African Journal of Biotechnology Vol. 9(45), pp. 7674-7682, 8 November, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1112
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Climatic factors interference with the occurrence of *Beauveria bassiana* and *Metarhizium anisopliae* in cultivated soil

Khalid A. Hussein[1,3], Mohamed A. A. Abdel-Rahman[2], Ahmed Y. Abdel-Mallek[1], Saad S. El-Maraghy[1] and Jin Ho Joo[3]*

[1]Botany Department, Faculty of Science, Assiut University, Egypt.
[2]Plant Protection Research Institute, A.R.C., Egypt.
[3]Department of Biological Environment, Kangwon National University, 192-1 Hyo-Ja Dong, Chuncheon, Kangwon-do 200-701, South Korea.

Accepted 5 October, 2010

Description of method and recommendation of laboratory and field procedures for the isolation of soil borne entomopathogenic fungi (specifically *Beauveria bassiana* and *Metarhizium anisopliae*) is presented. Baiting technique method was used for screening of occurrence of indigenous populations of entomopathogenic fungi. Totally, 2068 alive greater wax moth larvae (*Galleria mellonella* L.) were used to trap entomopathogenic fungi present in the soil. Site selected was the experimental farm of Assiut University; only 105 larvae were infected by entomopathogenic fungi representing 5.08% mortality. *B. bassiana* caused 85.71% of the mortality represented by 90 isolates. *M. anisopliae* caused 14.29% of the mortality giving only 15 isolates of *M. anisopliae*. Data showed that *B. bassiana* seems to be the most economically important entomopathogenic fungi inhabiting soil cultivated with wheat and cotton plants. The highest number of the isolates was recorded during spring and autumn seasons. The relationship between the incidence of *B. bassiana* and *M. anisopliae* and the selected weather factors was statistically analyzed using multiple regression analysis.

Key words: Entomopathogenic fungi, *Galleria mellonella*, baiting technique, soil.

INTRODUCTION

The Deuteromycetes, *Metarhizium anisopliae* and *Beauveria bassiana* are common soil-borne entomopathogenic fungi that occur worldwide (Klingen et al., 1998; Mietkiewski and Tkaczuk, 1998). The origin of the entomopathogenic lifestyle may have arisen several times from a common saprophytic ancestor inhabiting soil and leaf litter (Spatafora and Blackwell, 1993). Asexually produced fungal spores or conidia are generally responsible for infection, and are dispersed throughout the environment in which the insect hosts are present.

In contrast to bacteria and viruses that pass through the gut wall from contaminated food, fungi have a unique mode of infection. When conidia of the entomopathogenic fungi land on the cuticle of a suitable host, they attach and germinate, initiating cascades of recognition and enzyme activation reactions both by the host and the fungal parasite (Samson et al., 1988). Conidia of Hyphomycetes such as *Metarhizium* and *Beauveria* spp. are hydrophobic and are passively dispersed from infected cadavers; hyphomycete fungi may also form over-wintering structures based on compressed hyphae (sclerotia) or thick-walled resting spores (chlamydospores) (Shah and Pell, 2003). The biological control strategy in farming practices and environmental manipulations are adopted to enhance the living conditions for specific natural enemies of pests. However, in order to manipulate the environment for the benefit of populations of the entomopathogens, knowledge of fundamental aspects of the ecology of the fungi considered is necessary (Meyling and Eilenberg, 2007).

*Corresponding author. E-mail: jhjoo@kangwon.ac.kr.

A fungus introduced through attractant baited traps (Stimac and Pereira, 1997) has the potential to replicate and spread to produce epizootics in insect population (Fuxa et al., 1998).

The main intentions of this study are to: (1) Isolate indigenous entomopathogenic fungal species from field-collected samples of soil; (2) study the correlation between viability of those fungi in the soil and the estimated weather factors; (3) describe the methods to obtain knowledge of the community of entomopathogenic fungi in soils.

MATERIALS AND METHODS

Soil sampling

Soil samples were randomly sampled every 15 days from wheat and cotton fields on an area of ca 4200 m^2 (one acre) of Assiut University gardens. Wheat is usually planted in November and harvested by May, and cotton is planted during April and harvested by October. No insecticides or herbicides have been used in the study area. Wheat soil samples were taken from December to May; both cotton soil samples were from May to November. Ten soil samples were taken every 15 days, soil samples were taken at maximum depth of 10 cm. Each collected sample was transferred into a labeled collecting muslin bag and transferred into the laboratory. Each soil sample was stored in an individual 10 clean and dry plastic bag. A total of 230 soil samples were taken during the study.

Rearing of greater wax moth

To initiate a culture of the greater wax moth, *Galleria mellonella* pupae were collected from naturally infested stored honeybee combs and kept at 30 ± 1°C and 75 ± 5% relative humidity (RH) till adult emergence. Twenty adults (including male and female, 10 for each) were introduced into a glass jar (10 L) containing blokes of old bee wax (about 5 x 5 x 1.5 cm), and covered with gauze. After month's copulation and oviposition, pieces of wax blocks having *G. mellonella* eggs were transferred into another class jar (2 L capacity) until hatching. The newly hatching larvae were fed on old bee wax until pupation and provided with additional amounts of sterilized bee wax when needed (Hussein, 2007).

