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**Efficacy of Bee-Propolis in the Control of *Colletotrichum lindemuthianum*  
(Sacc. and Magn.) Briosi and Cav. *In vitro***

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**Abstract:** In the present study *Colletotrichum lindemuthianum* was isolated from leaves of infected cowpea (variety IT 90K-76) plants showing characteristic anthracnose symptoms. The efficacy of 12 different concentrations of ethanolic extract of bee-propolis prepared by serial dilution with distilled water from crude extract and incorporated into Saboraud dextrose agar plus acetic acid medium on the mycelial growth of *Colletotrichum lindemuthianum* (*in vitro*) was studied. Infra-red analysis of the propolis extract was carried out using Ultra-violet photospectrometer. Data obtained were subjected to analysis of variance (ANOVA) and treatment means were separated using Duncan's New Multiple Range Test. Fungitoxicity of the propolis extract concentrations were expressed as percentage inhibition of mycelia growth. Mycelial growth was not significantly ( $p \leq 0.05$ ) reduced in media containing propolis extract of 1-5%. Media containing propolis extract of 6-10% inhibited mycelial growth for 2, 3, 17, 18 and 21 days respectively and also significantly ( $p \leq 0.05$ ) reduced mycelial growth. Media containing propolis extract of 20 and 30% proved fungicidal to *C. lindemuthianum*. Infra-red analysis revealed the likely presence of phenolics in the propolis extract.

**Key words:** Propolis, fungistatic, fungicidal, phenolics

## INTRODUCTION

Propolis, a bee product of plant origin, has a high antimicrobial effect which acts against a wide spectrum of fungi, bacteria and viruses, and unlike penicillin or other drugs, it is always effective because bacteria and viruses cannot build tolerances against it (<http://www.positivehealth.com/permit/Articles/Nutrition/Mizrah25.htm>). Bee propolis, starts as a sticky resinous sap which seeps from the buds and bark of certain trees. Bees gather this bee glue and carry it back to the hive where it is blended with wax flakes secreted from special glands on the underside of the bee's abdomen (<http://www.texasdrone.com/propolis.htm>). It is used in and around the hive for sterilizing their home against infection by bacteria, fungi, mould and viruses and to protect them from the elements of weather. Like bee-pollen, propolis is a bee product that can however not be clearly defined as it varies from sample to sample, which is a natural outcome of the collection process. Propolis-collecting bees will use resins from a large variety of trees and other plant species and these naturally will differ in their qualitative and quantitative chemical composition. Nevertheless, different propolis samples share considerable similarity in their physical and overall general chemical nature and this makes a description of the properties of propolis possible (<http://www.positivehealth.com/permit/Articles/Nutrition/mizrah25.htm>).

In view of the problems of toxicity, persistence and broad-spectrum action associated with the use of synthetic fungicides, coupled with the pressure to withdraw many of them from agricultural use,

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research for newer and safer and botanical fungicides has become necessary. Crude extracts from plant materials, for instance, have been found to significantly inhibit the mycelial growth of many pathogenic fungi (Shetty *et al.*, 1989; Owolade and Osikanlu, 1999). This study was therefore undertaken to evaluate the antifungal potential of propolis extract on the growth of *C. destructivum* O'Gara (Bailey *et al.*, 1996; Latunde-Dada *et al.*, 1996; Sherriff *et al.*, 1994, 1995, Sreenivasaprasad *et al.*, 1996) *in vitro*, with a view to identifying its potential, possible development and use in the control of this fungus on field crops.

## MATERIALS AND METHODS

### Sources of Materials and Isolation of Test Fungus

Propolis was obtained from bee-hive boxes belonging to the University of Agriculture, Abeokuta, Nigeria. The test pathogen, *C. lindemuthianum* (Sacc. and Magn., Briosi and Cav.) was isolated from the leaves of cowpea var: IT 90K-76 plants showing characteristic anthracnose symptoms (tan to brown, sunken and lenticular lesions) on acidified (0.3% v/v acetic acid) Saboraud dextrose agar (SDA) in sterile Pyrex Petri-dishes. The organism was identified with the aid of colony and hyphal characteristics and guides from Illustrated Genera of Imperfect Fungi (Barnette and Hunter, 1972).

