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Bulbs mycoflora and their relation with three stored product mites

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Abstract

The distribution of moulds on stored and field onion and garlic plants infested by bulb mites in Assiut area (Egypt) was studied using PDA medium at 28 °C. Among 40 host samples and the three mite species tested no significant difference was noted in the contamination by moulds. A total of 20 species appertaining to 11 genera were identified from the tested mites and their habitats. The predominant moulds on all samples were "storage moulds" from the genera *Aspergillus* (*A. niger*, *A. versicolor*) and *Penicillium* (*P. chrysogenum*, *P. funiculosum*, and "field moulds" among which *Alternaria*, *Cladosporium*, *Fusarium* (and its teleomorphs) and *Setosphaeria* were encountered most frequently. One fungus well known facultative pathogen was obtained: *Beauveria bassiana*. The tested mites transfer *A. niger*, *N. haematococca*, *R. stolonifer* and *P. chrysogenum* outside their bodies while, *A. flavus* and *A. ochraceus* transfer through their digestive tracts along with the foods. Individuals of all mites could survived till the end of the experiment on all fungal species tested except *A. niger*, *A. ochraceus* and *A. sydowii*. Among 48 isolates screened for their ability to produce chitinase, about 83% of the isolates could produce this enzyme. Most of the positive isolates (17 isolates) had moderate producers

Key words: Entomopathogenic fungi, Acaridia mites. insect fungi, chitin-degrading enzyme, stored and field fungi, vectors

Introduction

From an ecological standpoint the fungi that invade storage bulbs could be conveniently divided into two general groups, field and storage fungi [1]. Field fungi are those that invade bulbs as they are developing on the plants in the field or after the bulbs have matured but before they are harvested. Common genera of field fungi are *Alternaria*, *Cladosporium*, *Fusarium* and *Cochliobolus*. Storage fungi are those that grow on products in storage and comprising mainly several group species of *Aspergillus* plus a few of *Penicillium*. Many species of the above fungi are plant pathogens and are host specific [2].

Many Astigmata mite species inhabit substrates associated with plants or fungi, while some exploit living plant tissues. The bulb mites, *Rhizoglyphus robini* Claparede, 1869 and *Histiostoma onioni* Eraky and Shoker (1994) and copra mite, *Tyrophagus putrescentiae* Shrank 1781 are considered the most injurious Acaridia mite species attacking a wide variety of materials such as stored products, decaying organic matters, ornamented bulbs, roots of some plants [3–6] and may cause large scale damage [7–10]. The micro fungi may either constitute a food supplement for the mite, or the fungi may have an indirect effect by decomposing plant tissue thus making it more accessible for the mites [11, 12]. Also, some mites play an important role in carrying and transmitting of moulds to different crop plants [13].

Chemical control of mites is very important, both for agriculture and for sanitation. Many synthetic acaricides have been developed, but mites rapidly develop resistance to such compounds [14]. The possible use of entomophathogenic fungi in the biological control of plant-parasitic insects has been extensively studied [15]. So it is very useful to get more information about the mycoflora associated with different insect-pests and their relation between each other.

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The aim of the present study is to isolate and identify the moulds externally and internally associated with some mites infesting bulb crop plants and their hosts, and the possible relationship between those mites and the transmission of parasitic or saprophytic fungi. A preliminary study on chitin-degradation by some fungi that has been isolated in the current study was also conducted.

Materials and methods

Isolation of storage and field fungi

Ten samples (250 g) each of stored onion (*Allium cepa*) and garlic (*Allium sativum*) were collected from different markets in Assiut city. Also, ten samples, each of onion and garlic plants, were gathered from different pre-harvested onion and garlic fields from the agricultural farm at Assiut University. Samples were transferred into the Mycological Laboratory and kept at 4 °C for fungal analysis. It was done by cutting the roots and the bulb parts into segments (1 cm^2). The dilution-plate method [16] was employed for isolation of fungi. Aliquots of 1 ml each of the final desired dilutions was transferred to sterile Petri-dishes in which cooled agar medium was poured.

