

# Occurrence of moulds from bee pollen in Central Italy – A preliminary study

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## Abstract

The present study aimed to preliminarily evaluate the occurrence of fungi in 40 specimens of trapped pollen collected from April – September 2015 in 40 apiaries from Tuscany (Central Italy). Cultural and microscopical examinations allowed the recognition of *Cladosporium* sp., *Alternaria* sp., *Humicola* sp. *Mucoraceae* and *Acremonium* sp. *Penicillium brevicompactum*, *Penicillium chrysogenum*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger* and *Aspergillus terreus* were also identified. Yeasts and *Fusarium* spp. were not isolated. All pollen specimens were positive for at least one fungal isolate. Total CFU per gram ranged from 4–568. *Aspergillus* and *Penicillium* were obtained from 8 (20%) and from 22 (55%) pollen samples, respectively, associated in 4 cases (10%). The recovery of storage fungi such as *Aspergillus* and *Penicillium* in trapped pollen presents a potential risk for human health and attention should be paid to all stages of the post-harvest process.

## Key words

toxigenic fungi, bee pollen, moulds, *Aspergillus*, *Penicillium*

## INTRODUCTION

Apiculture (beekeeping) is the practice of the management of honey bees in hives, principally for the honey and pollination (ecosystem services). Hives of bees also produce other products such as wax, royal jelly, propolis and pollen [1]. Bee pollen consists of plant pollens collected by worker bees combined with plant nectar. Field bees transport the bee pollen to the hive and usually store it as bee bread, compacted into the honeycomb cells, where it is used as food for all the colony drone bees. The beekeepers harvest bee pollen for human food by special traps where the foraging bees lose a part of bee pollen transported to colony. Bee pollen contains essential amino acids, vitamins, minerals, fats and oils, carbohydrates and antioxidants [2]. For these reasons, its use in the human diet is very highly appreciated; furthermore, it has been reported to have several therapeutic applications [3, 4, 5].

There is growing concern about the sanitary quality of food. To meet the demand for bee-pollen and to prevent harm to consumer health, permanent controls in the beekeeping chain and the implementation of good production and processing practices by beekeepers and warehouses are essential [6].

Bees do not collect pollen from grass; however, they may occasionally collect fungal spores from mouldy plants [5] and the occurrence of potentially toxigenic fungal species, as well as of mycotoxins, has been recorded [7, 8, 9, 10, 11].

To the best of the knowledge of the authors of this study, only few researchers have carefully investigated the mycological features of this substrate, and because of the lack of such information from Italy, the aim of the present study was a preliminary evaluation of the occurrence of fungi in samples of pollen obtained from Italian producers in Central Italy.

## MATERIALS AND METHOD

Forty specimens of trapped pollen were culturally examined for fungi. All the samples (50 grams) were collected from April – September 2015 in 40 apiaries from Tuscany (Central Italy). Trapped pollen was randomly collected by one beehive per apiary. In all cases, the traps were emptied daily and the samples of pollen placed in sterile plastic boxes, transported to a laboratory and processed within 24 hours.

Pollen aliquots of 5 g were diluted in 10 ml of sterile saline and seeded onto Malt Extract Agar (MEA) plates, then maintained at 25 °C for 10 days. All cultures were performed in triplicate. Colony forming units (CFU) per gram were calculated for each recovered fungi.

When required, subcultures on MEA and Czapeck-Dox agar for further identification were obtained. Fungal colonies were identified on the basis of their macro- and microscopic features [12, 13, 14, 15]. *Aspergillus* and *Penicillium* were identified at species level.

## RESULTS

Cultural and microscopical examinations allowed the recognition of *Cladosporium* sp., *Alternaria* sp., *Humicola* sp., *Mucoraceae* and *Acremonium* sp. Furthermore *Penicillium brevicompactum*, *Penicillium chrysogenum*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger* and *Aspergillus terreus* were also identified. All pollen specimens were positive for at least one fungal isolate. Total CFU per gram were ranging from 4–568. *Cladosporium* sp. was the most frequently recovered mould (from 27 specimens), followed by *P. chrysogenum* (from 18 specimens).

Potentially toxigenic fungal species belonging to genera *Aspergillus* and *Penicillium* were obtained from 8 (20%) and from 22 (55%) pollen samples, respectively, in association in 4 cases (10%). More detailed data about qualitative and quantitative mycological findings are shown in Table.

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**Table 1.** Qualitative and quantitative results of culture from pollen samples

