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Antifungal activity of some plant extracts against *Macrophomina phaseolina*

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Abstract

Macrophomina phaseolina is a prevalent pathogen that causes charcoal decay in various crops, posing a considerable challenge to sustainable agriculture. This research employed the poisoned food method to examine the in vitro antifungal properties of plant extracts against *M. phaseolina*, with the goal of identifying eco-friendly substitutes for synthetic fungicides. The ethyl acetate fraction of the *Moringa peregrina* ethanol extract exhibited the greatest antifungal activity compared to the other evaluated extracts. The EC50 value was established at 1.31 g/L, with a 77% inhibition of mycelial growth noted at a concentration of 2 g/L. The ethanol extract of *Artemisia herba-alba* markedly reduced microsclerotia production, resulting in a 44% decrease in mycelial proliferation at a concentration of 1.5 g/L. Conversely, other plant extracts demonstrated limited to negligible antifungal activity, with inhibition rates ranging from 0 to 22%. The results indicate that *M. peregrina* and *A. herba-alba* could serve as viable natural sources for the development of biodegradable, non-toxic, and effective fungicides. Additional research is needed to investigate and improve these promising natural alternatives for sustainable disease management in agriculture.

1. Introduction

Macrophomina phaseolina, a soilborne fungus, is responsible for charcoal rot disease, a significant threat to a wide range of crops. This pathogen infects over 500 plant species across 100 families [1], including economically important crops such as legumes, vegetables, fruits, and fiber plants [2][3]. In Egypt, it is considered a primary fungal pathogen affecting sunflower [4]. Symptoms in young *Phaseolus vulgaris* plants manifest as irregular black lesions. In mature plants, the disease leads to wilting and vascular blockage due to the production of black or gray microsclerotia [1].

The overuse and prolonged application of chemical fungicides pose significant risks to the environment, potentially disrupting ecosystems and resulting in the emergence of fungicide-resistant fungal strains. Research has increasingly highlighted the potential of natural, plant-derived compounds as a sustainable and safer alternative to synthetic fungicides. These botanical extracts offer a promising source of non-toxic, systemic, and readily degradable biological agents for controlling fungal pathogens [5][6]. Studies have revealed that bioactive compounds in these extracts can directly inhibit the growth of these fungal pathogens [5], or trigger defense responses in host plants, ultimately reducing disease severity [7][8].

Moringaceae family consists of 10 to 12 species belong to only one genus called *Moringa* [9-11]. These species are traditionally used to treat common diseases such as malaria, hypertension, fever, asthma, and diabetes [12][13]. Moringa species metabolites such as isothiocyanates, thiocyanates, nitriles, and thiocarbamates [13-15] have fungicidal, bactericidal, cancer chemo preventive, and hypotensive properties [16-18].

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This research evaluated the fungicidal effectiveness of several plant extracts, specifically *Moringa peregrina*, among other plant species *Melia azedarach*, *Teucrium polium*, *Artemisia herba-alba*, *Verbascum thapsus*, *Pergularia tomentosa*, *Crataegus pentagyna*, *Cnicus benedictus*, and *Atropa belladonna*, against the pathogen *Macrophomina phaseolina*.

2. Materials and Methods

2.1. Plant material preparation and extraction

The plant material was collected from Syria and Iran (Table 1) and shade-dried at a temperature of 25±3°C. The dried parts (100g each) of *Moringa peregrina*, *Melia azedarach*, *Teucrium polium*, *Artemisia herba-alba*, *Verbascum thapsus*, *Pergularia tomentosa, Crataegus pentagyna, Cnicus benedictus* and *Atropa belladonna* berries were subsequently ground using a manual grinder and extracted with 1 liter of 96% ethanol for 24 hours. Following this, the crude ethanol extract was concentrated and evaporated using a rotary evaporator at 40 °C [19]. *Moringa peregrina* ethanol extracts were partitioned into five solvent fractions (ethyl acetate, *n*-butanol, *n*-hexane, chloroform, and methanol) using a separating funnel. Each fraction was obtained by suspending the initial extract in 250 mL of water and extracting it with 250 mL of the respective solvent. Fractioned extracts were dried under vacuum using a rotary evaporator at 40 °C and all extracts were stored in the dark at 4°C until they were used in the antifungal assays.

Table 1. The plants and plant material used in the study

Plant name	Family	Used part	Country
Melia azedarach	Meliaceae	leaves	Syria
Teucrium polium	Lamiaceae	leaves	Syria
Artemisia herba-alba	Asteraceae	leaves	Syria
Verbascum thapsus	Scrophulariaceae	leaves	Iran
Moringa peregrina	Moringaceae	leaves	Iran
Atropa belladonna	Solanaceae	Berries	Iran
pergularia tomentosa	Apocynaceae	Stem	Iran
Crataegus pentagyna	Rosaceae	leaves	Iran
cnicus benedictus	Asteraceae	leaves	Iran

2.2. The tested pathogen

The study used *Macrophomina phaseolina* isolate Mph44, previously identified and characterized by Mahdizadeh *et al*. (2011) [20]. This isolate was initially recovered from melons exhibiting charcoal rot symptoms in Khorasan region, Iran. The pure cultures were obtained through hyphal tip technique [21] and maintained on sterile toothpicks at room temperature, and cultured on PDA at 4 °C for further use.

