Entomopathogenic fungi associated with certain scale insects (Hemiptera: Coccoidea) in Egypt

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ABSTRACT

Scale insects (Hemiptera: Coccoidea) are economically important pests in Egypt. Entomopathogenic fungi are considering principal pathogens among piercing sucking insects including scales. Very little attention was paid to the potential of fungal pathogens for scales in Egypt. In the following compilation, data on the record of fungal pathogen of the scale insects in Egypt, their host insects, host plants, locality distribution and identify are summarized. Assess of these entomopathogens in biocontrol experiments against scales and practical application under laboratory, field conditions and commercials utilization are also included. The aim of this review is to overview on the pathogenic fungal-scales relationships in Egypt.

Keywords: Entomopathogenic, scale insects, Egypt

INTRODUCTION

Scale insects (Hemiptera: Coccoidea) are notorious pests, on perennial plants; as well as ornamentals and crops (Williams and Watson 1988). Scale insects comprising 140 valid species names in 12 families in Egypt (Abd-Rabou, 2012). They are plant-sap feeding insects belong to Hemiptera closely related to the aphids, whiteflies and leaf hopper (Cook et al., 2002). Scales cause damage by sucking plant fluids from leaves, stems and sometimes roots. Some species feed on the underside of leaves, which can appear as stippling or chlorotic lesions. Heavily infested plants look unhealthy and produce little new growth and can cause extensive leaf yellowing, premature leaf drop (defoliation), branch dieback and plant death (Kosztarab, 1990).

Fungi have many interesting relationship with scale insects. Sooty mold fungi grow on a honeydew substance which is excreted from those insects due to their feed on a plant sap. The sticky and sugary substance honeydew excreted in large amounts coating leaves, stems and fruit stimulating the growth of sooty mold (Vandenberg et al., 2007). Genera of Septobasidium fungus has been a symbiotic relationship associated with coccid scale insects (McRitchie, 1991). Septobasidium covers, protects, and allows increases of scales populations while parasitizing a small proportion of apparently living scales with coiled haustoria that absorb nutrients from the host plant’s phloem indirectly through the scale’s hemocoel (Couch, 1931). Furthermore, entomopathogenic fungi are another group which infects insects being the fungal group most exploited commercially for biological control purposes. Generally fungi are principal pathogens among Homopteran piercing sucking insects including scale insects (Hajek & St. Leger, 1994 and Humber, 2008). The first noted of entomopathogenic fungi on scales was on armored scale insects (almost certainly Chionaspis salicis) in France in 1848 (Evans & Prior, 1990; c.a. peach, 1921). Moreover, Hall (1982) mentioned first described of the Deuteromycetes Lecanicillium muscarium (Verticillium lecanii) (Zimmermann) Viégas was on coffee scale from Java in the late 1800's. According to Evans and Hywel-Jones, 1997, Webber (1894)
provided the first epithet which reflected the true host relationship of the fungus and Coccidae. He studied the fungi associated with scales of Citrus in Florida that their insect-pathogenic natures became apparent and recognized that *Aschersonia turbinata* Berk. was a pathogen of *Ceroplastes floridensis* Comstock and that *A. cubensis* Berk. and Curt. was pathogenic to *Lecanium hesperidum* (= *Coccus hesperidum* L.).