Baiting technique

Soil was thoroughly mixed and homogenized by hand. Soil samples were crushed manually in normal glass mortar, and sieved via 2 mm pored strainers to obtain fine dust. After that, 70 g of each soil sample were put in clean glass sterilized Petri dish of 12 cm diameter, and then 25 ml of distilled and sterilized water was added. Ten larvae were released into a Petri dish (Zimmermann, 1986). This step was replicated ten times for ten soil samples, thus each test exploited 100 *G. mellonella* larvae. The Petri dishes were incubated at 25°C and examined everyday for 15 days. The dead larvae were transferred to clean and sterilized Petri dishes of 9 cm diameter. To elevate the moisture content, wet filter papers were added and to maintain the moisture content, distilled drops of water were used. The Petri dishes containing the dead larvae were incubated also at the same temperature. The dead larvae were inspected daily to observe the growth of entomopathogenic fungi.

Isolation and identification of fungi

Dead larvae with condense growth of fungi were examined under a dissecting microscope as soon as possible to observe external symptoms and fungal reproductive structures produced. Hyphal tips from external growth mycoses cadavers were taken by tiny fine tip of a sterile isolation needle and inoculated on appropriate potato dextrose agar (PDA) or Sabouraud dextrose agar (SDA) PDA in Petri dishes at 25°C. Insect cadavers (Figure 1; Plates A and D) or portion of mycelium were mounted on slides, stained with lacto phenol cotton blue prior to direct microscopic examination (Domsch et al., 1980) and was used for fungi identification. The isolated fungal species identification were approved and preserved by Assiut University Mycological Center (AUMC) members.

RESULTS

Survey of entomopathogenic fungi from soil, Assiut

During the present investigation, two species namely: *B. bassiana* and *M. anisopliae* belonging to family Moniliaceae, order Moniliales were identified. The morphological characteristics of the identified fungi species were described as follows:

B. bassiana

Colonies were found to be growing slowly, wooly, at first white but later often becoming yellow to slightly pinkish (Figure 1; Plate B). Conidiogenous cells arose either singly, in whorls from vegetative hyphae, or more commonly in clusters from swollen stalk cells. Conidiogenous cells differentiated into a subglobose to ellipsoidal or cylindrical venter and a filiform, zig-zag shaped (Figure 1; Plate C), and denticulate rhachis arising by sympodial elongation. Conidia hyaline, globose or ellipsoidal with a rounded or slightly pointed base were also observed. Also, conidiogenous apparatus formed dense clusters of swollen stalk cells, which consist of a subglobose to flask-shaped venter 3 - 6 × 2.5 - 3.5 µm, and zig-zag shaped. Conidiogenous cell length 8.7 µm; conidiogenous cell width 2.9 µm; hyphae width 2 - 3 µm; and conidia subglobose 2 - 4 µm diameter. The longest period was recorded for *M. anisopliae* but the shortest was observed for *B. bassiana* at 30°C.

M. anisopliae

Colonies growing rather slowly, at first floccose, later becoming olivaceous green (Figure 1; Plate E) due to abundant conidiation; reverse yellowish to brownish. Conidiophores aggregated in dense tufts, with repeated, more or less verticillate branching; phialides in a dense, parallel arrangement (Figure 1; Plate F). Phialides clavate, 9 - 14 µm long, with rounded apex. Conidia were produced in long chains, cylindrical, 5 - 8 x 2.5 - 3.5 µm, thick-walled and yellowish-green in mass. According to

