Activity in vitro of the oils of RosmarinusOfficinalis and LavandulaOfficinalis on the Mycelial growth of three lettuce mushrooms

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ABSTRACT- The objective of this study is to test the antifungal activity of essential oils from the PAM against *Rhyzoctoniasolani*, *Botrytis cinerea* and *Alternariaalternata*, agents of alteration of the vegetables. Each strain is a causal agent of Cryptogamic diseases that produce a high mortality of the lettuce in nursery and Orchard plants. The extraction of oils from Rosemary and lavender showed important fungicide activity in vitro against all Ley and foliar fungi studied. These species inhibit the development of the mycelium to varying concentrations. Periodic observations have shown that oils of *lavandula* showed an inhibitory effect more powerful on most of the strains, and this in the early days of incubation, compared to the witness. At the same time, the study provides toxicological tests of fungicides industrialists against the same strains, to compare two activities. Chromatographic analysis and calculation of performance have undergone the tested essential oils.

Index Terms- Essential oils, *Rosmarinusofficinalis*, *Lavandulaofficinalis*, antifungal activity.

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I. INTRODUCTION

Fungi that affect produce have a major economic impact because of the considerable deterioration produced in these crops like lettuce that is often consumed raw. Among these fungi, there are *Rhizoctoniasolani* (Kuhn 1858), *Botrytis cinerea* (Pers 1974) and *Alternariaalternata* (Keissler 1912), that respectively cause rots of collet Cryptogamic diseases and the disease. Mushrooms settle, necrosis (injury, water deficit, deficiency or excess of a mineral element), on the collar of the seedlings after causing damping-off and behave in parasite on healthy tissue. Several fungicides are used today because of their toxicity. However, a serious problem arises on the effectiveness of these chemicals that could generate a phenomenon of resistance among pathogenic fungi [1]. To do this, the search for new substances with active principles of the molecules that are naturally present in plants can register as a green low cost solution [2].

The flora of the Morocco has a rich and diverse vegetation including aromatic and medicinal plants. Some of them contain essential oils with antibacterial properties [3; 4; 5] and proven antifungals [6; 7; 8; 9]. More recently, it also recognizes their anti-cancer properties [10].

The essential oils of many plants have become popular in recent years and their bioactive principles conquered several industrial sectors [11, 12]. However, despite their large spectra of application, their use in the fight against Cryptogamic diseases remains little studied [13, 14]. *Rosmarinusofficinalis* plant is encountered in the Saharan Atlas, the High Atlas, the Middle Atlas (abundant in the Valley of Skoura-Mdez and on the SE of the Middle Atlas oriental reverse), thegir and in the mountains of the Eastern Morocco (abundant on the BeniSnassene and Debdou-Jerada mountains [15]. Its essential oils are used as a preservative in food products to protect them against any microbial deterioration. So, they have strong activity on filamentous fungi and protozoa [16]. The *LavandulaOfficinalis* plant is a sous-arbrisseau of the Labiatae family, the kind consists of approximately 28 species which are almost of Mediterranean origin. The natural properties of its essential oils make

promising conservation agents for the food industry [17].

This work is based on two main objectives: (i) characterize the chemical composition of the essential oils of a. officinalis and l. officinalis. (ii) study of the activity antifungal essential oils of these two species to three responsible Cryptogamic diseases in lactuca sativa strains.

II. MATERIAL AND METHODS

2.1. Plant material

Rosmarinusofficinalis samples were harvested randomly then dried for ten days in the shade before their use. *Lavandulaofficinalis* plant was harvested during the summer, plant material has been washed and dried in a place that is well ventilated at room temperature.

2.1.1. Fungal material

Three fungal species tested in this study are *Rhizoctoniasolani*, *Botrytis cinerea* and *Alternariaalternata*. These molds come from the Faculty of sciences of Kenitra, Morocco mycotheque. They have been isolated from lettuce deteriorated and identified based on their morphological and microscopic characters [18]. The strains are regularly maintained by transplanting on a basis agar.of Malt extract

2.1.2 Extraction of essential oils from plants

Extraction of essential oils was made by distillation in a device of type Clevenger. This is the method proposed by [19] since it provides better returns. Three distillations have been made by boiling of fresh plant material with 1 l of water, a topped ball of a column connected to a refrigerant. The essential oil yield was determined with the material dry, estimated from 3 samples of 30 g dried for 48 hours in an oven at 60 ° C. The essential oil obtained was stored at 4 ° C in the dark in the presence of anhydrous sodium sulfate. It is diluted in methanol (1/20, v/v) before proceeding to the CG and CG/SM analysis.

2.1.3. Chromatographic analysis

Chromatographic analyses by gas chromatograph gas "GIC" to electronic type pressure regulation Perkin Elmer (Clarus 580).

