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The accompaniment of microorganisms inside honeybee (*Apis mellifera)* and wasps (*Vespa onrientalis)*

Muna K. Marzoq, Amel S. Abdulredha*

Department of Biology , College of Education for Pure Science , University of Basrah, Basrah, Iraq

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Abstract

The current investigation was performed with the aim to explore the relationship between insects and microorganisms and to highlight on the microorganisms that presence inside the insects belong to hymenoptera, especially honeybee *Apis mellifera* and wasps *Vespa onrientalis*, as well as to identify the relationship nature between microorganisms and insects. The insects were collected from two regions in Basrah and after sterilization, they were examined under dissecting microscope to find the ectoparasites also dilution method used to isolate bacteria and fungi. The results showed the presence of ectoparasitic female mite *Varroa destructor* on the honeybee body only and absent in wasps. Also the results revealed the presence of lactose fermenter bacteria inside honey bee only while the yeasts were associated with honeybee and wasps.

* **Corresponding Author:** Amel S. Abdulredha ✉ amelabdulredha@yahoo.co.uk

Introduction

The relationships or interactions between different species organisms are described as their beneficial, neutral or harmful reaction with each other. Symbiosis considered as mutualistic relationship occur between two organisms living together and one or both species benefit (Gibson and Hunter, 2010). The positive interaction between insects and microorganisms are studied rarely because most studies focus on negative interaction such as competition and predation. So many important questions about mutualistic relationship between microorganisms and insects still should be explained (Hofstetter *et al.*, 2006; Ganter, 2006).The order Hymenoptera is classified into two suborder: the Apocrita and the Symphyta (sawflies). Bees, wasps and ants related to the suborder Apocrita which play an important roles in terrestrial ecosystem functioning (Wilson, 1971).The relationship between fungi and member of this group has been recorded in many species including ants, bee, wasps and bumblebees(Brysch-Herzberg, 2004; Engel *et al.*, 2012;De Vega and Herrera, 2012; Davis *et al.*,2012;Mendes *et al.*, 2012).

The relationship between bacteria and insect is always transmitted vertically directly from the mother to the offspring, whereas fungal symbiosis with insects is facultative or horizontally transmitted infection (not a parent – child relationship) that transmitted between members of the same species. Researchers found that bacteria found in special cells called ˝mycetocytes˝located in the homocoel lwhile fungal cell living extracellulary in specialized structures in the hemolymph or in fat bodies (Douglas, 1998; Klebzig*et al.*, 2009; Gibson and Hunter, 2010).The honey bee *Apis mellifera* L. is one of the most important domesticated insects in crop pollination. Microbes associated with honey bee may range from pathogenic to non-pathogenic forms. Symbionts bacteria have been studied widely and characterized due to the present of bacteria on every multicellular organism , so beneficial bacteria that benefit their insect hosts have received particular attention (Janson *et al.*,2008; Gibson and Hunter,2010).In addition honeybees are exposed to a different parasites, e.g., *Nosemaceranae* and *Varroa destructor*(Amdam *et al.*,2004; Fries ,2010).*Varroa* are major ectoparasites that feed on the body fluids of larvae, pupa, and adults honey bees.These external parasites are completely depended on colonies of honey bee for reproduction and survival, therefore they don't have the ability to live separately from honey bees for more than a few days. These parasites will feed on the hemolymph of adult honey bees after piercing their soft membranes located between exoskeleton segments. So these mites may create conditions favoring transfer of microorganisms within bee colonies by damage host integument. However, the damage to the bee colonies is not associated with the mite parasitic action but it obtains from its action as vector increasing the transference of viral diseases such as deformed wing viruses and acute paralysis viruses that are considered as the major causes of colony collapse disorder on honey bee colony (Sara and Ezio, 2016).

Social wasps are also associated with fungi especially yeasts, the most studied case is the relation between wasps and *Saccharomyces cerevisiae*. In spite of this yeast has been associated with human activities, their spread and survival in nature is depending on wasps. It's found that queens of *Polistes*spp. and*Vespa crabro*be ableto carry *S. cerevisiae* from autumn season to spring and transfer it to their progeny and thus play a role in dispersal and variety of this species (Stefanini*et al.*, 2012).According to few Local studies in this line , this study has been conducted to explore the relationship between microorganisms and insects.

Materials and methods

Honeybees and Wasps collection

live adults honeybees were collected from Karmat Ali quarter near Shatt Al-Arab river while live adults wasps were collected from Al-Fayhaa quarter . The insects were captured with nylon net and put in clean tubes. After captured, the insects were stored in the fridge for 15 minutes to anesthetize the insects. All captured insects were brought to the entomology laboratory and classified.

Isolation of microorganisms

After insect's classification, the samples were brought to laboratory of mycology. All media and the tools that used in isolation of microorganisms were autoclaved and sterilized before using.

Isolation of parasites: the insects were examined under dissecting microscope searching for ectoparasites.

