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STUDIES ON SOME FUNGAL DISEASES INFECTING THE BROOD IN HONEY BEE COLONIES

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ABSTRACT

The present study deals with isolation and purification of five different species of fungi from infected honey bees. These fungi were comprimized due to the differential in colour and shape. They were classified and identified according to the following symptoms as follows:

a. Black coloured fungi which were identified as *Aspergillus niger.*
b. Cotton fibre like fungi which identified as *Ascosphaera apis.*
c. Green coloured fungi which were identified as *Aspergillus flavus*
d. Gray coloured fungi which were identified as *Tieghemiomyces sp*
e. White coloured ftmgi which were identified as *Curvularia sp*

Pathogenicity tests were carried out on these species of fungi. The obtained results revealed that *Ascosphaera apis.* and *Aspergillus flavus* had pathogenic effects, while the other three fungi *Aspergillus niger, Tieghemiomyces sp* and *Curvularia sp* were non-pathogenic. The effects of entomopathogenic fungi on different developmental stages of honey bee were taken into consideration to find out the most susceptible larval instar and stages for these fungal Infections. It is concluded that, the first three larval instars were more resisted to fungal infection than the other instars.

Key words: Honey bee, Fungal diseases, *Ascosphaera apis, Aspergillus flavus*

INTRODUCTION

In recent years brood fungus diseases, i.e. chalk brood mainly caused by *Ascosphera apis* and stone brood which caused by several species of fungi belonging to genus *Aspergillus* have drastic damage in apiaries.

Different subjects concerning the fungal diseases had been studied by many authors: infection level of fungal diseases **(Befus-Nogel *et al* 1992)**; identification of fungal species **(Alonso Rodriguez *et al* 1992 and Lee and Chang 1993)**, varroa mite as vector of pathogenic microrganisms **(Liu *et al* 1988)**, effect of exposing the colonies to insecticides on appearance of fungal diseases **(Koenig, 1987)**, selecting genetic lines of bees with hygienic behaviour **(Spvak and Gillian, 1993)** and effect of infection on colonies

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production (**Rosenthal *et al* 1992**). Recently, honey bee brood diseases began to appear in Delta, especially in the Northern Governorates, to spread into the upper regions of Egypt. The diagnosis of such diseases are chalk brood and stone brood (**Zidan *et al* 1999**).

The present study aimed to throw more light on the nature of fungal diseases infecting honey bee by isolation, purification and identification. Furthermore, pathogenic tests were concluded to evaluate the efficiency of fungal spores that cause infection.

MATERIAL AND METHODS

Production of limited Age of Honey Bee Larvae

Honeybee colonies, each headed with first hybrid Carniolan queen were selected to obtain the required ages of larvae. On May 4, 1996, all the brood and honey combs of the chosen colonies were removed and transferred to queenright colonies after shaking the adult bees. Two honey and pollen combs were left in each colony as a source of food. An empty and clean comb was marked and inserted between the two combs for egg laying. The marked combs were left in the colonies for 24 hours, then transferred in the experimental colonies. The same procedure repeated every day five times to obtain different ages of eggs and limited ages of the following instars and stages.

Infection of Different Larval Ages

Seven colonies, each headed by first hybrid Carniolan queen, were prepared for this study. Every colony had five marked brood combs, each had a limited larval instar, i:e., the first frame had 1st instar larvae, the second frame had 2nd instar ones... etc.

On May12, 1996, samples of stone brood mummies were collected from naturally infected colonies. They were divided into two groups according to their colour; dark-stony and white-stony mummies. Mummies of each group were pourded separately and 5 grams of the resulted powder were added to one of the following regimes.

a: 45 g sugar powder
b: 45 g of pollen grains were added to the same amount of water to obtain pollen paste.
c: 45 g sugar powder were added to the same amount of water to obtain sugar syrup.

The experimental colonies were divided into two groups. The first group was treated with dark pounded mummies while the second was treated with the white pounded mummies. Untreated colony was provided and received 45 grams of sugar solution only in feeder.

Isolation of fungi from infected individuals

To isolate and identify the fungal pathogens, five replicates from each colony were randomly taken in sterilized glass vials by using sterilized forceps. The first sample was taken on May 13, 1996 and the second in the next day. The rest of the samples were taken every day for the first six samples, then every three days until the 18th day post treatment.