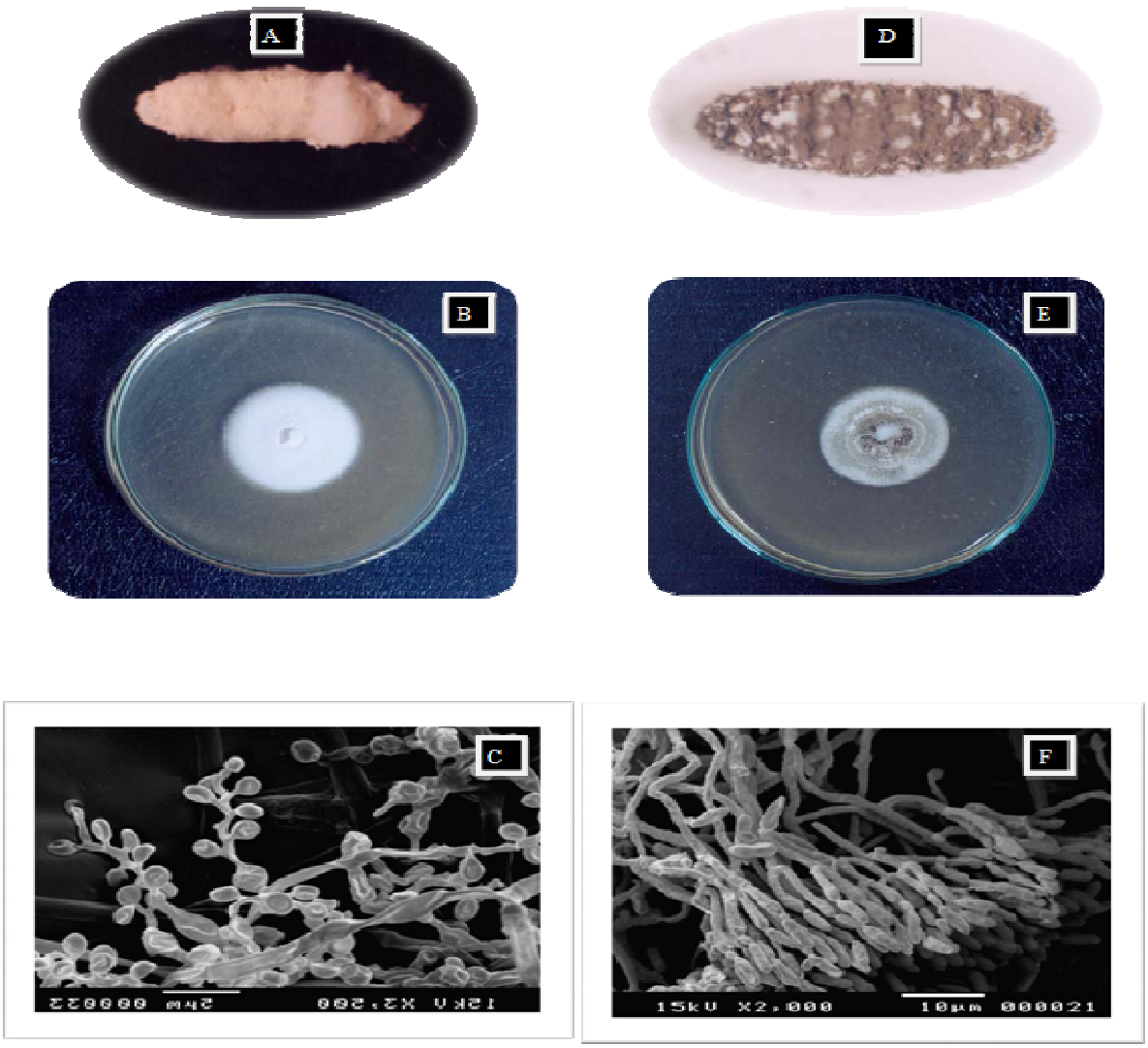

Figure 1. Plates A and D show the *G. mallonella* mycosis; B and E, 7-days old cultures of *B. bassiana* and *M. anisopliae*, respectively; C and F, scanning electron microscope photos of *B. bassiana* and *M. anisopliae*, respectively.

Table 1. Morphological characters of *B. bassiana* and *M. anisopliae* isolated from soil, Assiut, Egypt.

Fungi species	Length	Width	Ratio (L/W)
B. bassiana	2.74±0.36	2.03±0.26	1.31
M. anisopliae	7.52±0.79	2.90±0.54	2.59

conidial measurements of the previous fungi species, the mean spore size (length / width ratio) was measured.

The average conidial length of *B. bassiana* was 2.74 ± 0.36 µm, and width was 2.03 ± 0.26 µm. The conidial length of *M. anisopliae* was 7.52 ± 0.79 µm, while the width was 2.90 ± 0.54 µm. The data obtained from analysis of variance for *B. bassiana* and *M. anisopliae* spore size measurements showed significant differences between the two fungi species in Table 1.

Steps of infection

Data in Table 2 shows the number of days required for *G. mellonella* infection by *B. bassiana and M. anisopliae* isolated from soil of Assiut, Egypt. The results indicated that the time needed from baiting to the death of larvae were 9.33 and 11.53 days for *B. bassiana and M. anisopliae*, respectively. The infected (dead) larvae were collected and individually put in closed moist Petri dishes

Table 2. Number of days required for *G. mellonella* infection by *B. bassiana* and *M. anisopliae* isolated from soil, Assiut, Egypt.

Fungi species	Time (in days) elapsed					
	From bait to death		From death to fungi growth		Total	
	Mean SE	Range	Mean SE	Range	Mean SE	Range
B. bassiana	9.33	17 May	1.29	2 January	10.62	6.19
M. anisopliae	11.53	17 May	1.33	1 - 2	12.87	18 June

Table 3. Incidence of *B. bassiana* and *M. anisopliae* isolated from soil by bating technique, Assiut, Egypt.

