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Yeasts and filamentous fungi inhabiting guts of three insect species in Assiut, Egypt

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Abstract

This work aimed to evaluate for the first time in Egypt the biodiversity of mycobiota that inhabit the guts of three insect species collected from Assiut Governorate. 50 adult insect samples (28 worker honey bees, 11 black beetles and 11 red palm weevils) were analyzed. 68 species and three varieties were recovered of which 49 species and 2 varieties were filamentous fungi and 19 species + one variety were yeasts. The number of taxa recovered from red-palm weevils and honey bees was almost equal, while lower number was isolated from beetles. However, a higher number of yeast species was obtained from the gut of red-palm weevils than those obtained from honey bees or black beetles. Some filamentous species were recovered from the guts of the three insect species (*Aspergillus niger, A. parasiticus, A. terreus, Cladosporium cladosporioides, Penicillium chrysogenum*), while others were reported from one or two insect species. However, none of yeast species was regularly recovered from the three insect guts, but two insect species may share the same yeast species in their guts. Other yeast species were restrictedly isolated from guts of one insect species. Some gut samples were fungi-free. To our knowledge, some of the isolated yeast species are being reported here for the first time from insect guts. On the other hand, ITS sequence data from several strains did not match well with those of known described species, and are probably new species.

Keywords – Beetles – honey bees – phenotypic and genotypic characteristics – red-palm weevils

Introduction

Yeasts have been isolated frequently from the gut or surface of insects that feed on a variety of materials, including basidiomycete fruiting bodies, woody substrates, ephemeral flowers and nectar exudates (Kurtzman 2001, Lachance et al. 2001a, b, 2005, Rosa et al. 2003, Suh et al. 2003, 2004b, 2005a, b, Teixeira et al. 2003; Suh & Blackwell 2004, 2005, Pimentel et al. 2005, Nguyen et al. 2006). Yeasts have been also described as endosymbionts in mosquito populations, lacewings, beetles and homoptera (Ganter 2006, Urubschurov & Janczyk 2011). Gut-inhabiting fungi are known

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to be essential for the nutrition of many insects (Nardon & Grenier 1989, Vega & Dowd 2005), the insects also rely on yeasts for various metabolic functions, including synthesis of amino acids, vitamins, lipids, sterols and pheromones, degradation of nutritional substrates, and detoxification of compounds (Suh et al. 2003, Vega & Dowd 2005, Starmer & Lachance 2011). Yeast isolations from the gut of insects have led to discovery of a large number of new species of yeasts (Suh et al. 2003, 2004a, b, 2005b, 2006, Suh & Blackwell 2004, 2006, Berkov et al. 2007, Rivera et al. 2009, Grunwald et al. 2010, Houseknecht et al. 2011, Calderon & Berkov 2012). Despite these efforts, the diversity of gut-inhabiting yeasts remains understudied.

On the other hand, many species of filamentous fungi have been isolated from the guts of several insect species (Gilliam et al. 1974a, Rios-Velasquez & Hamada 2002, Gilliam 1997, Moraes et al. 2000, 2004, Marti et al. 2007, Vale et al. 2008, Wang et al. 2010, Chahbar & Mohamed 2014, Rojas-Jimenez & Hernandez 2015). The isolation of these fungi from insect guts suggests that these insects rely on these fungal species to assimilate the complex polysaccharides (cellulose and hemicelluloses) and phenylpropane polymers (lignin) (Rojas-Jimenez & Hernandez 2015) that comprise the secondary cell walls of woody plants and constitute the basic nutrition source for a large number of insects (Perez et al. 2002, Berenbaum & Eisner 2008).

Molecular techniques used since 2000 have greatly enhanced the number of new species identified. Molecular techniques, combined with microbiological and physiological tests, are being used to characterize yeast isolates and species. Most of the analyses have used rDNA sequences, however, we now know that there are no universal criteria to distinguish between genera (Barriaga et al. 2011).

The aim of this work was to identify and evaluate the biodiversity of yeasts and filamentous fungi that might inhabit the guts of three insect species collected from Assiut Governorate, Egypt. The identification was based on phenotypic characteristics in case of filamentous fungi and physiological and genotypic characteristics in case of yeasts.

Materials & Methods

Collection of insects

A total of 50 adult insect samples of three different species were collected, 28 samples from honey bees (*Apis mellifera* L., order: Hymenoptera, family: Apidae), eleven samples from black beetles (*Pterostichus melanarius* Illiger, order: Coleoptera, family: Carabidae), and eleven samples from red palm weevils, RPW (*Rhynchophorus ferrugineus* Olivier, order: Coleoptera, family: Curculionidae). Workers of honey bees from beehives (in June 2014) and red-palm weevils (in July 2013, February and March 2014) were kindly supplied by the Faculty of Agriculture, Assiut University, while black beetles were collected in July 2013, May and June 2014 from some farms in Assiut Governorate, Egypt. Insects obtained were put in separate clean containers (bottles or plastic bags) and kept for 3 days with slightly moistened filter paper so that surface debris is removed.

Dissection of insects to obtain guts

Insect samples were put each in a 250 ml conical flask with 95 % ethyl alcohol for 1-2 minutes and were then washed several times with sterile distilled water for surface sterilization. They were dissected using forceps (or pins) in a sterile Petri dish, and the gut was removed aseptically from body.

Isolation of yeasts and filamentous fungi from insect guts

The gut is transferred into a sterile 1.5 ml Eppendorf tube containing 200 µl of 0.7 % sterile saline solution. The gut is crushed with a pipette tip, and all the solution (including gut pieces) was spread with a loop over the surface of YM agar and acidified YM agar plates (at least three replicate-plates for each medium type and each gut sample). The inoculated plates were incubated at 25 °C for 3-7 days (Suh & Blackwell 2004). The gut plates were checked every day and the developing colonies were observed under a microscope to check yeasts and filamentous fungi. A water mount of a piece

of the colony was made and observed under a microscope if necessary. Bacterial colonies often developing on YM (distinguishable from yeasts by their cell sizes) were not considered. A single colony was picked up, and streaked three times by crossing the lines onto a fresh acidified YM (single colony isolation).