• For the control of the oils of lavender, the analysis is done on a molten silica capillary column, length 60 m, diameter of 0.25 mm. The stationary phase is Macrogol 20000. The carrier gas used is helium with a flow rate of 1.5 ml/min. The temperature of the column is automatically set at 70 ° C for 15 minutes, then 70-180 ° C in an interval of 15-60 min. The injector and detector temperatures keep a constant value of 220 ° C.

• Control of essential oils of Rosemary is realized on a molten silica capillary column, length 30 m, diameter of 0.25 mm. The carrier gas used is helium with a flow rate of 1,0 ml/min. The temperature of the column is programmed automatically to 50 ° C for 10 min, then from 50 to 200 ° C during 75 min and finally to 200 ° C for 25 min. The injector and detector temperatures keep a constant value of 200 ° C.

Density was measured by a hydrometer type METTLER TOLEDO 30 PX. The refractive index was determined by a Refractometer NAR-1TLIQUID type and the specific rotatory power by ATAGO AP300.

2.2. Chemical fungicide tested

Two fungicides to different impacts were also tested in this study and characteristics are listed in table1.

Table 1: Characteristics of the fungicides tested

Common name	Trade name	Active matter	Chemicalfamily
Mancozèbe	DITHANE M45	80 %	Carbamates
Azoxytrobine	ORTIVA 25sc	250g/l	Hétérocycles Azotés

These two molecules are used by farmers for fighting the diseases studied. According to the index plant health official of the years 2012 and 2014 from Morocco, the mancozeb is homologated on garden produce while the azoxytrobine is recommended in particular against downy mildew of lettuce.

2.3. Antimicrobial activity

In the flasks, each containing 50 ml of agar, sterilized in the autoclave (20 min at 121 ° C) and cooled to 45 ° C, we add 10, 20, 40, 60, 80, 100 and gasoline plant or the fungicide testing 120µl solubilized in water so as to obtain the following final concentrations 0.2; 0.4; 0.8; 1.2; 1.6; 2 and 2.4 µl/ml (v/v). After stirring, the content is poured into Petri dishes of 90 cm in diameter. The witness is made under the same conditions but without treatment. Seeding is done by deposit of fragments of 1 cm 2 in diameter, taken from the periphery of a Mycelial mat from a one week old young culture in agar. Incubation is done in the dark for 7 d at 25 ° C. Each test is repeated three

times. Assessment of the likely antifungal activity of the tested products was revealed by two perpendicular diameters of Mycelial growth measures every day for a week.

2.4. Statistical analysis

The data obtained have been a statistical analysis. This is done by using the Student's t test. The value found by calculating the t can say that people are different with a risk of error p such as

p> 0,05 = the difference is not significant.

0,05> p > 0,01 = the difference is significant.

0,05> p > 0,001 = the difference is highly significant.

p< 0,001 = the difference is very significant.

The data were processed using the software "SYSTAT 12". A test of comparison of means was made whenever there was a significant effect of the factor studied by ANOVA

III. RESULTS AND DISCUSSION

3.1. Antifungal activity of essential oils

3.1.1. Performance of essential oils

HEs average yields were calculated based on dry vegetable matter from the leaves of each plant. They are 1.1% and 1.4% respectively for the lavender and Rosemary.

Various authors have reported variable yields between 1 36±0, 2% for *Lavandula O.* [20] and 1.79% for *Rosmarinus O.*, [21] have shown that the average yield in *LavandulaOfficinalis* HEs from the Morocco is 1.12%. According to [22], several factors can influence the variability of returns in essential oils. [22] also indicated that the plant is not dried in good conditions may deteriorate, and thereafter, losing all of its essential oils. Thus, [23] reported that the essential oil of Rosemary leaves dried in the shade for a week is 4 times higher than that of the fresh plant. Physicochemical data of the oils used in this study are given in table 2.

Table 2: Physicochemical parameters of the tested essential oils

Essential oils	Density	Refractive index	Angle of rotation	Compounds identified (%)
Rosemary	0.915	1.469	+5.0	100
Lavender	0.888	1.462	-9.7	92

3.1.2. Quantitative and qualitative analysis

The results of the chromatographic analysis of essential oils helped to identify 11 compounds from oils of *Rosmarinus O.* with the predominance of the 1-8 cineole (41.3%) and the camphor (23%); And 10 compounds for oils of *Lavandula O.* including linalyle acetate (42.3%) linalool (30.4%) are the majority. It is to point out that the tested species have in common the following compounds (camphor, 1-8 cineole and the α-teripeol) to variable contents. Figure (1,2) and (3,4) tables following show respectively the chromatographic profile and the chemical composition of each of the tested essential oils: (a) the lavender; (b) the Rosemary

