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Essential Oils against *Aspergillus Parasiticus*: Sustainable Farming for Health

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ABSTRACT

Medicinal plants from various botanical families were collected. Their essential oils were extracted from the leaves using water distillation. Three different concentrations of the essential oil were prepared (5, 10, 15 µl/Petri dish). The inhibitory efficacy of the essential oils against the Aspergillus parasiticus fungus was evaluated in the laboratory, and the results were statistically significant (P <0.05) in inhibiting fungal growth compared to the control.

The percentage inhibition of colony diameter growth rates exhibited an inverse relationship with the applied doses of the respective essential oils. For Artemisia herba-alba Asso oil, the inhibition percentages were (77.47±0.01, 62.82±0.03, 51.1±0.04) % at concentrations of (15, 10, 5) µl/Petri dish, respectively. Similarly, for Rosmarinus officinalis oil, the inhibition percentages were (74.08±0.06, 61.44±0.04, 26.92±0.07) % at the corresponding concentrations. Hence, the essential oils can potentially be utilized as an effective alternative without side effects to combat this fungal disease in the near future.

Keywords: *Artemesia herba alba Asso, Rosmarunis officinalis, Aspergillus parasiticus, Essential Oil.*

1- INTRODUCTION

Many fungal species prevalent worldwide are significant destroyers of food materials, both during their vegetative life cycle and storage, rendering them unfit for human consumption by delaying their nutritional value and the fungi's ability to produce mycotoxins (Scherm et al.,2013). The latter are defined as compounds with diverse chemical structures and low molecular masses, characterized by a wide range of genotoxic, carcinogenic, mutagenic, teratogenic, or estrogenic effects, causing acute and chronic diseases in all elements of the food chain from the primary producer to the ultimate consumer (Zain, 2011; Assunção et al.,2016).

The Increasing number of resistant and pathogenic fungi, coupled with the failure of synthetic fungicides to control or eradicate them, has necessitated the search for new anti-cancer, anti-resistance, and alternative treatments (Mesnage et al.,2014; Nicolopoulou-Stamati et al.,2016). Furthermore, their residues surpass humans as the most interactive entities with them, thus exerting negative effects on both living and non-living components of the environment (da Cruz et al.,2013). All these points have paved the way for medicinal plants with their extracts to emerge as effective alternatives, as they have been classified as recognized as safe by the Food and Drug Administration (FDA) (Kedia et al.,2014).

Essential oils with their turbocharged and phenolic compounds such as ogenol, thymol and carvacrol attack lipophilic mycotoxin cells with low partial weight leading to structural and functional damage by disrupting membrane permeability and cell osmosis balance. This may also prevent certain enzymes' action, including mitochondrial enzymes that go into the process of ATP synthesis such as lactate and malate enzymes as well as inhibiting H + activity –ATPase. In view of that, stopping these processes leads to acidification and cells death (Ahmad et al. 2013, Kalagatur et al., 2015; Prakash et al., 2015; Grata, 2016).

The Intended goal of this study is to explore the potential use of essential oils as a means to combat the fungus Aspergillus parasiticus. This objective reflects a pursuit towards developing a sustainable effort aimed at achieving healthy agriculture and utilizing renewable natural resources. By utilizing essential oils as a natural fungicide, this effort aims to reduce reliance on chemical pesticides, which may be detrimental to the environment and public health. Consequently, this endeavor contributes to enhancing healthy and sustainable agriculture, along with increased utilization of renewable natural resources.

2- MATERIAL AND METHODS

2-1 Plant Material: The inhibitory efficacy level of the essential oil of *Artemisia herba alba* Asso and the essential oil of *Rosmarunis officinalis* was tested. The aerial parts were collected from the Tassala region, Mila Province, Algeria, at the end of March 2022, with geographic coordinates 36°34'31''N 5°59'31''E (Google Earth).

2-2 Fungal Isolates: The fungal isolate Aspergillus parasiticus was obtained from the Laboratory of Microbial Systems Biology (LBSM) at the Higher School of Teachers, Kouba, Algeria.

