ANTIFUNGAL PROPERTY OF BEE PROPOLIS COLLECTED FROM THE PROVINCE OF SOUK AHRAS (NORTHEAST ALGERIA)

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Abstract. Fungal resistance is a growing problem in protecting human and plant health. Natural bee products, including propolis, are efficient antifungal agents. As the biological activities of the propolis from the northeast regions of Algeria are poorly investigated, the present study aimed to determine the antifungal activity of ethanolic propolis (EPE) and acetone propolis (APE) extracts from two regions (sample N°1 and sample N° 2) in the province of Souk Ahras (northeast Algeria). Herein, four propolis extracts (two extracts (EPE1,APE1) of the Sample N°1 and two extracts (EPE2, APE2) of Samle N° 2) were tested for their antifungal activity against two phytopathogenic fungi species (*Botrytis cinerea* and *Fusarium oxysporum*) based on the determination of the radial growth of the fungus. Results showed that both propolis extracts (EPE and APE) generally exhibit very good antifungal effects against the studied phytopathogenic microorganisms. However, they do not have the same effect on phytopathogenic fungi. The extract of sample No. 2 (EPE2, APE2) had the best antifungal activity, which were 73% and 71%, respectively. In conclusion, the study suggests Algerian propolis collected from the province of Souk Ahras as a potent natural antifungal agent against *Botrytis cinerea* and *Fusarium oxysporum*.

Keywords: propolis; antifungal activity; *Fusarium oxysporum* f. sp *lycopersici* (FOL); *Botrytis cinerea*; Souk Ahras.

INTRODUCTION

Pathogenic fungi were known for ages to be the common causes of infectious diseases, affecting plants and the general population's health [10]. Fungi and their toxic metabolites, including mycotoxins, are among the major food contaminants occurring through the food chain during plant cultivation, food processing, and food storage and transportation [9]. The monitoring process of synthetic chemicals remains the main measure to reduce the fungal disease risks, but many of them are gradually becoming ineffective due to the resistance mutations development, and the new physiological agent. Due to the various pollutants able to induce phytotoxicity and biological imbalance in humans and plants [10, 15, 16, 32] as well as the small number of available antifungals and the increased resistance to antifungal agents, scientists paid attention to the search for alternatives [18]. Hence, pathogenic fungi are generally harmful to human health and crops, where consequently the need of using fungicide chemicals becomes of the highest priority, however, the fungal resistance limits their uses [26]. The fungal resistance may result from cellular and molecular factors, including genetic alterations and the overexpression efflux pumps and resistance proteins in the fungi [28]. As a result, two research approaches have been adopted, either through developing new molecules not yet affected by resistant microorganisms, or finding products that can through their own or synergistic effect, restore sensitivity to existing fungicides, and subsequently restore their therapeutic usefulness [10] to play a crucial role in healthcare [31]. In recent years, the pharmaceutical industries have paid great attention to safer, alternative, and effective chemotherapeutic agents [22]. The use of natural products as an alternative against fungal infections is currently being discussed [20], and hence a considerable amount of sold medicines around the world are originally derived from natural products, including plants, and bee products (bee venom and propolis) [29]. Natural products have been used in traditional medicine to treat many diseases owing to their active molecules having many therapeutic actions, as well as, being models for the synthesis of many drugs [4], and effective antimicrobial agents [24]. Propolis is an important therapeutic alternative from an economic, safety, and pharmacological point of view [4], and has been used in ancient Egyptian mummification rituals [8]. It is a mixture of beeswax and resins collected by bees from buds, leaves, and plant secretions. Propolis is not only used in the bee hive as a building material but also as a way to maintain and reduce the bacterial and fungal concentrations in the hive [2]. In the last few years, propolis has received great attention from researchers due to its effective biological activities and healing properties [23]. As reported, propolis is mainly composed of pharmacologically bioactive molecules, including flavonoids, aromatic acids, diterpenic acids, and phenolic compounds [15]. Additionally, propolis extracts also were found to inhibit the growth of food-contaminating fungi and can replace synthetic fungicides [9], and hence, it becomes a powerful antifungal agent [17]. The anticancer [29, 30] antioxidant [27], and anti-inflammatory [28] activities of propolis have been investigated, but the beneficial effects as a potent antifungal agent of Algerian propolis, in particular collected from the northeast regions have been poorly elucidated. Therefore, the present study investigated the *in vitro* antifungal activity of two different propolis extracts from the province of Souk Ahras (northeast Algeria).