Isolation of fungi associated with mites

Three species of the Acaridia mites were used: *Tyrophagus putrescentiae*, *Rhizoglyphus robini* and *Histiostoma onioni*. All tested mite species were grown on sterilized segments of onion bulbs. The fungal flora present on internal and external surfaces of the studied mites were estimated by aseptically placing ten individuals of each mites species on the surface of solid medium in each Petri-dish. Potato-dextrose agar (PDA Difco) was used throughout the above determinations. Rose-bengal (30 ppm) and chloramphenicol (25 mg/l) were used as bacteriostatic agents [17]. Two plates were used for each sample. All plates were incubated at 28 ± 2 °C for 7–10 days, during this period the developed fungi were identified and counted per g for the first experiment and per 20 mite individuals in each sample for the second one.

Transmission of fungi by mites

For estimation of transmission of fungi on the surfaces of the tested mites or through the elementary tract, the mites were classified into two groups; the first group were transferred directly from the growing fungi (without sterilization) onto the surfaces of plates with cooled potato dextrose agar medium. The second group previously grown on fungi were surface sterilized in ethyl alcohol 95% followed by washing several times with distilled water, also transferred onto the cooled medium. The plates were incubated at 28 °C for 10 days and the growing fungi were identified.

Survival of mites on some fungal cultures

Eleven species of common fungi isolated during the current study were selected for this experiment. The species were grown on potato dextrose agar medium in Petri-dishes for 10 days. Surface sterilized adults of each mite species (5 individuals) were transferred to each fungal culture. The plates were incubated at 24 °C with 80–90% relative humidity. Medium without fungus served as control. The plates were examined at 3, 5, 7, 9 and 15 days of incubation to check for mite survival.

Chitinase production by some isolated fungi

Forty-eight isolates of fungal species isolated in the present work were grown on a basal medium (PDA) of which glucose was supplemented with 0.3% (w/v) chitin (Sigma). The inoculating cultures were incubated at 28 °C for 10 days. After the growing periods, the clear zones around the fungal colony were measured and the measuring zone giving the chitinolytic power of the isolate and rated as + for weak producers; ++ for moderate producers and +++ for high producers.

Results and discussion

The distribution of moulds on internal and external surfaces of the Acaridia species and their infested crop plants was studied using three mite species and two bulb plants on potato-dextrose agar at 28 °C. The present study provides isolation and identification for 20 fungal species representing 11 genera from the tested mites in stored or field habitats (Table 1). There is a remarkably low incidence of diverse fungal contamination of the analyzed samples and there is no specific fungal association with mites and their infested plants. *Aspergillus niger*, *A. versicolor*, *Fusarium oxysporum*, *Gibberella fujikuroi* (anamorph = *Fusarium moniliforme*), *Nectria haematococca* (anamorph = *Fusarium solani*),

Table 1. Total counts (Tc/g), and percentage frequency (F%) of stored and field fungi isolated from onion and garlic plants and from three mites on potato dextrose agar at 28 °C.

Organisms	Stored moulds				Field moulds				Mite moulds on potato dextrose agar					
	Onion		Garlic		Onion		Garlic		*T. putrescentiae*		*R. robini*		*H. onioni*	
	TC	F%	TC	F%	TC	F%	TC	F%	TC	F%	TC	F%	TC	F%
Alternaria	–	–	–	–	800	90	500	70	–	–	–	–	–	–
A. alternata (Fr.) Keissler	–	–	–	–	800	90	–	–	–	–	–	–	–	–
A. tenuisima (Kunze ex Pers.) Wiltshire	–	–	–	–	–	–	500	70	–	–	–	–	–	–
Aspergillus	5100	100	3000	100	1750	100	800	60	450	100	750	75	950	100
A. flavus Link	850	40	650	50	–	–	–	–	–	–	–	–	–	–
A. fumigatus Fresenius	250	30	150	30	–	–	–	–	–	–	–	–	–	–
A. niger Van Tieghem	4000	100	2100	100	1550	100	800	60	450	100	650	75	850	100
A. ochraceus Wilhelm	–	–	–	–	–	–	–	–	–	–	100	50	50	25
A. versicolor (Vuill.) Tiraboschi	–	–	100	20	200	20	–	–	–	–	–	–	50	25
Beauveria bassiana (Balsamo) Vuill.	–	–	–	–	–	–	–	–	150	75	200	100	150	50
Cladosporium cladosporioidos (Fres.) De Vries	–	–	–	–	1300	100	200	30	–	–	–	–	–	–
Fusarium oxysporum Schlecht	250	30	100	20	1800	60	550	20	150	75	250	75		
Gibberella fukikuroi (Sawada) Ito	100	20	200	30	450	30	100	20	50	25	350	50	500	75
Humicola grisea Traaen	–	–	–	–	100	20	150	30	–	–	–	–	–	–
Nectria haematococca Ber. & Br.	100	20	150	30	100	20	150	30	100	50	250	75		
Penicillium	1250	60	850	70	950	60	350	30	–	–	350	50	400	75
P. aurantiogriseum Dierckx	50	10	–	–	50	10	–	–	–	–	–	–	–	–
P. chrysogenum Thom	450	30	350	30	–	–	–	–	–	–	350	50	400	75
P. funiculosum Thom	700	50	300	40	–	–	350	30	–	–	–	–	–	–
P. oxalicum Currie & Thom	50	10	–	–	900	60	–	–	–	–	–	–	–	–
P. variabile Wehmer	–	–	100	20	–	–	–	–	–	–	–	–	–	–
Rhizopus stolonifer (Ehrenb.) Lindt	350	60	–	–	150	20	350	70	200	100	–	–	–	–
Setosphaera rostrata Leonard	–	–	–	–	450	40	400	50	–	–	–	–	–	–
Total counts	7150		4300		7850		3550		1100		2150		2000	
Number of genera: 11	6		5		10		10		7		6		4	
Number of species: 20	11		11		11		10		7		7		6	

