-Sokhna (N=11)	Oyoun Mousa (N=11)	Por Tawfiq (N=18)	El Gablayate (N=27)	Amer (N=27)	Elomda (N=27)	Alraid (N=20)	Al-shalofa (N=25)	Shandora (N=34)	Al-Dowlia (N=31)	Al-Zeraien (N=32)	Al-shabab (N=30)	Kabreet (N=25)	Genifa (N=36)	
Aspergillus flavus	0.00	8.33	4.76	3.70	7.41	3.70	5.00	4.00	8.82	9.68	12.50	3.33	8.00	8.33	10.00
Aspergillus niger	0.00	0.00	4.76	3.70	0.00	3.70	0.00	4.00	2.94	6.45	0.00	3.33	8.00	0.00	4.74
Alternaria spp.	9.09	0.00	0.00	3.70	3.70	3.70	5.00	4.00	5.88	9.68	3.13	3.33	8.00	2.78	7.37
Beauveria bassiana	0.00	0.00	0.00	0.00	0.00	3.70	0.00	4.00	2.94	3.23	6.25	0.00	4.00	8.33	4.74
Cladosporium oxysporum.	0.00	8.33	4.76	7.41	3.70	7.41	5.00	4.00	2.94	6.45	3.13	0.00	0.00	2.78	6.32
Helminthosporium sp.	0.00	0.00	4.76	0.00	0.00	0.00	0.00	4.00	5.88	3.23	3.13	0.00	0.00	8.33	4.21
Fusarium spp	0.00	8.33	4.76	3.70	7.41	7.41	5.00	8.00	8.82	6.45	6.25	10.00	4.00	11.11	10.00
Metarhizium anisopliae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.94	6.45	6.25	0.00	0.00	8.33	3.16
Paecilomyces fumosoroseus	0.00	0.00	0.00	3.70	0.00	3.70	5.00	4.00	5.88	6.45	12.50	0.00	4.00	13.89	7.37
Penicillium spp.	0.00	0.00	4.76	0.00	0.00	3.70	5.00	4.00	5.88	6.45	6.25	0.00	0.00	5.56	5.26
Stamphylium sp.	0.00	0.00	4.76	0.00	0.00	3.70	5.00	4.00	0.00	3.23	3.13	3.33	0.00	5.56	4.74
Trichoderma sp.	0.00	0.00	0.00	0.00	0.00	3.70	5.00	4.00	5.88	6.45	3.13	3.33	4.00	8.33	5.79
Verticillum lecnii	0.00	0.00	0.00	0.00	3.70	7.41	0.00	0.00	2.94	6.45	3.13	0.00	4.00	8.33	4.74
Nematodes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.23	6.25	0.00	4.00	8.33	3.16

Table (3): Pathogenicity test (Koch's Postulates) results from entomopathogenic isolates on *Tenebrio molitor*

Organism	No. of isolates	Mortality rate (%)	Day after infection
Aspergillus flavus	19	43.1	7
Aspergillus niger	9	13.3	7
Alternaria spp.	14	31.2	7
Beauveria bassiana	9	91.4	3
Cladosporium oxysporum.	12	76.5	3-4
Helminthosporium sp.	8	22.4	6-7
Fusarium spp.	19	27.3	6-7
Metatrhizium anisopliae	6	92.6	3
Paecilomyces fumosoroseus	14	91.6	3-4
Penicillium spp.	10	31	5-6
Stamphylium sp.	9	17	5-6
Trichoderma sp.	11	51.2	4-5
Verticillum lecanii	9	84.3	4-5
Nematodes	6	34.4	5-7
Control	-	0.0	7