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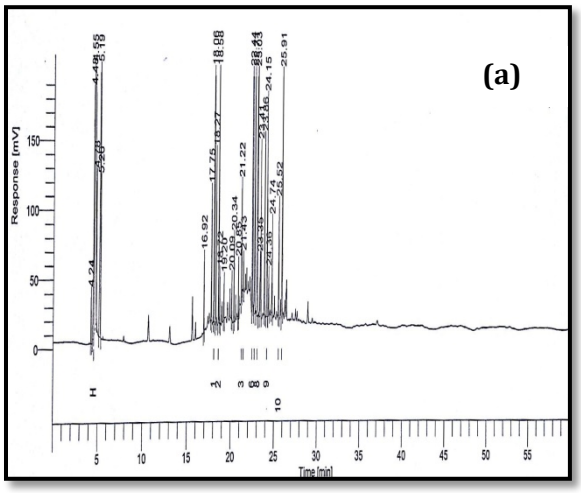

Figure 1 : Spectrum of a chromatogram of the essential oils of Lavandula O.

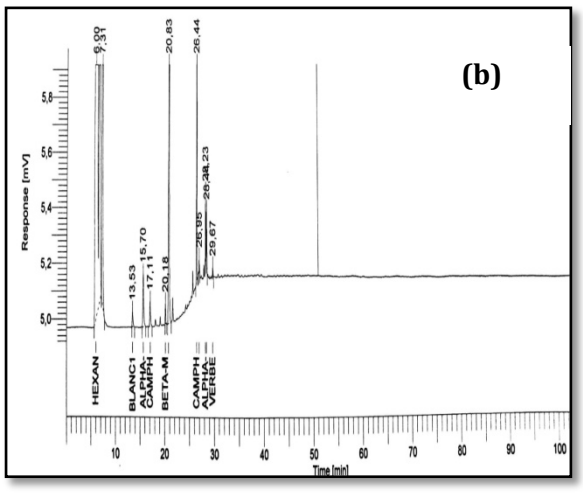

Figure 2 : Spectrum of the chromatogram of the essential oils of Rosmarinus O.

Table 3 : Chemical composition of essential oils of Lavandula O.

Composés de lavande	% Aire
Limonène	0,6
Cineole	2,7
3-Octanone	2,1
Camphre	0,6
Linalol	30,4
Acetate de Linalyle	42,3
Terpinén-4-ol	6,1
Acetate de lavandulyle	5,0
Lavandulol	0,3
Alpha-terpinéol	1,9

Table 4 : Chemical composition of essential oils of Rosmarinus O.

Composés du romarin	% Aire
Alpha-pinène	11,3
Camphène	4,5
Bêta-pinène	8,2
Bêta-myrcène	1,2
Cinéole	41,3
p-cymène	2,2
Camphre	23,0
Acetate de Bornyle	5,5
Alpha-terpinéole	1,7
Bornéole	1,0
Verbenone	0,1

These data are different from those reported by different authors. [24] analyzed the oils of Lavandulaofficinalis provenance Costantine (Algeria) and found the linalyl acetate (15,26%), linalool (10.68%), 1,8-cineole (10.25%), γ-terpinene (11.2%) and camphor (11.25%). [25] analyzed the chemical composition of the essential oils fromLavandulaofficinalis flowers collected from the mountain of Kozjak (Macedonia), they found 32 components with predominance of linalool (25.7%), linalyl acetate (23.2%), lavandulyl acetate (12.4%) with the presence of the terpene mono components and sesquiterpeniques hydrocarbons and oxygenated derivatives . [26]. studied the composition of the essential oil from *Lavandulaofficinalis* flowers grown in Uttarakand (India), they identified 37 monoterpeniques compounds. The major compounds were: linalyl acetate (47,56%), (28.06 percent) linalool, lavandulyl acetate (4.34%) and α-teripeol (3.7%). The variations in the chemical composition of essential oils, the qualitative and quantitative point of view, may be due to environmental factors, the part of plant used, the age of the plant and the period of the vegetative cycle or even genetic factors. [27] also pointed out that the difference in composition is probably due to various conditions environmental, genotype, geographical origin, the harvest period, the place and the drying time and the method of extraction.

3.1.3 Analysis of antifungal activity of essential oils

The graphics below show the antifungal activity of plant species tested on the three strains in the two culture media.