MATERIALS AND METHODS

Preparation of propolis extracts

The raw propolis of Apismellifera was collected by the grid (propolis collectors) method between July and September 2019 from beehives in different sites (Bendada & Khedara) in the province of Souk Ahras (northeast Algeria) (Fig. 1). The propolis samples (sample N° 1, sample N° 2) of each sampling site was gathered, cleaned, and placed into hermetically closed plastic bags against light, and then stored in the freezer at -18°C until use. The raw propolis was beforehand, ground into a granulated powder using a food grinder, and the extracts were afterward, prepared by simple maceration. Here, 10 g of two powder samples were extracted with 100 mL of two organic solvents (ethanol 70% and acetone 70%, v/v) for 72 h and stirred in a dark bottle at room temperature. The process has been conducted a minimum of three times. The extracts were then filtrated using Whatman N^{o}.1 filter paper, and the filtrate was concentrated and evaporated under pressure in a rotary evaporator at 40°C to form four extracts two ethanolic extrats (EPE1, EPE2) and two acetone extracts (APE1, APE2). Of note, the resulting crude extracts were kept in the freezer at 4°C until analysis.

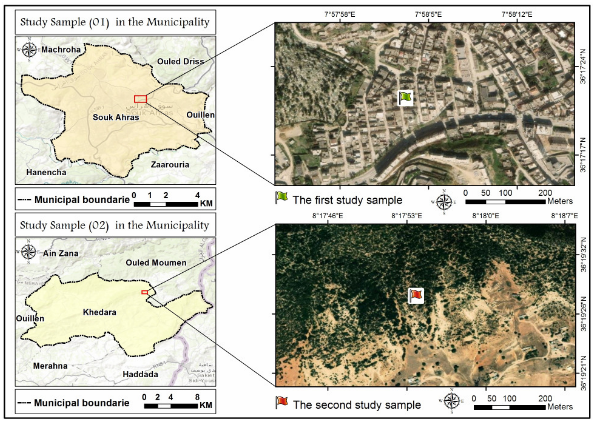

Figure 1. Propolis collection sites

Microorganisms

The performed study focused on two fungi, namely *Fusarium oxysporum* f. sp *lycopersic*i (FOL) strain 4287, *Botrytis cinerea*, obtained from the mycological collection of the Mycology Laboratory, National Research Centre for Biotechnologies Constantine, UV 03 BP E73 Constantine, Algeria. Cultures of each fungus were maintained on Potato Dextrose Agar (PDA), stored in PDA slants at 5°C for further use, and subcultured every 15 days, as described elsewhere [12].

Preparation of culture medium

The antifungal activity of the natural extracts was tested *in vitro* on PDA medium as previously described [10]. The concentration of propolis extract were based on the performed preliminary tests. In brief, a volume of 1 mL of dimethylsulfoxyde (DMSO) solution containing 100 mg of the freezed propolis was added to 100 mL autoclaved and cooled PDA medium at 60°C and then distributed in 7 cm-diameter Petri dishes. Similarly, the positive control was prepared as the sample by mixing 1 mL of DMSO with 100 mL of PDA medium, while the negative control contains the PDA medium without any other products [21, 25].

In vitro antifungal test

The two phytopathogenic agents were tested for fungal toxicity by evaluating of their mycelial growth inhibition. The measurement of the fungus radial growth on PDA medium, containing the extracts to be tested, was used as a method to determine the inhibitory activity of propolis. Before each series of experiments, the microorganisms were rejuvenated on the PDA medium, and the incubation was done at 25°C and in the dark for five days. After solidification, each petri dish was inoculated with a mycelial disk of 5 mm in diameter from the growth front of the young fungal cultures and, aseptically placed in the center of the Petri dish including PDA and propolis extracts. Four repetitions per condition were retained for each concentration. The plates were then sealed with parafilm and incubated at 25ºC in the dark. For each fungal species and each extract concentration, the mycelial growth diameters were measured at a millimetric scale at two perpendicular points 5 days after inoculation. Growth inhibition is defined as the ratio of mycelial growth at various concentrations of propolis extracts compared with that of control. Consequently, the antifungal effect was determined by measuring the percentage reduction in Diametrical Fungal Growth calculated for each isolate according to the formula (1) [3, 5, 21].

$$\textbf{(\%) I=(C-T/C)} \times \textbf{100} \ldots (1)$$

where "**I**" is the inhibition rate in%, “**C**” is the radial growth of the phytopathogenic agent in mm on PDA medium with DMSO (control), and “**T**“ is the radial growth expressed in mm of the phytopathogenic agent on PDA medium containing the tested sample.