Table (4): Chemical analysis of the studied soil samples

Location	pH	Cations Meg/l				Anions Meg/l				Macro-nutrient ppm			Micro-nutrient ppm			
		K^+	Na^+	Mg^{++}	Ca^{++}	So_4^-	Cl^-	Hco_3^-	CO_3^-	K	P	N	Fe	Mn	Zn	Cu
Al-Sokhna	8.04	0.39	26.61	1.10	14.27	11.83	29.71	0.84	0.00	188.14	4.00	30.00	2.07	1.13	1.01	0.16
Oyoun Mousa	8.15	0.12	0.87	2.32	7.20	3.68	5.67	1.15	0.00	102.23	7.46	25.76	1.19	1.31	0.80	0.28
Portwafiq	7.74	0.43	2.77	1.67	3.43	1.83	5.63	0.90	0.00	600.33	11.43	36.67	3.38	11.48	3.43	1.13
Elgabalayte	7.90	0.44	2.98	1.84	3.52	2.64	5.28	0.92	0.00	431.80	11.30	30.00	4.26	2.72	4.12	0.28
Amer	7.85	0.35	5.75	3.50	32.00	19.75	20.40	1.45	0.00	388.75	21.38	47.50	2.25	2.73	3.54	2.34
Alomda	7.85	3.30	1.87	9.73	9.07	17.00	5.63	1.33	0.00	476.12	16.38	34.66	6.31	6.52	1.62	2.28
Alshalofa	7.72	0.31	95.00	5.53	30.33	36.54	93.20	1.10	0.00	464.30	16.23	38.50	1.20	3.04	5.08	1.50
Shandors	7.75	0.30	11.07	3.47	13.33	21.93	5.20	1.03	0.00	429.78	14.74	43.53	3.19	6.37	1.89	1.53
Kabreet	7.98	0.57	8.98	4.50	9.54	11.26	11.20	1.12	0.00	292.66	14.47	79.80	10.26	8.15	4.85	2.06
Genifa	7.82	0.64	128.7	18.0	21.57	75.17	92.28	1.32	0.00	347.58	15.26	44.67	1.71	2.64	1.29	0.83
Alraid	8.00	0.12	8.11	10.6	11.56	8.10	21.37	0.96	0.00	337.44	13.86	49.61	1.89	0.86	0.99	0.45
Aldowlia	8.06	0.38	36.13	2.10	17.78	14.60	40.33	1.45	0.00	260.50	10.80	50.00	2.36	0.83	2.28	0.31
Alzeraien	7.78	0.10	2.50	2.50	14.23	14.63	3.87	0.83	0.00	92.33	16.00	23.33	1.91	2.90	2.77	1.31
Alshabab	8.13	0.20	22.73	9.27	14.80	18.80	26.97	1.23	0.00	217.75	1.37	30.00	2.08	2.67	1.43	0.77

Table (5): Physical contents of the studied soil samples

Location	Particle size distribution %			Texture class
	sand	silt	clay	
Alsokhna	73.5	23.1	3.4	sandy
Oyoun Mousa	71.8	24.1	4.1	sandy
Portwafiq	73.9	20.7	5.4	sandy
Elgabalayte	76.8	21.2	2.0	sandy
Amer	67.5	25.2	7.3	sandy
Alomda	30.3	22.3	47.4	clay
Alshalofa	73.8	23	3.2	sandy
shandoura	72.6	21.2	6.2	sandy
Kabreet	69.2	25.4	5.4	sandy
Genifa	68.2	24	7.8	sandy
Alraid	61.3	30.1	8.6	sandy
Aldowlia	36.8	37.2	26	loam clay
Alzeraien	68.8	25.5	5.7	sandy
Alshabab	68.2	27.1	4.7	sandy

locations. Higher frequencies occurred in Genifa and Aldowlia districts and lower frequencies occurred in the other districts may be due large variations of the edaphic factors in the studied locations. The genera of *Beauveria, Metarhizium, Paecilomyces* and *Verticilium* were specific entomopathogens, so in nature they cause high mortality rates within a short period of time, while the mortality rates caused by other pathogens varied.

Obtained results were compatible with those in other parts of the world such as in Spain, Finland, China, USA and Syria (Keller and Zimmerman, 1989, Vanninen, 1996, Meyling and Eilenberg 2006, Quesada *et al.*, 2007, Sun and Liu, 2008 and Ahmad *et al.*, 2011). Different frequency rates for existing of the entomopathogenic agents in different regions of the world have mostly resulted from the variations of the environmentally and geographical characteristics of search areas, soil types and ecosystems.