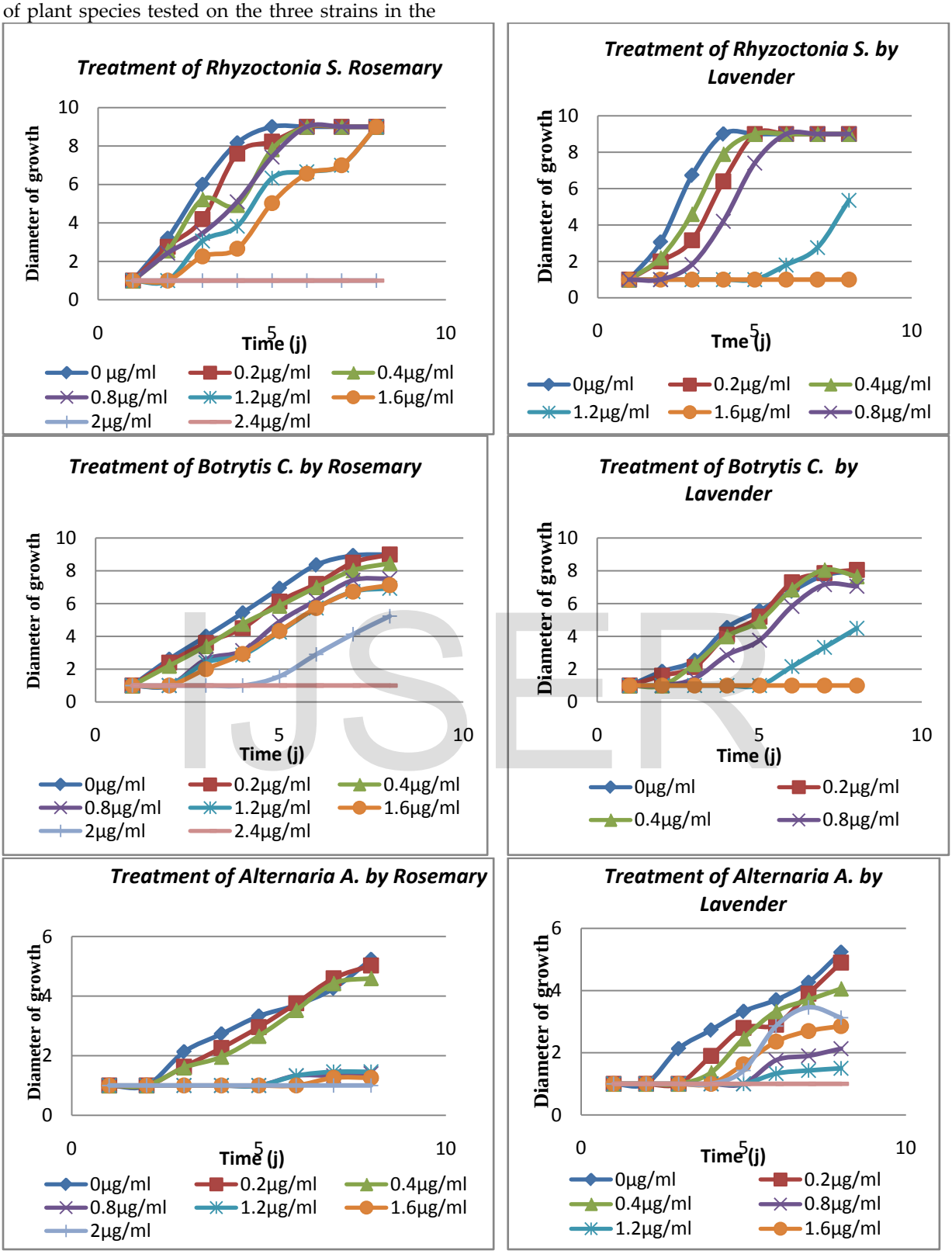

Figure 4: Effects of the HEs on the Mycelial growth of all three strains on extract of Malt

