
ANTIFUNGAL ACTIVITY OF PROPOLIS EXTRACTS AGAINST POSTHARVEST FUNGI OF MANGO AND OKRA FRUITS IN MAKURDI

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Abstract

Horticultural crops such as mango and okra fruits are susceptible to several diseases due to poor postharvest handling and storage. Fungal pathogens lead to post-harvest deterioration of many fruit vegetables produced in Nigeria. This study was carried out to evaluate the antifungal activity of propolis extracted from ethanolic extracts against the postharvest fungi of okra and mango fruits during storage in Makurdi. Two varieties of mango and okra fruits each that showed signs of deterioration were collected from the stored samples for laboratory experiments between May and June, 2024. Propolis' phytochemical composition was investigated both qualitatively and quantitatively using various conventional techniques. Data collected on the radial growth were subjected to analysis of variance (ANOVA) using GENSTAT and mean was separated using F-LSD at 5% level of significance. The study demonstrated that Nigerian propolis extracts obtained from Makurdi had considerable antifungal action against okra and mango fruit fungi. Thus, propolis extract could be a promising treatment for postharvest rot disease in okra and mango. However, further research is needed to establish the efficacy of propolis extract in the management of postharvest rot disease in real-world situations, particularly during long-distance transit. The study also showed that *Aspergillus* spp. and *Penicillium* spp., *Aspergillus niger*, and *Fusarium* spp. were isolated from decaying okra and mango fruits as these fungi are capable of causing postharvest diseases in healthy okra and mango fruits. The EEP at 90%EEP could significantly inhibit the radial growth of these organisms. Accordingly, the extract can be used as an alternative to the synthetic preservatives. Therefore, the fungicides-producing companies should take measures to produce large quantities of propolis that can be used against postharvest fungi in Benue, Nigeria.

Keywords: Antifungal, Propolis, postharvest rot, Okra, Mango fruits.

1. Introduction

Horticultural crops such as mango and okra fruits are full of compounds that are good for human health, like phenolic compounds and their antioxidative properties. They are good for their health benefits and help treat diseases like tumors and heart attacks (Parr and Bolwell, 2000; Rautiainen *et al.*, 2012; Baswal *et al.*, 2020). So, to protect human health and the environment, the use of chemicals that are generally recognised as safe (GRAS) is getting more attention around the world. This is to keep these compounds that are good for your health in fresh horticultural produce (Suleria *et al.*, 2015; Baswal *et al.*, 2020).

After bananas, grapes, apples, and oranges, mangos are the world's fifth largest fruit production (Bally *et al.*, 2009; Okoth *et al.*, 2013). It's one of the most widely used fruits for food, juice, flavor, aroma, and color, as well as a popular element in novel functional foods dubbed "super fruits" (Kittiphoom, 2012). The mango fruit includes practically all of the vitamins known to man. Okra is also the most major fruit vegetable crop and a calorie source (4550 kCal/kg) (Ekunwe *et al.* 2018) that tops other vegetables (Babatunde *et al.* 2007; Ekunwe *et al.* 2018). It's crucial to preserve okra's health benefits.

Agrochemicals help producers produce, protect, and preserve fresh products (Berk, 2016). Misuse of chemical fungicides can lead to resistant fungus strains, reducing their efficacy (Hao *et al.*, 2011). Agrochemical efficacy decreases over time, and excessive use has been debated by scientists, consumers, and the media, decreasing consumer acceptance (Sharma *et al.*, 2009; Koch *et al.*, 2017). Growers must reduce postharvest losses and eliminate pesticide side effects to assure food security and safety. The scientific community is currently developing agrochemical-free human measures (Palou *et al.*, 2008).

The development of new technologies for controlling the ripening process of fruits allows us to extend their shelf life, reduce distribution losses, and supply high-quality fruit to the market.

