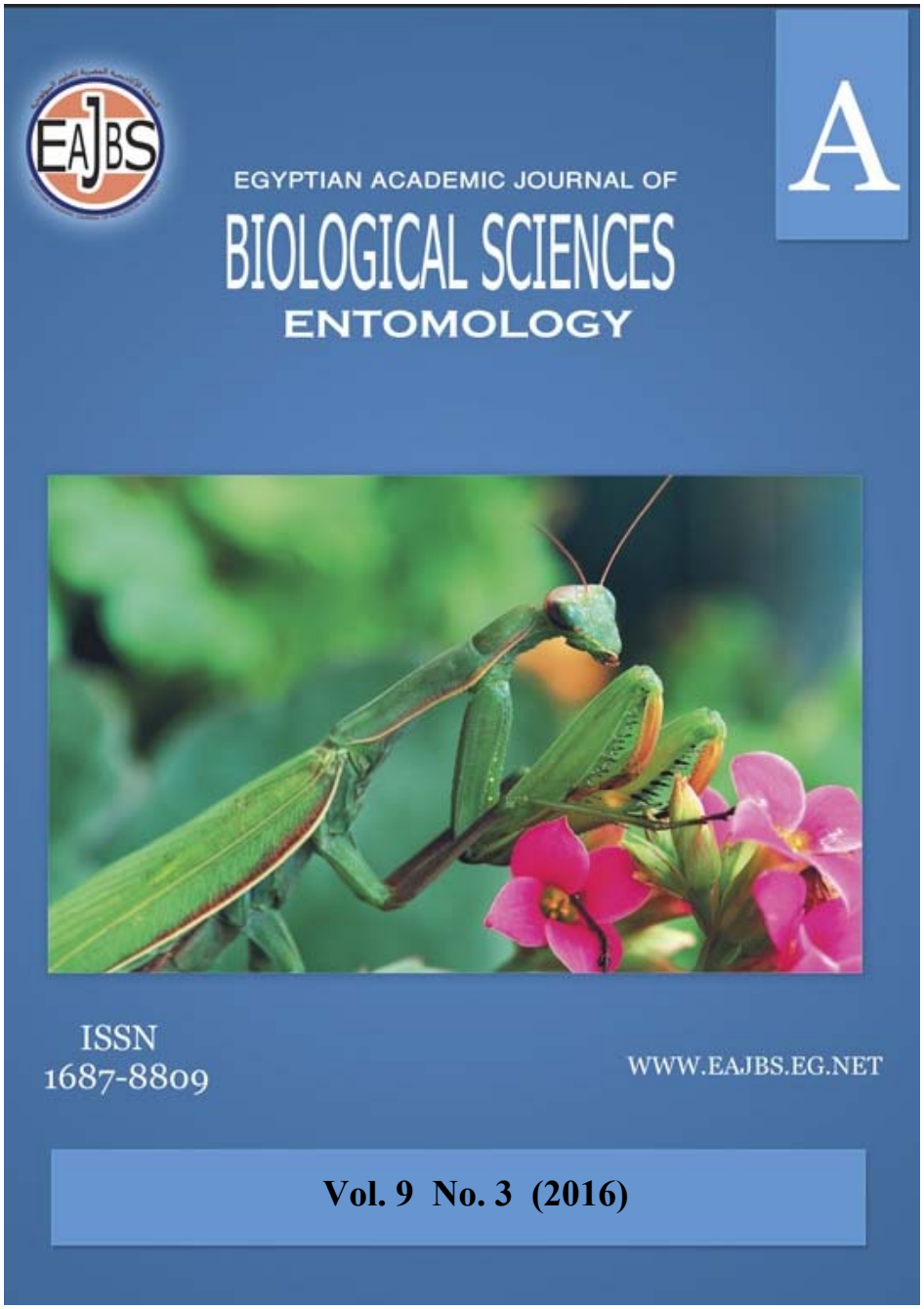

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.
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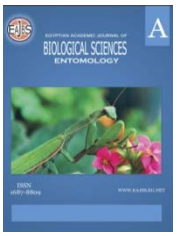
Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol.9 (3)pp. 69- 74(2016)*

Egypt. Acad. J. Biolog. Sci., 9(3): 69–74 (2016)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
www.eajbs.eg.net



Propolis Different Methods Extract, Quality Analysis, and Evaluation of its Antimicrobial Activity

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ARTICLE INFO
Article History
Received:1/7/2016
Accepted:10/8/2016

Keywords:Propolis antimicrobial activity ,

ABSTRACT

This study is conducted to test the Propolis different methods extract solution on the growth of bacteria (*Erwinia carotovora* and *Bacillus subtilis*) and rice blast fungi (*Magnaporthe oryzae).*

It tested two different concentrations (5% and 10%) for eight samples representing Ethyl alcohol extract, olive oil extract, water extract, and Petroleum ether extractor on bacteria and fungi growth.