In preparing propolis extract, propolis was cut into small bits (5-10 mm diameter) and put into a 250 mL Erhlenmeyer flask. Ethanol was then added, enough to submerge the propolis in the flask. The flask was covered at the mouth with foil paper which was held tightly in place with a rubber band. The mixture was shaken vigorously for 30 min and left to stand for 7 days to allow for thorough extraction. The resultant extract was filtered through Whatman No. 1 filter paper into a 250 mL Erhlenmeyer flask. The ethanol in the light-brown filtrate (supernatant) was subsequently removed by evaporation over a hot-water bath at 80°C. The beaker was then removed from the hot-water bath and left to stand overnight to allow any residual ethanol to evaporate at room temperature. The resultant extract (light-brown, slightly sticky viscous liquid) was used to prepare twelve concentrations (1-10, 20 and 30%) of the extract by adding sterile distilled water respectively to each of 1-10, 20 and 30 mL of the undiluted extract.

In preparing the extract SDA medium, 1 mL each of the concentrations of each extract prepared as described above, was transferred aseptically into sterilized petri dishes using sterilized pipette. Ten milliliter of molten acidified SDA was then added into each petri dish. Each plate was gently rotated on the table to ensure even mixture of the extract and medium. A set of petri dish containing 10 mL of acidified SDA mixed with 1ml of sterile distilled water served as control. The mixtures were subsequently allowed to cool.

Each plate in three replicates was inoculated at the center with a 5 mm diameter mycelial disc taken from the edge of a 3 day old pure culture of the test pathogen and were incubated at room temperature to examine extract effect on mycelial growth.

Fungitoxicity was expressed as percentage inhibition of mycelia growth using the formula adopted from Awuah (1989);

$$Mp = \frac{M_1 - M_2}{M_1} \times 100\%$$

Where Mp = Percentage inhibition of mycelia growth.

M<sub>1</sub> = Mycelia growth in control plate.

M<sub>2</sub> = Mycelia growth in Petri dish containing particular concentration of extract.

Radial growth measurements were taken at 24 h interval as the mean growth along two axes on two pre-drawn perpendicular lines on the reverse side of each plate.

Infra-red analysis, chemical extraction and estimation of phenolic constituents of the propolis extract were carried out at Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, using UV spectrometer.

Data obtained from the above experiment were subjected to analysis of variance (ANOVA) and means were separated using New Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

Propolis extract at concentrations 1-5% did not significantly ( $p \leq 0.05$ ) reduce mycelial growth of the test fungus. Concentrations 6-10% inhibited mycelial growth for 2, 3, 17, 18 and 21 days before commencement of growth, respectively. They also significantly reduced mycelial growth at varying degrees (Table 1). The factor(s) responsible for the fungus' eventual growth at these concentrations (6-10%) is unclear. However, it could have been due to adaptation by the test fungus and/ or degradation of the active ingredient(s) in the propolis extract. No mycelial growth was observed from plates containing the 20 and 30% concentrations at 21 days after incubation. Re-inoculation of mycelial discs from the plates containing the 20 and 30% concentrations (after 28 days of incubation) in unamended medium produced no mycelial growth after 14 days of incubation. When fresh mycelial discs of the test fungus were re-inoculated unto unamended medium following incubation for 48 h on 10, 20 and 30% propolis extract-modified SDA, respectively, there was an observed mycelial growth 1 and 2 days post re-inoculation from mycelia discs from the 10 and 20% propolis extract-modified SDA, respectively (Fig. 1). No mycelial growth was observed from mycelia discs from the

