CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF *ORIGANUM GLANDULOSUM* AGAINST TWO *BOTRYOSPHAERIA* SPECIES

AMMAD Faiza[1],
AOUICH Ahmed [2]

1. Faculté des Sciences de la Nature et de la Vie, Département des Biotechnologies, Université de Blida1, BP 270, route de Soumaa, Blida 09000 – Algérie , Tel : 05.52 29 11 02, E-mail: sahraoui_a_f@yahoo.fr
2. Departement de Biologie, Ecole Normale Supérieure Kouba, BP. 92, 16050 Vieux-Kouba, Alger, Algéria.

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Abstract

The chemical composition of essential oils isolated from the leaves and flowers of Origanum glandulosum by hydrodistillation was analyzed by Gas chromatography (GC) showed the presence of 48 compounds, representing 96.73% of total oil, were identified. Oregano oil was found to be rich in carvacrol (48.42%), γ–Terpinéne (27.09%), Para-Cymène (16.9%) and β-Caryophyllène (3.45%) and characterized by relatively high amounts of phenol, monoterpenes hydrocarbons and sesquiterpenes. Results of the antifungal testing by in vitro volatilty assay showed that the oil significantly inhibit the growth of two spécies of genus : Botryosphaeria. The results of this study showed that the toxicity of the oregano oil vary with increasing concentration of applied doses on the one hand, and a relatively gradual efficiency versus time, which results in improved efficiency on the other.

Keywords: Antifungal activity, *Botryosphaeria,* Gas chromatography, *Origanum glandulosum*

INTRODUCTION

Fungi are the main microorganisms responsible for losses in agriculture, 83% of plant diseases are caused by fungi. Black dead arm (BDA) is one of the most destructive of the woody tissues of grapevine causing decline and loss of productivity of vines [1] and other hotes. This type of fungus colonises wood tissue and causes dieback, no effective control exists for the time against this kind of fungus. The difficulty in proposing methods to short-term control is linked to poor knowledge of wood diseases of the vine, very complex involving several fungi. It should be noted that since the closure in 2001 of sodium arsenite, no way Chemical control is available against these diseases wood, prophylactic methods (from the nursery to vineyard) are mainly recommended in order to limit the development of those fungi. The knowledge of wood diseases and their epidemiology have greatly progressed. In this context, we propose to extract the essential oil of oregano by hydrodistillation, analyze the chemical composition of the essential oil by chromatogrphie gas phase (GC) then perform an analysis of its antifungal against the species of *Botryosphaeria*.

MATERIALS AND METHODS

1. Plant material

The leaves and flowers of ***Origanum glandulosum*** were collected from the Larabba (Blida) in (Algeria) in April 2012. Identification was carried out using a determination key [2].

1.1. Isolation of the essential oils

The essential oils were extracted by hydrodistillation of dried plant material (100 g of leaves and flowers in 500 ml of distilled water) using a Clevenger-type for 5 h. The Bottles of oil are covered with aluminum paper to protect them from any negative effects of light and were stored in a refrigerator at a temperature of 4°C. Yield based on dried weight of the sample was calculated.

1.2. Analysis of the essential oils

Chromatographic analysis was performed using a gas chromatograph (GC) to separate complex mixtures of volatiles identified and quantified in a relatively short time [3].

2. Fungal material

The fungal material destined for evaluating the efficiency of treatments based on the essential oil of Oreganum, two fungal strain was obtained from a personal collection: *Botryosphaeria obtusa* and *Botryosphaeria dothidae*, were isolated from infected wood of grapevine and identified using a combination of morphological and cultural characters, and confirmed by molecular analysis.