2.3. Growth Inhibition tests

To evaluate the effect of each plant extract on mycelial growth of *M. phaseolina,* Poisoned food technique was used [22]. Dimethyl sulfoxide (DMSO) solvent was employed in the preparation of stock solutions for every extract or extract fraction and then mixed with sterilized autoclaved Potato Dextrose Agar (PDA) to achieve the ultimate concentration of 1.5 g/L in each Petri plate. A mycelial disc 6mm in diameter, cut out from the periphery of 3 to 5-day old cultures of fungus, was aseptically inoculated onto the center of the Petri plate containing the plant extract. The inoculated plates were incubated at 25°C and colony diameter was measured and recorded after 3 to 4 days. Percentage of mycelial growth inhibition was then calculated using the following equation [23]:

Mycelial growth inhibition (MGI) % = [C-T/C]×100

C is the diameter of the fungal colony (mean of three replicates) in negative control (solvent without extract), and T is the diameter of the fungal colony (mean of three replicates) in the presence of plant extract.

2.4. *Moringa peregrina* ethyl acetate fraction EC_{50}

A gradual concentration (250, 500, 800, 1000, 1500, 2000, and 3000 mg/L) was used to determine the half maximal effective concentration (EC_{50}) of *Moringa peregrina* ethyl acetate fraction. EC_{50} value was calculated using Graph pad Prism software.

2.5. Statistical analysis

Mean comparison and the variance analysis were conducted on SPSS software using One-way analysis of variance (ANOVA) and Tukey HSD test at significance level $p = 0.05$.

3. Results

The ethanol extract of *Moringa peregrina* demonstrated the greatest efficacy among all evaluated extracts. It exhibited a notable suppression of mycelial growth, achieving 64±2% at a concentration of 1.5 g/L. The ethanol extract of *Artemisia herba-alba* completely inhibited microsclerotia production, displaying significant activity with an inhibition ratio of 44±1.1% at a concentration of 1.5 g/L. Despite having a significant influence on microsclerotia production, the ethanolic extract of *Teucrium polium* and *Pergularia tomentosa* demonstrated weak fungicidal activity, with growth inhibition rates of 17.5±2.5% and 22±1% at a concentration of 1.5 g/L, respectively. Furthermore, *Melia azedarach* extract demonstrated a minimal inhibitory effect of approximately 3±2%. The ethanolic extracts of *Atropa belladonna*, *Verbascum thapsus*, *Crataegus pentagyna*, and *Cnicus benedictus* demonstrated no significant impact on the mycelial growth of the pathogen (Fig. 1).

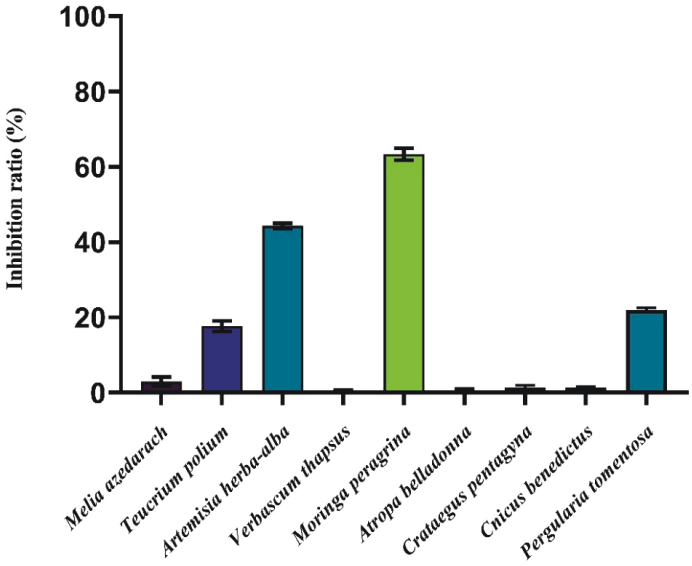

Figure 1. Growth inhibition ratio (%) for the ethanol extracts of the studied plants (1.5 g/L) against M. phaseolina

The ethanol extract derived from *Moringa peregrina* was divided into five separate fractions. The antifungal efficacy of these fractions, specifically their effect on the mycelial proliferation of *M. phaseolina*, was thoroughly investigated. The ethyl acetate fraction demonstrated a significant effect on the inhibition of mycelial growth (Fig. 2). This fraction markedly suppressed mycelial growth, attaining 77% inhibition at 2 g/L and demonstrating an EC_{50} value of 1.31 g/L (Fig. 3). The fractions derived from chloroform and methanol exhibited a moderate level of activity, inhibiting mycelial growth by approximately 32% and 25.6% at a concentration of 1.5 g/L, respectively. In contrast, the fractions derived from butanol and n-hexane demonstrated minimal efficacy. All fractions, except for the ethyl acetate fraction, demonstrated no effect on the reproduction of microsclerotia of *M. phaseolina*, while the ethyl acetate fraction exhibited a moderate degree of activity.