Media used for isolation of fungi

Two media were used: Yeast extract-malt extract-peptone-glucose agar, YM (Wickerham 1951) of the following composition was employed: (g/l) yeast extract 3.0, malt extract 3.0, peptone 5.0, glucose 10.0, agar 20.0. Chloramphenicol (250µg/ml) was used as a bacteriostatic agent. The medium is dispensed into containers and autoclaved at 121^{o} C for 15 minutes. The other medium was acidified yeast extract-malt extract-peptone-glucose agar, AYM (Wickerham 1951) of the same composition as YM but acidified by a predetermined volume of 1 N hydrochloric acid (approximately 0.7%, v/v) to give the desired pH of 3.7 to 3.8.

Phenotypic identification of the insect-associated filamentous fungi

The fungi were identified based on their macro- and microscopical features following the keys of Raper & Fennell (1965), Pitt (1979), Sivanesan (1987), Moubasher (1993), Zare & Gams (2004), Leslie & Summerell (2006) and Domsch et al. (2007).

Physiological characterization of yeast strains

Fermentation of sugars and oxidative utilization of carbon compounds were performed according to Barnett et al. (2000). Assimilation of nine nitrogen compounds (potassium nitrate, sodium nitrite, ethylamine, L-lysine, creatine, creatinine, D-glucosamine, imidazole, or D-tryptophan) was determined (Suh et al. 2008b). Growth at high osmotic pressure, in the presence of cycloheximide and production of extracellular starch-like compounds were also tested (Suh et al. 2008b). Identification keys of Barnett et al. (2000) were followed to assign each isolate to its species level. Confirmations of these identifications were carried out using the molecular technique.

Genotypic identification of yeast strains

The yeast strains were grown on YM plates and incubated at 25 °C for 2 days. A small amount of yeast growth was scraped off and suspended in 100 µl of distilled water and boiled at 100 °C for 15 minutes following the manufacturer's protocol (SolGent Company, Daejeon, South Korea). The samples were directly sent for extraction and sequencing or they were collected in a batch and stored at –70 °C before sending to Korea. Yeast DNA was extracted and isolated using SolGent purification beads at this company. Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using the universal primer ITS1 (5'- TCC GTA GGT GAA CCT GCG G -3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). Then amplification was performed using the polymerase chain reaction (PCR) (The GeneAmp® PCR System 9700 thermal cycler, Applied Biosystems, Foster City, California, USA). The PCR reaction mixtures were prepared using SolGent EF-Taq as follows: 10X EF-Taq buffer 2.5 µl, 10 mMdNTP (T) 0.5 µl, primer (F-10p) 1.0 µl, primer (R-10p) 1.0 µl, EF-Taq (2.5 U) 0.25 µl, template 1.0 µl, DW up to 25 µl. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95 °C for 15 minutes followed by 30 cycles of denaturation at 95 °C for 20 seconds, annealing at 50 °C for 40 seconds and extension at 72 °C for 1 minute, with a final extension step of 72 °C for 5 minutes.

The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. After that, the purified PCR products were reconfirmed (using size marker) by electrophoreses of the PCR products on 1% agarose gel. These bands were then eluted and sequenced. Each sample was sequenced in sense and antisense direction.

Contigs were created from the sequence data using the CLCBio Main Workbench program. The sequence obtained from each isolate was further analysed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained together with those

retrieved from the GenBank database were subjected to the Clustal W analysis using MegAlign software version 5.05 (DNASTAR Inc., Madison, Wisconsin, USA) for the phylogenetic analysis (Thompson et al. 1994).

Results

Total fungi recovered from guts of the three insect species investigated

The yeast isolates were characterized using phenotypic, physiological and molecular methods (Tables 1, 2), while filamentous fungi were identified using only phenotypic characteristics based on their macro- and microscopic features. Representative strains of the species recovered are deposited at Assiut University Mycological Center Culture Collection (AUMC) and ITS gene sequences of the yeast strains were deposited at National Center for Biotechnological Information (NCBI) and accession numbers are given for them (Table 2).

A total of 67 species and three varieties belonging to 34 genera were recovered on yeast extract malt extract agar (YM) and acidified yeast extract malt extract agar (AYM) from 50 gut samples of the three different insect species. From these, 48 species and two varieties assigned to 21 genera were filamentous fungi and 19 species + one variety belong to 15 genera were yeasts. The number of taxa recovered from honey bees (37 species and one variety belonged to 21 genera) and red-palm weevils (35 species and one variety related to 21 genera) was almost equal, while lower number was isolated from black beetles (20 species + one variety assigned to nine genera). However, higher number of yeast species (14 + one variety) was obtained from the gut of red-palm weevils than those obtained from honey bees (6 species) or black beetles (five species) (Table 3).

Fungi recovered from guts of honey bees

In the present investigation, thirty-eight species and one variety belonging to 21 genera were isolated from 28 gut samples of honey bees. Filamentous fungi were recovered from 21 samples and represented by 31 species, one variety and 16 genera while yeast fungi were isolated from 16 samples and represented by six species related to five genera.

Aspergillus (11 species) was isolated in high frequency from bee samples (18 out of 28 samples analyzed). *Aspergillus niger* was the most common species recovered from 12 samples, followed by *A. flavus* (7 samples) and *A. terreus* (5 samples). The remaining aspergilli were isolated from one or two samples only (Table 3).

Both *Cladosporium* (4 species) and *Penicillium* (2 species) were recovered in low frequency (6 samples each) and *C. cladosporioides*, *C. herbarum*, *P. chrysogenum* and *P. oxalicum* were the most common species. *Chaetomium* sp. was isolated from five samples, whereas *Scopulariopsis brevicaulis* was isolated from three samples. On the other hand, *Cochliobolus* (3 species) and *Mucor hiemalis* were recovered each from two samples, and *Bipolaris clavata, Fusarium verticillioides*, *Myceliophthora thermophila, Nigrospora oryzae*, *Papulaspora immerse*, *Pochonia suchlasporia* var. *catenata* and *Trichothecium roseum* were encountered each from one sample only.

From yeasts *Lachancea thermotolerans* was the most common in the gut of honey bees; it was isolated from ten samples. *Pichia* (represented by *P. kudriazevii*) and *Saccharomyces* (represented by *S. cerevisiae* and *Saccharomyces* sp. near to *S. cerevisiae*, less than 95% ITS sequence identities with the type strain) were recovered in seven samples each. *Wickerhamomyces subpelliculosus* was isolated from 4 gut samples while *Hanseniaspora opuntiae* was recovered from one sample only.

Fungi recovered from guts of black beetles

Twenty species and one variety belonging to nine genera were isolated from eleven gut samples of black beetles. Filamentous fungi were recovered from all samples and represented by 15 species and one variety belonging to five genera, while yeast fungi represented by five species related to four genera were isolated from four gut samples.