Sampling date	No. samples	No. alive larvae	Total dead larvae	Mortality (%)	Incidence of fungi			
					B. bassiana		*M. anisopliae*	
					No. isolates	%	No. isolates	%
December	10	50	2	4	1	2	1	2
	10	100	4	4	3	3	1	1
January	10	68	7	10.3	0	0	7	10.3
	10	50	0	0	0	0	0	0
February	10	50	2	4	2	4	0	0
	10	50	2	4	2	4	0	0
March	10	100	2	2	2	2	0	0
	10	100	0	0	0	0	0	0
April	10	100	19	19	18	18	1	1
	10	100	2	2	0	0	2	2
May	10	100	0	0	0	0	0	0
	10	100	1	1	1	1	0	0
June	10	100	1	1	0	0	1	1
	10	100	9	9	8	8	1	1
July	10	100	3	3	3	3	0	0
	10	100	3	3	2	2	1	1
August.	10	100	6	6	6	6	0	0
	10	100	3	3	3	3	0	0
September	10	100	7	7	7	7	0	0
	10	100	3	3	3	3	0	0
October	10	100	3	3	3	3	0	0
	10	100	20	20	20	20	0	0
November	10	100	6	6	6	6	0	0
Total	230	2068	105	100	90	85.71	15	14.29
%	-	100	5.08					

and incubated at 25°C. Thereafter, the first white and green mycelium on the larvae were noticed and increased gradually after 1 - 2 days. In general, the time needed for fungi to develope successfully from baiting to emergence over the larvae ranged from 6 - 19 days. Apparently, there were significant differences in the time needed for *B. bassiana* and *M. anisopliae*. The longest period was recorded for *M. anisopliae*.

Seasonal incidence of entomopathogenic fungi recovered

Table 3 shows the relative incidence of *B. bassiana* and *M. anisopliae* isolated from cultivated soil. Data indicated that out of 2068 live larvae of greater wax moth, only 105 larvae were infected by entomopathogenic fungi representing 5.08% mortality. 90 isolates (out of 105) of *B.*

Table 4. Effect of some meteorological factors on the incidence of *B. bassiana* and *M. anisopliae* isolated from soil, Assiut.

Sampling date	Fungi incidence			Meteorological factors						
				Temp. (ºC)			R. H. (%)		Soil temperature 5 cm (ºC)	
	B. bassiana	*M. anisopliae*	Total	Max.	Min.	Daily temperature	Max.	Min.	Max.	Min.
December 2004	2	2	4	23.9	8	15.94	88.46	21.8	19.16	2.73
	3	1	4	21.23	6.53	13.9	87.8	25.2	18.2	1.93
January 2005	0	10.3	10.3	19.76	6.2	13	86	32.73	18.13	1.83
	0	0	0	22.06	7.1	14.26	84.06	26	19.96	2.16
February	4	0	4	18.26	4.18	11.44	84.2	22.4	20.66	1.76
	4	0	4	26	10.26	17.93	87.38	24.23	22.3	5.96
March	2	0	2	25.16	9.83	17.51	85.93	23.73	26.9	8.03
	0	0	0	26.2	8.23	17.24	84.66	16.33	29.46	7.5
April	18	1	19	29.6	11.8	20.7	79.2	15.8	33.7	11.2
	0	2	2	34.9	15.3	25.1	82.7	15.4	36.9	15.1
May	0	0	0	33.2	16.6	24.5	72.8	14	38.2	16.4
	1	0	1	36.7	18.5	27	64.4	16.2	40.1	17.1
June	0	1	1	37.3	21.1	28.5	73.2	20.2	40.1	28.1
	8	1	9	37.6	20.9	29.3	78	16.8	41	29.2
July	3	0	3	39.7	22.3	31	82	17.5	42.9	30.4
	2	1	3	39.1	21.5	30.3	83.3	15	42.4	30.3
August	6	0	6	40	22.1	31	81.13	13.6	44.76	21.6
	3	0	3	36.4	20.7	28.5	86.1	29.5	41.3	20.4
September	7	0	7	35.6	19.3	27.5	84.8	19.2	36.3	18
	3	0	3	39.1	20.1	31.8	73.2	10.4	40.4	18.7
October	3	0	3	35.2	18.9	27	72.6	14.4	35.4	16.6
	20	0	20	29	15.2	23	71.8	16.8	30.1	12
November	6	0	6	24.76	11.4	18.1	75.8	20.6	24.5	17.26

Table 5. Dominance (%) and abundance (%) degrees of *B. bassiana* and *M. anisopliae* isolated from soil at Assiut.

Fungi specie	Dominance (%)	Abundance (%)
B. bassiana	85.71	73.91
M. anisopliae	14.29	26.09
Total	100	100

bassiana represented 85.71% and 15 isolates of *M. anisopliae* represented 14.29%. According to the seasonal incidence of the previous entomopathogenic fungi, data showed that, regardless of the fungal species, entomopathogenic fungi were recorded in 20 out of 23 soil samples constituting 87%. Mortality with these fungal pathogens was observed from December up to November and the percentages of mortality ranged between 1 and 20%. Also, these fungal species were observed over the year round in relatively low occurrence but the relatively higher occurrence was observed during April and October, which may be due to the optimum conditions (Table 4 and Figure 1).

Table 5 and Figure 2 show that *B. bassiana* seem to be the most important entomopathogenic fungi inhabiting soil cultivated with wheat and cotton plants at Assiut as indicated by the highest values of its dominance (85.74%) and abundance (73.91%) degrees. In contrast, the *M. anisopliae* had low values of dominance (14.29%) and abundance (34.78%) degrees. It is clear that *B. bassiana* was represented by 90 isolates and appeared almost in all collecting samples. In general, the highest number of the isolates was recorded during spring and autumn as shown in Figure 3. Whereas during the winter and summer months, the number of isolates were in low detectable level.