Bee samples of limited ages and stages were transferred to the laboratory of Plant pathology for fungal isolation on aqueous agar-agar medium (composed of 20 gm agar- agar and dissolved in one

litter of distilled water)in sterilized Petri-dishes. Each Petri-dish contained five individuals, which were distributed randomly in the dish. The dishes were incubated at 28 -30°C for 48-82 hrs until the appearance of fungal cultures around.. the infected specimens and the percentage of infected individuals as well as the number of growing fungal colonies (according to their growth shapes and colours) in each dish were recorded.

From each of the isolated specimens, a piece of agar-agar contained fungal cultures were taken. Each type of isolate (according to its growth shape and colour) was inoculated in Petri-dishes contained solid aqueous agar-agar medium by using sterilized inoculated needle. The inoculated dishes were incubated at 28 - 30°C for 5-6 days until the fungal colonies were appeared. A piece of apical hyphae was taken from each colony by means of a sterilized needle and inoculated in Petri-dishes contained solid agar-glucose or potato-dextrose medium (composed of 200 gm of potato extracts, 10 gm glucose or dextrose, 20 gm agar-agar, and one litter of distilled water). The inoculated dishes were incubated at 28 -30°C until reaching full growth of fungi in the dish. This procedure allows to obtain pure cultures of fungi from infected honey bee individuals.

The pure fungal cultures were preserved on oblique glucose-agar (or potato-dextrose) medium in test tubes and kept in refrigerator, The cultures were renewed in a period not more than one month.

Pathogenecity test

Pure cultures of fungi were continuously renewed to obtain 15 - 20 days old cultures. The fungal suspension was prepared from pure culture and the suspended spores were obtained from three-weeks old cultures. Fungal growth was suspended in sterilized water and adjusted to 3.2×10^8. Small amount of sucrose added to the suspension to activate the spores of *Aspergillus*. The fungal suspension sprayed directly on the frames containing different ages of larvae. Each fungus species was sprayed in one colony, each colony contained five frames covered with adult bees. Samples were taken every 24 hrs in sterilized vials. Fungi were isolated from the individuals of each sample in sterilized Petri-dishes contained solid aqueous agar-agar. The dishes were incubated at 28-30°C for 48-82 hrs until the appearance of fungal cultures around the infected specimens and the percentage of infected individuals was recorded.

RESULTS AND DISCUSSION

In the present study, five different fungal species were isolated and purified from the infected honey bee specimens. These fungi were isolated, according to the colony colour and shape, identified and classified as follows:

It is important to notice that *Aspergillus niger, Curvularia sp.* and *Tieghemiomyces sp.* which were detected as black coloured fungi, gray coloured fungi and white coloured and compact fungi are found to be non-pathogenic fungi. Therefore, these three species were neglected from the present work and the following studies were carried out on both *Ascosphaera apis* which appeared as white colour and cotton fiber like fungi, and *Aspergillus flavus* which detected as

No.	Fungal properties	Identification		
		Scientific name	Family	Order
1	White coloured and cotton fiber like fungi	*Ascosphaera apis*	Ascosphaeraceae	Ascosphaerales
2	Green coloured fungi	*Aspergillus flavus.*	Moniliaceae	Moniliales
3	Black coloured fungi	*Aspergillus niger*	Moniliaceae	Moniliales
4	Gray coloured fungi	*Curvularia sp.*	Dematiaceae	Moniliales
5	White coloured and compact fungi	*Tieghemiomyces sp.*	Sphaeropsidiaceae	Sphaeropsidiales

Green coloured fungi. The obtained results are summarized in Tables (1-7).

Percentages of infection in different honey bee stages

1- Treatment of the first instar larvae

After treating the first instar larvae with the powder of dark mummies, the infection did not detect in the second day (second instar larvae) (Table, 1). On the third day, the infection began to appear depending upon the spore carriers; 20% were infected with *A. apis* when pollen paste and sucrose syrup were used. When the treated larvae reached the fourth instar, the infection reached 40% with *A. apis* when pollen paste and sugar syrup were used. In the fifth instar treated larvae, the percentage of infection with the same species of fungi were 40% and 60% with sugar powder and sugar syrup, respectively. At the same age *A. falvus* began to appear with sugar powder and pollen paste, both at 20% level of infection. The highest level of infection caused by *A. apis* was observed on the ninth day after treatment (3 day old prepupa), when 100% and 25% infection were reached with sugar powder and pollen paste, respectively. Those percentages decreased in the pupal stage and began to appear at pronounced percentages in newly emerged adult; 20, 40 and 60% infection were reached with sugar powder, pollen paste and sugar syrup, respectively. However, the highest infection with *A.flavus* (80%) was reached 6 days after treatment after using pollen paste as fungal carrier.