Among piercing sucking insects, other pathogen that infect through the gut wall cannot be used in biological control (Hajek and St. Leger, 1994). That considered the entomogenous fungi a principal pathogen against these epidemics group due to their invading the host insect by contact, it also typically cause diseases in insects by invading the host directly via the cuticle, exoskeleton and orifices or wounds (Pedrini et al., 2007). Despite of Pettit in 1895 appears to be the first to have commented upon the biological control potential of entomopathogenic fungi in relation to Coccidae (Evans and Hywel-Jones, 1997) insufficient but promising serious attempts assessed the potential of fungi for control scale insects. Evans & Prior (1990) summarized the pathogens recorded on the diaspidid scale insects and mentioned the appearance of *V. lecanii* on *Aspidiotus nerii* (Buche’), *Aspidiotus* sp., *Pseudoulacospis pentagona* (Targioni-Tozzetti), *Chionaspis salicis*, *Lepidosaphes ulmi* (Linnaeus), *lepidosaphes* sp., *Mytilaxpis* sp. and *Unaspis citri* (Comstock). On the other hand, Marcelino et al. (2009) observed a natural epizootic in populations of diaspidid *Fiorinia externa*. A total of 121 fungal isolates of the entomopathogenic species: *Colletotrichum* sp., *L. muscarium*, *Beauveria bassiana*, *Metarhizium microspora*, and *Myriangium* sp. were obtained. Evans and Hywel-Jones (1997) reviewed entomopathogenic fungi species belonged to 17 genuses isolated from coccoid scales. In addition, Xie et al., (2010) found integument infection of four scale species, *Ceroplastes japonicus* Green, *Didesmococcus koreanus* Borchsenius, *Rhodococcus sariuoni* Borchsenius and *Coccus hesperidum* L. with the pathogenic fungus, *L. muscarium*. Likewise, differ species of entomopathogenic fungi were reported to be associated with mealybugs and were confirmed to be pathogenic. They included *Aspergillus parasiticus* Speare, *Cladosporium oxysporum* Berk. and M.A. Curtis, *Hirsutella sphaerospora* H.C. Evans and Samson, and *Neozygites fumosa* (Speare) Remaudière and Keller and *Isaria farinosa* (Holmsk.) (Delalibera et al. 1997; Leru 1986; Moore, 1988; Samways & Grech, 1986 and Demirci et al., 2011).

The aim of this review is to overview on the fungal-scales relationships in Egypt.

**RESULTS**

1. **Fungi, host insects, host plants and locality distribution:**

   Unfortunately little effort has been expended so far on associated the entomopathogenic fungi and scale insects in Egypt. The record presented in Table 1 identification of the fungal taxa which are associated with scale insects and involved in their population dynamics their host insects, host plants, occurrence and locality distribution. Some of these fungi were recorded for first time in Egypt. It seems that soft scales and mealybugs had the most fortunate in engagement with these microorganisms, while just two of diaspidids (*Aonidiella aurantii* and *Chrysomphalus ficus* Ashmead) were involved with entomopathogens. For the first time several species of the entomopathogenic fungi were recorded associated with the scale insects and mealybugs as shown in Table 1. The entomopathogenic fungi *Beauveria bassiana*, *Lecanicillium muscarium* (formally *Verticillium lecanii*), *Metarhizium anisopliae* and of *Paecilomyces fumosoroseus* consider successful biological control agents (Inglis et al., 2001). However, taxa of *Aspergillus*, *Penicillium Cladosporium,*
Conidiobolus are often encountered as necrophytic saprobes on cadavers and most species of Fusarium are not recommended for biological control regarding to their negative health effects or their weekly pathogenicity (Evans and Hywel-Jones, 1997). There are complex morphological variations between L. muscarium and Acremonium sp. (Steenberg & Humber, 1999) which was recorded on Icerya seychellarum. The Entomophthoralean Conidiobolus coronatus also was scored for the first time on I. seychellarum mealybug. Existence of different kind of conidiospores and resting spores is an evidence for occurrence of several species of Entomophthoralean species. For the entomopathogenic fungi to be expected to work, considerable effort needs to be invested in understanding the relationships between the host, the pathogen and the environment. Increasingly, research with insect fungi suggests that a single species may be a complex of strains that behave differently under different regimes (Evans and Hywel-Jones, 1997). However, screening may identify strains of a given species which are particularly successful against scale and mealybug pests over the range of environmental conditions found in the crop. The entomopathogens have to be collected, isolated and identified from their natural habitats for indigenous isolates adapted with the Egyptian conditions that can be used in Egyptian biological control strategies.

Table 1: Recorded of the fungal pathogen of the scale insects in Egypt.

