JJBS

Volume 4, Number 2, June 2011
ISSN 1995-6673
Pages 87 - 92

Jordan Journal of Biological Sciences

Association of Entomopathogenic and Other Opportunistic Fungi with Insects in Dormant Locations

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Received February 6, 2011; Accepted in revised form March 13, 2011

Abstract

A survey of entomopathogenic and other opportunistic fungi associated with seven naturally infected insect species live hidden in some plants at their hibernation sites at Gara mountain, Kurdistan region of Iraq was carried out. *Aspergillus flavus*, *A. niger* and *Beauveria bassiana* were detected with high isolation rates. Several other opportunistic pathogens including *Alternaria alternata*, *Curvularia* sp., *Fusarium* sp., *Humicola* sp., *Penicillum* sp., *Rhizopus stolonifer*, *Ulocladium atrum*, *Trichoderma* sp., and sterile mycelium were also isolated. *Beauvaria brongniartii* was isolated from Sunn pest (Eurygoster *integriceps*) for the first time in Iraq. A brief description along with photographs is provided for the newly recorded species.

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Keywords: Entomopathogenic fungi, opportunistic fungi, insects, Iraq.

1. Introduction

Fungal diseases of insects are common and widespread and often contribute to the natural regulation of insect populations (Samson *et al.*, 1988; Hajek and Leger, 1994). Entomopathogenic hyphomycetes have great potential as biological control agents against insects and as one component within integrated pest management systems. They are being developed worldwide for the control of many pests of agricultural importance (Ferron, 1985) and some are already available commercially for the control of various species of trips and aphids (Goettel *et al.*, 1990; Upadhyay, 2003).

As regards of insects, especially members of Hemiptera (Family: Scutelleridae and Pentatomide) live from summer to next spring hidden in some plants (e.g. *Acantholinon acerosum* (Willd.) Bioss) and after hibernation they get outside where reproduction proceeds (Paulian and Popove, 1980; Khanjani and Mirab, 2004). During their hibernation, insects are subjected to infection by entomopathogenic and other opportunistic fungi (Kubatove and Dorak, 2005).

In the Iraq Republic, the most common insect species found in dormant locations was *Eurygaster integriceps* Put. (Sunn pest) the highest percentage of its mortality was caused by the entomopathogenic fungus *Beauvaria bassiana* (Bals.)Vuill. (Assaf, 2007). KiliC (1976) showed ability of *B. bassiana* to kill up to 80% of the sunn pest. Comparable 80% mortality was induced by *B. bassiana* and *Fusarium* spp. entomopathogenic fungi associated with *E. integriceps* in dormant locations of Iraq were recorded by Ali (1995). Mohamad (2000) found the highest level of sunn pest infection by *B. bassiana* (80.18%) during October in the Safeen mountain (Irbil province).

The main aim of the current investigation is to extend our knowledge on the occurrence of entomopathogenic and other opportunistic fungi infecting insects in dormancy locations.

2. Materials and Methods

2.1. Insect collection

Sunn pests and other insects (Table 1) were collected from their hibernation sites at Gara mountain , Duhok governorate, North Iraq for two years between October to December of 2009-2010 consecutively. Collected insects were taken to the laboratory in clean bags for isolation of fungi.

2.2. Isolation of fungi

Collected insects were surface sterilized in 2% sodium hypochlorite solution for 3 minutes, rinsed in plenty of sterile distilled water, then dried by filter paper. Surface sterilized cadavers were plated onto potato dextrose agar (PDA) (Himedia Laboratories Pvt. Ltd. - India) containing 0.25 mg/ml chloramphenicol to inhibit growth of bacteria and incubated at 25°C. Hyphae of the fungi growing and sporulating on cadavers and on PDA medium were cut, transferred on fresh PDA plates and incubated at 25°C.

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Table 1. Insects specimens collected from Gare Mountain in the Duhok province-Iraq

Taxonomic group of insects (order, family)	Insect species	Number of Insects
Hemiptera, Pentatomidae	*Dolycoris baccarum* L.	107
Hemipetera, Scutelleridae	*Eurygoster integriceps* Put.	25
Coleoptera, Scarabaeidae	*Anomala* sp.	5
Orthoptera, Acrididae	*Acrotylus insubricus* Scop.	1
Hemiptera, Pentatomidae	*Aelia acuminate* L.	2
Hemiptera, Pentatomidae	*Apodiphus* sp.	3
Coleoptera, Coccinellidae	*Coccinella novemnotata* Herbft	6

2.3. Identification of fungal isolates

Identification of fungal isolates was mainly based on their morphological characteristics of their reproductive structures with the aid of relevant taxonomic keys (de Hoog, 1972; Samson *et al.,* 1988; Tzean *et al.,* 1997; Domsch *et al.,* 1980).

