



[Science Archives \(ISSN:2582-6697\)](http://www.sciencearchives.org)

Journal homepage: www.sciencearchives.org



Original Research Article

<http://dx.doi.org/10.47587/SA.2021.2413>



Isolation and diagnosis of fungi associated with beekeepers *Apis mellifera* and its effects on some plant pathogenic fungi

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Received: Oct 30, 2021 / Revised: Nov 20, 2021/ Accepted: Nov 27, 2021

Abstract

Fungi are real-nucleus organisms that are widely present in the environment, some of which are large, such as truffles and mushrooms, and others are small in size, and others are seen only by microscopes such as yeasts, fungi offer many benefits to modern society through their use in the pharmaceutical, beverage and food industries. The study was based on the isolation and diagnosis of fungi associated with beekeepers from different bees from different areas of Samarra district and the isolation lasted for 12 months. The results showed that there is a difference in the number and type of fungal insulation by the different parts of the insect's body on the one hand and the different chapters of the year on the other. Thirty-one fungal isolations from the body parts of the beekeepers' insect diagnosed microscopic isolations into 19 fungal races. Anti-all fungal isolation tests (31 mushrooms) with 5 nurses were conducted: *Alternaria solani*, *Fusarium oxysporum*, *Fusarium solanio* *Sclerotinia sclerotiorum* and *Pythium aphanidermatum* using the method of double implantation on the steel medium. This study aimed to isolate and diagnosing fungi accompanying the different parts of the beekeeper's body in Samarra and to assess the efficiency of isolated fungi in resisting some fungal pathogens.

Keywords *Apis mellifera*, *Aspergillus niger*, *A. solani*, mushrooms, plant fungi

Introduction

Fungi are real-nucleus organisms that are widely present in the environment, some of which are large, such as truffles and mushrooms, and others are small, and others are seen only by microscopes such as yeasts, fungi offer many benefits to modern society through their use in the pharmaceutical, beverage and food industries. On the other hand, it is one of the most important pathogens for humans, animals, and plants with the potential to cause devastating deaths and economic losses. Similarly, fungi also have a negative impact on agriculture, posing a serious threat to our food supplies. Due to the high incidence of fungal diseases affecting plants coupled with increased resistance of these fungi to pesticides at an alarming rate, the frequent and indiscriminate use of pesticides has caused many environmental problems, including pollution of water, soil, and food, as well as contamination of agricultural products and the elimination of beneficial microbiology in the soil, the

development of bio-control programmes for plant diseases, has become an urgent necessity to reduce the negative effects of pesticide use to include the use of bio-resistance by introducing bio-organisms. Anti-growth organisms of fungi, bacteria, yeasts, and their metabolic products (Tracy *et al.*, 2018).

Fungi also coexist with insects or form internal plant fungi and root colonies due to their presence in the soil, anti-plant diseases, and plant growth catalysts provide these newly understood features possibilities for using fungi in multiple roles where some fungal species can simultaneously suppress plant pathogens and parasitic nematodes as well as promote plant growth (Lacey *et al.*, 2015). Fungi and insects have coexisted over the past 400 million years, and their interactions have developed in different ways. (Mannino *et al.*, 2019). The coexistence of many fungi with insects may be intrusive or intrusive, and intrusion may be internally Endoparasites or externally Ectoparasites, and all insect ranks are at risk of fungal infection (Scholte *et al.*, 2004). Fungi are naturally present on honeybee insects, most of which cannot

penetrate the blood and injure the insect or grow in its cells, but the injury can occur as a result of ingestion of fungal spores and germination within the insect (Sharif, 2012).

Statments and others have indicated that honeybees may gain health benefits from fungi and their antimicrobial compounds. Western honeybee *Apis mellifera* is an important component of crop production and food diversity around the world, with *A. mellifera* and other *Apis* also playing a key role in the environmental stability of wild plants within endemic areas of Europe, Africa and Asia. from 75% of honeybee colonies. Bees are the main pollinators of insects for plants. Many seed and fruit farmers in the United States keep large numbers of bees or rent them from bees, spending several million dollars a year on pollination services (Tauber *et al.*, 2019). Honeybees are important pollinators for commercial blueberries in the southeastern United States, and blueberry producers often rely on bees to pollinate for high fruit production, as indicated by the results of a 3-year field study conducted by Dedej *et al.*, (2004) to test the hypothesis that the use of beehives equipped with dispensers containing bio-control product Serenade, a commercial combination of *Bacillus subtilis* bacteria that showed activity against floral infection In laboratory trials, the results of the study showed that using honeybees as pollinators to berries can reduce the incidence of mummy berries and that this is a wise practice that improves the benefits of pollination through the use of bees as a vital vector for the element used in bio-continnence.