In addition to the synthetic fungicides' usage, other techniques, such as propolis coating, are used to maintain the quality of harvested fruit during cold storage and shelf-life. Propolis is a natural resin substance (wax-like) in the form of sticky exudates that honeybees collect from various plants such as poplar, palm, pine, and leaf buds and then mix with salivary enzymatic secretions and pollen (Bankova, 2005; Sforcin, 2007; Abdulrahman *et al.*, 2012; Harfouch *et al.*, 2016). Propolis ranges in color from light yellow to green to dark brown (Salatino *et al.*, 2005). This bio-product has several uses inside the beehive, such as filling cracks, sealing spaces, maintaining temperature and humidity, and acting as an antiseptic to protect bee larvae and comb from microbial infections (Açikgoz *et al.*, 2005; Al-Hariri, 2011; Ahmed *et al.*, 2017). Propolis was described as having over 300 components, with the diversity of these compositions depending on geographical region, harvesting time and method, and solvent used in extractions (Afrouzan *et al.*, 2017). The majority of these compounds are important in the activation of many physiological processes in the plant (Rushdi *et al.*, 2014). Therefore, the aim of this study was to isolate and identify the pathogenicity of mango and okra pathogens and to evaluate the antifungal activity of propolis ethanolic extracts against the pathogens.

2. Materials and Methods

2.1 Collection of Samples

Mango and okra fruits showing symptoms of decay were collected in polythene envelopes from the stored samples and taken to the Botany Laboratory of the Benue State University

for isolation and identification of fungi pathogens as well as the antifungal efficacy of propolis extracts on the fungi pathogens.

2.2 Media Preparation

The medium used for isolation of fungi was Potato Dextrose Agar (PDA), which was prepared according to manufacturer's instruction. 40 g of powdered PDA medium was dissolved in 1 litre of sterile distilled water and sterilized by autoclaving at 121°C for 15 minutes under a pressure of 15 pounds per square inch (15lb/inch²). After cooling, it was dispensed into sterile petri dishes. Streptomycin was added to the potato dextrose medium to prevent the growth of fungi.

2.3 Isolation of fungi

Small sizes were cut from mango and okra fruits infected with rot and surface sterilized by dipping in 1% sodium hypochloride (NaOCl) solution for one minute. They were removed and rinsed in three changes of sterile distilled water then placed on sterile paper towel to dry. They were then placed on solidified Potato Dextrose Agar medium. Three replications were made for each sample. The inoculated plates were incubated at room temperature and observations were made for microbial growth. After 6-7 days of growth, sub culturing was done to obtain pure cultures of the isolates as reported by Liamngee *et al.* (2015).

2.4 Identification of fungi

The method reported by Agbatar *et al.* (2023) was used. Identification was done microscopically and macroscopically. For macroscopic characteristics, colony characteristics such as appearance, change in medium color and growth rate were observed. For microscopic characteristics, a drop of lactophenol cotton blue was placed on a grease free slide and a sterile wire loop was used to aseptically transfer a loopful of the fungal isolates onto the slide. A cover slip was placed on the slide in such a way as to prevent air bubbles. The slide was then viewed under a microscope at 10× and 40× objective. Shape of the conidia and conidiophores were taken note of. These features were matched with standards in Barnett and Hunter (1972) criteria, as described by Pérez-Álvarez *et al.* (2021).

2.5 Pathogenicity Test

Mycelia plugs of the fungal isolates from 5 days old pure cultures were used to inoculate the mango and okra fruits per pathogen. On appearance of symptoms, the tissues at the margin of the healthy and diseased parts were excised, sterilized and placed onto Potato Dextrose Agar (PDA) and incubated at room temperature for 5 - 7 days. At the end of the duration, morphological characteristics and growth patterns observed in each case were compared with the ones of the original isolates. One fruit each was used for each fungal isolate which was replicated three times and arranged in completely randomized design. Controls were mango and okra fruits inoculated with sterile PDA only. After 8-10 days of post inoculation, the area of rot was determined using the formula adopted by Ezeibekwe and Ibe (2010) as follows:

$$\text{Area of rot} = \pi dl$$

$$\text{Where } \pi = 22/7$$

$$d = \text{diameter of rot}$$

$$l = \text{depth}$$

2.6 Phytochemical Constituents Present in the Extracts of Propolis

The phytochemical composition of propolis was carried out qualitatively and quantitatively using different standard methods in order to identify the secondary metabolites present in the sample such as tannins, phenols, saponins, alkaloids, flavonoids, terpenoids, steroids and glycosides.

2.7 Data Collection and Analysis

Data collected on radial growth for both crops were subjected to Analysis of Variance (ANOVA) using GENSTAT statistical package 17.1DE and Fishers' least significant difference (F-LSD) test was used in separation of means significant at 5% level of probability.

