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### Honeybee Drone and Worker's Different Body Phases Extract Indebted Fungi and Bacteria Growth

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# ABSTRACT

This study was conducted to test the effect of honeybee's extract solution from various body phases compared with clover and citrus honey on the growth of bacteria (*Erwinia carotovora* and *Bacillus subtilis*) and Rice blast fungi (*Magnaporthe oryzae*).

Eight samples were tested representing: drone, worker, drone larvae, worker larvae, drone pupae, worker pupae, and honey (clover and citrus honey) on the growth of bacteria and fungi. The results of the experiment indicated that honey has high activity against bacteria and fungi growth. The highest effect of extracts was in *Bacillus larvae* by worker bee extract 2.5 c.m while less impact was 1 c.m in drone pupa and worker bee extract on both types of bacteria. At the top of the fungi, effect was 77.8% by drone larvae extract while less impact was 73.3% at worker bee, larvae of worker bee, and drone pupa. Compared with clover and citrus; honey was 77.8% inhibition.

# **INTRODUCTION**

Since early times, insects and the substances extracted from them were used as therapeutic resources in the medical systems of many cultures and used in folk medicine. Commonly considered to be disguising and lath animals, many insect species have been used live, cooked, ground, in infusions, in plasters, in salves, and as ointments, both in curve and prevented medicines, as well as in magic-religious rituals (Costa-Neto, 2002). Today, the world encourages the natural materials for cure. In Mexico, it is noticed that there are 43 species of insects employed in traditional medicine as ointments, pomades, or infusions. It were prepared and used in various ways in order to alleviate such ailments as stomach distress, kidney, liver disorders, nervous breakdowns, urogenital immunological, and glandular diseases. The Aztecs and several other indigenous groups had knowledge of what might be termed "medicinal insects" (De Asis, 1982; Meza, 1979; Sahagun, 1980). Many species of insects have played important roles in the mysticism and magic inherent in many Mexican cultures as well as in the treatment of a variety of illnesses (Aguirre, 1947; Clavijero, 1980). However, some references on the use of medicinal insects are even older. The Ebers papyrus, an Egyptian medical treads dated to the sixteenth century B.C., contains several accounts of medicines obtained from insects and

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spiders (Weiss, 1947). Silkworms were used in Chinese traditional medicine for at least three thousand years (Zimian *et al.*, 1997) and the larvae of certain flies have been recognized for centuries as beneficial agents for the healing of infected wounds (Sherman *et al.*, 2000).

# **MATERIALS AND METHODS**

Extract solution is often tested by antimicrobial activity as it is common in extract solution samples. The method of flow in agar (using cups, steel cylinder of paper disks) is use for screening samples against a range of microorganisms and the parameter for activity is the diameter of the inhibition zone. The inhibiting property of honeybee's different body phases towards bacterial and fungi growth was carried out according to Perez (1990).

# Samples

#### **Honeybee Different Body Phases**

Honeybees drone, worker, drone larvae, worker larvae, drone pupa, and worker pupa samples were collected in 2014. Twenty-five for each phase supplied from Sakha station apiary (Kafr El-Sheikh Governorate, Egypt), were stored at 18 °C until extraction.

#### **Honey Samples**

Clover honey samples were supplied from Sakha research station apiary, as citrus honey supplied from apiary located in Motobis city (Kafr El-Sheikh Governorate, Egypt)

#### Sample Extracts

Twenty-five individuals for each phase of honeybee were extracted as following: homogenate manually using a mortar in 50 ml of distilled water and were shacked with a shaker, during 24 h, at room temperature. After that period, the extractive solutions were filtered by filter paper.

#### Procedure

Double strength nutrient agar (standard nutrient agar) medium was cooled to  $45^{\circ}$ c and mixed with microorganism bur farm (bacteria and fungi) under full sterile condition until it gave wide good growth, then it was poured to sterile Petri dish then cooled to  $4^{\circ}$ c/24 hour. Autoclave the tube for 15 minutes at 15 pounds pressure. Wells were punched in the set agar with an agar punch in a regular grid pattern.