Because of the importance and spread of fungal diseases on field crops in the country in general and Salaheddine province in particular and the absence of a prior study to assess the efficiency of fungi associated with bee insect factors as vital control factors against plant nurses, this study was conducted to isolating and diagnosing fungi accompanying the different parts of the beekeeper's body in Samarra and to assess the efficiency of isolated fungi in resisting some fungal pathogens.

Materials and Methods

Sample collection

Samples were collected from various bees in Samarra district for a period of (December 12, 2019-December 12, 2020) for the purpose of isolating fungi associated with bee insect workers, diagnosing them both superficially and figuratively, and assessing their efficiency in inhibiting a number of plant-pathogenic fungi. The observations, which include the date of the sample and the name of the area from which it was collected, were recorded, and transferred to the laboratory for the purpose of isolation, purification, and diagnosis.

Insulation of insect-accompanying fungi

The samples were transferred to the laboratory and each insect was placed in a petri dish containing distilled water sterile for the purpose of washing and for one minute and

then transferred to other dishes containing sodium hypochlorite solution NaOCL at a concentration of 1% and for 30 seconds to dispose of fungi on the outside surface and then transferred to other dishes containing sterilized distilled water to wash them from the residue of sodium hypochlorate solution and then placed on sterile filtration sheets for drying. Each insect was then cut into five parts: head, chest, abdomen, legs, wings, with a sterile scalpel, then the parts were moved by sterile tongs and planted in petri container dishes on the transplant circles (PDA, SDA, ME A) And in fact 3 similar pieces in each petri dish with a diameter of 9 cm, and wrote the required notes on each dish and includes the number of the part and its name, the date of insulation and the name of the medium used and then hugged the dishes at a temperature of 25 ± 2 C° for 7 days

After the end of the incubation period and the growth of the fungus accompanying the insect, the insulation was purified by taking part of each developing fungal colony by a sterile lobe and transferred to a petri dish containing the PDA implant medium and incubated at a temperature of 25 ± 2 C° for 7 days (Chauhan and Jindal, 2020) After the growth of innate insulation was encoded with semantic keys, the developing insulation was given numbers, the first number indicating the part of the insect from which it was taken, the second number indicates the refined part and the third letter indicates the name of the isolated medium, and after the second incubation period the isolations were divided outwardly by the color and strength of the innate colony and divided on this basis into isolation.

Direct antibody test on the solid medium to assess the efficiency of fungi associated with beekeepers against plant pathogenic fungi

We tested the anti-fungus efficiency associated with isolated beekeepers with dual Culture Technique nurses with petri dishes containing PDA implant medium and to revise the center of each half dish with a disc (0.5 cm diameter) from nurses' colonies Each of the 7-day-old mushroom farm was taken by a sterile cork piercer and at the same time to be vaccinated in the middle of the other half with a tablet taken by isolated fungus colonies, and by three repeaters per isolation and incubated dishes at a temperature of 25 ± 2 After 7 days, the contrast was estimated according to the five-year standardization scale prepared by (Bell *et al.*, 1982), as follows:

- The growth of the fungus accompanying the insect covers the entire area of the dish without allowing the insulation of pathogenic fungi to grow.
- The growth of the insect-accompanying mushrooms covers three quarters of the dish, and the growth of pathogenic mushrooms covers the remaining quarter.
- The growth of the insect-accompanying mushrooms covers half the area of the dish and the pathogen's

growth covers the other half with no separation zone between the two colonies.

- The growth of the mushrooms accompanying the insect covers a quarter of the area of the dish while the nurse's growths cover three quarters of the dish.
- The insect-accompanying mushrooms do not grow, and the pathogen covers the entire area of the dish.

Antibody test using fungal spray to assess the effectiveness of bee-accompanying fungi against nurses

Malt Extract Broth was attended and distributed in 250 ml glass rotors, at a rate of 250 ml/dork, closed the glass rotors with tampons and sterilized with the electric bumper and after the sterilization process was completed left the rotor at a temperature The room to cool and then add the antibiotic chloramfinicol by (62.5g/250ml) per dork and vaccinate the rotor with 0.5 cm diameter tablets from each isolation of the fungus accompanying the bees, I hugged the rotor at 25+2 m temperature for 28 days taking into account the swing of the rotor every 2-3 days for the purpose of distributing innate growth, the filters were filtered after the end of the incubation period by filter paper (whatman No. 1) for the purpose of separating condenses and fungal threads from the fungal spray, then placing the spray in tubes discarded using centrifuge at 5000 rpm for 3 minutes to get rid of the micro impurities of the center and mushroom growths and to ensure the purity of the filter refilling process was restored Using a 0.22 µm sterile Millipore membrane filter, the spray was distributed in sealed tubes and kept in the refrigerator until use .The results were statistically analyzed by the application of Minitab statistical program, Ver.Ver17. Use the ANOVA test and compare the calculation averages of different transactions with the Dunkin' multi-border test with a probability level of 0.05 and 0.01. (Al-Rawie, 2002).