Simultaneous effect of some abiotic factors on the incidence of the fungi

The relationship between the incidence of *B. bassiana* and the selected weather factors was statistically tested using multiple regression analysis: $A = Y + B(x)$. The

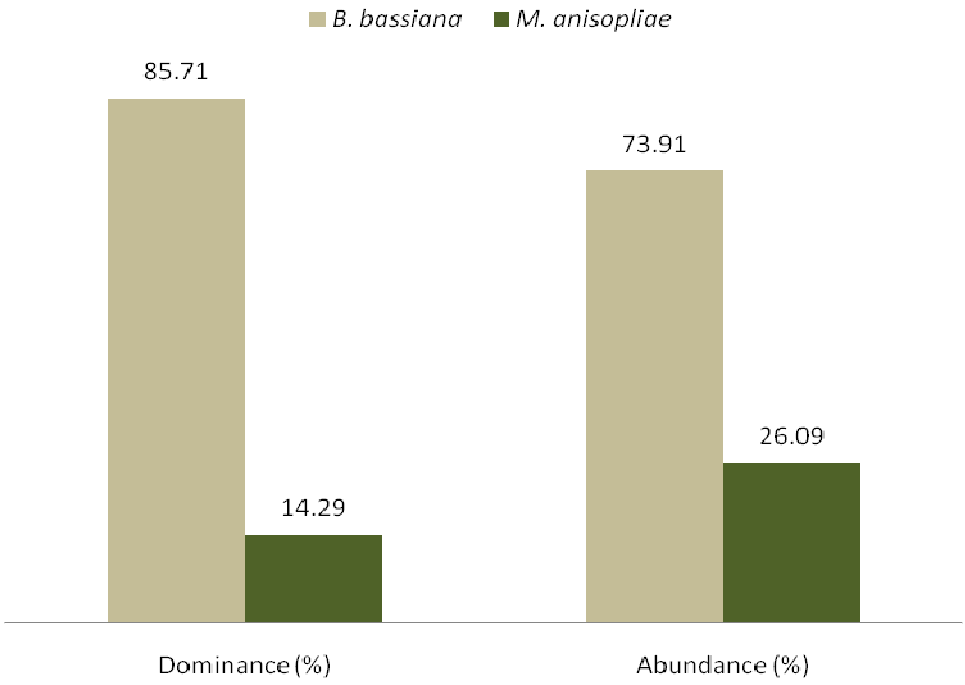

Figure 2. Dominance (%) and abundance (%) degrees of *B. bassiana* and *M. anisopliae* isolated from soil at Assiut.

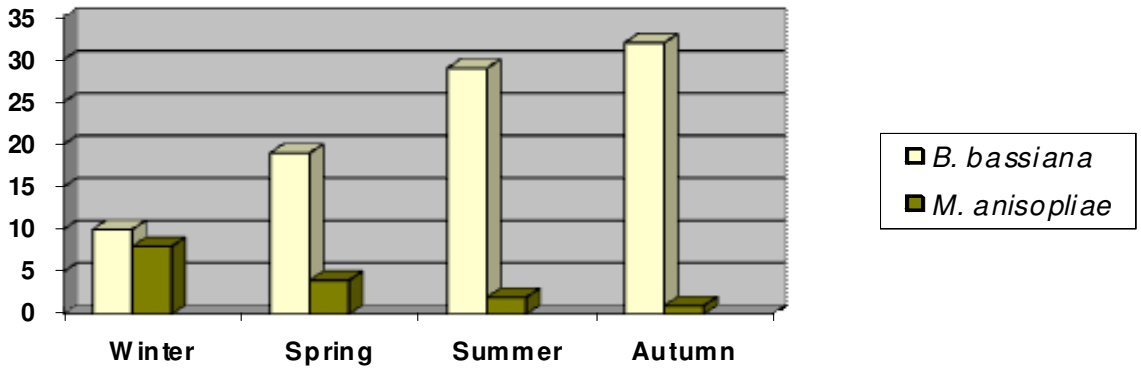

Figure 3. Seasonal occurrence of *B. bassiana* and *M. anisopliae.*

selected weather factors were maximum temperature (x1), minimum temperature (x2), maximum R.H (x3), minimum R.H. (x4), maximum soil temperature (x5) and minimum temperature (x6). Table 6 shows the multi regression analysis between the incidence of *B. bassiana* and the weather factors. Simple correlation analysis revealed a highly significant effect of maximum and mini-mum temperature and the minimum R.H. On the other hand, maximum R.H had an insignificant effect on the incidence of *B. bassiana.* However, coefficient of determi-nation (R2) was 0.3037 indicating that the mentioned tested variables were responsible together for 30.37% of the changes of *B. bassiana* in soil. Dropping one of each variable (Table 6), step by step from the input analysis data, explains the gradual representative efficiency of each variable on the changes of fungi existence. The studied variable can be arranged in descending order as follows: Maximum temperature, minimum temperature, minimum relative humidity, mini-mum soil temperature, maximum soil temperature and minimum RH, where their efficiencies were 9.47, 8.95, 7.79, 1.82, 1.43 and 0.89%, respectively.