Aspergillus (7 species and 1 variety) was recovered in ten samples out of eleven investigated. *A. niger* was also the most common species and was encountered in eight samples, followed by *A. terreus* (in 3 samples), and *A. parasiticus* and *A. taichungensis* (in 2 samples), while the remaining aspergilli were isolated each from one gut sample only (Table 3).

Cladosporium and *Penicillium* (3 species each) were recovered from six and five samples, respectively with *C. sphaerospermum* and *P. chrysogenum* being the most common. *Scopulariopsis chartarum* was isolated from two samples and *Chaetomium* sp. from one sample only.

From yeasts, *Candida fermentati* was isolated in two samples while *C. parapsilosis*, *C. tropicalis*, *Exobasidium* sp., and *Cyberindnera jadinii* were encountered each from one sample only.

Fungi recovered from guts of red-palm weevils

Thirty-five species and one variety belonging to 21 genera were recovered from eleven gut samples of red-palm weevils. Filamentous fungi were recovered from nine samples and represented by 21 species assigned to ten genera, while yeast fungi were isolated from all samples and represented by 14 species and one variety related to eleven genera (Table 3).

Cladosporium (3 species) and *Fusarium* (2 species) were isolated in moderate frequency (5 samples each). *C. cladosporioides* and *F. solani* were the most common species.

Aspergillus (6 species) was recovered from four samples. *A. niger* and *A. terreus* were isolated each from two samples. The remaining four aspergilli were isolated each from one sample only (Table 3).

Gondwanamyces (G. serotectus) and *Penicillium* (4 species) were recovered each from only two samples, while *Cunninghamella echinulata, Mucor circinelloides, Pseudallescheria boydii, Scopulariopsis japonicum, Stemphylium sarciniforme* and *Triadelphia* near to *T. disseminata* were encountered each from one gut sample.

Cyberlindnera jadinii was the most common yeast fungus isolated from all gut samples of red-palm weevil investigated, followed by *Prototheca zopfii* var. *hydrocarbonea* was also recovered in high frequency (9 samples). *Candida* (5 species) was also isolated in high frequency (7 samples) with *C. tropicalis* being the most common *Candida* species; it was isolated from four samples. *C. aaseri*, *C. pararugosa*, and *C. quercitrusa* were recovered each from 2 samples while *C. parapsilosis* was encountered from one sample only. *Saccharomyces cerevisiae* was isolated in moderate frequency (5 samples). *Reniforma* sp. near to *R. strues* (87% similarity with the type strain, Table 2, probably a new species) and *Meyerozyma carribbica* were isolated each from two samples while *Cryptococccus magnus*, *Debaryomyces nepalensis*, *Exobasidium* sp., *Saccharomycopsis fibuligera* and *Sterigmatomyces elviae* were encountered each from 1 sample only.

Overview on the gut mycobiota of the three insect species

The number of fungal taxa recovered from red-palm weevils and honey bees was almost equal, while lower number was isolated from black beetles. However, higher number of yeast species (14 + 1 variety) was obtained from the gut of red-palm weevils than those obtained from honey bees (6 species) or black beetles (5 species). Yeasts and filamentous fungi were isolated more frequently in guts of red palm weevil (100% and 81.81% of the samples respectively) than in honey bees (57.14% and 75%) and black beetles (36.36% and 100%). Some filamentous species were recovered from guts of the 3 insect species (*Aspergillus niger, A. parasiticus, A. terreus, Cladosporium cladosporioides, Penicillium chrysogenum*), while others were reported from guts of one or two insect species. However, none of yeast species was regularly recovered from the three insect guts, but two insects may share the same yeast species in their guts (*Candida parapsilosis, C. tropicalis, Cyberlindnera jadinii, Exobasidium* sp., *Meyerozyma carribbica* from both black beetles and red palm weevils; and *Saccharomyces cerevisiae* from both honey bees and red palm weevils). Other species were restrictedly isolated from guts of one insect species. To our knowledge, some of the isolated fungal species are reported here for the first time from guts of either adults of red palm weevil (*Gondwanamyces serotectus, Triadelphia* sp. near to *T. disseminata, Candida aaseri, C. pararugosa, C. quercitrusa, Cryptococcus magnus, Cyberlindnera jadinii, Debaryomyces nepalensis,*

Exobasidium sp., *Reniforma* sp. near to *S. strues*, *Saccharomycopsis fibuligera* and *Sterigmatomyces elviae* and *Prottheca zopfii* var. *hydrocarbonea*), from worker honey bees (*Hanseniaspora opuntia* and *Wickerhamomyces subpelliculosus*) or from black beetle gut (*Exobasidium* sp.) and are being considered in another publication. On the other hand, some strains are far from the known described species (based on their low ITS sequence similarities < 95% with those deposited in the Genbank) and probably some of these could be new species such as KX023220-KX023223, KX029122, KX029124, KX029125, KX015906, KX015907, KX015909-KX015911, KX029126, KX011609 and KX015891 (listed in Table 2). More studies on these strains are in progress.

Discussion

Total fungi recovered from guts of the three insect species

Phenotypic, physiological and molecular methods were used to characterize yeast isolates, while macro- and microscopic features were used for identification of filamentous fungi. Strains of the species recovered were deposited at Assiut University Mycological Center AUMC and ITS gene sequences of the yeast strains were deposited at NCBI. 68 species and three varieties belonging to 36 genera were recovered from gut samples of the three insect species investigated. The number of taxa recovered from honey bees and red-palm weevils was almost equal, while lower number was isolated from black beetles. However, higher number of yeast species was obtained from the gut of red-palm weevils than those obtained from honey bees or black beetles. The gut of insects of several families is found as a productive environment and is rich in yeasts that carry out the fermentation of cellobiose and xylose (Suh et al. 2003, Urbina et al. 2013). Urbina et al. (2013) reported the isolation of ascomyceteous and basidiomyceteous yeasts from the gut of the 16 species of passalid beetles collected in nine localities in Guatemala with the xylose-fermenting (X-F) yeasts were the most abundant taxa isolated. Also beetle guts (Callaham & Shifrine 1960, Suh et al. 2003, 2004a, 2005a,b, 2008a, Suh & Blackwell 2004, Houseknecht et al. 2011), honey bee guts (Gilliam et al. 1974a, b, Sandhu & Waraich 1985, Chahbar & Mohamed 2014) and guts from other insect species (Nguyen et al. 2007, Urubschurov & Janczyk 2011, Hui et al. 2012, 2013, Bagde et al. 2013, Urbina et al. 2013, Rojas-Jimenez & Hernanddez 2015) were reported as a good source of hyper-diverse source of yeasts and filamentous fungi.