Evaluation of antifungal activity

The studied antifungal activity of the plant extracts was evaluated according to the percentage of inhibition of the Diametrical Growth of the tillers (%):

- 30 to 40% means low activity,
- 50 to 60% means moderate activity,
- 60 to 70% means good activity,
- >70% means excellent activity [1, 10].

RESULTS

The antifungal activity of the propolis extracts was tested based on mycelial growth inhibition against two phytopathogenic fungal strains; *Fusarium oxysporum* f. sp *lycopersici* (FOL) strain 4287, *Botrytis cinerea* (Fig. 2). The results of the preliminary tests revealed that the propolis extracts have an effective antimicrobial activity. They have significant inhibitory potential on the diametrical growth of all fungi (Fig. 3). Where we found that the four (4) propolis extracts (EPE1, APE1, EPE2, APE2) at the inhibitory quantity of 100 mg of the prepared extracts exerted a varied antifungal activity on the tested fungal species. This sensitivity differs according to the strains and the extract, with different degrees. The analysis of the results of the antifungal tests carried out in vitro shows a remarkable and highly significant efficiency against the pathogens studied. The propolis extracts of sample N° 2 and , in particular, the ethanolic extract (EPE2) seems to be more effective than the other extracts against the tested fungal strains. The latter's show excellent activity against *Fusarium oxysporum* f. sp *lycopersici* with an inhibition rate of 73% and good activity against *Botrytis cinerea* with a percentage of 69%. While, the same extract of sample No 1 (EPE1) shows effective antifungal activity on the *Fusarium* strain with an inhibition percentage of 63%, and moderate activity against the *Botrytis* strain, which reaches 57%. The tested pathogens revealed a high sensitivity towards acetone extracts (APE1, APE2) but remain lower than that observed against ethanol extracts.

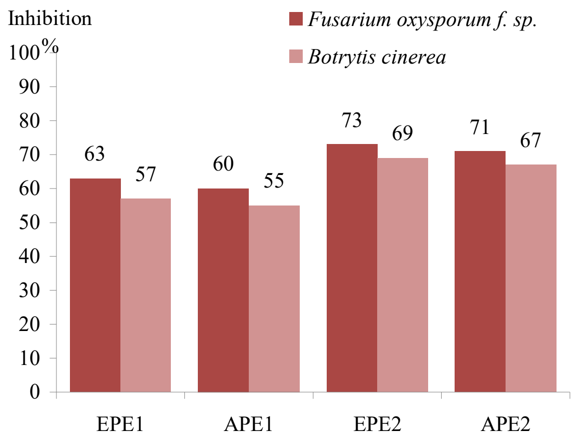

Figure 2. Activity of different extracts of propolis on the fungal strains tested (inhibition %) (EPE1: Ethanol Propolis Extract of Sample N° 1, APE: Acetone Propolis Extract of sample N° 1, EPE2: Ethanol Propolis Extract of Sample N° 2, APE2: Acetone Propolis Extract of sample N°2)

DISCUSSION

Natural products are the common source of complex and diverse chemical structures that can be principally applied in the pharmaceutical, food, cosmetics, and perfumery industries. Over the past two decades, the search for new substances with effective biological activity fighting microbial resistance has become one of the greatest scientific interests. In this context, A previous study has investigated the antimicrobial activity of natural products [10], in addition to our study investigating the fungicidal activity of Algerian propolis extracts against *Fusarium oxysporum* f.sp *lycopersici* (FOL) strain 4287 and *Botrytis cinerea*. To our knowledge, we are the first to examine the fungicidal activity of Algerian propolis extract against the selected phytopathogenic fungi; a few studies have also touched on proving the antifungal activity of propolis in general.

In this study, the propolis extracts exhibited a marked inhibitory activity against the tested species, as evidenced by the effectiveness of the extracts at the selected dose (100 mg/mL). The biological activity of the propolis extracts is likely related to the place of harvest, the geographical climate, and in particular, the richness of active compounds. Accordingly, the propolis has been reported to exert effective antifungal activity against *Fusarium oxysporum* [13] and *Botrytis cinerea* [29], while its beneficial activity can be influenced by the collections and origin conditions of propolis [6, 27, 28]. Further, the potential antifungal activity of propolis extracts has been studied, but with various testing methods. On the other hand, propolis extracts were found to have antimicrobial activity against other human pathogen fungal species such as *Candida albicans* and Dermatophytes. Also, ethanol extracts of Brazilian and Iraqi propolis were reported to have inhibitory activity against vaginal yeasts [4, 11]. Another study conducted on Romanian propolis revealed a fungicidal effect against *Candida albicans* strain ATCC 26790 isolated from tracheal secretions in hospitalized patients [27].