3 RESULTS AND DISCUSSION

Phytochemicals are non-nutrient plant chemical compounds or bioactive components that are responsible for protecting the plant against microbial infections or infestations by pests (Doughari *et al.*, 2009; Yang *et al.*, 2011; Abdullahi *et al.*, 2020; Petruzzi *et al.*, 2020; Paula *et al.*, 2021). In this study, preliminary phytochemical screening of the ethanol extract propolis revealed the presence of alkaloids, flavonoids, phenolic compounds, saponins, tannins, steroids terpenoids and glycosides (Table 1). The presence of flavonoids and phenolic compounds account for the free radical scavengers that prevent oxidative cell damage. Their activities could be attributed to their ability to neutralize and quench free radicals (Pourmorad *et al.*, 2006; Omale and Okafor, 2008; Ugwu *et al.*, 2013). Nurhamizah (2016) reported the presence of terpenoids, flavonoids and essential oils in Propolis. Ipavet *et al.*, (2022) obtained similar phytochemicals working with propolis sourced from Gboko, Benue state. Also, Abudllahi *et al.* (2020) and Duru (2021) obtained similar phytochemicals with ethanol extracted propolis sourced from Jigawa, Kano and Abia states, Nigeria.

Table 1: Phytochemical Composition of Nigerian Propolis Sourced from Benue State

Parameters	Presence of Phytochemicals
Alkaloid	++++
Flavonoid	++++
Saponins	++
Tannin	++++
Glycoside	++++
Terpenoids	++++
Steroids	++++
Phenol	++++

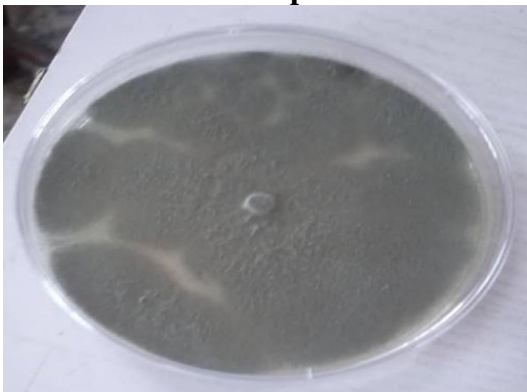
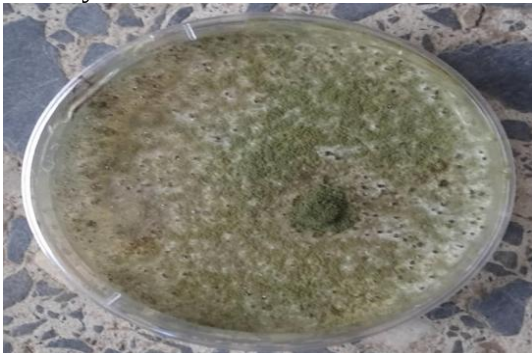
+ = indicates presence of phytochemicals; +++ = shows moderate concentration; ++++ = shows high concentration.

Three fungi namely *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*, were isolated from the decaying okra fruits in storage. For *A. fumigatus*, the colony was powderish green and velvety in nature; the Conidiophores were hyaline with cylindrical conidia (table 2). For *Aspergillus flavus*, the colony was green in color; the conidia were bluish green borne on long conidiophores. For *Aspergillus niger*, the colony was black in color and the conidia were black with transparent conidiophores. Some of these organisms have been previously reported as pathogens of okra fruit fruits by Abubakar *et al.* (2019) and Ezekiel and Sombie, (2014). They isolated and identified *Aspergillus fumigatus*, *Aspergillus flauus*, *Aspergillus niger* and 8 other fungi from decaying okra fruits in Kebbi, Nigeria. Ogunoye *et al.* (2023)

also isolated *Aspergillus niger*, *A. fumigatus*, *A. flavus* and 14 other species from rotting okra fruits in Owo, Ondo State.

The efficacy of propolis on the radial growth of fungi of okra fruits during storage revealed that ethanol extracted propolis (EEP) at higher levels reduced the radial growth of the okra fungi as compared lower level of 70% EEP and the control (figure 1). The study affirms that EEP regardless of the levels exhibited antifungal properties on the okra fungi. Urea *et al.* (2023) in their study had shown that flavonoids such as pinocembrin (flavanone) and galangin (flavonol), have antifungal activity against phytopathogens such as *Aspergillus niger*, *Penicillium notatum*, and *Fusarium spp.* In studies made with propolis from China, strong antifungal activity was demonstrated against citrus blue mold and green mold caused by *Penicillium italicum* and *P. digitatum*, respectively, with pinocembrin being identified as one of the main active components (Yang *et al.*, 2010; Yang *et al.*, 2011). The mode of action of pinocembrin is the inhibition of respiration in hyphal cells, which leads to a deficit of energy and damage to cell membranes, accelerating cell death (Peng *et al.*, 2012; Urrea *et al.*, 2023).