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<th>Fungi</th>
<th>Host insect</th>
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### Entomopathogenic Fungi Associated with Certain Scale Insects: Coccoidea in Egypt

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<th>Fungi</th>
<th>Host</th>
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<td><strong>Pulvinaria psidii</strong></td>
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<td><strong>Penicillium sp.</strong></td>
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* First record in Egypt.

(-) Genus not confirmed.

### Identify:

Recent article used the currently accepted name for a pathogen, but it is beyond the scope of an article aimed at entomologists to include all synonyms. Identification of fungi species was carried out using a colour Atlas of pathogenic fungi (Frey et al., 1979), key of Humber (1997) and Mycology on line ([http://www.mycology.adelaide.edu.au/](http://www.mycology.adelaide.edu.au/))

1. **(Acremonium) sp.**

Colonies of **(Acremonium) sp.** were slow growing, often compact and moist at first, becoming powdery, suede-like or floccose with age, and may be white, grey, pink, rose or orange in color. Hyphae are fine and hyaline and produce mostly simple awl-shaped erect phialides. Conidia are usually one-celled (ameroconidia), hyaline or pigmented, globose to cylindrical, and mostly aggregated in slimy heads at the apex of each phialide.

2. **Alternaria spp.**

**Alternaria** spp. usually branched acropetal chains of multicelled conidia are produced sympodially from simple, sometimes branched, short or elongate conidiophores. Conidia are obclavate, obpyriform, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical beak, pale brown, smooth-walled or verrucose.

3. **Aspergillus spp.**

**Aspergillus** spp. colour varies from yellow-green to dark green. Conidial heads are typically radiate, later splitting to form loose columns, biseriate but having some heads with phialides borne directly on the vesicle. Conidiophores are hyaline and...
coarsely roughened, the roughness often being more noticeable near the vesicle. Conidia are globose to subglobose, pale green and conspicuously echinulate.

3.1 *Aspergillus flavus*

Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, mostly 300-400 µm in diameter, later splitting to form loose columns, biseriate but having some heads with phialides borne directly on the vesicle. Conidiophores are hyaline and coarsely roughened, the roughness often being more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia.

4. *Beauveria bassiana* (Balsamo) Vuillemin

*Beauveria* colonies are usually slow growing, downy, at first white but later often becoming yellow. Microscopic examination showed that *B. bassiana* conidiogenous cell with globose bases and extended, denticulate rachis and the conidia is globose in shape (< 3.5-µm diameters). The spore balls representing dense clusters of large numbers of conidiogenous cell and conidia.

5. *Cladosporium* sp.

Colonies are rather slow growing, mostly olivaceous-brown to blackish brown but also sometimes grey, buff or brown, suede-like to floccose, often becoming powdery due to the production of abundant conidia. Vegetative hyphae, conidiophores and conidia are equally pigmented. Conidiophores are more or less distinct from the vegetative hyphae, are erect, straight or flexuous, unbranched or branched only in the apical region, with geniculate sympodial elongation in some species. Conidia are 1- to 4-celled, smooth, verrucose or echinulate, with a distinct dark hilum and are produced in branched acropetal chains. The term blastocatenate is often used to describe chains of conidia where the youngest conidium is at the apical or distal end of the chain. Note, the conidia closest to the conidiophore and where the chains branch, are usually "shield-shaped". The presence of shield-shaped conidia, a distinct hilum, and chains of conidia that readily disarticulate, are diagnostic for the genus *Cladosporium*.

6. *Conidiobalus coronatus* (Costantin) Batko

The microscopic examination showed that primary conidia are globose with elongated papilla forming secondary microconidia. Old conidia are villose.

7. *Fusarium* spp.

Colonies of *Fusarium* spp. fast growing, pink or light orange colored and had some times cottony aerial mycelium. Microscopic examination showed solitary or aggregated conidiophores, simple branched. Macro- and micro conidia produced from slender phialides. Macroconidia are hyaline, two- to several-celled, fusiform and curved, and microconidia are 1- to 2-celled aseptate, small and ovoid.

