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BIOACTIVITY OF SOME NATURAL PRODUCTS ON SOME BACTERIAL PLANT PATOGENS

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ABSTRACT

Bacterial plant diseases caused heavy yield and growth losses to agriculture crops worldwide. Among the most important bacterial plant diseases are soft rot, *Pectobacterium carotovorum* and crown gall bacteria, *Agrobacterium tumefaciens*. The application of chemicals to control both diseases affecting environment and consumers health. The antibacterial activity of three methanolic plant extracts , *Lantana camara , Rhazya stricta, Ruta chalepensis* and and Propolis as a nutral honey product on *A. tumenfaciens* and *P. carotovorum* were evaluated. The present study aims to use alternative methods to control these diseases with Propolis as a natural honey product and some plant extracts grown in Saudi Arabia. The propolis extract demonstrated strong inhibition activity against the tested plant pathogenic bacteria. The results showed that the inhibition zones were 14 mm and 13 mm at a concentration of 400 µg/ml for *P. carotovorum* and *A. tumefaciens* respectively. The results of *L. camara, Rh. stricta* and *R. chalepensis* at the same concentration were 13, 12 and 11 mm for *P. carotovorum* respectively, where the inhibition zones were 12, 11 and 11mm respectively for *A. tumefaciens*. The obtained results revealed that Propolis and these plant extracts are new promising potential sources of antibacterial compounds and good future practical applications for plant health. a promised alternatives to control bacterial diseases in field crops to avoid the use of chemical pesticides with harmful effects to environment and human health.

Keywords: Plant extracts, Propolis, *Pectobacterium carotovorum*, *Agrobacterium tumefaciens*, Antibiotics, Bioassy

INTRODUCTION

Bacterial plant pathogens and their control are a serious problem in agriculture practice. Among the most serious bacterial diseases is *Agrobacterium tumefaciens* causes crown gall disease, a plant tumor affecting wide range of plant species by transferring and integrating bacterial DNA(T-DNA) into plant genome(Holt *et al.,* 1994 and Lee, *et al.,* 2009). Also, soft rot bacteria, *Pectobacterium carotovorum* occur commonly on vegetable and agronomic crops in the field or on the commodity after harvest (Karwasra and Parasher,1990 and Wells *et al.,* 1993). Management strategies include the use of disease free seed and seedling, resistant cultivars and chemical spray(Kotan *et al.,* 2007). These strategies are not always effective when environmental conditions are optimal for diseases (Sahin and Miller, 1996). Spraying with antibiotics suggested to control bacterial diseases, have never been satisfactory and forbidden in many countries (Kotan *et al.,* 2007). The excessive use of chemical pesticides to control the bacterial plant diseases cause environmental and public health risks and disturb the ecological balance of beneficial organisms. Natural compounds of plant as a source of alternative strategy to prevent the spread of plant diseases, can be use as new pesticides (Basim and Basim,2003).

Zingiber officinale (ginger) is effective against many diseases that affect cultivated crops (Stoilova *et al.,* 2007). Khan and Khan (2007) revealed that *Rhazya stricta* has a antimicrobial effect specially fungal diseases. Organic solvent extracts showed antibacterial effect towards different strains of bacteria which causes many diseases to plants(Vaidya and Bhattarai (2009). Also, Harsha (2011) showed that ethanol extract of *Lantana camara* presented the best results for inhibition of the microbial growth. Dabur *et al.,* (2007) found that water extracts of *Acacia nilotica, Justicia zelanica, Lantana camara* and *Saraca asoca* exhibited good activity against all the bacteria tested. El Sayed et al., (2000) found that extract of *Ruta chalepensis* has antimicrobial effect. Also, *R. chalepensis* L. (Rutaceae) is widely used in folk medicine (Lee and Lee, 2011). Morover, Propolis has bactericidal and fungicidal properties and it is used as an alternative treatment for infections. The wide range of action of Propolis on various microorganisms is the result of the combined activities of flavonoids and aromatic compounds (Ivancajic *et al.,* 2010)

In the present investigation, the antibacterial activity of various plant and Propolis extracts on *P. carotovorum* and *A. tumefaciens* were evaluated.