Table 2: Macroscopic and Microscopic views of isolated Fungi from Decaying Okra fruits in Storage

Crop	Macroscopic View	Microscopic View
Okra	 Plate 1a. Macroscopic view of <i>Aspergillus fumigatus</i> Colony colour was powderish green and velvety in nature	 Plate 1b. Microscopic view of <i>Aspergillus fumigatus</i> Characteristics: Conidiophores were hyaline with cylindrical conidia
	 Plate 2a. Macroscopic view of <i>Aspergillus flavus</i> Characteristics: Colony colour was green	 Plate 2b. Microscopic view of <i>Aspergillus flavus</i> Characteristics: Conidia were bluish green borne on long conidiophores

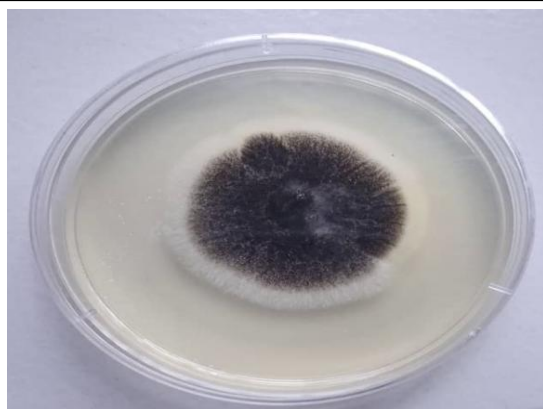


Plate 3a. Macroscopic view of *Aspergillus niger*
Characteristics: Colony colour was black

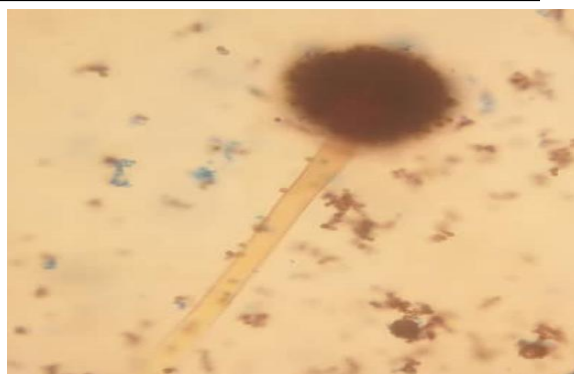


Plate 3b. Microscopic view of *Aspergillus niger*
Characteristics: Conidia were black with transparent conidiophores

