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Antifungal activity of essential oil of *Syzygium aromaticum* on *Rhynchosporium secalis*, the causal agent of barley Scald

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ABSTRACT

Scald caused by *Rhynchosporium secalis* is one of the most devastating barley foliar diseases worldwide. Morocco has not been spared from this scourge. Our research focused on the antifungal effects of clove essential oil. Essential oils are extracted from the flower buds of aromatic plants using hydrodistillation. According to this study, the obtained extraction rate (9.07%) is quite satisfactory, making the plant a valuable natural resource. The aroma essential oil was identified by gas chromatography, and its main component was eugenol with a content of more than 52%, followed by eugenol acetate (25.94%), caryophyllene (7.845%) and caryophyllene oxide (1.74%). Two R. secalis isolates were tested for antibacterial efficacy. For the two isolates *(Rs1 and Rs2)* at a low concentration of 0.4 µl/ml, complete inhibition was observed under the action of *S. aromaticum* essential oil. According to our results, the essential oil has an antifungal effect on Scald.

Keywords: Scald, *R.secalis*, *Syzygium aromaticum,* Essential oil, Antifungal activity, Extraction, Total inhibition.

INTRODUCTION

Along with net blotch, scald is one of the most destructive fungal diseases of barley leaves, especially in cool temperate climates. The causative agent of the disease is *R. secalis* (Oudem) J.J., which also affects rye and other grasses (1,2). The disease causes severe yield loss and reduces barley seed quality (3).

The overuse of fungicides has led to the emergence of resistant strains. The resistance of R. secalis to MBCs (benzimidazoles and carbamates) and DMIs (sterol demethylation inhibitors) was reported by Torriani in 2004 (4). In addition to the variable efficacy of fungicides against Rhynchosporiosis (which varies according to geographical location) (5), the threat of this disease can also be addressed by biocontrol of medicinal plant essential oils. They have a wide range of applications in therapeutics,

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cosmetics, agri-food, and more. Furthermore, the potential use of essential oils and their constituents as fungicides is enhanced by their biodegradability (6,7).

Eugenol is the main constituent (78%) of clove essential oil (8), which is the dried flower buds of the clove tree (*S. aromaticum* (L.)) harvested before opening; they have, inter alia, acaricidal properties (9), Antifungal, antibacterial, antiviral and anesthetic properties (10,11).

Following the results obtained by Essaouaadi et al 2021 (12), showing the superior efficacy of gifole extract against this pathogen, we opted to finalize the results by working on the essential oil of this plant against R.Secalis.This work aimed to extract essential oil from cloves and to characterize and evaluate the antifungal efficacy of this essential oil against R. secalis.

2-MATERIALS AND METHODS

2-1-Materials

The medicinal plant used is *Clove,* a plant available on the market throughout the year. It was supplied by a herbalist in the Dakhla region during March.

2-2-Fungal material.

The two *R. secalis* isolates, Rs1 and Rs2, are from Khémisset and Gharb respectively. The choice of these two isolates was based on the one hand on the evaluation of their severity in the field and on the geographical distribution.

2-2 Methods

Extraction of essential oil

The essential oil of *S. aromaticum* was extracted by hydrodistillation in a Clevenger apparatus. Distillation was carried out by boiling 100g of plant material with 1 L of water for 1 h 30 min in a 2 L flask with a 60 cm column connected to a refrigerator.

The yield of essential oil was calculated using the following equation (13)**:**

RHE(%)=MHE/Mmvs ˟ 100

Where:

RHE: yield of essential oil in (%)

MHE: mass of essential oil (g)

Mmvs: mass of dry plant matter (g)

Chromatographic analysis

Analysis was performed on a Hewlett Packard gas chromatograph (HP 6890 series) coupled to a Perkin Elmer (CG-SM) mass spectrometer. Fragmentation is done by electron impact at a field of 70 EV. The column used was an Rxi 5ms 5MS capillary column (30 m ˟ 0.25 mm). The layer thickness is 0.25 µm. The column temperature was ramped from 50 °C to 280 °C at a rate of 8 °C/min. The carrier gas was helium, and the flow rate was 1ml/min. Injection mode is divided into (leakage ratio: 1/20, flow rate 50ml/min). The device is controlled by a PerkinElmer computer system (version 6.1.0.1963), which manages the operation of the device and allows monitoring of the progress of the chromatographic analysis. It is also connected to the computer system that manages the NIST 2011 mass spectral library, which enables it to identify peaks and their percentages based on the retention time of each component.