3.Antifungal activity assays

Cultures of each of the fungi were maintained on potato dextrose agar (PDA) and were stored at 4°C. The fungal species used in this study were: *Botryosphaeria obtusa* and *Botryosphaeria dothidae* .Antifungal activity was studied by using a volatility assay (*in vitro*). The essential oil was dissolved in tween water solution (3%) and Whatman paper discs of 8 mm diameter, previously sterilized by autoclaving, are first impregnated and saturated with 30 µl of each dilution of the essential oil, and then deposited on the lid of the Petri dish returned due to a disc impregnated with the cover [4]. On the basis of essential oils obtained, we prepare three doses tested after dilution in Tween 80 (diluted 3%). (0.25, 0.50, 0.75%). The control consists of discs impregnated with the same volume of tween 80 (3%). All the petri dishes were immediately closed and sealed with parafilm to prevented evaporation of the oil. The estimation of mycelial growth was carried for 15 days from 5 days after treatment with the essential oil. For a better measure diameters of growth, all plates were taken digital pictures then treated with Image Tool Software (3.1), three (3) measures were selected for each diameter.

4.Statistical analysis

The results of antifungal potency were treated with Excel. by Systat Software ver.12, SPSS 2009. Hypothesis antifungal efficacy of essential oil was tested by analysis of variance (GLM) by software systat, ver.12, SPSS 2009.

RESULTS

1.Chemical composition

The oil yield obtained from hydrodistillation of ***Origanum glandulosum*** leaves and flowers was 0.98 %. 48compounds, representing 96.73% of the essential oil were identified; the chemical composition of our ***O. glandulosum*** oil was dominated by monoterpene fraction with a predominance of phenolic compounds. Like the essential oil from other oreganum species, the major compounds of the oil were (carvacrol or his isomer the thymol). The essential oil of Oregano was previously investigated in other studies, generally it was shown that carvacrol was the major components in the oil but with different levels. Similar results have been proved by [5] on the essential oil of thymus showing that chemical composition is dominated by monoterpenes and the highest proportion of carvacrol (74%) is obtained in full blossom. According to [6], these differences in the oil composition and yield may be due to several factors such as, climatic and geographic conditions, time of collection and extraction methods.

2. Antifungal activity of essential oils

The essential oils isolated from *Oreganum* organes were tested for their antifungal activity against two important agriculturally fungal species caused the grapevie dieback. These results showed that the oil significantly reduced the growth of the fungal species. The concentrations tested D3 (0.75%) and D2 (0.5%) showed the highest inhibitory effect compared to D1 (0.25%). Whereas no inhbition was register even after 15 days with the control. D3 and D2 are evolving to the average toxicity at the beginning of application of treatments to a high toxicity up to (8 days). While the dose D1 (0.25%) showed low toxicity at the beginning of its application to an average toxicity at the end of treatment (Figure 1).