Physical and chemical properties of soils

Data in Fig. (2) indicate that the electric conductivity in all the soil samples ranged from 1.67 to 11.28 mhos/cm, *i.e.*, from 0.53 to 3.61 as a total salinity (TSS). The salinity level was relatively high in all locations, highest at Alraid and lowest at Alomda. Furthermore, percentage of calcium carbonate ($CaCO_3$) varied from 3.0 to 41.0 in all locations. $CaCO_3$ rates were high in all locations, except at Kabreet (3.70%), Aldowlia (8.80%) and Alzeraien (7.83%) (Fig. 3).

Data in Table (4) present macronutrient (N.P.K.) that refers to fertility status of the studied soil samples. Potassium content recorded high levels in all the tested samples. Nitrogen content was greater (79.80 ppm) at Kabreet compared to the lowest (23.33 ppm) at Alzeraien. Phosphorus content was high (21.38 ppm) at Amer whereas it was very low (1.37 ppm) at Alshabab.

Micronutrient status in the samples that contained high levels of available iron and magnesium was recorded at Kabreet, whereas the low levels were found at Oyoun-Mousa followed by Aldwolia. Also, the samples were found containing high levels of zinc and copper, except that at Oyoun-Mousa and Alsokhna. The dominated salts of cations and anions in all the studied samples were the sodium (Na) and chloride (Cl), respectively (Table, 4).

Data in Table (5) show that clay and loam clay soils are dominant at Alomda and Aldowlia, respectively whereas sandy soil is dominant in the other locations. Organic matter in soil samples ranged from 0.01 to 0.35%. High percentage of organic matter (0.34%) was found in the samples of Aldowlia, followed descendingly by that of Kabreet (0.22%), Alshalofa (0.21%) and Alomda (0.20%), respectively. It ranged from 0.07 to 0.19% in the other regions (Fig. 3). Statistical analysis showed that correlation coefficients values of chemical and physical soil properties were insignificantly poor for correlated effects, except for calcium carbonate and magnesium (Mg) that showed highly significant correlations.

Many studies have examined the distribution and diversity of microorganisms in soil, but often such studies did not directly link the microbial information with the formation of edaphic properties even though such properties are increasingly being shown to have an important influence on the biogeographical patterns exhibited by soil bacteria and fungi (Fierer and Jackson 2006 and Lauber *et al.*, 2008). Obtained data showed that variability in edaphic factors could have significant effect on microbial community structure and should not be ignored. Composition of bacterial and fungal communities was most strongly correlated with specific soil properties. Soil pH was the best predictor of bacterial community composition across this landscape, while fungal community composition was most closely associated with changes in soil nutrient status.

Side effect of pesticides on insect-associated pathogens

Commonly pesticides used in the area under consideration were tested to clarify their effects on the growth of the fungi; *B. bassiana, M. anisopliae, P. fumosoroseus* and the nematodes *Heterorhabditis* spp. Data in table (6) showed that the herbicide Sencor achieved a very strong inhibition effect to the radial growth of the fungus *B. bassiana*, (92.59%), followed by Furdan, Sumthion, Vydate, Gallant, Table (6): Effect of certain pesticides on the growth of the fungi *B. bassaina*, *M. ansiopliae* *P. fumosoroseus* and the nematodes *Heterorbditis* spp.