8. (Gliocladium) sp.

The genus *Gliocladium* is often described as a counterpart of *Penicillium* with slimy conidia. Colonies are fast growing, suede-like to downy in texture, white at first, sometimes pink to salmon, becoming pale to dark green with sporulation. The most characteristic feature of the genus is the distinctive erect, often densely penicillate conidiophores with phialides which bear slimy, one-celled hyaline to green, smooth-walled conidia in heads or columns. Although, some penicillate conidiophores are always present, *Gliocladium* species may also produce verticillate branching conidiophores which can be confused with *Verticillium* or *Trichoderma*.

9. *Metarhizium anisopliae* (Metsch.) Sorokin

Mycelium often wholly covering affected hosts; conidiophores in compact patches; individual conidiophores broadly branched (candelabrum-like), densely...
intertwined; conidiogenous cells with rounded to conical apices, arranged in dense hymenium; conidia aseptate, cylindrical or ovoid, forming chains usually aggregated into prismatic or cylindrical columns or a solid mass of parallel chains, pale to bright green to yellow-green, olivaceous, sepia or white in mass. Conidia 7-9 µm long. Phialide is cylindrical


The genus *Paecilomyces* may be distinguished from the closely related genus *Penicillium* by having long slender divergent phialides and colonies that are never typically green. Colonies are fast growing, powdery or suede-like, gold, green-gold, yellow-brown, lilac or tan, but never green or blue-green as in *Penicillium*. Phialides are swollen at their bases, gradually tapering into a rather long and slender neck, and occur solitarily, in pairs, as verticils, and in penicillate heads. Long, dry chains of single-celled, hyaline to dark, smooth or rough, ovoid to fusoid conidia are produced in basipetal succession from the phialides.


*Penicillium* sp. chains of single-celled conidia (ameroconidia) are produced in basipetal succession from a specialized conidiogenous cell (phialide). Phialides may be produced singly, in groups or from branched metulae, giving a brush-like appearance known as a penicillus. The penicillus may contain both branches and metulae (penultimate branches which bear a whorl of phialides). All cells between the metulae and the stipes of the conidiophores are referred to as branches. Conidiophores are hyaline and may be smooth or rough-walled. Phialides are usually flask-shaped, consisting of a cylindrical basal part and a distinct neck, or lanceolate (with a narrow basal part tapering to a somewhat pointed apex). Conidia are globose, ellipsoidal, cylindrical or fusiform, hyaline or greenish, smooth- or rough-walled.

12. *Lecanicillium muscarium* (*Verticillium lecanii* (Zimmermann) Viégas)

*L. muscarium* colonies are suede-like, white to pale yellow in colour. Conidiogenous cell (phialides) in whorls on hyphae (verticillately branched), elongate and tapering uniformly from the base. Conidia hyaline, released into a slim droplet at apices of phialides, elongate, diameters ranged between 5.88-8.55 µm long and 1.88-2.81 µm wide conidia. conidiogenous cells 20-35 x 1.1-1.7 µm, secondary necks very uncommon.


Colonies are fast growing, at first white and downy, later developing yellowish-green to deep green compact tufts, often only in small areas or in concentric ring-like zones on the agar surface. Conidiophores are repeatedly branched, irregularly verticillate, bearing clusters of divergent, often irregularly bent, flask-shaped phialides. Conidia are mostly green, sometimes hyaline, with smooth or rough walls and are formed in slimy conidial heads (gloiospora) clustered at the tips of the phialides.

The limited previous offered some strong evidence about associated fungi with scale insect in Egypt. In the first place, scale insects appear to be the insect hosts most affected by the greatest number and diversity of fungal pathogens (Humber, 2008). These fungus/hemipteran associations offer some of the clearest insights into the evolution of entomopathogenicity. There is still much revision that needs to be done and work is in progress to fully review these fungi by making more collections, using modern techniques and with an emphasis on *in vitro* cultural investigations.