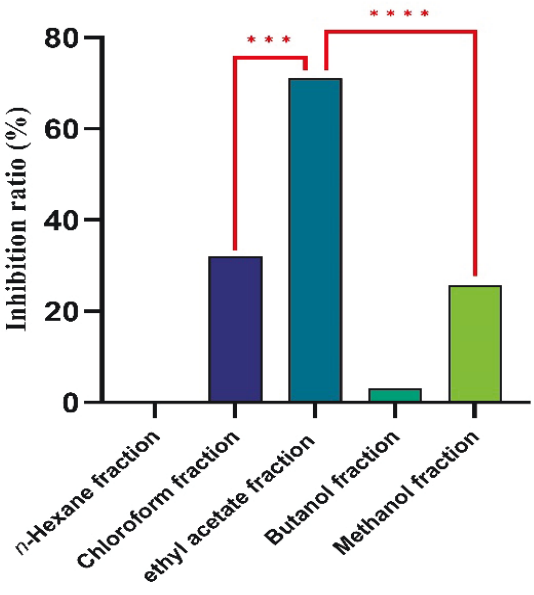

Figure 2. Growth inhibition ratio (%) for the ethanol extracts fractions of *Moringa peragrina* (1.5 g/L). The (*) indicates a statistically significant difference between fractions.

4. Discussion

This study evaluated the antifungal properties of a specific botanical species against *M. phaseolina*. The ethyl acetate fraction of *M. peragina* demonstrated significant effectiveness in inhibiting the mycelial growth of the specified pathogenic organism. *M. peregrina* extract was previously reported to exhibit antibacterial efficacy against Staphylococcus aureus, *S. epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Candida albicans*, *C. tropicalis*, and *C. glabrata* [24]. Numerous compounds derived from *M. peregrine* demonstrate significant biological activities, encompassing antimicrobial, antiviral, anticancer, and antioxidant properties [25]. Multiple previous studies have recorded the existence of antimicrobial properties in various parts of Moringa, including its roots, flowers, bark, seeds, and stem [26][27].

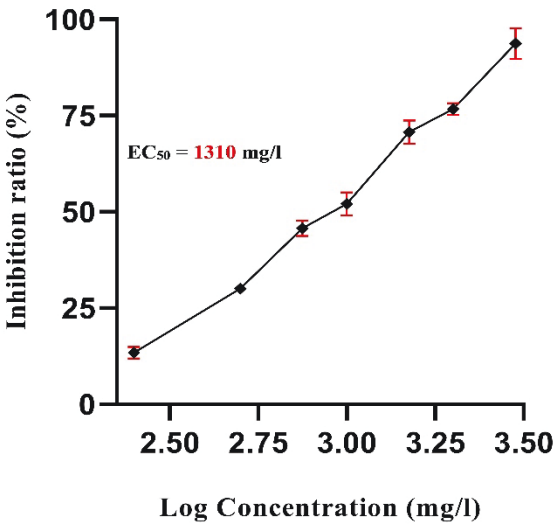

Figure 3. Growth inhibition ratio dose-response curve and the half maximal effective concentration (EC_{50}) for the ethyl acetate fraction obtained from *Moringa peragrina.*

Artemisia herba-alba extract has shown a significant impact on the production of microsclerotia, while the effects of other plant extracts were negligible. This discovery holds considerable significance in pathogen management, given the essential role of microsclerotia in the pathogen's life cycle. Prior literature has thoroughly recorded the significance of natural products in inhibiting microsclerotia production [28], likely due to the suppression of melanin synthesis within the fungus. It is plausible to suggest that the extract from *Artemisia herba-alba* can disrupt the melanin biosynthesis pathway in the fungus, thereby inhibiting microsclerotia formation.

The currently presented findings indicate that *M. peragina* and *Artemisia herba-alba* extracts may serve as environmentally friendly, plant-based pesticides against *M. phaseolina*, providing a viable alternative to traditional fungicides. Future research on botanical extracts may reveal novel natural compounds with exceptional antifungal properties, facilitating sustainable disease management in agriculture.

5. Conclusion

This study examined the antifungal properties of various plant extracts against *M. phaseolina*. The ethyl acetate fraction of *M. peregrina* extract demonstrated a remarkable ability to inhibit the growth of the pathogen. The findings indicate that the ethanolic extracts of both *M. peragina* and *Artemisia herba-alba* has potential as environmentally sustainable, plant-derived insecticides against *M. phaseolina*. This might offer a feasible substitute for the traditional fungicides presently employed. Nonetheless, further investigation is necessary to assess the feasibility of employing these extracts under field conditions.

Conflict of interest statement

The authors declared no conflict of interest.

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Data availability statement

The authors declared that all related data are included in the article.

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