Concerning *M. anisopliae*, (Table 7), the simple corre-lation coefficients of the minimum RH was highly significant followed by maximum and minimum air temperatures while those of maximum relative humidity and maximum and minimum soil temperatures were insignificant. The multi-regression analysis revealed that the six studied

Table 6. Multiple-regression analysis between the incidence of *B. bassiana* isolated from soil and meteorological factors during study period, Assiut, Egypt.

Variable removed	(r)	(R)	R^2 x 100	Decrease in R^2 x 100	Efficiency
Non	-	0.5511	30.37	-	-
Max. temp	0.4906	0.288	8.29	22.08	9.47
Min. temp	0.4802	0.3081	9.49	20.88	8.95
Max. RH	0.1705	0.5318	28.28	2.09	0.89
Min. RH	0.4547	0.3495	12.22	18.15	7.79
Max. soil temp	0.214	0.5198	27.02	3.35	1.43
Min. soil temp	0.2398	0.511	26.12	4.25	1.82

(r), Simple correlation coefficient; (R), multi correlation coefficient; R2, coefficient of determination; *, ** significant at P = 0.05 and 0.01, respectively.

Table 7. Multiple-regression analysis between the incidence of *M. anisopliae* isolated from soil and meteorological factors during study period, Assiut, Egypt.

Variable removed	(r)	(R)	R^2 x 100	Decrease in R^2 x 100	Efficiency
Non	-	0.5387	29.02	-	-
Max. temperature	0.2376	0.4977	24.77	4.25	4.2743
Min. temperature	0.2382	0.4975	24.75	4.27	4.2994
Max. RH	0.1743	0.5177	26.8	2.22	2.2379
Min. RH	0.4283	0.3615	13.07	15.95	16.0563
Max. soil temperature	0.1241	0.5283	27.91	1.11	1.1175
Min. soil temperature	0.1197	0.829	27.99	1.03	1.0394

Table 8. Multiple-regression analysis between the total incidence of entomopathogenic fungi isolated from soil and meteorological factors during study period in Assiut, Egypt.

Variable removed	(r)	(R)	R^2 x 100	Decrease in R^2 x 100	Efficiency
Non	-	0.4268	18.22	-	-
Max. temperature	0.388	0.1929	3.72	14.5	6.6164
Min. temperature	0.3766	0.2167	4.7	13.52	6.1701
Max. RH	0.0904	0.4188	17.54	0.68	0.3076
Min. RH	0.275	0.3395	11.52	6.7	3.0536
Max. soil temperature	0.1508	0.4039	16.31	1.91	0.8683
Min. soil temperature	0.1768	0.3947	15.57	2.65	1.2054

variables were responsible for 29.02% of the changes in the incidence of this fungi species in cultivated soil. Most of the changes in the incidence of these fungi (16.06%) out of the total of 29.02% were related to the minimum relative humidity variable.

Table 8 supported the former results for both fungal species *B. bassiana* and *M. anisopliae*, regarding the high significance of the simple correlation coefficient of the following variables: Maximum and minimum air temperatures, and minimum relative humidity. However, the coefficient of determination (R2) was 18.22% indicating that the tested mentioned variables were responsible for 18.22% of the changes in the incidence of the two-entomopathogenic fungi species. The aforementioned results indicated that maximum and minimum air temperatures and minimum relative humidity played the most important role in the incidence of the insect mycopathogens inhabiting cultivated soils at Assiut.

DISCUSSION

The traditional approaches do not exploit the indigenous reservoir of fungi that is already present in the cropping system in biological control with entomopathogenic fungi. Thus these approaches still apply the fungal material (usually conidia) to the cropping system, using an inundative or inoculative biological control strategy

(Eilenberg et al., 2001). Another biological control strategy is conservation biological control. Eilenberg et al. (2001) defined this strategy as "modifications of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effects of pests". Therefore, knowledge of the community of natural enemies in the agroecosystem, as well as the effect of the agronomical practices on these organisms is essential to use a conservation biological control strategy. Our results revealed that the maximum and minimum air temperatures and the minimum RH played the most important role in the incidence of the insect mycopathogens inhabiting cultivated soils.