Fungi recovered from guts of honey bees

As the health of honey bees and the quality of honey is affected by many microbes, thus there is the need of isolating and identifying these microbes so as to find out the means of controlling them. Insect gut microbiota plays essential role in the growth, development, pathogenesis and environmental adaptation of host insects (Bhat et al. 2014). Composition of the digestive microbiota of honey bees is the result of feeding pollen and nectar, but also it is a consequence of interaction among the bees in the hive (Glinski & Jarosz 1995).

In the current work, 38 species and one variety were isolated from 28 gut samples of honey bees. 32 filamentous fungal species were recovered from 21 samples while only 6 yeast species were recorded from 16 samples only. In this respect, 18 species of filamentous fungi (Gilliam et al. 1974a) and seven of yeasts (Gilliam et al. 1974b) were previously isolated from the intestinal tracts of 388 adult worker honey bees. Moreover, Gilliam (1997) stated that not all bees contained yeasts, and the numbers of yeasts varied from colony to colony.

Nine species of *Aspergillus* were reported, with *A. niger* (from 12 samples), *A. flavus* (7 samples) and *A. terreus* (5 samples) being the most common, followed by *Cladosporium cladosporioides*, *C. herbarum*, *Penicillium chrysogenum*, *P. oxalicum* and *Chaetomium* sp. On the other hand, species of *Scopulariopsis* (1 species), *Cochliobolus* (3) and *Mucor* (1) were recovered each from two or three samples, while other species were encountered each from one sample only. In harmony with the current results, filamentous fungi previously reported from the intestinal tract of worker honey bees include species of *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Bipolaris*, *Curvularia* and *Rhizopus* of which *Penicillium frequentans*, *P. cyclopium*, *Aspergillus*

flavus, A. niger, Alternaria tenuissima and *Cladosporium cladosporioides* were found most frequently (Gilliam & Prest 1972, Gilliam et al. 1974a, 1977, 1988a). However no filamentous fungi were recovered from the digestive tracts of 2 Algerian subspecies worker honeybees (*Apis mellifera intermissa* and *A. mellifera sahariensis*) during the two seasons (winter and spring) of the year 2012, but strains of these obtained from bee bread were identified to the generic level as *Penicillium, Aspergillus, Cladosporium, Alternaria* and *Mucor* (Chahbar & Mohamed 2014). From yeasts *Lachancea thermotolerans* was the most common yeast fungus in the gut of honey bees followed by *Pichia kudriazevii*, two *Saccharomyces* spp. and *Wickerhamomyces subpelliculosus* while *Hanseniaspora opuntiae* was recovered from one sample only. *L. thermotolerans* was recently isolated from the gut of honey bee (Mousavi et al. 2015). In the work of Gilliam *et al.* (1974b, 1977), guts of adult worker honey bees emerged different spectrum of yeast species in the following order of frequency: *Torulopsis magnoliae, T. glabrata, Candida parapsilosis, Hansenula anomala, Saccharomyces bailii* var. *osmophilus*, *Rhodotorula minuta* var. *minuta* and *Cryptococcus albidus* var. *aerius*. Also it was stated that intestinal yeasts were most frequently encountered in worker bees that live under stress conditions (Gilliam 1973, Gilliam et al. 1974b, 1977, 1979, 1988b) and intestinal yeasts were isolated from 21 bees from free-flying colonies in the fall (Gilliam et al. 1988a). Sandhu & Waraich (1985) obtained 473 yeast isolates from 328 stomach samples of seven different pollinating bee species (including *A. mellifera*) that were identified to 13 genera and 30 species, of these *Dekkera intermedia* and *Saccharomyces cerevisiae* followed by *Candida humicola, C. incommunis, C. ishiwadae, C. membranaefaciens, C. parapsilosis, Rhodotorula lactosa* and *Cryptococcus albidus* were the most common. However, Chahbar & Mohamed (2014) found no yeasts in the digestive tracts of two Algerian subspecies worker honeybees, but yeast strains were obtained from bee bread and are likely to belong to the genus *Saccharomyces*.

Fungi recovered from guts of black beetles

All gut samples of black beetles yielded 15 species and one variety of filamentous fungi however, four gut samples only yielded five yeast species. Suh et al. (2005a) stated that beetle gut is a hyper-diverse source of novel species since they isolated over 650 yeast strains over a three year period from the gut of beetles from 27 taxa and found that at least 200 of these were undescribed taxa. Some of the yeasts recovered were basidiomycetes, but the vast majority was ascomycete budding yeasts (Saccharomycetes).

Aspergillus niger was also the most commonly encountered species (from 8 samples) followed by *P. chrysogenum* (5), *A. terreus*, *C. sphaerospermum* (3) and *A. parasiticus*, *A. taichungensis* and *Scopulariopsis chartarum* (2 samples each) from black beetle guts, while other species were encountered each from only one gut sample. In their study on fungi associated with the guts of tropical wood-feeding beetle larvae of Coleoptera, Rojas-Jimenez & Hernandez (2015) obtained 92 strains of filamentous and yeast fungi and assigned them to 40 genera mostly related to Ascomycota (32 genera), then Basidiomycota (6) and Zygomycota (2) with *Trichoderma* being the most abundant fungus, and *Penicillium, Paecilomyces, Fusarium, Aspergillus* and *Mucor* were among the isolated fungi. In the studies of Moraes et al. (2000, 2004), 758 fungal strains were recovered from the digestive tract of many individuals of adult insects and nymphs of nine species triatomine bugs (Order: Hemiptera); they found that the genera with the greatest number of species were *Penicillium, Aspergillus, Acremonium, Cladosporium* and *Paecilomyces* and the most frequent species in both studies were *P. corylophilum, P. fellutanum, P. waksmanii, Aspergillus niger, A. awamori, Cladosporium herbarum* and *Paecilomyces variotii*. The digestive tract of *Triatoma infestans* (Hemiptera) yielded also 33 species of fungi of which *Penicillium* (15 species), *Aspergillus* (5) and *Cladosporium* (2) gave rise the greatest number of species (Marti et al. 2007). *Candida fermentati* was isolated in 2 gut samples of black beetles while *C. parapsilosis* and *C. tropicalis, Exobasidium* sp. and *Cyberindnera jadinii* were encountered each from 1 sample only.