The white mummies were less than in case of the powder of dark mummies. On the other hand, both species of fungi were recorded also. The existence and the level of infection depend upon the brood instar and bee stage as well as the fungal carrier. Generally, the highest infection level with *A. apis* (100%) was recorded 6 days after treatment (in the second day of prepupal stage). The corresponding value (60%) with *A. flavus* was, however, obtained 5 days after treatment (in the first day of prepupal stage), both were recorded when sugar powder only was used as fungal carrier.

2- Treatment of the second instar larvae

After treating the second instar larvae with the powder of dark mummies the infection did not appear in the second day

Table 1. Percentages of infected honey bees of different stages after treating the first instar larvae with the powder of both dark and white mummies during May-June, 1996 at Ain Shams University

Days after treatment	Instar or stages	Ages	*A. apis*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	2 day old	0	0	0	0	0	0	0	0
2	Larvae	3 day old	0	0	20 20*	0	20 20*	0	0	0
3	Larvae	4 day old	0	0	40 40*	0	40 40*	20 20*	0	0
4	Larvae	5 day old	40 40*	0	0	0	60 60*	0	0	0
5	Larvae	Cocoon spinning	20 20*	0	20 20*	0	0	0	0	0
6	Larvae	cocoon spinning	20 20*	100 100*	0	0	0	0	0	0
9	Prepu-pae	3 day old	100 100*	20 0*	40 25*	20 0*	20 0*	20 0*	20	20
12	Pupae	2 day old	20 80*	40 40*	80 80*	0	0	0	0	0
15	Pupae	5 day old	40 25*	20 20*	20 0*	20 0*	20 0*	20 0*	20	20
18	Adults	Newly emerged	20 20*	0	40 40*	0	60 60*	40 40*	0	0

Table 1. Cont.

Days after treatment	Instar or stages	Ages	*A. flavus*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	2 day old	0	0	0	0	0	0	0	0
2	Larvae	3 day old	20 0*	20 0*	20 0*	20 0*	0	20 0*	20 0	20 0
3	Larvae	4 day old	0	20 20*	0	0	0	20 20*	0	0
4	Larvae	5 day old	20 20*	0	20 20*	0	0	20 20*	0	0
5	Larvae	Cocoon Spinning	0	60 60*	0	0	0	0	0	0
6	Larvae	cocoon spinning	0	0	80 80*	0	0	0	0	0
9	Prepu-pae	3 day old	0	0	0	0	0	40 25*	0	0
12	Pupae	2 day old	40 40*	0	0	20 20*	0	0	0	0
15	Pupae	5 day old	0	0	0	0	0	0	0	0
18	adults	newly emerged	0	20 20*	0	0	0	20 20*	0	0

Where: * = values represent the corrected percentages of infected bees; D = dark mummies; W = white mummies

(third instar larvae (Table, 2). On the third day the infection with *A. apis* appeared at high level, reaching 60, 40 and 40% when sugar powder, pollen paste and sugar syrup were used as fungal carriers, respectively. The level of infection with this fungal species was fluctuated as the age of treated bees progressed. In case of *A. apis*, this fungus began to appear at the beginning of the prepupal stage with slight level reaching 20% only.

The percentages of infection with both *A. apis* and *A. flavus* after treating the second instar larvae with the powder of white mummies were less than in case of the powder of dark mummies. When infection appeared, it exceeded not more than 25% and in most cases, infection disappeared completely for both fungal species and with all carriers used in the present study.

3- Treatment of the third instar larvae

After treating the third instar larvae with the powder of the dark mummies, the infection began to appear in larvae 5 day old (2 days after treatment). *A. apis* was found to infect 20-60% of the tested larvae 4 days after treatment (in the second day of spinning cocoon), depending upon the fungal carrier, being 60, 20 and 40% when sugar powder, pollen paste and sugar syrup were used, respectively. The highest level of infection with this fungal species (100%) was recorded in pupae 4 day old when sugar powder was used. *A. flavus* was detected in two cases only and with slight percentages not more than 20% (Table, 3).