Isolation of bacteria

Insect samples were sterilized in alcohol 70% and then washed 3 times in sterile distilled water and homogenized in nutrient broth media. Serial dilutions (from 10^{-1} to 10^{-8}) were prepared and 0.1 ml of each dilution was transferred to petri dish containing sterile nutrient agar. All petri dish were incubated at 37° c for 1-2 days. After period of incubation, the petri dish were examined and the selected bacterial colonies were then purified by subculture them (Iskender *et al*., 2017).

Isolation of fungi: All samples were rinsed in a solution of sodium hypochlorite(1%) for 3 minutes and twice in sterile distilled water for 1 minutes , then transferred to a normal saline solution 0.85% . 0.1 ml of this solution was transferred to sabouraud dextrose agar (SDA) supplement with chloramphenicol as antibacterial.The petri were kept at 25 °c for 7 days. The fungi were identified using a light microscope. The fungal colonies were stained with blue lacto phenol for observation under microscope (Zarrin *et al*., 2007).

Results and discussion

Isolation of ectoparasites

All wasps and honeybees that studied in this work were examined under dissecting microscope, the examination showed the presence of the external parasite *Varroa destructor* female on the body of honeybees only while there is no ectoparasites on the wasps.

The ectoparasite female mites were reddish-brown in color, flat, having a button shape, with 8mm long and 1.5 mm wide, and have 8 legs (Fig. 1).

Fig. 1. *Varroa destructor* female mite, dorsal and ventral view.

The ectoparasites mite *Varroa destructor* is one of the main risk for apiculture. In spite of the conditions inside the honeybee brood cells from temperatures and humidity are not suitable for these parasites to influence their life cycle, these ectoparasites have become an important threat of the honey bee *Apis mellifera* (Maggi *et al*., 2010). Several researches have reported the presence of *Varroa* mites on the honey bee external body (Eickwort, 1990; Anderson and Sukarsih, 1996; Anderson and Trueman, 2000; Klimov *et al*., 2007).

Isolation of bacteria

All bacterial isolates were gram negative, enteric bacilli bacteria and when cultivated on MacConkey agar, all of them were lactose fermenter isolates except of one isolate was gram positive cocci bacteria (Fig. 2). This results might be due to the presence of mites on the external body of the bee and thus create a good conditions to the bacteria to invade the haemocoel and feed on it. Also these bacteria were lactose fermenters and this might help the bee in fermentation the sugars and assistance in honey synthesis and production. The presence of bacterial colonies insides honeybee has been demonstrated in several studies (Mathialagan *et al*., 2018; Pajor *et al*., 2018). Ugras (2017) found that *Lactobacillus* species, isolated from honeybee's stomach, considered as beneficial bacteria which have a role in strengthen the immune system of the bee against pathogens.

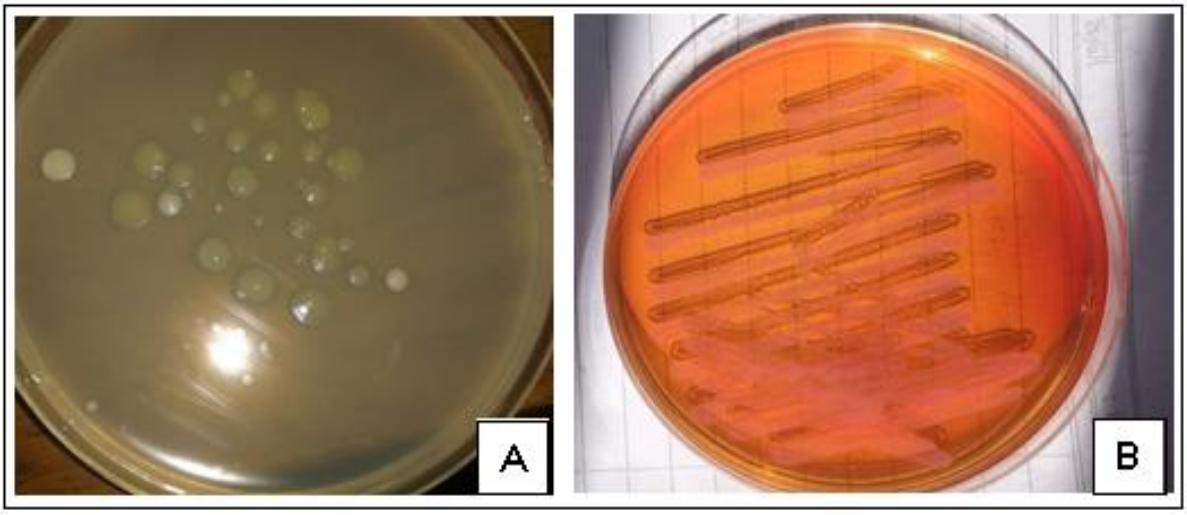

Fig. 2. A: bacterial colonies on nutrient agar B: lactose fermenter bacterial colonies on MacConkey agar.

Whereas there was no bacterial growth in the suspension that take from wasps .This results might be due to the absence of mite on the wasps external body that help the microorganisms to enter the insect body.