**I. Biological control**

In the following, laboratory and field studies on the use of entomopathogenic fungi against scales in Egypt are reviewed. The experiments were conducted with
local isolations, which can be easily produced in submerged culture or commercial products.

1. Laboratory tests

Attempts for practical application of entomopathogenic fungi, in biological control strategy are always preceded by laboratory tests. Laboratory tests provided valuable information on the activity of entomopathogenic fungi and their potential role of the insect-pathogen-environment interactions. Bioassay is central to the successful development of fungi as microbial control agents (Butt and Goettel, 2000). An important confederation in selecting the fungal isolates is virulence which it depends on the bioassay. Under laboratory conditions Ezz et al., 2008 studied the susceptibility of nymphal and adult stages of soft scale insect Saissètia coffeae to local entomopathogen isolate of B. bassiana. Bioassay revealed the pathogenicity of the fungus isolate to S. coffeae. Susceptibility of nymphal stage was higher than adult female stage to the tested. That reflected by the LC$_{50}$ values $1.57 \times 10^3$ and $9.14 \times 10^6$ spores/ml for nymph and adult female stages, respectively. LT$_{50}$ were detected on concentration $1 \times 10^8$ spores/ml recorded 8.63 and 10.37 days, for nymphs and adult females stages, respectively. The authors assumed principality of the tested pathogen isolate to the hemipterian S. coffeae. For screening the pathogenicity of B. bassiana on the mealybug, Ezz, 2004 treated I. seychellarum adults with fungus suspension in three concentrations $5 \times 10^6$, $5 \times 10^7$ and $1 \times 10^8$ spores/ml. Mortality rates after 32 days of treatment were 30, 45, and 60%, respectively, with LC$_{50}$ value $8.61 \times 10^6$ and LT$_{50}$ 26.97 days at concentration $1 \times 10^8$ spores/ml. The author pointed to the treated B. bassiana isolate against their original host I. Seychellarum indicate long period which it needed to conduct the bioassay procedure. On the other hand Sayed, 2008 recorded 71.4% mortality percentage 12 days post treatment of the pest with $3.2 \times 10^7$ spores/ml of B. bassiana suspension with LT$_{50}$ 7.2 days.

2. Application

The use of a microorganism in practice is not easy. The biggest problem is that it is very difficult to predict the effects of biological control agents before their release. The success of field trials depends on many factors that must be taken into consideration. Timing of application may be important, suitable weather, aiming the most susceptible pest stage and method of application. (Charnley & Collins, 2007).

The followings are attempts to employing entomopathogenic fungi to scales under Egyptian conditions.

2.1. Semi field

Examples of practical application of entomopathogenic fungi demonstrated by Rezk, (2009) who employed the local isolated entomopathogen L. muscarium on I. seychellarum at concentration $1.7 \times 10^7$ spores/ml on young citrus trees in greenhouse. Mealybug mortality rates reached 97.8% nine days post treatment. Utilization the pathogen against the green shield scale Pulvinaria psidii Maskell on guava young trees recorded 92.5% mortality rates during the same previous period. The previous pest treated with the entomopathogen B. bassiana under field conditions scored 72.5% reduction percentages.

2.2. Field

B. bassiana was applied under field conditions to P. psidii on guava trees. Reduction percentage of pest mortality was 75.6% after 8 weeks of treatment (Mohammed, 2010). Moreover, Ezz (2008) employed a local B. bassiana isolate against the soft scale S. coffeae infesting Cycas revolute palmlike. The investigation revealed appropriate the entomopathogen with demonstrating obvious effect in reduction percent of S. coffeae population. However the reduction percent began with
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low rates after 5 days from spraying. It increased afterward to reach high percentages in 30 days after spraying in both nymphaal and adult stages (74.10 and 69.70%, respectively).

