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VALORIZATION OF ESSENTIAL OIL EXTRACTION BY-PRODUCTS FROM TWO MEDICINAL AND AROMATIC PLANTS FOR ANTIFUNGAL ACTIVITY

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ABSTRACT

Traditional chemical fungicides remain widely used for disease control; however, concerns about environmental contamination, residue persistence, and pathogen resistance have intensified the search for sustainable alternatives. Plant-derived extracts, particularly from aromatic species, represent promising biopesticides due to their richness in bioactive metabolites, diverse antifungal mechanisms, and favorable ecological profiles. This study investigates the antifungal potential of aqueous plant extracts derived from essential oil extraction waste of *Artemisia sp*. and *Cymbopogon citratus* against *Pythium Ultitum*. After hydro-distillation of the powdered plant materials to extract essential oils, the residual biomass was reused to prepare aqueous extracts via maceration. Five concentrations (1%, 2%, 5%, 10%, and 20%) of each extract were evaluated using the poisoned food technique. Mycelial growth inhibition was significantly affected by extract concentration ($P < 0.001$). The highest inhibition was observed at 20%, reaching 100% for *C. citratus* and 76.42% for *Artemisia sp.* extracts. In contrast, the lowest inhibition was recorded at 1%, with values of 34.96 % for *C. citratus* and 28.21% for *Artemisia sp.*. On the other hand, the determination of flavonoids and total phenol contents was performed using the spectrophotometric methods with aluminum chloride ($AlCl_3$) and the Folin-Ciocalteu, respectively. Phytochemical analyses revealed that *C. citratus* extract contained higher flavonoid levels (10.59µg QE/mg dry extract) compared to

Artemisia (6.97µg QE/mg). However, total phenolic content was greater in Artemisia (92.28µg GAE/mg) than in *C. citratus* (67.50µg GAE/mg). These findings suggest that post-distillation plant residues retain significant antifungal activity and bioactive compound content, supporting their valorization as potential active ingredients in biopesticide formulations for the management of *P. ultitum*.

Keywords: *Antifungal activity, Artemisia sp, C. citratus,* plant extracts, *P. ultitum.*

INTRODUCTION

Pythium species are oomycetes that cause devastating diseases such as damping-off and root rot across a wide variety of crops, particularly under wet and poorly drained conditions. Their fast-spreading zoospores pose significant management challenges in irrigated and protected agriculture (Govers, 2024; Karunasinghe et al. 2025; Adhikari et al. 2024). Traditional chemical fungicides remain widely used for control; however, rising concerns over environmental contamination, residue accumulation, and the development of resistance have prompted exploration of eco-friendlier alternatives (Syed et al. 2020; Delai et al. 2024; Adhikari et al. 2024). In this context, aromatic plants and essential oils have garnered attention as promising biopesticides. These extracts are enriched in bioactive secondary metabolites such as terpenes, phenolics, and aldehydes with multifaceted modes of action against pathogens, making them attractive candidates for integration into sustainable disease management strategies. Moreover, their biodegradability and lower toxicity to non-target organisms further enhance their appeal in modern agroecological systems (Khadfy et al. 2024; Kumari et al. 2024; Qessaoui et al. 2023, 2024; Shelepova et al. 2024).

Various Artemisia species, including *A. annua* and *A. herba-alba*, have demonstrated significant antifungal efficacy. For instance, methanolic extracts from *A. annua* leaves have inhibited the growth of pathogens such as *Fusarium oxysporum*, *F. solani*, and *Sclerotinia sclerotiorum*, with minimum inhibitory concentrations comparable to conventional fungicides (Ma et al. 2019; Zhang et al. 2016). Meanwhile, research on *Artemisia herba-alba*, a Moroccan endemic, confirms that its essential oil and extracts possess both antifungal and antioxidant activities against post-harvest pathogens, including *Botrytis cinerea* and *Penicillium spp* (EL Hajli et al. 2024). Likewise, *C. citratus* (lemongrass or citronella) essential oil, rich in citral, geranial, and neral, shows strong antifungal activity (Khasanah et al. 2025; Kiełtyka-Dadasiewicz et al. 2024; Rahhal et al. 2024). It significantly inhibits *Pythium ultimum* at low concentrations IC_{50} values indicating potent efficacy against oomycetes (Myriama et al. 2018). Additionally, *C. citratus* essential oil demonstrates inhibitory effects against a broad range of fungal species (Irkin et al. 2009). The present study aims to investigate the antifungal potential of aqueous plant extracts derived from essential oil extraction waste of *Artemisia sp.* and *C. citratus* against *P. ultitum.*