Microbiological procedure

The minimum inhibitory concentration (MIC) of essential oils was determined by the method of Remmal et al. (1993) and Strani et al. (2001) (14,15). Since essential oils are not harmful to water and media, they are emulsified with a Tween 80 (0.05%) emulsifying solution to facilitate bacterial contact with the compound.

In Erlenmeyer flasks containing 25 mL of autoclaved PDA medium (121 °C for 2 min) and cooled to 45 °C, prepare initial dilutions by adding 10, 15, 20, and 25 µL of aromatic essential oils to give final dilution. Essential oils and Tween 80 in concentrations of 0.4, 0.6, 0.8 and 1 l/ml. shake the mixture well before pouring it into Petri dishes. A control containing only PDA was also prepared.

Inoculation was performed by depositing fragments of the fungal culture approximately 6.5 mm in diameter taken from 7-day-old mycelial mats. Incubate for 7 days at 22 °C with a 12 h photoperiod. Each experiment was repeated three times.

Statistical analysis

GraphPad Prism 9 program was used and data was analyzed by Microsoft Excel and design tables and comparison.

3-RESULTS

1-Yield and chemical composition of *S. aromaticum* essential oil

The yield of *S. aromaticum* essential oil extracted from cloves by hydrodistillation on a laboratory scale was 9.07%.

The analysis of the essential oil of *S. aromaticum* by gas chromatography made it possible to identify thirty terpene compounds cited in the table in order of retention time (Table 1; Figure 1).