Penicillium chrysogenum, *P. funiculosum*, and *Rhizopus stolonifer* were the most prevalent fungi (Table 1). Nearly all species of fungi recovered in the present work can be considered common saprophytic soil organisms. Also, some of the saprophytic fungi recovered here were isolated from different insects and their habitats and are known to be facultative insect pathogens [18–27]. The present results were in harmony with those noticed by Grant et al. [28] and studied the possible association of bacteria or fungi with honey bees (*Apis mellifera*) infested with tracheal mites (*Acarapis woodi*). They reported that few microorganisms were cultured from internal bee tissues, whereas numerous were cultured from the exterior surfaces of tracheal mite-infested and uninfested honey bee thoraces and heads.

The highest incidence rate was represented by genus *Aspergillus*. It was isolated from 60–100% of the samples analyzed comprising 40.9–71.3% of total fungi. Five species from the above genus were identified of which *A. niger* and *A. versicolor* were the most common species. They occurred in 60–100% and 20–25% of the samples: *A. flavus* (40% and 50% of the samples) and *A. fumigatus* (30% each) were detected only from stored materials, while *A. ochraceus* (25% and 50%) was isolated only from *R. robini* and *H. onioni* (Table 1). Similar observation was reported by Adebanjo and Shopeju [29] who indicated that the major fungal flora of stored vegetables were *A. flavus*, *A. niger* and *A. fumigatus*. Members of *Aspergillus* were also found to be associated with wide varieties of insects and their habitats such as: the European corn borer *Ostrinia nubilalis* larvae [30]; corn ear worm, *Heliothis zea* [31, 32]; dying and subterranean termite, *Reticulitermes flavipes* [21, 33]; adults of *Vespula vulgaris* [34]; Egyptian cotton leaf worm, *Spodoptera littoralis* [23]; house-dust mites [20]; different insects infesting various crop plants [22]; Tracheal mite infested honey bees [28] and vegetables contact with soil insects [35]. The presence of *A. flavus*, *A. ochracues* and other *Aspergillus* spp. on the vegetables at harvest and in storage is undesirable, since some of these organisms have been shown to produce mycotoxins. This aspect requires further investigation with a view to highlighting the possible health hazards it might pose [29].

Penicillium (represented by 5 species) was the second most commonly encountered genus, isolated from the later two mite species and all their habitats. It was occurred in 30–75% of the samples. From the five identified species *P. chrysogenum* (30–75% of the samples) and *P. funiculosum* (30–50%) were the most prevalent. *P. aurantiogriseum* (10% of the samples) and *P. oxalicum* (10–60%) were only isolated from stored and field onion. Whereas, *P. variabile* (20%) was recovered only from stored garlic (Table 1).ln this instance, several of *Penicillium* species were isolated from various insect stages and their environments [20, 21, 23, 26, 27, 29, 34, 36].