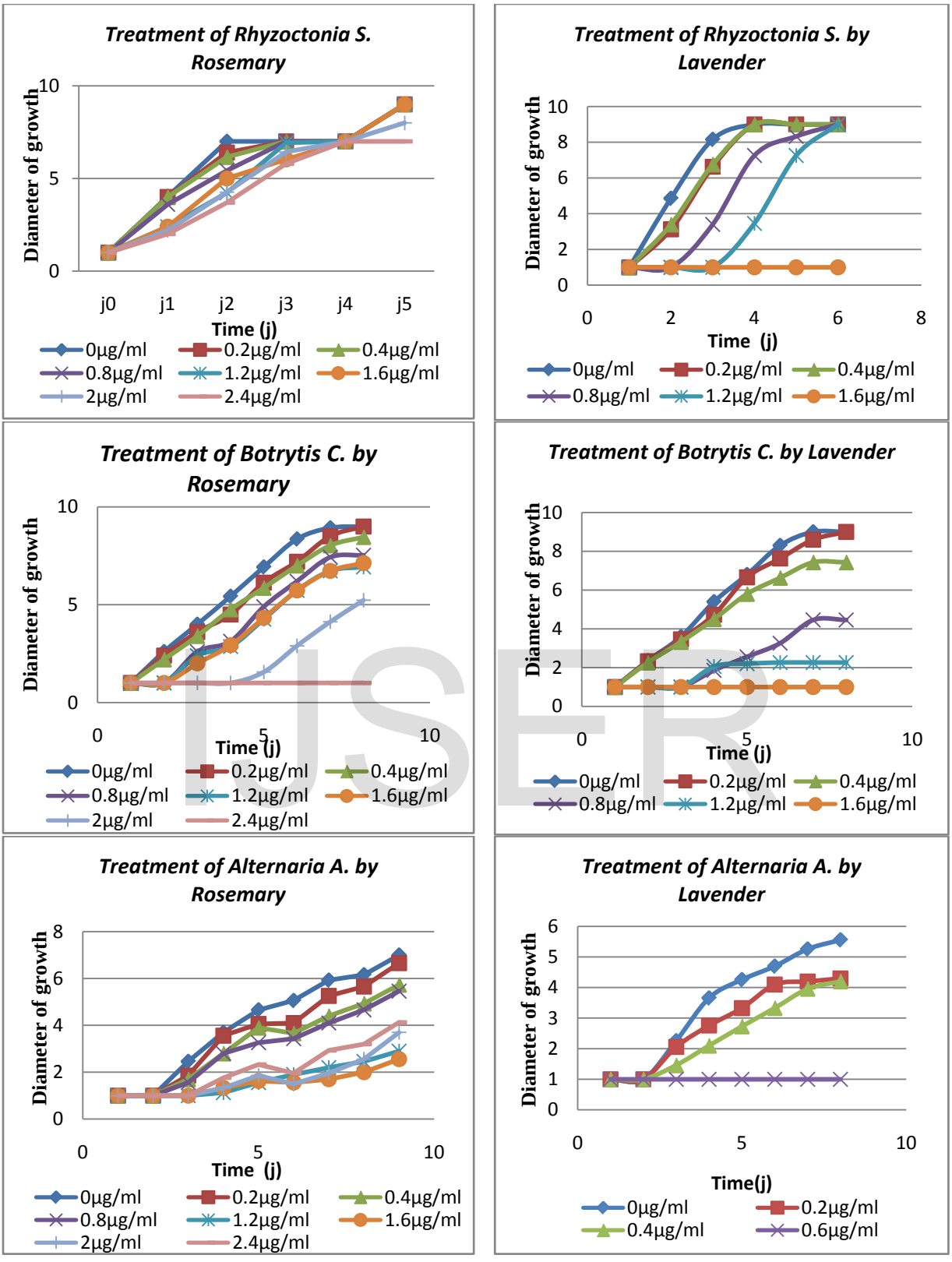

Figure 5 : Effects of the HEs on the Mycelial growth of all three strains on PDA

After the results of the study of antimicrobial activity, it is clear that essential oils of *Lavandula O.* have an effect fongitoxic on environment from Malt and PDA. Indeed, Botrytis and Rhyzoctonia experienced a slowdown of their growth from the concentration of 1.2V / v and complete inhibition at 1.6V / v of the oils of lavender on Malt extract. The same strains have slowed to 0.8v / v and inhibited at 1.6V / v respectively on PDA. As for the Mycelial development of A. alternata, it

decreased in a dose of 2.0V / v of the oils of lavender and stopped at 2.4v / v on Malt extract, whereas on PDA, we have seen a growth in 0.8v stop / v only. The graphs show that the essences of the rosmarinus o. manifest an antifungal effect on fungi but at doses compared to the oils of *lavandula O.* On malt extract, a total inhibition of Mycelial activity was observed at 2.4v / v for rhyzoctonia and botrytis and Alternaria 2µl/ml. On medium PDA.partial inhibition by Rosemary is dependent on its concentration. Indeed, you increase the volume of this gas in the culture medium increases the higher inhibition of Mycelial growth of different fungi. By comparing the spectra of growth of oils of lavender on the two culture media, we observed the same antifungal response in *R.solani* and *b. cinerea*. On the other hand, the strain *a. alternata* proved to be very sensitive to oils incorporated into PDAs, inhibition of growth was clearly seen at a dose of 0.8 µl/ml only.