Pesticides	Organism						
	Baeuveria bassiana		*Metarhizium anisopliae*		*Pacilomyces fumosoroseus*		*Heterorhabditis* sp.
	Radial growth cm	% Reduction	Radial growth cm	% Reduction	Radial growth cm	% Reduction	Radial growth cm % Reduction
Insecticides							
Actellic	3.33 c	62.96	4.03 cd	55.19	4.53 c	49.63	91.67 a
Sumithion	0.79 d	91.20	0.67 hi	92.59	0.75 e	91.67	90.00 a
Cidial	4.33 c	51.85	6.07 b	32.59	6.32 b	29.81	93.33 a
KZ oil	6.17 b	31.48	4.70 bc	47.78	4.85 c	46.11	40.00 bc
Fungicides							
Topsin	1.17 d	87.04	3.00 e	66.67	3.00 d	66.67	28.33 cde
Coside 101	1.08 d	87.96	2.83 e	68.52	2.72 d	69.81	33.33 bcd
Oxychlor cupper	1.80 d	80.00	0.38 i	95.83	0.79 e	91.20	21.67 de
Sulfur	5.50 b	38.89	5.50 bc	38.89	6.93 b	22.96	30.00 cde
Acaricides							
Ortus	8.50 a	5.56	8.72 a	3.15	8.83 a	1.85	45.00 b
Vertmeic	8.67 a	3.70	8.67 a	3.70	8.68 a	3.52	43.33 b
Nematicides							
Furdan	0.75 d	91.67	2.33 ef	74.07	2.75 d	69.44	93.33 a
Vydate 24 EC	0.92 d	89.81	1.83 fg	79.63	3.08 d	65.74	91.67 a
Herbicides							
Sencor	0.67 d	92.59	1.42 gh	84.26	1.33 e	85.19	20.00 e
Gallant	0.96 d	89.35	1.83 fg	79.63	1.17 e	87.04	21.67 de
L.S.D	1.16		0.84		1.29		10.95

Cosid 101, Topsin and cupper xychlor that caused superior reduction rates of 91.67, 91.20, 89.81, 89.35, 87.96, 87.04 and 80.96%, respectively . The pesticides, Actellic, Cidial, KZ oil and Sulfur had moderate reduction values 62.96, 51.85, 31.48, and 38.89%, respectively on the growth of *B. bassiana*. Tested acaricides; Ortus and Vertemic showed lowest effect (5.3 and 3.70%), respectively.

For the fungus *M. ansipoliae*, highest effect (growth reduction) was manifested by each of the fungicides, Cupper oxychlor (97.2 %), followed by the insecticide, Sumthion (92.59%), and the herbicide, Sencor (84.26%). The other insecticides showed moderate reductions; Gallant (79.63%), Vydate (79.63%), Furdan (74.07%), Kocide 101 (68.52%), Topsin (66.67%), Actellic (52.19%), KZ oil (47.78%), Sulfur (38.89%) and Cidial (32.59%). The tested acaricides; Vertmic and Ortus had very low effects on the fungus.

For the fungus *P. fumosoroseus*, highest effect (growth reduction) was manifested by each of the insecticides, Sumthion 91.67%, followed by the fungicide, chloride oxychlor (91.20%), the herbicides Gallant (87.04%) and Sencor (85.19%). However, the tested acaricides; (Vertmic and Ortus), the insecticide, Cidial and fungicide, Sulfur had lowest effects on the fungus. The other fungicides and nematicides showed moderate effects. The fungicide, Sulfur caused low effect (22.6%), whereas the tested nematicides and insecticides, except Kz Oil, caused the highest nematicidal activity, whereas the other pesticides showed low effects.

It is obvious that many pesticides tested might have caused great damages to the insect pathogens as biocontrol agents. The proportion of occurrence of biocontrol agents was not enough to set any effective pest control strategy based solely on such biocontrol agents; and many practices should be carried out. The most important is to avoid the use of harmful pesticides to these agents to conserve their populations in soil. Furthermore, such bioagents may be introduced artificially in the fields. The results of our respective experiment were largely in agreement with those obtained by El-Adawy *et al*., (2001) and Ahmed *et al*., (2007).