Isolation percentage of a particular species on insect was calculated using the formula:

Isolation percentage = (Number of fungal isolates of a particular species / Total number of isolates of all species) X 100

3. Results

A total of 149 cadavers belong to seven insect species were examined for the presence of fungi. Approximately 226 fungal colonies assigned to 12 different species and sterile mycelium were isolated (Table 2). The highest number of isolates (184 and 25) was detected from *Dolycoris baccarum* and *Eurygoster integriceps*, respectively.

Aspergillus flavus (33.70%), *Aspergillus niger* (26.63%), *Beauveria bassiana* (10.33%) and *Alternaria alternata* (9.78%) were the most common fungal species isolated from *Dolycoris baccarum*, whereas *B. bassiana* showed the highest isolation percentage (36.00%) on *Eurygoster intergriceps*, followed by *Ulocladium atrum* (20.00%) and *Rhizopus stolonifer* (16.00%). Fungi isolated from *Coccinella novemnotata* were in descending order, **B**. *bassiana* (33.33%), *R. stolonifer* (22.22%), followed by *A. alternate*, *A. flavus*, *A. niger* and *Trichoderma* sp. (11.11% each). *Beauveria* was not isolated from the other four insect species. *Beauveria brongniartii* was isolated from the sunn pest *Eurygoster integriceps* for the first time in Iraq. The newly recorded species is described and illustrated.

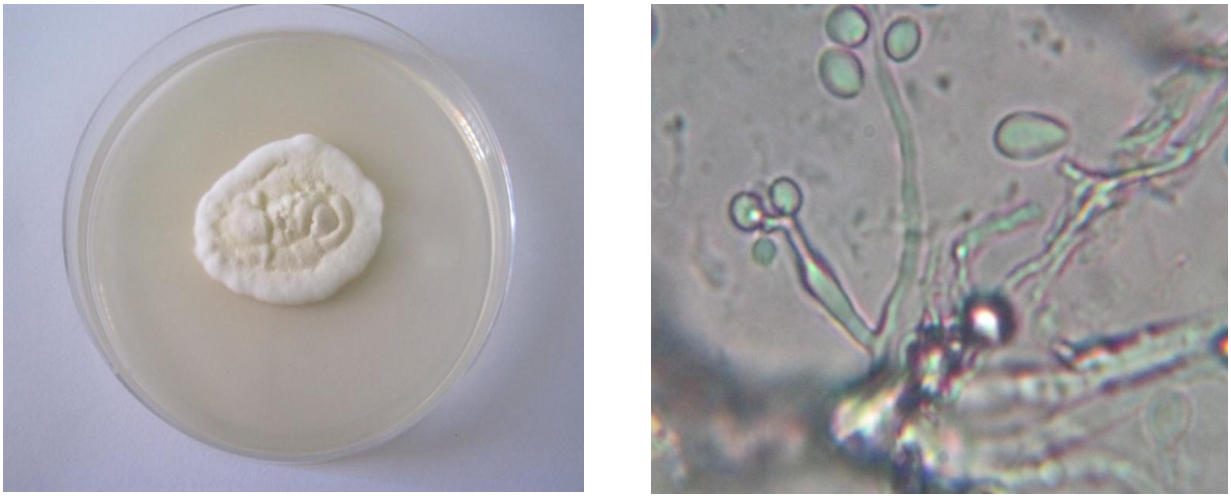
3.1. Phenotypical characterization of Beauvaria brongniartii *(Sacc.) Petch.*

Trans. Br. Mycol. Soc. 10:249.1924. Syll. Fung. 10:540. 1892. Fig. (1) A-B.

Fungal colony on PDA reached a radial of 37 mm after 25 days: floccose, velvety to powdery, at first white, later often becoming yellowish to pinkish. Reverse yellowish to orange. Hyphae hyaline, smooth-walled, 2- 4 µm wide, bearing groups of swollen lateral cells, globose, cylindrical to sub-cylindrical. Conidiogenous cells are arranged in small groups or solitarily along the hyphae consisting of globose to sub-globose basal part 3×3 µm and terminal cell; terminal cells mostly slender, rachis well developed 20 × 2 µm geniculate or irregularly bent, denticulate, denticles thinner than rachis. Conidia oblong to ellipsoidal, hyline, smooth-walled, base slightly apiculate, 2.5–4.8× 2.5–3 µm. Chlamydospores not observed. This description was in agreement with de Hoog (1972) and Tzean *et al.,* (1997).

Table 2. Isolation percentage of fungi and their association with insect species.