The same trend of infection, but with less level, was obtained after treating the third instar larvae with the powder of the white mummies. The highest percentage of infection was recorded for *A. apis* (40%) as compared with *A. flavus* (60%). On the other hand, pupae 4 day old was more susceptible to infection with *A. apis* as compared with the other tested instars and stages.

4- Treatment of the fourth instar larvae

After treating the fourth instar larvae with the powder of the dark mummies, the infection began to appear immediately in the second day and reach the highest level of infection with *A. apis* (40, 40 and 20% with sugar powder, pollen paste and sugar syrup, respectively) 4 days after treatment (in one day old prepupae). The same high level of infection with *A. apis* was also recorded in the newly emerged adult bees (20, 40 and 40% with sugar powder, pollen paste and sugar syrup, respectively). *A. flavus* appeared at slight level not exceeded 20% in few cases (Table, 4).

In case of white mummies, the percentages of infection with both *A. apis* and *A. flavus* after treating the fourth instar larvae were less than in case of the powder of the dark mummies.

5- Treatment of the fifth instar larvae

After treating the fifth (last) instar larvae with the powder of the dark mummies, the infection with either *A. apis* and *A. flavus* began to ppear in the second day. The highest infection with the former fungus was recorded 4 days after treatment (in 2 day old prepupae), then the infection fluctuated between disappearance and appearance with low degree till emergence. In case of *A. flavus*, the

Table 2. Percentages of infected honey bees of different stages after treating the second instar larvae with the powder of both dark and white mummies during May-June, 1996 at Ain Shams University

Days after treatment	Instar or stages	Ages	*A. apis*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	3 day old	0	0	0	0	0	0	0	0
2	Larvae	4 day old	60 60*	20 20*	40 40*	0	40 40*	0	0	0
3	Larvae	5 day old	20 20*	0	0	0	0	20 20*	0	0
4	Larvae	cocoon spinning	20 20*	0	40 40*	0	0	0	0	0
5	Larvae	cocoon spinning	0	20 20*	0	0	0	0	0	0
6	Prepu-pae	1 day old	60 60*	20 20*	20 20*	0	0	0	0	0
9		Transform	40 25*	20 0*	40 25*	20 0*	20 0*	20 0*	20	20
12	Pupae	3 day old	40 25*	20 0*	40 25*	40 25*	20 0*	20 0*	20	20
15	Pupae	6 day old	0	0	0	0	0	0	0	0
18	adults	2 day old	20 0*	20 0*	40 25*	20 0*	0	40 25*	20	20

Table 2. Cont.

Days after treatment	Instar or stages	Ages	*A. flavus*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	3 day old	0	0	0	0	0	0	0	0
2	Larvae	4 day old	0	0	0	0	0	0	0	0
3	Larvae	5 day old	0	20 20*	0	0	0	20 20*	0	0
4	Larvae	cocoon spinning	0 0	0	0 0	0	0	0	0	0
5	Larvae	cocoon spinning	0	0	20 20*	0	0	0	0	0
6	Prepu-pae	1 day old	20 20*	20 20*	0 20*	0	0	0	0	0
9		Transform	0	20 20*	0	20 20*	0	0	0	0
12	Pupae	3 day old	0	20 20*	0	0	0	0	0	0
15	Pupae	6 day old	0	0	0	0	0	0	0	0
18	adults	2 day old	0	0	40 40*	0	0	0	0	0

Where: * = values represent the corrected percentages of infected bees; D = dark mummies; W = white mummies

Table 3. Percentages of infected honey bees of different stages after treating the third instar larvae with the powder of both dark and white mummies during May-June, 1996 at Ain Shams University

Days after treatment	Instar or stages	Ages	*A. apis*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	4 day old	0	0	0	0	0	0	0	0
2	Larvae	5 day old	60 60*	20 20*	0	0	0	40 40*	0	0
3	Larvae	cocoon spinning	0	0	0	20 20*	0	0	0	0
4	Larvae	cocoon spinning	60 60*	0	20 20*	0	40 40*	0	0	0
5	Prepu-pae	1 day old	20 0	20 0*	20 0*	20 0*	20 0*	20 0*	20 0	20 0
6	Prepu-pae	2 day old	0	20 20*	20 20*	0	20 20*	0		
9	Pupae	1 day old	20 20*	0	40 40*	0	40 40*	40 40*	0	0
12	Pupae	4 day old	100 100*	40 40*	20 20*	40 40*	20 20*	0	0	0
15	Pupae	7 day old	20 20*	0	0	0	0	0	0	0
18	adults	3 day old	20 20*	0	0	0	0	20 20*	0	0

Table 3. Cont.