Results

The results of isolation and diagnosis showed the presence of many fungi associated with bee insect factors where 31 fungal species dating back to 19 species were diagnosed, including two yeasts isolated from the body parts of bee insect workers, as in table (1).

Exactly 31 was isolated, diagnosed fungal species belonging to 19 species, from different body parts of the bee insect (head, chest, abdomen, wings, and legs) and the results showed that isolated fungi belong to six people and the vast majority of them belonged to the missing fungi Deuteromycota and by 83.87% of the number of species and 73.68% of the number of races followed by the insethical fungi Zygomycota, from which two species were isolated, Rhizopus stolonifern and recomosus Mucor, accounting for 6.45% of the number of species and 10.52% of the number of races followed by the egg fungus Oomycota where isolated from them one species due to the sex Pythium and cystic fungi Ascomycota isolated from them one race belongs to yeast Saccharom Basidomycota, represented by the

rhodotorula yeast genus, appeared in equal proportions, which are 3.22% of the number of races and 5.26 of the number of species. The results of the insulation of the fungi included in the figure (1) show that the highest rate of innate growth was of the legs at 80.64%, which may be due to the friction of the legs with wherever the insect stands as well as having legs in it a basket collecting pollen that may carry some of the fungal spores and each insect has 3 pairs of legs and the least of which was the fungi isolated from the abdominal area and head with 25.80% and 54.83 %respectively.

Table 1. Fungi isolated from the body parts of beekeepers' insect

No.	Fungi
1	<i>Alternaria alternata</i> (Fries) Keissler
2	<i>Alternaria citri</i> Ellis& Pierce
3	<i>Alternaria solani</i> Sorauer
4	<i>Aspergillus flavus</i> Link
5	<i>Aspergillus fumigatus</i> Fresenius
6	<i>Aspergillus niger</i> van Tiegh.
7	<i>Aspergillus ocraceus</i> Wilhelm.
8	<i>Aspergillus parasiticus</i> Speare
9	<i>Aspergillus tamarii</i> Kita
10	<i>Aspergillus sp</i>
11	<i>Biopolaris spicifera</i> (M.B.Ellis)Tsuda&Ueyama
12	<i>Cladosporium cladosporioides</i> (Fresn.) de Vries
13	<i>Cladosporium herbarum</i> (Pers.) Link ex Gray
14	<i>Curvularia lunata</i> Cochliobolus Drechsler
15	<i>Epicoccum nigrum</i> Link
16	<i>Fusarium oxysporum</i> f.sp.lycopersci
17	<i>Fusarium solani</i> (Mart.) Sace
18	<i>Hendersonula toruloide</i> Natrass
19	<i>Mucor recomosus</i> Fres
20	<i>Penicillium chrysogenum</i> Thom
21	<i>Penicillium corylophilum</i> Dierckx
22	<i>Penicillium sp</i>
23	<i>Pythium aphanidermatum</i> (Edson) Fitz
24	<i>Rhizopus stolonifern</i> (Ehrenb. Ex Fr.) Lind.
25	<i>Rhodotorula sp.</i>
26	<i>Saccharomyces sp.</i>
27	<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary
28	<i>Sclerotium sp.</i>
29	<i>Talaromyces radicus</i> A.D. Hocking & Whitelaw.
30	<i>Trichoderma harzianum</i> Rifai
31	<i>Ulocladium chartarum</i> (Preuss) Simmons

The results showed very clear differences in fungi associated with bee insect agents in their effect on the growth of *A. solani* pathogenic mushrooms, with *A. niger* *A. fumigatus* and *T. hazarianum* showing the highest anti-pathogenic mushrooms *A. solani* at an anti-1 degree. On the double farm with a inhibition rate of 100% and showed a very clear superiority over the rest of the other isolations as in the figure (2), followed by the isolation of mushrooms *A. parasiticus* with an opposite score of 1.5 and a inhibition rate of 87.5%.