Fusarium oxysporum, *Gibberella fujikuroi*, *Nectria haematococca* (20–75% of each sample) and *Rhizopus stolonifer* (20–100%) were also isolated from the surfaces of most mite species and their host plants (Table 1). On the other hand, *Alternaria alternata* (90% of the samples), *A. tenuissima* (70%), *Cladosporium cladosporioides* (100% and 30%), *Humicola grisea* (20% and 30%) and *Setosphaeria rostrata* (40% and 50%) were only identified from field onion and garlic plants (Table 1). The previous species and others were also, isolated in different frequencies of occurrences from various insects and their environments [21–23, 27].

Beauveria bassiana, a well known entomopathogenic fungus was only isolated from the three mite species tested and not encountered from their habitats. It was recovered from 75, 100 and 50% of the samples constituting 13.6%, 9.3% and 7.5% of total fungi isolated from *T. putrescentiae*, *R. robini* and *H. onioni*, respectively (Table 1). This species and other species of the genus *Beauveria* were found to be associated with other insects as reported by MacLeod [37] who isolated numerous *Beauveria* isolates from 70 insect and 4 rodent species. Also, Gambino and Thomas [34] isolated *B. bassiana* from the adults of *Vespula vulgaris*. Ismail and Abdel-Sater [23] isolated *B. alba* from the last two larval instars, pupae and adults of the Egyptian cotton leaf worm *S. littoralis*. Hernandezcrespo and Santiagoalvarez [24] reported that the entomopatogenic fungi *B. bassiana* and *Metarhisium anisopliae* have been found in natural populations of the Moroccan locust *Dociostaurus maroccanus* and other species of Acaridoidea in southern Spain. Also, Steinhaus [38], Thomas and Poinar [18], Kmitowa [19], Leatherdale [39] found that this species attacks many insects.

Concerning to the ability of the tested mites for carrying and transmitting moulds, results in Table 2 revealed that the studied mites could transfer *Aspergillus niger*, *Nectria haematococca*, *Rhizopus stolonifer* and *Penicillium chrysogenum* outside their bodies. On the other hand, the mites could transfer *Aspergillus flavus* and *A. ochraceus* in their digestive tracts along

Table 2. Fungal species transferred by the mites with sterilized or non sterilized surfaces on PDA plates.

Mite species	Surface sterilized			Not-sterilized		
Fungal species	*R. robini*	*T. putrescentiae*	*H. onioni*	*R. robini*	*T. putrescentiae*	*H. onioni*
Aspergillus flavus	+	+	−	−	−	−
A. niger	+	+	−	+	+	+
A. ochraceus	+	−	−	−	−	−
Nectria haematococca	−	+	−	−	−	+
Penicillium chrysogenum	+	−	−	+	−	+
Rhizopus stolonifer	−	+	+	+	+	+

Table 3. Survival periods (3, 5, 7, 9 and 15 days) of three Acaridida mite species on some fungal species grown on PDA at 25 °C and 80–90% R. H.

Mites	*R. robini*					*T. putrescentiae*					*H. onioni*				
Fungal species	3	5	7	9	15	3	5	7	9	15	3	5	7	9	15
Alternaria alternata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
A. ochraceus	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+
A. sydowii	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+
Cladosporium cladosporioides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gibberella fujikuroi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillium chrysogenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. funiculsum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhizopus stolonifer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ulocladium alternariae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Control	+	+	−	−	−	+	+	+	+	+	+	+	−	−	−

+, Survival.
−, Not survival.

with the foods (Table 2). These results agreed with data recorded by Bulla et al. [13] who reported that insects can act as a vectors of mouldy diseases and can inoculate crop plants with spoilage organisms. Also, Sinha et al. [11], and Chirila et al. [12] indicated that the microfungi may either constitute a food supplement for the mites or the fungi and may have an indirect effect by decomposing plant tissue thus making it more accessible for the mites. The same results were reported by Ragunathan et al. [40] who stated that the saprophytic fungi are mechanically carried in the alimentary canal of the insects along with the foods. Also, Maraun et al. [26] studied the effects of *Panphytophagous oriebatid* mites on the recovery of the microbial community in F. layer materials. They noticed that oriebatid mites enhanced the recovery of the disturbed systems. It is concluded that the accelerated recovery of the microbial metabolism was caused by dispersal of spores of oriebatid mites and by grazing on microbial populations. The results show that mites act as a good vector for carrying and transmitting moulds not only outside their bodies but also in their digestive tracts along with the food. So, the mites play an important role in the development of moulds in plant and the subsequent the disease or spoilage of stored foodstuffs.