These antimicrobial activities could be explained by the molecular interaction of functional groups of the chemical profiles of the HEs with the wall of the mushrooms, which causes deep lesions. But that doesn't mean that a synergistic effect between several constituents of the essential oils of the two species can also be taken into account in this activity. Lavender essential oils have antibacterial and antifungal characteristics very interesting on microorganisms [28]. Our findings are consistent with several authors who have found similar effects on other germs like *Shigellasonnei* and *Penicilliumdigitatum* which have been inhibited by these oils to 500v/1/v. in the study of [22]this oil has proven its antifungal activity against *Aspergillusflavus, Rhizopusstolonifer, Mucor spp., Trichoderma spp., Alternaria spp., Fusarium spp.* and*Penicilliumspp* on PDA environment. This effect is attributed to the content of oil and which can be connected to the majority compounds, mainly in the cineole and camphor.

3.2. Antimicrobial activity of chemical fungicides

Two figures below show the different reactions developed by the three strains tested towards the chemical fungicides two and this on malt extract and PDA (figure 3 and 4)

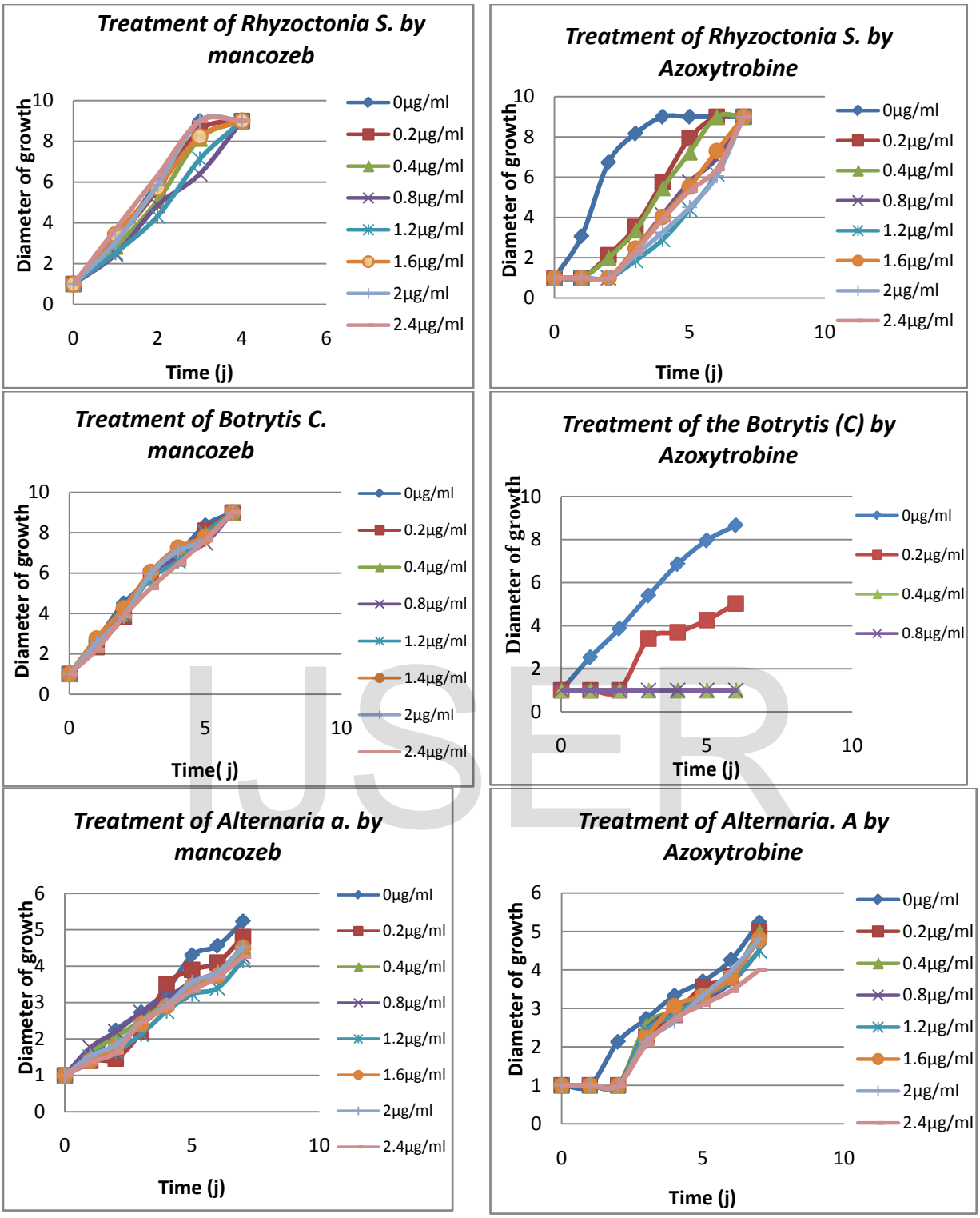

Figure 3 : Effects of two fungicides on Mycelial growth of all three strains on malt extract