Sample	Fungal genera/species (Colony Forming Units per gram)
1	<i>Cladosporium</i> sp. (50), <i>Alternaria</i> sp. (32)
2	<i>Alternaria</i> sp.(61)
3	<i>Cladosporium</i> sp. (38), <i>Alternaria</i> sp. (16)
4	<i>Penicillium chrysogenum</i> (32)
5	<i>Penicillium chrysogenum</i> (20)
6	<i>Cladosporium</i> sp. (43)
7	<i>Cladosporium</i> sp. (64), <i>Humicola</i> sp. (14)
8	<i>Aspergillus flavus</i> (19)
9	<i>Aspergillus nidulans</i> (8), <i>Cladosporium</i> sp. (47), <i>Humicola</i> sp. (12)
10	<i>Aspergillus niger</i> (34), <i>Cladosporium</i> sp. (58), <i>Humicola</i> sp. (7)
11	<i>Alternaria</i> sp. (44), <i>Cladosporium</i> sp. (43), <i>Humicola</i> sp. (4)
12	<i>Penicillium chrysogenum</i> (79), <i>Cladosporium</i> sp. (65)
13	<i>Cladosporium</i> sp. (30)
14	<i>Cladosporium</i> sp. (64), <i>Alternaria</i> sp. (53)
15	<i>Penicillium brevicompactum</i> (43)
16	<i>Penicillium brevicompactum</i> (98), <i>Cladosporium</i> sp. (24)
17	<i>A. niger</i> (21)
18	<i>Penicillium brevicompactum</i> (24), <i>Cladosporium</i> sp. (12)
19	<i>Penicillium chrysogenum</i> (61)
20	<i>Penicillium chrysogenum</i> (72), <i>Cladosporium</i> (34)
21	<i>Penicillium chrysogenum</i> (100), <i>Cladosporium</i> (24)
22	<i>Penicillium chrysogenum</i> (231), <i>Cladosporium</i> (9)
23	<i>Penicillium chrysogenum</i> (136)
24	<i>Penicillium chrysogenum</i> (178), <i>Penicillium brevicompactum</i> (4)
25	<i>Cladosporium</i> sp. (42), <i>Penicillium brevicompactum</i> (156)
26	<i>Penicillium chrysogenum</i> (172), <i>Cladosporium</i> sp.(36), <i>Aspergillus terreus</i> (8)
27	<i>Aspergillus niger</i> (8), <i>Penicillium chrysogenum</i> (235), <i>Alternaria</i> sp.(8), <i>Cladosporium</i> sp.(62)
28	<i>Aspergillus terreus</i> (16), <i>Penicillium chrysogenum</i> (88)
29	<i>Alternaria</i> sp (50), <i>Cladosporium</i> sp. (104)
30	<i>Penicillium chrysogenum</i> (84)
31	<i>Cladosporium</i> sp. (44), <i>Penicillium chrysogenum</i> (52)
32	<i>Acremonium</i> sp. (48), <i>Alternaria</i> sp. (79), <i>Cladosporium</i> sp. (25)
33	<i>Penicillium chrysogenum</i> (484)
34	<i>Mucoraceae</i> (120), <i>Cladosporium</i> sp. (492), <i>Alternaria</i> sp. (278)
35	<i>Cladosporium</i> sp. (428), <i>Penicillium chrysogenum</i> (544)
36	<i>Mucoraceae</i> (143), <i>Cladosporium</i> sp. (344), <i>Alternaria</i> sp.(62)
37	<i>Cladosporium</i> sp. (488)
38	<i>Cladosporium</i> sp.(404), <i>Penicillium chrysogenum</i> (486), <i>Penicillium brevicompactum</i> (340)
39	<i>Penicillium chrysogenum</i> (568), <i>Aspergillus flavus</i> (24)
40	<i>Cladosporium</i> sp. (262), <i>Mucoraceae</i> (65)

## DISCUSSION

The results of the presented study show the presence of moulds in all samples of the bee pollen examined. A few literature papers can be compared with our work. *Penicillium* spp., *Aureobasidium* sp. and *Rhizopus* sp. were reported as the dominant species in corbicular almond pollen from Arizona in the USA [7]. In Europe, Gonzales et al. [8] recovered *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., *Rhizopus* spp. and *Fusarium* spp. from 89.9%, 90%, 80%, 80% and 53.3% of specimens from commercially available bee pollen. In Slovakia, *Mucoraceae*, *Fusarium* sp., *Aspergillus* spp., *Alternaria* spp. and *Paecilomyces* spp. were isolated [16]. Furthermore cultures from differently treated pollen (frozen, dried and UV irradiated) in various areas of Slovakia yielded *Mucoraceae*, *Alternaria*, several *Aspergillus* species, *Cladosporium cladosporioides* and many *Fusarium* species [10]. Estevinho et al. [17] refer to the isolation of moulds and yeasts from 60% of specimens from Portuguese bee pollen. The data in the presented study regarding fungal genera are in general agreement with Gonzales et al., [8] and with Kakaniova et al. [10]. However, the results of the present survey differ from these authors in that *Fusarium* spp. was not isolated. Yeasts were also not isolated, although some authors [6, 8] report the occurrence of these fungi in 96.6% and 57.7% of bee pollen, respectively.

Bee pollen is reported as a substrate that stimulates mycotoxins' production [18] if the beekeeper does not provide adequate and prompt drying [19]. Hygiene measures on the pollen production chain, such as drying, can prevent mould growth and the possibility of mycotoxin biosynthesis. Hence, mycotoxins from *Fusarium* were identified from not properly dried pollen specimens [10, 11], and Gonzales et al. [8] demonstrated the occurrence of mycotoxin-producing fungi in bee pollen.

Values of humidity of freshly trapped pollen are usually low, ranging from 6.7% – 20.6% RH, corresponding to a water activity ( $a_w$ ) of 0.3–0.7. Storage conditions are very important for health and for the quality standards of pollen. Considering that minimal  $a_w$  requirements of some common toxigenic moulds are near 0.70 [20, 21], the range of  $a_w$  of fresh bee collected pollen includes compatible conditions for the growth of moulds and yeasts.

Toxigenic fungi are classified as field fungi and storage fungi [22]. *Fusarium* is the main toxigenic field fungal genus, while *Aspergillus* and *Penicillium* are storage fungi. In the presented study, the lack of isolation of *Fusarium* from fresh untreated bee pollen is striking, and could suggest a low environmental occurrence of these fungi. Conversely, the presence of both *Aspergillus* and *Penicillium* suggests that attention should be paid to all stages of the post-harvest process. A further step will be the investigation of contamination by mycotoxins in bee pollen from Italy.

In conclusion, the recovery of storage fungi such as *Aspergillus* and *Penicillium* in trapped pollen presents a potential risk for human health, and attention should be paid to all stages of the post-harvest process.



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