C. fermentati and other three new *Candida* species (Suh and Blackwell 2004), *Candida* ambrosiae and 16 new *Candida* species (Suh et al. 2004b) were isolated from the digestive tract of

basidiocarp-feeding beetles. Species of *Sugiyamaella* and *Candida* (including a new *Candida* species) were isolated from the gut of wood-ingesting beetles (Houseknecht et al. 2011).

In their study on fungi associated with the guts of tropical wood-feeding beetle larvae of Coleoptera, Rojas-Jimenez & Hernandez (2015) obtained two strains of *Trichosporon* and two strains of *Geotrichum*. *Pichia stipitatis*-like yeasts were consistently isolated from the gut of wood-ingesting passalid beetles (Suh et al. 2003). Isolates from the digestive tracts of plant-associated beetles and other insects were identified as *Candida orthopsilosis, C. maltosa, C. pseudorhagii, C. parapsilosis, C. tropicalis, C. neerlandica, Lodderomyces elongisporus* and seven new *Candida* species near to *C. albicans* (Suh et al. 2008a); also nine new *Candida* species near *C. membranifaciens* were isolated from the gut of a variety of insects (including beetles, lacewings, fishflies, craneflies and a cockroach) (Suh et al. 2005b). Novel species of *Candida* (Kurtzman & Robnett 1998, Hui et al. 2012) and *Wickerhamomyces* (Hui et al. 2013) were described from guts or frass of wood-boring insect larvae or ambrosia beetle.

Fungi recovered from guts of red-palm weevils

The red palm weevil (RPW) is widely considered the most damaging insect pest of palms in the world, even in all the countries where it has been accidentally introduced (Cox, 1993). Since the 1980s it has rapidly expanded its geographical range westwards. It reached Saudi Arabia and the United Arab Emirates in about 1985, spreading throughout the Middle East and it had been introduced to Egypt in 1992 (Cox 1993), with thousands of healthy trees being damaged or lost (El-Sebaey 2004). RPW larvae feed within the apical growing point of the palms, producing a wet fermenting frass inside the tunnels (Butera et al. 2012) creating extensive damage to palm tissues and weakening the structure of the palm trunk; the resulting damage is often only visible long after infestation, when palms are close to death (Ferry & Gomez 2002, Faleiro 2006, Dembilio & Jacas 2011).

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Table 1 Physiological comparison of the strains tested of the recorded yeast species.

Ascomyceteous species: **1** *Candida aaseri* AUMC 10247, **2** *C. fermentati* (Saito) Bai (teleomorph: *Meyerozyma caribbica*) AUMC 10242, **3** *C. krusei* (Castellani) Berkhout (teleomorph: *Pichia kudriavzevii*) AUMC 10226, **4** *C. parapsilosis* AUMC 10243, **5** *C. pararugosa* AUMC 10248, **6** *C. quercitrusa* AUMC 10249, **7** *C. tropicalis* AUMC 10245, **8** *Cyberlindera jadinii* AUMC 10246, **9** *Debaryomyces nepalensis* AUMC 10261, **10** *Hanseniaspora opuntiae* AUMC 10221, **11** *Lachancea thermotolerans* AUMC 10222, **12** *Saccharomyces cerevisiae* AUMC 10265, **13** *Saccharomyces cerevisiae* AUMC 10229, **14** *Saccharomyces cerevisiae* AUMC 10235, **15** *Saccharomycopsis fibuligera* AUMC 10266, **16** *Wickerhamomyces subpelliculosus* AUMC 10236; Basidiomyceteous yeasts: **17** *Cryptococcus magnus* AUMC 10254, **18** *Exobasidium* sp. AUMC 10262, **19** *Reniforma strues* AUMC 10263, **20** *R. strues* AUMC 10264, **21** *Sterigmatomyces elviae* AUMC 10267; and **22** *Prototheca zopfii* var. *hydrocarbonea* AUMC 10268.

Test /Species strain number	**Code**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Fermentation (D-glucose)	F1	-	+	+	+	+	w	+	+	-	-	+	+	+	+	-	+	-	-	-	-	-	-
Assimilation of carbon compounds																							
D-glucose	C1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	C2	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	+	+
L-sorbose	C3	+	+	-	+	+	+	+	+	+	-	+	-	-	-	-	-	+	-	-	-	+	-
D-glucosamine	C4	+	-	w	-	-	+	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-
D-ribose	C5	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	+	-	-	+	-
D-xylose	C6	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	-	+	+	-
L-arabinose	C7	+	+	-	+	+	-	+	+	+	-	-	-	-	w	-	d	+	+	-	-	+	-
L-rhamnose	C9	-	+	-	w	-	-	w	-	+	-	-	-	-	-	-	-	+	nt	-	+	+	+
Sucrose	C10	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
Maltose	C11	+	+	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
α, α-trehalose	C12	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Methyl-α-D-glucoside	C13	+	+	-	+	-	+	+	+	+	w	+	+	+	+	+	+	+	+	-	+	-	d
Cellobiose	C14	+	+	-	-	+	-	+	+	+	+	+	-	-	-	+	+	+	+	-	-	+	-
Salicin	C15	+	+	-	-	-	-	+	+	+	+	-	+	-	-	+	+	+	+	-	w	+	-
Arbutin	C16	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	w	+	-
Lactose	C18	+	-	-	-	-	-	-	-	+	-	-	-	w	-	-	nt	+	-	-	w	+	-
Raffinose	C19	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-
Melezitose	C20	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	d
Inulin	C21	+	+	+	+	+	+	+	+	+	+	w	+	-	-	+	-	+	+	+	+	+	+