Isolation of fungi

The results of the study showed that fungi were associated with both honeybee and wasps. All the fungal isolated colony were related to yeasts as showed in Fig. 3.

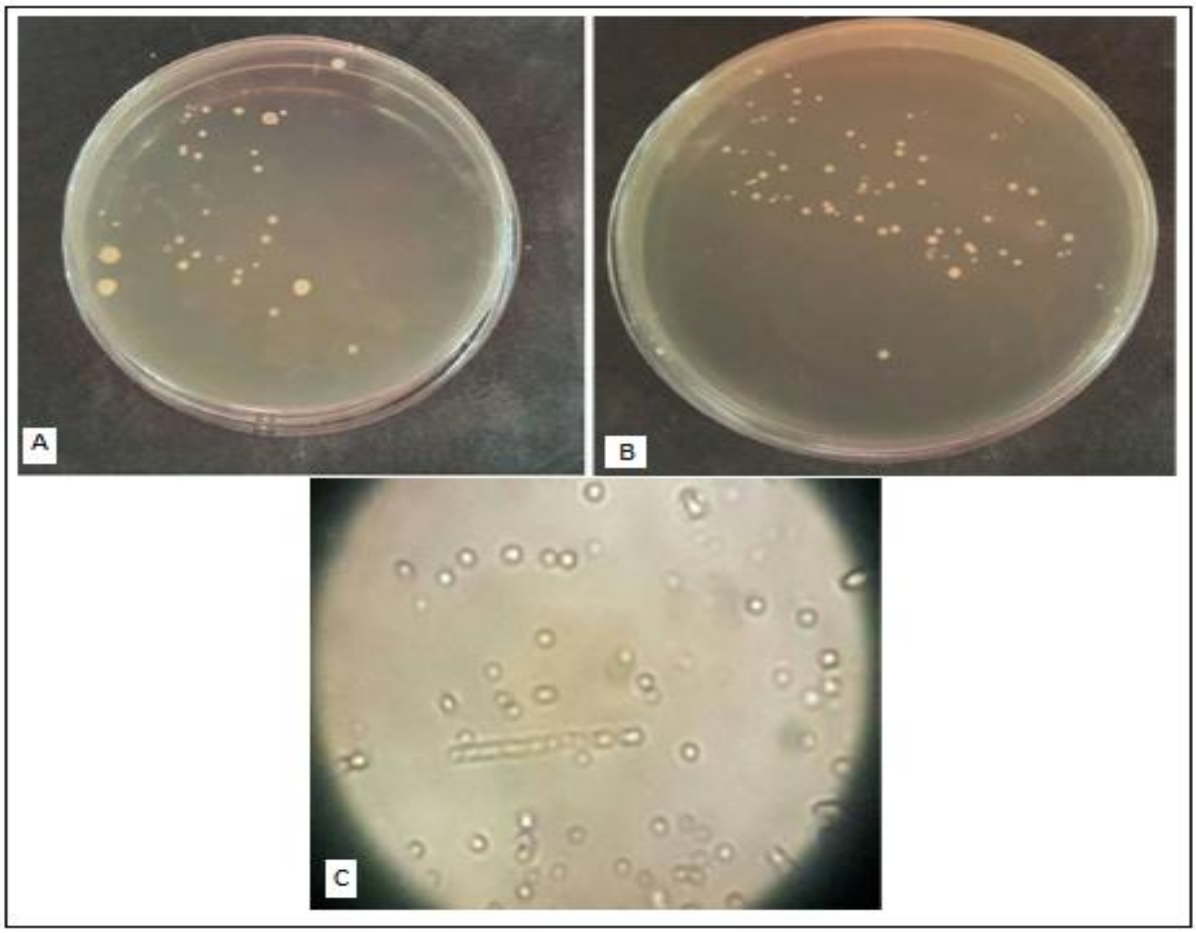

Fig. 3. Fungal colonies on SDA isolated from: A, *Apis mellifera* B, *Vespa onrientalis*. C, Single yeast cells under microscope 40x.

The association between yeast and honeybee has been reported, especially species of *Starmerella*, *Cryptococcus*, *Candida* and *Metschnikowia* (Rosa *et al.,* 2003). Yeast can create and discharge enzymes that increase, keep and maintain pollen and also have an assumed role as producers of antifungal substances that keep the hive save from infections and diseases (Gilliam, 1997; Rosa *et al.,* 2003).

The presence of yeast inside wasps also found in this results and this finding agreed with many researchers (Stefanini *et al.*, 2016; Blackwell and kurtzman, 2016).

The scientists have found that distinct strains of yeast combine and reproduce inside the guts of wasps, and this suggest to be help in yeast biodiversity which have important implications for ecology and industry (Stefanini *et al.*, 2016; Blackwell and kurtzman, 2016). The role of yeast as chemical indicators for wasps has been showed. The researchers indicate that volatile compounds produced by yeast attracted more individuals of wasps than unbaited traps, suggesting that wasps might use these compounds as indicators of nutritional sources (Davis *et al.*, 2012, 2013).

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