Table 1: Effect of propolis extract on mycelial growth of *C. lindemuthianum* after 3 days of growth at  $28 \pm 2^\circ\text{C}$  (*in vitro*)

| Propolis extract Conc. (%) | Days after incubation | Radial mycelial growth (cm)* | Percentage inhibition of mycelia growth | No. of days of inhibition of mycelia growth |
|----------------------------|-----------------------|------------------------------|-----------------------------------------|---------------------------------------------|
| 1                          | 3                     | 8.63 <sup>ab</sup>           | 4.17                                    | 0                                           |
| 2                          | 3                     | 8.35 <sup>b</sup>            | 3.47                                    | 0                                           |
| 3                          | 3                     | 8.55 <sup>ab</sup>           | 5.00                                    | 0                                           |
| 4                          | 3                     | 8.38 <sup>b</sup>            | 3.12                                    | 0                                           |
| 5                          | 3                     | 8.75 <sup>a</sup>            | 2.78                                    | 0                                           |
| 6                          | 5                     | 1.33 <sup>d</sup>            | 84.62                                   | 2                                           |
| 7                          | 6                     | 1.40 <sup>d</sup>            | 84.44                                   | 3                                           |
| 8                          | 20                    | 3.90 <sup>c</sup>            | 54.91                                   | 17                                          |
| 9                          | 21                    | 3.95 <sup>c</sup>            | 56.11                                   | 18                                          |
| Control                    | 3                     | 8.65 <sup>ab</sup>           |                                         |                                             |

\*Values are means of three replicates



Fig. 1: Re-inoculated mycelial discs of *C. lindemuthianum*, following transfer from 10, 20 and 30% propolis extract-modified medium unto unamended medium, showing mycelial growth (2 days post re-inoculation) from disc of the 20% propolis extract-modified medium. Mycelia from disc from the 10% propolis extract-modified medium is already 2 days into growth

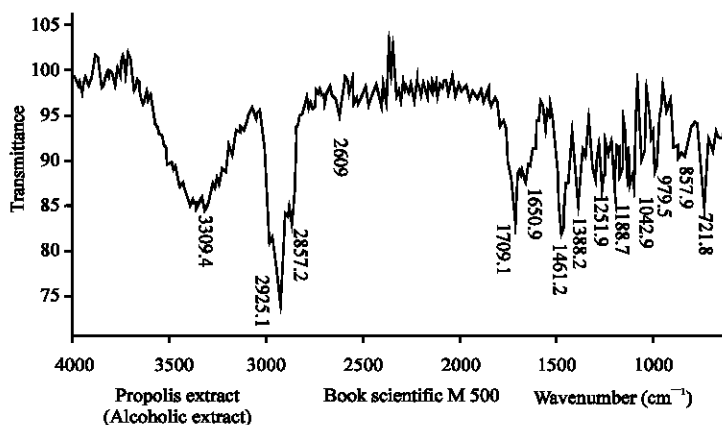


Fig. 2: Infra-red analysis of propolis extract showing possible presence of phenolics

30% propolis extract-modified SDA. This suggests that the 30% concentration of propolis extract is fungicidal on *C. lindemuthianum*. Depending on the duration of exposure of the test fungus to it, the 20% concentration can be fungistatic or fungicidal, elucidating therefore the importance of concentration and duration of exposure of *C. lindemuthianum* to the fungitoxic compound(s) in bee propolis in the antifungal result obtained.

The result of the infra-red analysis of propolis extract (Fig. 2) indicates the possible presence of phenolics. This was subsequently confirmed from the result of chemical extraction of the phenolic constituents, estimated at 0.221%. Ethanolic extracts of bee propolis have been reported to contain antifungal and antibacterial substances, mostly of phenolic origin (<http://www.apitherapy.org/determiningquality2.htm>). The phenolic constituent of bee propolis used in this study may therefore serve to explain the observed antifungal activity by the bee propolis extract *in-vitro*.

This study shows that bee-propolis extract is a potential source of fungicidal substance(s) for the control of *C. lindemuthianum*, although the efficacy of the extract is yet to be evaluated under field condition. If the efficacy is confirmed in field trials, this extract could serve as alternative to synthetic fungicides and possibly provide a new knowledge base useful toward the development of an effective and safer fungicide for control of the *C. lindemuthianum* on the field.

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