Test /Species strain number	Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Soluble starch	C22	-	-	-	-	-	-	+	-	+	w	-	-	-	-	+	+	+	-	-	+	-	-
Glycerol	C23	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Meso-erythritol	C24	+	w	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-	-	+	-
Ribitol	C25	+	-	-	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	-	-	+	-
Xylitol	C26	+	+	-	+	+	+	+	+	+	-	+	-	w	-	-	+	+	+	-	-	+	-
D-glucitol	C28	+	+	-	+	+	+	+	+	+	-	+	d	d	-	+	+	+	+	-	w	+	+
D-mannitol	C29	+-	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	-
Galactitol	C30	-	-	-	-	w	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
Myo-inositol	C31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	w	+
Glucono-d-lactone	C32	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	w	w	+	-
2-keto-D-gluconate	C33	+	+	+	+	+	+	+	+	+	+	+	+	w	+	w	-	+	+	+	+	+	+
D-gluconate	C35	+	+	+	-	-	+	+	+	+	+	w	-	-	-	+	+	+	+	+	+	+	-
D-glucuronate	C36	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	+
D-galacturonate	C37	-	w	-	-	nt	+	-	-	+	w	-	-	-	-	-	-	+	+	-	-	+	-
DL-lactate	C38	w	+	+	-	+	-	-	+	+	-	w	+	-	-	-	+	-	+	-	+	+	+
Succinate	C39	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	-	+	+	+
Citrate	C40	+	+	+	+	+	+	+	+	+	-	-	-	w	-	+	+	+	-	+	w	+	-
Methanol	C41	-	-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	w	-	+
Ethanol	C42	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Propane 1,2 diol	C43	-	-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	-	-
Butane 2,3 diol	C44	-	+	w	+	+	-	-	+	-	-	-	-	-	-	+	+	+	+	w	-	+	+
Quinic acid	C45	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-	+	+	-
Nitrogen compounds																							
Nitrate	N1	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	nt	-	-	-	-
Nitrite	N2	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	nt	-	-	-	-
Ethylamine	N3	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	-	nt	+	+	+	+
L-lysine	N4	+	+	+	+	+	+	+	+	+	w	w	-	-	-	+	+	w	nt	+	+	+	+
Creatine	N6	-	-	-	nt	-	-	nt	-	+	-	-	-	-	-	-	-	-	nt	-	-	-	-
Creatinine	N7	-	-	-	nt	-	-	nt	-	+	-	-	-	-	-	-	-	-	nt	-	-	-	-
D-glucosamine	N8	+	+	w	+	+	+	+	+	+	-	w	+	w	w	+	w	+	nt	+	+	+	+
Imidazole	N9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nt	-	-	-	-
D-tryptophane	N10	+	+	+	+	+	+	+	+	+	w	+	-	-	-	+	-	w	nt	+	+	+	+

Test /Species strain number	Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Miscellaneous																							
0.01% cycloheximide	O1	-	-	+	-	nt	-	+	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-
0.1 % cycloheximide	O2	-	-	-	-	nt	-	+	-	+	+	-	-	-	-	w	-	-	-	-	-	-	-
50% D-glucose	O4	+	-	-	+	+	+	+	-	+	+	+	-	+	-	-	+	-	nt	nt	nt	nt	-
60% D-glucose	O5	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	nt	nt	nt	nt	-
10% NaCl	O6	+	-	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-	nt	nt	nt	nt	-
16% NaCl	O7	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	nt	nt	nt	nt	-
Starch formation	M1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: growth; w: weak growth; d, delayed growth, -: no growth, nt: not tested.

Table 2 Assiut University Mycological Centre accession number (AUMC) of yeast strains isolated from insect guts with accession GenBank numbers given together with the closest match in the GenBank database and sequence similarity in percent to the match as inferred from Blastn searches of ITS sequences.

AUMC number	Isolation source	Accession GenBank Number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species
10221	Honey bee	KX015895	751	H2S2K5=FM199954	742/745(99.60)	*Hanseniaspora opuntiae*
				CBS 8733^{T}= AJ512435	665/671(99.11)	
10222	Honey bee	KX015896	683	CBS 6340=CU928180	674/678(99.41)	*Lachancea thermotolerans*
				UniFGKT2= KT029797	637/637(100)	
				NRRL Y-8284^{T}=NR_111334	639/642(99.53)	
10223	Honey bee	KX015898	682	CBS 6340=CU928180	676/678(99.71)	*Lachancea thermotolerans*
				NRRL Y-8284^{T}=NR_111334	639/642(99.53)	
10224	Honey bee	KX015903	682	CBS 6340=CU928180	674/677(99.56)	*Lachancea thermotolerans*
				UniFGKT2= KT029797	637/637(100)	
				NRRL Y-8284^{T}=NR_111334	639/642(99.53)	
10225	Honey bee	KX015901	513	ATCC 24210^{T}=AY939808	501/508(98.62)	*Pichia kudriavzevii*
10226	Honey bee	KX015902	514	ATCC 24210^{T}=AY939808	505/511(98.83)	*Pichia kudriavzevii*
10227	Honey bee	KX023220	475	ATCC 24210^{T}=AY939808	425/470(90.43)	*Pichia kudriavzevii*
10228	Honey bee	KX023221	471	ATCC 24210^{T}=AY939808	385/417(92.32)	*Pichia kudriavzevii*

AUMC number	Isolation source	Accession GenBank Number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species
10229	Honey bee	KX023222	817	CBS 1171^{T}=NR_111007	682/730(93.42)	*Saccharomyces cerevisiae*
10230	Honey bee	KX023223	488	CBS 1171^{T}=NR_111007	427/461(92.63)	*Saccharomyces cerevisiae*
				CBS 432^{T}=AJ229059	441/476(92.65)	*Saccharomyces paradoxus*
10231	Honey bee	KX029121	668	CBS 432^{T}=AJ229059	498/521(95.59)	*Saccharomyces paradoxus*
				CBS 1171^{T}=NR_111007	482/505(95.45)	*Saccharomyces cerevisiae*
10232	Honey bee	KX029122	568	CBS 1171^{T}=NR_111007	464/536(86.57)	*Saccharomyces cerevisiae*
				CBS 432^{T}=AJ229059	478/552(86.59)	*Saccharomyces paradoxus*
10233	Honey bee	KX029123	819	CBS 1171^{T}=NR_111007	728/763(95.41)	*Saccharomyces cerevisiae*
				CBS 432^{T}=AJ229059	716/746(95.98)	*Saccharomyces paradoxus*
10234	Honey bee	KX029124	658	CBS 1171^{T}=NR_111007	466/499(93.39)	*Saccharomyces cerevisiae*
				ATCC MYA-4449^{T}= NR_111355	481/518(92.86)	*Saccharomyces kudriavzevii*
10235	Honey bee	KX029125	727	CBS 432^{T}=AJ229059CBS	546/626(87.22)	*Saccharomyces paradoxus*
				1171^{T}=NR_111007	529/608(87.01)	*Saccharomyces cerevisiae*
10236	Honey bee	KX015897	637	PMM08-1561-AL= KP132906	631/637(99.06)	*Wickerhamomyces*
				NRRL Y-1683^{T}=NR_111336	578/580(99.66)	*subpelliculosus*
10237	Honey bee	KX015899	639	PMM08-1561-AL= KP132906	632/639(98.90)	*Wickerhamomyces*
				NRRL Y-1683^{T}=NR_111336	580/580(100)	*subpelliculosus*
10238	Honey bee	KX015900	640	PMM08-1561-AL= KP132906	622/626(99.36)	*Wickerhamomyces*
				NRRL Y-1683^{T}=NR_111336	579/580(99.83)	*subpelliculosus*
10239	Honey bee	KX015904	637	PMM08-1561-AL= KP132906	633/637(99.37)	*Wickerhamomyces*
				NRRL Y-1683^{T}=NR_111336	579/580(99.83)	*subpelliculosus*
10240	Black beetle	KX011596	586	CBS 2022^{T}=EU568913	586/586(100)	*Meyerozyma caribbica*
10241	Black beetle	KX011597	586	CBS 2022^{T}=EU568913	586/586(100)	*Meyerozyma caribbica*
10242	Black beetle	KX011598	589	CBS 2022^{T}=EU568913	589/589(100)	*Meyerozyma caribbica*
10243	Black beetle	KX011602	524	ZA039=FJ662416	524/524(100)	*Candida parapsilosis*
				CBS 604^{T}=AY391843	522/523(99.81)	
10244	Black beetle	KX011603	520	UZ31_13= KM36151	512/520(98.46)	*Candida tropicalis*
				ATCC 750=AY939810	510/518(98.46)	
10245	Black beetle	KX015905	480	LY2=KJ535096	461/471(97.88)	*Candida tropicalis*
				CBS 94^{T}=NR_111250	452/468(96.58)	