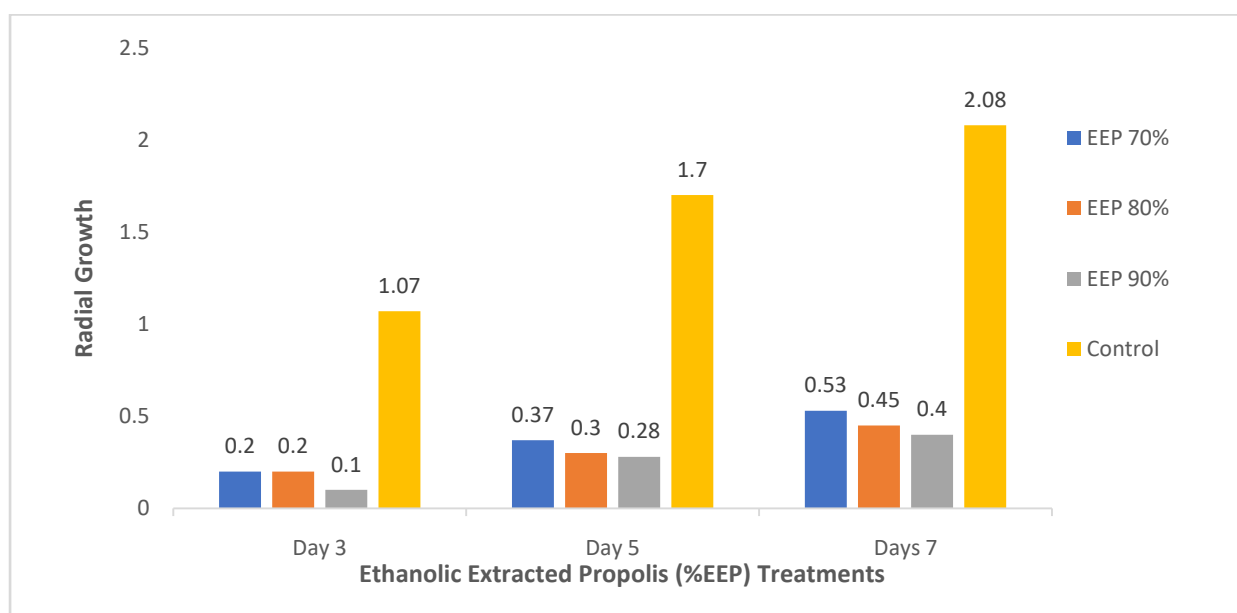


Figure 1: Effect of EEP on the Radial Growth of Fungi of Okra fruits during Storage in Makurdi

Table 3 showed that three fungi namely *Penicillium spp*, *Aspergillus niger*, and *Fusarium spp*. were isolated from the decaying mango fruits in storage. For *Penicillium spp*, the colony was green in color with white edges; the Conidiophores produced fingerlike structure. For *Aspergillus niger*, the colony was blackish in color; the conidia were black with transparent conidiophores. For *Fusarium spp.*, the colony was cottony white in color and the Conidia were oval and curved with a smooth tapered end. Some of these organisms isolated and identified have been previously reported as pathogens of mango fruits by Ishwa *et al.* (2024) and Keta *et al.* (2020). They isolated and identified *Aspergillus flavus* and *penicillium notatum* from decaying mango fruits sold in Kebbi, Nigeria. Ishwa *et al.* (2024) isolated and identified two fungi of *Aspergillus niger* and *Rhizopus stolonifer* that causes decay of mango fruits in Makurdi

The radial growth test revealed that three organisms could grow on mango fruits namely *Penicillium spp*, *Aspergillus niger*, and *Fusarium spp*. With *A. niger* having the highest occurrence during the 7 days of inoculation. *Fusarium spp* recorded the lowest occurrence and

the difference was significant. However, the radial between *Penicillium spp* and *A. niger* were at par at 5 to 7days of inoculation (table 4). Ishwa *et al.* (2024) and Bukar *et al.* (2009) reported that *A. niger* caused the highest area rot in mango and orange fruits from Makurdi and Kano metropolis. Furthermore, the ability of *A. niger* to induce the highest rot in this study could be due to its high broad-spectrum host specificity and its virulence nature on the host plant. This affirms the recent study of Ishwa *et al.* (2024) working on mango fruits. Moreso, Twuamsi *et al.* (2014) explained that due to the broad spectrum of *Aspergillus spp.*, the organism can infect a wide range of crops. Furthermore, the mango fruits provide the optimum requirements in term of nutrients for the increased proliferation of *A. niger*.

On mango fruits, the efficacy of ethanolic extracted propolis (EEP) suppressed the radial growth of fungi during storage. Table 4 also revealed that ethanol extracted propolis (EEP) at higher levels reduced the radial growth of the mango fungi as compared lower level of 70%EEP and the control. The study affirms that EEP regardless of the levels exhibited antifungal properties on the mango fruits fungi. This finding agrees the report of Urea *et al.* (2023) who had shown that propolis, have antifungal activity against phytopathogens such as *Phlyctema vagabunda*. Working on propolis from China, they reported a strong antifungal activity that was demonstrated against citrus blue mold and green mold caused by *Penicillium italicum* and *P. digitatum*.