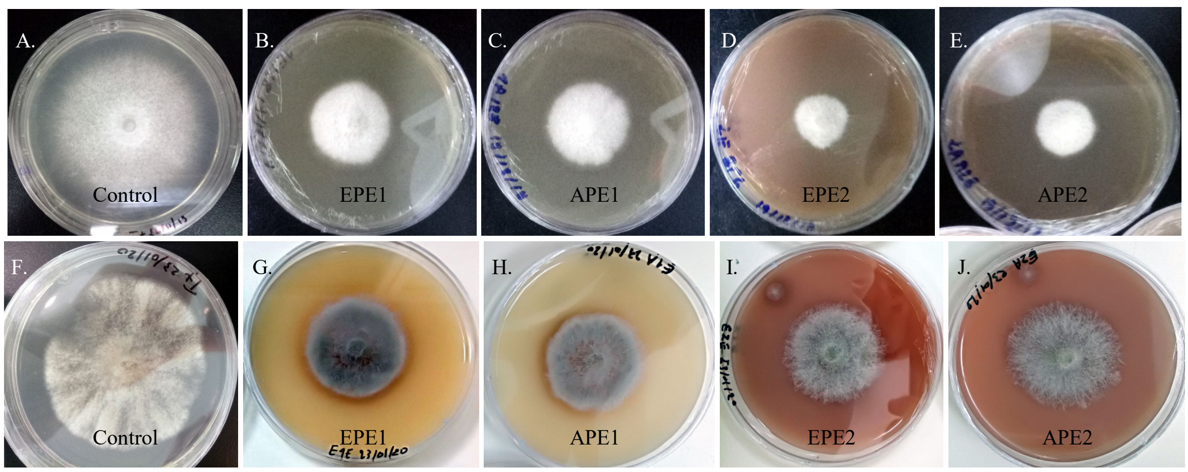

Figure 3. Inhibition rate of propolis extracts against *Fusarium oxysporum* (A-E) and *Botrytis cinerea* (F-J), after 5 days of incubation

In addition, a marked antifungal activity was reported in using Turkish propolis against yeasts isolated from positive blood cultures [15], and against yeasts isolated from mycoses [16]. Similarly, a significant antifungal activity of propolis was collected from beehives in the holy city of Karbala against dermatophytes and *Candida albicans* [2]. In parallel, the Mexican propolis showed an effect on 10 different clinical isolates of Candida [20]. It can be suggested that the inhibitory activity of propolis on the two phytopathogenic strains is significant compared to the activity of the tested plant extracts, including Moroccan medicinal plants against *Fusarium* and *Botrytis* [10], *Thymus vulgaris* L. [7] and *M. vulgare* [14] against *Fusarium* and *Botrytis* [26]. On top of that, 20 plant species were tested against the *Fusarium* strain [21], and the essential oil of *Origanum compactum* was reported to have antibacterial activity against the same strain. This high fungicidal potency may be explained by the effective role of the bioactive molecules, including phenols and flavonoids against the pathogenic fungis [31]. Another study has proved high antifungal activity against *Candida albicans* associated with high concentrations of terphenyl esters [19]. In fact, Pathogens develop difficulty in their resistance to such a mixture of components with, apparently, different mechanisms of action [7].

In conclusion, based on the results, this work allowed us to evaluate the antifungal activity of Algerian propolis, specifically Souk Ahras, against two types of phytopathogenic fungi*: Fusarium oxysporum* and *Botrytis cinerea*. Ethanolic propolis (EPE) and acetone (APE) extracts showed effective antifungal capacity against these filamentous fungi and were found to have a strong inhibitory effect against the growth of each of the fungal strains tested. Thus, the effective effect of propolis makes it a potential source of bioactive molecules with different applications. This study is considered the definitive first step in the search for an inexpensive and environmentally friendly natural remedy for fungal diseases. It may be useful for the pharmaceutical industry to produce a natural fungicide capable of controlling fungal plant pathogens and as a preservative in agricultural diets as it inhibits the growth of plant fungi and thus protects crops, as well as the development of food additives. Thus, the potential antifungal activity of the Algerian propolis would have to be supported by the phytochemistry analysis using HPLC and LCMS/MS methods, as it is not yet fully investigated, to reveal the exact amount of the propolis components, as well as to examine separately their antifungal activity against the common pathogenic fungi.

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Conflict of interest. There is no actual or potential conflict of interest concerning this article.

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