Galleria baiting method (Zimmermann, 1986) has been found to be a very sensitive method for detection of entomopathogenic fungi in soil samples (Keller et al., 2003). By baiting soil samples with larvae of *G. mellonella* (Meyling and Eilenberg, 2006), detailed surveys of the occurrences of entomopathogenic fungi were conducted in the soil of an organically farmed field and the associated hedgerow. In the agricultural field soil, *B. bassiana* was the most common fungus (Meyling and Eilenberg, 2006). Similarly, in the present study, *B. bassiana* seems to be more dominant than *M. anisopliae* in the soil cultivated with wheat and cotton plants. The cultural practices include reduced tillage regimes and enhanced *B. bassiana* levels in the soil (Bing and Lewis, 1993; Hummel et al., 2002). That is why *B. bassiana* persisted after the establishment within an area. However, *B. bassiana* rely on repeated infections of susceptible hosts to maintain high density levels in soils (Fargues and Robert, 1985), as demonstrated for *Beauveria brongniartii* (Saccardo) Petch (Kessler et al., 2004). Steenberg (1995) found that only one larva was infected with *Metarhizium flavoviride*, while Mietkiewski et al. (1997) detected the species at very low frequencies in arable soils from southern UK using *G. mellonella* as bait larvae. In the present study, *B. bassiana* was locally abundant while *M. anisopliae* was locally rare. Furthermore, there is evidence that *M. anisopliae* is less virulent in the field than in the laboratory (Benjamin et al., 2002), and it indicates that the environmental conditions may diminish the pathogenicity of *M. anisopliae* to infect *G. mellonella* in the soil. Thus, knowledge of the local species composition of entomopathogenic fungi in the soil is necessary when evaluating the potential for this group of natural enemies as a reservoir for controlling pest insects in a specific agroecosystem. Our survey detected 90 isolates of *B. bassiana* which represented 85.71% and only 15 isolates of *M. anisopliae* represented 14.29% of the death of the same larvae of *G. mellonella*. Thus, *B. bassiana* exhibits more dominance and viability than *M. anisoliae* and that may be due to some environmental causes; *B. bassiana* can infect many species of insects and can grow on artificial media or some kinds of soil (Li, 1988). It is believed that these hosts and survival niches supplied the necessary nutrients for growth (Wang et al., 2005).

Also, *B. bassiana* could persist for relatively long periods, 12 weeks in sterilized waters (Wang and Shimazu, 2006). To utilize this fungus as a microbial insecticide, dynamics of the fungus after application should be considered since it could persist in certain niches for a relatively long time (Shimazu et al., 2002). Moreover, soil-dwelling mites were shown to be potential vectors of *B. bassiana* (Renker et al., 2005).

M. anisopliae could also survive for a long time in soil (Mikuni et al., 1982; Yaginuma, 1990). Inoculated conidia of *M. anisopliae* persisted significantly better up to one year in the rhizosphere of *Picea abies* in comparison with the bulk soil (Bruck, 2005). Thus survival outside the host may be critical for the ability of *M. anisopliae* to control insect pests in the soil (Roberts and Leger, 2004). However, *in* vitro experiments further showed that the fungicide triadimefon inhibited the growth of *B. bassiana*, but fields previously treated with this product showed a higher frequency of occurrence of the fungus in soil samples than in samples from untreated control soils (Mietkiewski et al., 1997; Chandler et al., 1998).

Temperature and relative humidity are the major factors affecting the ability of fungi to survive, propagate, infect and kill their host (Goettel et al., 2000; McCoy et al., 2003). Our results indicated that the temperatures and the RH played very important role in the occurrence of the insect mycopathogens inhabiting cultivated soils at Assiut. Through specific management strategies that provide optimal conditions for the entomopathogenic fungi in the soil, these natural enemies of insects can be included in the suppression of pests in a conservation biological control strategy (Landis et al., 2000; Eilenberg et al., 2001). Indeed, temperature, moisture and UV-radiation seems to be most important for *B. Bassiana* survival (Meikle et al., 2003).

Acknowledgments

This study was supported by a grant from the Research Institute of Agricultural Science, Kangwon National University. The authors are very grateful to Prof. Ahmed Moharram and to the members of Assiut University Mycological Centre (AUMC), for their cooperation in assurance the fungi identification and preservation of the isolates.

REFERENCES

Benjamin MA, Zhioua E, Ostfeld RS (2002). Laboratory and field evaluation of the entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycetes) for controlling questing adult *Ixodes scapularis* (Acari: *Ixodidae*). J. Med. Entomol. 39: 723-728.

Bing LA, Lewis LC (1993). Occurrence of the entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin in different tillage regimes and in *Zea mays* and virulence towards *Ostrinia nubilalis* (Hubner).

Bruck DJ (2005). Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. Biol. Control, 32: 155-163.

Chandler D, Mietkiewski RT, Davidson G, Pell JK, Smits PH (1998). Impact of habitat type and pesticide application on the natural occurrence of entomopathogenic fungi in UK soils. IOBC-SROP Bull. 21: 81-84.

Domsch KH, Gams W, Anderson T (1980). Compendium of soil fungi. Academic Press. London, 1-2: 859-405.

Eilenberg J, Hajek A, Lomer C (2001). Suggestions for unifying the terminology in biological control. Biocontrol, 46: 387-400.

Fargues J, Robert PH (1985). Persistence of conidia of 4 entomopathogenic Hyphomycetes in soil, Beauveria bassiana (Bals) Vuill *Metarhizium anisopliae* (Metsch) Sor, *Nomuraea rileyi* (F) Samson and *Paecilomyces fumosoroseus* Wize, in controlled conditions. Agronomie, 5: 73-80.

Goettel MS, Inglis GD, Wraight SP (2000). In: Field manual technique in invertebrate pathology, Lacey LA and kaya HK (eds.). Kluwer Academic Publisher, Netherlamds.3 Fungi, pp. 255-282.