The results of the experiments indicate that petroleum ether at concentration of 10 % on *Erwinia carotovora* recorded the highest inhibition zone 4.83 cm, while less impact was 2 c.m in *Erwinia carotovora* at concentration of 10 % by ethyl alcohol and 5 % distilled water. At the top of the fungi effect was 71.43 % by Distilled water at concentration of 5 and 10 % while less impact was 2.39 % at Ethyl alcohol at concentration of 10%.

INTRODUCTION

Propolis is a sticky natural hive bee product, composed mainly of beeswax and plant resins derived from plant exudates collected by honeybees. Due to biological and pharmacological activities, it is extensively used in folk medicine. Therefore, its chemical composition varies owing to the geographic and plant origins of these resins. In regions of temperate climate, such as Europe and North America, *Apis mellifera* bees get resins mainly from the buds of species of *Populus* (Greenaway *et al*., 1990; Garcia *et al*., 1993) and the main bioactive components are flavonoids. *Apis mellifera* line their hives and even cover dead invaders with propolis, due to its antimicrobial properties (Brumfitt *et al*., 1990). In equatorial, tropical and sub-tropical climates countries such as Brazil, the plant origin and chemical composition of propolis is far more varied. In addition to the introduced honeybee species, *A. mellifera*¸ there are hundreds of species of native Brazilian stingless bees that also mix plant resins with wax (cerumen), sometimes clay (geopropolis), use this as construction material, as defense against predators and disease (Dutra *et al*., 2008). Propolis has been used as a popular remedy for several centuries, mainly due to its antimicrobial properties present in propolis from different origins (Kujumgiev *et al*., 1999), but it is also taken orally and applied externally for a series of diseases, ranging from tumors to parasites (Marcucci 1995).

Brazil is an important supplier of propolis on the world market (Pereira *et al*., 2002).

The present study designed to investigate the antimicrobial activity of propolis oil, distill water, alcohol, and petroleum ether extract on the bacteria and fungi growth.

MATERIALS AND METHODS

Propolis is often tested by antimicrobial activity as it is common in propolis samples. The method of flow in agar (using cups, steel cylinder of paper disks) is used for screening samples against a range of microorganisms and the parameter for activity is the diameter of the inhibition zone. The inhibiting property of propolis towards bacterial and fungi growth was carried out according to Perez (1990).

Propolis Samples

Propolis samples were collected in 2014. Supplied from Qallin city (Kafr El-Sheikh Governorate). Propolis was stored at 18 ^{o}C until extraction.

Propolis Extracts

Ethyl Alcohol Extract

Fifty grams of propolis were extracted in 500 and 1000 ml of 70 % ethyl alcohol and were shacked with a shaker, during 24 h, at room temperature. After that period, the extractive solutions were filtered by filter paper.

Oil Extract

Fifty grams of propolis were extracted by heat in 500 and 1000 ml of olive oil, were shacked with a shaker during 24 h, at room temperature. After that period, the extractive solutions were filtered by filter paper.

Water Extract

Fifty grams of propolis were extracted with 500-and1000 ml of distilled water and were shaken with a shaker during 24 h, at room temperature. After ten days, the extractive solutions were filtered by filter paper.

Petroleum Ether

Fifty grams of propolis were extracted with 500 and 1000 ml of 70% petroleum ether and were shaken 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°c and mixed with Bactria (bur farm) under full sterile condition until it gave wide good growth then it was poured to sterile Petri dish then cooled to 4°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.

Propolis solution samples were tested at a concentration of 5 % &10 % and control (sterile water-alcohol-olive oil-petroleum ether) in the middle of the dish on a sterile condition for antibacterial activity. After incubation for 48 hours, digital calipers were used to measure the clear zone by taking the square of the diameter of the area of inhibition.

RESULTS AND DISCUSION

Data in Table 1 and Figure 1 showed that petroleum ether at concentration of 10% on *Erwinia carotovora* recorded the highest inhibition zone 4.83 cm. While the lowest *Erwinia carotovora* inhibition zone 2.00 cm was recorded at concentration of

10% by ethyl alcohol and distilled water 5%. Olive oil at concentration of 5% on *Bacillius subtlis* recorded the highest inhibition zone 3.67cm. While the lowest *Bacillius subtlis* inhibition zone were recorded 0.00 cm at concentration of 10% by Petroleum ether. The difference between concentrations' Mean is not significant in all treatments so the differences between treatments and control were significant. Ethyl alcohol at 5% showed inhibition effect more than 10% on both bacteria type. Distilled water 10% and Olive oil 10% inhibited more than 5% at both bacteria type. While Petroleum ether 5% inhibited more than 10% on both bacteria type.