For isolations *A. flavus*, *H. toruloide*, *M. recomosus* and *P. aphanidermatum*, the degree of contrast was 2 and the inhibition rate was 75%, followed by insulation, *A. tamarii*, *C. lunatao*, *E. nigrum* and *R. stolonifern* with an opposite score of 2.5% and an inhibition rate of 62.5%. Insulations *A. alternata*, *A. citri*, *A. ocraceus*, *Aspergillus* sp, *B. spicifera*, *C. cladosporioides*, *C. herbarumo*, *F. oxysporum* and *F.*

Solani, *P. chrysogeniumo*, *P. corylophilum*, *penicillium* sp, *Sclerotium* sp, *T. raduciso*, *U. chartarum* showed a 3% anti-ism score and an inhibition rate of 50%. *Rhodotorula* sp and *S. sclerotiorum* in both were antithesis at 3.5% and inhibition rate of 37.5%, while yeast *Saccharomyces* sp gave the lowest contrast score of 4 and an inhibition rate of 25%. As shown in figure (2).

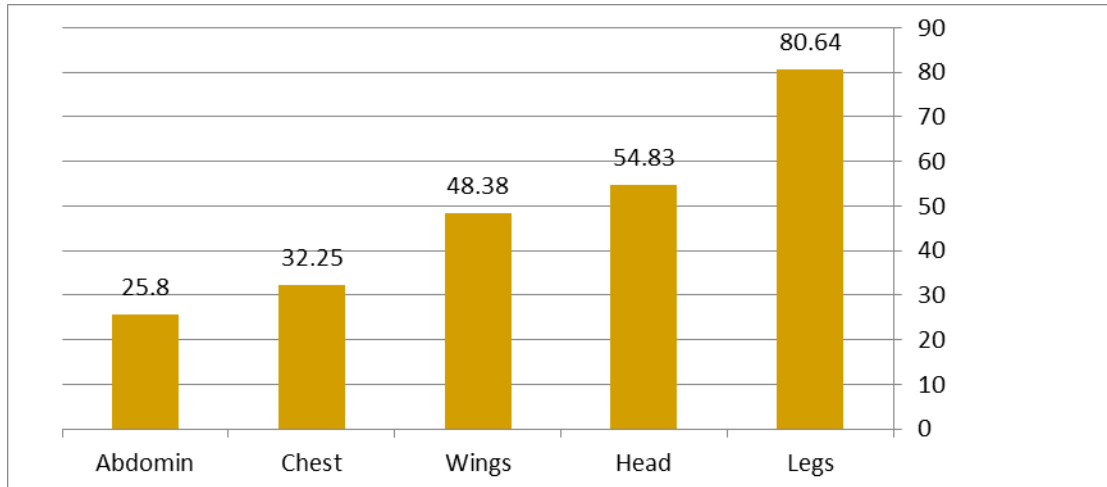


Fig 1. The growth rate of fungi on the body parts of insects factoring bees

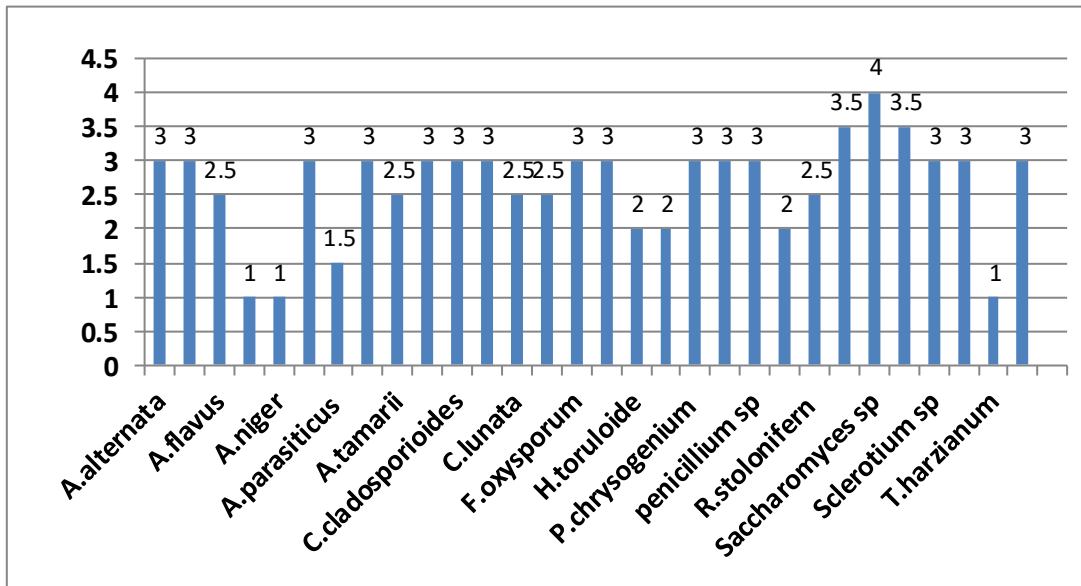


Fig 2. Anti-fungus insulation associated against *A. solani* pathogenic mushrooms