Methods:

Extraction of Oil and Yield Determination:

The essential oil was extracted using the water distillation method, utilizing a Dean Starck-type distillation apparatus. The yield was determined by processing 100 g of the plant material in a 500 ml capacity flask. The sample was stored in a sealed vial at 4 degrees Celsius away from light.

2-3 Effect of oil on fungus growth (direct contact method):

Potato Dextrose Agar (PDA) was aseptically dispensed into 9 cm diameter Petri dishes, and a specified volume of pure essential oil was added at designated concentrations (5, 10, 15 µl/Petri dish).

The contents were thoroughly mixed by swirling the plates in all directions to ensure complete coverage of the agar surface and homogeneity of the essential oil-agar mixture. The disk diffusion method was employed, wherein a 5 mm diameter fungal disk was aseptically excised from the active growth zone of the culture and inoculated at the center of each Petri dish.

The Inoculated plates were incubated in darkness at 30°C for 7 days, with a control plate containing PDA without essential oil. Subsequently, the following calculation was performed (Kordali et al., 2003).:

Percentage inhibition of radial growth = [(R1 – R2)/R1] x 100

Where R1 is the radial growth of the fungus in the control plate, and R2 is the radial growth of the fungus in the treated plate.

This methodology allows for the evaluation of the antifungal efficacy of essential oils at varying concentrations against the test fungus by quantifying the inhibition of radial mycelial growth compared to an untreated control.

3-Results

3-1 Oil Yield

Essential oil yield in Table 1

Table 1: Extraction yield and color of essential oils from the plant of wormwood and rosemary.

Gendre	Odor	Color	Famille	Yield (%)
Artemisia herba alba Asso	Camphorous scent	Pale yellow	*Astéracées*	1.5
Rosmarunis officinalis	Camphorous scent	Transparent	*Lamiacées*	0.9

The water distillation of 100 g of dried material from the white sage plant *"Artemisia herba alba"* yielded a pale-yellow oily sample with a yield of 1.5% (Table.1). A previous study reported a yield of 0.95% for 100 grams of white sage plant (Bezza et *al*.,2010), while another study by Zaim et *al*., (2012) reported a yield of 1.2% in their investigation on the effect of *Artemisia herba-*

alba essential oils on the survival of adult grasshoppers (Euchorthippus albolineatus). Additionally, Bouchikhi-Tani et *al*. (2018) noted a yield for white sage herb. The variation in yields can be attributed to the vegetative cycle during which the white sage plant was harvested. Sample of essential oil from the "*Rosmarinus officinalis*" plant with colorless oil and a yield of (0.9%) (Table.1). Kiran et *al*., (2015) reported a range of essential oil yield extracted from fresh weights of rosemary plant ranging between (0.84 and 0.97%). This yield is consistent with findings from previous studies (Boutabia, 2016), which can be attributed to the active synthesis and storage of active compounds in the leaves and flowering tops of the rosemary plant during the harvesting period, leading to variability in proportions

The variation in yield of essential oils can be attributed to several factors. The extraction method used can significantly impact the quantity of oil extracted from different plant parts (roots, stems, flowers, fruits, or seeds), as well as the efficiency and proficiency of the extraction apparatus, as noted by Bagheri et al. (2014). Climatic conditions or the specific microclimate of the plant region also play a role (Khammassi et *al*.,2018), along with genetic factors (Dobravalskytė et al.,2013), harvest season, and geographic origin of the species (Díaz-Maroto et *al*.,2005). Additionally, the stage of maturity (Telci et *al*.,2009) and storage conditions of the sample (exposure to light, excessive temperature, etc.) can impact yield, as observed by Sayed-Ahmad et *al*. (2017).

3-2 The antifungal activity of tested essential oils.

The inhibitory efficacy of essential oils against fusarium wilt varied according to concentration and type of oil used, with a preference for Artemisia herba alba Asso oil (Table 2)."

Table (2): Percentage of inhibition of colonies of *Aspergillus parasiticus* fungus according to different concentrations (mean ± SD) and growth on PDA medium.