Extracted solution samples were tested in the middle of the dish on a sterile condition for antibacterial activity. After incubation for 48 hours, digital callipers were used to measure the clear zone by taking the square of the diameter of the area of inhibition.

#### RESULTS

Table (1) showed that the highest inhibition percentage was in both honey (citrus and clover) 77.8 followed by drone pupa and adult worker 75.6. However, the least inhibition percentage was in larva, adult drone, and worker pupa 73.3. No significant difference between all treatments, the significant difference was found between all treatments and control.

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Treatments	linear growth	inhibition %		
Adult worker	1.83	75.6 a		
Adult drone	2.00	73.3 a		
worker Larva	1.67	77.8 a		
drone Larva	2.00	73.3 a		
worker Pupa	2.00	73.3 a		
drone Pupa	1.83	75.6 a		
Clover honey	1.67	77.8 a		
Citrus honey	1.67	77.8 a		
Control	7.50	В		
L.S.D 5%	0.363			

Table 1: Fungi Inhibition percentage treated with honeybees' drone and worker's different body phases and honey (clover and citrus).

Means having the same letter are not significantly different according to Duncan's multiple range test at 0.05 level

Data in Table (2) and Figure (1) showed that, the highest inhibition percentage was in both honey (citrus and clover) 4.13 and 3.93 followed by drone larvae 2.25. However, the least inhibition percentage fond in drone pupa 1 cm.

Static analyses indicated that, the significant different between all treatments and control were high. As there are no significant different between both of honey (citrus and clover) Generally, *Erwini carofora* showed the highest inhibition percentage in all treatments .The highest inhibition percentage in both honey type 4.5 and 4 while in bacillus 3.87 and 3.77. The least on was in pupa of drone 1 in both of bacterial.

 Table 2: Effect of honeybee's drone and worker's different body phases extract on the growth different bacterial

Treatment	Erwinia carotovora	Bacillus subtilis	Mean
Adult worker	2.00	1.50	1.75 c
Adult drone	1.50	2.00	1.75 c
worker Larva	1.50	1.50	1.50 c
drone Larva	2.00	2.50	2.25 b
worker Pupa	2.00	1.00	1.50 c
drone Pupa	1.00	1.00	1.00 d
Clover honey	4.00	3.87	3.93 a
Citrus honey	4.50	3.77	4.13 a
Control	0.00	0.00	0.00 e
Mean	2.06	1.90	

Means having the same letter are not significantly different according to Duncan's multiple range test at 0.05 level



Fig. 1: Inhibition percentage values of different bacterial treated with honeybees drone and worker's different body phases and honey (clover and citrus).

#### DISCUSSION

We think that it may be that the extract bodies of the various stages of honeybees, as well as honey, have many anti-bacteria and fungus functions. The search for new antimicrobial substances exhibiting minimal side effects is warranted because of the severe side effects of some drugs currently in use.

Many others discussed the new antimicrobial substances and found that:

The hind legs of grasshoppers were crushed and mixed with water, then drunk as a powerful diuretic to treat kidney diseases. The infusion, which is said to have refreshing properties, reduces swelling (De Asis, 1982). Rural people in the State of Oaxaca today use grasshoppers to treat certain intestinal disorders, Locusls and Schislocerca pp. These insects were pulverized and eaten as a dietary supplement to alleviate nutritional deficiencies (FAO, 1973) and to fortify the blood. It was also reported to be helpful in cases of post childbirth anemia and in lung diseases, e.g., asthma and chronic cough. Crickets legs were prepared like those of grasshoppers and were employed as a diuretic for dropsy (edema) (Barajas, 1951; Conconi, 1982; De Asis, 1982). Bugs' (Hemiptera Pentatomidiea) Euchislus spp., Edessa spp., Alize. sp.; and Stink bugs (Hemiptera, CorMae), Acanthocephala spp.; and (Leaf-footed) bugs (Xomitl Jumiles) in Nahuatl, the oil of these bugs obtained in these four taxa was applied externally in treating Scrofula and other tubercular diseases and was also used for kidney, liver, and stomach ailments. When alive, these bugs are a powerful analgesic and anaesthetic against toothache and rheumatic and arthritic pain or to alleviate gastrointestinal diseases It was also used to treat goiter and was recommended for those with a weak constitution and as an aphrodisiac (Ancona, 1933; De Asis, 1982; Taylor, 1975). The mass of boiled mealy bugs was sometimes ingested to alleviate the effects of poisonous mushrooms and other fungi, or diarrhea and to dean the teeth (Herrera, 1871). DactyJopius coccus, known as "grana" mealy bug, is mostly used as an agent to color or redden tissue or foods. It also can be boiled to produce a sticky mass and used as a skin treatment, a tooth powder to dean teeth. and in the treatment of caries (Lopez, 1971; Meza, 1979).