MATERIAL AND METHODS

Plant extracts preparation

The preparation of plant extracts for both *Artemisia sp*. and *C. citratus* was conducted in the laboratory of the Plant Protection Regional Center of the National Institute of Agronomic Research, Agadir, Morocco. After hydro-distillation of the powdered plant materials to extract essential oils, the residual biomass was reused to prepare aqueous extracts via maceration. The extraction by cold water maceration was carried out using the protocol of Romani et al., (2006). Powder (20 g) was macerated at room temperature for 2.5 h with 200 mL of distilled water and stirred by a magnetic agitator. The mixture was filtered using a muslin cloth. The filtrate was centrifuged at 4000 rpm for 20 min. The supernatant was concentrated using a Martin Christ Gefriertrocknungsanlagen GmbH freez-dryer. The plant extracts were then stored in a refrigerator at 4 °C.

Antifungal activity tests

The antifungal activity of the plant extracts was evaluated using the inhibition test for mycelial growth. The appropriate volumes of each aqueous extract were added to sterile liquid PDA culture medium to obtain final concentrations (C1 = 1%, C = 22%, C3 = 5%, C4 = 10% and C5 = 20%). After solidification in Petri dishes, the plates were inoculated with the fungus using a 5 mm diameter agar disk taken from one-week-old cultures (Kasmi et al., 2017). The agar plates were then incubated at 25 °C for six days. Radial growth was determined by measuring the diameters along two perpendicular axes (Askarne et al., 2012). Antifungal activity was expressed as a percentage of mycelium growth inhibition, calculated using the following formula:

$$\text{Percentage of mycelial growth inhibition} = \frac{\text{Control diamete} - \text{Plant extract diameter}}{\text{Control diameter}} \times 100$$

Determination of total flavonoids

The determination of total flavonoid content in the plant extracts was performed using the spectrophotometric method with aluminum chloride ($AlCl_3$) cited by Djeridane et al., (2006). One milliliter of each extract and standard (quercetin) was added to 1 mL of a 2% $AlCl_3$. After 10 minutes of incubation, the absorbance was measured at 430 nm using a spectrophotometer, visible, ONDA v-10 plus (CHINA). A calibration curve was performed in parallel under the same operating conditions using quercetin at different concentrations (0 - 100 µg/mL). The results were expressed as µg quercetin equivalent/mg dry extract (µg QE/mg DE).

Determination of total soluble phenols

The total soluble phenols content in the plant extracts was determined using the Folin-Ciocalteu spectrophotometric method. In a test tube, 0.5 mL of each extract was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted 1/10. After 4 minutes, 2 mL of sodium bicarbonate (7.5%) was added to the mixture. After 1 hour of incubation at room temperature, the absorbance was measured at 765 nm using a spectrophotometer, visible, ONDA v-10 plus (CHINA). A calibration curve was performed in parallel under the same operating conditions using gallic acid at

different concentrations (0 - 100 µg/mL). Results were expressed as µg gallic acid equivalents/mg dry extract (µg GAE/mg DE) (Pavlović et al., 2021).

Statistical analysis

The data obtained were analyzed by ANOVA using IBM SPSS Statistics 26. The comparison of means was performed using the Tukey test ($p<0.05$).

RESULTS AND DISCUSSION

Antifungal activity of plant extracts

The results of the in vitro antifungal activity of both plant extracts against *P. ultitum* are shown in Figure 1. The mycelial growth inhibition was significantly affected by extract concentration ($P < 0.001$). The highest inhibition was observed at 20%, reaching 100% for *C. citratus* and 76.42% for Artemisia extracts. In contrast, the lowest inhibition was recorded at 1%, with values of 34.96 % for *C. citratus* and 28.21% for Artemisia. Similarly, Aourach et al. (2021) tested the effect of aqueous extracts of lemongrass (*C. citratus*) against the growth of *Fusarium oxysporum* f. sp. lentis. The results obtained showed that the extract exhibited the highest antifungal activity. A concentration of 0.05 mg/mL inhibited 67% of conidial germination and 77% of sporulation, while 5 mg/mL restrained 70% of mycelial growth and 20 mg/mL stopped the pathogen almost completely. On the other hand, Parveen et al. (2014) showed that *A. absinthium* at the highest concentration, standard solutions (100%), was found to be the most effective against *A. alternata* and caused the highest inhibition in the mycelial growth (79.75%).