Essential Oils	**Percentage of inhibition (%)**		
concentration	**5 µl/ Petri dish**	**10 µl/ Petri dish**	**15 µl/ Petri dish**
***Artemisia herba alba* Asso**	**51,1±0,04**	**62,82±0,03**	**77,47±0,01**
Rosmarinus officinalis	**26,92±0,07**	**61,44±0,04**	**74,08±0,06**

High inhibitory efficacy was recorded for oil "*Artemisia herba alba* Asso", followed by oil "*Rosmarinus officinalis*", The percentage of inhibition of the growth rate of colony diameters was inversely proportional to the doses of the respective essential oils applied. For "*Artemisia herba alba* Asso oil, it was (77.47±0.01, 62.82±0.03, 51.1±0.04) % (image 1, Table.2) while for *Rosmarinus officinalis* oil, it was (74.08±0.06, 61.44±0.04, 26.92±0.07) % (image 2, Table.2)

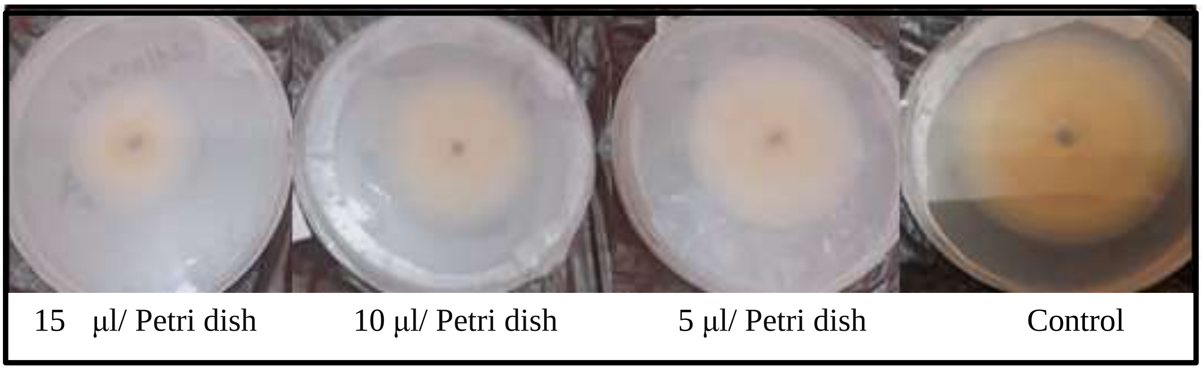

Image (1): Colonies of Aspergillus parasiticus strain under the influence of different concentrations of *Artemisia herba alba* Asso oil on the seventh day**.**

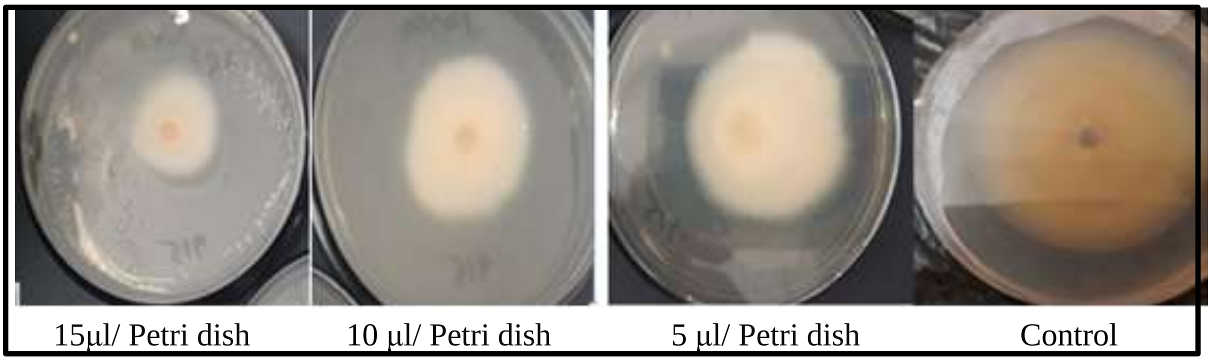

Image (2): Colonies of *Aspergillus parasiticus* strain under the influence of different concentrations of *Rosmarinus officinalis* oil on the seventh day**.**