Table 3 shows survival of the three mite species grown on common fungi isolated in the current study on potato dextrose agar medium. The results revealed that individuals of all mites tested survived till the end of the experiment (15 days) when culture on all fungal species cultivated on PDA except *A. niger*, *A.*

ochraceus and *A. sydowii*. The individuals of three mite species could not survive at all when grown on *A. niger*. Also *T. putrescentiae* could not survive when grown on *A. ochraceus* and *A. sydowii* but the other two individuals mites (*R. robini* and *H. onioni*) could survived on those two species (Table 3). This indicates that *T. putrescentiae* was more sensitive than the other two species of mites. These results were greatly similar to those obtained by Sinha and Harasymek [41]. They reported that the two mite species, *Glycyphagus domesticus* and *Acarus siro* survived on *Syncephalastrum racemosum*, *Absidia spinosa* and *Curvularia geniculata* but failed to survive on *Aspergillus ochraceus*, *Gliocadium roseum* and *Penicillium* sp. On the other hand, this finding does not agree with result of Andersen [20] who reported that the greatest increase in the population of *D. petronyssinus* occur if *A. amstelodami* grown on the medium, but the largest growth of the population of *D. farinae* occurs when *A. niger* is growing on the medium. Also, Bronswijk and Sinha [36] found that *Dermatophagoides* sp. eats conidia and mycelium of microfungi if present in the nourishment of the mites. The same authors stated that *D. farinae* can not survive or reproduce on any of the 45 species of fungi isolated from stored grain and house-dust or skin-scales. It was, however, confirmed that *D. farinae* eats certain microfungi such as *P. cyclopium*, *P. funiculosum*, *Cladosporium cladosporioides* and *Alternaria alternata*.

Forty-eight isolates appertaining to 11 species and 8 genera isolated in the present work were screened for their ability to produce chitinase enzyme (Table 4). The results revealed that 40 isolates (83.33% of total isolates) could produce this enzyme. Among the positive isolates 11 isolates (27.5%) achieved high enzyme producers. Seventeen isolates (42.5%) showed a reasonable rate of growth on the medium supplemented with chitin. The other 12 isolates (30%) gave weak producers. Insect cuticle-degrading enzymes (especially chitinase) are believed to be important in the infection mechanisms of entomopathogenic fungi [15]. So, the ability of conidia of entomopathogenic fungus to adhere, germinate and then penetrate the cuticular integument, resulting in mycelia growth and death of the insect pest, is an intriguing biological process [42]. These results were greatly similar to those obtained by Ismail and Abdel-Sater [23]. They tested 42 isolates, recovered from the Egyptian cotton leaf worm, *Spodoptera littoralis* and its environment for chitinase production. They noticed that most of the isolates (32) showed a reasonable rate (++) of growth.

Table 4. Chitinase production by some common fungal species.

Organisms	NIT*	NIP**	Degree of hydrolysis		
			+	++	+++
Alternaria alternata	5	3	1	2	–
Aspergillus niger	10	9	1	2	6
A. ochraceus	4	3	2	1	–
A. sydowii	3	3	–	3	–
Cladosporium cladosporioides	2	2	1	1	–
Fusarium oxysporum	6	4	2	2	–
Gibberella fujikuroi	3	2	1	–	1
Penicillium chrysogenum	7	6	1	4	1
P. funiculosum	3	3	1	–	2
Rhizopus stolonifer	2	2	1	1	–
Ulocladium alternariae	3	3	–	1	2
Total isolates	48	40	11	17	12

* Number of isolates tested.
** Number of isolates positive.
+++, High producers; ++, Moderate producers; +, Weak producers.

Six isolates gave a higher rate (+++), while the rest (4) of the isolates showed little and scarce growth (+). Also, other entomopathogenic species were found to be chitinase producers such as *Beauveria bassiana* [43] and *Metarrhizium anisopliae* [44]. On the other hand, chitosan (a deacetylated derivative of chitin) was shown to reduce the growth in vitro of numerous fungi exception of Zygomycetes [45], or some soil born phytopathogenic fungi such as *Fusarium solani* (Teleomorph = *Nectria haematocca* in present work) and *Colletotrichum lindemuihianum* [46].

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