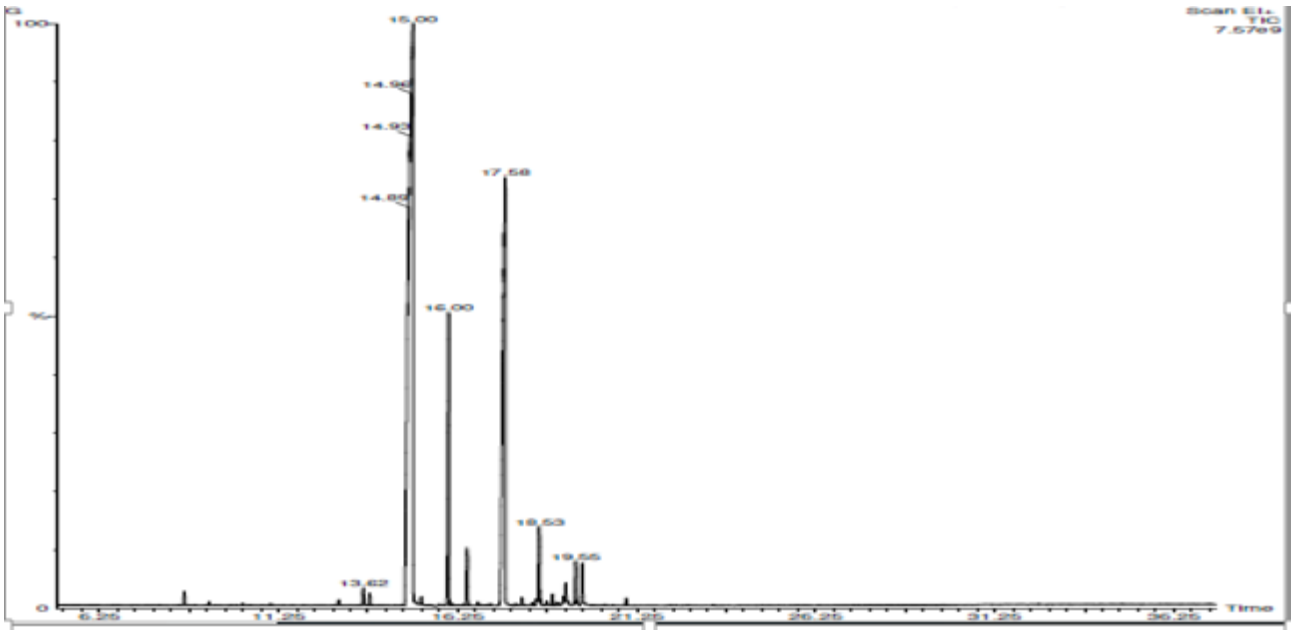

Figure 1: Chromatogram obtained during gas chromatography (GC)

Table 1: Chemical composition of the C*love* essential oil studied.

N	RT	Compound name	%
1	8.618	o-Cymene	0.277
2	9.314	ç-Terpinene	0.069
3	10.256	3-Thujanone	0.048
4	12.928	Phenol, 4-(2-propenyl)-	0.130
5	13.616	Thymol	0.372
6	13.795	Carvacrol	0.273
7	15.000	Eugenol	52.515
8	15.238	alfa-Copaene	0.157
9	15.755	Isocaryophyllene	0.049
10	15.834	Nerolidylacetate	0.053
11	16.001	Caryophyllene	7.845
12	16.518	Humulene	1.206
13	16.818	ç-Muurolene	0.094
14	17.164	Guaia-1(10),11-diene	0.059
15	17.585	Eugenol acetate	25.945
16	17.689	à-Cubebene	0.046
17	17.872	à-Calacorene	0.077
18	18.047	Diepicedrene-1-oxide	0.186
19	18.339	Caryophyllenyl alcohol	0.088
20	18.469	Alloaromadendrene oxide-(1)	0.123
21	18.531	Caryophyllene oxide	1.736
22	18.577	9á-Acetoxy-3,4,8-trimethyltricyclo[6.3.1.0(1,5)]dodec-3-ene	0.090
23	18.748	Isoaromadendrene epoxide	0.121
24	18.902	Humulene epoxide	0.246
25	19.023	Caryophyllene	0.078
26	19.211	Alloaromadendrene oxide-(1)	0.279
27	19.269	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl	0.640
28	19.548	Isoaromadendrene epoxide	0.935
29	19.732	Isoaromadendrene epoxide	0.995
30	20.961	Benzyl Benzoate	0.154
Total			94.886

RT: Retention Time

These findings show that 94.886% of the elements of clove essential oil represent the total of the obtained constituent percentages. The primary ingredients in this oil are eugenol (52.515%), eugenol acetate (25.945), secondary ingredients like caryophyllene (7.845%), and minor ingredients like isoaromadendrene epoxide (1.93) and humulene (1.206%).

2-Antimicrobial activity of S. aromaticum essential oil against R. secalis

Table 2 summarizes the findings of the antifungal activity of S. aromaticum essential oil on

the two isolates of R. secalis, the causative agent of Scald in barley (Rs1 and Rs2).

Table 2: Antifungal activity of the essential oil of *S.aromaticum* against two isolates of *R.secalis*

	0.4uḷ/ml	0.6uḷ/ml	0.8uḷ/ml	1uḷ/ml	control
Rs1	-	-	-	-	+
Rs2	-	-	-	-	+

-:Inhibition (-) and : Growth (+)

This study of the antifungal activity of *S.aromaticum* essential oil showed that a concentration of 0.4uḷ/ml was sufficient to completely inhibit the growth of the two *R.secalis* isolates Rs1 and Rs2 (Figure 2).