Days after treatment	Instar or stages	Ages	*A. flavus*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	4 day old	0	0	0	0	0	0	0	0
2	Larvae	5 day old	0	0	0	20 20*	20 0	0	0	0
3	Larvae	cocoon spinning	0	20 0*	0	20 0*	20* 0	20 0*	0	20
4	Larvae	cocoon spinning	0	0	20 20*	0	0	0	0	0
5	Prepu-pae	1 day old	0	0	0	0	0	0	0	0
6	Prepu-pae	2 day old	0	20 20*	0	0	0	0	0	0
9	Pupae	1 day old	0	20 0*	0	20 0*	0	20 0*	0	20
12	Pupae	4 day old	0	0	0	0	0	0	0	0
15	Pupae	7 day old	0	20 0*	0	20 0*	0	20 0*	0	20
18	adults	3 day old	0	0	0	60 60*	0	0	0	0

Where: * = values represent the corrected percentages of infected bees; D = dark mummies; W = white mummies

Table 4. Percentages of infected honey bees of different stages after treating the fourth instar larvae with the powder of both dark and white mummies during May-June, 1996 at Ain Shams University

Days after treatment	Instar or stages	Ages	*A. apis*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	5 day old	20 20*	0	20 20*	0	0	0	0	0
2	Larvae	cocoon spinning	20 20*	0	20 20*	0	20 20*	20 20*	0	0
3	Larvae	cocoon spinning	60 60*	0	0	20 20*	0	0	0	0
4	Prepu-pae	1 day old	40 40*	80 80*	40 40*	0	20 20*	0	0	0
5	Prepu-pae	2 day old	20 20*	0	0	0	0	20 20*	0	0
6	Prepu-pae	3 day old	0	0	60 60*	20 20*	0	0	0	0
9	Pupae	2 day old	40 25*	20 20*	40 25*	20 0*	20 0*	60 50*	20	20
12	Pupae	5 day old	20 0*	20 20*	20 0*	40 25*	80 75*	20 0*	20	20
15	adults	Newly emerged	20 20*	0	40 40*	0	40 40*	0	0	0

Table 4. Cont.

Days after treatment	Instar or stages	Ages	*A. flavus*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	5 day old	20 20*	0	0	0	0	0	0	0
2	Larvae	cocoon spinning	0	20 20*	0	0	0	0	0	0
3	Larvae	cocoon spinning	20 0*	80 75*	20 0*	20 0*	20 0*	20 0*	20	20
4	Prepu-pae	1 day old	0	0	0	0	0	20 20*	0	0
5	Prepu-pae	2 day old	0	0	20 20*	0	0	0	0	0
6	Prepu-pae	3 day old	0	0	0	0	0	0	0	0
9	Pupae	2 day old	0	20 20*	0	0	20 20*	0	0	0
12	Pupae	5 day old	0	0	20 20*	0	0	0	0	0
15	adults	Newly emerged	0	0	0	0	0	0	0	0

Where: * = values represent the corrected percentages of infected bees; D = dark mummies; W = white mummies

signs of symptoms of the disease appeared on few cases. On the other hand the percentages of infection with *A. flavus* after treating the fifth instar larvae with the white mummies were recorded in few cases and not exceeded 20% (Table 5).

From the fore-mentioned results it could be concluded that *A. apis* is more abundant and distributed in the Egyptian apiaries than *A. flavus* On the other hand, all instar larvae were found to be susceptible to fungal infection but with different degrees; i.e., the infection appeared in the early instar larvae 2 days after treatment, while in late instars the infection appeared 4 days after treatment. In most cases, the signs by *A. apis* appeared on larvae during spinning their cocoon, prepupae and young pupae.