Alzahrani *et al.* (2012) found four varieties of honey of different botanical origins were effective against *Staphylococcus aureus oxar* and *S. aureus oxas*, *Pseudomonas aeruginosa* and *Candida albicans*. The antimicrobial activity of honey attributed to several properties of honey including its osmotic effect. Sheikh *et al.* (1995) reported that honey is effective against dermatophytes (*Microsporum ferrugineum, Trichophytonlong feuseus, T. mentagrophyte, T. semmie,* and *T. tonsurance*), parasitic fungi (*Allescheria boydii*), and saprophytes (Mucormucaralis).

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#### **ARABIC SUMMERY**

تأثير مستخلص أجسام نحل العسل والاطوار الغير الكاملة للشغالات والذكور علي نمو بكتريا الباسيلس والارونيا وفطر اللفحة في الارز

# أشرف شريف فتحي - مروة بسيوني محمود مركز البحوث الزراعية – معهد وقابة النباتات

أجريت هذه الدراسة لمعرفة تأثير المستخلص المائي للأطوار المختلفة لنحل العسل مقارنة مع عسل البرسيم والموالح على نمو البكتيريا الإيروينية والباسلس والفطر المسبب لمرض اللفحة بالأرز.

لختبرت ثماني عينات تمثل المستخلص المائي لكل من الشغالات، الذكور، يرقات الشغالات، يرقات الذكور، عذارى الشغالات، عذارى الذكور والعسل (برسيم - موالح) على نمو البكتيريا والفطريات. وأشارت النتائج أن العسل له فعالية عالية ضد نمو البكتيريا والفطريات، وقد سجل أعلى تأثير لمستخلص أجسام الشغالات على نمو بكتيريا والنعال وأشارت على نمو البكتيريا والفطريات وأشارت على نمو بكتيريا والفطريات وأشارت النتائج أن العسل له فعالية عالية ضد نمو البكتيريا والفطريات، وقد سجل أعلى تأثير لمستخلص أجسام الشغالات على نمو بكتيريا الباسيلس ٢٠٥ من الكثيريا والفطريات، وقد سجل أعلى تأثير لمستخلص أجسام الشغالات على نمو بكتيريا الباسيلس ٢٠٥ من كان أقل تأثيرا ٢ ٢. مستخلص عذارى الشغالات والذكور على كلا النوعين من البكتيريا. أعلى تثبيط للفطريات كان لمستخلص يرقات الشغالات ٨ ٧٢٪ ، في حين كان أقل تأثيرا ٣٠ ٣٣٪ لمستخلص كلا من الشعالات معالية من البكتيريا. أحلى تثبيط للفطريات كان لمستخلص يرقات الشغالات ٨ ٧٢٪ ، في حين كان أقل تأثيرا ٣٠ ٣٣٪ لمستخلص كلا من الموالح والذيرا ٣٠ ٢٠٠٠ مع معلي معاريات معالي ٢٠٠٠ معالية من المعالات معالية من البكتيريا. ألمان ٢٠٠ معلى كان أقل تأثيرا ٢٠٠ معالي من المعالات والذكور على كلا النوعين من البكتيريا. أعلى تثبيط للفطريات كان لمستخلص يرقات الشغالات ٨ ٧٢٪ ولي حين كان أقل تأثيرا ٣٠ ٣٠٠ المعالات ٢٠٠٠ معالية معالي معالية معالي معالي معالي معالي معال الموالح والذين ٣٠ ٢٠٠٠ معالي الموالح والذين سجلانية تثبيط ٨ ٧٢٪.