MATERIALS AND METHODS

Bacterial isolates

Pectobacterium carotovorum and Agrobacterium tumefaciens isolates used in this study were obtained from of plant pathology dept., Faculty of Agriculture, Alexandria University, Egypt. The bacterial cultures preserved in Loria Broth and 15% glycerol solution at -80C for using in further studies. Nutrient agar (NA) medium was used for maintenance of the tested bacterial organisms. Mueller Hinton agar (MHA) was used in all bioassays applying the disc diffusion method.

Preparation of plant extracts:

Three plants which were tested are: *L. camara, Rh. stricta* and *R. chalepensis*. About 0.5 kg of each plant material was collected from local environment in Jeddah, Saudi Arabia. The plant materials were washed and air dried at laboratory before grinded into powder. Fifty gram of each plant material was dissolved in 1000ml of Methanol and left for 24h and filtered with filter paper. The solutions were evaporated using rotary evaporator until the dry extracts were obtained. The suitable solvent Dimethyl sulfoxide (DMSO) was used to obtain the concentrations of the extracts for antibacterial assays 1000, 2000, 3000 and 4000 µg/ml.

Propolis preparation

Propolis was obtained from Agriculture station, King Abduaziz University- Hada Elsham, Saudi Arabia. Then crushed well , soak in methanol for 27 hours and stirring several times a day. Propolis was filtered using filter papers whattman No. 1. The solution was saved in the refrigerator for 24 hours followed by the filtration process by using filter paper No. 50. Mehanol

was evaporated or removed from Propolis by Rotary evaporator until the Propolis was obtained in dense resin.

Antibacterial assays

The agar disc diffusion method was employed for the determination of antimicrobial activities of plant and proplis extracts by serial dilution of extracts (1000, 2000, 3000 and 4000 µg/ml) (NCCLS, 1997). A suspension of the tested bacteria (0.1ml of 10 (FU/ml) was spread on the media plates. Filter paper(5 mm in diameter) were lauded with 20 µl of the extract and placed on the inoculated plates and after staying at 4º C for 2h. The plates were incubated at 30 ºC for 24 h. The diameter of inhibition zones (IZs) were measured in millimeters. Negative control was prepared using DMSO solvent. Streptomycin (20 µg/disc) was used as a positive control with the tested bacteria. All tests were performed in duplicate.

Statistical analysis

Statistical analysis of variance were carried out using SAS, 1999. Values of p≤ 0.05 were considered as significantly different.

RESULTS AND DISCUSSION

Three plant extracts and Propolis the sticky product of honey bees were tested for antibacterial activity against plant pathogenic bacteria, *P. carotovorum* and *A. tumefaciens*. According to test results, all application showed good activity against all tested bacteria on petri plates assays (Table1 and 2). The most gained results were obtained from Propolis extract and *Lantana camara* extracts. Antibacterial activity of Propolis extract at concentration of 4000 µg/ml was highly significant with strong inhibition zone of 1.36 , 1.35 mm followed by *Lantana camara* with 1.30, 1.24 mm for pathogenic bacteria of *P. carotovorum* and *A. tumefaciens* respectively. At the same concentration of 4000 µg/ml, *Rhazya stricta and Ruta chalepensis* showed 1.19, 1.14 mm and 1.09 , 1.13 mm of inhibition zone respectively. Positive control streptomycin showed 1.073 and 0.901 mm at the same concentration. The inhibition effect of Propolis, *L. camara* and *Rh. stricta* were stronger than of positive control of streptomycin.