3-2-1 *Artemisia herba alba* Asso

The statistical results revealed significant differences at a significance level of 0.05 between the highest concentration (15 µl/ Petri dish) and the lowest concentration (5 µl/ Petri dish) with a p-value of P=0.005. Additionally, significant differences were observed between these concentrations and the control factor with highly significant differences (P=0.000). Significant differences were also found between the second concentration (10 microliters per petri dish) and the control factor with highly significant differences (P=0.001), and between the last concentration (5 microliters per petri dish) and the control factor with a significant difference (P=0.015).

To analyze further, we categorized the concentrations into three homogeneous subgroups based on the least significant difference $\bar{X}$: the first subgroup comprised concentrations of 15 µl/ Petri dish and 10 µl/ Petri dish, the second subgroup comprised concentrations of 10 µl/ Petri dish and 5 µl/ Petri dish, while the third subgroup included the control experiment.

Previous studies have revealed varying inhibitory effectiveness. Ghanmi et *al*. (2010) reported inhibitory effectiveness of white wormwood oil against three fungal strains, including

Aspergillus niger. Similarly, Houicher et *al*. (2015) reported moderate inhibitory effectiveness of essential oil from *Artemisia campestris* against various fungi, including *Aspergillus niger*. These findings are similar to what was observed in our study. A study conducted by Ghita et *al*. (2019) demonstrated high antifungal activity using essential oils, with white wormwood oil exhibiting the highest inhibitory activity against *Aspergillus niger* with an inhibition zone of 23.6 mm.

Gacem et *al*. (2019) reported on the antifungal activities of three medicinal plants from the Algerian steppes and desert, including white wormwood. They found that the methanolic extract of white wormwood at various concentrations (25 to 100 mg/ml) exhibited weak sensitivity towards both *Aspergillus parasiticus* and *Aspergillus ochraceus*, with inhibition percentages ranging from 0% to 5% and from 6.19% to 29.25%, respectively. These results are considerably lower compared to what we have found in our study.

Hanan Y. Aati et *al*. (2020) highlighted good to moderate effectiveness of three types of wormwood plants: *Artemisia absinthium*, *Artemisia scoparia*, and *Artemisia sieberi*, against *Aspergillus parasiticus.*

Bidgoli (2021) conducted a study to evaluate the antimicrobial activity of essential oil from *Artemisia persica* Boiss. They found that the most sensitive fungal strains to the oil were *Aspergillus parasiticus* and *Aspergillus ochraceus*. The sensitivity of the oil increased with *Aspergillus flavus* and *Aspergillus nidulans* with increasing concentrations. However, *Artemisia persica* oil exhibited moderate inhibitory sensitivity towards *Aspergillus niger* and *Aspergillus fumigatus*. These results are consistent with similar studies conducted by Hammami et *al.* (2013) and Selles et *al*. (2013).

3-2-2 *Rosmarinus officinalis* oil.

The results of the statistical study revealed an effect between inhibition and concentrations, with significant differences observed among concentration means. The highest concentration (15 µl/ Petri dish) showed significantly higher differences compared to the lowest concentration (5 µl/ Petri dish), supported by a p-value of P=0.000. Additionally, significant differences were found between the moderate concentration (10 µl/ Petri dish) and both the lowest concentration and the control experiment, with p-values of P=0.01 and P=0.001 respectively. The lowest concentration (5 µl/ Petri dish) exhibited highly significant differences with the highest concentration (P=0.000) and significant differences with the moderate concentration (P=0.01).

Based on the least significant difference test $\bar{X}$, concentrations can be divided into two consistent subgroups. The first subgroup includes concentrations ranging from 15 to 10 µl/ Petri dish, while the second subgroup consists of the lowest concentration (5 µl/ Petri dish) and the control experiment.

Several previous studies have corroborated the findings of our current study. Moghtader et *al*. (2011) highlighted the antifungal effects of mountain savory essential oil against *Aspergillus flavus*, the causative agent of yellow mold. Yang et *al*. (2011) demonstrated the high efficacy of rosemary essential oil *(Rosmarinus officinalis*) against *Aspergillus niger*, a black mold fungus.