According to **Gochnauer (1963)**, fungus infections of honey bee colonies appeared in colonies with excessive hive moisture. Chalkbrood disease caused by *A. apis* occurs during summers in apiaries located in moist places (**Dallmann, 1966**). Honey bee larvae 3-4 day old and those aged 2 days after sealing were most susceptible to *A. apis* infection (**Bailey, 1976**). This is in agreement with the results of the present study. *A. apis* might survive in the soil and find its way into the food chain of honey bees, and may be transmitted to larvae via contaminated brood food. Worker and queen bees could transmit the disease.

6- Treatment of the adult workers

In this experiment adult of worker honey bees were treated 5grm of the powder of dark and white mummies and examination was made after certain periods. The obtained results are recorded in Table (6).

After treating the adults with the powder of dark mummies the infection with *A. apis* start to appear 4 days after treatment at low degree (25%). The infection increased to reach the maximum at 6 days after treatment when pollen paste or sugar syrup was used as fungal carriers. The infection fluctuated towards the end of the experiment. While in case *A. flavus,* the opposite trend could be noticed, symptoms appeared immediately in the second day and fluctuated towards the end of the experiment.

After treating the adult workers with the powder of the white mummies, the infection with *A. apis* appeared at low level and in few cases; in most cases this fungus did not detect. But in case of *A. flavus,* a pronounced level of infection was noticed in most cases and at any age to reach the maximum (40, 20 and 40% infection when sugar powder, pollen paste and sugar syrup were used) 6 days after treatment. The infection was decreased towards the end of the experiment and 15 days after treatment, the infection completely disappeared.

It is important, from the fore-mentioned results to notice that *A. apis* appeared on larvae at higher levels than on adults and the opposite was, however, true for *A. flavus,* which appeared on adults at higher levels than on larvae. This phenomenon may need extensive studies to explain the susceptibility of different mode of action of both *A. apis* and *A. . flavus.*

Effect of larval instar on percentages of infection with different pathogenic fungus species

This experiment was carried out to find out the effect of larval instar on the

Table 5. Percentages of infected honey bees of different stages after treating the fifth instar larvae with the powder of both dark and white mummies during May-June, 1996 at Ain Shams University

Days after treatment	Instar or stages	Ages	*A. apis*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	cocoon spinning	0	0	20 20*	0	0	0	0	0
2	Larvae	cocoon spinning	20 20*	60 60*	0	0	0	40 40*	0	0
3	Prepu-pae	1 day old	20 20*	40 40*	40 40*	0	20 20*	0	0	0
4	Prepu-pae	2 day old	20 20*	40 40*	100 100*	0	40 40*	0	0	0
5	Prepu-pae	3 day old	0	0	60 60*	0	0	0	0	0
6	Pupae	transform	40 25*	20 0*	20 0*	20 0*	20 0*	60 50*	20	20
9	Pupae	3 day old	20 0	20 0*	40 25*	20 0*	20 0*	20 0*	20	20
12	Pupae	6 day old	20 0	40 25*	20 0*	40 25*	40 25*	20 0*	20	20
15	adults	2 day old	0	0	40 40*	20 20*	20 20*	0	0	0

Table 5. Cont.

Days after treatment	Instar or stages	Ages	*A. flavus*							
			Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
			D	W	D	W	D	W	D	W
1	Larvae	cocoon spinning	20 20*	0	0	0	0	0	0	0
2	Larvae	cocoon spinning	20 20*	0	0	0	20 20*	0	0	0
3	Prepu-pae	1 day old	0	20 20*	0	0	0	0	0	0
4	Prepu-pae	2 day old	0	0	0	20 20*	0	0	0	0
5	Prepu-pae	3 day old	0	0	0	0	0	0	0	0
6	Pupae	transform	20 20*	0	0	0	0	0	0	0
9	Pupae	3 day old	0	0	0	20 20*	0	0	0	0
12	Pupae	6 day old	0	0	0	0	0	0	0	0
15	adults	2 day old	0	0	0	0	0	0	0	0

Where: * = values represent the corrected percentages of infected bees; D = dark mummies; W = white mummies

Table 6. Percentages of infected adult workers of honey bees after treating with the powder of both dark and white mummies during May-June, 1996 at Ain Shams University

Days after treatment	*A. apis*							
	Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
	D	W	D	W	D	W	D	W
1	20 0*	0	0	20	0	0	0	0
2	20 0*	20 0*	20 0*	20 0*	20 0*	20 0*	20	20
3	20 0*	20 0*	20 0*	20 0*	20 0*	20 0*	20	20
4	20 0*	20 0*	20 0*	20 0*	40 25*	40 25*	20	20
5	40 0*	40 0*	40 0*	40 0*	60 33*	40 0*	40	40
6	20 0*	20 0*	60 50*	20 0*	60 50*	20 0*	20	20
9	0	0	40 40*	0	0	60 60*	0	0
12	20 0*	20 0*	20 0*	20 0*	80 75*	20 0*	20	20
15	40 0*	20 0*	20 20*	20 20*	0	20 20*	0	0

Table 6. Cont.