Diversity indices

The insect-associated pathogens obtained from the soil samples of the same region can be treated as a pathogen community. To elucidate the major variation patterns, parameters of the pathogens community of the studied locations including; species richness, species diversity index, evenness were calculated. Richness (S) was a measure of the number of different kinds of organisms present in a particular area. The Obtained data revealed that the maximum species diversity from different locations of Suez governorate was found at Genifa, Aldowlia and Elzeraien; however Alsokhna and Oyoun-Mousa had minimum species diversity (Fig. 5). Species richness (S) at Aldowlia was the highest value (15), followed by Genifa (14), Alzeraien (14), Shandoura (13), Alshalofa, Alomda(12), Elraid (9), Portwafiq (8), Alshabab and Elgabalayte (7), Amer (6), Oyoun-Mousa (4) and Alsokhna (2). However, diversity depends not only on richness, but also on evenness. Evenness is a measure of the relative abundance of different species making up the richness of an area. The values of evenness index (*J*) ranged between 0.81 – 0.99. Species evenness was highest (0.99) at Alshalofa and lowest (0.81) at Alsokhna.

Shannon diversity index is an index applied to biological systems derived from a mathematical formula used in communication area by Shannon in 1948 (Mandaville, 2002). It's the most preferred index among the other diversity indices. The values of Shannon diversity index ranged between 0.56 and 2.51. The lowest value was for Alsokhna and the highest was for Genifa. The lowest values indicate that there were unstable and degradation of habitat structure.

Simpson's Diversity Index (1–D) measures the probability of two individuals randomly selected from a sample belong to the same species (or some category other than species). It is a diversity indices derived by Mandaville (2002). The values of index ranged between 0.50 and 0.99. Alsokhna district had the lowest value while Alshalofa had the highest value (Fig. 4)

The Shannon index compares samples of different sizes to assess number and abundance of species present. The Shannon index is essentially a measure of randomness. In general, overall diversity values for richness, evenness, Shannon and Simposen indices in Suez governorate that calculated as; 15, 0.93, 2.51 and 0.90, respectively demonstrated that the combined collections included a greater amount of diversity.

Potential use of biodiversity indices might be sensitive to any changes in the species data that may occur at any district. Diversity indices provide important information about rarity and commonness of species in a community. Studies of biodiversity in agro-ecosystems and the delivery of ecosystem services to agricultural production have usually ignored the contribution of entomopathogens in the regulation of pest populations (Gurr *et al.*, 2003 and Tscharntke *et al.*, 2005). However, entomopathogens are among the natural enemies of pests in agro-ecosystems. An improved understanding of the ecology of indigenous populations of these beneficial organisms is a prerequisite for evaluation of their contributions to pest control and for predicting the impact of agricultural practices on their populations. Consequently, to understand more about the insect-pathogen dynamics in the soil, studies on the natural occurrence and distribution of insect pathogens in different soil types and in different geographical regions are necessary. Ecological studies on occurrence of all insect-associated pathogens in the soil are needed too.

The present work documented occurrence of entomopathogenic agents in various habitats and localities and also yield of new species and isolates of the bioagents at Suez Governorate, Egypt. At the same time, advantage of using native isolates of bioagents in biocontrol programs is likely to be an enhanced ecological compatibility and also in significant reduction impact on non-target organisms when compared with exotic isolates. Accordingly, control potentials of the new isolates will be evaluated in the future against natural hosts under local conditions.

In general, the present findings are early steps toward using these bioagents for pest control. Likewise, biological control agents also have effects on non-target insect communities. It is clear that more work is still needed to assess their impact, at least on beneficial insects. It would ultimately provide a baseline decision-making strategy using the insect pathogens in the integrated control program against targeted pests.

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REFERENCES

Ahmad, M., S. Al-Moughrabi and A. Haj Hassan 2011. Survey of entomopathogenic fungi in soils of different Ecosystems in Lattakia. Arab Journal of Plant Protection, 29: 171-178.

Ahmed S. A., A. A. El-Saedi, H. M. Ahmed and A. M. Sayed 2007. Entomopathogenic fungi a promising bio-control agent for *Icerya seychellarum* (Homoptera: Margarodidae) in Egypt. Proc. 2nd Inter. Conf. Ent. Soc. Egypt, Vol. II (39-53).