Table 3: Macroscopic and Microscopic views of isolated Fungi from Decaying Okra fruits in Storage

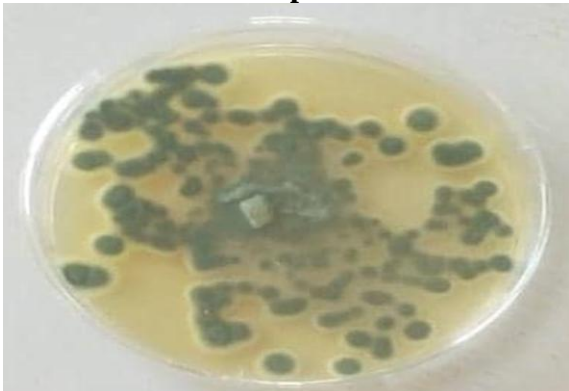
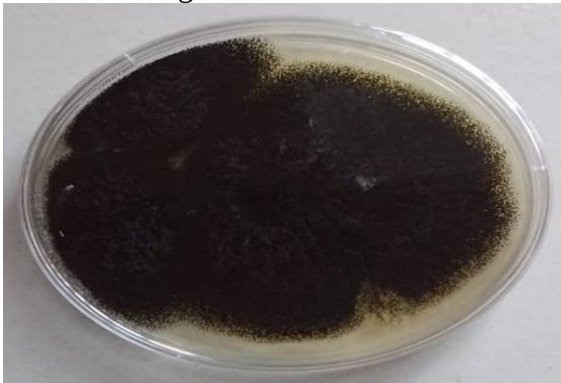
Crop	Macroscopic View	Microscopic View
		
	Plate 1a. Macroscopic view of <i>Penicillium spp.</i> Characteristics: Colony colour was green with white edges	Plate 1b. Microscopic view of <i>Penicilliumsp</i> Characteristics: Conidiophores produced fingerlike structure
Mango		
	Plate 2a. Macroscopic view of <i>Aspergillus niger</i> Characteristics: Colony colour was black	Plate 2b. Microscopic view of <i>Aspergillus niger</i> Characteristics: Conidia were black with transparent conidiophores



Plate 3a. Macroscopic view of *Fusarium* spp.

Characteristics: Colony colour was cottony white

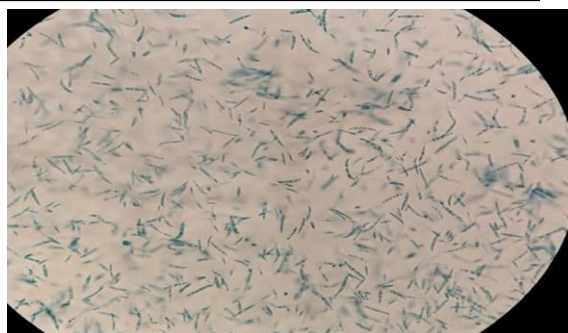


Plate 3b. Microscopic view of *Fusarium* spp.

Characteristics: Conidia were oval and curved with a smooth tapered end

Table 4: Effect of EEP on the Radial Growth of Fungi of Mango fruits during Storage in Makurdi

Treatment	Radial Growth		
	Day 3	Day 5	Days 7
Fungi A	0.68	1.00	1.33
Fungi B	0.73	1.06	1.35
Fungi C	0.55	0.83	1.20
F-LSD ($P \leq 0.05$)	0.06	0.07	0.07
EEP (%)			
70	0.67	0.93	1.24
80	0.64	0.90	1.15
90	0.43	0.61	0.83
Control	0.88	1.43	1.96
F-LSD ($P \leq 0.05$)	0.07	0.08	0.08

EEP = *Ethanollic Extracted Propolis*; Fungi A = *Penicillium* spp.; Fungi B = *Aspergillus niger*; Fungi C = *Fusarium* spp.

4. CONCLUSION

This study showed that the Nigerian propolis extracts sourced from Gboko had significant antifungal activity against okra and mango fruits fungi. Thus, the use of propolis extract could be a promising alternative for the control of postharvest rot disease in okra and mango. However, more studies are required to demonstrate the effect of propolis extract in the control of postharvest rot disease in real conditions, especially under long distance transportation. The study also showed that *Aspergillus* spp. and *Penicillium* spp., *Aspergillus niger*, and *Fusarium* spp. Were isolated from decaying okra and mango fruits as these fungi are capable of causing postharvest diseases in healthy okra and mango fruits. The EEP at 90%EEP could significantly inhibit the radial growth of these organisms. Accordingly, the extract can be used as an alternative to the synthetic preservatives. Therefore, the fungicides-producing companies should take measures to produce large quantities of propolis that can be used against postharvest fungi in Benue, Nigeria.

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DECLARATIONS

Authors have declared that no competing interests exist.

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