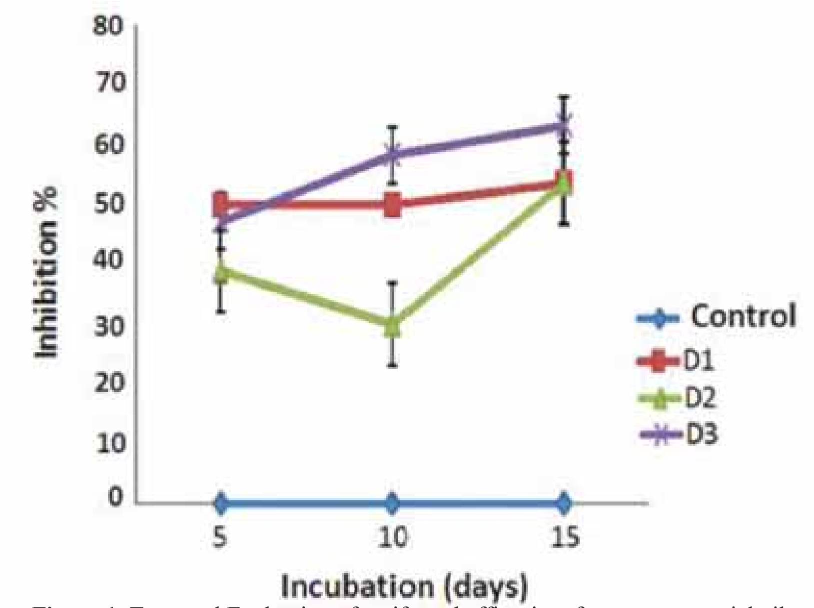

Figure 1: Temporal Evaluation of antifungal efficacity of oregano essential oil

The results of the effect of the essential oil of oregano on two strains of Botryosphaeria exhibits a net efficiency, it results in the inhibition of mycelial growth of the strains (28.5 mm, 27 mm). This tendency is verified by testing the variance or difference was highly significant (F-ratio=52.45, p=0.000, p <0.01%). The doses of treatment indicate the existence of a highly significant difference in inhibition of mycelial growth rate with the respective values (F-ratio=15.974; p=0.000; p<0.01%) (Figure 2a), whereas the different periods showed an insignificant effect (F-ratio=0.768; p=0.472; p>5%) (Figure 2b).

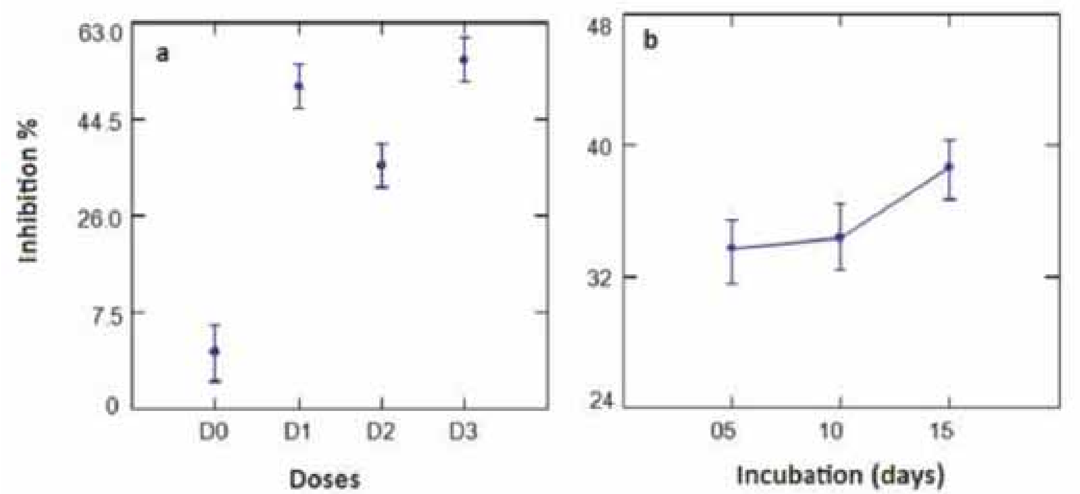

Figure 2: Effect of essential oil (dose (a), time (b) on *Botryosphaeria* species

DISCUSSION AND CONCLUSION

The results indicate that higher concentrations inhibit more efficiently than when diluted. According the statistical analysis, the oils exhibited different degrees of inhibition on the growth of tested fungi; the two sepices were the most sensitive to the action of the oil. Generally, *Origanum* species are known to possess an antifungal activity. In this study, the essential tested was considered rich phenol, monoterpenes and sesquiterpenes.

The chemical results indicated that *O.glandulosum* essential oils were characterized by the relatively high content of carvacrol, which is known to possess an important antifungal activity. According to [7] showed that the antimicrobial nature of essential oil apparently with their high phenolic content in particular thymol and carvacrol, they proved that the more phenols are high levels essential oils are more effective and they have a broad spectrum of activity against filamentous fungi and insects [8]. [9] showed that thymus rich in phenol groups posses an important antifungal activity against *d'Aspergillus fumigatus*. For these reasons the antifungal activity of our oil was attributed to the presence of phenol , sesquiterpenes and monoterpenes and the synergism between components does play an important role. Indeed, many authors showed that phénols were not the only responsible of the activity, all of the chemical composition should be taken into account [10]. Lahlou [11], reported that the activity of essential oil is higher than that of its majority composed tested separately.

As a result, the plant secondary compounds, possess several modes of action of the fungal strain, but in general, their action takes place in three phases: the attack of the wall by the plant extract, resulting in an increase permeability and losing of cellular constituents, the acidification of the inside of the cell blocking the production of cellular energy and synthesis of structural components and finally destruction of genetic material leading to the death of fungi [12].

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