Bidochka M. J, J. E. Kasperski and W. Gam 1998. Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. Canadian Journal of Botany, 76: 1198-1204.

El-Adawy, A. M., E. S. El-barogy, M. H. S. Naeim,

M. A. A. Essa, M. H. El-hamawi and T. A. Sharkawy 2001. Factors affecting the natural occurrence of some insect biocontrol agents in cultivated soil in Ismailia governorate. Egypt. J. Agric. Res., 79(2):419-430.

Fierer, N. and R. B. Jackson 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626–631.

Gardner, W. A. and G. K. Story 1985. Sensitivity of *Beauveria bassiana* to selected herbicides. J. Econ. Ent., 78:1275-1279.

Chandler D., D. Hay and A. P. Reid 1997. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Applied Soil Ecol., 5: 133-141.

Gee, G. W. and J. W. Bauder 1986. Particle-size analysis. p. 383–411. In A. Klute (ed.). Methods of soil analysis. Part 1st and 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Goettel, M. S. and G. D. Inglis 1997. Fungi: Hyphomycetes. Manual of Techniques in Insect Pathology (ed. by L.A. Lacey), pp. 213-249. Academic Press, San Diego, USA.

Gurr, G. M., S. D. Wratten and J. Luna 2003. Multi-function agricultural biodiversity: pest management and other benefits. Basic and Applied Ecology, 4,107–116.

Inglis G. D., M. S. Goettel, T. M. Butt and H. Strasser 2001. Use of hyphomycetous fungi for managing insect pests. In: Fungi as biocontrol agents: progress, problems and potential. Ed. by Butt TM, Jackson C, Magan N, CAB International, Wallingford, UK, 23–69.

Jackson, M. L. 1967. Soil chemical analysis. Prentice Hall of India Private Limited, New Delhi.

Keller, S. and G. Zimmerman 1989. Mycopathogens of soil insects. Pages, 239–270. In: Insect–Fungus Interactions. N. Wilding, N.M. Collins, P.M. Hammond and J.F. Webber (eds.). Academic Press, London.

Lauber, C. L., M. S. Michael and M. A. Bradford and N. Fierer 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry, 40:2407–2415

Magurran, A. E. 2004. Measuring Biological Diversity. Blackwell Publishing, Oxford, p. 256.

Mandaville, S. M. 2002. Benthic Macroinvertebrates in Freshwaters-Taxa Tolerance Values, Metrics, and Protocols. Project H-1. Soil & Water Conservation Society of Metro Halifax. http://chebucto.ca/Science/SWCS/SWCS.html

Meyling, N. V. and J. Eilenberg 2006. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agro-ecosystem. Agriculture, Ecosystems and Environment, 113: 336–341.

Quesada-Moraga, E., J. A. Navas-Cortes, E. A. A. Maranhao, A. Ortiz-Urquiza and C. Santiago-Alvarez 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological Research, 111: 947–966.

Samson, R. A., H. C. Evans, and J. P. Latge 1988. Atlas of entomopathogenic fungi. 187 pp. Springer-Verlag, Berlin, Germany.

Sun, B. D. and X. Z. Liu 2008. Occurrence and diversity of insect-associated fungi in natural soils in China. Applied Soil Ecology, 39: 100–108.

Tscharntke,T., A. M. Klein, A. Kruess, I. Steffan-Dewenter and C. Thies 2005. Landscape perspectives on agricultural intensification and biodiversity ecosystem service management. Ecology Letters, 8, 857–874.

Vanninen, I, 1996. Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycol. Res., 100, 93–101.

Woodering, J. L., and H. K. Kaya 1988. Steinernematid and Heterorhabditid nematodes: A handbook of techniques. Southern Cooperative Series Bulletin 331.30 pp. Arkansas Agricultural Experiment Station, Fayetteville.

Zimmermann, G. 1986. The 'Galleria bait method' for detection of entomopathogenic fungi in soil. J. Appl. Entomol., 102, 213–215.