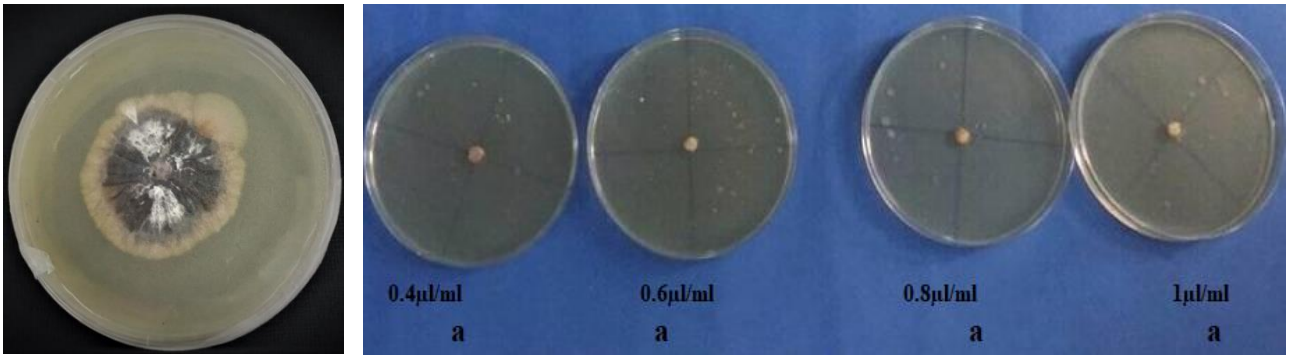

Figure 2: Effect of the essential oil of *S.aromaticum* on the diametrical growth of isolate of *R.secalis* throughout 7 days.

4-DISCUSSION

An estimated yield of 9.067% of clove essential oil was produced. Other authors have reported yields that are significantly different; for example, Redriguez et al. (2014) used the identical hydrodistillation technique in their investigation and came up with a value of 2.20% (16). The simplicity of our hydrodistillation apparatus and the loss of oil in the aqueous phase of the distillate are likely to be blamed for the difference in output.

The nature of the aromatic plants as well as the species, harvesting period, cultivation practices, extraction process, meteorological conditions (heat, cold), geography (height, soil type, sun exposure), and extraction technique all affect essential oil production differently. Eugenol, which makes up more than 52% of the S. aromaticum essential oil, serves as the primary constituent and is followed by Eugenol acetate (25.94%), caryophyllene oxide (1.74%), and caryophyllene (7.845%) (Table 1). These findings are comparable to those of Chaieb et al. (2007), who demonstrated that the primary constituents in the essential oil of S. aromaticum are eugenol and eugenol acetate (17). Additionally, Houari A.D.E. (2015) demonstrated that the components of S. aromaticum essential oil are almost identical to those discovered in our work, but in different proportions (18).

At a modest concentration of 0.4 l/ml, the essential oil of *S. aromaticum* caused a complete suppression in both R. secalis isolates. This is in line with Eugénia P. (2009), who demonstrated the high fungicidal activity of eugenol and the essential oil of S. *aromaticum* against Candida albicans, dermophytes, and Aspergillus sp. This research reveals that Clove oil may be advantageous in the therapeutic management of cutaneous-mucosal candidiasis in particular, such as vulvovaginal candidiasis when compared to Fluconazole, a systemic antifungal medicine approved for the

treatment of candidiasis (19).

The inactivation of fungal enzymes with the SH (Hydrogen Sulphide) group in their active site is the basis for the phenolic toxicity to phytopathogenic fungi [4]. Phenolic terpenes also alter the permeability of the leakage of intracellular components by attaching to the amine and hydroxylamine groups of microbial membrane proteins (20).

Our findings are in agreement with those of Matusinsky et al., (2015) who demonstrated the in vitro antifungal activity of five essential oils from five medicinal plants, namely Pimpinella anisu, Thymus vulgaris, Pelargonium odoratissimum, Rosamarinus officinalis and Foeniculum vulgare on fungal pathogens affecting Cereals, viz: Oculimacula yallundae, Microdochium nivale, Pyrenophora teres, Fusarium culmorum and Z.tritici on Wheat (21).

Therefore, S. aromaticum is a more considerate of the environment and the health of users and consumers source of novel compounds in the quest for bioactive molecules against phytopathogenic fungi of natural origin. To utilize fewer chemical products, it can be incorporated into integrated pest management programs.

CONCLUSION

The positive outcomes of our in vitro experiments allow us to conclude that the essential oil of S. *aromaticum* offers a new opportunity for the natural management of Scald in barley.

Conflict of interest:

None

Fund:

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