Table 1: different propolis extraction methods effecting Bactria growth.

Treatment	Inhibition zone (cm)		
	Erwinia carotovora	*Bacillius subtlis*	Mean
Ethyl alcohol 10%	2.00	2.37	2.18 c
Ethyl alcohol 5%	2.67	3.00	2.83 abc
Control (cm)	0.00	1.00	0.50 d
Distilled water 10%	3.00	2.17	2.58 bc
Distilled water 5%	2.00	1.50	1.75 c
Control (cm)	0.00	0.00	0.00 d
Olive oil 10%	4.00	3.00	3.50 ab
Olive oil 5%	3.43	3.67	3.55 ab
Control (cm)	2.00	2.00	2.00 c
Petroleum ether 10%	4.43	3.00	3.72 a
Petroleum ether 5%	4.83	0.00	2.42 c
Control (cm)	0.00	0.00	0.00 d

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

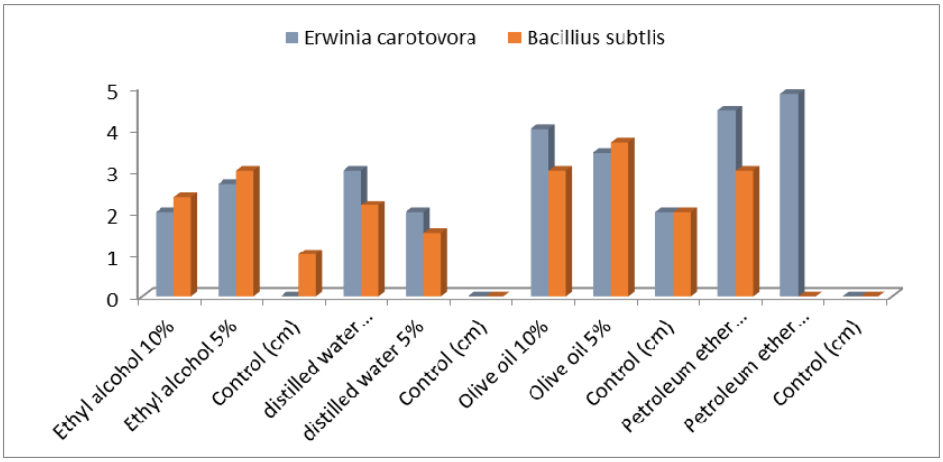

Fig.1: different propolis extraction methods effecting Bactria growth

Data in Table 2 and Figure 2 showed that Distilled water at concentration of 5 and 10% recorded the highest inhibition zone 71.43 % followed by Olive oil at concentration of 10%. While the lowest inhibition zone 2.39% was recorded on Ethyl alcohol 10% followed by Ethyl alcohol 5%. The difference between concentrations' Means is not significant in all treatments so the difference between treatments and control were significant. The difference between Ethyl alcohol and Petroleum ether in both concentrations was not significant. The difference between both of them and all treatments was significant.

Table 2: different propolis extraction methods effecting Rice blast Fungi (*Magnaborthe oryzae*) growth

Treatment	linear growth cm	Inhibition %
Ethyl alcohol 10%	6.83	2.39 c
Ethyl alcohol 5%	7.00	2.33 c
Control (cm)	7.00	0 c
Distilled water 10%	2.00	71.43 a
Distilled water 5%	2.00	71.43 a
Control (cm)	7.00	0 c
Olive oil 10%	3.33	52.39 b
Olive oil 5%	3.17	55.81 b
Control (cm)	7.00	0 c
Petroleum ether10%	7.17	4.44 c
Petroleum ether10%	7.17	4.44 c
Control (cm)	7.50	0 d

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

Bacillius subtlis was affected with Olive oil at concentration of 5% more than the other treatment. As the Petroleum ether, concentration of 10% had the lowest effect. *Magnaborthe oryzae* was affected with Distilled water at both concentration more than the other treatment. While the ethyl alcohol had the lowest effect. This may be due to solvent properties and different type of solvent. Many researchers have studied the propolis.