AUMC number	Isolation source	Accession GenBank Number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species
10246	Black beetle	KX015906	528	CNRMA10.725=KP131996 CBS 1600^{T}=NR_111211	405/520(77.89) 389/499(77.96)	*Cyberlindnera jadinii*
10247	Red palm weevil	KX011604	617	WM 03.488=KP131656 CBS 1913^{T}=NR_077069	606/607(99.84) 579/580(99.83)	*Candida aaseri*
10248	Red palm weevil	KX011600	417	PMM10-923-L= KP131807	415/416(99.75)	*Candida pararugosa*
10249	Red palm weevil	KX011605	605	10H1064=KF220648 CBS 4412^{T}=AM158924	601/604(99.50) 597/605(98.68)	*Candida quercitrusa*
10250	Red palm weevil	KX015890	618	10H1064=KF220648 CBS 4412^{T}=AM158924	613/616(99.51) 609/619(98.38)	*Candida quercitrusa*
10251	Red palm weevil	KX015889	521	ZA038= FJ662410 ATCC 750=AY939810	520/521(99.81) 515/516(99.81)	*Candida tropicalis*
10252	Red palm weevil	KX015907	488	ZA038= FJ662410 ATCC 750=AY939810 CBS 94^{T}=NR_111250	452/492(91.87) 445/482(92.32) 439/476(92.22)	*Candida tropicalis*
10253	Red palm weevil	KX015908	493	ATCC 750=AY939810 CBS 94^{T}=NR_111250	475/485(97.94) 471/480(98.13)	*Candida tropicalis*
10254	Red palm weevil	KX015893	654	AUMC 7246=JQ425371 CBS 140^{T}=NR_130655 CBS 8361=AF444388	653/655(99.69) 557/558(99.82) 557/558(99.82)	*Cryptococcus magnus*
10255	Red palm weevil	KX011601	572	CNRMA10.725=KP131996 CBS 621= EU568909 CBS 1600^{T}=NR_111211	551/565(97.52) 545/564(96.63) 510/522(97.70)	*Cyberlindnera jadinii*
10256	Red palm weevil	KX015892	572	CNRMA10.725=KP131996 CBS 621= EU568909 CBS 1600^{T}=NR_111211	544/560(97.14) 535/560(95.54) 506/524(96.56)	*Cyberlindnera jadinii*
10257	Red palm weevil	KX015909	521	CNRMA10.725=KP131996 CBS 1600^{T}=NR_111211 CBS 621= EU568909	455/513(88.69) 449/503(89.26) 452/513(88.11)	*Cyberlindnera jadinii*
10258	Red palm weevil	KX015910	351	CBS 1600^{T}=NR_111211	261/328(79.57)	*Cyberlindnera jadinii*
10259	Red palm weevil	KX015911	536	CBS 621=EU568909 CBS 1600^{T}=NR_111211	415/526(78.90) 403/510(79.02)	*Cyberlindnera jadinii*

AUMC number	Isolation source	Accession GenBank Number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species
10260	Red palm weevil	KX029126	392	CNRMA10.725=KP131996 CBS 621=EU568909	322/390(82.56) 298/363(82.09)	*Cyberlindnera jadinii*
10261	Red palm weevil	KX011606	645	CBS5921^{T}=JN942654	639/643(99.64)	*Debaryomyces nepalensis*
10262	Red palm weevil	KX011608	573	IBL 03150=DQ682574 PC1=KM403454	571/572(99.83) 569/574(99.13)	Exobasidiomycetidae sp. Exobasidiales sp.
10263	Red palm weevil	KX011609	583	CBS 8263^{T}=NR_073314	518/594(87.21)	*Reniforma strues*
10264	Red palm weevil	KX015891	592	CBS 8263^{T}=NR_073314	525/601(87.35)	*Reniforma strues*
10265	Red palm weevil	KX011599	776	CBS2888= KY109257 CBS 1171^{T}=NR_111007	770//770(100) 749/753(99.47)	*Saccharomyces cerevisiae*
10266	Red palm weevil	KX015894	636	AUMC 9092= KU052788 CBS 329.83^{T}= AF218988	635/635(100) 370/370(100)	*Saccharomycopsis fibuligera*
10267	Red palm weevil	KX011607	642	CBS 5922^{T}=NR_073301	638/638(100)	*Sterigmatomyces elviae*
10268	Red palm weevil	KX015912	751	UP-PT-P63=HG515044	636/639(99.53)	*Prototheca zopfii* var. *hydrocarbonea*

Table 3 Comparison between the frequencies of occurrence of fungal species recovered from guts of the three different insects (honey bees, black beetles and red-palm weevil insects).