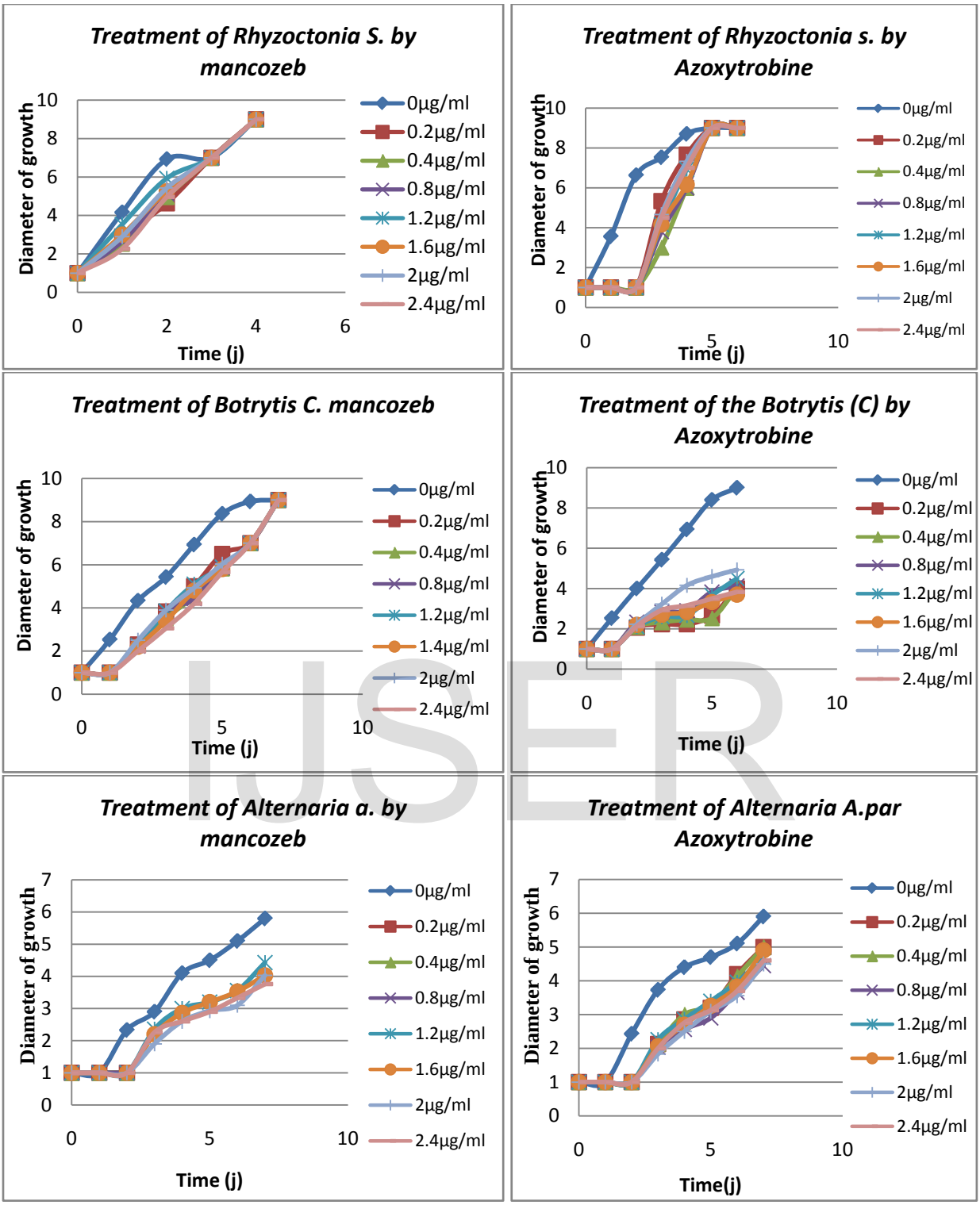

Figure 4 : Effects of two fungicides on Mycelial growth of all three strains on PDA

According the figures on growth of the strains, we raised the resistance of the strain *r. solani* to these fungicides; 4 days after treatment, Mycelial growth is similar to that observed at the witness. What explains that the active substances, mancozeb and azoxytrobine, do not affect the proliferation of the fungus in vitro. However, the strain of *Alternariaalternata* is inhibited by the two chemicals and especially the mancozeb on PDA environment where the inhibition rate reached more than 30% to j7. For *Botrytis cinerea*, the azoxytrobine has exercised strong antifungal activity against this germ and especially on Malt extract community.