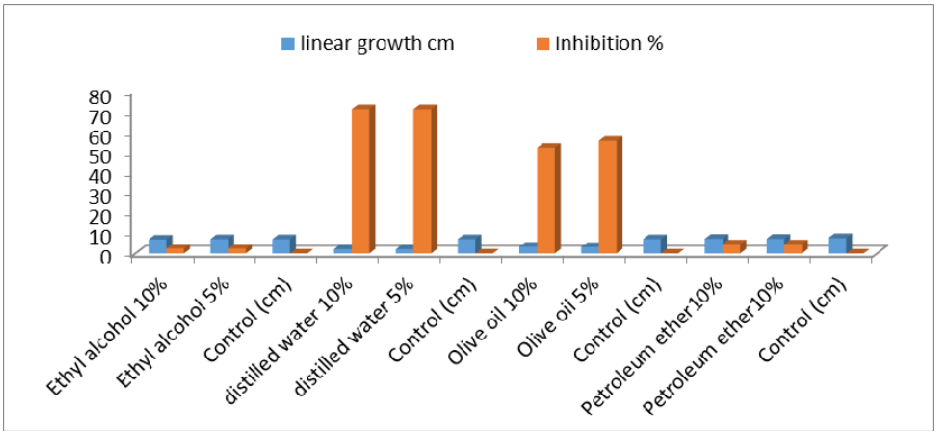

Fig. 2: different propolis extraction methods effecting Rice blast Fungi (*Magnaborthe oryzae*) growth.

From the obtain results it could be suggested that:

Erwinia carotovora was affected with petroleum ether at concentration of 10% more than the other treatment. As the ethyl alcohol had the lowest effect in extracting methods and its bacteria growth inhibition properties.

Propolis antimicrobial activities are documented against different bacteria (Sforcin *et al*., 2000), yeasts (Sforcin *et al*., 2001), virus (Gekker *et al*., 2005; Bufalo *et al*., 2009), and parasites (Freitas *et al*., 2006). In vitro, propolis may act directly on microorganisms, and in vivo it may stimulate the immune system, activating the mechanisms involved in the microorganisms killing. Propolis may also show synergistic effects with antimicrobial drugs, and its association to commercially disposable drugs is a field of interest to the development of new products by the pharmaceutical industry.

Oksuz *et al*. (2005) verified a synergistic activity between ciprofloxacin and propolis in the treatment of experimental *Staphylococcus aureus* keratitis. *Orsi et al*. (2006) reported that propolis diminished the resistance of the bacteria wall to antibiotics (amoxicillin, ampicillin, and cefalexin) and had synergistic effects with antibiotics acting on the ribosome (chloramphenicol, tetracycline, and neomycin). Nevertheless, propolis does not seem to interact with the antibiotics acting on the DNA (ciprofloxacin and norfloxacin) and folic acid (cotrimoxazole) (Orsi *et al*., 2006).

Liberio *et al*. (2009) published a review dealing with the effects of propolis on Streptococcus mutans group, suggesting the potential of propolis or its compounds as cariostatic agents and for the development of biotechnological products to control caries and other infectious diseases. Santos *et al*. (2008) evaluated the clinical efficacy of a new Brazilian propolis gel formulation in patients diagnosed with denture stomatitis, verifying the complete clinical remission of palatal edema and erythema and suggesting that this gel was efficient and could be an alternative topical choice for the treatment of denture stomatitis.

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ARABIC SUMMERY

اختبار طرق مختلفة لاستخلاص العكبر(البروبوليس) بقياس قدرت المستخلص على تثبيط نمو الميكروبات (البكتريا-الفطر)

أشرف شريف فتحي شريف

محطة البحوث الزراعية بسخا - قسم بحوث النحل- معهد بحوث وقاية النباتات

هذه الدراسة أجريت لاختبار طرق مختلفة لاستخلاص البروبوليس على نمو البكتيريا الإيروينية والباسيلس(*Bacillus subtilis & Erwinia carotovora*) والفطر المسبب لمرض اللفحة في الارز (*Magnaporthe oryzae*). تم اختبار أربع مذيبات وهي الكحول الإيثيلي، زيت الزيتون، المياه، والأثير البترولي بتركيزين لكل مذيب (5٪ ،10٪) على نمو البكتيريا والفطر.

وتشير النتائج أن الأثير البترولي في تركيز 10٪ على الإيروينية كاروتوفورا سجل أعلى منطقة تثبيط 4.83 سم بينما كان أقل تثبيط 2 سم في الإيروينية كاروتوفورا في تركيز 10٪ من الكحول الإيثيلي و 5٪ من الماء المقطر. سجل الماء المقطر في تركيز 5 و 10٪ أعلى تثبيط على الفطر كان 71.43٪ ، في حين كان أقل تثبيط 2 .39٪ في الكحول الإيثيلي 10٪.