Days after treatment	*A. flavus*							
	Sugar powder		Pollen paste		Sucrose syrup		Untreated check	
	D	W	D	W	D	W	D	W
1	20 20*	20 20*	40 40*	20 20*	20 20*	0	0	0
2	0	40 40*	20 20*	20 20*	0	20 20*	0	0
3	0	40 40*	40 40*	40 40*	20 20*	0	0	0
4	40 40*	20 20*	20 20*	20 20*	40 40*	20 20*	0	0
5	20 20*	0	20 20*	20 20*	0	0	0	0
6	40 40*	40 40*	20 20*	20 20*	20 20*	40 40*	0	0
9	0	20 20*	20 20*	20 20*	0	20 20*	0	0
12	0	0	0	60 60*	0	20 20*	0	0
15	20 20*	0	40 40*	0	20 20*	0	0	0

Where: * = values represent the corrected percentages of infected bees; D = dark mummies; W = white mummies

Table 7. Percentages of infection after treating different instar larvae with different fungal species during April-May, 1997 at Ain Shams University

Treatment	Treated larvae					Mean ± s.e.
	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	
	After 2 days					
Ascosphaera apis	0	0	0	40	50	18.00 ± 11.12
Aspergillus flavus	0	0	0	80	70	30.00 ± 18.41
Aspergillus niger	0	0	0	0	0	0.00 ± 0.00
Curvularia sp	0	0	0	0	0	0.00 ± 0.00
Tieghemiomyces sp	0	0	0	0	0	0.00 ± 0.00
	After 4 days					
Ascosphaera apis	0	0	0	80	50	20.00 ± 16.58
Aspergillus flavus	0	0	25	90	100	43.00 ± 21.73
Aspergillus niger	0	0	0	10	0	0.00 ± 0.00
Curvularia sp	0	0	0	0	0	0.00 ± 0.00
Tieghemiomyces sp	0	0	0	0	0	0.00 ± 0.00
	After 6 days					
Ascosphaera apis	80	70	50	35	20	58.75 ± 10.98
Aspergillus flavus	0	55	80	100	100	67.00 ± 18.65
Aspergillus niger	0	0	0	0	0	0.00 ± 0.00
Curvularia sp	0	0	0	0	0	0.00 ± 0.00
Tieghemiomyces sp	0	0	0	0	0	0.00 ± 0.00
	After 8 days					
Ascosphaera apis	50	55	55	80	60	60.00 ± 5.23
Aspergillus flavus	10	25	100	100	100	67.00 ± 20.31
Aspergillus niger	0	0	0	0	0	0.00 ± 0.00
Curvularia sp	0	0	0	0	0	0.00 ± 0.00
Tieghemiomyces sp	0	0	0	0	0	0.00 ± 0.00

percentage of infection with the isolated fungus species through the pathogenicity test. The data obtained summarized in Table (7).

Results show that the first instar larvae treated with the spores of different fungi, the disease began to appear 6 days after treatment which caused by *A. apis* (80%). 8 days after treatment, 50 and 10% were recorded for both *A. apis* and *A.flavus*, respectively. This means that *A. apis* began to appear earlier than *A. flavus*.

The same trend could be applied for the percentages of infection for both *A. apis* and *A. flavus*. Means of 70, 55% were recorded 6 days after treatment and 55, 25%were recorded 8 days after treatment when the second instar larvae were treated with the five isolated fungi spores. respectively.

However, the opposite was true for the third instar larvae, e.g. *A flavus* began to appear earlier (4 days after treatment at the rate of 2S%) while *A. apis* began to appear 6 days after treatment. Means ranged 50 - 80% infection were recorded 6 days after treatment and 55 -100% were recorded 8 days after treatment for both *A. apis* and *A . flavus* respectively.