Many plant pathogenic bacteria have acquired resistance to pesticides (White *et al.,* 2002). Also, some pathogenic bacteria have developed resistance to some antibiotics such as ampicillin and penicillin (Rodriguez *et al.,* 1997). Thus there is an urgent need to search for alternative approaches for control of plant pathogenic microorganisms. In this study, there were significant antibacterial activity shown using Propolis and *Lantana camara* extract. It found that Propolis is rich in in many components like flavonoids , phenols and alkaloids which are effective against bacteria (Hendi, *et al.,* 2011). These extract work as potential control agents for management of soft rot and crown gall diseases.

Table 1: Antibacterial activity of the plant extracts of *L. camara, Rh. stricta, R. chalepensis* and proplis using agar disc diffusion method against *P. carotovorum*

Extracts	Concentration (µg/ml)			
	1000	2000	3000	4000
Propolis	10 IZ	11	13	14a*
Lantana camara	10	11	12	13b
Rhazya stricta	0.73	0.88	11	11c
Ruta chalepensis	0.86	0.93	10	10d
Positive control Streptomycin	0.63	0.86	0.97	10d
Negative control	0.00	0.00	0.00	0.00

***Means having the letter within the column are not significantly different at p≤=0.05**
IZ: Diameter of inhibition zone (mm) including disc diameter of 5 mm

Table 2: Antibacterial activity of the plant extracts of *L. camara, Rh. stricta, R. chalepensis* and proplis using agar disc diffusion method against *A. tumefaciens*

Extracts	Concentration (µg/ml)			
	1000	2000	3000	4000
Propolis	0.97IZ	11	12	13a*
Lantana camara	0.70	10	11	12b
Rhazya stricta	0.70	0.93	10	11c
Ruta chalepensis	0.68	0.90	10	11c
Positive control Streptomycin	0.65	0.75	0.87	0.90d
Negative control (DMSO)	0.00	0.00	0.00	0.00

***Means having the letter within the column are not significantly different at p≤=0.05**
IZ: Diameter of inhibition zone (mm) including disc diameter of 5mm

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النشاط الحيوى لبعض المنتجات الطبيعية على بعض الممرضات البكتيرية النباتية

نجيب مرعى المسعودى
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تسبب الأمراض النباتية البكتيرية التى تصيب النبات خسائر كبيرة للمحاصيل الزراعية على مستوى العالم. ومن أهم هذه الأمراض البكتيرية مرض العفن الطرى *Pectobacterium carotovorum* والتدرن التاجى *Agrobacterium tumefaciens* . وأستخدام المواد الكيماوية فى مكافحة هذه الأمراض يؤثر على البيئة وعلى صحة المستهلكين لهذه المحاصيل. ولذا اجريت هذه الدراسة لدراسة التأثير الأبادى الحيوى لبعض المنتجات الطبيعية الموجودة فى المملكة العربية السعودية مثل المستخلص الميثانولى لصمغ النحل Probolis واالمستخلص الميثانولى لكل من اللانتانا كمارا *Lantana camara* و الحرمل *Rhazya stricta* والشذاب *Ruta chalepensis* وذلك على كل من العفن الطرى والتدرن التاجى. أظهرت النتائج ان مستخلص صمغ النحل كان له تاثيرا تثبيطيا عالى المعنوية على كل من التدرن التاجى والعفن الطرى كالتالى 14 , 13 مم لكل من العفن الطرى والتدرن التاجى على الترتيب عند المعاملة بتركيز 4000 ميكروجرام/ مل. أما بالنسبة للمستخلصات النباتية فقد كان المستخلص االميثانولى لنبات اللانتانا كمارا هو الأعلى يليه كل من الحرمل والشذاب وكانت النتائج كالتالى لنفس التراكيز وهى 13 , 12 و 11 مم للعفن الطرى على الترتيب. بينما كانت النتائج للمستخلصات النباتية على التدرن التاجى هى 12, 11 و11 بالترتيب. ومن النتائج المتحصل عليها نجد ان المستخلص الميثانولى لصمغ النحل و كذلك المستخلصات النباتية المستخدمة ربما تكون واعدة كبدائل آمنة لصحة النبات.

قام بتحكيم البحث

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