1. Commercial

Non-local commercial treated under Egyptian field conditions biopesticide product Bio-power® based on the entomopathogenic fungi B. bassiana shared the L. muscarium bioproduct Bio-Catch® the highly effect against P. psidii on the guava trees followed by the commercials Priority® (Paecilomyces fumosorosea) and Bio-magic® (Metarhizium anisopliae) thought 8 weeks of inspection period. The same trend continue expressed with Latania scale insect Hemiberlesia lataniae (Signort), Florida wax scale Ceroplastes floridensis Comstock and the mealubug I. seychellarum on both citrus and guava trees (Rezk, 2009). Generally Commercialization of entomopathogenic fungi has been restricted to those species that are amenable to mass production in vitro on economical substrates (Wraight et al., 2007). Over 75% of entomopathgenic fungi products based on the hypocrealean fungi such as M. anisopliae, B. bassiana, Isaria fumosorosea, and B. brongniartii (Faria and Wraight, 2007). In Egypt two biocontrol products based on entomopathogen B. bassiana have been commercialized, Bio-flay® and Biosect®. In Egypt, Agriculture pesticide committee (APC), Ministry of Agriculture and Land Reclamation is subjected to bioproudacts registration. Depended on indigenous strains adapted to the Egyptian conditions suitable elements in Egyptian biological control strategies preferable than exotic pathogen.

CONCLUSIONS

Since a major to invade their insect hosts by contact entomopathogenic fungi are most candidates microbial control agent of Hemiptera including scale insects. The review observed fungal-scales association in Egypt flora. Many genera of the entomopathogenic fungi were observed isolated and identified from the scale insects some of them for first time in Egypt. Effort in survey of these pathogens throughout its scale hosts with modern identification techniques should be attended. Moreover, indigenous strains adapted to the local conditions which it suitable elements in Egyptian control strategies makes fungal collection is a considerable notion. Limited evaluation of entomogenous fungi under both laboratory and Egyptian field conditions demonstrated ability of these pathogens to infected and control scale pests. Thus, it is imperative to extended estimations of different species of fungi among wide scale taxa, and selection of fungal strains with rapid kill. Attention must be focused on employ the entomopathogenic fungi alone or in compatibility with other scales biocontrol methods. This leads to point at important of test safety of these pathogens on wide range of scales natural enemies. Production and formulation are critical to the commercial development of a fungal biocontrol agent. Depend on native fungal strains to development production and formulation must be an attractive goal. Insights gained from these studies will result in the effective use of these promising organisms as an integral part of Egyptian agricultural systems.

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Entomopathogenic fungi associated with certain scale insects Hemiptera: Coccoidea in Egypt


ARABIC SUMMARY

الفطريات الممرضة للحشرات المرتبطة بالحشرات القشرية في مصر

نهلة عبد العزيز عز

معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - جيزة - مصر

الحشرات القشرية تعد من الآفات ذات الضرر الاقتصادي في مصر. وتتمثل الفطريات الممرضة للحشرات القشرية في بعض الأفلاط مثلاً في بعض الأنواع من الفطريات الممرضة للحشرات القشرية.( אתרי מציאתי) كما أنها تتضمن أيضًا عدة أنواع أخرى من الفطريات الممرضة للحشرات القشرية، مثل الفطريات الممرضة للحشرات القشرية والصودا، والطحالب، والبكتيريا، والبلاستيا، أو بعض الفطريات الممرضة للحشرات القشرية، والتي تساهم في علاج بعض أنواع الفطريات الممرضة للحشرات القشرية، وتشمل بعض الفطريات الممرضة للحشرات القشرية، وهي تساعد في توزيع الفطريات الممرضة للحشرات القشرية، وتم استخدام هذه الفطريات الممرضة للحشرات القشرية، والتي تكون في بعض الأحيان في التحريض على بعض أنواع الفطريات الممرضة للحشرات القشرية، وتم استخدام هذه الفطريات الممرضة للحشرات القشرية، والتي تكون في بعض الأحيان في التحريض على بعض أنواع الفطريات الممرضة للحشرات القشرية.