Discussion

Our study found that missing fungi are the most present on insect bodies and may be due to the widespread spread of these fungi for their ability to adapt to inadequate environmental conditions and their ability to grow in various natural and industrial agricultural circles alike due to their limited food needs as well as their production of large numbers of colonies (Seifert *et al.*, 2008).

This study is consistent with what Barbosa *et al.*, (2018) found that isolating 21 species of fungi, including 6 *penicillium* species and 4 *Talaromyces* species of bee nests, pollen, and honey, consistent with Elsevier and Prest results (1972) who isolated 38 fungal culture from the intestinal contents of bee workers, including four species belonging to the *Penicillium* genus, two species of the *Aspergillus* genus and two species of *Cladosporium*, and this result surpassed what Gilliam and Morton (1974) found, who isolated 18 species. Some kind of fungus from the intestines of bees, it is in line with the Study of Al-Jumaili (2017), which isolated 28 species of fungi from mosquitoes dating back to 14 species, the majority of which were due to missing fungi. It is consistent with Ghanmi (2016), isolating 39 species of fungi from mosquito larvae *Cx. quinquefasciatu* and agreeing with the results of Kassiri *et al.*, (2015), who isolated 28 species of fungi from the domestic fly, the most important of which are *Aspergillus* spp, *Penicillium* spp, and *Rhodotorula* sp.

The highest rate of innate growth was from the legs at 80.64%, which may be due to the friction of the legs with any place where the insect stands, as well as having legs in which the pollen collection basket, which may carry some fungal spores, and each insect has 3 pairs of legs and the lowest percentage was fungi isolated from the abdominal area where it reached 25.80%. Fungi isolated from the head area appeared at 54.83% and we can explain this because the head contains the mouth and is in direct contact with flowers to absorb nectar from it and flowers may be infected with some fungi and move to the parts of the mouth and the rest of the head, followed by fungi isolated from Wings are 48.38%, followed by fungi isolated from the chest area and 32.25%, and these results are consistent with some studies that have indicated that fungi are able to stick to insect skin and penetrate the skin through condescending. With insect skin through unspecified interactions (Pedrini, 2017). Batta (2018) also noted that fungi can be used oral and respiratory as an alternative to penetrating the skin of the host insect, which increases the possibility of fungi to coexist with insects.

The results showed very clear differences in fungi associated with bee insect agents in their effect on the growth of *A. solani* pathogenic mushrooms, as the isolations *A. niger*, *A. fumigatus* and *T. hazarianum* showed the highest anti-pathogenic effect against *A. solani* pathogenic mushrooms with an opposite score of 1 on the double farm and in proportions. A 100% inhibition that showed very clear superiority over the rest of the other isolations, as in figure (2). According to Bell *et al.*, (1982), *T. hazarianum* has a direct intrusion mechanism on the fungal yarn of pathogenic mushrooms by circumventing its filaments and analyzing its walls by releasing its analyzed enzymes, as well as the mechanism of competition for space and food and its rapid growth, as well as its production of antibiotics that negatively affect the growth of pathogenic fungi and limit their reproduction (Zewain and Hamad, 2020). Panaccione and Coyle (2005) stated that mushrooms *A. fumigatus* produce secondary metabolism substances such as 1,2,3, trimethoxy benzene, and dimethoxy phenol-2.5 which is the reason for its high ability to contrast with some types of fungi and pathological bacteria.

Conclusion

Isolating insect-accompanying fungi from different parts of the body individually and on several transplant circles and in different months of the year has an important role to play in obtaining the largest number of fungi. Bees have been found to be one of the most important factors in the transfer of pathogenic fungi from one plant to another, as well as bioconfictionation factors. The presence of the type *Aspergillus niger* in all parts of the insect's body and in all months of the year.

Consent for publication

The author declares that the work has consent for publication.

Funding support

The author declares that they have no funding support for this study.

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How to cite this article

Muhammad, G.A. & Hammoun, A.H.M. (2021). Isolation and diagnosis of fungi associated with beekeepers *Apis mellifera* and its effects on some plant pathogenic fungi. *Science Archives*, Vol. 2 (4) 343-348. <http://dx.doi.org/10.47587/SA.2021.2413>

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