Chemical control is defined by the use of fungicides to destroy, weaken or suppress the

fungus. The results obtained in vitro show that *Rhyzoctoniasolani*, have resistance to fungicides mancozeb and azoxytrobine, this would be due to inadequate dose of fungicide products made in vitro, and which are lower than the doses approved on the field. On the other hand these same fungicides gave very satisfactory results on the inhibition of both species *Botrytis cinerea* and Alternaria*alternata*. Also we can admit in our tests, we have tested our fungicide chemical vitro on growing strains, we assume that the absence of a significant inhibition returns to this probability. Earlier work showed that the mancozeb has a great preventive inhibitory activity against the helminthosporioses, rot and rusts [29]. His fongitoxicite is mainly attributed to its ability to generate the isothianate, which mainly inactive group thiol metabolites and enzymes in the cells. (Ragsdal, 1992), and so the fact to test in vitro is not considered a means of effective struggle against the three strains.

3.3. Statistical analysis of treatment with essential oils ANOVA

The statistical study by ANOVA with SYSTAT 12 software gives many results presented in table 4. The variables considered are the treatment (Rosemary, lavender), middle (PDA, Malt extract) and Concentration (0 µg/ml; 0, 2µg/ml; 0, 8µg/ml; 1.2 µg/ml; 1.6 µg/ml; 2 µg/ml and 2.4 µg/ml). the source of variation is the species (*Rhyzoctoniasolani*; *Alternariaalternata*; *Botrytis cinerea*) this procedure performs an analysis of variance to several factors for the variable 'species '. It displays various tests and charts to determine which factors have a statistically significant effect on this variable. It also tests if there is significant among factors interactions where there are sufficient data. F-tests in the ANOVA table allows us to identify significant factors. For each significant factor, tests on multiple scopes indicate you averages which are significantly different from each other. ANOVA table decomposes the variability of number in contributions due to various factors. The contribution of each factor is measured after elimination of the effects of the other factors. The values of probabilities test the statistical significance of each factor. If the P-value is greater than 0.05 significance level, we keep the hypothesis null (no significant difference between the samples). If instead the value of probability is less than 0.05, we conceive that there is a significant statistical difference between variables. Table 4 summarizes data from the ANOVA for all species for which variables treatment (Rosemary, lavender), middle (PDA, Malt extract) and Concentration (0 µg/ml; 0, 2µg/ml; 0, 8µg/ml; 1.2 µg/ml; 1.6 µg/ml; 2 µg/ml and 2.4 µg/ml) have significant effects.

An exhaustive analysis of the evidence leads us to conclude that all the variables are significantly different fungal strain to another, in other words that each of the studied parameters can influence deeply the resistance of the species in the inhibitor treatment of the essential oils of *Rosmarinusofficinalis, Lavandulaofficinalis*

Table 4: Summary table of the different variables studied by ANOVA

	Rhyzoctoniasolani		*Alternariaalternata*		*Botrytis cinerea*	
	Sum of squares	Probability	Sum of squares	Probability	Sum of squares	Probability
TRAIT	6,304	0	6,255	0	19,468	0
Medium	7,37	0	7,313	0	0,134	0,01
CONC	12,584	0	12,504	0	39,872	0
TRAIT*Medium	5,227	0	5,186	0	4,912	0
TRAIT*CONC	1,557	0	1,557	0	5,081	0
MILIEU*CONC	1,532	0	1,532	0	0,884	0
TRAIT*Medium*CONC	0,623	0	0,623	0	3,514	0

IV. CONCLUSION

In this work, we found a good activity of the oils of lavender to Rosemary; at low dilutions, the development of the strains was inhibited. Thus the culture medium is a factor involved in antifungal activity, its composition, the compounds in oils and the nature of the fungus are all elements that interfere in a way that allows an acceleration or delay of the Mycelial growth inhibition.

However, the fungus b. cinerea escaped to this probability.

Indeed, on the basis of the results, we can conclude that - the *AlternariaAlternata* strain is sensitive to the fungicide products and Rhyzoctoniasolani is strongest.

-The strain of *AlternariaAlternata* is sensitive to essential oils tested on the two culture media.
-The tested plant species have net and effective fungicide effects on growth in vitro of the three strains in a way variable but much more important than the chemicals. The antifungal activity of the *Lavandula* oils is best compared to that of *Rosmarinus* with important zones of inhibition.
In turn, the statistics revealed that the involvement of several components (nature of the treatment, the nature of the culture medium, and its concentration in the determination of the power of resistance of fungal strains to essential oils of *Rosmarinusofficinalis*, *Lavandulaofficinalis*.
Essential oils and their components are beginning to have a lot of interest as a potential source of molecules natural bioactive [30]. Our two oils from the PAM showed significant against the different test fungi inhibitory activity. Inhibition of the isolates was observed to *rosmarinus*and low doses of essences of lavender. This work illustrates one of the modes of likely use of these species in the field of biological control.

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