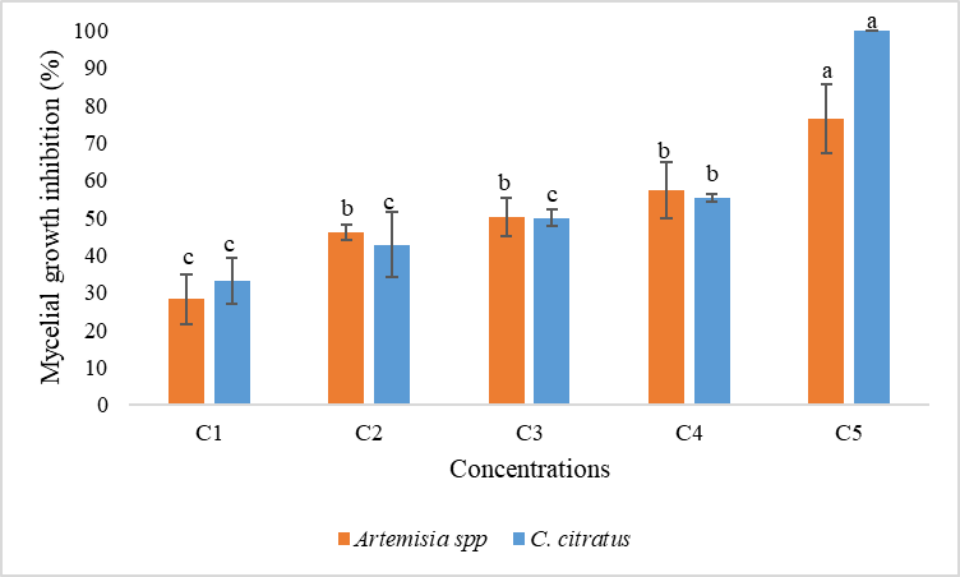

Figure 1. In vitro antifungal activity of *Artemisia sp* and *C. citratus* extracts against mycelial growth of *Pythium spp*. By series, values with the same letters are not significantly different according to the Tukey test at 5%.

Flavonoids and total phenols determination

The results obtained for the analysis of flavonoid and total phenol content are presented in Table 1. The findings demonstrated a significant difference between the plant extracts (P-value<0.001). The phytochemical analyses revealed that *C. citratus* extract contained higher flavonoid levels (10.59 µg QE/mg dry extract) compared to Artemisia (6.97 µg QE/mg). However, a higher total phenolic content was observed in Artemisia (92.28 µg GAE/mg) compared to *C. citratus* (67.50 µg GAE/mg). In comparison with other studies, Tazi et al. (2024) demonstrated that the value of total phenolic and flavonoid contents obtained in aqueous extracts of *C. citratus* were 4.60 ± 0.29 mg/g and 0.57 ± 0.07 mg QE/g, respectively, which is lower than the findings of the present study. Conversely, Elazzouzi et al. (2022) demonstrated that the total phenols and flavonoid contents exhibit significant variation between the various extracts of *Artemisia ifranensis*. This observation is corroborated by the low values recorded in the residual aqueous fraction. Indeed, the results indicate that all *A. ifranensis* extracts are richer in total phenols (21,15; 35,81; 33,33; 11,93 mg GAE/g Extract, respectively) than in flavonoids (10,76; 31,85; 17,15; 2,61 mg QE/g Extract). Research has indicated that the bioactive compounds vary depending on the specific part of the plant used, the extraction method employed, the type of solvent used, and the geographical location (Sepahpour et al. 2018; Unuigbe et al. 2019; Godwin et al. 2014).

Table 1. Flavonoids and total phenolic contents in plant extracts

Plant extracts	*Flavonoids (µg QE/mg DE)*	*Polyphenols (µg GAE/mg DE)*
Artemisia sp.	6.97 ± 0.05^{b}	92.28 ± 2.31^{a}
C. citratus	10.59 ± 0.29^{a}	67.50 ± 5.55^{b}
P-value	<0.001	<0.001

By column, values with the same letters are not significantly different according to the Tukey test at 5%.

CONCLUSION

In this study, we investigated the antifungal potential of aqueous plant extracts derived from essential oil extraction waste of *Artemisia sp*. and *C. citratus* against *P. ultitum.* The results obtained suggest that post-distillation plant residues retain significant antifungal activity and bioactive compound content, supporting their valorization as potential active ingredients in biopesticide formulations and as sustainable, plant-based alternatives for managing Pythium infections, especially in systems seeking lower chemical input and enhanced environmental stewardship.

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