Moreover, a study evaluating the effectiveness of rosemary essential oil (*Rosmarinus officinalis*) in controlling post-harvest diseases and indigenous fungal algae in grapevine (*Vitis labrusca L.*) reported a high efficacy of rosemary oil. The inhibition rate was 97.7% against Aspergillus flavus and 98.9% against Aspergillus niger (Larissa et *al*., 2013).

Boris et *al*., (2014) found that *Rosmarinus officinalis* plant oil was more effective against Aspergillus niger, a black mold fungus. Similarly, Mahmoud et *al*. (2014) indicated inhibition against Aspergillus niger. Additionally, the parasitic mold was reduced by 4% in dry weight at the highest concentration of *Rosmarinus officinalis* oil.

Koç et *al.*, (2018) reported good inhibitory effectiveness against Aspergillus parasiticus and Aspergillus flavus with inhibition zones ranging from 0 to 15 mm and 0 to 17 mm, respectively, which is consistent with our findings. Despite some minor differences, which could be attributed to various factors affecting the performance of essential oils, such as environmental acidity, the presence of fats that may reduce the activity of hydrophobic compounds, and proteins that may cause interactions between some compounds, thus reducing their activity.

Similarly, Bomfim et *al*., (2020) reported high inhibitory effectiveness with high concentrations of Rosmarinus officinalis oil (ranging from 100 to 2000 µl/ml) against Aspergillus flavus, showing a reduction in its growth rate from 0% to 93.2% depending on the concentration. These results are consistent with what we have previously discussed.

Other studies have shown contrasting results to what we have found. Prakash et *al.* (2015) reported moderate effectiveness of *Rosmarinus officinalis* oil against various Aspergillus species, including *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. candidus*, *A. sydowi*, and *A. fumigatus*. The inhibition rates varied depending on different concentrations (ranging from 0.25 to 1.25 µl/ml) and each fungal species (from 39.58% to 100%). Despite the variation in concentrations, the results trended similarly to our study, indicating that "the higher the concentration, the higher the effectiveness."

On the other hand, Gömöri et *al.* (2018) reported weak inhibitory effectiveness against Aspergillus parasiticus. Similarly, Lorán et *al*. (2022) reported weak inhibitory effectiveness of Rosmarinus officinalis oil against *Aspergillus parasiticus*.

This effectiveness is suggested to be due to the concentration of essential oils on fungal hyphae, resulting in membrane disruption that leads to leakage of cytoplasmic components, loss of rigidity, and integrity of the cell wall, ultimately causing collapse and death (El Badawy et *al*., 2014).

4- Discussion

The synergistic effect between secondary compounds plays a crucial role in this microbial cell collapse. This effect has generated debate, resulting in two different interpretations:

- The first argues for the possibility of these secondary compounds penetrating the fungal organism, then binding to the active sites of fungal enzymes containing a (SH-) group and reacting with them.

- The second suggests that secondary compounds cause membrane disruption, inducing instability and oxidation (Bakkali et *al*., 2008; Soumanou et *al*., 2016).

Many studies have demonstrated the effectiveness of oxygenated monoterpene compounds responsible for the antifungal activity in oils. This explains the effectiveness of oils such as rosemary oil, which contains 70% oxygenated terpene compounds (Dias et *al*., 2017; Danielli et *al*., 2019).

Indeed, the variation in effectiveness can also be attributed to the concentrations used. Many studies have indicated that biological activities are concentration-dependent. Consequently, as the concentration of essential oils increases, the active component therein also increases, leading to enhanced biological activity of the oil (Silva et *al*., 2012).

5- Conclusion

The antifungal activity of the essential oils revealed a high sensitivity towards Aspergillus parasiticus, with inhibitory rates exceeding 70% for both oils. This highlights the potential of essential oils exhibiting effective antifungal activity, which could be utilized in combating fungal diseases in the future and safely incorporated into food product manufacturing processes.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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