Hummel RL, Walgenbach JF, Barbercheck ME, Kennedy GG, Hoyt GD, Arellano C (2002). Effects of production practices on soil-borne entomopathogens in western North Carolina vegetable systems. Environ. Entomol. 31: 84-91.

Hussein Kh (2007). Studies on insect mycopathogens isolated from soil. M.Sc. Thesis, Assiut University, Egypt.

Keller S, Kessler P, Schweizer C (2003). Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metarhizium anisopliae*. Biocontrol, 48: 307-319.

Kessler P, Enkerli J, Schweizer C, Keller S (2004). Survival of *Beauveria brongniartii* in the soil after application as a biocontrol agent against the European cockchafer Melolontha melolontha. Biocontrol, 49: 563-581.

Klingen I, Eilenberg J, Meadow R (1998). Insect pathogenic fungi from northern Norway baited on Delia oralis (Diptera, *Anthomyiidae*) and *Galleria mellonella* (Lepidoptera, *Pyralidae*). IOBC wprs Bulletin, 21: 121-124.

Landis DA, Wratten SD, Gurr GM (2000). Habitat management to conserve natural enemies of arthropod pests in agriculture. Ann. Rev. Entomol. 45: 175-201.

Li ZZ (1988). Insect hosts list of *Beauveria bassiana*. In: Study and Application of Entomogenous Fungi of China, Beijing, Academic Periodical Press, 1: 241-245. (in Chinese).

McCoy C, Quintela ED, De-Fria M (2003). Environmental persistence of entomopathogenic fungi. In: Factors affecting the survival of entomopathogens, Baur ME, Fuxa JR (eds.) p. 8.

Meikle WG, Jaronski ST, Mercadier G, Quimby PC (2003). A distributed delay routine-based simulation model of Beauveria bassiana conidial stability in response to environmental stressors. Biocontrol, 48: 561-578.

Meyling NV, Eilenberg J (2006). Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric. Ecosyst. Environ. 113: 336-341.

Meyling NV, Eilenberg J (2007). Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol. Control, 43: 145-155.

Mietkiewski RT, Pell JK, Clark SJ (1997). Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: field and laboratory comparisons. Biocontrol Sci. Technol. 7: 565-575.

Mietkiewski RT, Tkaczuk C (1998). The spectrum and frequency of entomopathogenic fungi in litter, forest soil and arable soil in Poland. IOBC/WPRS Bull. 21: 41-44.

Mikuni T, Kawakami K, Nakayama M (1982). Survival of an entomogenous fungus, *Metarhizium anisopliae*, causingthe muscardine disease of the silkworm, *Bombyx mori*, in the soil of mulberry plantation, J. Seric. Sci. Jpn. (in Japanese with English summary) 51: 325-331.

Renker C, Otto P, Schneider K, Zimdars B, Maraun M, Buscot F (2005). Oribatid mites as potential vectors for soil microfungi: study of mite-associated fungal species. Microb. Ecol. 50: 518-528.

Roberts DW, St. Leger RJ (2004). *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: Mycological aspects. Adv. Appl. Microbiol. 54: 1-70.

Samson RA, Evans HC, Latg JP (1988). Atlas of entomopathogenic fungi. Springer, Berlin Heidelberg New York.

Shah PA, Pell JK (2003). Entomopathogenic fungi as biological control agents. Appl. Microbiol. Biotechnol. 61: 413-423.

Shimazu M, Sato H, Maehara N (2002). Density of the entomopathogenic fungus, *Beauveria bassiana* Vuillemin (Deuteromycotina: *Hyphomycetes*) in forest air and soil. Appl. Entomol. Zool. 37: 19-26.

Spatafora JW, Blackwell M (1993). Molecular systematics of unitunicate perithecia ascomycetes: the Clavicipitales-Hypocreales connection. Mycologia, 85: 912-922.

Steenberg T (1995). Natural occurrence of *Beauveria bassiana* (Bals.) Vuill. with focus on infectivity to *Sitona* species and other insects in lucerne. Ph.D. Thesis, The Royal Veterinary and Agricultural University, Denmark.

Stimac JL, Pereira RM (1997). Controlling cockroaches, carpenter ants, and pharaoh ants using strains of *Beauveria bassiana.* US Patent, 5: 683-689.

Wang B, Ding DG, Fan MZ, Li ZZ (2005). Community structure of entomogenous fungi in *Beauveria*-inoculated Masson's pine forest, Chin. J. Appl. Ecol. 16: 2357-2360.

Wang B, Shimazu M (2006). Density dynamics of an entomopathogenic fungus, *Beauveria bassiana* introduced into fresh water (Bulletin of FFPRI), 5: 227-233.

Yaginuma K (1990). Detection of fungi pathogenic to peach fruit moth, *Carposina niponensis* Walsingham from soil, Bull. (in Japanese with English summary). Fruit Tree Res. Stn. 17: 77-89.

Zimmermann G (1986). The *Galleria* bait method for detection of entomopathogenic fungi in soil. J. Appl. Entomol. 102: 213-215.