Treating the fourth or fifth instar larvae with the two isolated fungus pathogens, the infection appears 2 days after treatment. being higher *A flavus* than in *A. apis*. The former reached 100% infection 4 days after treatment, while the later was found to be not exceeded 80% after 8 days from application.

It is important to notice that the three other fungus species namely, *Aspergius niger, Curvularia sp.* and *Tieghemiomyces sp.* were not detected in the pathogenecity test, because these fungi are considered non-pathogenic.

From the fore-mentioned results it could be concluded that the fourth instar larvae are considered the most susceptible instar for fungus infection.

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دراسات على بعض الأمراض الفطرية التى تصيب الحضنة فى طوائف نحل العسل

[٣١]

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أجريت هذه الدراسة بمنحل كلية الزراعة جامعة عين شمس. حيث تم عزل و تنقية وتعريف خمسة أنواع من الفطريات (عن طريق قسم أمراض النبات بالكلية) من موميات الحضنة التى جمعت من داخل الخلايا لطوائف نحل العسل التى ظهر بها أمراض الحضنة طبيعيا. هذه المومياوات قسمت إلى قسمين حسب لونها, مومياوات بيضاء وأخرى داكنة اللون حيث طحنت مومياوات كل قسم على حدى حيث تم تنميتها على بيئات غذائية معقمة. ظهرت خمسة أنواع من الفطريات قسمت كمايلى.

١- فطريات ذات لون اسود وقد عرفت بأنها *Aspergillus niger*

٢- فطريات ذات لون ابيض وقد عرفت بأنها *Ascosphera apis*

٣- فطريات ذات لون اخضر وقد عرفت بأنها *Aspergillus flavus*

٤- فطريات ذات لون رمادى فاتح وقد عرفت بأنها *Curvularia sp.*

٥- فطريات مدمجة ذات لون ابيض وقد عرفت بأنها *Tieghemiomyces sp.*

وقد ثبت من اختبارات القدرة المرضية لهذه الأنواع الخمسة من الفطريات أن

الأنواع الأول والرابع والخامس هى أنواع رمية لا تسبب اى أمراض لنحل العسل, بينما النوع الثانى *A. apis* يسبب مرض تحجر الحضنة والنوع الثالث *A. flavus* يسبب مرض الحضنة الطباشيرى , لذلك تم تجاهل الثلاث فطريات الرمية و تركزت الدراسة على نوعى الفطر المسببين لأمراض حضنة نحل العسل .

وجد ان الإصابة بفطر *A. apis* كانت اكثر انتشارا من الإصابة بفطر *A. flavus* فى المناحل المصرية, كما ظهر ان جميع الأعمار اليرقية كانت اكثر حساسية للإصابة بالأمراض الفطرية عن الأطوار التالية (قبل العذراء و العذراء و الحشرة الكاملة حديثة الخروج) وان اختلفت الإصابة باختلاف العمر اليرقى. هذا وفى معظم الحالات التى درست ظهرت أعراض الإصابة بفطر *A. apis* على اليرقات أثناء غزلها للشرنقة وفى طور ما قبل العذراء وفى العذراء حديثة العمر.

عند معاملة الشغالات البالغة بمسحوق مومياوات سوداء ظهر المرض بفطر *A. apis* بعد أربعة أيام بدرجة منخفضة (٢٥%) ثم زادت درجة الإصابة حتى وصلت أقصاها بعد ستة أيام وخصوصا عند استعمال عجينة حبوب اللقاح كمادة حاملة للجراثيم. أما بالنسبة لفطر *A. flavus* فقد ظهرت أعراض الإصابة أبان اليوم التالى للمعاملة مباشرة وتأرجحت نسب الإصابة ما بين ارتفاع و انخفاض إلى نهاية التجربة. وعند المعاملة بمسحوق مومياوات بيضاء ظهر المرض بفطرى *A. flavus* و *A. apis* ولكن بدرجة اقل كثيرا عنة فى حالة استخدام مسحوق المومياوات السوداء.

ويمكن القول أن المرض الناتج عن الإصابة بفطر *A. apis* قد ظهر على اليرقات بدرجة أعلى منة على الحشرات الكاملة ، بينما كان العكس فى حالة الإصابة بفطر *A. flavus* كما أن العمر اليرقى الرابع كان اكثر الأعمار اليرقية حساسية للإصابة الفطرية . وعموما هذه النتيجة تحتاج إلى مزيد من الدراسة.

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