Insect species	Associated fungus species	No. of isolated fungi	Isolation percentage (%)
Dolycoris baccarum L.	*Alternaria alternate*(Fr.) Keissl.	18	9.78
	Aspergillus flavus Link	62	33.70
	Aspergillus niger Tiegh.	49	26.63
	Beauvaria bassiana (Bals.) Vuill.	19	10.33
	Curvularia sp.	1	0.54
	Fusarium sp.	1	0.54
	Humicola sp.	2	1.08
	Penicillium sp.	3	1.63
	Rhizopus stolonifer (Ehrenb.) Vuill.	13	7.07
	Sterile mycelium	16	8.70
Eurygoster integriceps Put.	*Aspergillus niger* Tiegh.	2	8.00
	Beauvaria bassiana (Bals.) Vuill.	8	36.00
	Beauvaria brongniartii (Saccardo) Petch	1	4.00
	Penicillium sp.	3	12.00
	Rhizopus stolonifer (Ehrenb.) Vuill.	4	16.00
	Ulocladium atrum Preuss	5	20.00
	Sterile mycelium	2	8.00
Anomala sp.	*Rhizopus stolonifer* (Ehrenb.) Vuill.	1	50
	Penicillium sp.	1	50
Acrotylus insubricus Scop.	*Aspergillus niger* Tiegh.	1	100
Aelia acuminata L.	*Aspergillus flavus* Link	2	100
Apodiphus sp.	*Alternaria alternate* (Fr.) Keissl.	3	27.27
	Aspergillus flavus Link	2	18.18
	Aspergillus niger Tiegh.	1	9.09
	Mucor sp.	2	18.18
	Penecillium sp.	1	9.09
	Rhizopus stolonifer (Ehrenb.) Vuill.	2	18.18
Coccinella novemnotata Herbft	*Aspergillus niger* Tiegh.	1	11.11
	Aspergillus flavus Link	1	11.11
	Alternaria alternata (Fr.) Keissl.	1	11.11
	Beauvaria bassiana (Bals.) Vuill.	3	33.33
	Rhizopus stolonifer (Ehrenb.) Vuill.	2	22.22
	Trichoderma sp.	1	11.11



A B

Figure 1. *Beauvaria brongniartii* . (**A**) Twenty five day old colony on PDA; (**B**) Conidiogenous cells and Conidia. Scale bar of B = 10 µm.

4. Discussion

Most insect-associated fungi found in Iraq during this study have been reported from other parts of the world (Va¨nninen *et al.,* 1989; Va¨nninen, 1995; Meyling and Eilenberg, 2006). A total of 12 species and sterile mycelium were detected in the cadavers of different insects. Regarding entomopathogenic fungal species, *B. bassiana* was the most frequently isolated fungus from three insect taxa and with a relatively high isolation percentage. This result is in agreement with several other studies indicating that *B. bassiana* has a relatively broad host range and is encountered from different ecosystems (Doberski and Tribe, 1980; Vanninen, 1995; Tzean *et al*., 1997; Jankevica, 2004; Kubatova and Dvorak, 2005; Meyling and Elenberg, 2006). *B. bassiana* seems has a wide distribution over the country and has been repeatedly isolated from different insects as well as from different soils in Iraq (Khalaf *et al*.,1997, 1998; Al-Doski, 2007; Abdullah and Mohamed Amin, 2009). The genus *Beauveria* contains a number of species, all of which are pathogenic to insects (Zimmerman, 2007). *B. brongniartii*, a newly recorded in Iraq is detected in one occasion on the sunn pest, *Eurygoster integriceps*. The scarcity of this fungus is largely attributed to its very limited host range and its poor saprophytic competitive ability (Keller *et al.*, 2003: Kessler *et al.,* 2004). The description of our isolate is in agreement with de Hoog (1972). It is very close to *B. bassiana*, but can be separated by its conidial shape and size (de Hoog, 1972: Tzean *et al.*,1997).

Aspergillus flavus and *A. niger* isolated in the present study have previously been found in significant incidence rates on different insect species by several authors (Hermandez-Crespo *et al.,* 1997; Gunde-Cimerman *et al.*, 1998: Balogun and Fagade, 2004). The two species were reported as pathogens to the larvae and pupeae of *Musca domestica* L. under laboratory conditions (Khalaf *et al*., 1997, 1998). These two species were also isolated from populations of the subterranean termite *Microcerotermes diversus* in Basrah, Iraq (Abdullah *et al.,* 2001,2002).

Fusarium sp. was detected from *Dolycoris baccarum* cadaver in one occasion. *Fusarium* species have also been isolated from larvae and adult insects and were reported as insect pathogens (Claydon and Grove, 1984; Sur *et al*., 1999), and as soil opportunistic pathogen to insects in several studies (Ali-Shtayeh *et al.,* 2002; Sun and Liu, 2008; Sun *et al*.,2008; Abdullah and Mohamed Amin,2009).

In this study, several other fungal species including *Alternaria alternata*, *Curvularia* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus stolonifer* , *Trichoderma* sp., *Ulocladium atrum* and sterile mycelium were detected from dead cadavers of different insect species. These species are considered as secondary colonizers unless proven their pathogenicity. However, isolates from the *Mucor*, *Penicillium* and *Trichoderma* genera were considered as opportunistic pathogenic fungi of insects (Gunde-Cimerman *et al*.,1998; Ali-Shtayeh *et al.,*2002, Sun *et al.,*2008 and Abdullah and Mohamed Amin, 2009).

The high isolation percentage of *B. bassiana* and other opportunistic fungi from the cadavers of sunn pests and other insects suggests that probably these fungi display an important role in regulating insect populations of the two important pests during their dormancy at hibernation sites.

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