Fungi \ Insects	Honey bees		Black beetles		Red-palm weevils	
	N=28	%	N=11	%	N=11	%
Filamentous fungi	21	75	11	100	9	81.81
Aspergillus	18	67.85	10	90.90	4	36.36
A.brasiliensis Varga, Frisvad & Samson	1	3.57				
A. flavus Link	7	25				
A. flavus var. *columnaris* Raper & Fennell			1	9.09		
A. fumigatus Fresenius			1	9.09		
A. lacticofeatus Samson & Frisvad					1	9.09
A. montevidensis Talice & J.A. Mackinnon	1	3.57				
A. nidulans (Eidam) G. Winter	2	7.14				
A. niger van Tieghem	12	42.86	8	72.72	2	18.18
A. ochraceus Wilhelm	1	3.57			1	9.09
A.parasiticus Speare	1	3.57	2	18.18	1	9.09
A. proliferans G. Smith	1	3.57	1	9.09		
A. recurvatus Raper & Fennell	1	3.57				
A. sydowii (Bainier & Sartory) Thom & Church	2	7.14				
A. taichungensis Yaguchi, Someya & Udagawa			2	18.18	1	9.09
A. terreus Thom	5	17.85	3	27.27	2	18.18
A. versicolor (Vuillemin) Tiraboschi			1	9.09		
Chaetomium sp.	5	17.85	1	9.09		
Cladosporium	6	21.43	6	54.55	5	45.45
C.chlorocephalum (Fresen.) E.W.Mason & M.B. Ellis	1	3.57			1	9.09
C. cladosporioides (Fresen.) G.A. de Vries	2	7.14	2	18.18	5	45.45
C. herbarum (Pers.) Link	2	3.57				
C. oxysporum Berkeley & Curtis	1	3.57	1	9.09		
C. sphaerospermum Penzig			3	27.27	1	9.09
Bipolaris clavata Alcorn	1	3.57				
Cochliobolus	2	7.14				
C. australiensis (Tsuda & Ueyama) Alcorn	1	3.57				
C. lunatus R. Nelson & Haasis	1	3.57				
C. pallescens (Tsuda & Ueyama) Sivanesan	1	3.57				
Cunninghamella echinulata (Thaxter) Thaxter					1	9.09
Fusarium	2	7.14			5	45.45
F. proliferatum (Matsushima) Nirenberg	1	3.57				
F. solani (Martius) Saccardo					4	36.36
F. verticillioides (Saccardo) Nirenberg	1	3.57			1	9.09
Gondwanamyces serotectus van der Linde & Jol. Roux					2	18.18
Mucor	2	7.14			1	9.09
M. circinelloides van Tieghem					1	9.09
M. hiemalis Wehmer	2	7.14				
Myceliophthora thermophila (Apinis) Oorschot	1	3.57				
Nigrospora oryzae (Berkeley & Broome) Petch	1	3.57				
Penicillium	6	21.43	5	45.45	2	18.18
P. chrysogenum Thom	4	14.28	5	45.45	2	18.18
P.crustosum Thom					1	9.09
P. griseofulvum Dierckx					1	9.09

Fungi \ Insects	Honey bees		Black beetles		Red-palm weevils	
	N=28	%	N=11	%	N=11	%
P. oxalicum Currie & Thom	2	7.14				
P. puberulum Bainier			1	9.09	1	9,09
P. purpurogenum Stoll			2	18.18		
Papulospora immersa Hotson	1	3.57				
Pochonia suchlasporia var. *catenata* (W. Gams & Dackman) Zare & W. Gams	1	3.57				
Pseudallescheria boydii (Shear) McGinnis *et al.*					1	9.09
Scopulariopsis	3	10.71	2	18.18	1	9.09
S. brevicaulis (Sacc.) Bainier	3	10.71				
S. chartarum (G. Smith) Morton & J. Smith			2	18.18		
S. japonicum Udajawa *et al.*					1	9.09
Stachybotrys chartarum (Ehrenberg) Hughes	1	3.57				
Stemphylium sarciniforme (Cavara) Wiltshire					1	9.09
Steril mycelia (dark)			1	9.09		
Triadelphia sp. near to *T. disseminata* Madrid & J. Edathodu					1	9.09
Trichothecium roseum (Persoon: Fries) Link	1	3.57				
Yeasts	16	57.14	4	36.36	11	100
Candida			1	9.09	7	63.63
C. aaseri Dietrichson ex van Uden & H.R. Buckley					2	18.18
C. parapsilosis (Ashford) Langeron & Talice			1	9.09	1	9.09
C. pararugosa Nakase, Komagata & Fukazawa					2	18.18
C. quercitrusa S.A. Meyer & Phaff					2	18.18
C. tropicalis Berkhout			1	9.09	4	36.36
Cryptococccus magnus (Lodder & Kreger) Baptist & Kurtzman					1	9.09
Cyberlindnera jadinii (Sartory, R. Sartory, Weill & J. Mey.) Minter			1	9.09	11	100
Debaryomyces nepalensis Goto & Sugiyama					1	9.09
Exobasidium sp.			1	9.09	1	9.09
Hanseniaspora opuntiae Cadez, Poot, Raspor & M.T. Sm.	1	3.57				
Lachancea thermotolerans (Filippov) Kurtzman	10	35.71				
Meyerozyma caribbica (Vaughan-Mart., Kurtzman, S.A. Mey. & E.B. O'Neill) Kurtzman & M. Suzuki			2	18.18	2	18.18
Pichia kudriavzevii Boidin, Pignal & Besson	7	25				
Reniforma sp. near to *R. strues* Pore & Sorenson					2	18.18
Saccharomyces	7	25			5	45.45
S. cerevisiae Meyen ex E.C. Hansen (>95%)	2	7.14			5	45.45
Saccharomyce sp. near to *S. cerevisiae* (<95%)	7	25				
Saccharomycopsis fibuligera (Lindner) Klöcker					1	9.09
Sterigamtomyces elviae Sonck & Yarrow					1	9.09

Fungi \ Insects	Honey bees		Black beetles		Red-palm weevils	
	N=28	%	N=11	%	N=11	%
Wickerhamomyces subpelliculosus (Kurtzman) Kurtzman, Robnett & Basehoar-Powers	4	14.28				
Prototheca zopfii var. *hydrocarbonea* (Kockova´-Kratochvılova & Havelkova) Pore					9	81.81
Number of genera (36)	23		9		21	
Number of species (68+3 varieties)	38+1		20+1		35+1	

N = number of insects from which yeast or filamentous fungal species were recovered.

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